

Ambr® 250

Software Manual



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Revision Record

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Draft 0.02	26 October 2012	RBSO	Version for internal review
Draft 0.03	3 January 2013	DRL	Added manual liquid handling options
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1 INTRODUCTION

This document provides a comprehensive description of the Ambr® 250 software.

Important note:



This software described herein is designed to control an Ambr® 250 system. Other documents describe how to operate the system safely and how to get the best results from the system.

In particular the safety manual – TAP-9351-06-013 ambr 250 Safety Manual – explains the special safety procedures that must be followed when working with Ambr® 250.

1.1 Roadmap

Section 2 provides an overview of the logical concepts used in the system.

Section 3 then describes how to operate the software to define processes, to run the system and to view the results of completed experiments.

Sections 4 describes the parts of the software for defining the process and section 5 then describes the specific steps that can be used within the process definition.

The subsequent sections cover the remainder of the user interface following the general groupings of functionality within the interface.

Section 6 describes the set of pages which walk the operator through the stages of setting up a run on the system.

Section 6.15 describes the pages used to load and unload bottles, tube racks, plates and pipette tips.

Section 9 describes the pages for the direct control and monitoring of bioreactors.

Section 10 describes the pages showing graphs and tables with the results of running an experiment.

Section 11 describes the pages for controlling and monitoring the liquid handler.

Section 12 describes the pages for controlling and monitoring the Analysis Module.

Section 13 describes the pages for controlling and monitoring the pH station.

Section 14 describes the pages for controlling and monitoring the Cell Counter.

Section 15 describes the pages for controlling and monitoring the Flex2.

Section 16 describes the pages for controlling and monitoring the Biomass.

Section 17 describes the pages for controlling and monitoring the spot reader.

Section 18 describes the pages for controlling and monitoring the system supervisor that monitors various system wide properties of the system.

Section 19 describes the Notes feature for adding notes to the record of a process.

Section 20 describes some reports that can be displayed with details of the labware required for a run.

Section 21 describes aspects of the configuration of the system that the user can edit including if and to whom to send emails.

Section 22 describes how to export log files, which may be of use when troubleshooting.

Section 23 describes the control and operation of the mid-run priming feature.

Section 24 describes how to insert simulated data into the system when running in emulated mode.

1.2 Conventions

Labels from the user interface are shown **in this font**.

1.3 Glossary

ACW	Anti-clockwise
ambrAM	Analysis module that can be integrated into an Ambr® 250 or Ambr® 15 system to perform pH and other measurements.
CER	Carbon evolution rate – the rate at which the culture in the bioreactor is evolving carbon dioxide. Ambr® 250 systems fitted with off-gas sensors and running microbial cultures are able to calculate the CER.
Context menu	A menu of options relating to a particular part of the user interface. Accessed typically using the right mouse button.
CW	Clockwise
Dead volume	The dead volume in a piece of labware is the volume of liquid that cannot be removed from a bottle or from a well in the plate because the pipette tips cannot reach the absolute bottom of any labware
Design of Experiment	In general usage, design of experiments (DOE) or experimental design is the design of any information-gathering exercises where variation is present, whether under the full control of the experimenter or not. Within Ambr® 250 Design of Experiments refers to experiments varying parameters

	across the bioreactors in a systematic fashion.
DO	Dissolved oxygen tension: The amount of oxygen in solution as a percentage of air saturation
DOE tag	A label applied to a step parameter facilitating editing values of the parameter for Design of Experiment scenarios.
IMAP	A protocol for receiving emails
Keyboard wedge barcode reader	A barcode reader that acts like a keyboard. No barcode reader is supplied with an Ambr® 250 system, but any keyboard wedge barcode reader that sends the barcode followed by the return character can be used when loading labware.
Mandrel	A tool component that can be used to grip other moving tool components – specifically the bit of the liquid handler that is inserted into a pipette tip.
NaN	Not a number, numeric data that can be interpreted as a value that is undefined or unrepresentable
Nominal inoculation volume	A specified volume that can be used: <ul style="list-style-type: none"> • As the volume to have in the bioreactor just after it has been inoculated • As the volume to use when normalising OUR and CER results
OPC	Open Platform Communications, a series of standards specifications for use in process control and manufacturing automation applications.
OUR	Oxygen uptake rate – the rate at which the culture in the bioreactor is consuming oxygen. Ambr® 250 systems fitted with off-gas sensors and running microbial cultures are able to calculate the OUR.
pH	Standard measure of acidity
PID loop	Proportional-Integral-Derivative Control Loop where the output is set as a function

	of the error, the integral of the error and the rate of change of the error.
Process	A complete definition of an experiment to be run on an Ambr® 250 system.
Protocol	A list of steps and pump assignments that can be applied to one or more bioreactors that should follow a similar run.
SMTP	Simple mail transfer protocol – a standard protocol for sending emails
Step	Specifies an action to be performed by a bioreactor associated with the protocol containing the step.
Supervisor	<p>Part of the software performing automatic control of an element of the system.</p> <p>There is a supervisor for each bioreactor which is responsible for periodically polling the data for the bioreactor.</p> <p>There are also supervisors for the spot reader; a liquid handler if present; for a pH station if present; and a supervisor for general aspects of the system such as generating alert messages.</p>

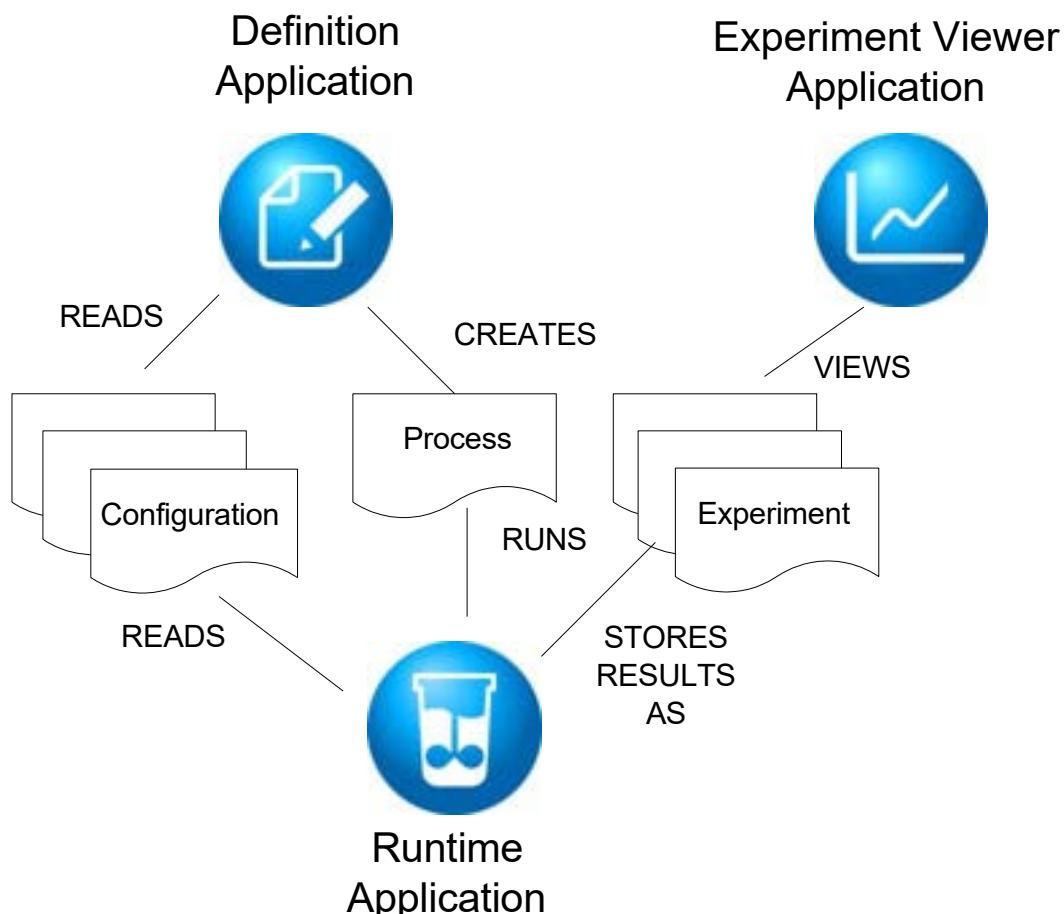
2 CONCEPTS

This section describes the core concepts and features of the Ambr® 250 system modelled by the software.

2.1 Configurations, Processes and Experiments

The Ambr® 250 software comprises three main applications: the definition, runtime and experiment viewer applications that interact with the configuration, process and experiment in different ways (as represented in the diagram below).

- The definition application is used to create and edit processes ahead of an experiment.
- The runtime application uses processes to control the hardware. This is typically done using a process definition created in advance, but runtime also allows the process to be altered as the experiment progresses.
- The experiment viewer application lets one view the results of completed experiments.



2.1.1 Configurations

A configuration defines details of an Ambr® 250 system and would not normally need to be changed by a user. The configuration includes many details including:

A configuration defines details of an Ambr® 250 system. The configuration includes many details including:

- How many bioreactors are part of the system
- How the bed of the system is laid out
- Whether the system has a liquid handler
- Which communication ports are to be used to talk to components of the system
- Which experiment is in progress at the moment
- ...

The configuration is stored as a folder of files. The figure below shows some of the files in a sample configuration.

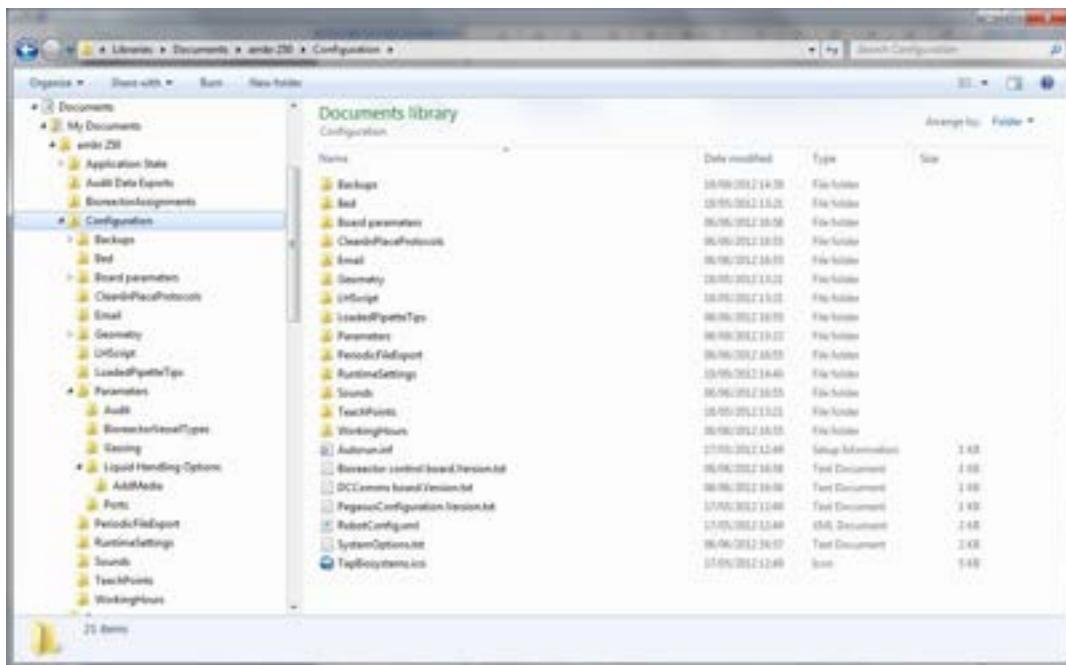


Figure 1 Configuration files

The definition application uses the configuration to know what options are valid for the process being edited. The same process can be edited using different configurations but aspects of the process may only be valid with a particular configuration. The definition application lets the user choose the configuration to be used.

The runtime application uses the configuration to control how it talks to the hardware. On a system connected to hardware there should only be a single configuration. The configuration files are stored in the \Configuration folder on a USB stick mounted on the DC/Comms board of the system.

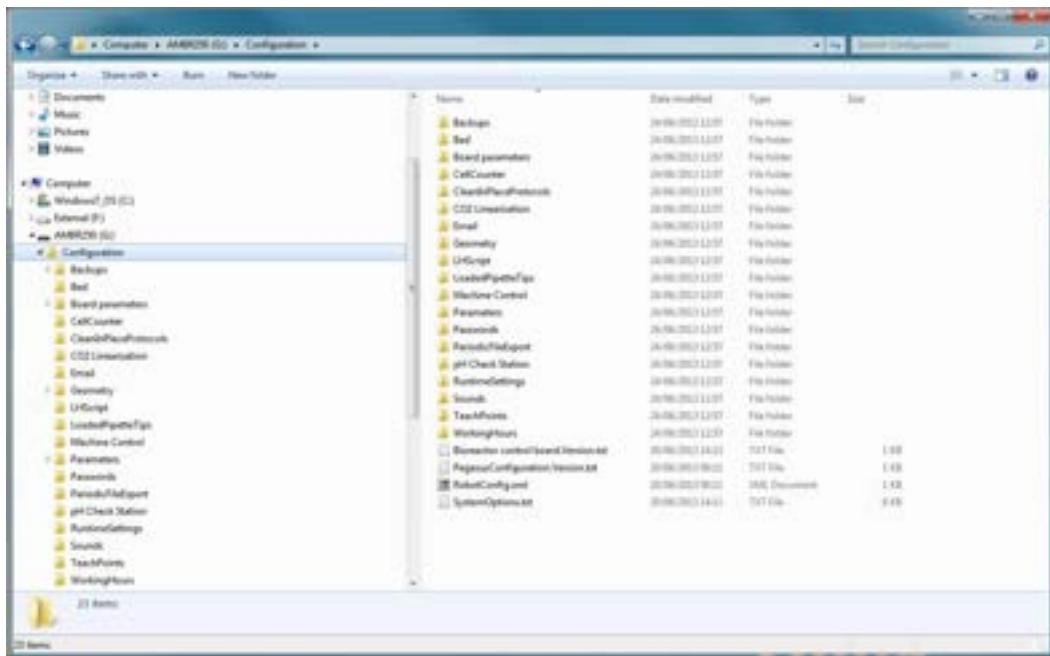


Figure 2 Configuration files on USB stick (runtime)

The experiment viewer uses a copy of the configuration stored within the experiment results.

If the Ambr® 250 software is to be used offline then a copy of the configuration files should be placed in the My Documents\ambr 250 folder on the system. The system looks within the ambr 250 folder for all folders with 'Configuration' in their name when looking for configurations. (If the runtime software is to be used offline with a configuration then the configuration must be edited to indicate to the runtime software that there is no hardware attached to the system.)

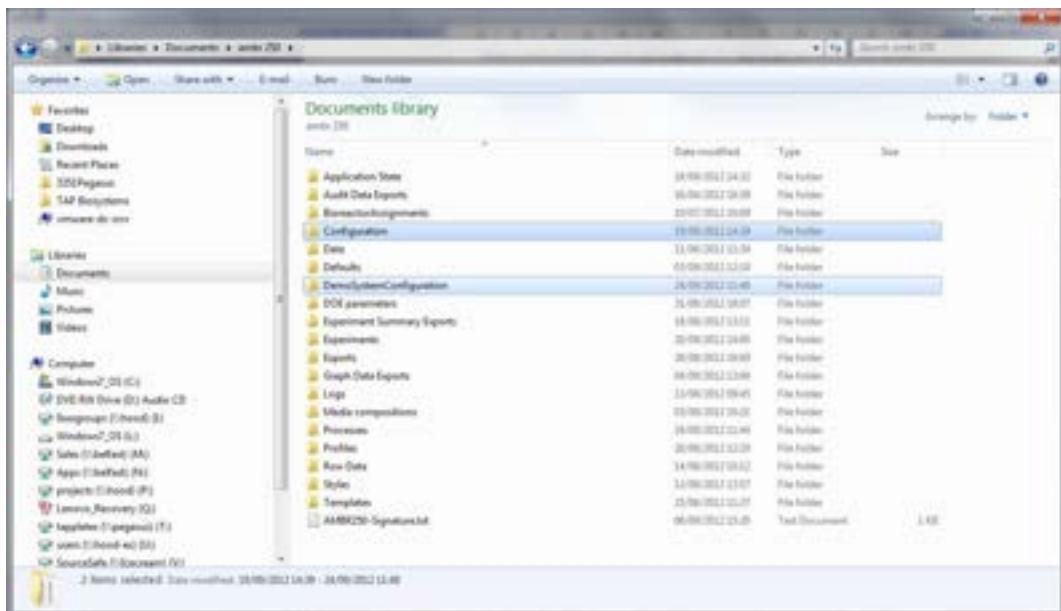


Figure 3 System with two configurations installed

2.1.2 Processes

The process defines how the hardware will be operated and includes details such as:

- What conditions and set points (pH, T, DO) will be used in individual bioreactors and the timing of their implementation
- Instructions for adding liquids to bioreactors and taking samples from bioreactors
- What liquids are to be used in the pumps
- The control loops that will be used
- Pumping regimes for feed solutions.
- What cells will be used in which bioreactors
- Definitions of alarm conditions that will be monitored

Processes can be defined in advance of a run using the **ambr250 Definition** application and are stored as single files. By default processes are saved in the ambr 250\Processes folder, but they can be saved anywhere.

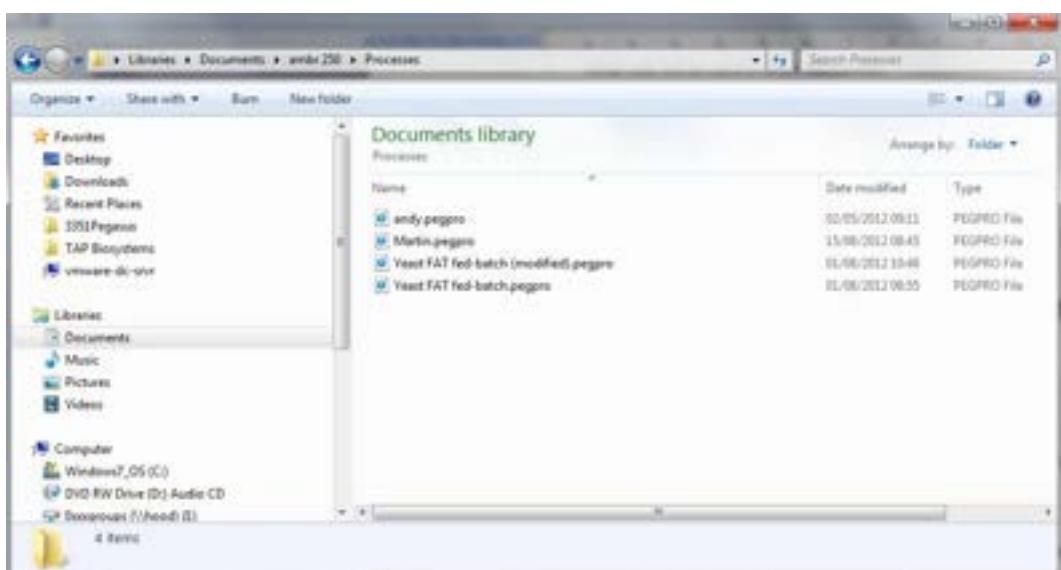


Figure 4 Processes stored as single files

2.1.3 Experiments

An experiment contains and records all the information the system needs to run and report back on a culture. The Ambr® 250 system is always running an experiment and logging data that can be accessed later. New experiments can be started either by creating a blank experiment or by creating an experiment using a saved process.

2.2 Protocols and Steps

A process definition contains a number of protocols.

Each protocol defines settings for a subset of the bioreactors in the system. Specifically the protocol:

- defines the sequence steps to be followed by the bioreactors in order to run the desired process
- defines some key volumes
- defines the allocation of pumps to different roles

Bioreactors assigned to the same protocol share the same definition of the steps to follow but can have different parameters – for example they can have different pH set points.

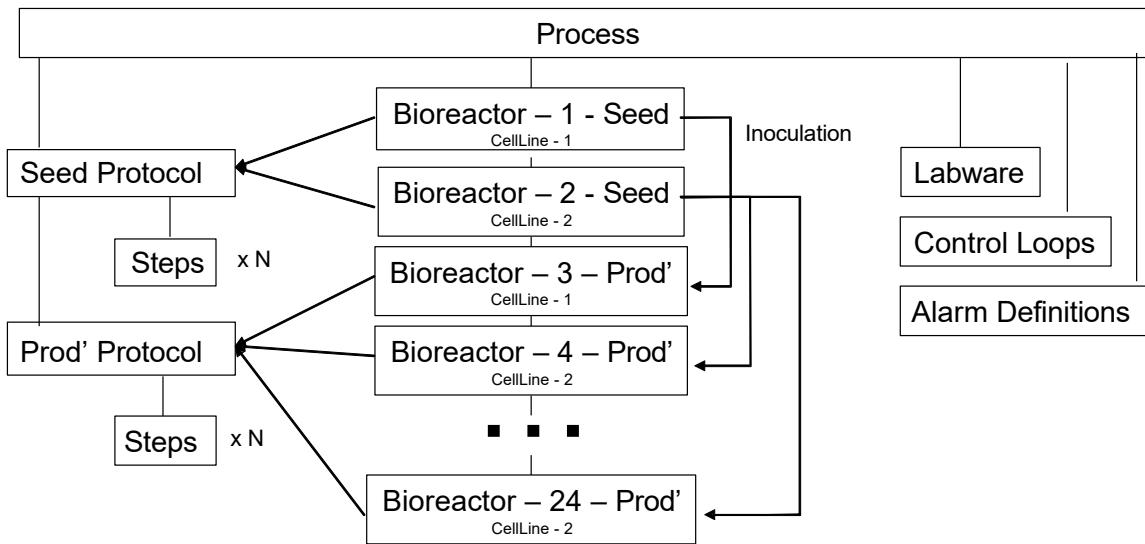


Figure 5 Protocols and Bioreactors

The steps define planned actions to be taken to run the system. There are five basic sorts of steps:

- ‘Toggle’ steps – these turn things on and off in a process and will operate in the background of the software until a new step indicates otherwise. e.g: set points (temp.; pH; DO); control loop; monitoring of sensors (DO reader)
- Pumped liquids – these activate pumping operations and like ‘Toggle’ steps will operate in the background of the software, e.g. pumped bolus shots; feeding regimes
- Liquid handling operations – these will remain active until the operation has been completed for that bioreactor, e.g. bolus additions; sampling
- Process ‘structural’ steps – these control the timings of when other steps will occur, e.g. ‘wait 8 hrs’; conditional steps, prompts for the user to respond to an action
- Grouping steps – these group steps together to break up a long series of steps or to support multiple parallel series of steps.

2.2.1 Parallel Blocks

The steps for a bioreactor are executed in turn with the next step not starting until after the previous step has finished. A **Parallel Block** can be created for multiple steps to happen in parallel for the same bioreactor. This is typically done where the order of the steps should be different depending on how long different things take to happen.

The figure below shows some steps with a parallel block. The system will execute step 1, then steps 2,3,4,5 and will then start the parallel block. Steps 7 and steps 6.1 are then both available to be executed. Once step 7 is complete step 8 will be free to start and once step 6.1 is complete step 6.2 will be free to start. Step 8 is no longer dependant on step 6.1 or 6.2

completing.

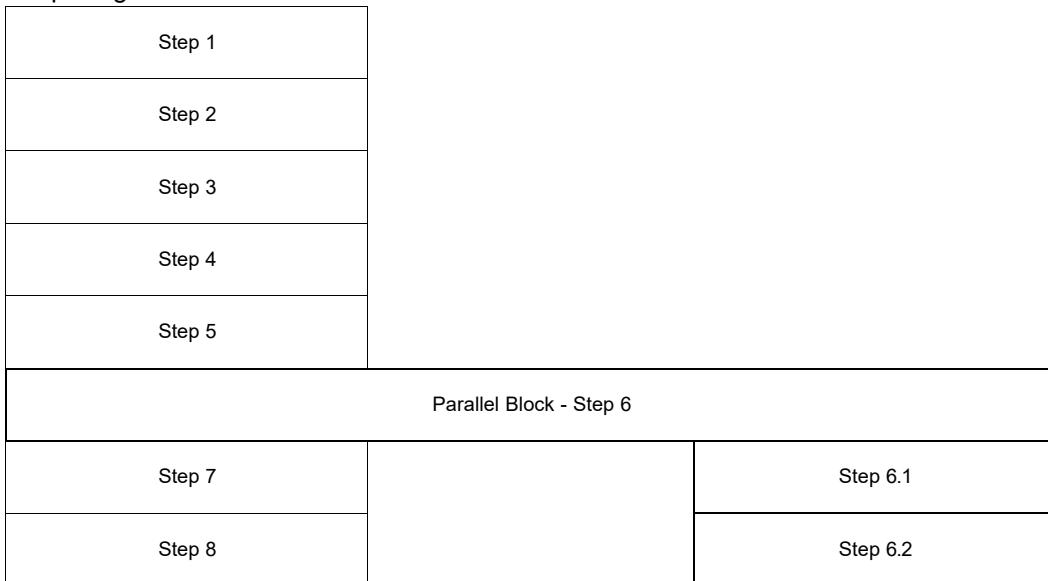


Figure 6 Steps with **Parallel Block**

2.2.2 Phases

The sequence of steps can be divided up into **Phases**. Phases:

- provide a grouping of steps to make the process more readable
- allows the user to define a step not to start until an interval of time after the start of the phase – for example one could have a step to stop pumping that started 100 hours after the start of a phase.

A simplified process with phases is shown below.

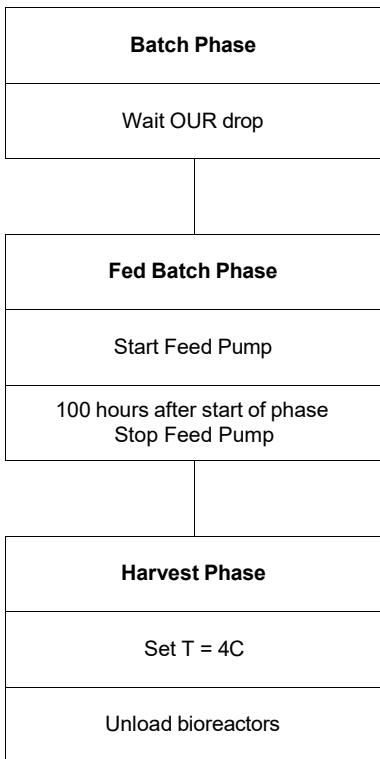


Figure 7 Simplified process with Batch, Fed Batch and Harvest Phase

2.3 Set points and Control loops

The control of the bioreactors is defined by their set points – that is the target values to be achieved for aspects of the bioreactor.

Each bioreactor has a number of parameters for which set points can be defined:

- Temperature
- Stir speed and direction
- Gassing rates
- pH
- DO
- Pumping

Some of these parameters are directly controlled by system. The control boards for the bioreactors control the heaters and chillers to maintain the temperature; the motor to control the stirring; the valves to control the gassing; and the syringes to control the pumping.

Control of other parameters is done via more generic control loops which in turn set the values of the directly controlled set points. Different control loops can be defined and used depending on the application. Typically one control loop is defined to pump or gas acid to control pH downwards; one control loop is defined to pump a base to control pH upwards; a third control loop sets the stir speed and the gassing rates in order to control DO.

Switching on the value of a set point that is not directly controlled requires the control loop that will be used to effect the set point to be specified. Except for pH where a separate acid and base control loop can be specified just a single control can be specified.

Additional control loops can be defined that are declared to run a condition rather than to control a specific set point. These additional control loops can be enabled or disabled by steps in the process. A typical use for such a control loop is to pump antifoam in response to a signal from a foam sensor on the system.

Switching on a set point and associated control loop(s) whether built into the system or defined by the user disables the values previously set for the things set by the control loop. For example:

- 1) Set Stir Rate set point to 3000 rpm
- 2) Enable DO control with stir rate as one of the outputs of the control loop. The stir rate is set by the control loop.
- 3) Disable DO control – the stir rate is now turned off

2.3.1 Set point profiles

General set points can be defined in a number of ways. (There is a different set of options for pumping that are described in the next section.)

A set point can be defined:

- To be off
- To have a specified value
- To have a value calculated from an expression
- To ramp from the value of the set point at the moment the set point was altered to another value at either:
 - A fixed rate
 - Or over a fixed interval
- To follow a profile specified as a list of time/value pairs

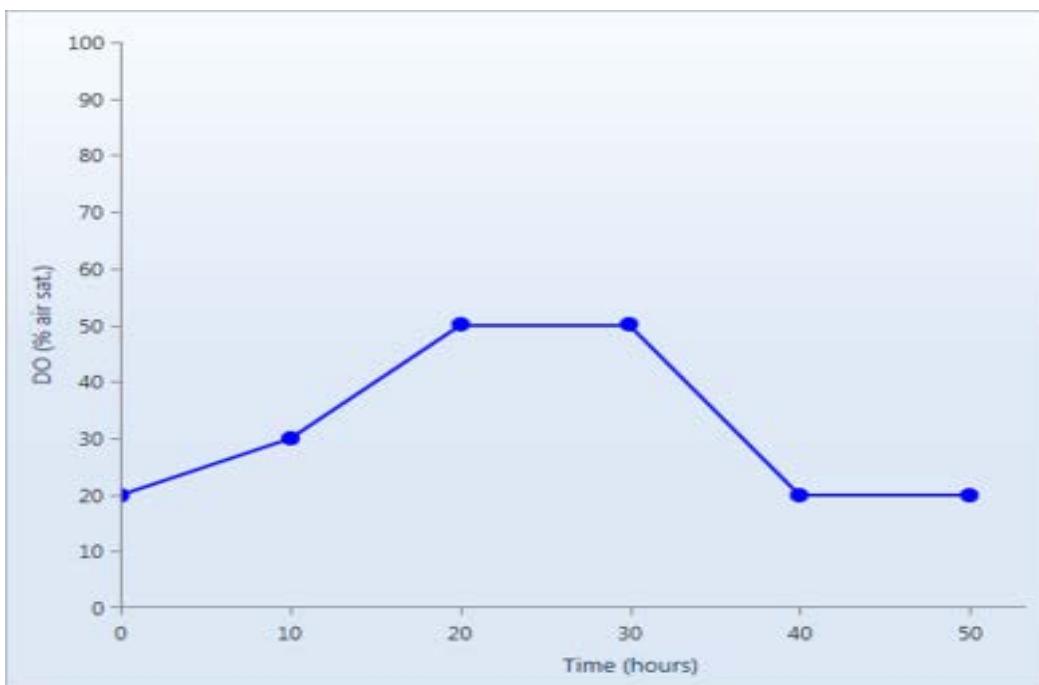


Figure 8 Simple profile defined by values at different times

2.3.2 Control Loops

The system supports a number of types of control loops.

- PID loops with one or more levels of cascade
- Loops that deliver a bolus when a condition is true
- Loops that set the value of a set point based on a lookup table

2.4 Custom Variables

Custom variables are user defined parameters that can be either calculated values where the value of the variable is recalculated each time the bioreactor polls values or can be for data entered by the user.

Custom variables can be associated with a set point. In that case the set point can be set with a profile just like the in-built set points.

2.5 Pumping

Each bioreactor has four pumps.

Typically the matching pumps on adjacent bioreactors are connected to a single tube line connected to a single container e.g. pump 'A' on every bioreactor might be connected to the single acid container. More general arrangements are possible e.g. blocks of four bioreactors can each be connected to their own feed containers.

The tubing connected to the pumps can be reused from run to run or can be replaced for each new run.

The overall procedure to set up a pump is:

- 1) connect tubing to pump if required
- 2) clean/sterilize tubing and pump if required
- 3) prime tubing so that the tubing and pump are completely full of liquid if required
- 4) connect bioreactor to pump by coupling the liquid manifold on the bioreactor to the fixed manifold on the system
- 5) if required prime the bioreactor inlet by pumping liquid so that the meniscus of the liquid is just at the top of the bioreactor

Step 1) is not required if the tubing is being reused.

Steps 2 and 3) are not required if the liquid in the tubing is being reused.

Step 5) is only required if fine control of the volume of liquid pumped initially is required. Moving the liquid to the top of the bioreactor reduces uncertainty from variations in the volume of bioreactor inlet tubing and filter between the liquid manifold and the end of the feed line inside the bioreactor.

2.5.1 Pump setup

The user can define the role of the pumps associated with a protocol – e.g. one pump can be assigned to pump acid and another to pump feed. Steps within a protocol refer to pumps by their role e.g. “Pump 10mL of Feed#1”

The name of the liquid fulfilling the role can be different for each bioreactor e.g. Feed#1 could be Glucose for bioreactor 1 and could be Glycerol for bioreactor 2. All the pumps attached to the same tube line must have the same role and the same liquid name.

The pump setup also defines whether the bioreactor inlet should be manually primed or whether the volume of bioreactor inlet tubing and filter between the liquid manifold and the end of the feed line inside the bioreactor should be assumed to have its nominal value.

2.5.2 Pumped liquid classes

The properties of the liquids to be pumped are defined in “Pumped liquid classes”. These define the limits for how quickly the liquid can be pumped through any filter on the bioreactor inlet and how quickly the pump can be refilled.

If issues are found with cavitation inducing gas bubbles in the tubes feeding the pumps then a slower refill rate should be considered

2.5.3 Tubing

The layout of the tubing to the pumps can be defined in the software.

The layout is then available when setting up the tubing on the machine.

The layout is also used when priming the tube lines to know how many bioreactors are sharing a tube line and therefore how fast the pumps can refill themselves.

2.5.4 Clean/sterilize in place

Protocols can be defined to clean/sterilize in place tube runs and pumps.

The protocols comprise pumping defined volumes of liquid or air through the pumps and tubing with suitable pauses whilst sterilizing agents have their effect.

2.5.5 Profiles

The flow rate of a pump works similarly to other set points, but has a different set of options for control which include:

- turning the pump off
- pumping at a fixed rate
- pumping a fixed volume of liquid (bolus)
- pumping at a rate increasing or decreasing linearly with time
- pumping at a rate increasing or decreasing exponentially with time
- following a profile specified as a list of time/value pairs

2.6 Gassing

This section describes the controls available for gassing in Ambr® 250. The specific controls available on an individual system will depend on the configuration of that system and if multiple gassing options are part of that configuration on which gassing option is chosen. Typically not all of the controls will be available within a single gassing option.

If the system supports both headspace gassing and sparge gassing then the controls available for headspace and for sparge gassing are configured independently.

2.6.1 Gassing parameters

This section describes the normal options for setting the desired gas flows.

One gas is designated as the Primary gas for the system. This gas is controlled by the set point Gas flow (XXX/mix) where XXX is the name of the primary gas e.g. **Gas flow (Air/mix)**

The other gasses connected to the system can be configured to have an added flow e.g. **O₂ added flow** and/or a mix flow e.g. **O₂ mix**.

An added flow is simply added in parallel with the other gas flows.

A mix flow replaces the corresponding percentage of the primary gas flow. A mix flow is ideal when control of dissolved oxygen is to be done by progressively replacing air with oxygen whilst maintaining a constant total gas flow rate and therefore VVM (vessel volumes per minute).

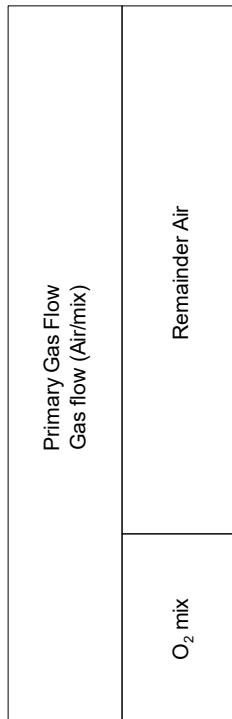


Figure 9 Division of primary flow with mix and added flow – a percentage of the air flow is replaced by the O₂ mix. The CO₂ added flow is added to the air and O₂ flows.

From the various gas flow set points (**Gas flow (Air/mix)**, **O₂ mix**, **CO₂ added flow**, ...) the system computes the set points for each gas. The bioreactor PCB computes from the set points how open the valves should be. The gasses are connected to a mass flow sensor and the PCB computes a separate correction factor for each gas to adjust the valve open time to more accurately match the requested flow rate.

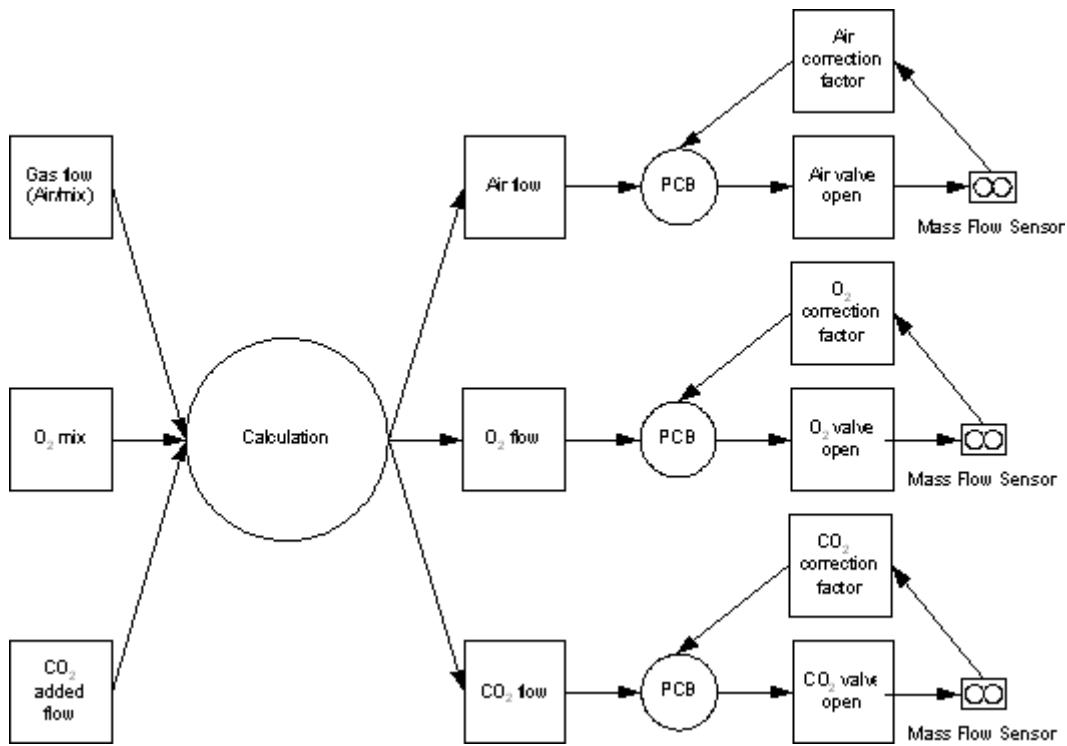


Figure 10 Gas parameter flow

2.6.2 Gassing control

Gassing is controlled by valves that are switched on for a defined percentage of a cycle time to control the flow of the gas attached to the valve. The longer the valve is on the higher the gas flow. The flow of gas may be monitored by a mass flow sensor and the percentage of time the valve is on adjusted automatically to provide the flow requested by the user.

Under some circumstances the system may be unable to deliver the requested gas flows.

If the requested gas flow requires the valve to be on for more than 95% of a cycle then the flow will be limited.

If the aggregate time that all the valves are on is more than 95% of a cycle then the system will be unable to make use of the mass flow sensor which is shared between multiple gasses.

If headspace gassing is used then the valve time is shared between the headspace gassing and the sparge gassing.

If excessive pressure is detected in the bioreactor then the PCB will stop gassing as long as the pressure continues.

It is possible to have gassing options that limit the values of the gassing set points so that the gassing should be achievable subject to any blocking of sparge tubes or filters. It is also possible to have gassing options that allow more flexibility in what can be specified at the risk of allowing the process to request a gas flow that cannot be delivered.

2.6.3 Carbon dioxide flushing

The system contains a feature to avoid issues with carbon dioxide being left in the sparge tube.

If carbon dioxide has been gassed to a bioreactor then when the bioreactor is asked to stop gassing a pulse of nitrogen if available or else air is gassed to the bioreactor to flush out the carbon dioxide.

2.6.4 Background sparge flow

The system contains a feature to provide a flow of sparge gas when no other gassing is happening to reduce the incidence of liquid being pushed up the sparge tube. When no gassing is requested a short pulse of the primary gas is generated periodically.

2.7 Off-gas Analysis

The Ambr® 250 system can be configured with sensors that monitor the oxygen and carbon dioxide percentages in the gas coming off the bioreactor.

Using these percentages together with the gas flows going into the bioreactor the system can estimate the OUR and CER of the culture.

OUR and CER calculation is not applicable for mammalian cultures where gas flow rates are low and where carbon dioxide being dissolved or evolved from the buffering solution would distort the results.

The system initially calculates the total uptake/evolution rate of the culture in mmol/h. This is then normalised to the volume selected by the user from: the actual culture volume as estimated by the system; a nominal inoculation volume; the actual inoculation volume as estimated by the user.

When a liquid handler is used then the gas in the headspace and in the exhaust lines is disturbed when the liquid handler removes the cap from the bioreactor. Offgas results are filtered from the time of the cap being removed until an interval after the cap has been replaced to reduce the risk of incorrectly triggering any actions in response to the disturbance.

Table 1 Off-gas variables

Variable	Description	Units
CER	The carbon evolution rate normalized by the volume specified in the 'System Options'. Results are filtered to ignore fluctuations after the cap is removed from a bioreactor.	mmol/L/h
CER - integrated	The integrated carbon evolution normalized by the volume specified in the 'System Options'	mol/L
CER - raw, unnormalized	The total carbon evolution rate of the culture. Results are NOT filtered to ignore fluctuations after the cap is removed from a bioreactor.	mmol/h

CER - unnormalized	The total carbon evolution rate of the culture. Results are filtered to ignore fluctuations after the cap is removed from a bioreactor.	mmol/h
CER - unnormalized, integrated	The total amount of carbon dioxide evolved by the culture.	Mol
Normalisation volume	The volume used to normalise OUR and CER values	ML
Off-gas calculations suppressed	True during the interval that off-gas calculations are suppressed between taking the cap off a bioreactor to a certain time after the cap has been replaced	
Off-gas CO ₂ %	The measured percentage of carbon dioxide (volume/volume) in the off-gas	%
Off-gas H ₂ O%	The calculated percentage of water (volume/volume) in the off-gas	%
Off-gas O ₂ %	The measured percentage of oxygen (volume/volume) in the off-gas	%
OUR	The oxygen uptake rate normalized by the volume specified in the 'System Options'. Results are filtered to ignore fluctuations after the cap is removed from a bioreactor.	mmol/L/h
OUR - integrated	The integrated oxygen uptake normalized by the volume specified in the 'System Options'	mol/L
OUR - raw, unnormalized	The total oxygen uptake rate of the culture. Results are NOT filtered to ignore fluctuations after the cap is removed from a bioreactor.	mmol/h
OUR - unnormalized	The total oxygen uptake rate of the culture. Results are	mmol/h

	filtered to ignore fluctuations after the cap is removed from a bioreactor.	
OUR - unnormalized, integrated	The total amount of oxygen evolved by the culture.	Mol

2.8 Alarms

Alarms monitor a specified condition and when the condition is not met an action is activated..

The actions available include:

- alerting the user
- stopping the liquid hander
- stopping gassing on a bioreactor
- stopping stirring on a bioreactor
- stopping liquid handling on a bioreactor
- stopping pumping on a bioreactor

The system automatically defines some alarms and the user can define additional alarms.

2.9 Supervisors

The components of the system are run by ‘supervisors.’ An Ambr® 250 system may contain:

- a bioreactor supervisor for each bioreactor
- a system supervisor that monitors and controls aspects of the enclosure such as laminar air flow
- a spot reader supervisor that reads spots in the bioreactors
- a liquid handling supervisor that runs the liquid handler
- a pH check station supervisor that monitors and control a pH check station (optional)
- a cell counter supervisor that monitors and controls a cell counter (optional)

The basic operation of these supervisors is described more fully below.

In normal operation the supervisors should be running and are only stopped in order to create the next experiment or to bring the system to a stop.

The liquid handler may be paused without disturbing other aspects of the system to allow safe access to the bioreactors, samples or labware

2.9.1 Bioreactor supervisors

When running, a bioreactor supervisor periodically:

- queries data from its bioreactor

- updates calculated variables
- deals with any clean in place or priming operation
- runs the steps in the process
- updates the set points and calculates the current values of set points following profiles
- runs the control loops
- runs the alarms
- write the new values of set points to the bioreactor

When the supervisor is stopped it sends instructions down to the bioreactor to stop gassing, pumping, stirring and controlling temperature. Typically the bioreactor supervisors are started the first time it is necessary to do some pumping on the system and not stopped again until the end of the experiment.

2.9.2 System supervisors

The system supervisor works in the same general way as the bioreactor supervisor.

When running on a system with the appropriate hardware it:

- monitors laminar air flow
- monitors errors from the system DC/Comms board
- monitors pump supplying wash fluid to the syringe pumps
- controls freezer
- controls magnetic stirrer

The bioreactor pumps are disabled if there is a problem with the pump supplying wash fluid to the syringe pumps. The system supervisor therefore always has to be running whilst the bioreactors are running.

The liquid handler is paused if there is a problem with the laminar air flow. The system supervisor therefore always has to be running whilst the liquid handler is running

2.9.3 Spot reader

The spot reader when running periodically reads the spots on each bioreactor where spot reading is enabled.

The spot reader also – on systems with appropriate hardware – monitors the gas pressures being delivered by the regulators inside the system and raises a warning message if the pressure is too high or too low.

Gassing to bioreactors is disabled by a hard-coded alarm if the gas pressure is too low. The spot reader therefore always has to be running whilst the bioreactors are running.

2.9.4 Liquid handler

The liquid handling supervisor controls the liquid handler.

Steps and control loops that need liquid handling put an action describing the required liquid handling into a queue.

When running the liquid handling supervisor looks at the actions in the queue and runs the highest priority action available. The actions in the queue have a numerical priority and have a time when the action became available. The actions are sorted first by the numerical priority with the highest number taking precedence and then by the time with the oldest action among the actions with the same numerical priority taking precedence.

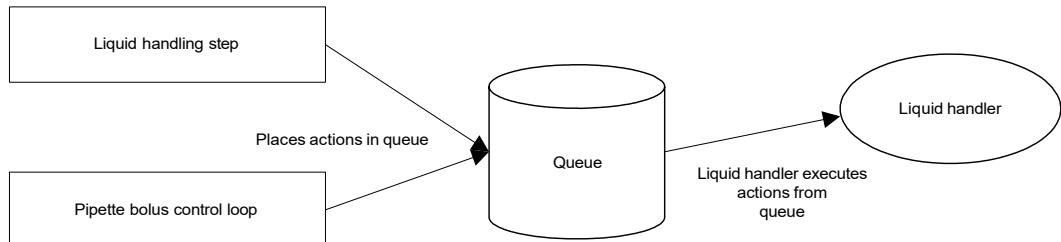


Figure 11 Liquid handler queue

Just before the action is run some final checks are made that the system is in a position to perform the action. If the checks fail then the action is marked as being in error and the liquid handling supervisor will look at other actions it might be able to perform.

How the liquid handling is done is defined by a hierarchy of definitions:

- “Liquid handling methods” define a sequence of movements of the pipette head and the piston in the liquid handling head. Different speeds and pauses are used to optimize the liquid handling for particular scenarios.
- “Pipetted liquid classes” define for a particular sort of transfer such as from one bioreactor to another bioreactor which liquid handling methods and what sort of tip should be used.
- Individual steps or control loops specify which “Pipetted liquid class” to use.

2.9.4.1 Clean/sterilize in place

A procedure is defined that can be used to clean/sterilize the liquid handler mandrels and piston.

2.9.5 pH station

The pH station supervisor when running:

- calibrates the pH station ahead of samples
- places the pH station into storage solution when it is not being used
- optionally controls the temperature of the pH station to match the temperature of the samples to be taken.

2.9.6 Cell counter

The cell counter supervisor when running periodically monitors the state of the cell counter and checks for cell count actions in the liquid handler queue.

The supervisor will automatically ready the cell counter for a cell count and perform a clean of the cell counter between cell counts if necessary. If no cell counts are due for an extended period the supervisor will shut down the cell counter.

2.9.7 Flex2

The Flex2 supervisor when running:

- periodically monitors the state of the Flex2
 - checks for analysis actions in the liquid handler queue.

The supervisor will automatically update the analysis panel configuration from the Flex2 on starting the supervisor.

2.9.8 Biomass

The biomass supervisor when running checks the state of the biomass monitoring devices.

299 Chillers

The chillers supervisor monitors the state of the chillers attached to the system. If the system is configured for dual chillers it is responsible for switching between the chillers on the duty cycle and failover to the standby chiller if the duty chiller faults.

2.9.10 Washflow monitor

The washflow supervisor when running checks the state of the washflow around the individual liquid pump blocks

2.9.11 Spectrometer

The Spectrometer supervisor when running monitors the state of the spectrometer server and checks for spectrometer actions in the liquid handler queue.

2.10 Messages



Figure 12 Messages for liquid handler

Supervisors and other parts of the system can generate messages indicating problems or providing information about what they are doing.

The left hand column shows the severity of the error. The icon for the severity flashes if an error represents a continuing condition. The icon stops flashing when that condition goes away.

Details... shows technical details of the error. These details should be included if an issue needs to be raised because of the problem.

2.11 Labware

The plates, bottles and tube racks to be used by the liquid handler must be defined as part of the process.

The labware is assigned a role from:

- Liquid source
- Sample
- Waste
- Inocula
- Multiple uses

which controls where the labware is available and what parameters are required on the labware.

Each piece of labware is assigned a position which is where the system will ask the user to load the labware. The position can be changed as required.

The system looks at the steps and the control loops referencing the labware to work out when the labware is needed. The system prompts the user to load the labware before it is needed and to unload the labware when it is no longer needed.

The system similarly tracks how many pipette tips are likely to be needed and prompts the user to load and unload pipette tips as required.

At the start of an experiment the system assumes:

- **Plates, bottles and tube racks from previous runs have been unloaded and nothing has yet been loaded for the current run**
- **The pipette tips loaded on the system have not been changed**

2.12 Forward modelling

The system models what steps will happen when in order to give feedback on what the process will do and to be able to prompt when labware should be loaded and unloaded.

The model is based on some simplifying assumptions and is therefore an approximation. The predictions about the near future are more accurate than predictions about the more distant future. The predicted time for steps on future days is likely to change unless the steps have been specifically planned to happen at a particular time in the definition of the process.

Steps that wait for a condition to happen are assigned a minimum and a maximum wait time. The system considers two scenarios: one where all waits take the minimum assigned time and one where all waits take the maximum assigned time.

Actions taken by the liquid handler have a simple estimate made of their duration. This estimate does not take into account any special liquid handling within the steps that can make a material difference to their speed.

Actions from control loops are ignored when considering how long it will take the liquid handler to perform the actions from the steps in the process.

The system assumes that the liquid handler performs its actions following its standard priority algorithm, but when many actions are queued at a similar time the simulation may queue actions in a different order than the real system will queue the actions. The overall time to finish steps is typically not affected by this, but the order in which different bioreactors will complete liquid handling steps is typically different in reality from the model.

2.13 Working hours

The system has a concept of working hours. These are used:

- When prompting to load or unload labware the system will preferentially prompt for the action to take place inside working hours.
- When sending emails different destinations may be used inside and outside of working hours

2.14 Email interface

The system can:

- Send emails with alerts or status messages
- Receive emails and send a status message in response

2.15 Periodic file export

The system has a file export facility that can be used to send data from the system to a corporate database or LIMS system.

Data is exported for a bioreactor if the periodic file export is validly configured for the system as a whole and export has been explicitly enabled for the individual bioreactor.

2.16 OPC

The system can optionally be supplied with the ability to communicate over OPC.

The OPC interface is a separately licensed feature that is not enabled by default.

See the separate document **TAP-9351-06-100 OPC Interface Manual** Draft for more details if required.

3 RUNNING THE AMBR® 250 PROGRAMS

3.1 Licensing

The software requires a licence file from Sartorius to run. The licence file authorises running of the software and may also enable particular optional features within the software.

When the software is run on a machine it will display a code.



Figure 13 License dialog

Email this code to royston-support@sartorius.com (UK/EU/RoW) or NA_TAP-Support@sartorius.com (USA/Canada) who will supply you with a licence file to enable the software on that particular machine.

Use the **Load new signature file...** or place the licence file in the [My Documents]\ambr 250 folder to enable the software to run.

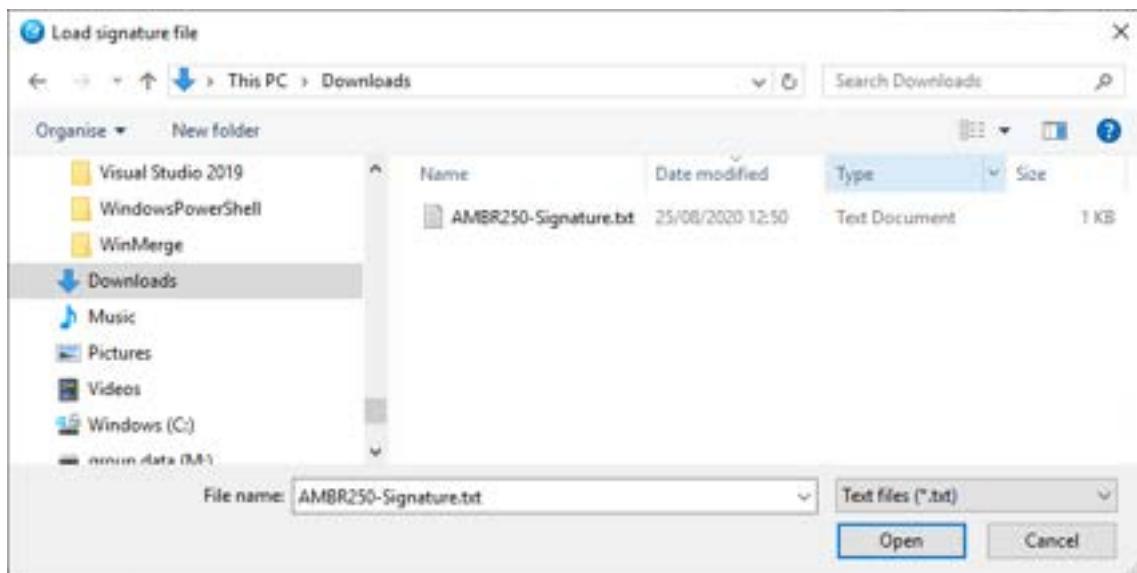


Figure 14 Load signature file dialog

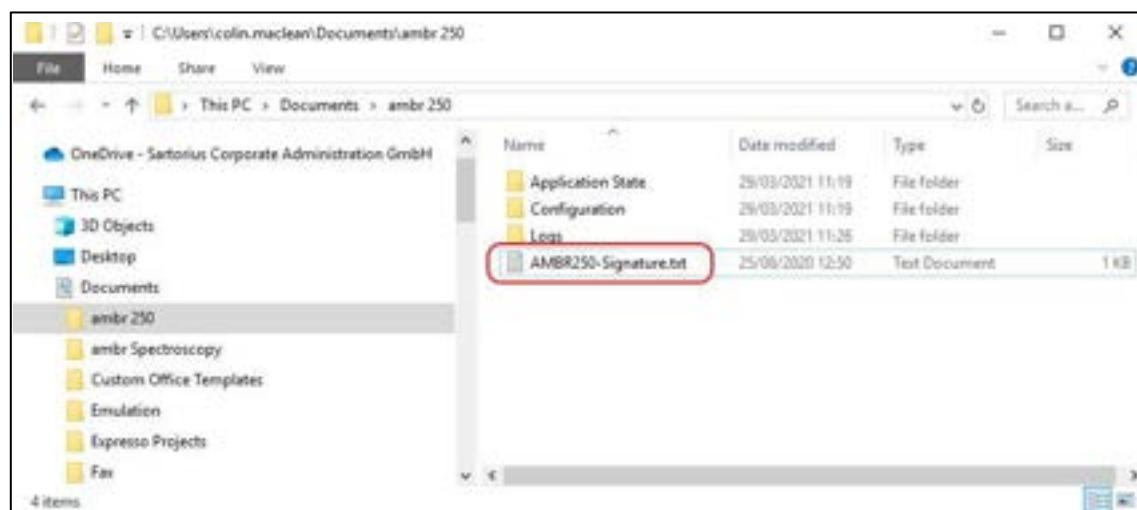


Figure 15 ambr 250 folder with license file in the correct location

3.2 User interface features

This section describes the common features of the core Ambr® 250 programs.

The three core programs – definition, runtime and experiment viewer – all have the same structure. The applications differ in which pages of information are available and what is editable.

3.2.1 Main Window

The figure below shows the main window of the runtime application.

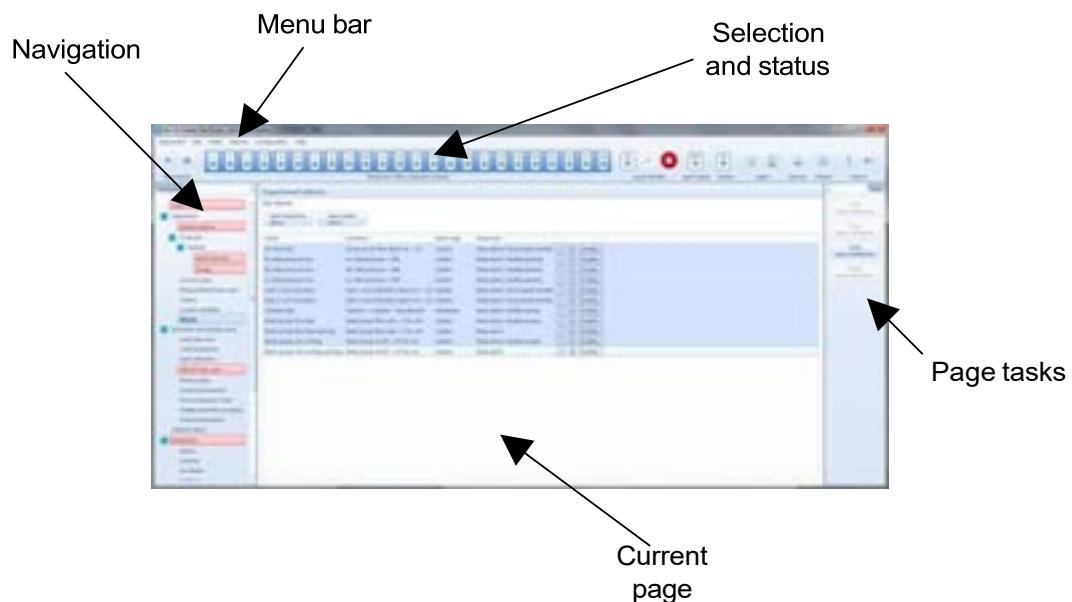


Figure 16 Runtime application main window

The main window contains:

- A menu bar
- A top area with an image for each bioreactor to allow selection of bioreactors and at runtime to show the status of the bioreactors. Additional controls are shown in the top bar for other aspects of the system at runtime.
- A navigation panel for selecting the page to display
- A current page displayed in the central part of the window
- When applicable a panel on the right showing tasks for the current page

3.2.2 Navigation

The navigation panel shows the pages available in the application and highlights pages which need attention.

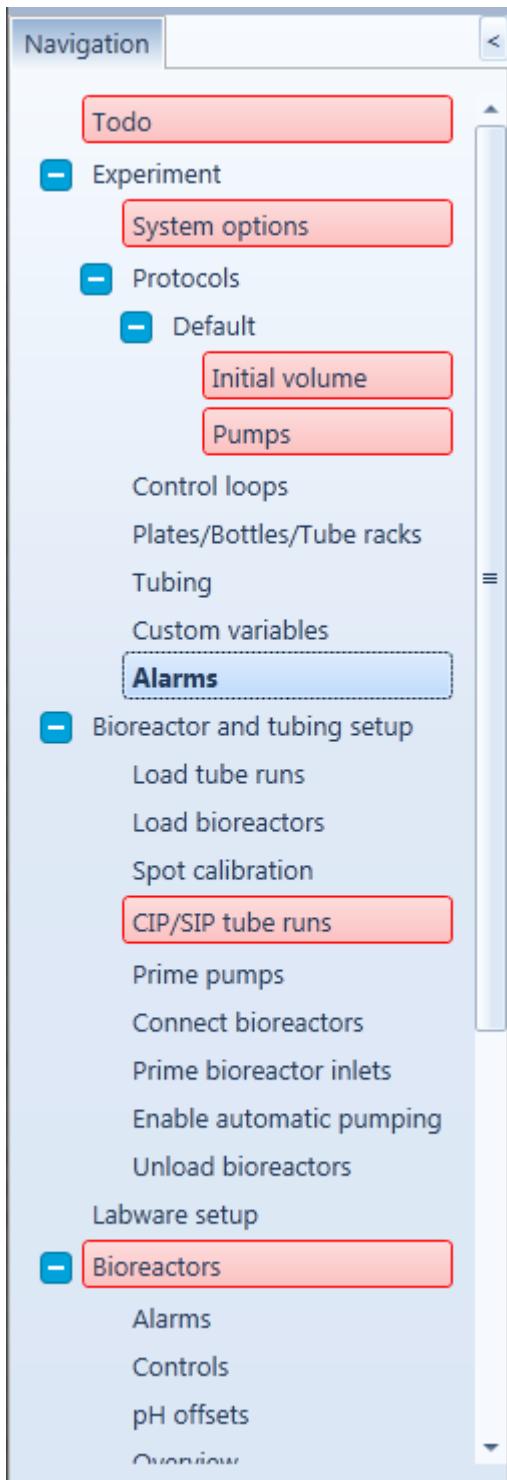


Figure 17 Navigation panel

The currently selected page is shown in bold.

Click on the required page to select a page.

Pages where there is invalid data, errors shown or something needing to be done are highlighted in pink.

The highlight flashes for pages where there is something to be done now for the run to progress.

3.2.2.1 Editing and Deleting Items

To edit an item in a list you can:

- click on the edit button () for the item
- double click on the item

To delete an item in a list you can:

- click on the delete button () for the item
- use the Delete option from the Edit menu
- use the Delete key

3.2.3 Undo and redo

Undo and redo are supported for changes to the process definition.

This feature is supported both in the definition and runtime applications. Changes to steps as part of undo and redo are runtime are subject to the same restrictions as making the changes by hand. For example if a step has been started it will no longer be possible to undo edits made to the step.

The options Undo process changes and Redo process changes are shown on the pages where undo and redo are supported.

Undo and redo can also be invoked from the main menu or using the standard keyboard shortcuts of Ctrl-Z and Ctrl-Y.

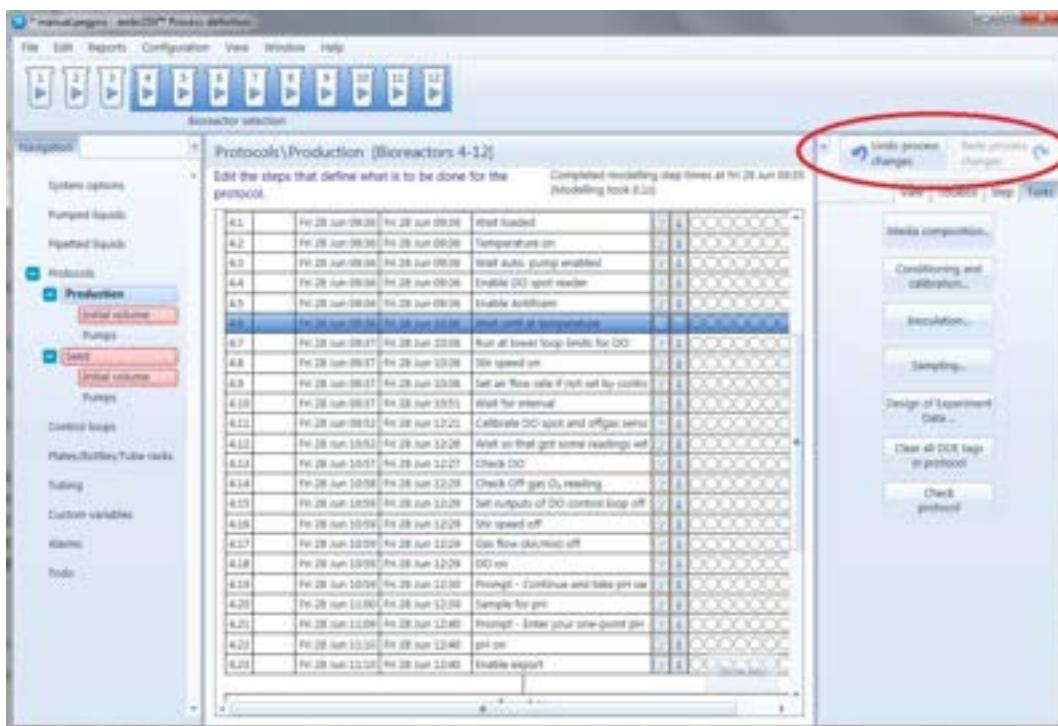


Figure 18 Undo and redo process changes

3.2.4 Defaults

Defaults can be saved and loaded for various aspects of the process definition.

- The System options page which includes the gasses to be used on the system and the sort of bioreactor vessel to use
- Pipetted liquid classes
- Pumped liquid classes
- Pump setup
- Control loops
- Tubing setup
- Custom variables
- Alarms (note that only user created alarms are included in the saved defaults)
- Sample destinations (see **Sampling wizard**)

Note that defaults may be saved from the definition, runtime and experiment viewer applications, but can only be loaded from within the definition application.

Each page that supports saving and loading defaults has a Saved settings tab with options **Load** and **Save**.

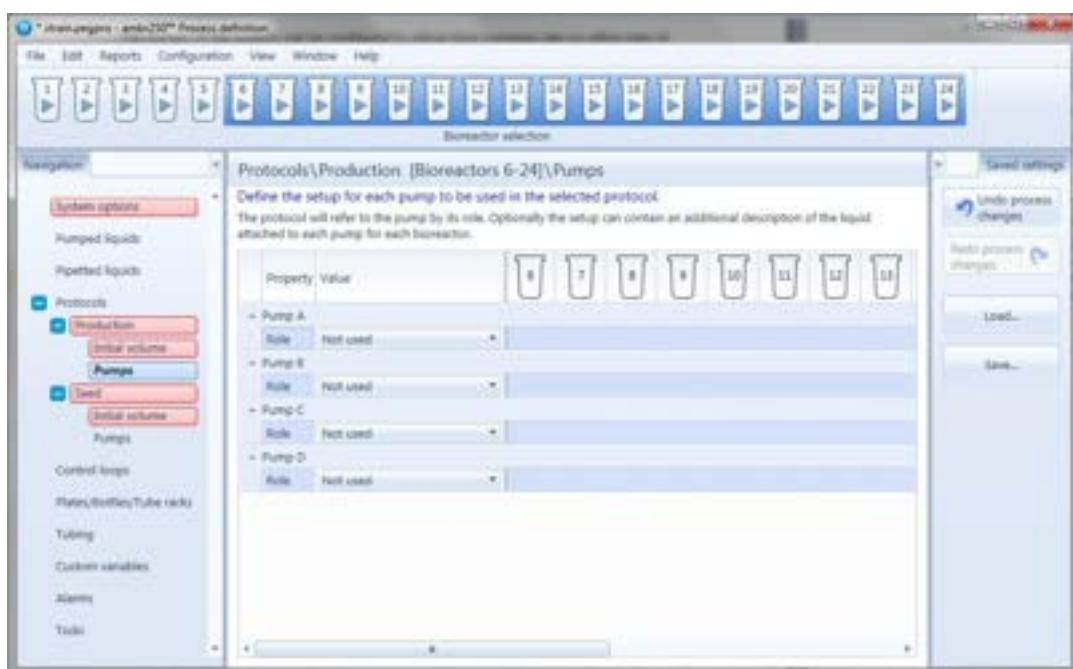


Figure 19 Sample window with **Saved settings** tab

Load displays a window with a list of defaults that have been saved on the system plus any defaults built into the software.

Loading settings replaces all of the relevant settings with the set of entries in the default settings.

The **Load** option is only available in the definition application.

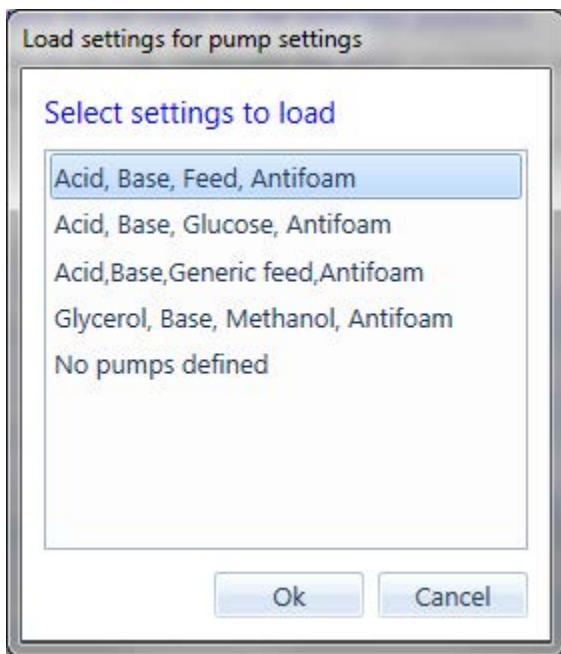


Figure 20 Load settings window

Save displays a window that prompts for the name to give the set of defaults.

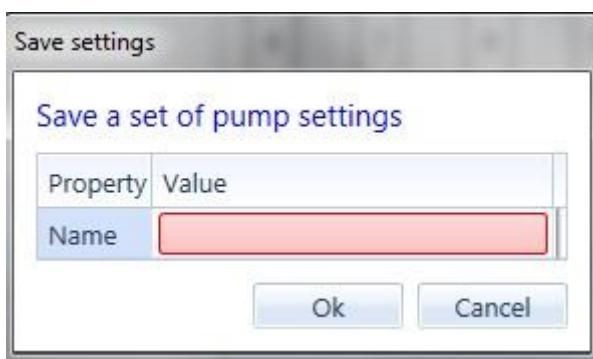


Figure 21 Save settings window

Defaults are saved as files in the **Defaults** folder within the ambr 250 root folder. The files can be deleted or renamed as required to manage the defaults on the system. The system looks in specific folders within the **Defaults** folder for different sorts of defaults, so files should not be moved between folders.

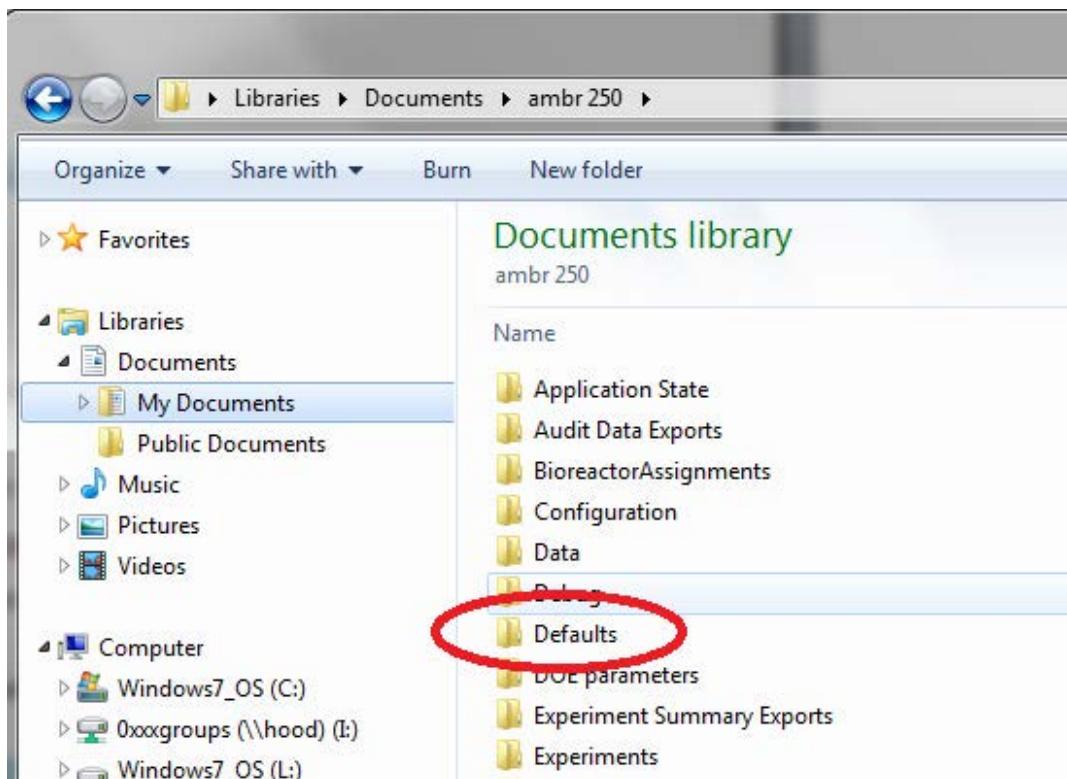


Figure 22 Location of defaults files

3.2.5 Selection

The Ambr® 250 software allows items to be selected by clicking on individual items.

Where multiple selections are allowed Shift-click can be used to select a range of items and Ctrl-click can be used to toggle the selection of an item.

The selection bar shows the bioreactors in the system; which bioreactors are selected; and in the runtime application the status of the bioreactors.

There is at any time one selection of bioreactors that can be changed when the system is displaying a page where the selection of bioreactors is relevant.

When the selection is not relevant – for example when the **Protocols** page that applies to all the bioreactors is selected – the selection is shown as locked.



Figure 23 Locked selection - all bioreactors applicable

When working on an individual protocol the selection is locked and shows the bioreactors that are part of the protocol.

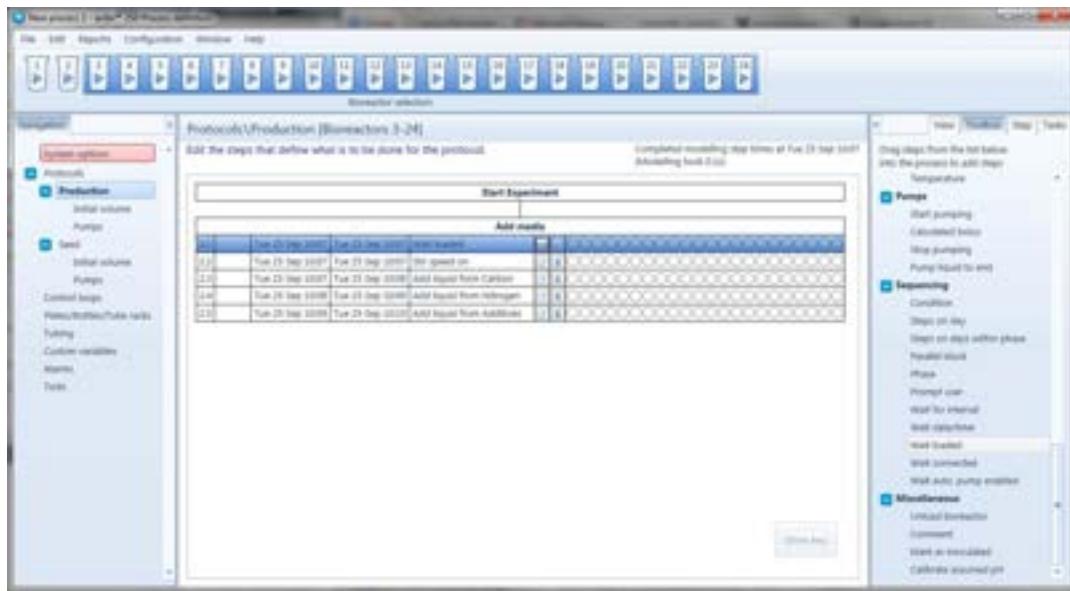


Figure 24 Locked selection editing protocol for bioreactors 3-24

When the page displays data for the selected bioreactors the selection is not locked and can be edited.

There are two sorts of pages displaying data for the selected bioreactors:

- pages that display data for all the selected bioreactors e.g. Graphs/Results
- pages that display data for the single primary selected bioreactor e.g. Controls

The primary selected bioreactor is indicated by a dark blue background. The other selected bioreactors are indicated by a lighter background.

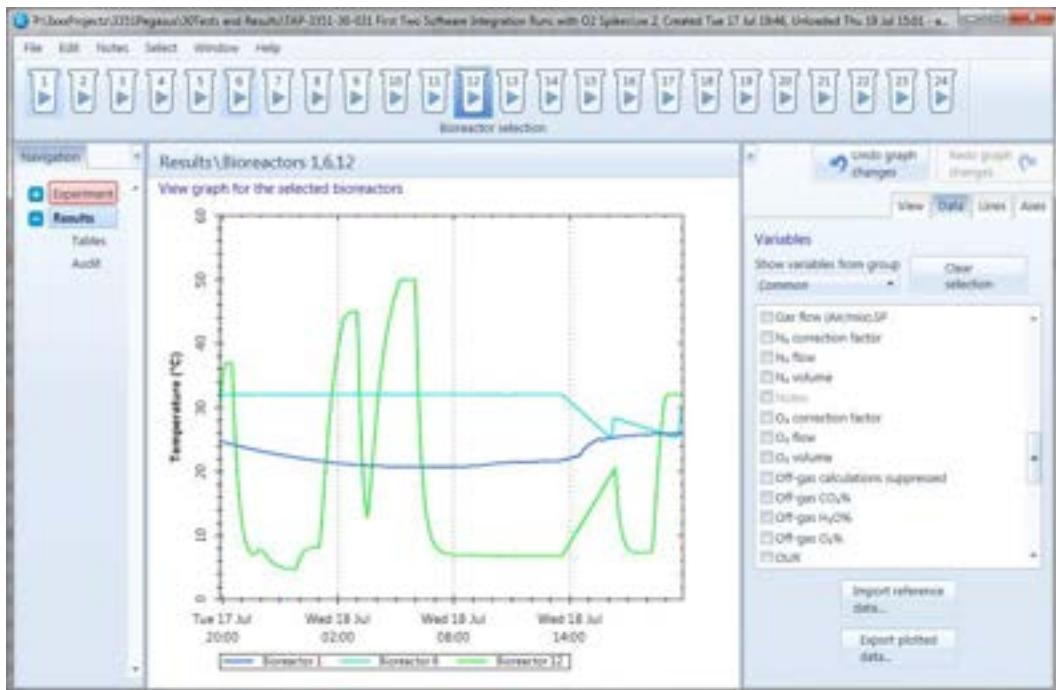


Figure 25 Results page showing data for multiple bioreactors



Figure 26 Controls page showing data for primary selected bioreactor

3.2.5.1 Selecting bioreactors with the mouse

To select a bioreactor click on the bioreactor to select.

To select a range of bioreactors:

- 1) Click on the first bioreactor in the range to be selected
- 2) Shift-click on the last bioreactor in the range to be selected

To toggle the selection state of a bioreactor Control-click on the bioreactor

3.2.5.2 Selecting bioreactors from the menu

The **Select** menu is displayed when the selection is editable.

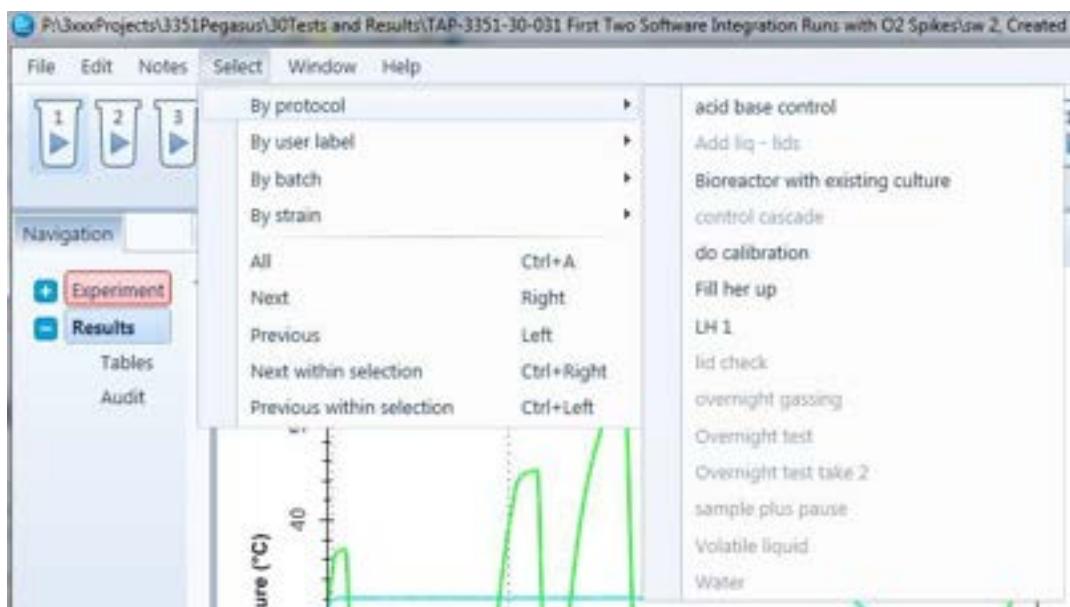


Figure 27 Select menu

The Select menu contains options:

- **By protocol** - selects all bioreactors that are assigned to a particular protocol
- **By user label** - selects all bioreactors with a particular user label
- **By batch** - selects all bioreactors with a particular batch
- **By strain** - selects all bioreactors with a particular strain
- **All** – selects all the bioreactors
- **Next** – selects the bioreactor to the right of the currently selected bioreactor
- **Previous** – selects the bioreactor to the left of the currently selected bioreactor
- **Next within selection** – makes the primary selected bioreactor the next bioreactor within the overall selection which is not changed
- **Previous within selection** – makes the primary selected bioreactor the next bioreactor within the overall selection which is not changed

3.2.6 Validation

Incomplete or missing process information is shown with a pink highlight.

It is OK to save a process with incomplete information. The system will ensure that information is completed and therefore validated before it is used.

Hovering over incomplete data with the mouse will display details of what the problem is.

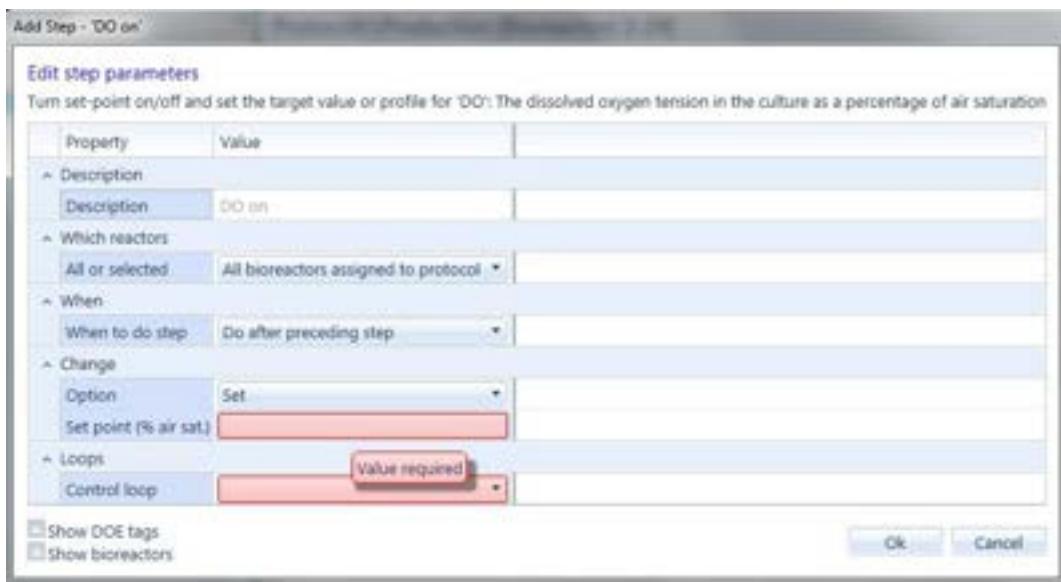


Figure 28 Step dialog showing missing data for the set point value and the control loop to be used.

3.2.7 Tooltips

Extra information is available in many screens by hovering over parts of the screen.

Examples include:

- What the problem is with an incomplete or missing value
- Extended descriptions of parameters and set points
- The status of parts of the system

3.2.8 Grid data entry model

Ambr® 250 uses a common system to allow easy entry of data that is typically the same for all of the bioreactors in a protocol or system but can sometimes vary.

The windows and pages where this applies use a grid with a default column and a column for each bioreactor that the data applies to. The columns for the bioreactors may be hidden by default if the data is the same for all of the bioreactors.

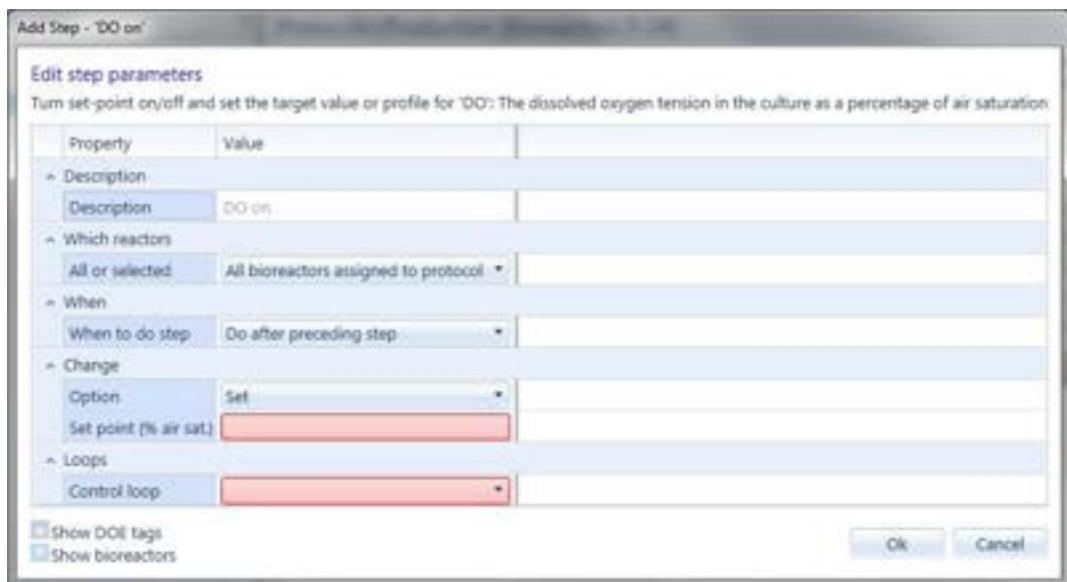


Figure 29 Step dialog with bioreactor columns hidden

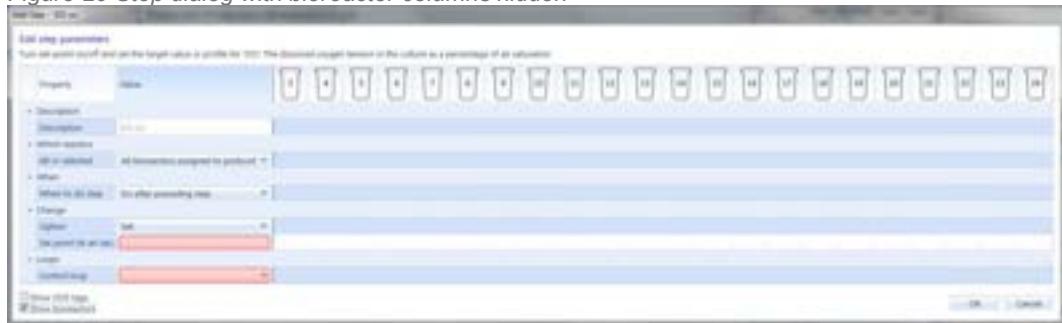


Figure 30 Step dialog with bioreactor columns displayed

The figures above show a step dialog with the bioreactor columns hidden and then displayed. Data for **Set point (% air sat.)** can be set individually for each bioreactor. The cells that are non-editable all have a blue background; the cells that are editable have a white background.

A default value that applies to every bioreactor can be entered in the **Value** column.



Figure 31 Default value applied to all bioreactors

The default value can be overridden for selected bioreactors.

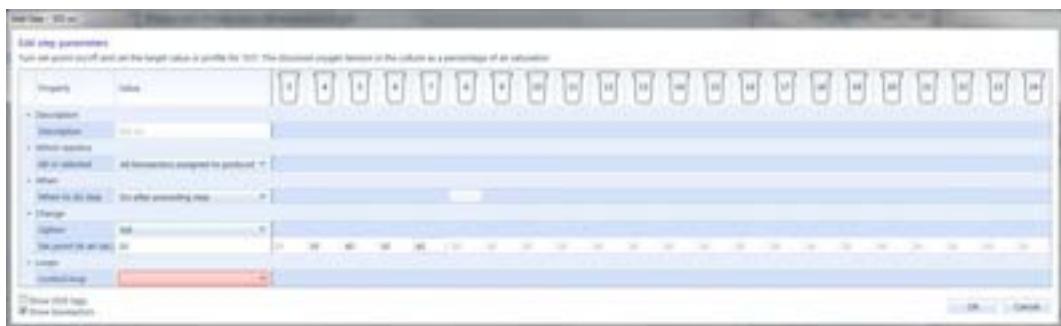


Figure 32 Default value overridden for bioreactors 4,5,6 and 7

If the default value is overridden for all the bioreactors then it will not be used, and can have any value without being considered invalid by the system.



Figure 33 Value overridden for all bioreactors

3.2.8.1 Cell Context menu

Cells in the grid may have context menu entries. These can be displayed by right-clicking on the cell.

Add Step - 'DO on'

Edit step parameters

Turn set-point on/off and set the target value or profile for 'DO': The dissolved oxygen concentration.

Property	Value
Description	DO on
All or selected	All bioreactors assigned to protocol
When to do step	Do after preceding step
Option	Set
Set point (% air sat.)	NOT A VALID ENTRY BUT OVERRIDDEN 20

Figure 34 Single value - **Clear cell** context menu option

The **Clear cell** menu option clears the value of the selected cell.



Figure 35 Multiple values context menu options

The **Clear all overrides** option clears the value of all of the overrides for different bioreactors.

The **Copy row to clipboard** option copies the data for the row to the clipboard and the **Paste row from clipboard** option copies data from the clipboard to the row.

The **Export row to file** and **Import row from file** options transfer data from the row to or from a file.

HINT: Copy or export the data to get a sample of the expected format

HINT: Copy data into Excel to make use of the quick editing functions available there.

Options with a list of tick boxes for different bioreactors have a context menu to quickly set multiple values.

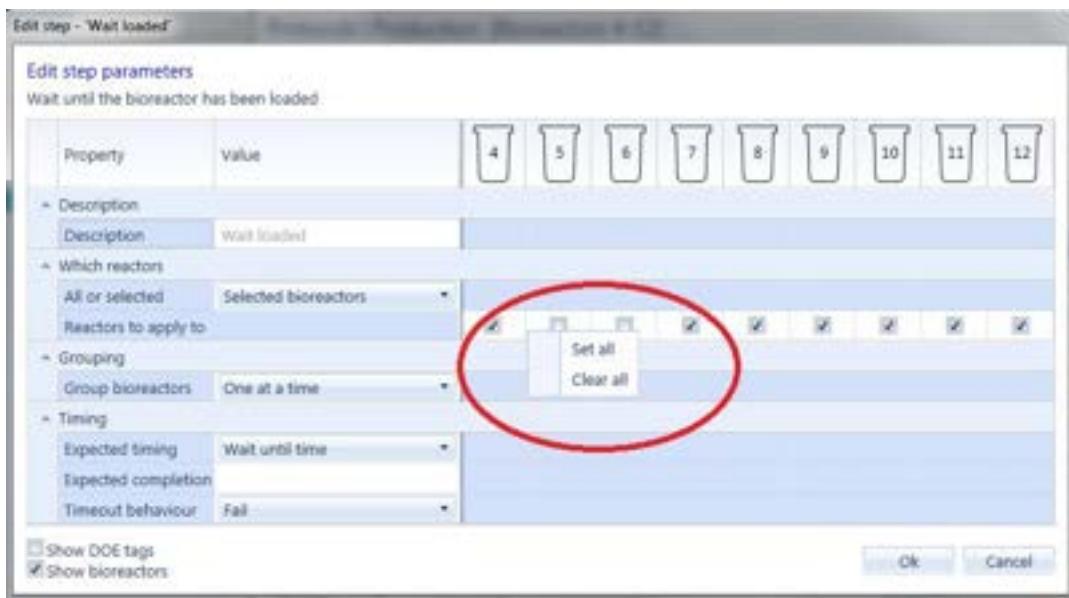


Figure 36 Set all and Clear all options

Set all selects all of the reactors.

Clear all deselects all of the reactors.

One or the other or both options may be present depending on which reactors are already selected.

3.2.8.2 Headers

Grid header cells contain a context menu.

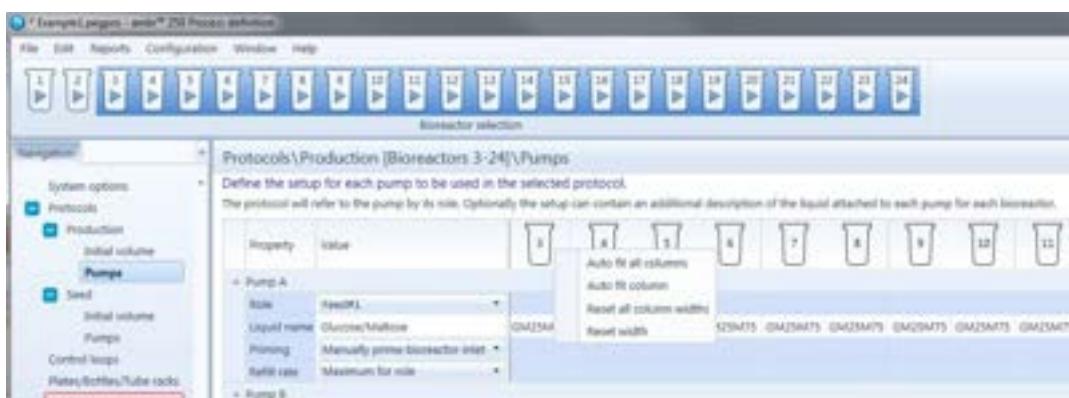


Figure 37 Grid header context menu

The context menu has options:

- **Auto fit all columns**
- **Auto fit column**
- **Reset all column widths**
- **Reset width**

Double clicking on the boundary between two column headers will also auto fit column widths.

3.2.9 Edit menu

The Edit menu has options for **Cut**, **Copy**, **Paste** and **Delete**.



Figure 38 **Edit** menu

The Cut/Copy/Paste/Delete options apply to the currently selected items on a page. They can be applied to:

- Protocols
- Steps
- Labware
- Alarm definition
- Custom variable definitions
- Control loop definitions

Copied data can be pasted not just within the application that it was copied from but also to the other applications if they are running. For example one could copy a control loop definition from an experiment being viewed in the experiment viewer into a new process being defined in the definition application.

3.2.10 Help menu

The Help menu provides help and information about the software.

Within the menu:

- **Help** displays this manual as a help document. The Search feature within the viewer can be particularly useful.
- **Manuals** shows the folders where copies of this and other manuals are stored.
- **Export log files...** enables files containing details of the software operation to be exported.
- **About** displays details about the version of software being run.

3.2.11 Print settings dialog

The **Print settings...** dialog allows the printer used for printing reports to be selected together with the paper size, orientation and margins.

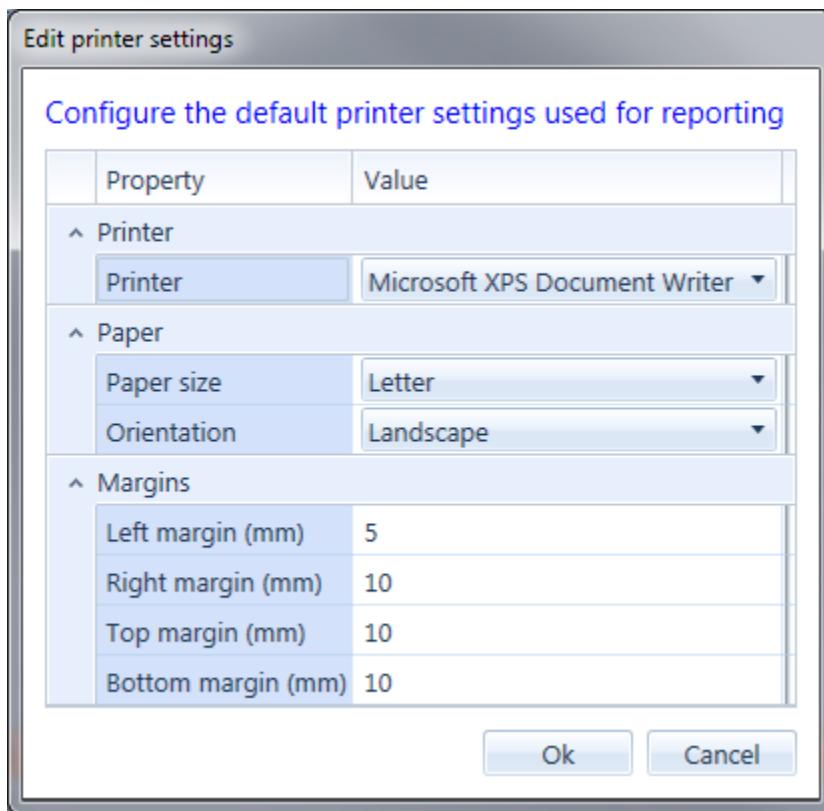


Figure 39 Configure printer settings dialog

3.2.12 Advanced features

The Ambr® 250 software supports the ability to switch features on and off to provide a simpler user interface for those who do not need the features while providing the features for those who need them. The settings for these advanced features are stored as part of the process definition and can be edited within either the definition application or the runtime application.

To change which features are on or off select **Advanced features** from the Configuration options.



Figure 40 Advanced features application within definition application.

The **Advanced features** window switches features of the system on and off and provides an explanation of each feature.

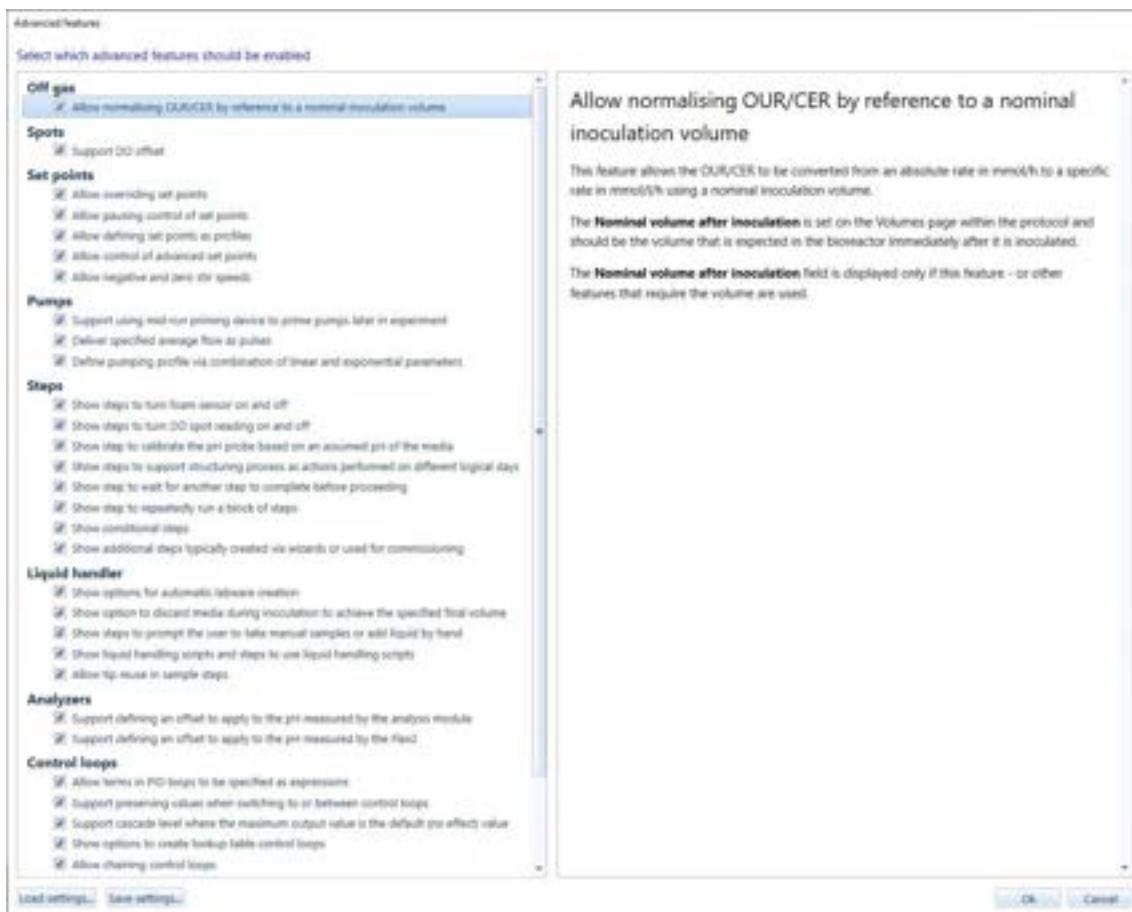


Figure 41 Advanced features window

Click on a feature to see details of that feature.

Set or clear the checkbox for the feature to turn that feature on or off when the window is closed.

Use **Load settings** and **Save settings** to load default or previously saved selections of options and to save sets of options.

Features of the system that are controlled as advanced features are highlighted in this manual with the symbol below.



Figure 42 Advanced features icon

3.3 Definition Application

This section describes the basic operation of the definition application. Subsequent sections describe the details of creating and editing specific elements of a process definition.

The definition application is used to create or edit process definitions.

3.3.1 Initial Window

On starting the application a window is displayed that lets one:

- edit an existing process definition
- create a new process definition either using sets of defaults or based on a template

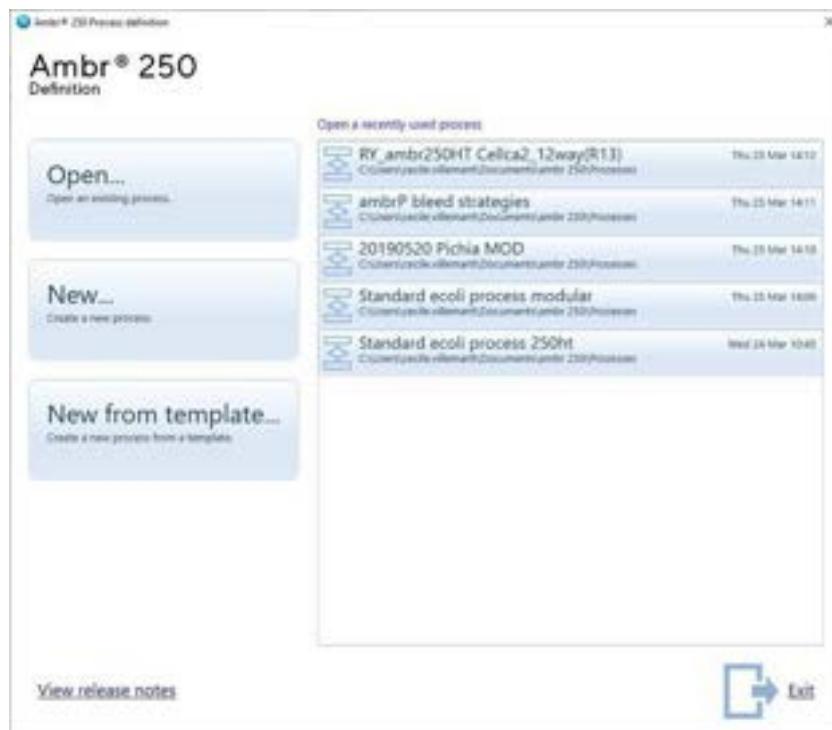


Figure 43 Initial definition screen

3.3.1.1 Choose configuration

If there are multiple configurations on the PC the system will display a window with the configurations present. Choose the required configuration and press return.

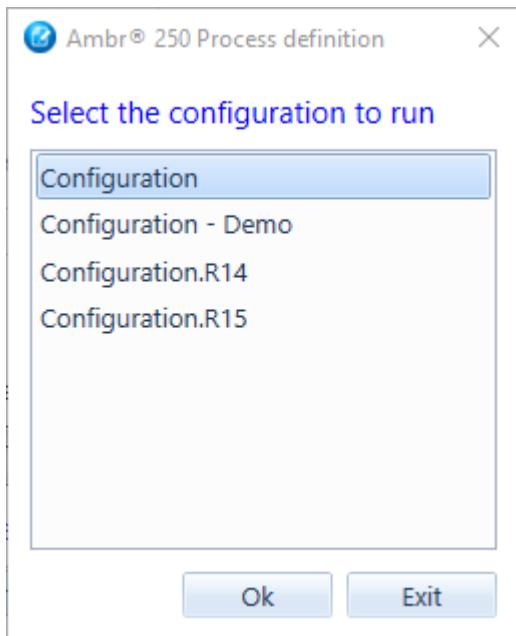


Figure 44 Configuration selection window

The selected configuration tells the software about the system that the process will run on. The configuration includes details such as how many bioreactors the system has and whether the system has a liquid handler.

All the processes open in the definition application at one time will all be using the same configuration.

3.3.1.2 Open an existing process definition

Choosing to open an existing process definition presents a file open window for choosing the process to edit.

If the process has been used recently then the process will be in the list of recent processes and can be opened by clicking on its entry.

3.3.1.3 Create a new process definition

Creating a new process displays a window to select an initial set of defaults to use. After selecting the defaults the system displays a window that allows simple creation of the initial protocols and bioreactor assignments if required.

Create new process

Select initial defaults for aspects of the process definition

Property	Value
System options	Mammalian (filters) (factory default)
Pumped liquid classes	Default (factory default)
Pipetted liquid classes	Default (factory default)
Liquid handling scripts	Analysis module (factory default)
Control loops	Mammalian Headspace - Air, O ₂ , CO ₂ (factory default)
Tubing setup	Default (factory default)
Custom variables	Mammalian (factory default)
Alarm definitions	No extra alarms (factory default)
Sample destinations	All (factory default)
Advanced features	Steps on days (factory default)

Ok **Cancel**

Figure 45 Initial defaults to create a new process

Having chosen the defaults the **Create protocol or protocols** window will be displayed. See section 4.5.1 below for more details of this window.

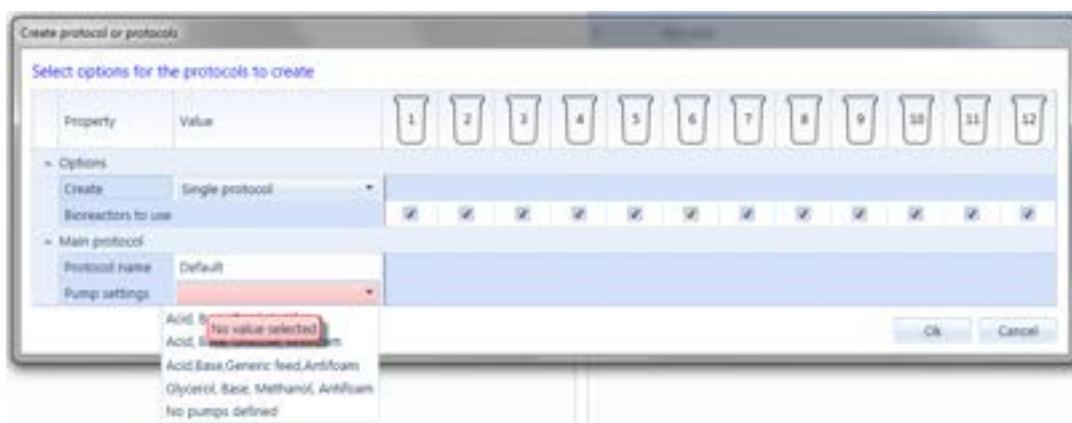


Figure 46 **Create protocol or protocols** window for a single protocol

3.3.1.4 Create a new process definition from a template

Creating a new process definition from a template opens an existing process and creates a copy as a new process that is ready to be saved to disk.

A template is nothing but a process that has been saved typically in the Templates folder.

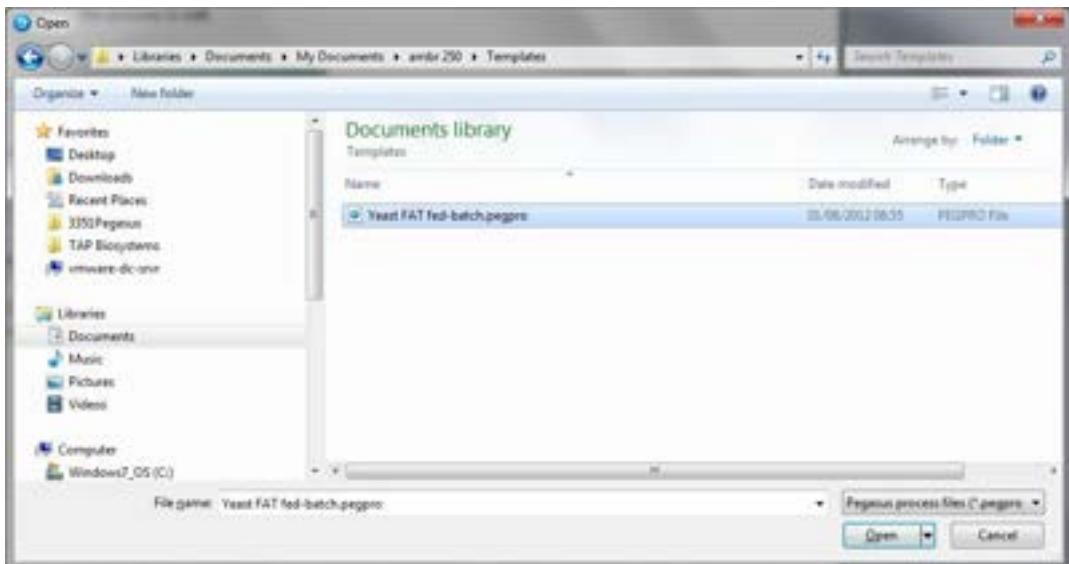


Figure 47 Window for creating a new process definition from a template

3.3.2 File Menu

The **File** menu on the main process definition window allows additional processes to be opened at the same time.

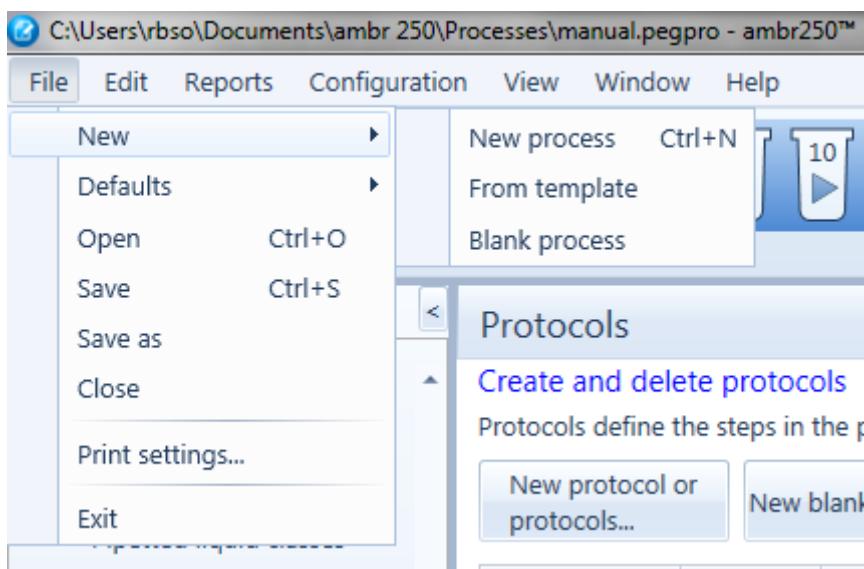


Figure 48 File menu

The **File** menu offers the options:

- **New\New process** – creates a new process starting with the selection of defaults for different aspects of the process
- **New\From template** – creates a new process definition from a template
- **New\Blank process** – creates a new blank process definition
- **Defaults\Load default settings** – allows updating aspects of the process from the defaults saved on the system and built into the software.

- **Open** – opens an existing window definition
- **Save** – saves the process definition as a file
- **Save as** – saves the process definition as a new file
- **Close** – closes the process definition in the current window. The application exits when the last process definition is closed.
- **Print settings...** – displays the print setting dialog to allow selection of a printer, paper size and margins
- **Exit** – exits the definition application

3.3.2.1 Load default settings

The **Load defaults** window can be displayed from the menu option **Defaults → Load default settings** to replace multiple sets of defaults.

When opened for an existing process the option **No change** is the default for each option. Only the settings where a set of defaults is selected instead of **No change** will be updated.

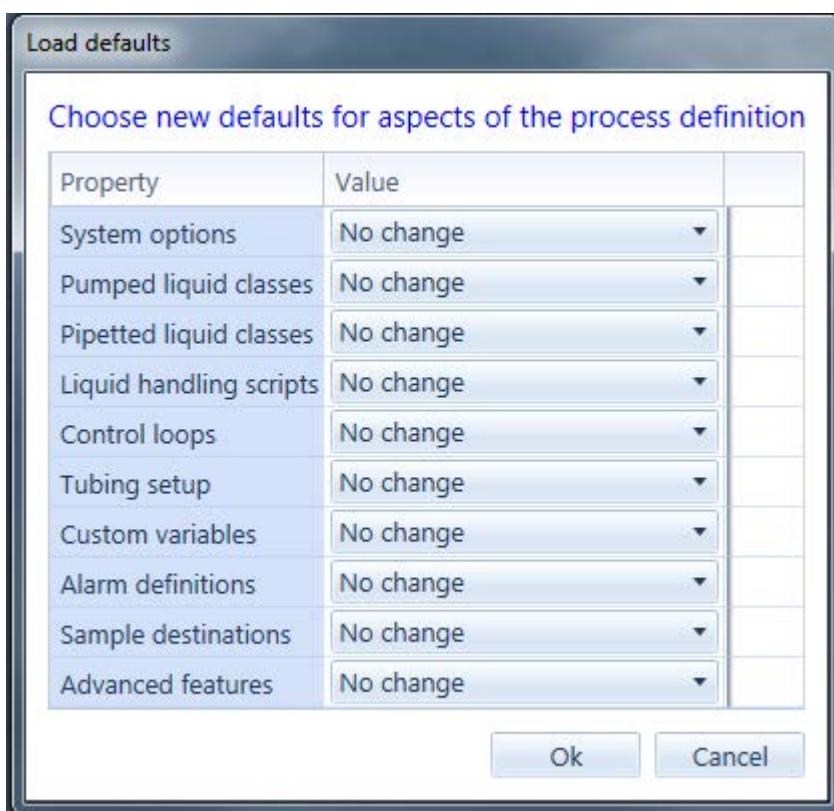


Figure 49 **Load defaults** window

3.3.3 Window

The **Window** menu allows switching between the open processes.

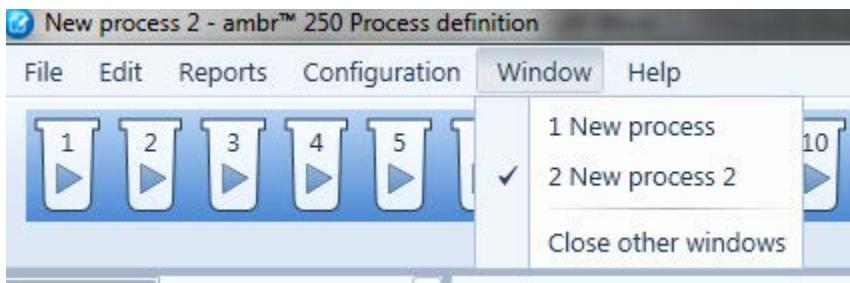


Figure 50 **Window** menu

The **Window** menu indicates which process is open in the current window.

To switch to another process select that process from the menu.

To close the other open windows select **Close other windows**.

3.4 Runtime Application

When started, the runtime application checks if there are multiple configurations installed on the computer. If there are multiple definitions – this should only be the case off line and not on the hardware – then a window is displayed to select the configuration to be used.

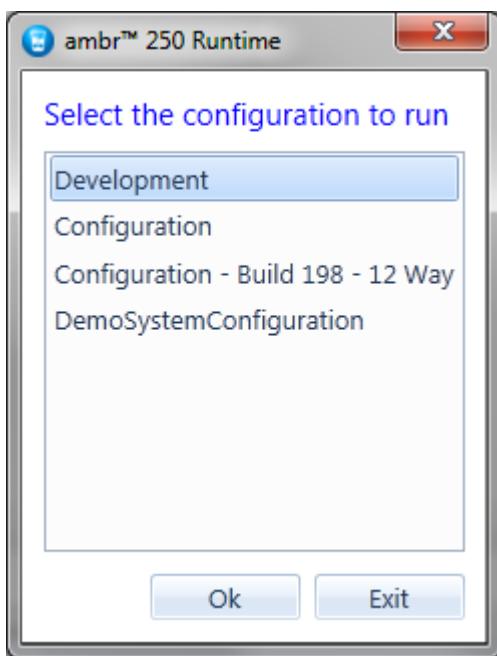


Figure 51 Choice of configurations

Once the configuration is known the runtime application displays a screen with options to start a new run or continue with the existing run.

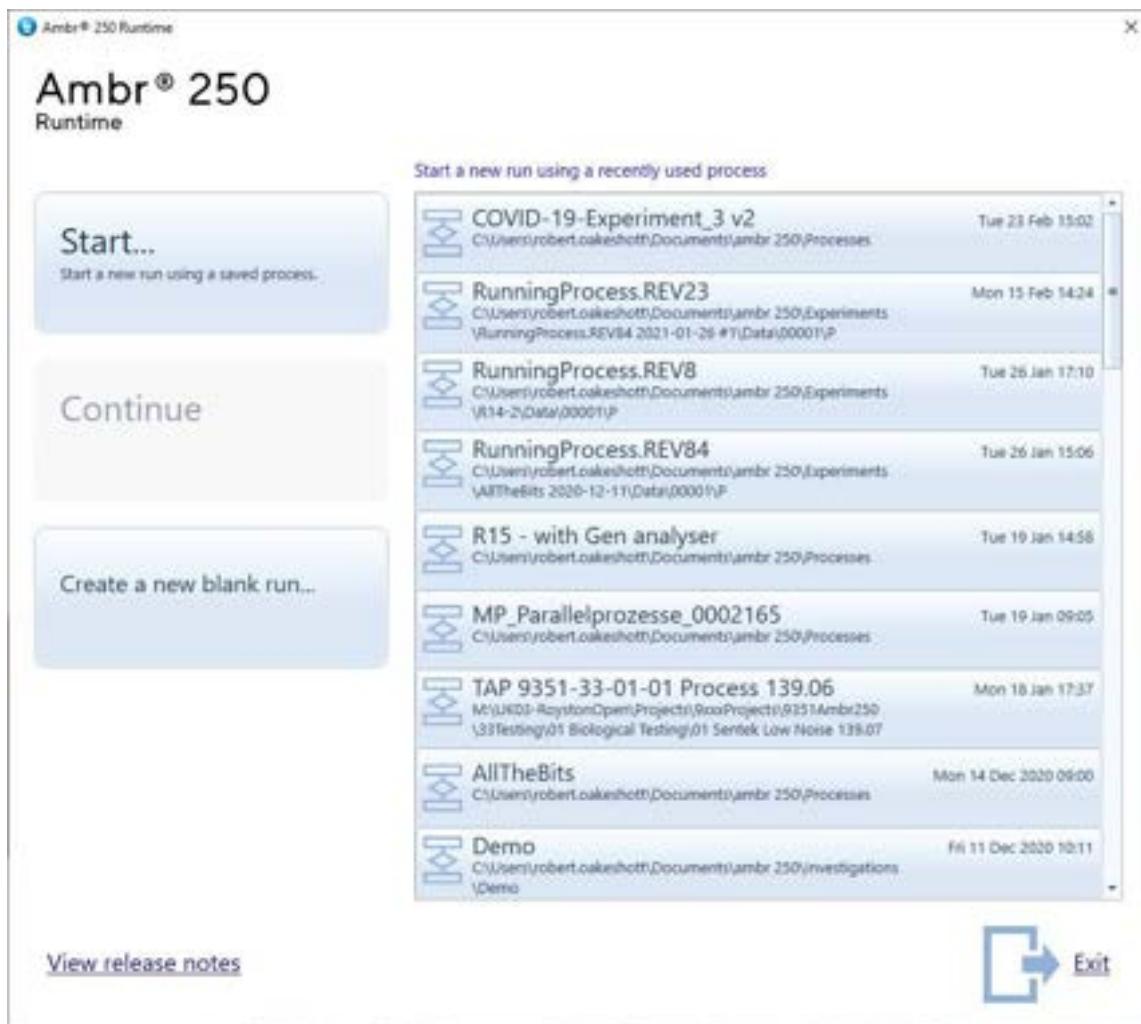


Figure 52 Initial screen of runtime application

Press **Start** to start a new experiment based on a process that has been defined previously. If the process has been used recently then you can click on the entry in the list of recent processes instead.

Press **Continue** to load the experiment that was last being run and display the **Todo** page.

Press **Create a new blank run** to create a new experiment with a minimal process definition.

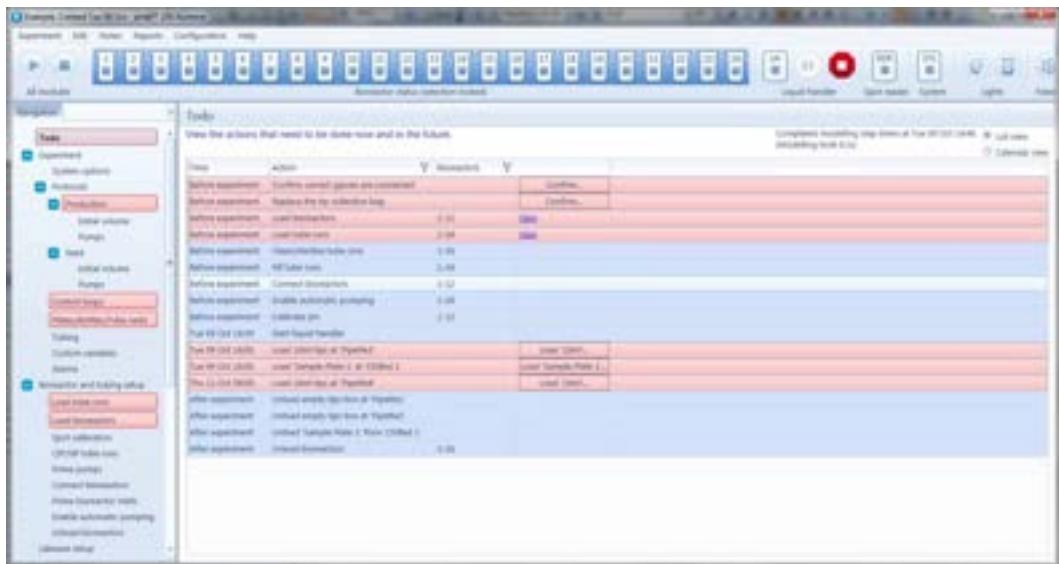


Figure 53 Initial view of the runtime application

Press **View release notes** to see the release notes for the currently installed software version.

3.4.1 Top bar controls

At the top of the screen are a set of top bar controls that are visible at all times. The controls available on individual systems will vary depending on their configuration.

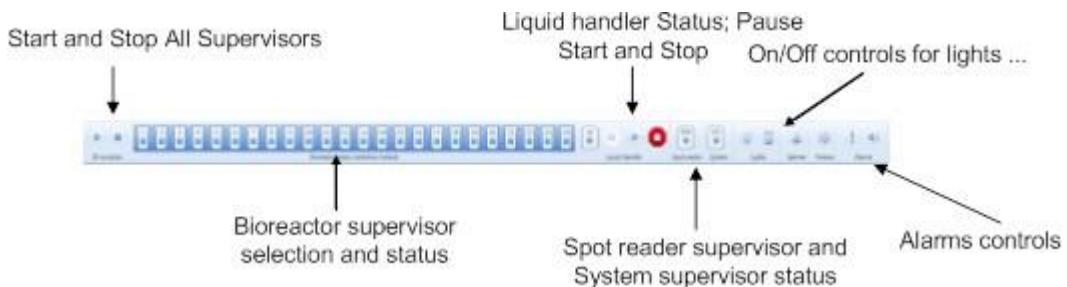


Figure 54 Top bar controls

3.4.1.1 Starting and stopping the system

The **Start all the supervisors** and **Stop all the supervisors** buttons can be used to start and stop the complete system.

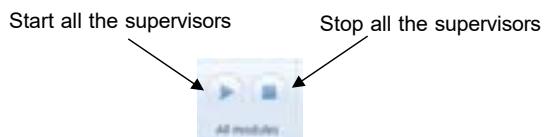


Figure 55 Start and stop buttons

Pressing the **Start all the supervisors** button displays the **Start system** window shown below.

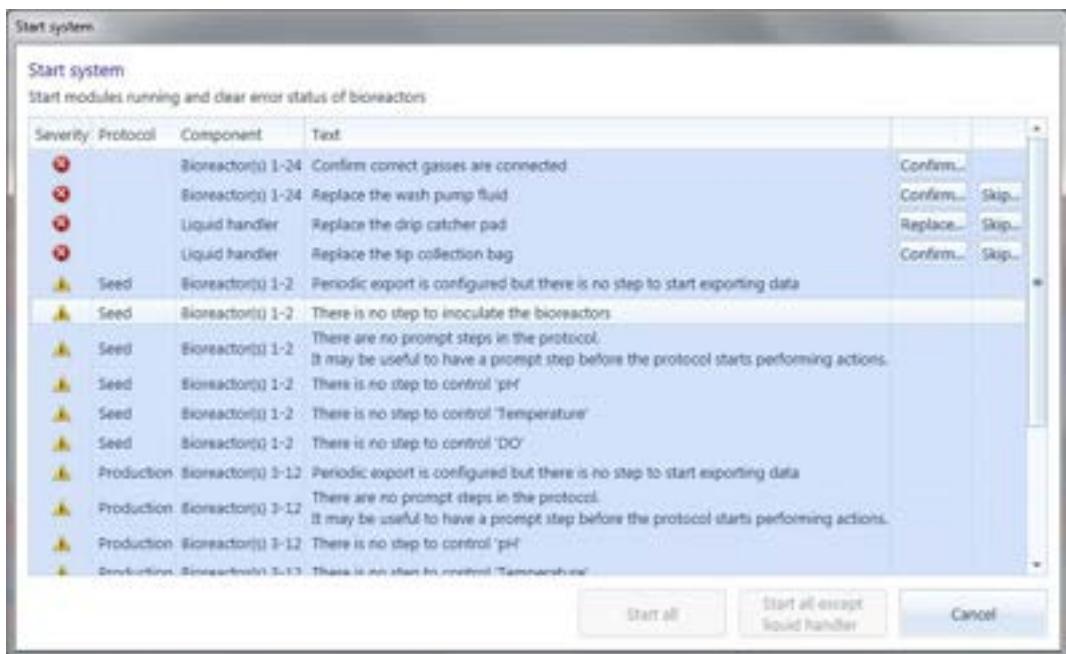


Figure 56 Start system window

The **Start system** window shows:

- Any errors that must be addressed before one or more of the supervisors can be started. Where the issue can be addressed from this window then options for addressing the issue are displayed.
- Any warnings that do not stop the supervisors being started but may indicate mistakes in the process definition.

Start all starts all of the supervisors in the system.

Start all except liquid handler starts all of the supervisors except the liquid handler. Since the liquid handler must be stopped when loading labware or bioreactors it is common to start the rest of the system first and later to start the liquid handler supervisor.

Pressing the **Stop all the supervisors** button posts a window asking for confirmation and then stops all of the supervisors. Stopping the supervisors stops all of the activity on the system including gassing, stirring and pumping bioreactors and reading the state of the bioreactors. Typically the supervisors are only stopped when an experiment has been completed and a new experiment is to be created – either as a main experiment or as a placeholder for cleaning the system down between experiments.

3.4.1.1.1 Liquid handler pre-run checks

When the liquid handler is being started the system presents the option to perform pre-run checks on the loaded labware before starting process liquid handler operations.

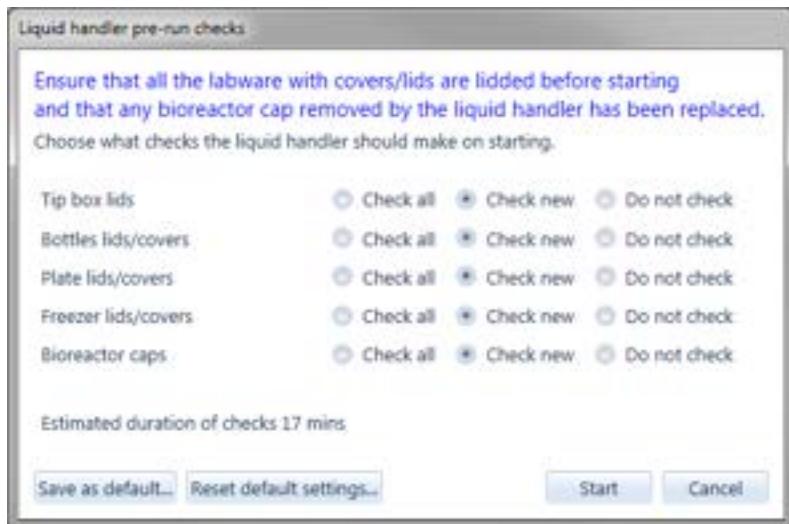


Figure 57 Liquid handler pre-run checks dialogue

The radio buttons on the dialog allow the user to select the labware items to be checked.

- **Check new** – newly loaded or refilled labware items are checked
- **Check all** – all labware items are checked even if they have previously been checked
- **Do not check** – labware items are not checked

An estimation of the duration the checks will take is displayed. This will vary depending on the number of items to check and if the robot needs initialization.

Save as default... – saves the current selection as the default.

Reset default settings... – restores the current selection to the default selection

Cancel – exits the dialogue without starting the liquid handler.

Start – starts the liquid handler, or if items require checking displays a summary dialogue of the items that will be checked by the liquid handler.

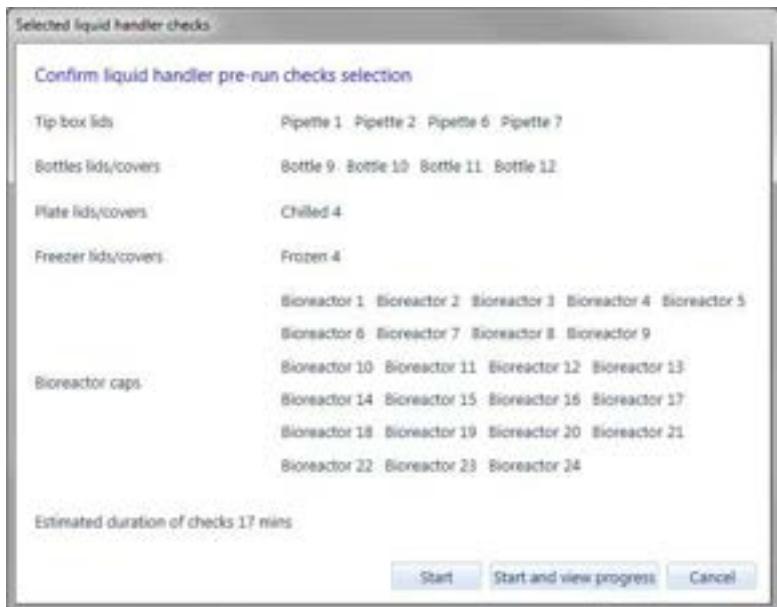


Figure 58 Selected liquid handler checks summary dialogue

Start – starts the liquid handler and adds to the liquid handler queues the labware items that require checks.

Start and view progress – starts the liquid handler and adds to the liquid handler queues the labware items that require checks and displays the **Liquid handler** page so that the queued actions can be viewed.

Cancel – exits the dialogue and returns to the check selection dialogue.

The screenshot shows the 'Liquid handler' interface. At the top, there's a status bar with buttons for 'Stop', 'Running' (highlighted in green), 'Autostart/hold', 'Change strip grid...', 'Wipe liquid handler tools...', and 'Sterilize in place...'. Below this is a table titled 'List' with columns 'Time', 'Task', and 'Details'. The table lists several actions, many of which are preceded by a small blue circular icon with a white question mark. A red oval highlights the 'Clear check actions...' button at the bottom of the table.

Time	Task	Details
Thu 06-Oct 18:17:14	Replace Cap : Labware Type [CULTURE VESSEL] Location [Reservoir] Well [1]	
Thu 06-Oct 18:17:17	Finished running queued item	
Thu 06-Oct 18:17:17	Running queued item [Check Bioreactor 10]	
Thu 06-Oct 18:17:17	Remove Cap : Labware Type [CULTURE VESSEL] Location [Reservoir] Well [1]	
Thu 06-Oct 18:17:20	Replace Cap : Labware Type [CULTURE VESSEL] Location [Reservoir] Well [1]	
Thu 06-Oct 18:17:23	Finished running queued item	
Thu 06-Oct 18:18:44	Disable liquid handler	
Thu 06-Oct 18:20:02	Enable liquid handler	
Thu 06-Oct 18:20:02	Running queued item [Check Bioreactor 1]	
Thu 06-Oct 18:20:02	Remove Cap : Labware Type [CULTURE VESSEL] Location [Reservoir] Well [1]	
Thu 06-Oct 18:20:04	Replace Cap : Labware Type [CULTURE VESSEL] Location [Reservoir] Well [1]	
Thu 06-Oct 18:20:08	Finished running queued item	
Thu 06-Oct 18:20:08	Running queued item [Check Bioreactor 2]	
Thu 06-Oct 18:20:08	Remove Cap : Labware Type [CULTURE VESSEL] Location [Reservoir] Well [1]	

View and re-prioritize the liquid handler's queued actions. **Clear check actions...**

Figure 59 Liquid handler page

The **Clear check actions...** button can be used to remove check actions not yet started from the liquid handler queue. A confirmation dialogue is displayed.

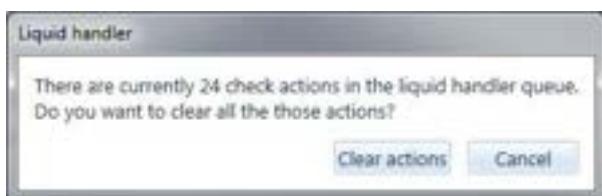


Figure 60 Clear check actions confirmation dialogue

Clear actions – removes all pending check actions from the liquid handler queue

Cancel – closes the dialogue.

When all checks have been completed the following dialogue is displayed



Figure 61 Pre-run checks complete dialogue

If e-mails alerts are configured on the system then subscribed users will receive a notification that the checks have been successful.

3.4.1.1.2 Automatic start of bioreactors

The system automatically starts bioreactors that were running when the software was stopped so long as the software is restarted within a configurable time. As well as the bioreactors all the supervisors except for the liquid handler are started.

To adjust the time or to inhibit automatic starting add or edit the parameter **Max_Autostart_Time_Minutes** in the SystemOptions.txt file.

To disable autostart set the value to a negative time.

3.4.1.2 Supervisor status

The status of the bioreactor and other supervisors is shown on the top screen.

Supervisors can be:

- Running – the supervisor is polling and looking for work. The supervisor shows the start symbol and if there have not been any faults or alarms the supervisor will be green.



Figure 62 Running supervisor

- Stopped – the supervisor is not doing anything. The supervisor shows the stop symbol.



Figure 63 Stopped supervisor

- Initialising – the supervisor is preparing to be running. The supervisor shows the start symbol and is coloured orange.



Figure 64 Initialising supervisor

- Stopping – the supervisor is switching things off as required ready to be Stopped.



Figure 65 Stopping supervisor

- Disabled – the supervisor is stopped and has been marked as disabled to indicate that no attempt should be made to start the supervisor or wait for the supervisor



Figure 66 Disabled supervisor

If a supervisor is Running or Stopped and a fault has occurred or an alarm has been raised then the supervisor will be shown in a red colour.



Figure 67 Supervisor with error

If there are unacknowledged faults or alarms then the label for the supervisor will also flash.

More details of the state of the supervisor are displayed by hovering over the supervisor symbol with the mouse or by navigating to the pages for the supervisor.

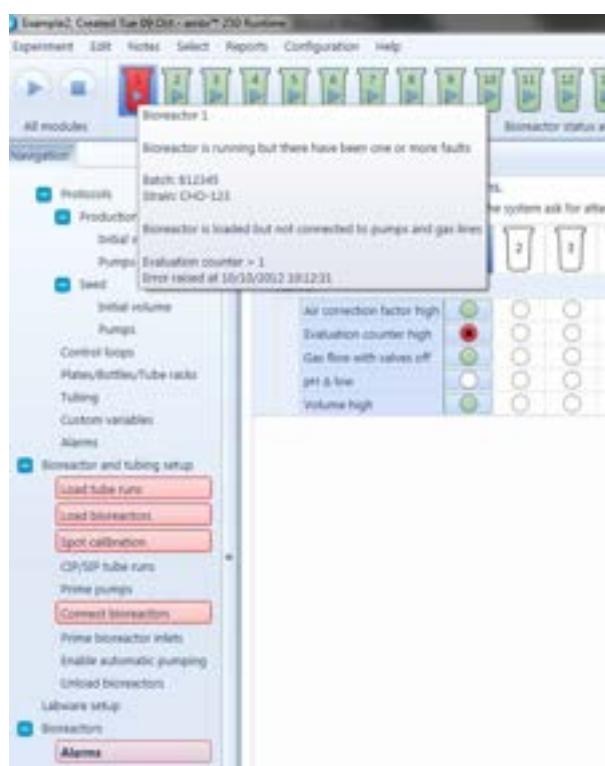


Figure 68 Additional details of supervisor state

3.4.1.3 Liquid handler

The liquid handler has some additional controls.

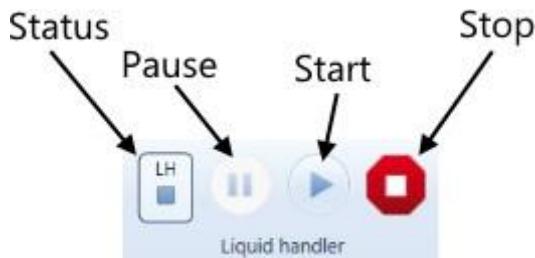


Figure 69 Liquid handler controls

There is an indication of the liquid handler status.

There is a **Pause** button, **Start** button and a **Stop** button.

The **Pause** button makes the liquid handler finish what it is doing, replace any covers it has taken off plates, bottles or tube racks and then stop. The Pause button should be used to make the liquid handler safe before loading or unloading labware or bioreactors.

The **Start** button starts the liquid handler. The start button can be used to restart the liquid handler after it has been paused or stopped.

The **Stop** button stops the liquid handler as quickly as possible. If the liquid handler was doing something the system then displays the **Abandon Liquid Handler Process Step** window – otherwise the system just displays a confirmation that the liquid handler is stopped.



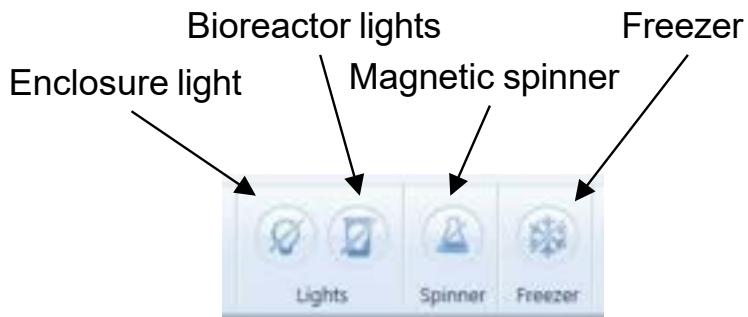
Figure 70 Abandon Liquid Handler Process Step window

Choosing **Abort Process** will abandon whatever the liquid handler was doing.

Choosing **Continue** will allow the liquid handler to continue. Note that the liquid handler will assume that nothing has been changed while it was stopped so errors may occur if the liquid handler has been moved.

3.4.1.4 Miscellaneous controls

Depending on the system configuration one or more miscellaneous controlling buttons may be displayed.



Enclosure light switches the lights in the laminar air flow cabinet on and off.

Bioreactor light switches the lights in the bioreactors on and off.

The bioreactor and enclosure lights are turned off after configurable intervals. To adjust the default time before the lights are turned off add or edit the parameters

Enclosure_Lights_Off_Time_Minutes and **Bioreactor_Lights_Off_Time_Minutes** in the SystemOptions.txt file. A context menu (right mouse click or long touch) is available with a choice of times to keep the lights on for.

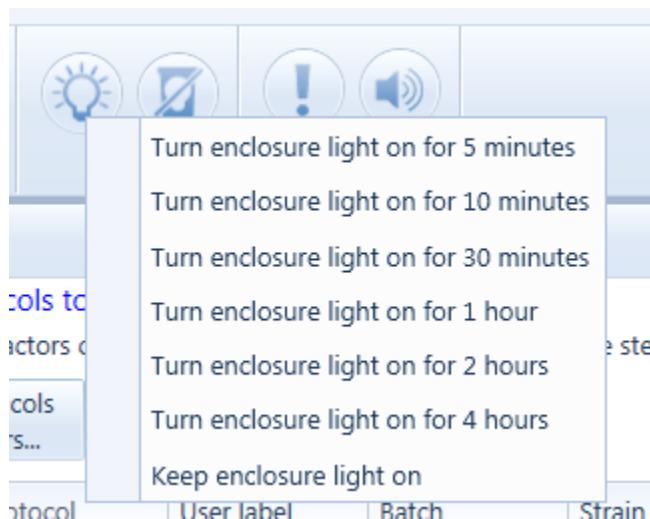


Figure 71 Context menu with choice of times to keep lights on for.

Magnetic spinner switches the motor for the magnetic spinner that is present under specified bottle locations on certain machines on or off. The speed on the spinner can be adjusted on the Labware setup page.

Freezer switches additional cooling for freezable locations on and off. The temperature for those locations spinner can be adjusted on the Labware setup page.

3.4.1.5 Alarms controls

The **Alarms** controls indicate when the system needs attention and control whether the system makes a noise to indicate whether it needs attention.

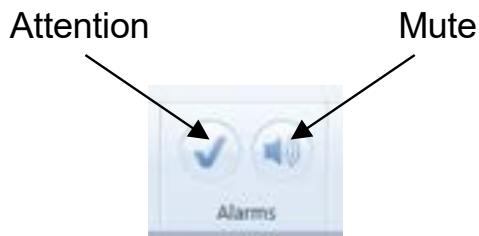


Figure 72 **Alarms** controls



Figure 73 **Alarms** controls showing that attention is required and that sounds have been muted.

The **Attention** button indicates if the machine needs attention and opens the **Attention** window to show details of any faults and alarms. The **Attention** window is also opened automatically if the system needs attention and the mouse and keyboard have been idle for a while.

The **Mute** button allows disabling for an interval any sound that the system is configured to make in response to fault conditions. Pressing the **Mute** button again enables the sounds.

3.4.1.6 Attention window

The Attention window shows a list of reasons why the machine needs attention. While the Attention window is open the screen flashes to draw attention to the user interface and if the system is configured to make a sound when there is a problem, then the system will be making that sound.

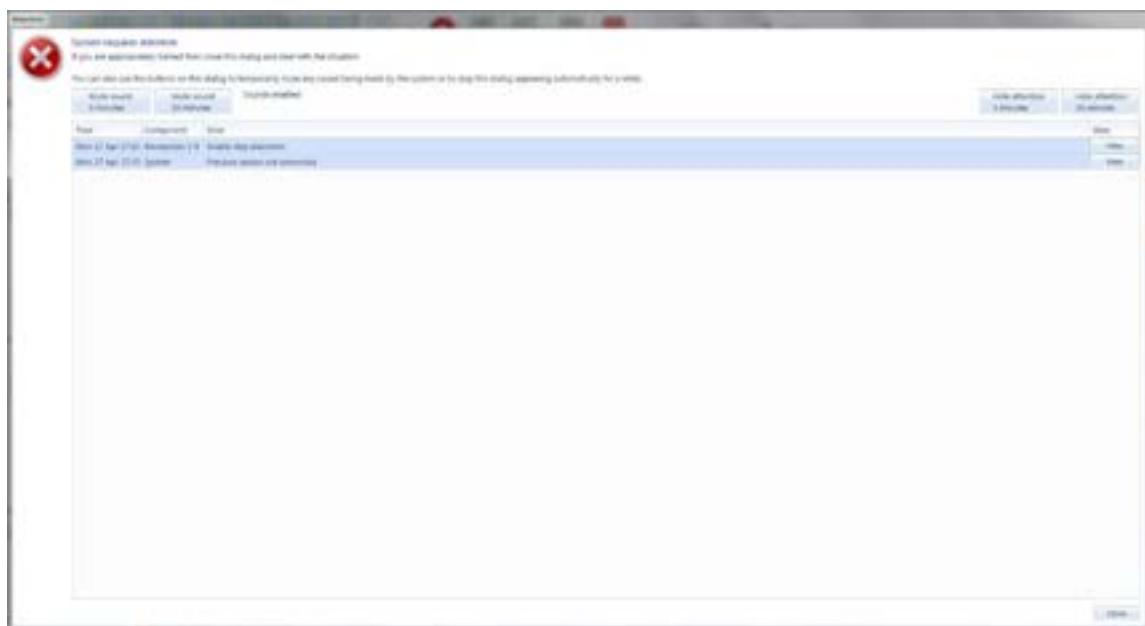


Figure 74 **Attention** window

As well as the list of problems the Attention window has controls to suppress warnings for an interval.

Mute sound 5 minutes stops the system making sounds because it has a problem for 5 minutes.

Mute sound 30 minutes stops the system making sounds because it has a problem for 30 minutes.

Hide attention 5 minutes hides the Attention window and stops the machine redisplaying it automatically for 5 minutes.

Hide attention 30 minutes hides the Attention window and stops the machine redisplaying it automatically for 30 minutes.

Pressing **View** for an item switches to the most relevant page in the user interface and highlights where possible the relevant item on the page.

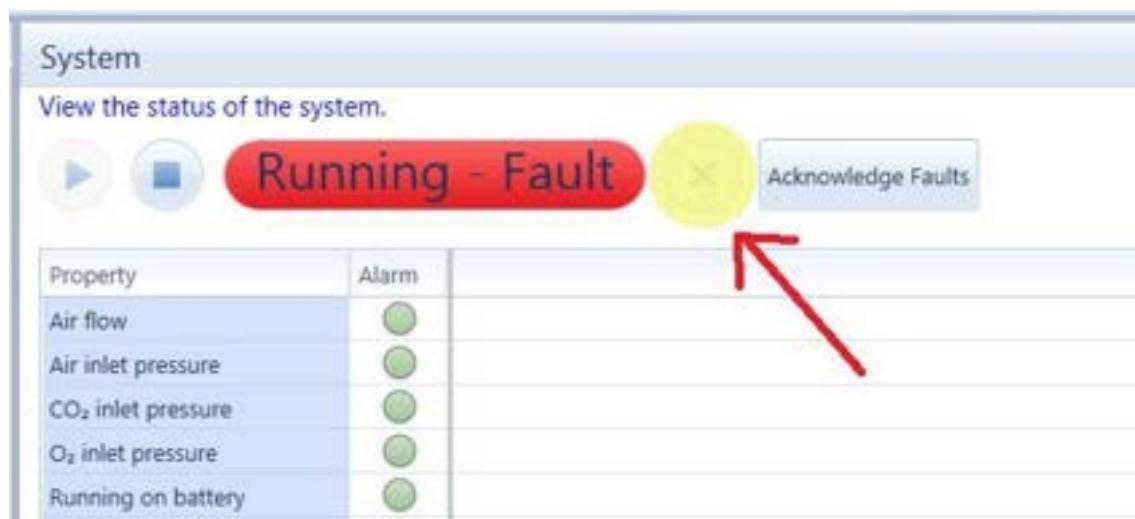


Figure 75 Item highlighted on page

3.4.1.7 Closing runtime software

When closing the runtime software the choice is offered between:

Stop all and exit stops all the bioreactors, the spot reader and other modules before closing the software. Stirring, gassing, pumping and temperature control are all stopped.

Continue basic control and exit leaves the bioreactors running with their current settings. Control managed by the runtime software (DO, pH) stops: an average of the recent output is used for gassing and stirring; pumps controlled via a PID loop are turned off.

An option has been added to exit the software and leave the bioreactors running with as much control as is supported without the software. In particular temperature control continues, but DO and pH controls do not. Before exiting, the software issues a set of stirring, gassing to reflect the recent average outputs of the control loops and turns off pumps controlled by control loops.



Figure 76 Option to continue basic control

To remove the option **Continue basic control and exit** set the option **Allow_Exit_And_Keep_Running** in the SystemOptions.txt file to **false**.

3.4.2 Todo

The Todo page shows the actions that need to be done now and in the future to progress the current experiment.

Actions that can be done now have a button to do the action or a link to do the page where the action can be done.

Todo			
View the actions that need to be done now and in the future.			
Time	Action	Y	Completed
Before experiment	Connect correct gases are connected		Complete...
Before experiment	Replace the wash pump fuse		Complete...
Before experiment	Replace the stirrer switch plug		Replace...
Before experiment	Replace the tip collector tray		Complete...
Before experiment	LOAD BIOPROCESSOR	1-12	Now
Before experiment	Load tube rack	1-14	Now
Before experiment	Characterize tube type	1-16	
Before experiment	PB tube runs	1-18	
Before experiment	Connect bioreactor	1-19	
Before experiment	Enable automatic pumping	1-20	
Before experiment	Calibrate pH	1-22	
Pt-19-Nov-18:40	Start liquid handler		
Pt-19-Nov-18:40	Load Carbon 12 at Bottle 12		Load Carbon...
Pt-19-Nov-18:40	Load Carbon 12 at Bottle 12		Load Carbon(12...)
Pt-19-Nov-18:40	Load Carbon 12 at Bottle 12		
Pt-19-Nov-18:40	Unload Carbon 12 from Bottle 12		
Pt-19-Nov-18:40	Load Nitrogen at Bottle 12		Load Nitrogen...
Pt-19-Nov-18:40	Load Nitrogen 12 at Bottle 12		Load Nitrogen(12...)
Pt-19-Nov-18:40	Unload Nitrogen 12 from Bottle 12		
Pt-19-Nov-18:40	Load Sample Plate 12 at Chilled 12		Load Sample Plate(12...)
After experiment	Unload Additives 12 from Bottle 12		
After experiment	Unload Carbon 12 from Bottle 12		
After experiment	Unload Carbon 12 from Bottle 12		

Actions linking to screen when can do page

Actions that can be done from this page

Actions that will be required later

Figure 77 Todo page

Actions are shown with the time the action is required. This can be:

- **Before experiment** – the action is needed before starting the main body of an experiment.
- **NOW** – not having done the action is holding up the progression of the experiment

- A calculated time – the action can best be done at the indicated time. The time takes into account working hours – e.g. the system will try to schedule enough tip loading actions in working hours that none are needed out of hours.
- **After experiment** – the action is needed to tidy up after the experiment.

The **List view** and **Calendar view** options switch between the normal list view and a calendar view showing actions with a definite time attached to them along with what the liquid handler will be doing.

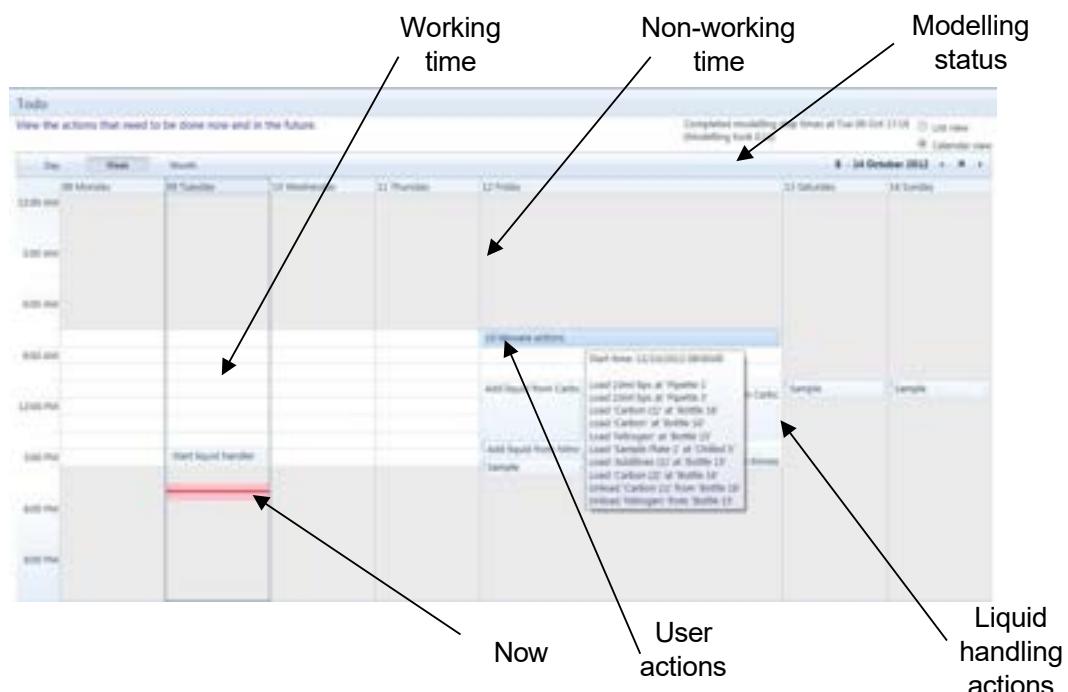


Figure 78 Calendar view

The calendar view shows the current time – “Now”, working time and non-working time with different backgrounds.

Entries are shown for user actions and for the liquid handling actions. Multiple actions at similar times can be grouped together. Hovering over items will show additional details of the item.

The status of the modelling on which the view is based is shown. In normal operation the model is updated once a minute.

3.4.3 Experiment

The **Experiment** page shows the current experiment.

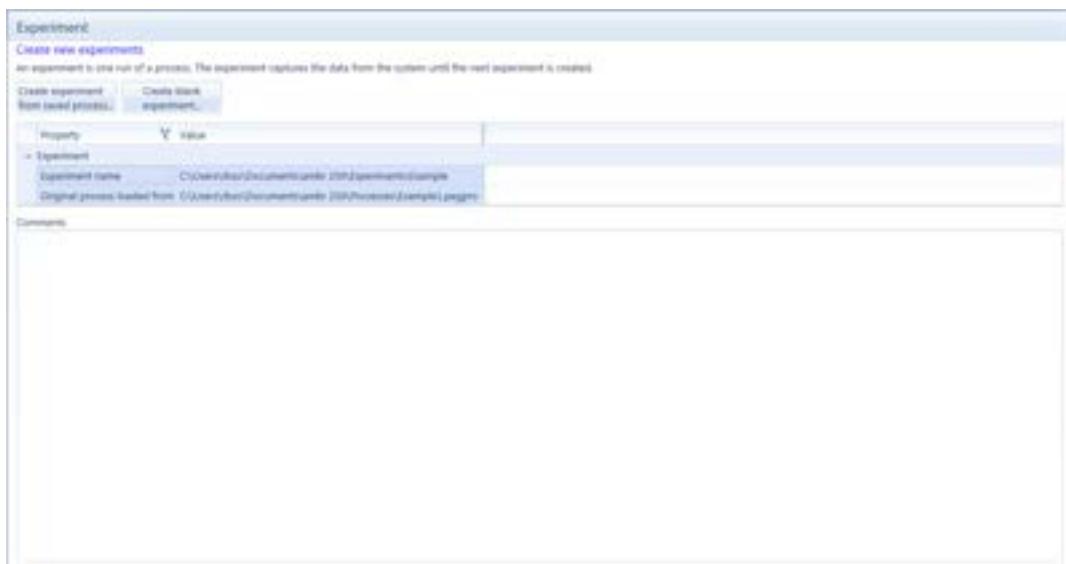


Figure 79 **Experiment** page

To start a new experiment all supervisors must be completely stopped.

The computer running the Ambr® 250 software must be set up so that it does not apply patches and reboot automatically during the course of an experiment. Most IT groups will want to ensure that the computer is patched to the appropriate version of Windows. Therefore before continuing and starting a new experiment it is advisable to:

- 1) Exit the Ambr® 250 software
- 2) Reboot the computer installing any windows patches that are due
- 3) Log in and restart the software

Once the system is stopped and if desired the software has been restarted then select either **Create experiment from saved process...** or **Create blank experiment...**

Choosing **Create experiment from saved process...** displays a window asking for the process to run and the folder in which to store the results of the experiment.

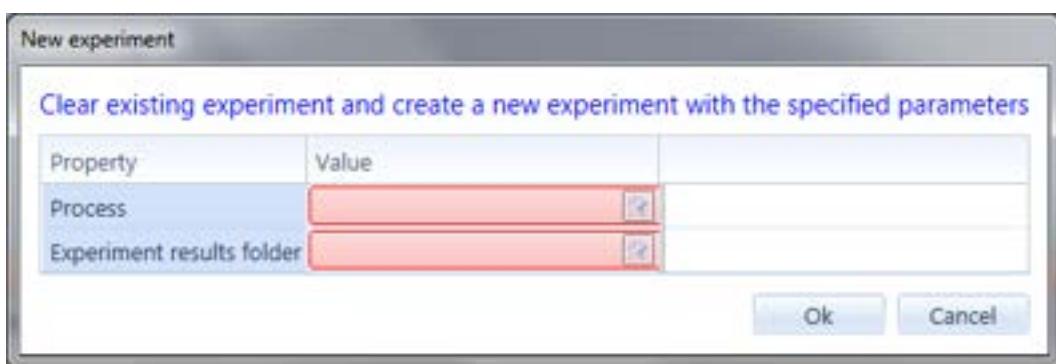


Figure 80 **New run** window

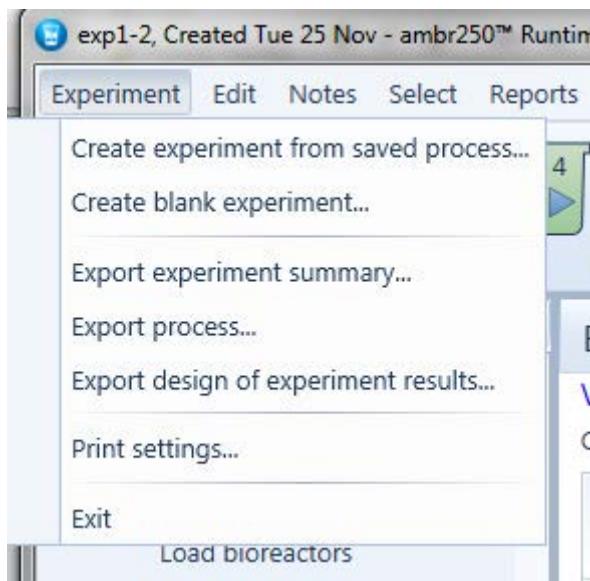
The **Process** can be selected from the processes stored in the system.

Processes can either be .pegpro files – typically found in ...\\ambr 250\\Processes – or .ambr250wpkpkt files from the design of experiment application which are typically in ...\\ambr 250\\Processes\\Investigations or in ...\\ambr 250\\Processes\\DOE work packets.

The **Experiment results folder** can either be chosen using a folder browser or a name of a folder can be typed into the option and the experiment will be saved in that folder within the ambr 250\Experiments folder.

Choosing **Create blank experiment...** displays a window asking for the folder in which to store the results of the experiment. The default is to store the results in an automatically named folder in the ambr 250\Experiments folder. **Create blank experiment...** can be a good option for storing clean-up and miscellaneous activity on the system between full experiments.

The **Create experiment from saved process...** and **Create blank experiment...** options are also available from the **Experiment** menu.



3.4.3.1 Export experiment...

This option exports the definition and all or a subset of the data associated with an experiment.

The option can be used to:

- export a copy of a running experiment for examination on another system
- export a subset of the data for an experiment to reduce the amount of space required on disk or when sending the experiment over the internet
- export the experiment to a single compressed '.zip' file

The **Export experiment** option is available from the **Experiment** menu in the runtime application and from the **File** menu in the experiment viewer application.

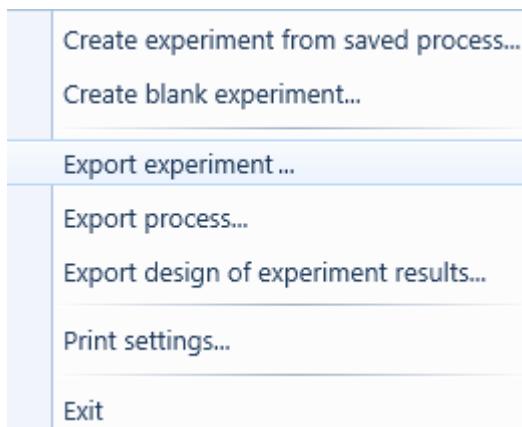


Figure 81 Export experiment menu item

A window is displayed where the options for the export can be chosen:

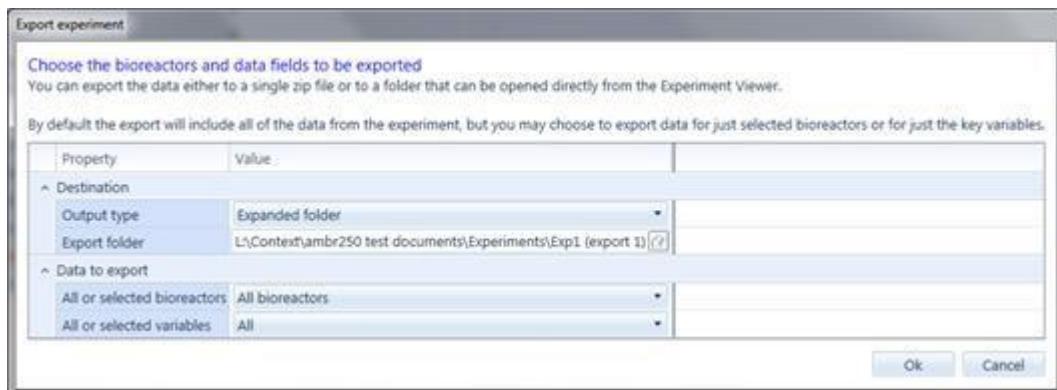


Figure 82 Options for exporting experiment summary

Output type allows export either to a single compressed file or as an expanded folder of files ready for opening in the experiment viewer application.

Export file or **Export folder** specifies where to store the exported data.

All or selected bioreactors allows either data for all bioreactors to be exported or data for just selected bioreactors.

If the option **Selected bioreactors** is chosen then the bioreactors to be included in the export must be selected in the **Which bioreactors** option.

All or selected variables allows either data for all variables to be exported or data just for key variables.

3.4.3.1.1 Opening an exported experiment

To open the data from a zipped experiment first expand the zip file into a convenient location.

Once expanded the data from an experiment can be opened in the experiment viewer application just like any other experiment.

If the export contains only data for selected bioreactors then only those bioreactors can be selected.

The experiment viewer shows in a light-grey variables for which there is no data present – either because the variable was not included in the export or because there was no data for the variable when the export was performed.

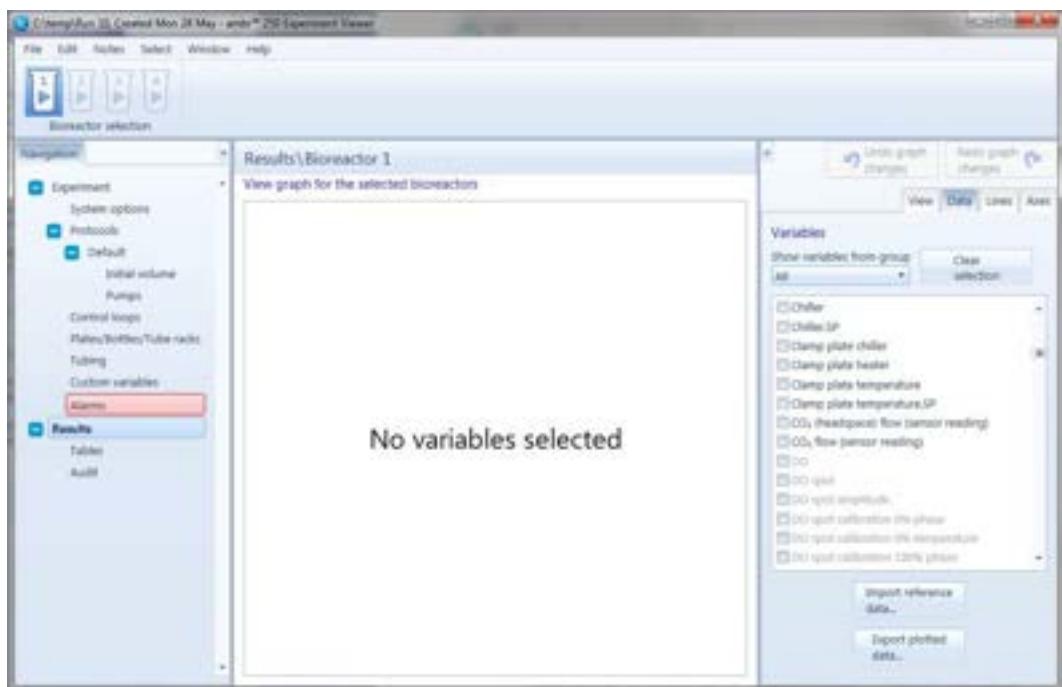


Figure 83 Experiment export only includes data for bioreactor 1. Export does not contain data for DO, DO spot etc.

3.4.3.2 Export process

The **Export process** option allows the process within an experiment to be saved to a file. The option is available from the **Experiment** menu in the runtime application and from the **File** menu in the experiment viewer application.

When the option is selected a window is shown for selecting where to save the process file.

3.4.3.3 Export design of experiment results

Export design of experiment results is used to export responses for an experiment created with the **Ambr® 250 Design of Experiment** application.

The **Export design of experiments** screen will be displayed showing a summary of the Ambr® 250 variables that have been associated with the DOE responses and their values.

Bioreactor	DOE expression	Cell count
Bioreactor 1	N013	96
Bioreactor 6	N024	96
Bioreactor 7	N024	96
Bioreactor 8	N065	96
Bioreactor 9	N05	96
Bioreactor 10	N06	96
Bioreactor 11	N025	96
Bioreactor 12	N044	96
Bioreactor 13	N045	96
Bioreactor 18	N054	96
Bioreactor 17	N025	96
Bioreactor 19	N055	96
Bioreactor 19	N049	96
Bioreactor 20	N050	96
Bioreactor 21	N030	96
Bioreactor 22	N080	96
Bioreactor 23	N077	96
Bioreactor 24	N032	96

Figure 84 Ambr® 250 Export design of experiment results

Selecting the **Save results...** button allows the results to be saved.

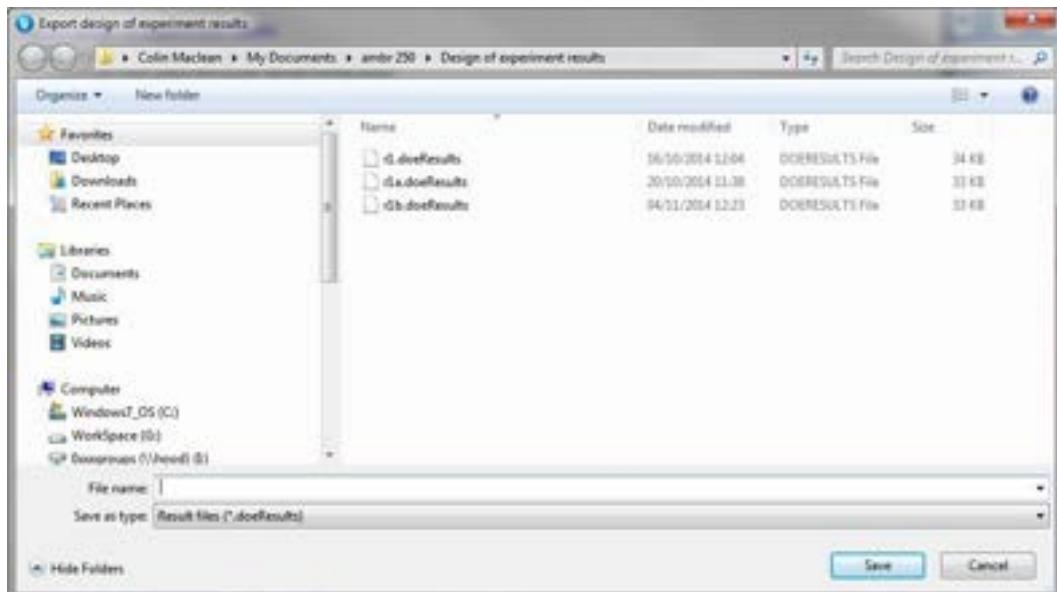


Figure 85 Save results... screen

Results are saved in the ...\\ambr 250\\Design of experiment results directory with a .doeResults extension. The Results can subsequently be imported into the **ambr250 Design of Experiments** application.

3.4.4 Interacting with the Process

Beneath the **Experiment** page in the navigation panel are the pages with the definition of the process to be run.

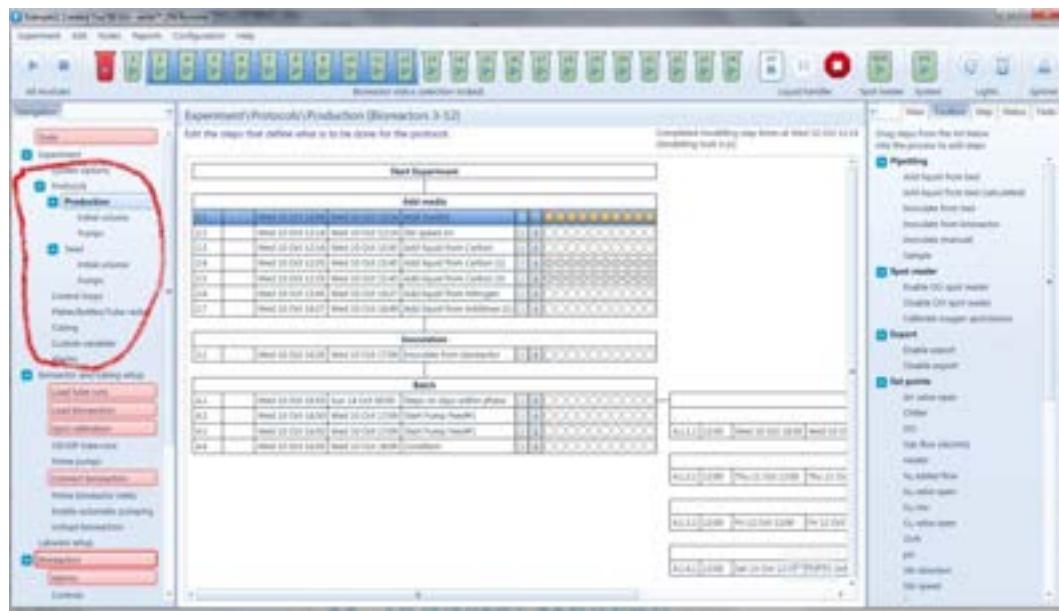


Figure 86 Process definition pages under the **Experiment** page

With some restrictions the process can be edited during the course of an experiment.

In particular you can:

- Define additional labware
- Change the parameters of control loops
- Add and edit steps that have not yet begun

The interactions with steps are described below.

3.4.4.1 Step status

The status of the steps for each bioreactor is shown on the Steps screen. Additional information about the status of a step can be seen by hovering over the status indicator or by viewing the **Status** panel for the selected step.

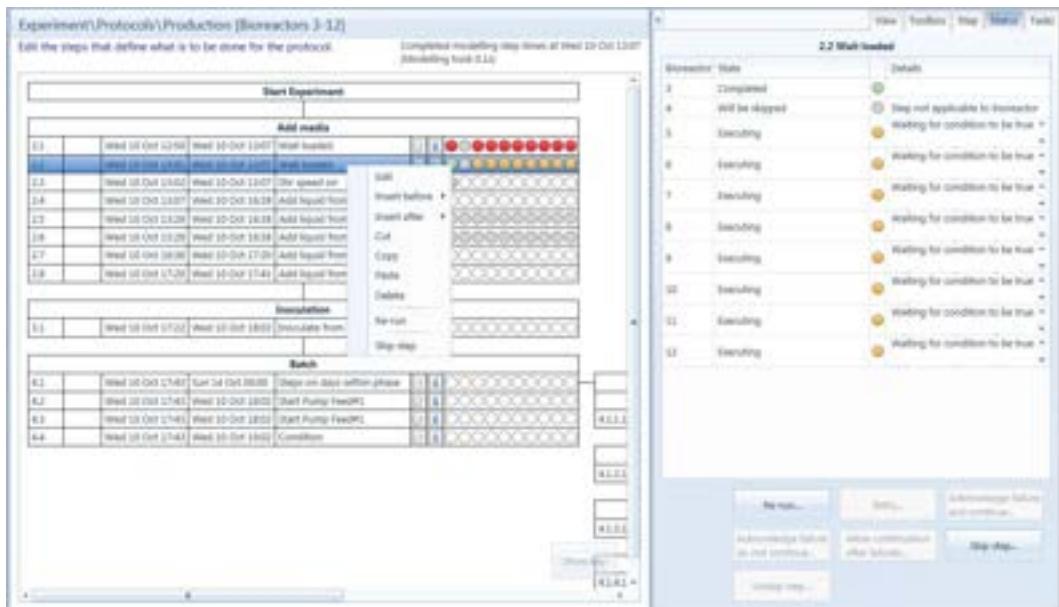


Figure 87 Status panel and context menu on selected step

The status indicators show:

- Step has not been done yet
○
- Step is in progress
○
- Step has completed successfully
●
- Step has or will not be done for this bioreactor
○
- Step has failed or has been skipped
●
- Step needs attention
● (flashing)

Depending on the state of the step the Status panel may have options enabled for intervening with the step. The same options can be seen if a context menu is displayed for the step.

Re-run... and **Retry...** create a new step that can be edited.

Re-run... creates another copy of the step to be executed by the bioreactors that have completed the step. The copy is created directly after the step if that is valid or otherwise inside a Parallel Block. The Edit steps window is displayed for the copy.

Retry... creates another copy of the step to be executed by the bioreactors that have failed the step. The copy is created directly after the step if that is valid or otherwise inside a Parallel Block. The Edit steps window is displayed for the copy.

The remaining options update the status of a step. A window is displayed to confirm the action and select which bioreactors the action should apply to.

Acknowledge failure and continue... acknowledges a failed step for a bioreactor and allows subsequent steps after the failed step to be executed.

Acknowledge failure do not continue... acknowledges a failed step for a bioreactor but does not allow subsequent steps to be executed.

Allow continuation after failure... allows steps after a failed step to be executed. (Allow continuation after failure... provides a way to continue if Acknowledge failure do not continue... is chosen in error.)

Skip step... marks the step to be skipped for one or more bioreactors.

Unskip step... undoes the marking of a step to be skipped.

When the **Show step to repeatedly run a block of steps** feature has been selected **Pause block** and **Resume block** pause and resume operation of a repeating block.

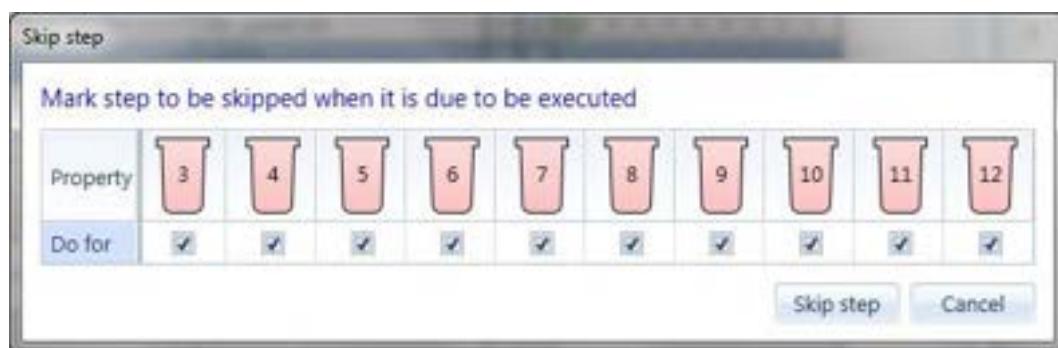


Figure 88 Confirmation window for **Skip step**

3.4.4.2 Step context menu

The options **Re-run**, **Retry** etc. are also offered from a context menu on the step.

Some additional short cuts are provided on this context menu and are described below.

3.4.4.2.1 Responding to prompt

Where a prompt is associated with a step (**Add liquid (manual)**, **Inoculate (manual)**, **Sample (manual)**, **Prompt user**, **Unload bioreactor**) then the prompt can be accessed from a context menu on the step.

If there are different prompts for different bioreactors then a choice of bioreactors is presented.

Choosing from the context menu displays the same window that would be shown from the **Todo** page.

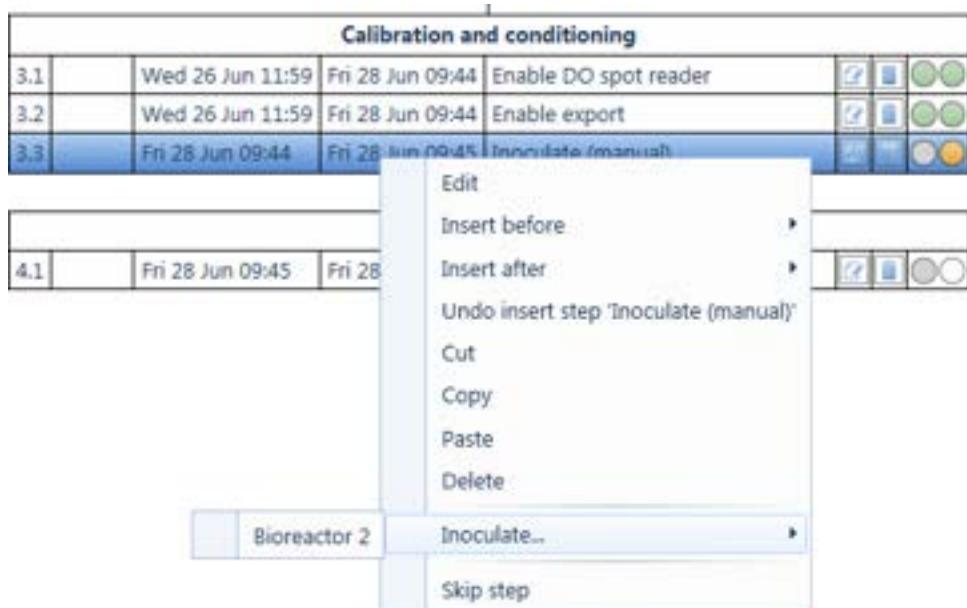


Figure 89 Context menu on step to respond to prompt

3.4.4.3 Editing steps

Editing steps once a process has started is subject to certain restrictions.

Parameters that have been used by the system cannot be changed. Once a step has completed for a bioreactor the parameters affecting that bioreactor cannot be edited. Once a step has completed for all bioreactors then it is read-only.



Figure 90 Edit step window for a step completed by a bioreactor

Steps cannot be inserted in the process out of order. Once the process has passed a point in the sequence of steps you cannot insert a step. If the process has passed a point for some bioreactors then a step can be inserted but that step will only be executable by the bioreactors that have not passed that point in the process.

While a step is being edited the system cannot execute the step. Leaving a window open editing a step will stop the process at that point until the window is closed.

3.5 Experiment Viewer Application

The Experiment Viewer application allows viewing data from completed or exported experiments.

On starting the Experiment Viewer a window is displayed to allow an experiment to be opened.

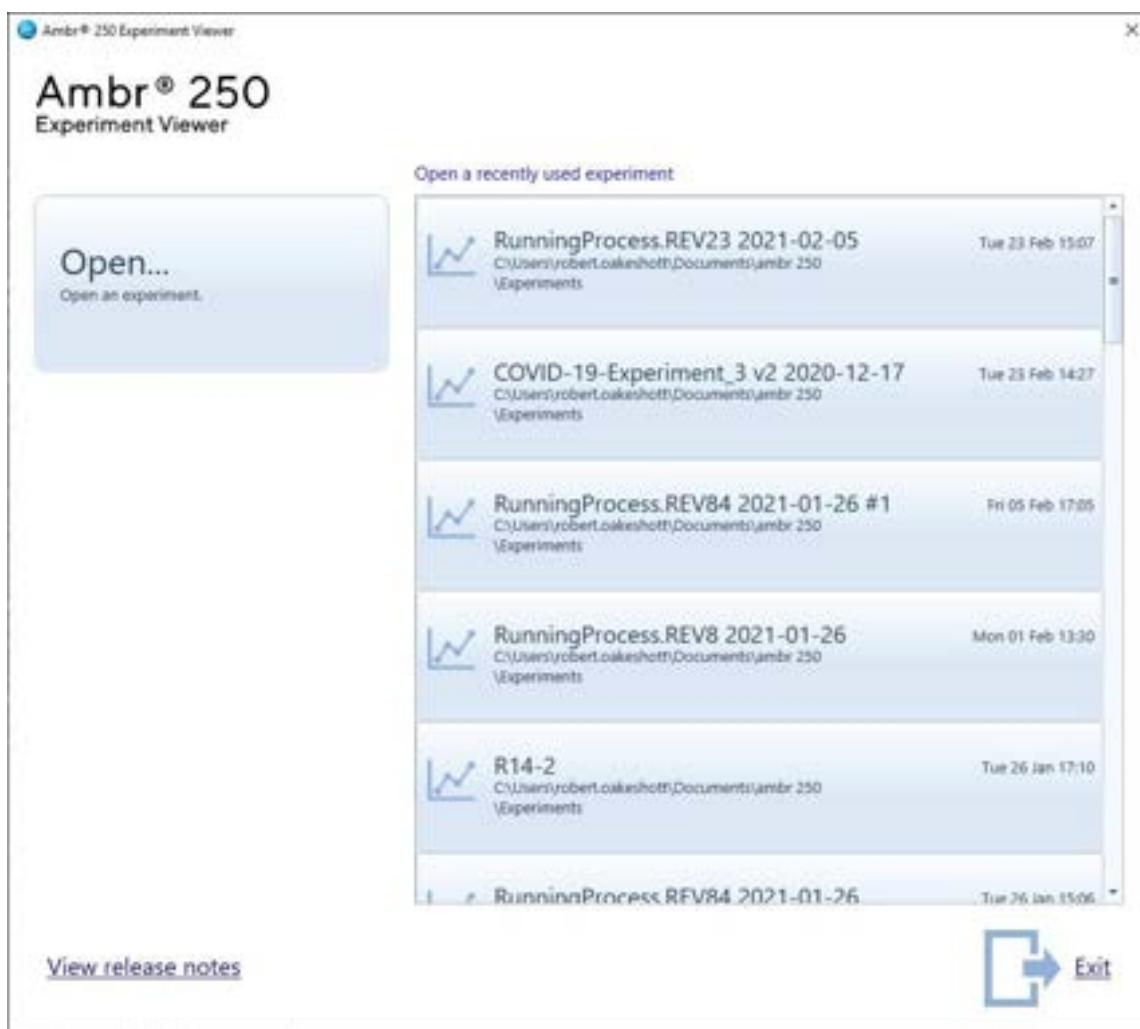


Figure 91 Initial window

If the experiment has been used recently then click the entry in the list of recent experiments to open it.

For other experiments selecting **Open** opens a window to select an experiment. Choose the top folder of the experiment (under which are the Process, Configuration and other folders).

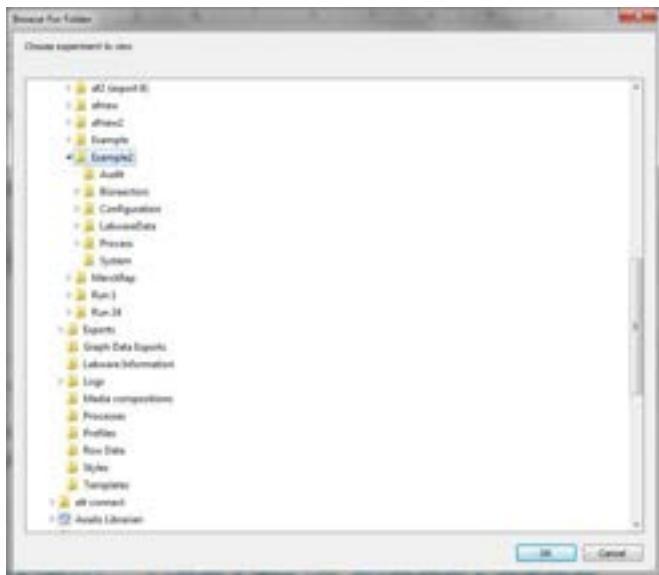


Figure 92 Opening the **Example2** experiment

Having selected the experiment to view the main window is displayed.

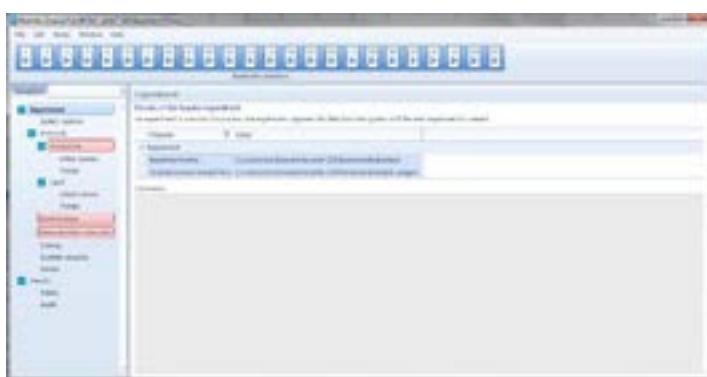


Figure 93 Experiment Viewer application

The Experiment Viewer shows a subset of the pages in the Runtime application.

Editing the process definition is disabled with the exception of the ability to edit the custom variables.

Additional experiments can be opened from the **Open experiment** option on the **File** menu.

Should a problem occur loading the data for an experiment then an additional **Errors** page is added which contains details of the problem.

3.6 Common process definition options

This section describes some common options that are used in multiple places within the process definition.

- Expressions which can be used inside conditions; to specify the values of selected options in steps and control loops; and to specify the values of calculated variables.
- Conditions which can be used inside wait condition steps to control the overall process of a bioreactor and in control loops to define when to deliver boluses.
- Liquid handling options which can be used inside steps and inside control loops.

- Labware options
- Profiles
- Comments

3.6.1 Expressions

Expressions can be used as parameters in various parts of the application.

See **TAP-9351-06-022 ambr 250 expressions guide** for details.

3.6.2 Conditions

Conditions test whether something is true about a general expression, and are used in:

- Pipette bolus control loops
- Pump bolus control loops
- Condition steps

The conditions have the same options in each case that are described here.

Condition	
Expression	Deviation 
Comparison	Greater than 
Compare against	Value 
Value	0.01
Delay time	5m

Bolus	
-------	--

Figure 94 Simple condition

The figure above shows a simple condition testing that Deviation > 0.01 continuously for 5 minutes.

An **Expression** defines the value tested by the condition.

A comparison is made against either another value or against a past value of the expression.

Comparison can be:

- **Greater than**
- **Greater than or equal**
- **Less than**
- **Less than or equal**
- **Equal to**
- **Fallen by**
- **Fallen by percent**
- **Risen by**
- **Stable**

- Range
- Change by

If Comparison is **Greater than**, **Greater than or equal**, **Less than**, **Less than or equal**, or **Equal to** then the comparison can be made against a **Value** or against another **Expression** according to the **Compare against** parameter.

- When **Equal to**, **Less than or equal** or **Greater than or equal** is selected then the **Max. difference** between the values that should be counted as equality must be entered. The **Delay time** specifies how long the comparison must be continuously true for before the Condition as a whole is considered to be true. If the **Delay time** is not set then the condition as a whole is true whenever the comparison is true.

Condition	
Expression	Deviation
Comparison	Greater than
Compare against	Expression
Expression	2 * 'pH.Upper SP'
Delay time	5m

Figure 95 Comparison of the values of two expressions

If the Comparison is **Fallen by**, **Fallen by percent**, **Risen by**, or **Change by** then it is necessary to specify more parameters.

Fall or **Rise** or **Change** specifies how much the expression should have fallen, risen or changed by.

Change interval type specifies over what interval the change is looked for. Depending on context the options may include:

- **Over interval** – the change is looked for between the specified **Change interval** ago and now
- **Since phase started** – the change is looked for between the start of the current Phase and now
- **Since condition started** – the change is looked for between the start of testing the condition and now
- **'Minimum since condition started'** or **'Maximum since condition started'** - the change is looked for between the minimum (for a Rise) or maximum (for a Fall) value between the start of testing the condition and now'

Condition	
Expression	Deviation
Comparison	Fallen by
Fall	20
Change interval type	Over interval
Change interval	10m
Smoothing mode	Min
Smoothing interval	1m
Delay time	5m

Figure 96 Testing for Deviation to have fallen by 20 over a 10 minute interval.

Condition 1	
Expression	DO
Comparison	Risen by
Rise	20
Change interval type	Since phase started
Smoothing mode	None
Delay time	

Figure 97 Testing for DO to have risen by 20 since the start of the phase

The test for the value rising or falling may either be made against the raw data – a **Smoothing mode** of **None** – or may be made against smoothed data.

The options for averaging the data are:

- **None** – the most recent values are used
- **Min** – the minimum value in the smoothing interval is used
- **Max** – the maximum value in the smoothing interval is used
- **Mean** – the average (mean) value in the smoothing interval is used

When smoothing is being used the **Smoothing interval** over which to smooth the data must be specified.

The figure below shows how the parameters fit together:

- The data is sampled over two intervals each **Smoothing interval** in duration and offset by the **Change interval**
- The minimum value of the data in each interval is chosen
- The difference between the values in the each interval is compared to the required rise

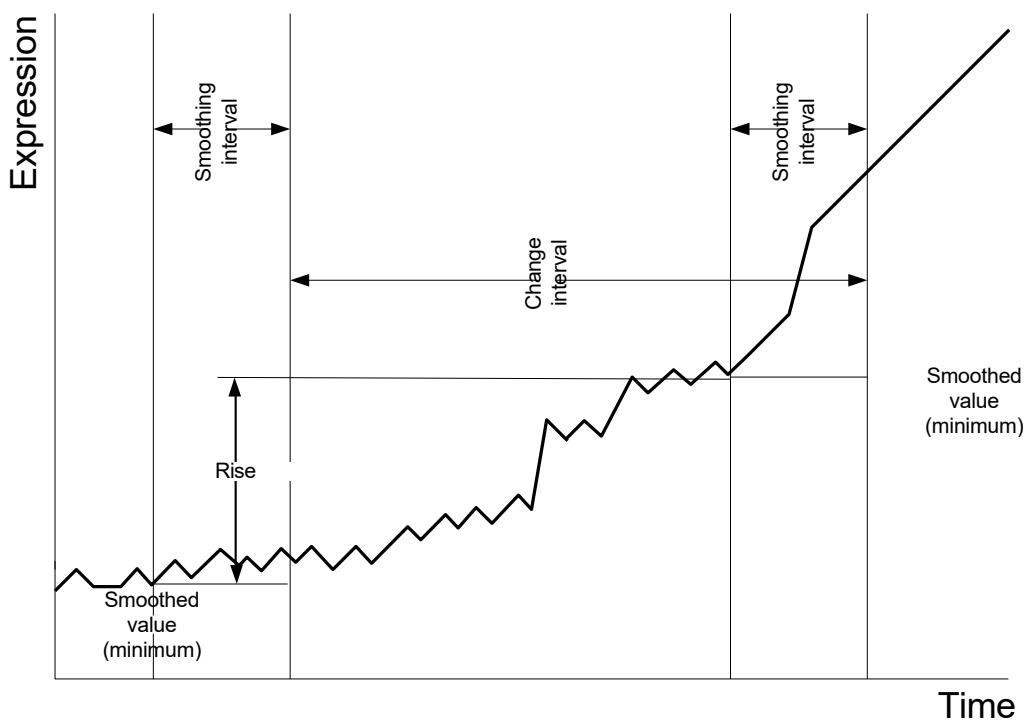


Figure 98 Smoothing interval, Change interval and Rise

If the **Comparison** is **Stable** by then it is necessary to specify the **Max. range** and the **Smoothing interval**. The condition evaluates to true when the range of the **Expression** over the **Smoothing interval** is less than or equal to the **Max. range**.

Condition 1	
Expression	pH
Comparison	Stable
Max. range	0.2
Smoothing interval	5m
Delay time	

Figure 99 Parameters when **Comparison** is **Stable**

If the **Comparison** is **Range** by then it is necessary to specify the **Min. range** and the **Smoothing interval**. The condition evaluates to true when the range of the **Expression** over the **Smoothing interval** is greater than or equal to the **Min. range**.

Condition 1	
Expression	'Foam sensor'
Comparison	Range
Min. range	35
Smoothing interval	10m
Delay time	2m

Figure 100 Parameters when **Comparison** is **Range**

3.6.2.1 Combining multiple conditions

Where multiple conditions are offered – for example in a **Wait condition** step – then the conditions can be combined using **Or** or **And**.

Edit step - 'Wait condition'

Edit step parameters

Wait until a condition is true for a suitable interval

Property	Value
Description	Wait condition
Which reactors	
All or selected	All bioreactors assigned to protocol
Action	
Action	Wait until condition is met
If fail or timeout	Do next steps
Significance of failure	
Grouping	
Group bioreactors	One at a time
Timing	
Expected timing	Wait for duration
Minimum wait time	0h
If condition satisfied early	Continue immediately
Maximum wait time	30m
Clauses	
Number of clauses	2
Applies to	This bioreactor
Condition 1	
Expression	'pH cell temperature'
Comparison	Equal to
Compare against	Expression
Max. difference	0.1
Expression	'pH cell temperature.SP'
Condition 2	
Combine as	Or
Expression	'Optical density'
Comparison	Greater than
<input type="checkbox"/> Show DOE tags	
<input type="checkbox"/> Show bioreactors	
<input type="button" value="Ok"/> <input type="button" value="Cancel"/>	

Figure 101 Wait condition step set to proceed if either condition passes

In bolus control loops where 3 conditions can be used then AND takes priority over OR so “True AND False OR False” would be interpreted as “(True AND False) OR False” and would be evaluated as false.

3.6.3 Error handling options

Liquid handling steps have **Error handling** options to allow the system to continue with other steps in the process even if the liquid handler has a problem.

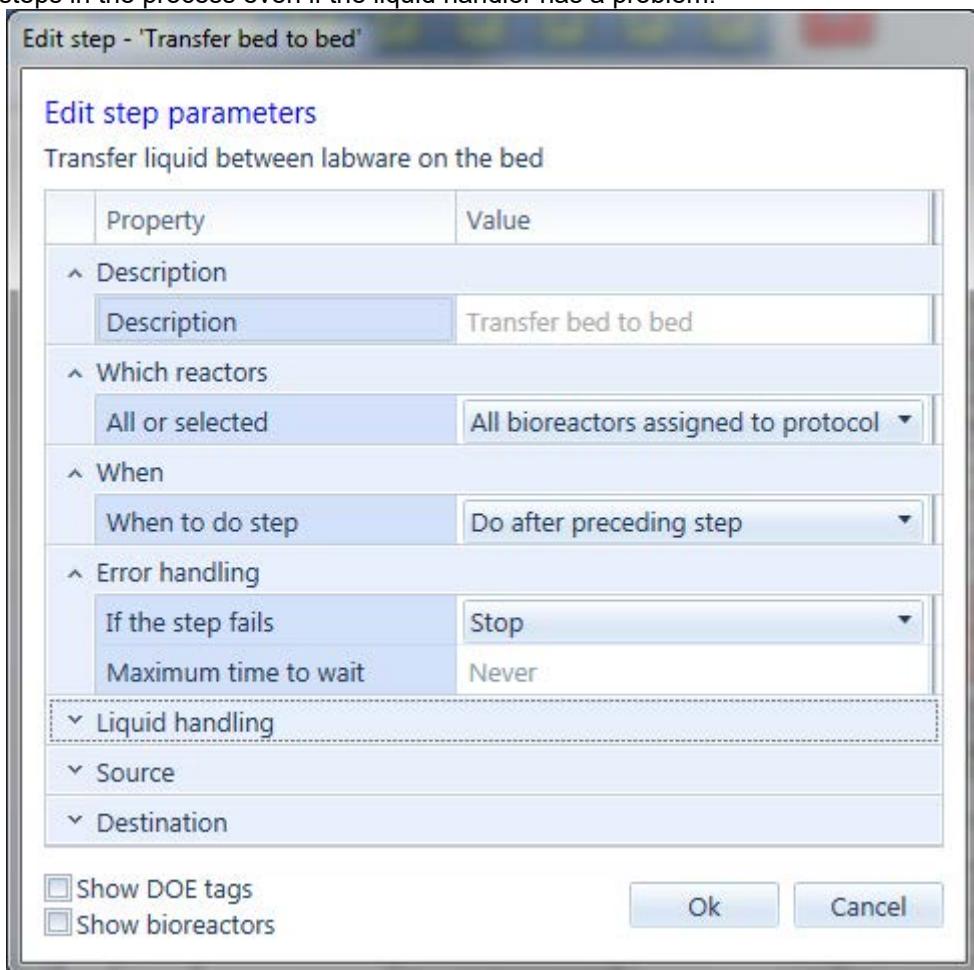


Figure 102 Liquid handling step with **Error handling** options

3.6.3.1 Option to continue to next step

If the step fails specifies whether in the event of the step failing subsequent steps should be executed – **Do next steps** – or subsequent steps should not be executed – **Stop**.

Maximum time to wait specifies a timeout after which this step will fail. Take care if using this feature: setting this timeout to too low a value will cause steps to fail if the liquid handler is busy – even if there is no problem with the system.

3.6.3.2 Option for liquid handler to try next action after simple errors

An option has been added to pipetted liquid classes to allow the behaviour of the liquid handler after an error to be adjusted.

If the liquid handler has a problem offers the choice of:

- Ask user for instructions causes the system to present a dialog with options for the user after a problem has occurred and the system has failed to resolve the problem using its in built logic for retrying individual actions.
- Continue with next action where practical allows the system to attempt to continue automatically in the presence of some errors. To do so the system must make assumptions as to the nature of the error and how to continue. Continuing may result in other harder to recover errors later – or may allow the system to successfully complete its other tasks.

When Continue with next action where practical is selected and certain errors occur the system displays an error message allowing the standard recovery dialog to be displayed by clicking Show other recovery options. If the user clicks Attempt to continue with next action or does not click anything then the system will attempt its automatic continuation.



From release 4 of the software the following errors have special handling:

- If the liquid handler cannot de-lid a tip box then it presents a dialog to ask if it should test for a lid at the corresponding lid location. If the liquid handler finds a lid there it assumes it came from the tip box and continues accordingly. If there is no lid there then the liquid handler tries any other loaded tip boxes.
- If the liquid handler detects that it has lost a tip before an aspirate or dispense then it disposes of the tip and attempts to continue with the next piece of liquid handling available. If it had marked the current liquid handling as started then the current liquid handling is marked as failed – if not then the system will typically reattempt the current liquid handling.

3.6.4 Liquid handling options

This section describes the **Liquid handling** options that are available both within **Pipetting** steps and in the **Pipette bolus** control loop. Different sets of options are available in different contexts and the most specialised options are described in the particular contexts where they are valid.

^ Liquid handling	
Priority	50
Liquid class	Default
Selected delivery specification	MEDIA; MEDIA; V >= 0.25
Reuse tips	<input checked="" type="checkbox"/>
Multiple dispenses from one aspirate	<input type="checkbox"/>

Figure 103 Liquid handling options from a pipetting step

Liquid handling	
Priority	50
Liquid class	Default
Selected delivery specification	MEDIA; MEDIA; V >= 0.25
Reuse tips	<input type="checkbox"/>

Figure 104 Liquid handling options from a pipette bolus control loop

Steps and control loops requesting liquid handling place an action into a queue. The liquid handler executes the actions from the queue in priority order. The **Priority** option specifies the parameter of the actions. Use a higher number for actions that should take priority – for example if the actions from the control loop are the most urgent things then set their priority to a higher priority than has been chosen for other actions.

The **Liquid class** specifies how the transfer is to be done. The system selects the delivery specification from the liquid class that best fits the volume to be transferred and the labware types involved. **Selected delivery specification** displays the description of the selected delivery specification. Unless overridden that description shows the aspirate and dispense methods used along with the minimum volume for the delivery specification.

The delivery specification specifies the size of tip to be used and the methods used by the liquid handler to aspirate liquid and dispense liquid.

Some steps may have additional parameters so that different liquid classes can be used for different transfers.

Reuse tips specifies whether aspirates from the same source should reuse the same tip. Aspirates from different sources will always use different tips. For aspirates from the same source to the same bioreactor to reuse tips then both the **Reuse tips** option must be selected and the bioreactors must be grouped **All together** or **Group by source**



Reuse tips is shown for Sample steps only if the **Allow tip reuse in sample steps** option is selected in the **Advanced features** window.

The **Multiple dispenses from one aspirate** option allows the liquid handler to suck up liquid into the tip and then dispense that liquid to multiple destinations.

3.6.4.1 Gas hold up

Steps that aspirate liquid from active bioreactor have a gas hold up parameter to help deal with transferring the aerated liquids in the bioreactor.

Gas hold up (%) – percentage of the volume in the source bioreactor taken up by gas within the liquid.

The amount aspirated by the liquid handler is increased so that the required volume of liquid is aspirated to account for the gas in the liquid. The amount dispensed by the liquid handler is not changed and the liquid handling methods should include sufficient time for the gas held up in the liquid to have settled out within the tips. Suppose **Gas hold up (%)** is 20% and 5mL is to be transferred.

The aspirate volume will be $5 * 100/(100-20) = 6.25\text{mL}$

The gas will rise up in the tip leaving ideally 5mL of liquid at the bottom of the tip that can then be dispensed.

If the Gas hold up value chosen is too high then some liquid will be left in the tips at the end of each transfer.

If the Gas hold up value chosen is too low then there will be too little liquid for the transfer and air will be dispensed into the destination instead of the desired liquid.

3.6.5 Labware options

This section describes the labware options that are available both within **Pipetting** steps and in the **Pipette bolus** control loop. The options are presented for each distinct source or destination required.

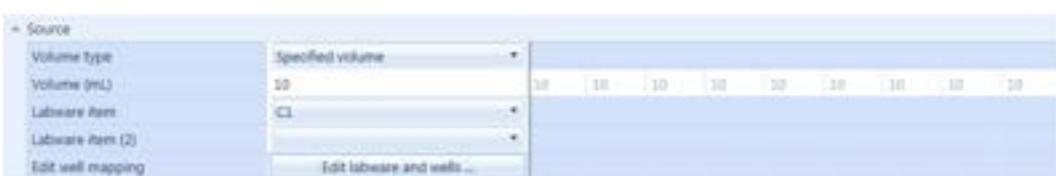


Figure 105 Labware options with a single bottle selected

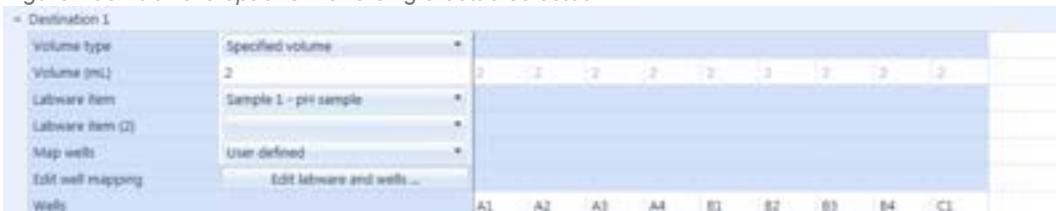


Figure 106 Labware options with plate selected

The options include:

- If applicable **Volume type** allows the selection of either **Specified volume** to transfer a specified volume or **Calculated volume** that allows the **Volume** to be specified by an expression.
- If applicable the **Volume** to transfer to or from the labware for each bioreactor
- If the **Volume** is a **Calculated volume** then **Minimum volume** and **Maximum volume** specify the allowed transfer range. Transfers greater than the maximum volume are taken as an indication of an error. **What to do if volume is low?** specifies what do with volumes that are less than the minimum volume or are negative.
- The **Labware item** that is the source or destination of the transfer. The available labware items are the labware items with the appropriate **Role** e.g. for a **Sample** step the items are chose with a **Role** of **Sample**.
- An optional second item (**Labware item (2)**) that allows two different labware items to be used for the different bioreactors
- **Map wells** selects how different bioreactors are mapped to different wells in plates. The option is displayed when the **Labware item (2)** option is chosen or when a plate or tube rack is chosen as the labware. The options are:
 - **By absolute position** – the position of the bioreactor is used to lookup the well and labware item from a predefined well mapping

- **By relative position** – the position of the bioreactor within the context is used to lookup the well and labware item from a predefined well mapping. Within a protocol using bioreactors 3,4 and 6 the relative positions used would be 1, 2 and 3 respectively.
- **User defined** – the well and labware item is specified explicitly.
- **Wizard defined** – the well and labware item is specified explicitly as the result of a wizard. To edit the mapping change **Map wells to User defined**. The sample wizard will recognise steps that are using a mapping other than **Wizard defined** and will not update those steps.
- **Edit well mapping** displays another window for editing these labware options
- **Which labware item?** specifies which labware item will be used for each bioreactor.
- **Wells** specifies which well is used for each bioreactor. If **By absolute position** or **By relative position** is used then **Wells** presents a choice of mappings from bioreactor position to well and shows the result of that mapping. If **User defined** is used when Wells lets the well for each bioreactor be chosen independently.

The **Edit well mapping** option displays the window shown below.



Figure 107 Edit well mapping window. Both bioreactors 3 and 4 are mapped to the same well in this example.

The window repeats the labware options and adds a graphical display of the mapping.

Each well in the mapping shows the bioreactors associated with that well. The bioreactor numbers are shown in bold if they belong to the context of this window and in a lighter colour if they belong to another context.

Dragging from a bioreactor column to a well or from a well to a bioreactor column will choose that well for that bioreactor.

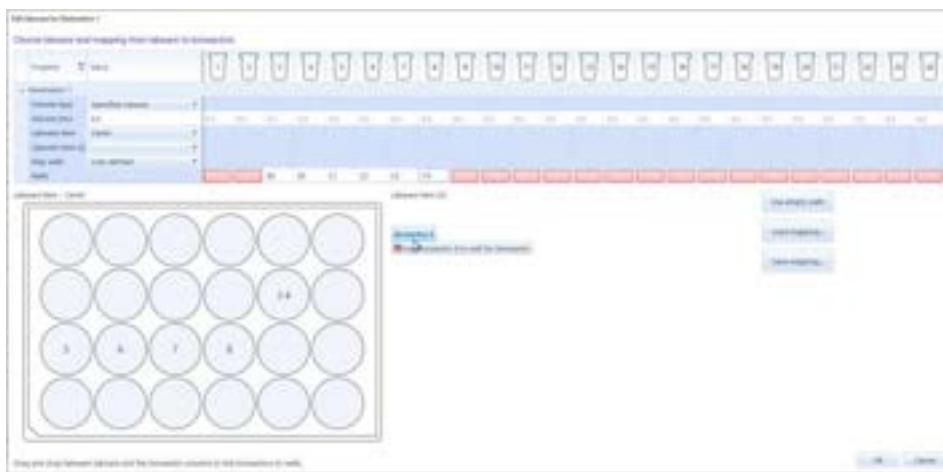
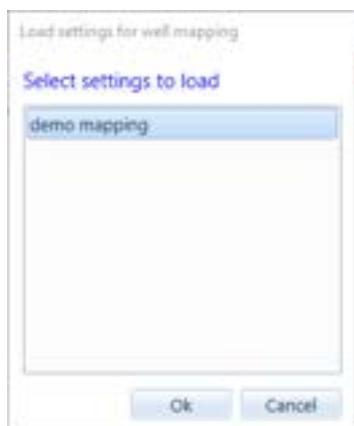


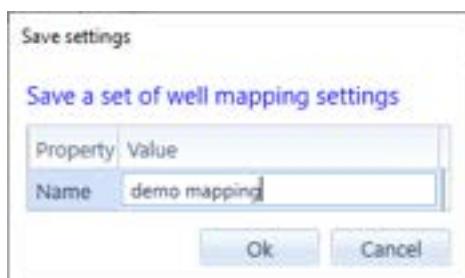
Figure 108 Dragging to link well to bioreactor

The **Use empty wells** option can be used to try and assign an otherwise unused well to each bioreactor.

The **Load mapping...** option shows a dialogue that allows a previously saved well mapping to be loaded.



The **Save mapping...** option shows a dialogue that allows the current well mapping to be saved.



3.6.6 Profiles



Profiles are an advanced feature enabled by the **Allow defining set points as profiles** or the **Show options to create lookup table control loops** options in the **Advanced features** window.

Profiles are used:

- In steps setting set points or starting pumping to specify the value of the output as a function of time
- In Lookup table control loops to specify the value of the output as a function of the input

The figure below shows the edit profiles dialog.

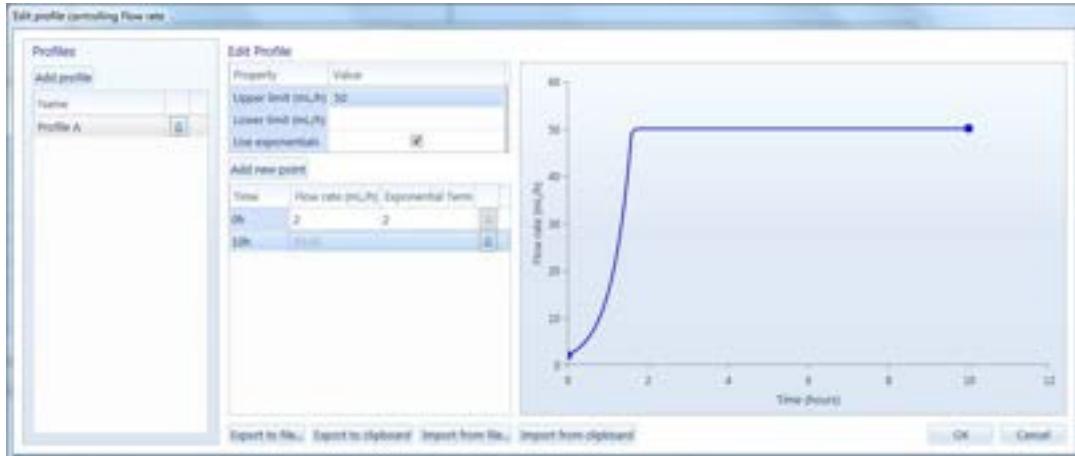


Figure 109 Edit profiles dialog

To edit a profile:

- 1) give the profile a name
- 2) edit and add points to the profile defining the values at different times

Add new point adds a new point at the end of the profile. (Or for a lookup table profile just before the fixed last point.)

A context menu on the points in the list has options **Add point after** and **Add point before** to add points into the profile.

The initial point in a profile for a set point can have its value left blank. In this case the profile will start with the current value of the set point when the profile is applied.

Once time runs beyond the end of the profile the final point in the profile will continue to be used.

Depending what the profile is for it may be possible to define the profile using exponential terms. To enable this option check the **Use exponentials** option. When the Use exponentials option is selected each point can optionally have an exponential term associated with it. The value of the profile going forward from that point is the value at the time of the point multiplied by $\exp(\text{time from point} \times \text{exponential term})$.

The point following an exponential term can have either the value of the profile or the time of the point unspecified. The position of the point is then inferred from the previous point.

If the value calculated from the profile would exceed the limits of what it is profiling then the profile is clipped to the valid limits. The lower and upper limit scan be adjusted using the **Lower limit** and **Upper limit** options.

The figure below shows a simple profile demonstrating these aspects.

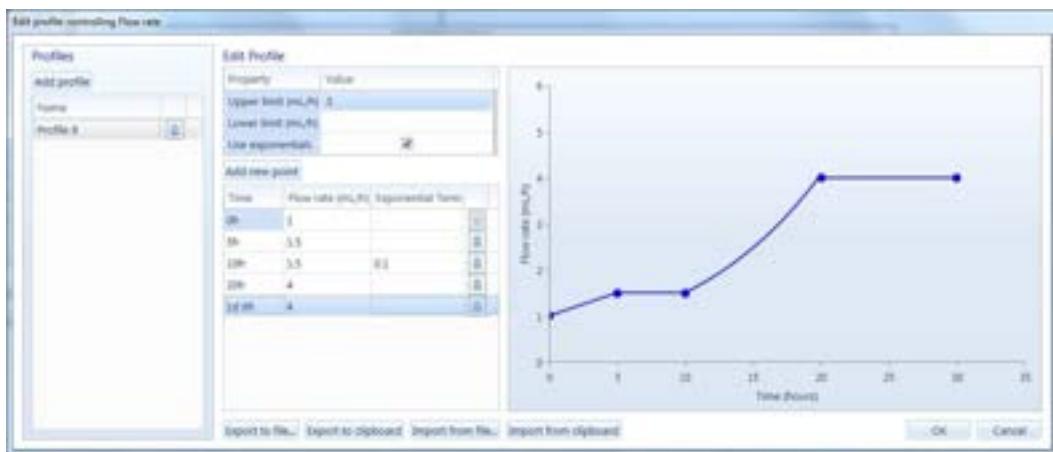


Figure 110 Profile with linear section, exponential section and clipping

If the profile is for pH then it is possible to define separate profiles for the lower and upper set points.

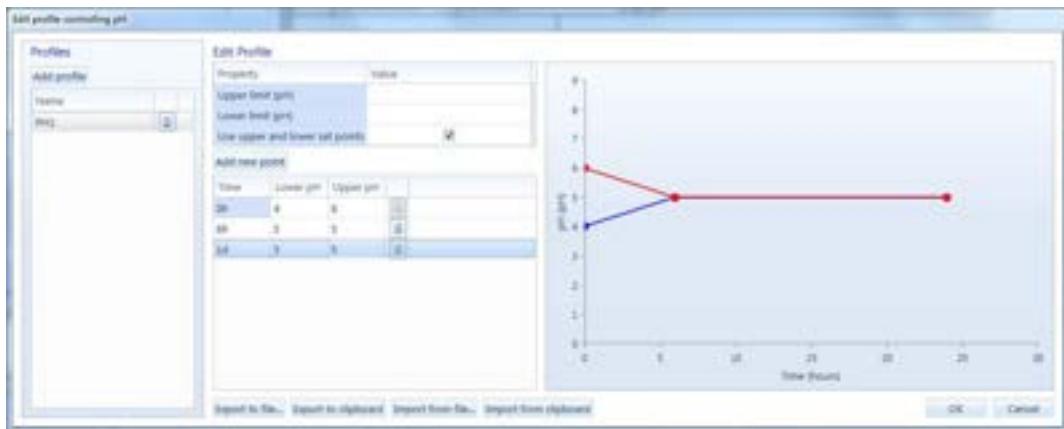


Figure 111 pH profile with lower and upper setpoint

If the profile is for a **Lookup table** loop then the points map from x (what the loop is controlling) to y (the output of the loop).

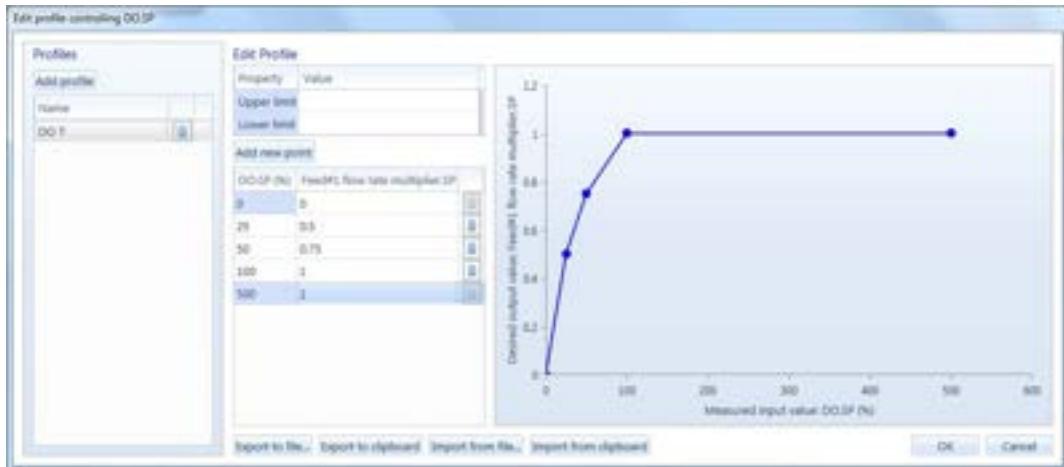


Figure 112 Lookup table loop profile

The **Export to file...**, **Export to clipboard**, **Import from file...** and **Import from clipboard** options export and import the contents of the currently selected profile.

3.6.7 Comments

Parts of the definition that do not have a more custom description have a **Comments** field that can be used to describe the item.

Edit custom variable 'Glucose_Addition'

Edit parameters for variable

Property	Value
^ Description	
Description	Glucose_Addition
Comments	Amount of glucose added to bioreactor.
^ Definition	
Axis title	Glucose_Addition
Units	Custom
Custom units	mL
Display format	1 decimal place
Minimum value	0.0
Maximum value	100.0
Has set point	<input type="checkbox"/>
Is calculated	<input type="checkbox"/>

Ok Cancel

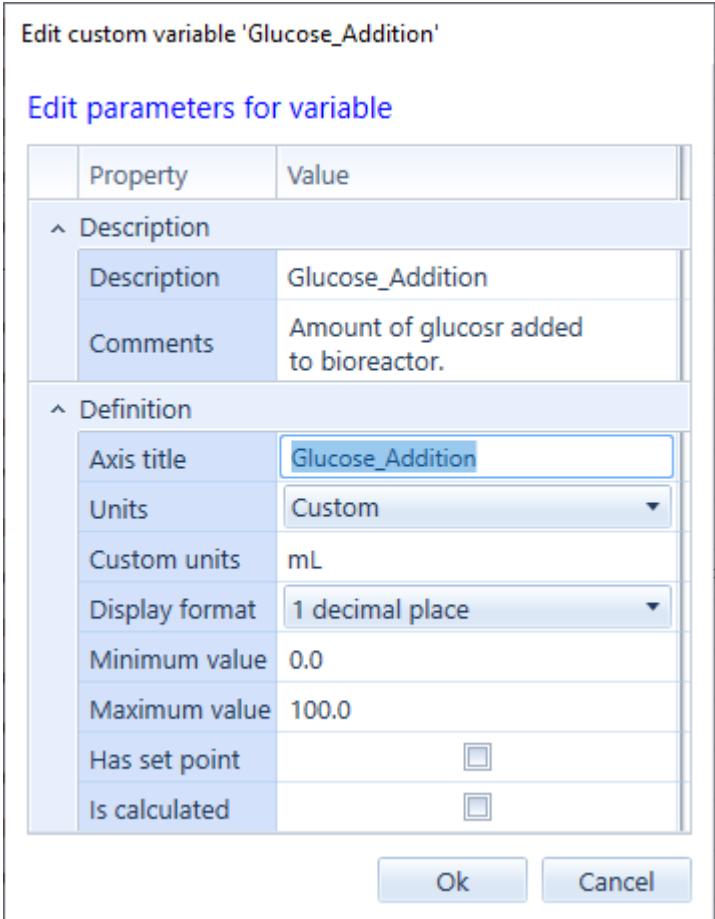


Figure 113 Custom variable with comment field

4 PROCESS DEFINITION

This section describes the features and controls for defining an Ambr® 250 process.

The operation of the Ambr® 250 machine is controlled by a definition of the process it is to run. Typically the initial definition of the process is created offline using the definition application and loaded onto the machine when an experiment is started. Aspects of the definition can be edited, subject to some restrictions, as the experiment runs.

4.1 System options

The **System options** page holds options for the overall setup and running of the system. The available options depend on the configuration of the Ambr® 250 system and so not all of the options here will be shown on all systems.

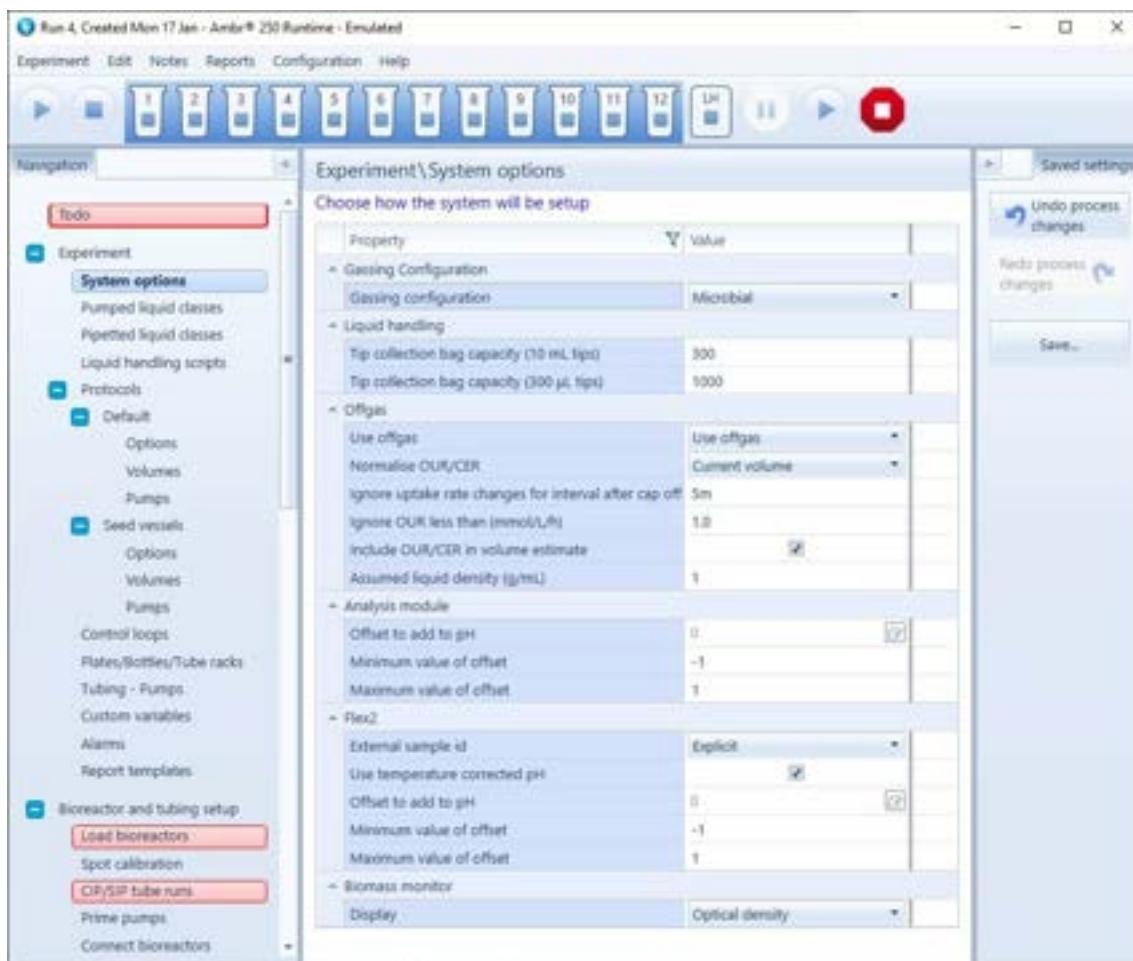


Figure 114 System options page

4.1.1 Gassing Configuration

Gassing configuration selects the gassing options, i.e. which gasses are connected to the system; which set points should be available to control the gassing and what the valid values of those set points are; whether headspace and/or sparge gassing is supported and whether OUR/CER calculations are supported.

4.1.2 Vessel Type

Bioreactor vessel type selects the type of bioreactor vessel to be used with the system.

Selecting a vessel type that includes external condensing enables the use of external condensing when the system supports this.

Selecting a perfusion vessel type enables perfusion when the system supports this.

4.1.3 Perfusion filter priming

If the **Bioreactor vessel type** supports perfusion then the **Perfusion filter priming** options will be available.

Permeate volume specifies the volume of liquid to pump to prime the outside of the hollow filters. **Permeate flow rate** specified the rate at which to pump while priming.

4.1.4 Offgas

Use offgas – when unchecked offgas sensors will be ignored and no offgas values recorded.

If the **Bioreactor vessel type** supports external condensing then the **Use external condenser** option will be available. This should be checked if the external condenser is to be used and not checked if the external condenser will be bypassed and internal condensing used instead.

Normalise OUR/CER offers options for the volume to be used when converting a total uptake/evolution rate in mmol/h to a normalised rate in mmol/l/h. Options are:

- **Actual inoculation volume** – the volume that the system calculated to be in the bioreactor when the bioreactor was inoculated
- **Current volume** – the current volume in the bioreactor
- **Nominal inoculation volume** – the volume entered as the **Nominal inoculation volume**.



The **Nominal inoculation volume** option is only offered if the **Allow normalising OUR/CER by reference to a nominal inoculation volume** option is selected in the **Advanced features** window.

Ignore update rate changes for interval after cap off allows the time for which changes to OUR/CER are ignored after a cap has been taken off and replaced by the liquid handler to be customised. The option is only shown on systems that have a liquid handler.

Include OUR/CER in volume estimate chooses whether or not to include the integrated oxygen uptake and carbon dioxide evolution in the volume estimate.

Assumed liquid density specifies the density used when allowing for the effect of oxygen uptake and carbon evolution on the culture volume. The calculation of OUR and CER is used to compute a total number of grams of oxygen added to the culture and grams of carbon dioxide evolved from the culture. This density is used to convert that weight to a volume of liquid. The default value is typically adequate. Adjusting this density typically represents a small correction to what is already a small correction to the volume of the culture.

4.1.5 Liquid handling

The liquid handling options allow entering the number of tips that the tip collection bag can hold. The user is prompted to change the tip collection bag once it holds more tips than this.

Tip collection bag capacity (10 mL) tips specifies the number of 10 mL tips that the bag can hold.

Tip collection bag capacity (300 µl) tips specifies the number of 300 µl tips that the bag can hold.

4.1.6 Analysis module

An offset can be applied to the raw result from the analysis module. The pH measured from a sample from a bioreactor differs from the pH inside the bioreactor because of out-gassing from the sample and other factors. The magnitude of these factors depends on the procedure used to take the sample; the procedure used to measure the pH; the composition of the media; the gassing of the media and other factors. The offset can be used to provide a better correlation between the pH measured by taking and measuring a sample using the analysis module and the pH measured in another way.



The **Offset to add to pH**, **Minimum value of offset** and **Maximum value of offset** options are only offered if the **Support defining an offset to apply to the pH measured by the analysis module** option is selected in the **Advanced features** window.

Offset to add to pH defines an expression that is added to the pH measured by the Analysis module to provide a corrected pH reading to apply to the measured bioreactor. If the result of the offset is outside the range between the **Minimum value of offset** and **Maximum value of offset** then the offset is limited to that range.

Within the expression for the offset the special variable **RawpHFromAnalysisModule** may be used to refer to the uncorrected pH from the analysis module. This would be useful if the offset were itself to be pH dependent.

When the offset feature is being used the results are recorded as follows:

- The diagnostic variable **pH measured (correction)** records the offset used and the details of the calculation of the offset. To see the details examine the variable using the **Table** view.
- The diagnostic variable **pH measured (raw)** records the pH measured by the analysis module before the application of any offset.

View data

[View data points for pH measured \(correction\)](#)

Time	pH measured (correction) (pH)	Note
Tue 08 Dec 2015 17:22:31	4.86876916885376	pH offset definition = Unadjusted Expression = Unadjusted pH Unadjusted pH => 4.86876 pH offset = 0.487
Tue 08 Dec 2015 17:28:47	0.486732006072998	pH offset definition = Unadjusted Expression = Unadjusted pH Unadjusted pH => 4.86732 pH offset = 0.487
Tue 08 Dec 2015 17:48:15	0.48651065826416	pH offset definition = Unadjusted Expression = Unadjusted pH Unadjusted pH => 4.86510 pH offset = 0.487
Tue 08 Dec 2015 18:09:20	0.486384201049805	pH offset definition = Unadjusted Expression = Unadjusted pH Unadjusted pH => 4.86384 pH offset = 0.486
		pH offset definition = Unadjusted Expression = Unadjusted pH Unadjusted pH => 4.86384 pH offset = 0.486

[Close](#)

Figure 115 View of pH offset calculations

4.1.7 pH station

Set temperature automatically enables or disables automatic control of the pH station temperature.

Temperature tolerance specifies the difference allowed between the temperature of the liquid in the bioreactor and the temperature of the pH station. The system will ensure the temperature of the pH station is in this range before taking a measurement subject to limits placed on the temperature of the pH station.

Minimum temperature sets the minimum temperature to which the system will attempt to set the pH station. The minimum temperature must be selected to be higher than the ambient temperature for the pH station to be able to reach the temperature.

The maximum temperature of the pH station is restricted by a limit coded into the system.

The feature to control the temperature works as follows.

If the temperature selected for the pH station is good for something in the liquid handler's queue then the temperature is left unchanged. Otherwise choose as the ultimate target temperature the temperature that would be optimum for the first item in the liquid handler's queue that uses the pH station. If there is an interim temperature that would be good for another item in the liquid handler's queue then set that interim temperature as the target instead.

If there is nothing in the queue and there is no need to recalibrate the pH station then set the temperature of the pH station based on the samples predicted to arrive at the station.

4.1.8 Flex2

External sample id specifies the option for if and how to provide an external sample identifier for the flex readings.

Use temperature corrected pH specifies that the temperature corrected pH returned from the sample reading on the Flex should be used as the reference pH for adjusting the bioreactor pH probe.

An offset can be applied to the pH result from the Flex2. The pH measured from a sample from a bioreactor differs from the pH inside the bioreactor because of out-gassing from the sample and other factors. The magnitude of these factors depends on the procedure used by the External Sample Module (ESM) to take the sample; the procedure used to measure the pH by the Flex2; the composition of the media; the gassing of the media and other factors. The offset can be used to provide a better correlation between the pH measured by taking and measuring a sample using the Flex2 and the pH measured in another way.



The **Offset to add to pH**, **Minimum value of offset** and **Maximum value of offset** options are only offered if the **Support defining an offset to apply to the pH measured by the Flex2** option is selected in the **Advanced features** window.

Offset to add to pH defines an expression that is added to the pH measured by the Flex2 to provide a corrected pH reading to apply to the measured bioreactor. If the result of the offset is outside the range between the **Minimum value of offset** and **Maximum value of offset** then the offset is limited to that range.

4.1.9 Biomass monitor

Display specifies the biomass measure that will be displayed on the bioreactor controls and overview screens. Options are:

- **None** – no measure displayed
- **Dry cell weight** – the biomass measure in g/L of dry cells
- **Wet cell weight** – the biomass measure in g/L of wet cells
- **Optical density** – the biomass measure as optical density

4.2 Pumped liquid classes

Pumped liquid classes provide a way to manage the limitations on pumping and pump refill rates for different liquids.

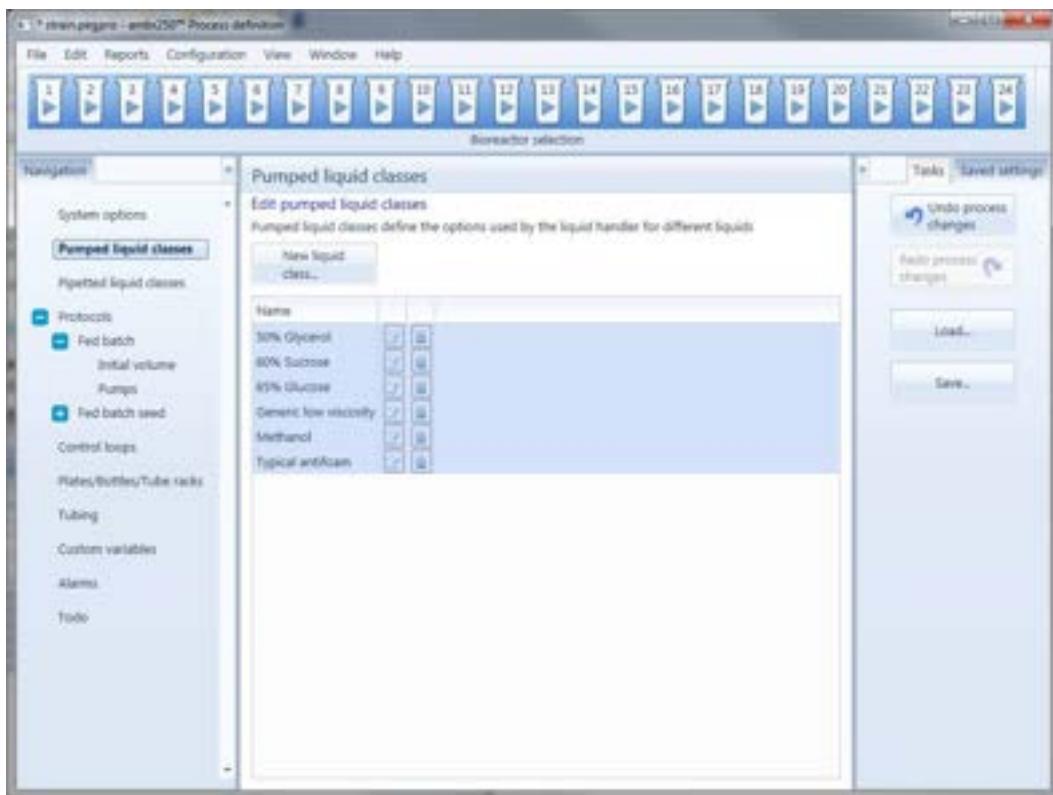


Figure 116 Pumped liquid classes page

Edit liquid class '60% Sucrose'

Edit details of liquid class

Property	Value
Description	
Name	60% Sucrose
Flow limits	
Maximum flow rate with filter (mL/h)	1
Maximum refill rate for the pump	Slow

Ok Cancel

Figure 117 Pumped liquid class

Each **Pumped liquid class** defines the following options:

Name – the name of the liquid.

Maximum flow rate with filter – how fast the liquid can be pumped through a bioreactor inlet filter. Attempting to pump too fast can cause leaks or burst filters.

Maximum refill rate for the pump – how fast the liquid can be sucked into the pump. If too high a refill rate is used then issues can arise with cavitation and bubbles in the tubing connected to the pumps.

4.3 Pipetted liquid classes

Pipetted liquid classes provide a way to manage the multiple options that control the liquid handler.

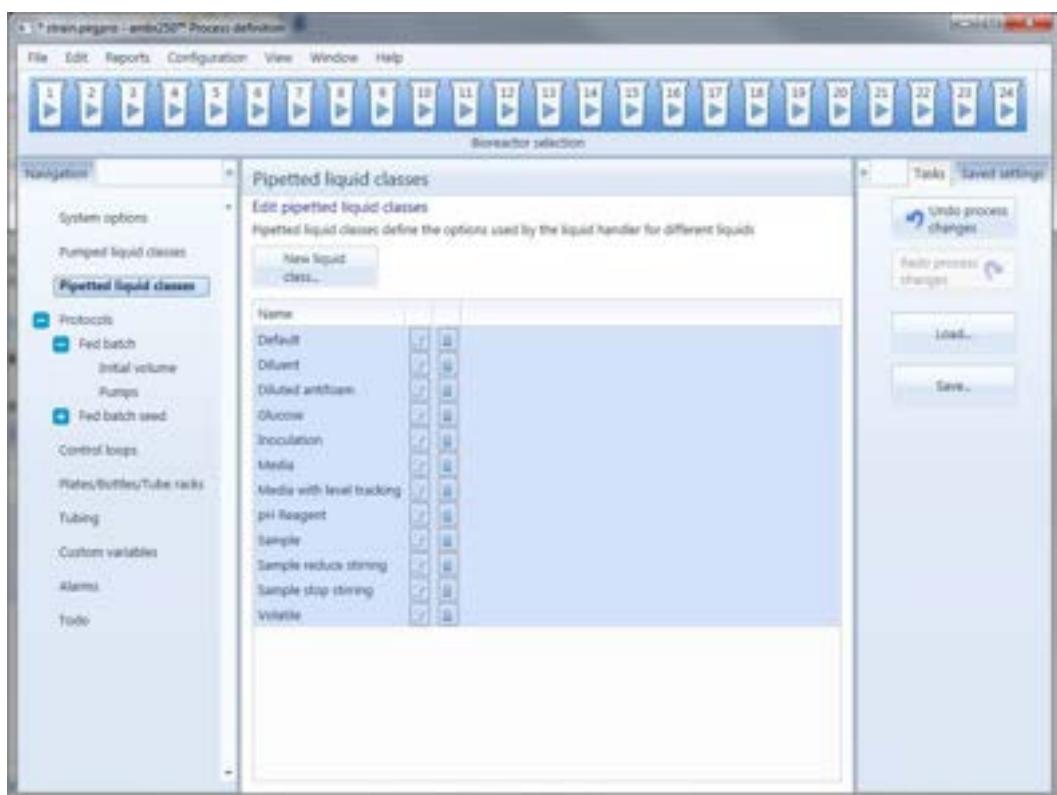


Figure 118 **Pipetted liquid classes** page

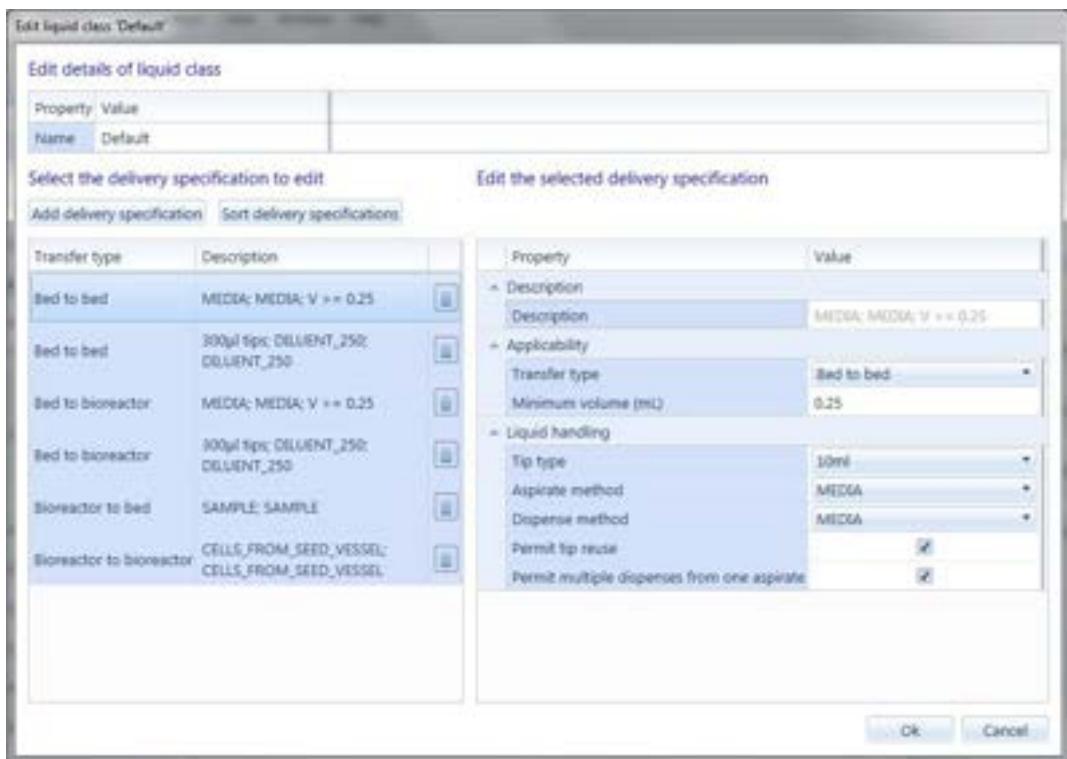


Figure 119 Pipetted liquid classes page

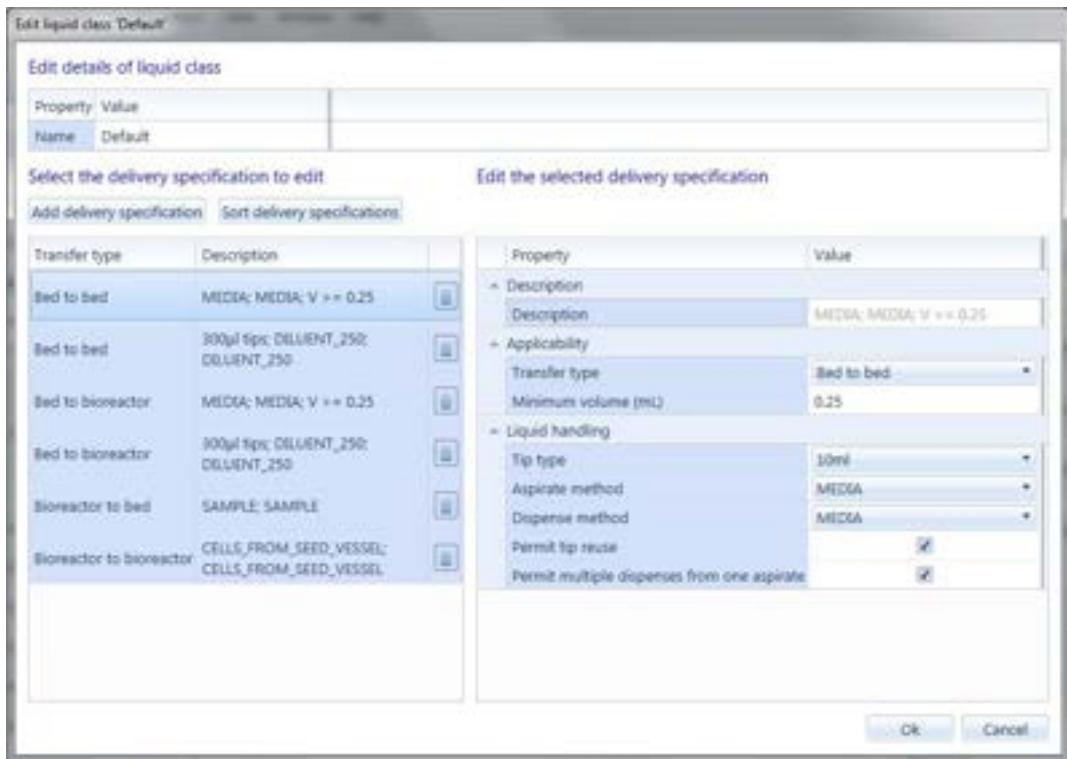


Figure 120 Pipetted liquid class

A **Pipetted liquid class** contains a number of delivery specifications. Each delivery specification defines the Aspirate method, Dispense method and other parameters to use for a certain Transfer type.

Within a liquid handling step the user specifies a **Liquid class**. The system uses the parameters of the step including the volume to be transferred and the sort of labware used in the step to select the delivery specification to use for that step. Where the liquid handling step performs multiple different transfers different liquid classes may be specified for each sort of transfer.

The **Name** of the liquid class can be edited.

If the system has been configured for the **Wash tips before disposal** (optional feature) the user can select that tips are washed before they are ejected into the waste bin.

Property	Value
Name	Non viscous
Wash tips before disposal	<input checked="" type="checkbox"/>

Select the delivery specification to edit

Add delivery specification Sort delivery specifications

Transfer type	Description
---------------	-------------

Figure 121 Wash tips before disposal option

4.3.1 Delivery specification

Each delivery specification has a **Description** which can be left as its automatically generated value or can be edited.

Transfer type specifies whether the delivery specification applies to a transfer:

- **Bed to bed** – between labware and analysers on the bed of the liquid handler
- **Bed to bioreactor** – from the bed to the bioreactor
- **Bioreactor to bed** – taking liquid from the bioreactor for a sample or disposal
- **Bioreactor to bioreactor** – transferring liquid from one bioreactor to another

Minimum volume specifies the minimum volume of liquid that the delivery specification can be used for. The system will select the delivery specification with the highest minimum volume that is consistent with the required transfer.

The **Tip type** option specifies the size of tip to be used for the transfer. Ambr 250 supports:

- **300µl** tips
- **10ml** tips
- **10ml wide bore** tips

The **Aspirate method** and the **Dispense method** specify the liquid handling methods used to aspirate liquid and dispense liquid respectively.

Permit tip reuse indicates whether the method can be used for multiple transfers from the same source. The final decision as to whether to reuse tips or not is made in the individual steps.

Permit multiple dispenses from one aspirate indicates whether the method can be used to dispense to multiple destinations from one aspirate. The final decision as to whether or not to do this is made in the individual steps.

Bed to bioreactor delivery specifications also have the option **Preaspire liquid**. If selected then when the delivery specification is used the liquid handler will:

- aspirate some water
- take in an air gap
- aspirate the liquid to be transferred

The **Preaspire liquid** option is intended to help with the transfer of volatile liquids such as methanol.

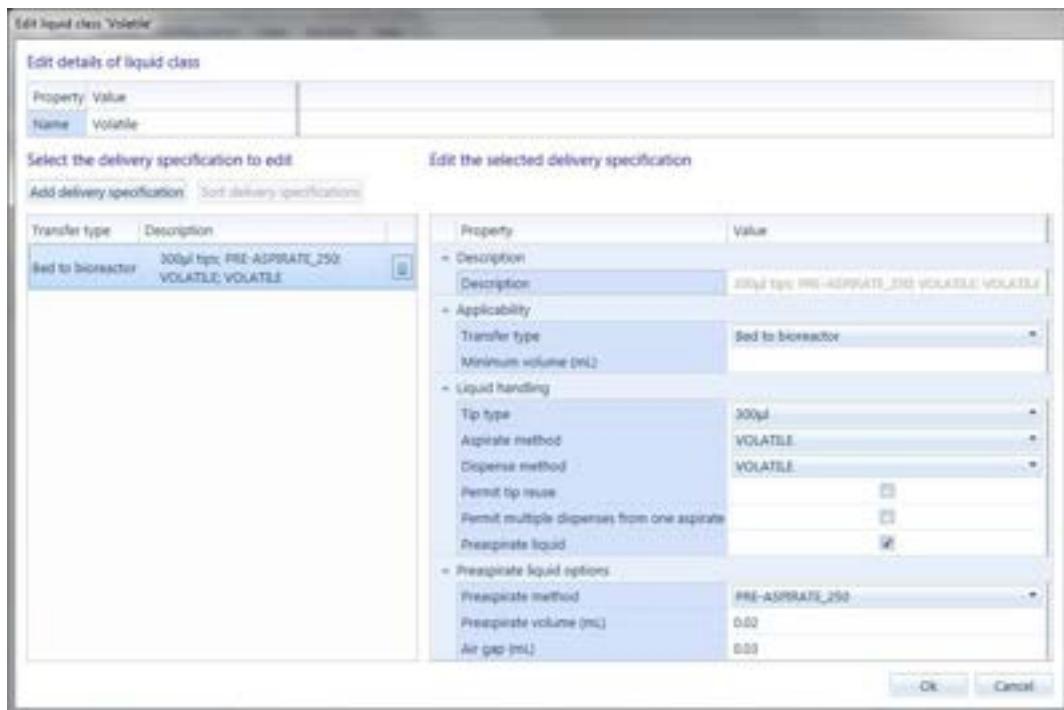


Figure 122 Pipetted liquid class using Preaspire liquid

When **Preaspire liquid** is selected: **Preaspire method** specifies how the water is aspirated; **Preaspire volume** specifies the volume of water aspirated and **Air gap** specifies the size of the air gap between the water and the liquid to be transferred.

Note: only **300μl** tips can be used with the **Preaspire liquid** option

Note: the definition of the **Dispense method** within the system configuration may be defined to dispense both the first and second liquid to the destination or may be defined to just dispense the first liquid. The volumes dispensed are assumed to be small and just the second liquid is included in the accounting of how much liquid is in the bioreactors.

4.4 Liquid handling scripts



Liquid handling scripts are enabled by the **Show liquid handling scripts and steps to use liquid handling steps** option in the **Advanced features** window.

Liquid handling scripts allow advanced liquid handling operations that are not supported via the standard steps such as **Add liquid from bed** or **Sample**.

Liquid handling scripts allow temporary overrides to the bioreactor control to be performed as well as allowing aspirate and dispense operations.

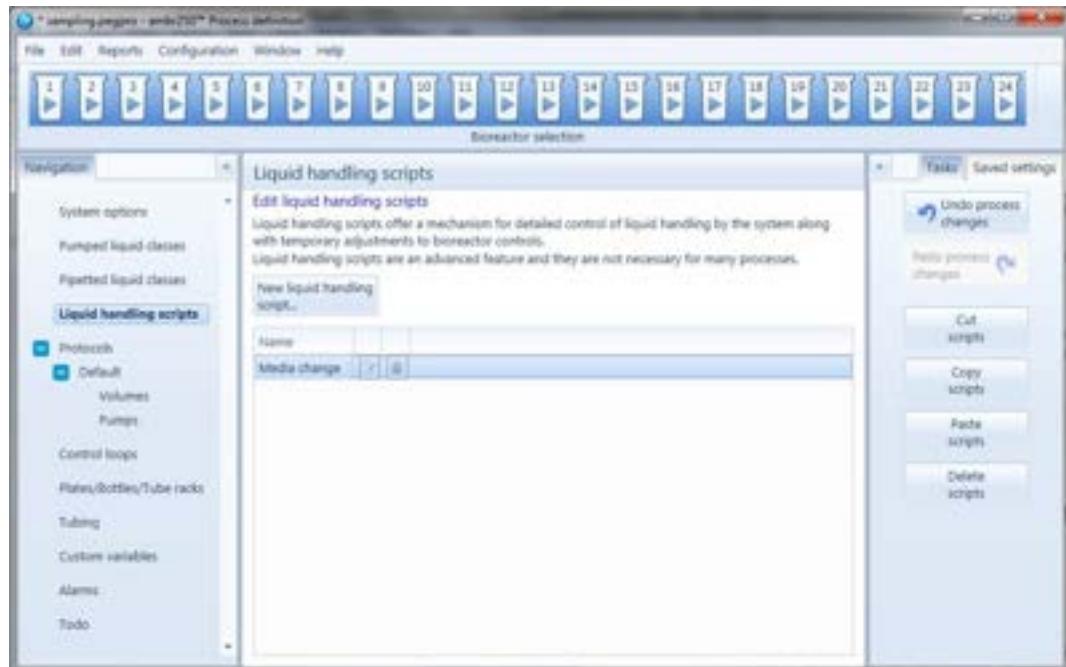


Figure 123 Liquid handling script page

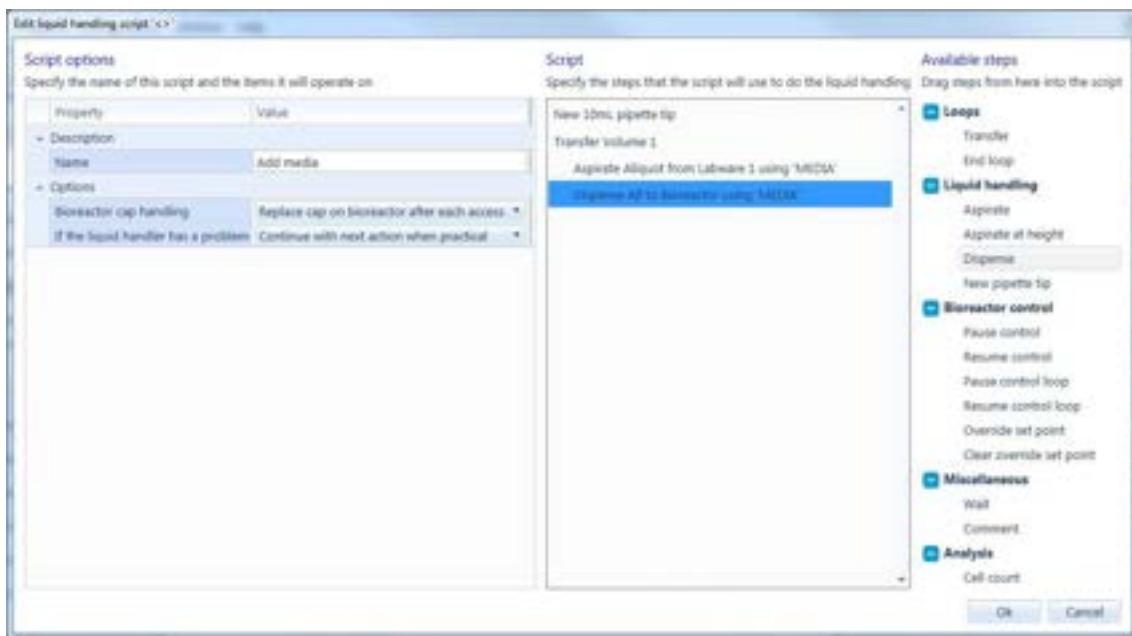


Figure 124 Edit liquid handling script window

Each script has the following basic options:

- **Name** provides a name for the script
- **Bioreactor cap handling** allows a choice of
 - **Replace cap on bioreactor after each access** causes the system to replace bioreactor caps after each access to a bioreactor.
 - **Keep holding cap when possible** causes the system to keep bioreactor caps held in its gripper when possible. This can speed up scripts involving multiple accesses to a bioreactor but introduces more risks of contamination and of dripping from the cap.
- **If the liquid handler has a problem** allows the response of the liquid handler to errors to be adjusted.
 - **Ask user for instructions** causes the system to present a dialog with options for the user after a problem has occurred and the system has failed to resolve the problem using its built logic for retrying individual actions.
 - **Continue with next action where practical** allows the system to attempt to continue automatically in the presence of some errors. To do so the system must make assumptions as to the nature of the error and how to continue. Continuing may result in other harder to recover errors later – or may allow the system to successfully complete its other tasks.

The main body of the script comprises a list of steps.

To edit the script you can:

- Drag steps from the list on the right hand side of the window
- Drag steps to move them within the list
- Click on steps to select them
- Double click on steps in the list to edit them

- Use keyboard to edit and delete steps: the standard short-cuts Del, Ctrl-C, Ctrl-X and Ctrl-V are available.
- Use context menu options to add, edit, move and delete steps.

Script

Specify the steps that the script will use to do the liquid handling

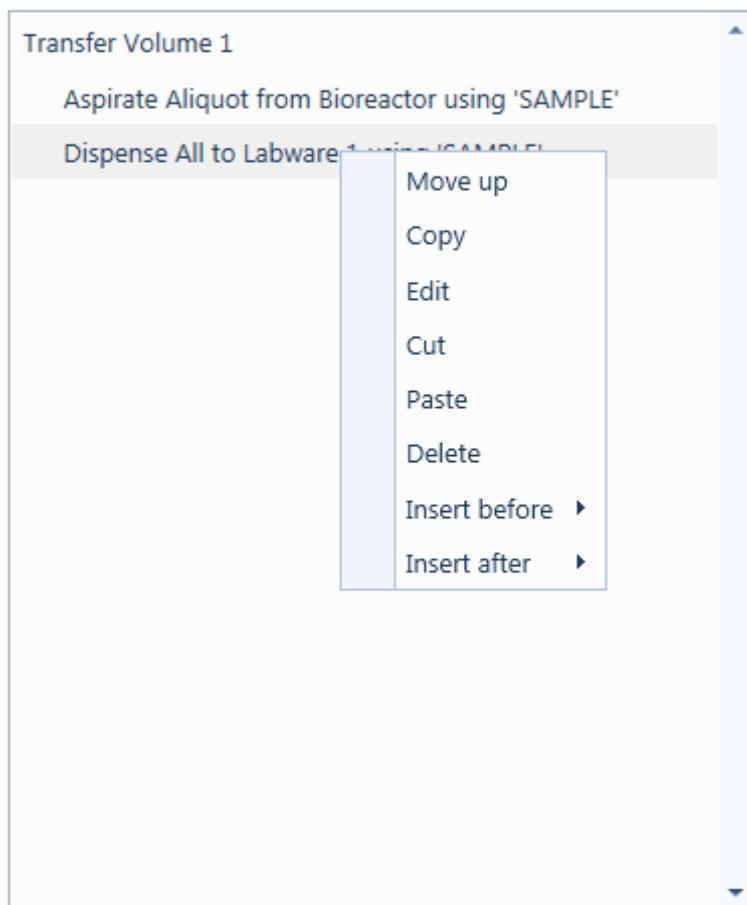


Figure 125 Context menu options on a step

4.4.1 Liquid handling script steps

The individual steps have their own options as described below.

4.4.1.1 Transfer

The **Transfer** step starts a block of steps that will be repeated as necessary to transfer a specified volume.

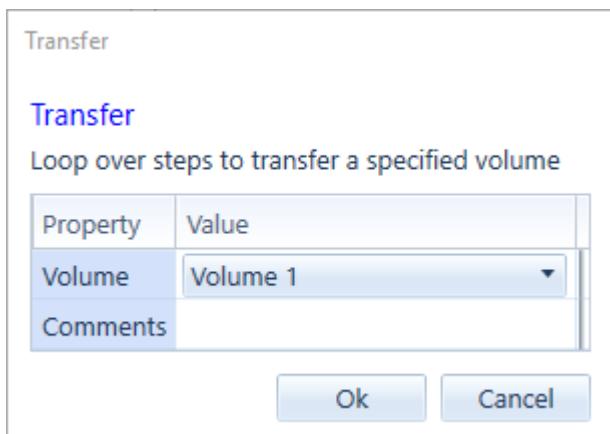


Figure 126 Transfer step

The **Transfer** step has two parameters, **Volume**, that defines which volume parameter will be used to define the volume to transfer. That parameter will be given a value by the **Run liquid handling script** step that uses the script. The **Comments** parameter can be used to comment on the purpose of the step.

The block of steps for the Transfer step must contain a step that aspirates a default volume of '**Aliquot**'. The figure below shows a typical block transferring **Volume 1** of liquid from **Labware 1** to the bioreactor.

```
Transfer Volume 1
Aspirate Aliquot from Labware 1 using 'MEDIA'
Dispense All to Bioreactor using 'MEDIA'
```

Figure 127 Simple transfer block

4.4.1.2 End loop

The **End loop** step ends the block of steps contained in a **Transfer**.



Figure 128 End loop step

The **End loop** step is only required if additional steps are needed after the **Transfer** loop. The **Comments** parameter can be used to comment on the purpose of the step.

4.4.1.3 Aspirate

The **Aspirate** step tells the liquid handler to go to a location and aspirate a specified volume of liquid.

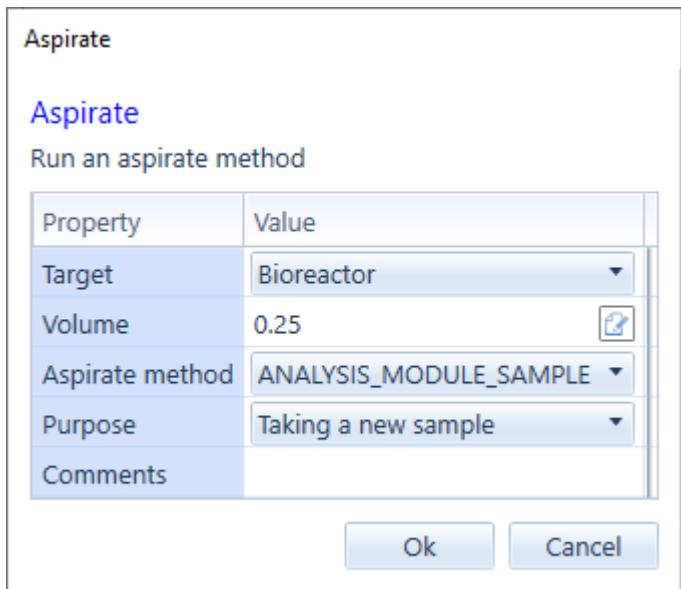


Figure 129 **Aspirate** step

The **Aspirate** step has the options:

- **Target** – the location to aspirate from
- **Volume** – the volume to aspirate which may be specified explicitly or may be left as the default blank value which is interpreted as **Aliquot** when the volume is derived from an enclosing **Transfer** step. The amount that the liquid handling method is asked to aspirate is increased to take account of any gas hold up value specified for the **Target** in the **Run liquid handling step**.
- **Aspirate method** – the level liquid handling method to use
- **Purpose** – the purpose of the aspirate step. Purpose values are either **Taking a sample** or **Other**. When **Taking a sample** is selected the time of the aspirate is noted and is used as the time of the sample for the results returned from subsequent analysis.
- **Comments** – comments on the step.

The liquid handler automatically removes and replaces lids and caps as required.

4.4.1.4 Aspirate at height

The **Aspirate at height** step tells the liquid handler to go to a bioreactor and aspirate a specified volume of liquid from a specified height. The aspirate is allowed to happen however little liquid the system thinks is in the bioreactor. After the aspirate the volume of the bioreactor is reduced by the volume aspirated unless the volume would be less than the specified dead volume when the volume is not reduced below that value.

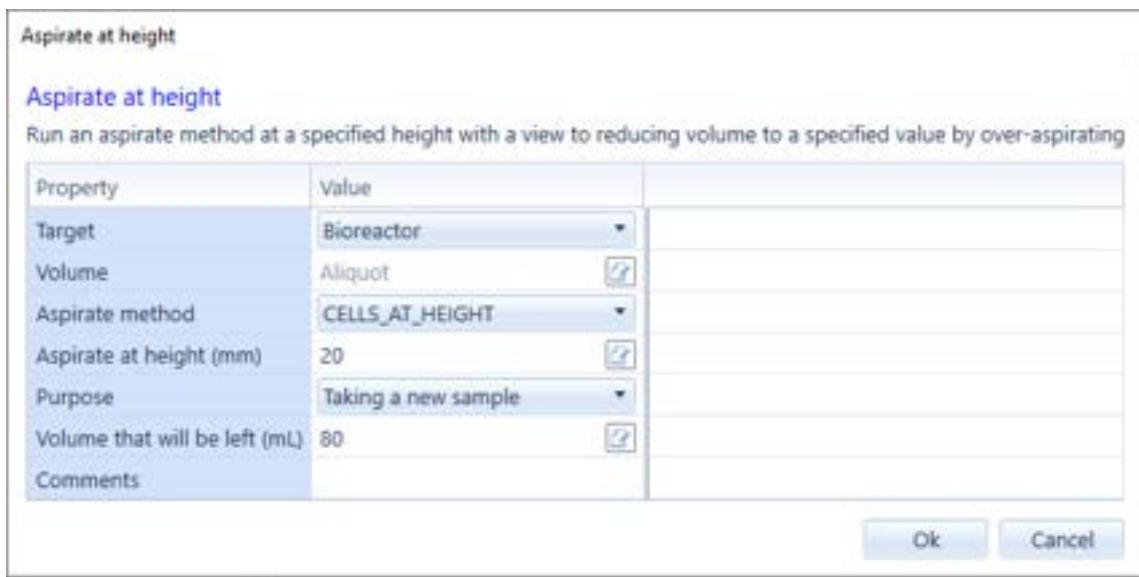


Figure 130 *Aspirate at height* step

The **Aspirate at height** step has the options:

- **Target** – the location to aspirate from
- **Volume** – the volume to aspirate which may be specified explicitly or may be left as the default blank value which is interpreted as **Aliquot** when the volume is derived from an enclosing **Transfer** step. The amount that the liquid handling method is asked to aspirate is increased to take account of any gas hold up value specified for the **Target** in the **Run liquid handling step**.
- **Aspirate method** – the level liquid handling method to use
- **Aspirate at height** - the offset above the 'BOTTOM' height from which to aspirate. Provides the height for a HEIGHT line within the liquid handling method.
- **Purpose** – the purpose of the aspirate step. Purpose values are either **Taking a sample** or **Other**. When **Taking a sample** is selected the time of the aspirate is noted and is used as the time of the sample for the results returned from subsequent analysis.
- **Volume that will be left** – the volume of liquid (dead volume) that will remain in the bioreactor however many times this aspirate method is used. The relationship between the height at which the aspirate is done and this dead volume will depend on how the liquid in the bioreactor is gassed and stirred and must be determined by trial and error.
- **Comments** – comments on the step.

The liquid handler automatically removes and replaces lids and caps as required.

4.4.1.5 Dispense

The **Dispense** step tells the liquid handler to go to a location and dispense a specified volume of liquid.

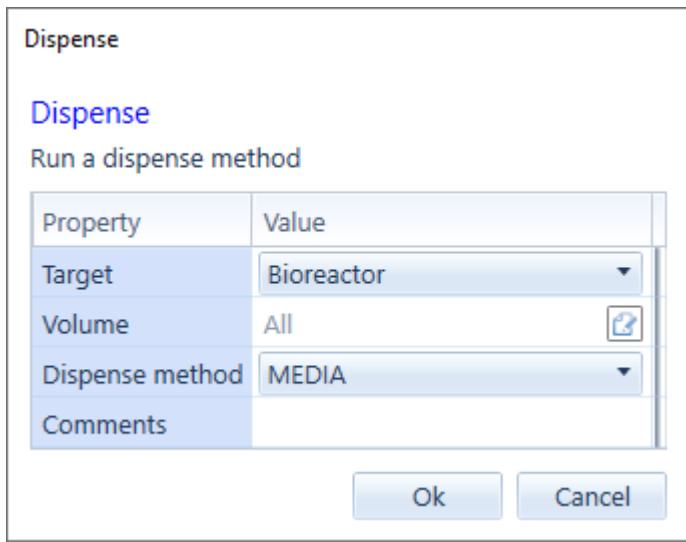


Figure 131 Dispense step

The **Dispense** step has the options:

- **Target** – the location to dispense to
- **Volume** – the volume to dispense which may be specified explicitly or may be left as the default blank value which is interpreted as **All** when all the liquid in the tip is dispensed. It is assumed that before dispensing the volume, the gas taken up into the pipette with the liquid has been allowed to rise to the top – and so the volume dispensed is correct.
- **Dispense method** – the level liquid handling method to use
- **Comments** – comments on the step.

The liquid handler automatically removes and replaces lids and caps as required.

4.4.1.6 New pipette tip

The **New pipette tip** step causes the liquid handler to pick a new pipette tip.

```
Transfer Volume 1
New 10mL pipette tip
Aspirate Aliquot from Labware 1 using 'MEDIA'
Dispense All to Bioreactor using 'MEDIA'
```

Figure 132 Script with a **New pipette tip step** which will use a new tip for each aliquot.

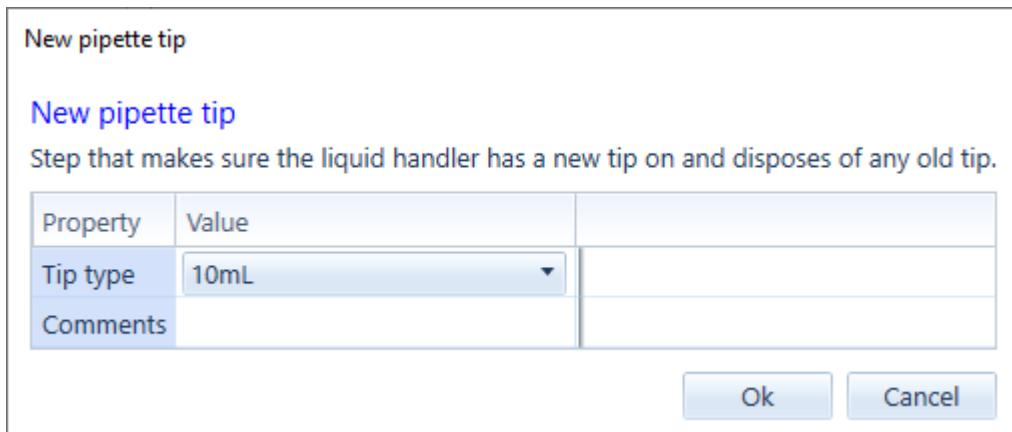


Figure 133 New pipette tip step

The **New pipette tip** step has the options:

- **Tip Type** – specifies the type of pipette tip to pick.
- **Comments** – comments on the step.

4.4.1.7 Eject pipette tip

The **Eject pipette tip** step gets rid of any tip that the liquid handler is holding. This can be a useful optimisation if the liquid handler will be going on to wait for an analyzer to finish its processing.

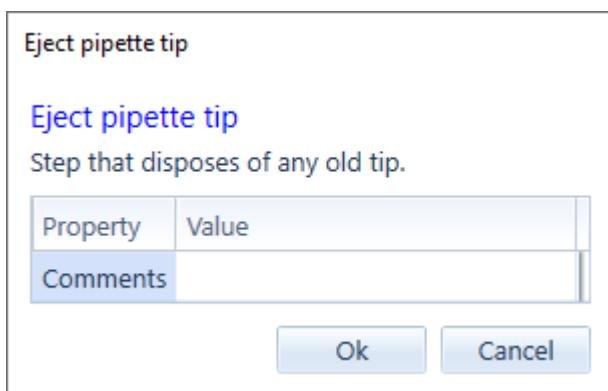


Figure 134 Eject pipette tip step

The **Eject pipette tip** step has the options:

- **Comments** – comments on the step.

4.4.1.8 Pause control

The **Pause control** step allows the operation of the control loops for DO, pH, Temperature and for custom set points to be suspended temporarily.

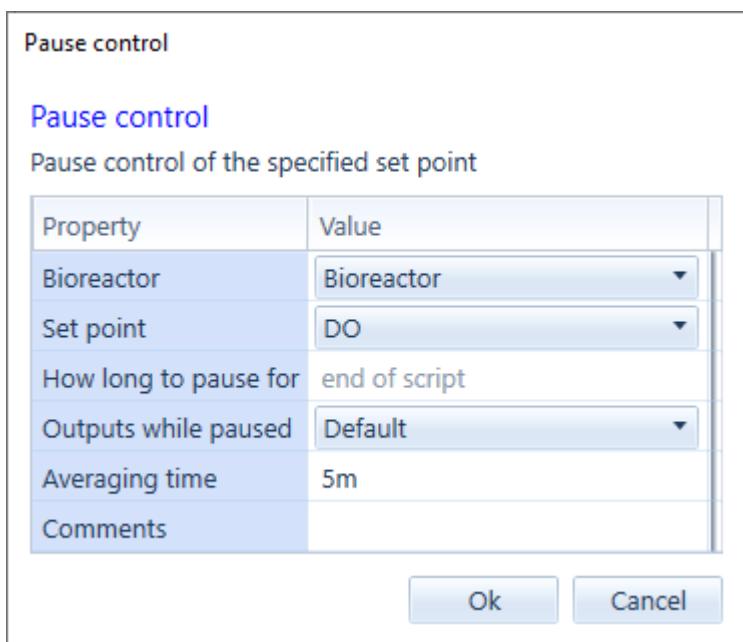


Figure 135 Pause control step

The **Pause control** step has the options:

- **Bioreactor** specifies whether the step applies to the bioreactor the **Run liquid handling script** step is being run for or whether it applies to that bioreactor's seed bioreactor.
- **Set point** specifies the property whose control should be paused
- **How long to pause for** allows the duration that control is paused for to be specified. The default is for control to be paused until the liquid handling script ends.
- **Outputs while paused** is applicable for control loops that are part of the process definition – and not for **Temperature** where heating and cooling are always turned off during a pause – and has options **Default**, **Off** and **Average**. **Off** turns the outputs off for the duration of the pause. **Average** sets the outputs to their average interval over the last **Averaging time**. **Default** turns pump outputs off and sets other outputs to their average value.
- **Comments** – comments on the step.

4.4.1.9 Resume control

The **Resume control** step explicitly resumes normal control of a set point. (Normal operation will otherwise resume after a specified interval or at the end of the script.)

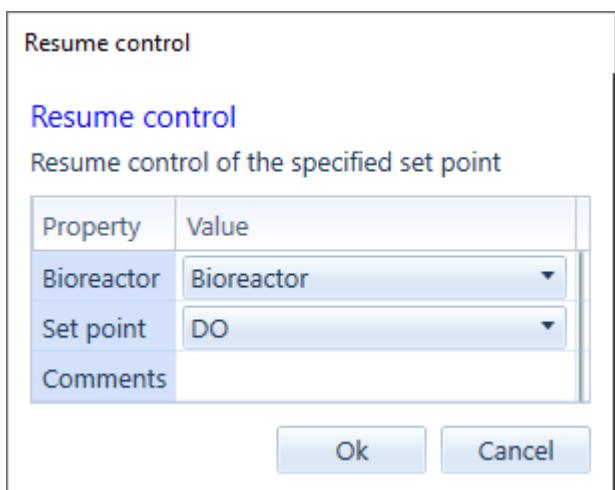


Figure 136 **Resume control** step

The **Resume control** step has the options:

- **Bioreactor** specifies whether the step applies to the bioreactor the **Run liquid handling script** step is being run for or whether it applies to that bioreactor's seed bioreactor.
- **Set point** specifies the property whose control should be resumed.
- **Comments** – comments on the step.

4.4.1.10 Pause control loop

The **Pause control loop** step allows the operation of a specific control loop to be suspended temporarily.

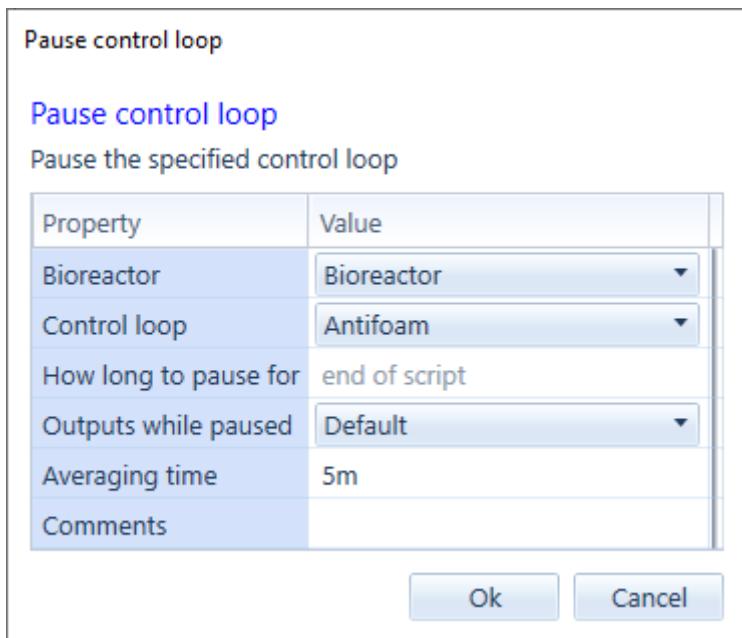


Figure 137 **Pause control loop** step

The **Pause control loop** step has the options:

- **Bioreactor** specifies whether the step applies to the bioreactor the **Run liquid handling script** step is being run for or whether it applies to that bioreactor's seed bioreactor.
- **Control loop** specifies the control loop that should be paused
- **How long to pause** for allows the duration that control is paused for to be specified. The default is for control to be paused until the liquid handling script ends.
- **Outputs while paused** has options **Default**, **Off** and **Average**. **Off** turns the outputs off for the duration of the pause. **Average** sets the outputs to their average interval over the last **Averaging time**. **Default** turns pump outputs off and sets other outputs to their average value.
- **Comments** – comments on the step.

4.4.1.11 Resume control loop

The **Resume control loop** step allows normal operation of a control loop to be resumed explicitly. (Normal operation will otherwise resume after a specified interval or at the end of the script.)

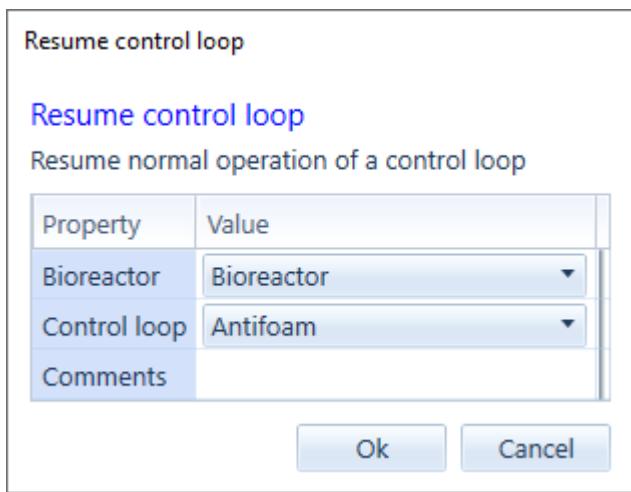


Figure 138 *Resume control loop* step

The **Resume control loop** step has the options:

- **Bioreactor** specifies whether the step applies to the bioreactor the **Run liquid handling script** step is being run for or whether it applies to that bioreactor's seed bioreactor.
- **Control loop** specifies the control loop that should be resumed
- **Comments** – comments on the step.

4.4.1.12 Override set point

The **Override set point** step allows a set point to be temporarily set to a specified value.

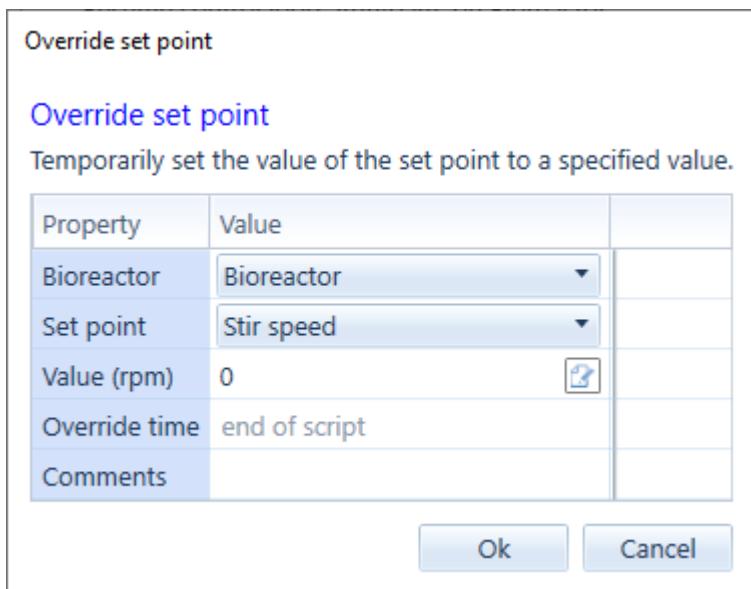


Figure 139 **Override set point** step

The step has the options:

- **Bioreactor** specifies whether the step applies to the bioreactor the **Run liquid handling script** step is being run for or whether it applies to that bioreactor's seed bioreactor.
- **Set point** specifies the property whose control should be overridden. The available set points are key set points controlled directly by the system.
- **Value** specifies the value to assign to the set point.
- **Override time** allows the duration that control is overridden for to be specified. The default is for control to be overridden until the liquid handling script ends.
- **Comments** – comments on the step.

4.4.1.13 Clear override set point

The **Clear override set point** step allows the override of a set point to be cleared explicitly. (Normal operation will otherwise resume after a specified interval or at the end of the script.)

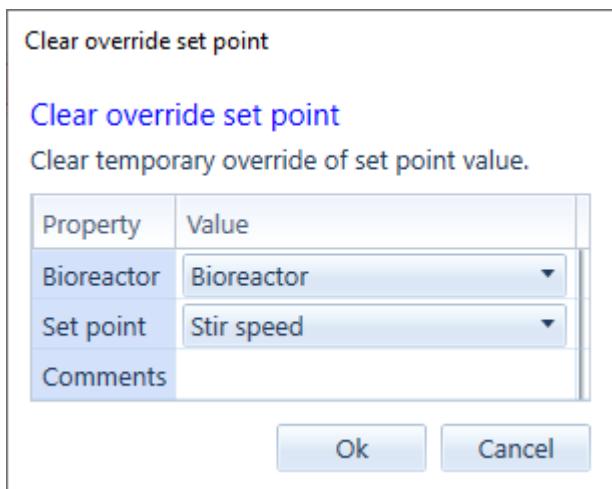


Figure 140 **Clear override set point** step

The **Clear override set point** step has the options:

- **Bioreactor** specifies whether the step applies to the bioreactor the **Run liquid handling script step** is being run for or whether it applies to that bioreactor's seed bioreactor.
- **Set point** specifies the property whose control should be resumed.
- **Comments** – comments on the step.

4.4.1.14 Wait

The **Wait** step causes the liquid handler to wait for the specified duration.

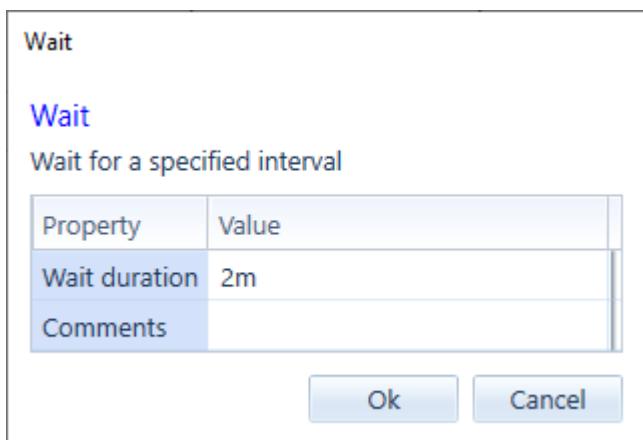


Figure 141 **Wait** step

The **Wait** step has the options:

- **Wait duration** – specifies how long to wait for.
- **Comments** – comments on the step

4.4.1.15 Comment

The **Comment** step just displays text to make the script clearer.

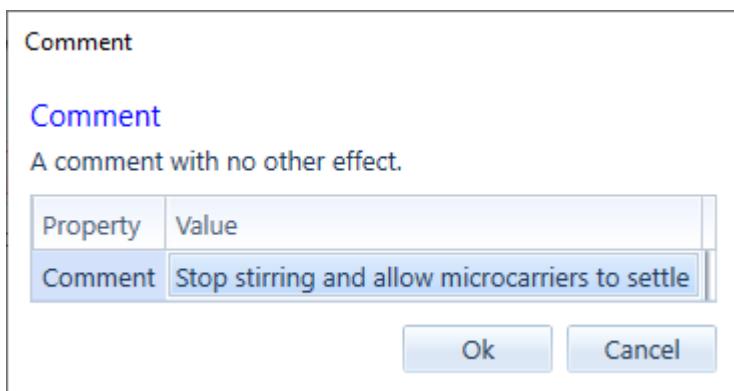


Figure 142 **Comment** step

Comment is the text displayed in the script definition.

4.4.1.16 Cell count

The **Cell count** step gets the cell reader to do a cell count. The script should fill the cell counter with the liquid to be counted before this step.

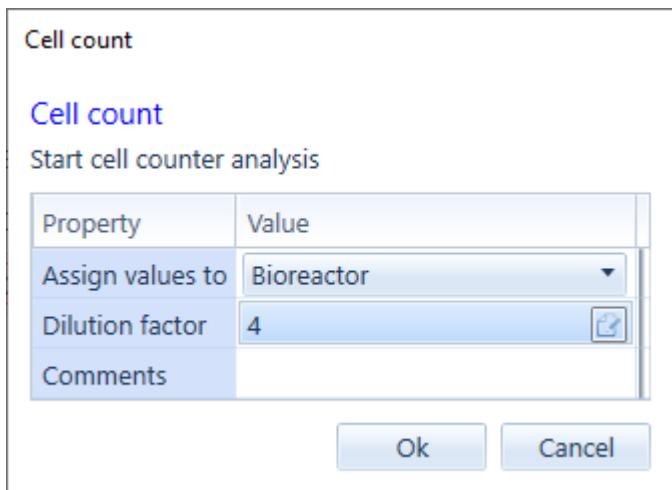


Figure 143 Cell count line

Assign values to specifies the logical source of the cells to be counted and defines where the results should be stored:

- **Bioreactor** stores the results as the cell count of the bioreactor running the step.
- **Labware** stores the results as the “Seed –“ readings of the bioreactor running the step.
- **Seed bioreactor** stores the results as the cell count of the seed bioreactor of the bioreactor running the step and as the “Seed –“ readings of the bioreactor running the step.

Dilution factor specifies the factor by which the raw readings from the cell counter should be multiplied to get the best estimate of the cell count.

Comments parameter allows comments on the step.

4.4.1.17 Wait for analysis module

The **Wait for analysis module** step waits for the analysis module to be idle or ready to perform the next action.

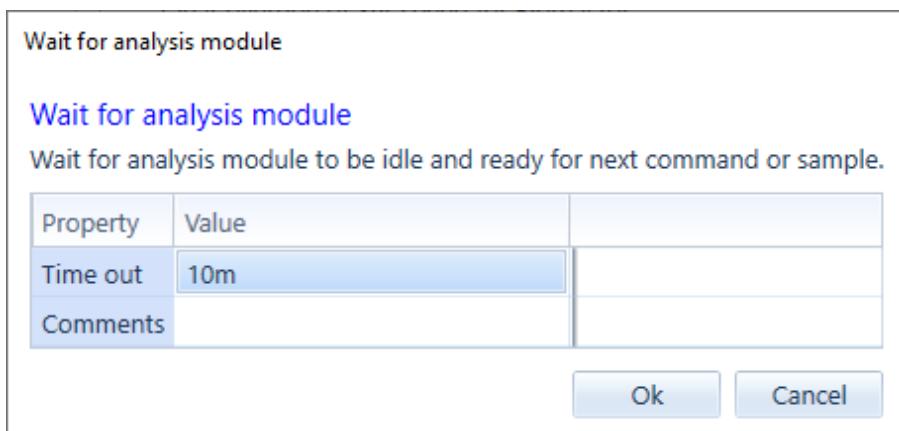


Figure 144 Wait for analysis module line

Time out specifies the maximum time to wait for the Analysis Module to become idle or ready before moving to the next step.

Comments parameter allows comments on the step.

4.4.1.18 Analyse with Analysis Module

The **Analyse with Analysis Module** step gets the Analysis Module to do a reading.

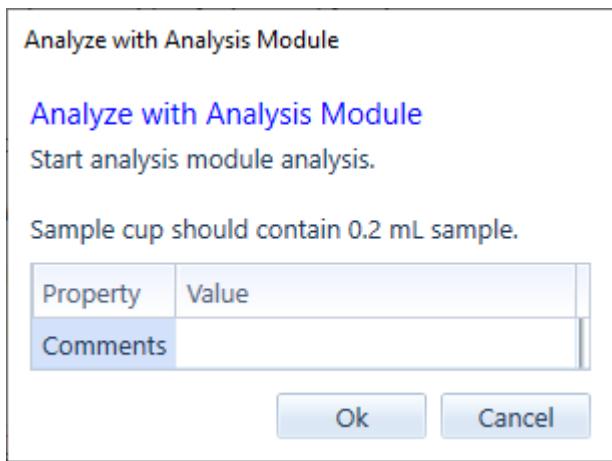


Figure 145 Analyse with Analysis Module line.

There should be a 0.2mL sample in the analysis module sample cup before running this step.

Comments parameter allows comments on the step.

4.4.1.19 Analyse with Flex2 (part 1 of 2)

The **Analyse with Flex2 (part 1 of 2)** step gets the Flex2 to do a sample cup wash as the first required part of a Flex2 analysis.

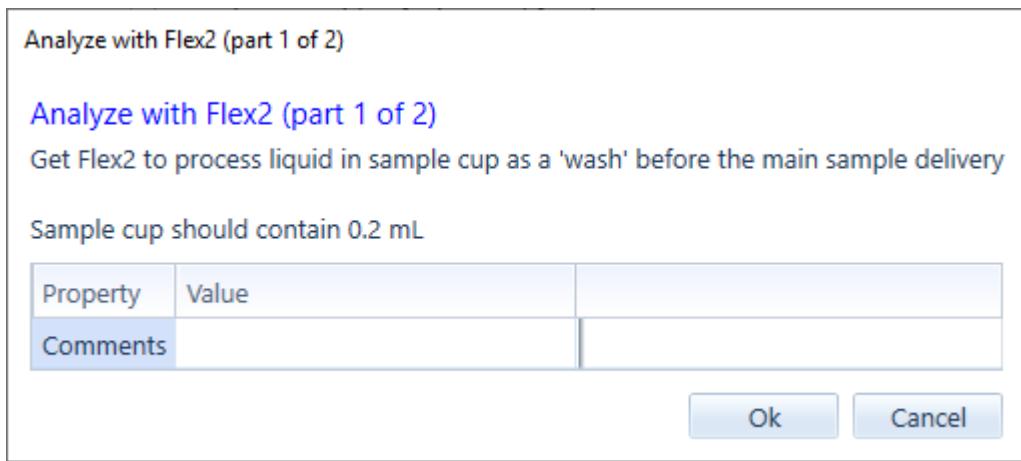


Figure 146 Analyse with Flex2 (part1 of 2) line

There should be a 0.2mL sample in the Flex2 sample cup before running this step.

Comments parameter allows comments on the step.

4.4.1.20 Analyse with Flex2 (part 2 of 2)

The **Analyse with Flex2 (part 2 of 2)** step gets the Flex2 to do an analysis of the sample.

There should be a 0.475 mL sample in the Flex2 sample cup before running this step.

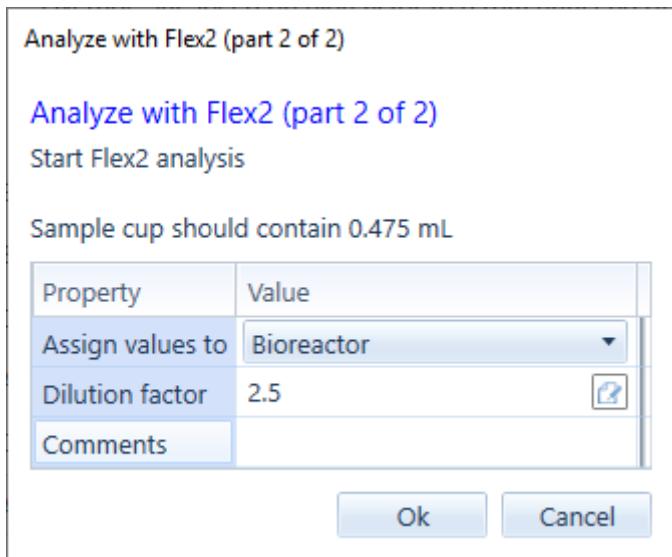


Figure 147 Analyse with Flex2 (part 2 of 2) line.

Assign values to specifies the logical source of the sample and defines where the results should be stored:

- **Bioreactor** stores the results as the analysis of the bioreactor running the step.
- **Labware** stores the results as the “Seed –“ readings of the bioreactor running the step.
- **Seed bioreactor** stores the results as the analysis of the seed bioreactor of the bioreactor running the step and as the “Seed –“ readings of the bioreactor running the step.

Dilution factor specifies the factor by which the raw readings from the Flex2 cell counter should be multiplied to get the best estimate of the cell count.

Comments parameter allows comments on the step.

4.4.1.21 Move liquid to flow cell with analysis module

The **Move liquid to flow cell with analysis module** gets the analysis module to transfer sample in the analysis module sample cup into the spectrometer flow cell for analysis.

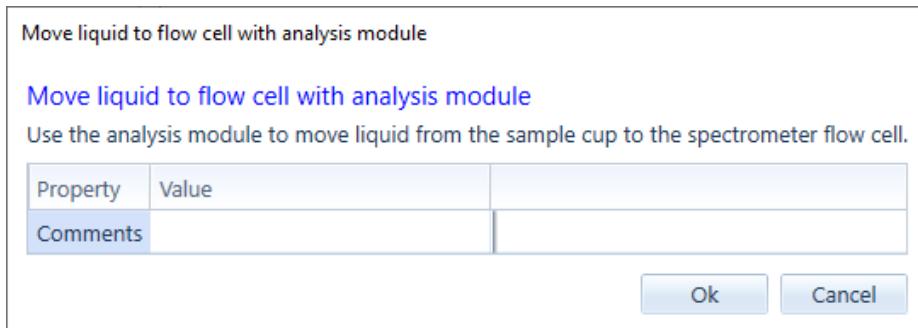
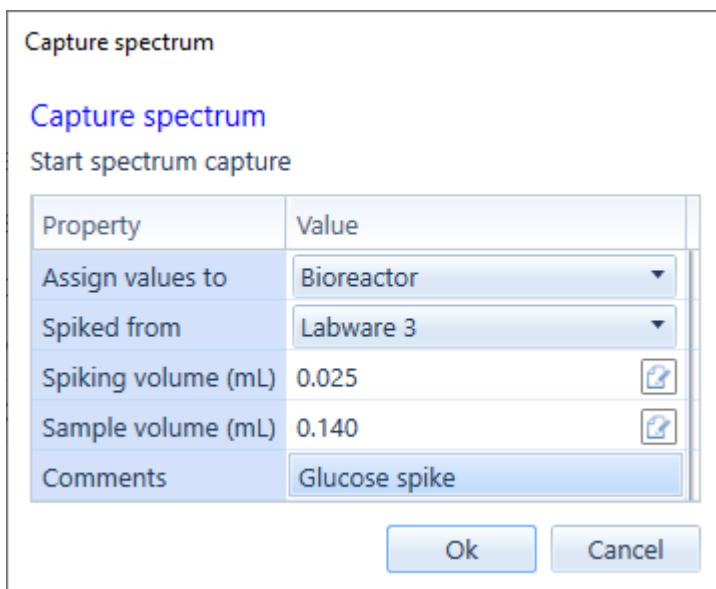


Figure 148 Move liquid to flow cell with analysis module line

The **Comments** parameter allows comments on the step.

4.4.1.22 Capture spectrum

The **Capture spectrum** step gets the spectrometer to capture a spectrum of the sample in the flow cell.



Assign values to specifies the logical source of the sample and defines where the results should be stored:

- **Bioreactor** stores the results as the analysis of the bioreactor running the step.
- **Labware** stores the results as the “Seed –“ readings of the bioreactor running the step.
- **Seed bioreactor** stores the results as the analysis of the seed bioreactor of the bioreactor running the step and as the “Seed –“ readings of the bioreactor running the step.

Spiked from specifies the logical source of the liquid used to spike the sample with:

- **Not spiked** sample has not been spiked. No spiking data is assigned to the capture.
- **Labware** data from the selected labware item is assigned to spiking data for the capture.
- **Spiking volume (mL)** the volume of spiking liquid taken from the labware used to spike the sample.
- **Sample volume (mL)** the volume of the sample.
- **Comments** – comments on the step

4.4.1.23 Wait for spectrometer

The **Wait for spectrometer** step waits until the spectrometer is not busy performing a capture.

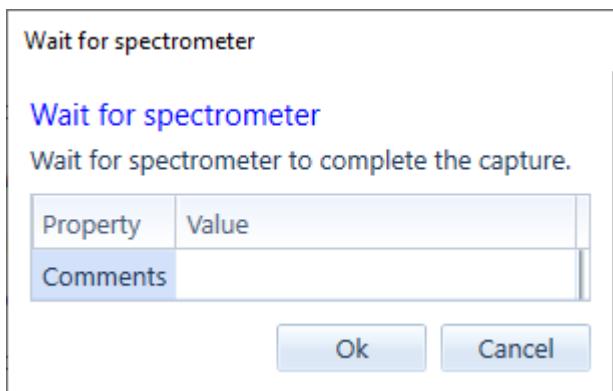


Figure 149 Wait for spectrometer line

The **Comments** parameter allows comments on the step.

4.4.2 User entered roles for labware and volumes

Roles can be entered in the liquid handling script step for any labware and volumes used in the script.

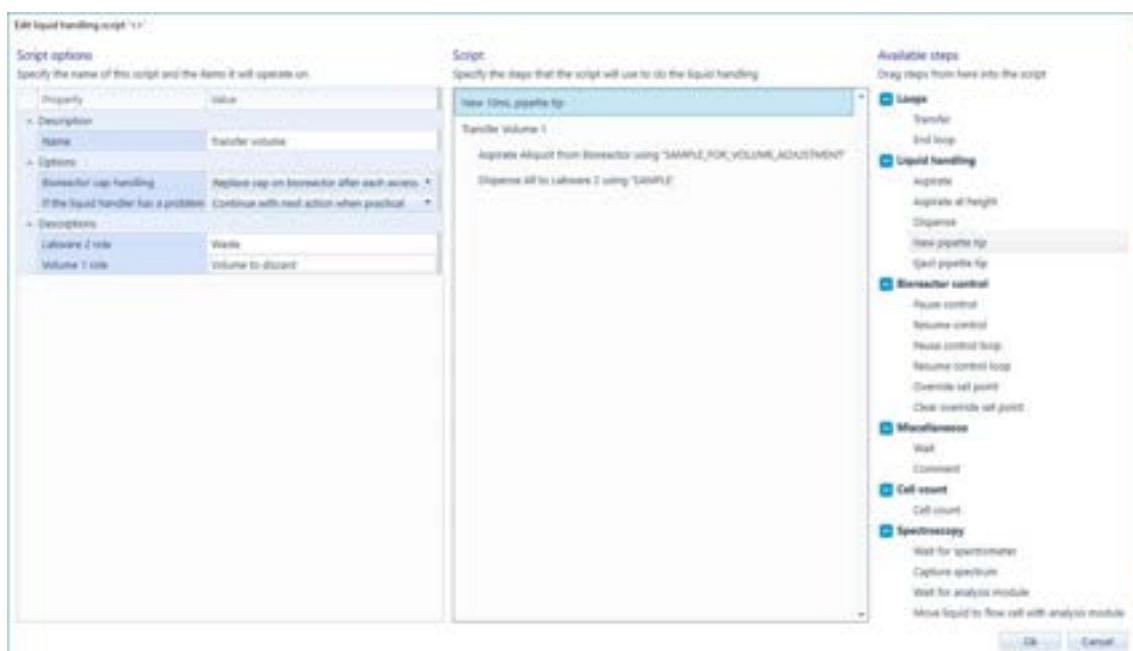


Figure 150 Step specifying role for labware and volume

Any role entered is then visible when defining the step to run the liquid handling step.

Add step - 'Run script 'Transfer volume''

Edit step parameters

Run a liquid handling script on the liquid handler

Property	Value
^ Description	
Description	Run script 'Transfer volume'
^ Which reactors	
All or selected	All bioreactors assigned to protocol ▾
^ When	
When to do step	Do after preceding step ▾
^ Error handling	
If the step fails	Stop ▾
Maximum time to wait	Never
^ Liquid handling	
Priority	50
Liquid handling script	Transfer volume ▾
Gas hold up (%)	
^ Grouping	
Group bioreactors	One at a time ▾
^ Volume to discard	
Volume type	Specified volume ▾
Volume (mL)	
^ Waste	
Labware item	
<input type="checkbox"/> Show DOE tags	Ok
<input type="checkbox"/> Show bioreactors	Cancel

Figure 151 Run liquid handling script step with role specified for labware and volume

4.4.3 Calculated parameters

Most parameters within steps in the liquid handling script can be expressions. For example the volume to aspirate or the dilution to include in a cell count.

In addition to general variables, these expressions can reference generic parameters for the script. The values for these parameters can then be set from the Run Liquid Handling Script step. The generic parameters are Parameter 1, Parameter 2 and Parameter 3.



Figure 152 Expression editor with variables filtered to just show generic parameters

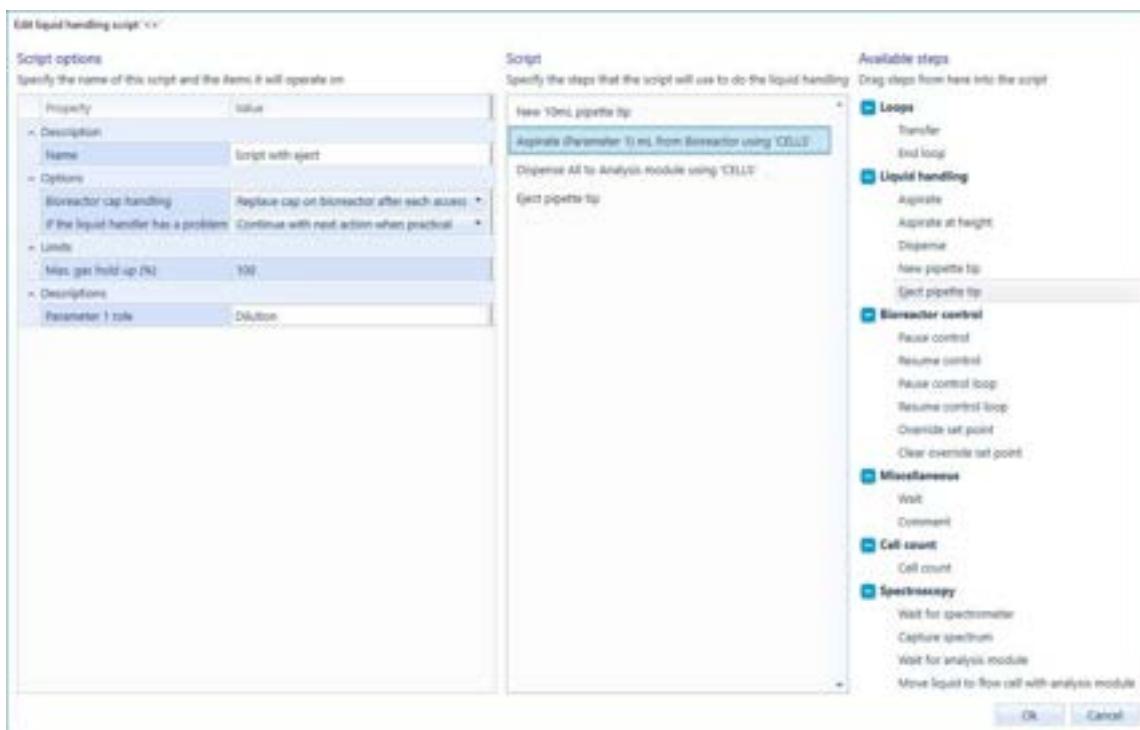


Figure 153 Script with aspirate volume defined by an expression



Figure 154 Run liquid handling script step that assigns a value to the Dilution parameter

4.4.4 Overlapping liquid handling scripts

The system allows overlapping liquid handling scripts that:

- 1) Override bioreactor set points and/or pause bioreactor control loops.
- 2) Wait for an interval.
- 3) Do some liquid handling and implicitly or explicitly clear the overrides.

Overlapping allows the wait for one bioreactor to start while liquid handling and waiting is in progress for other bioreactors.

To use the option first create a script that has the potential for interleaving.

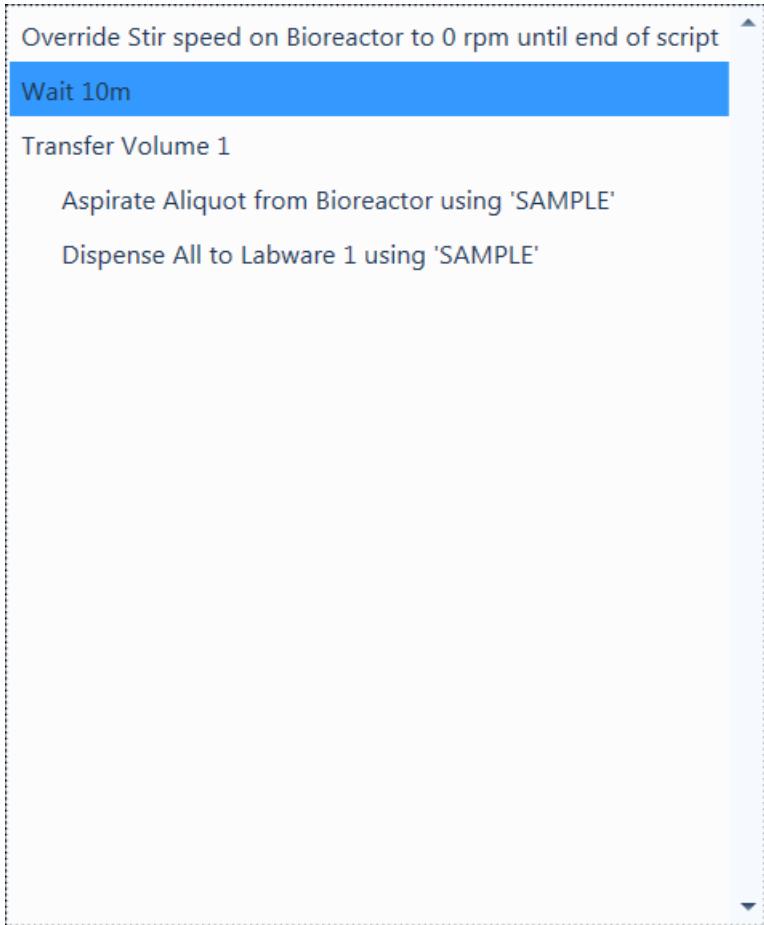


Figure 155 Liquid handling script suitable for interleaving.

To make use of interleaving then in the **Run liquid handling script** step:

- 1) choose for **Group bioreactors** the option **All together**
- 2) Select the **Overlap operations** option
- 3) Enter into **Time to allow to run script** the total time to allow for the script to run including both the pause part of the script and the liquid handling part. The time should be large enough to allow for the largest volumes that will be used in the step, and the use of the bioreactors furthest from the other labware.

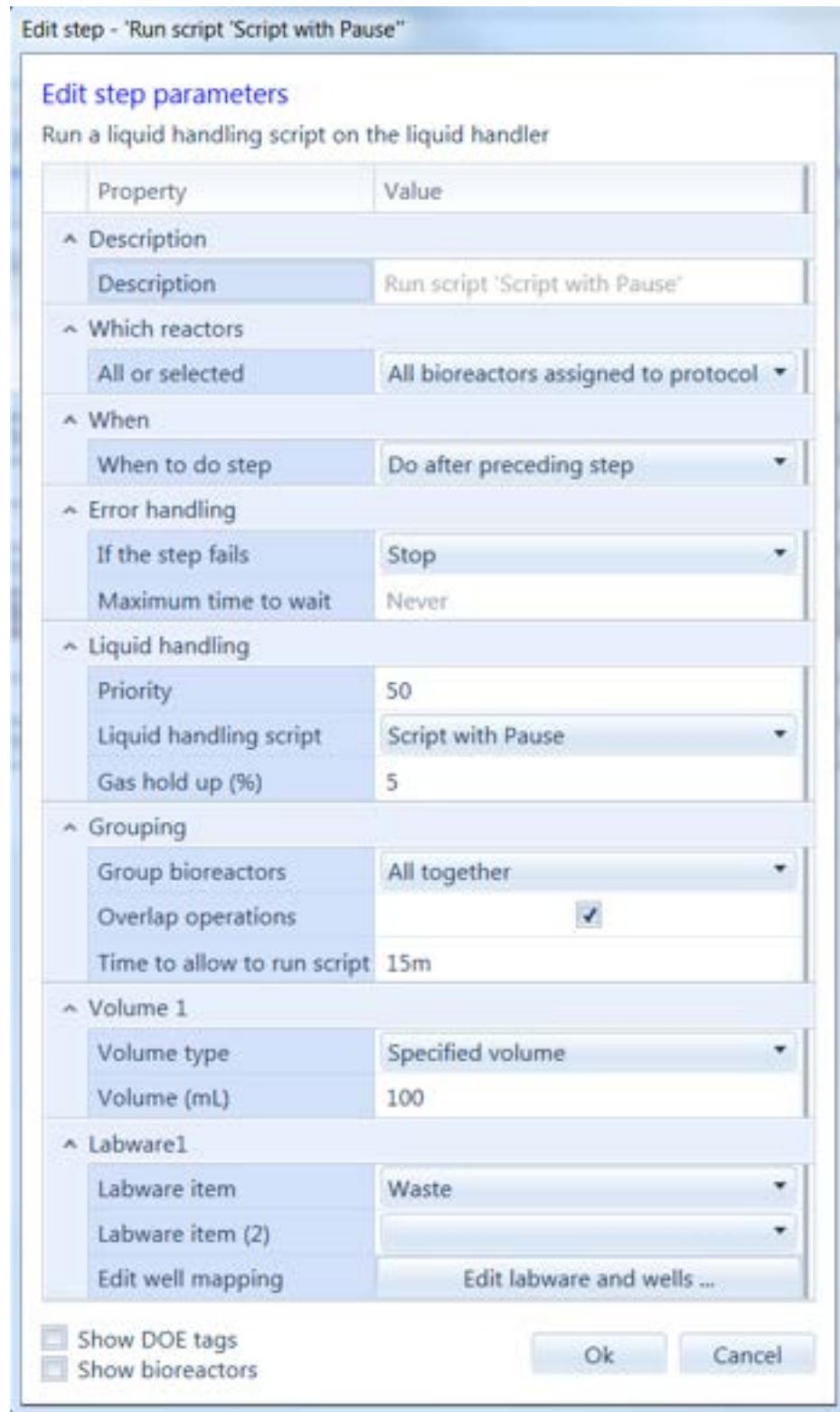


Figure 156 Step with **Group bioreactors**, **Overlap operations** and **Time to allow to run script** options set to allow overlapping.

With these options selected the liquid handler will overlap the scripts for different bioreactors where possible. While running scripts for a bioreactor the liquid handler checks when the scripts

that are already started should finish according to the **Time to allow to run script** option and starts the next script as appropriate.

The figure below shows how this works.

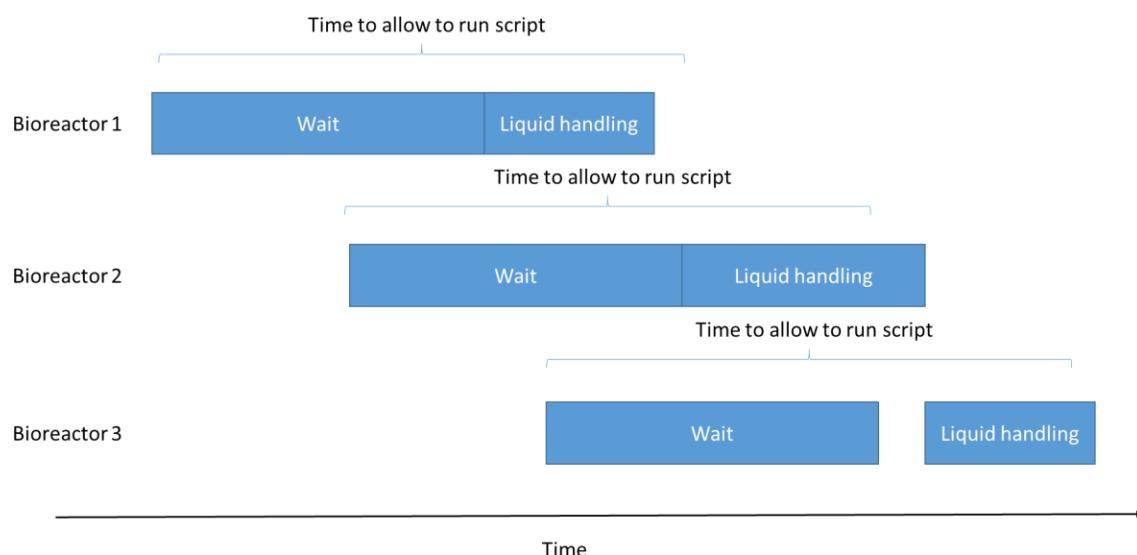


Figure 157 Overlapping scripts

- 1) The liquid handler starts running the script for bioreactor 1
- 2) The liquid handler starts the script for bioreactor 2
- 3) The liquid handler completes the script for bioreactor 1. The script takes a little less time than was allowed by **Time to allow to run script**. While running bioreactor 1's script the liquid handler started bioreactor 3's script.
- 4) The liquid handler completes the wait part of bioreactor 2's script and completes the rest of the script for bioreactor 2. The script takes a little more time than was allowed by **Time to allow to run script**.
- 5) The liquid handler completes bioreactor 3's script. Because bioreactor 2's script took longer to run than was allowed there is an extra interval before bioreactor 3's liquid handling starts.

The Liquid handler page shows how long scripts take to run. This information can be used to set a suitable value for **Time to allow to run script**.

Liquid handler

View status of the liquid handler

Running

Acknowledge Faults Change drip pad...

Time	Text
Mon 25 Sep 10:09:21	SUCK BACKLASH RATE 200UL/SEC
Mon 25 Sep 10:09:21	SUCK 50 RATE 200UL/SEC
Mon 25 Sep 10:09:22	Move to clear height above column 1 and row 1
Mon 25 Sep 10:09:22	Script line 2: Transfer Volume 1
Mon 25 Sep 10:09:22	Script line 2: Transfer Volume 1
Mon 25 Sep 10:09:23	801 Actual time 1m 27s; Time allowed N/A
Mon 25 Sep 10:09:23	Script line 2: Transfer Volume 1
Mon 25 Sep 10:09:23	Finished running queued item
Mon 25 Sep 10:09:33	Relid : Labware Type [10ML TIP BOX] Location [Pipette 1] LidLocation[Pipette lid 1]
Mon 25 Sep 10:09:48	Relid : Labware Type [1L BOTTLE] Location [Bottle 9] LidLocation[Bottle lid]
Mon 25 Sep 10:10:08	Running queued item 'Run script 'WaitTest''
Mon 25 Sep 10:10:08	Script line 1: Override Stir speed on Bioreactor to 0 rpm until end of script
Mon 25 Sep 10:10:08	Script line 2: Wait 1m
Mon 25 Sep 10:10:11	Paused until 10:11:11

6)

7) Figure 158 Liquid handler page with detail of how long a script took to run.

4.5 Protocols

The protocols page allows protocols to be created and bioreactors assigned to protocols.

Under the protocols page are the pages with the definitions of the steps and other aspects of the individual protocols.

Child pages with steps for protocols

The screenshot shows a software interface for managing protocols. On the left, there is a sidebar titled 'Protocols' with several options: System settings, Standard Liquid Handler, Advanced Liquid Handler, Standard Liquid Handler, Protocols, Bioreactors, and Help. Below this is a tree view labeled 'Child pages with steps for protocols' which includes 'Protocol steps' and 'Protocol steps for steps'. The main area displays a table of protocols with columns for Name, Version, Last Used, and Status. The 'Edit' and 'Delete' buttons are located at the top right of the protocol table. A large red arrow points from the 'Edit' button to the 'Edit' link in the sidebar. Another large red arrow points from the 'Delete' button to the 'Delete' link in the sidebar.

Figure 159 Protocols page with child pages for steps

To create a protocol either:

- a) Use the **New protocol or protocols** button which displays a screen to create protocols and make a simple assignment of bioreactors to those protocols or
- b) Use the **New blank protocol** button.

To edit an existing protocol use the Edit button.

To delete a protocol use the Delete button.

To assign protocols to bioreactors use the **Assign protocols to bioreactors...** button.

4.5.1 Create protocol or protocols

The Create protocol or protocols window is displayed:

- After completing the **Create new process** window
- Using the **New protocol or protocols** option on the **Protocols** page.

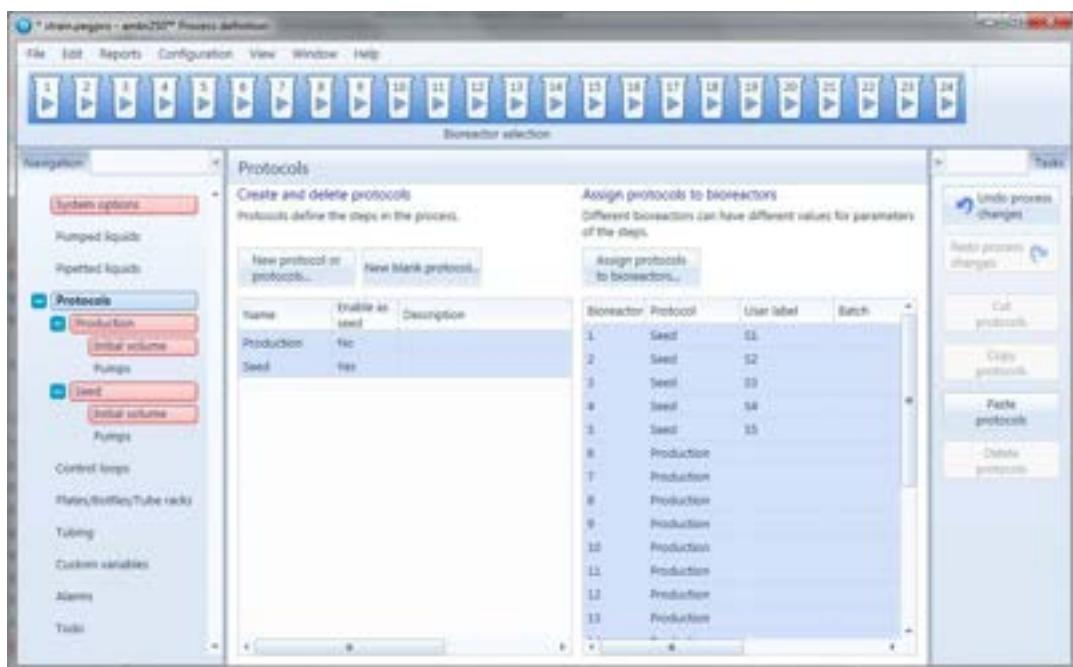


Figure 160 **Protocols** page with **New protocol or protocols** option

The **Create protocol or protocols** window creates either:

- a **Single protocol**
- a **Seed/production pair**
- Or a **Single seed protocol**

Use the **Create** option to choose what to create.

If the system does not have a liquid handler then the **Create** option is not shown and only a **Single protocol** can be created.

Use **Bioreactors to use** to choose from the bioreactors not already allocated to protocols which bioreactors to use.

For each protocol to be created choose the **Pump settings** for the protocol from the defaults on the system.

If required change the names of the protocols from the defaults provided.

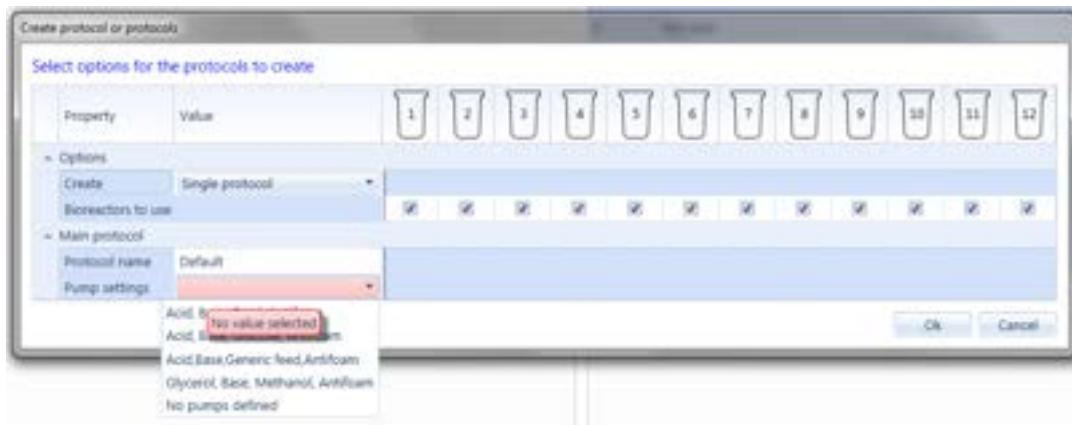


Figure 161 **Create protocol or protocols** window for a single protocol

When creating a Seed/production pair it is also necessary to specify the mapping of seed to production bioreactors.

Use the **Production bioreactors per seed**, **Seed position** and **Use seed bioreactors** options to change the mapping. If none of the in built mappings is suitable then apply the settings from the window and then use the Assign protocols to bioreactors option to set up the required mapping.

Seed bioreactors shows which bioreactors have been designated as seeds.

Seed bioreactor shows which bioreactors in the Main protocol have been assigned to which seed bioreactor.



Figure 162 **Create protocol or protocols** window for a Seed/production pair

4.5.2 Edit protocol window

Creating a '**New blank protocol**' or editing an existing protocol displays a window with details of the protocol.

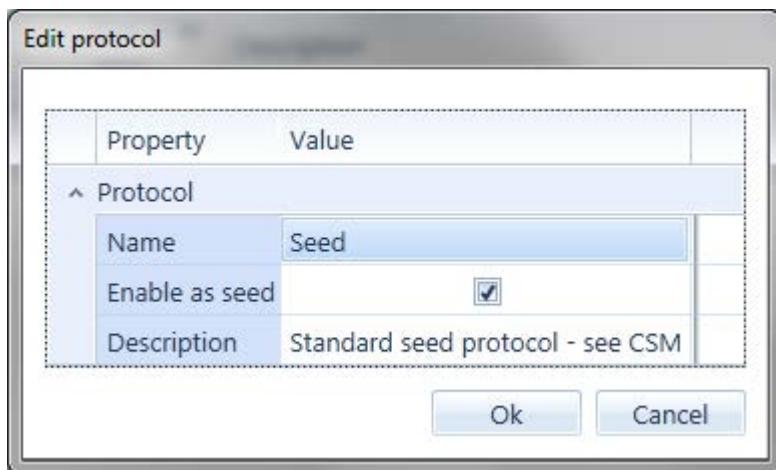


Figure 163 Edit protocol window

Name is a name for the protocol

Enable as seed indicates that the bioreactors assigned to the protocol are intended to be used as seeds for other bioreactors.

Description allows the entry of additional text describing the protocol.

If the system does not have a liquid handler then the **Enable as seed** option is not shown.

4.5.3 Assign protocols to bioreactors

The assign protocols to bioreactors window is used to indicate which bioreactors should follow which protocol.

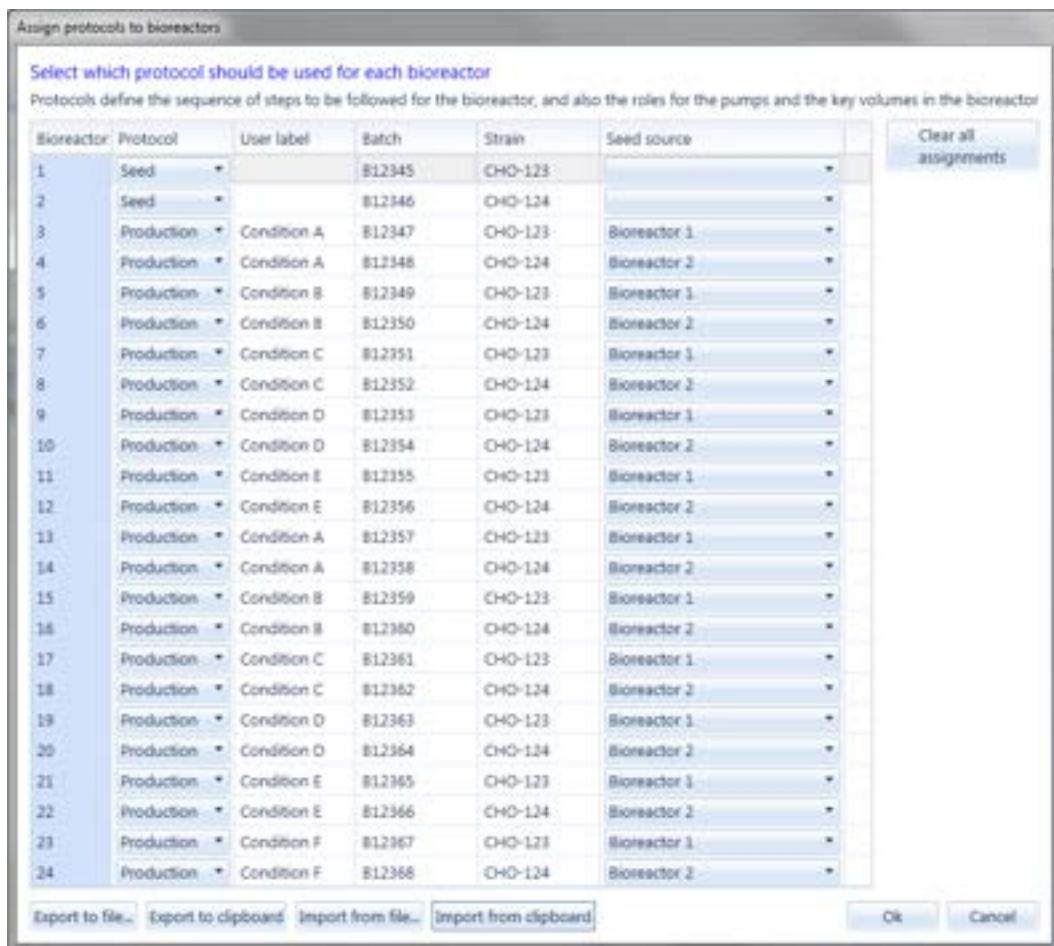


Figure 164 Assign protocols to bioreactors window

For each bioreactor the user can:

- select the **Protocol** for the bioreactor
- enter a **User label** for the bioreactor. This can be any text that is helpful for identifying the bioreactor
- enter a **Batch** for the bioreactor. This is typically a unique id for a run. The Ambr® 250 allows entry of such an id but does not perform any validation that the id is unique or is valid within a particular naming convention.
- enter a **Strain** for the bioreactor. This is typically an identifier for the strain of cell-line in the bioreactor.
- select a bioreactor to be the **Seed source** for this bioreactor. This seed bioreactor can be referred to in conditions and can be used in a step to inoculate a bioreactor from its **Seed source**. The bioreactors offered are the bioreactors allocated to protocols that are designated as **Enable as seed**.

If the system does not have a liquid handler then the Seed source option is not shown.

The Clear all assignments button will clear all the entries in the window.

The options **Export to file...**, **Export to clipboard**, **Import from file...** and **Import from clipboard** allow external data to be exported from the dialog or imported into the dialog.

4.5.4 Design of experiment data

Pressing **Design of experiment data** displays the **Edit DOE parameters** dialog which shows parameters that have been tagged in steps and control loops with DOE tags as well as some well-known properties of bioreactors such as **User label**, **Strain** and **Batch**.

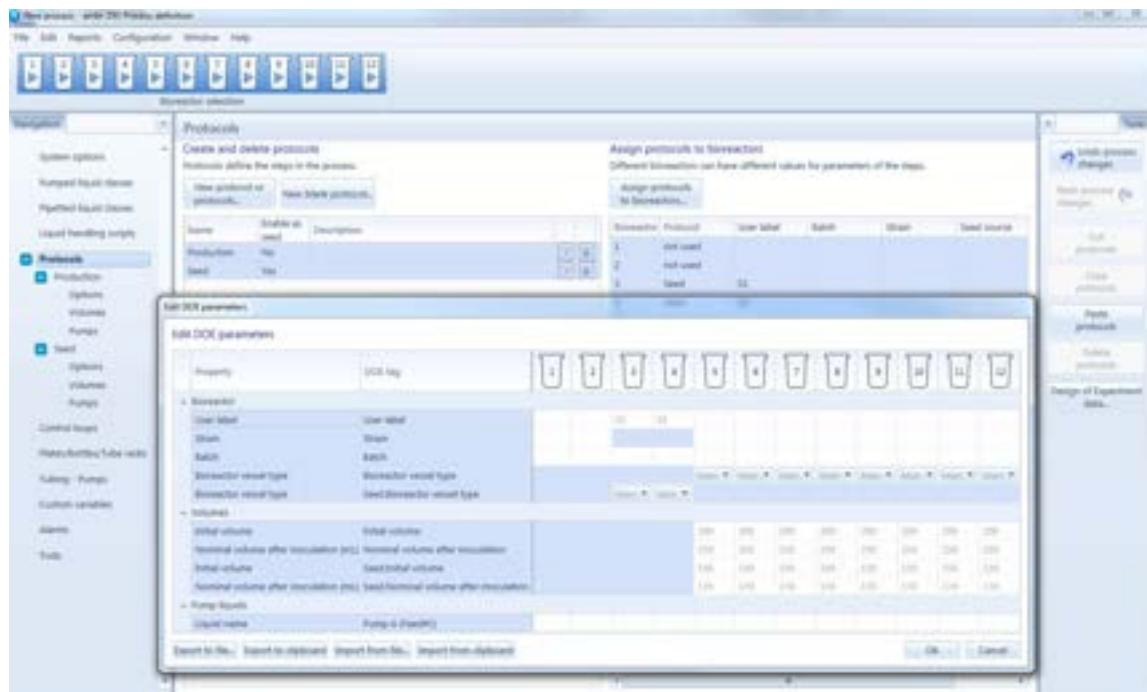


Figure 165 Edit DOE parameters dialog

The **Export to file...**, **Export to clipboard**, **Import from file...** and **Import from clipboard** options export and import the contents of the window. Data is matched to the window by the **DOE tag**.

Where parameters necessarily have the same value for different bioreactors editing the value for one bioreactor will change the value for all the linked bioreactors. Values that will be changed together are highlighted when clicking on a cell.

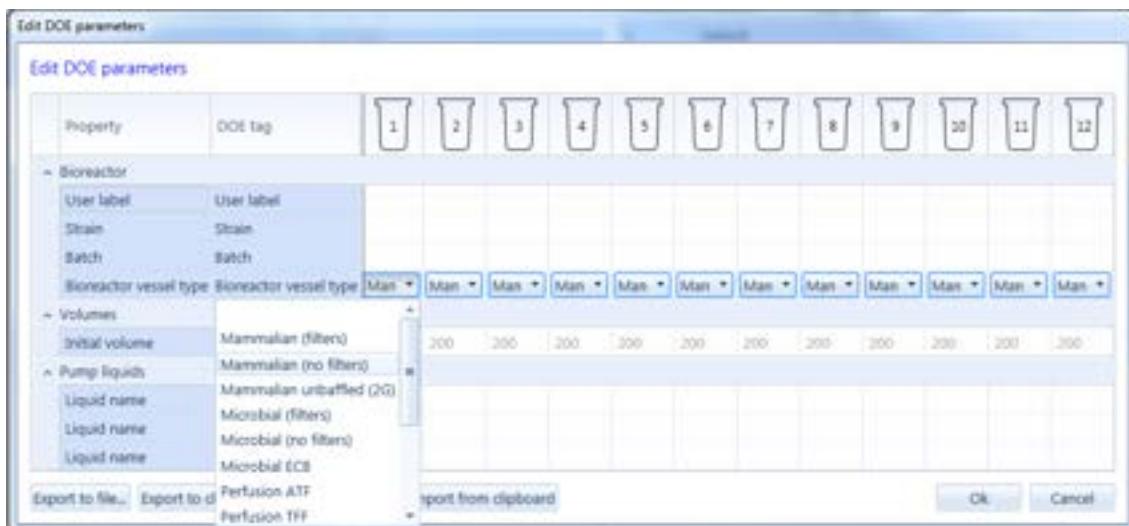


Figure 166 Editing linked parameters. In this case all bioreactors belong to the same protocol and so must have the same bioreactor vessel type.

4.5.5 Steps

The top page for each protocol defines the steps in that protocol.

The panel has a number of panels to aid editing the steps.

The central part of the page shows the steps in the protocol.

Each step can be edited using the edit and delete buttons on the step.

Once an experiment has started to run there are restrictions on editing or deleting steps that have started and on inserting steps ahead of steps that have started.

4.5.5.1 Editing steps

Initially the **Toolbox** is displayed.

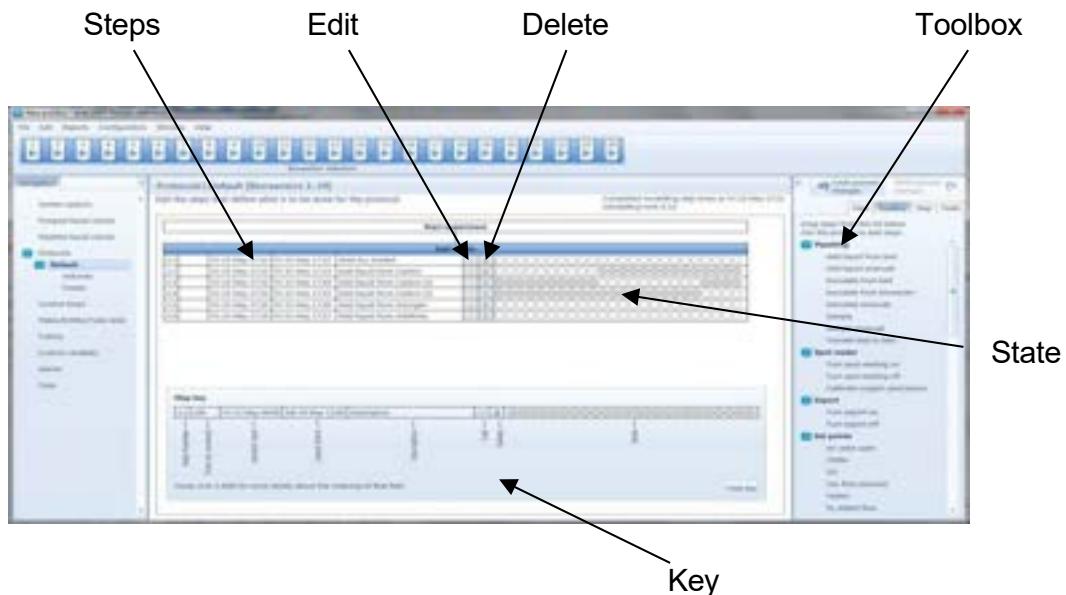


Figure 167 Steps page showing Toolbox

Steps can be added by dragging the step from the toolbox onto one of the insertion points that is highlighted in green. When the step is dropped a window will be opened for editing the parameters of the step. The various parameters of the different steps and the window for editing a step are described in section 5 Steps and Step Parameters below.

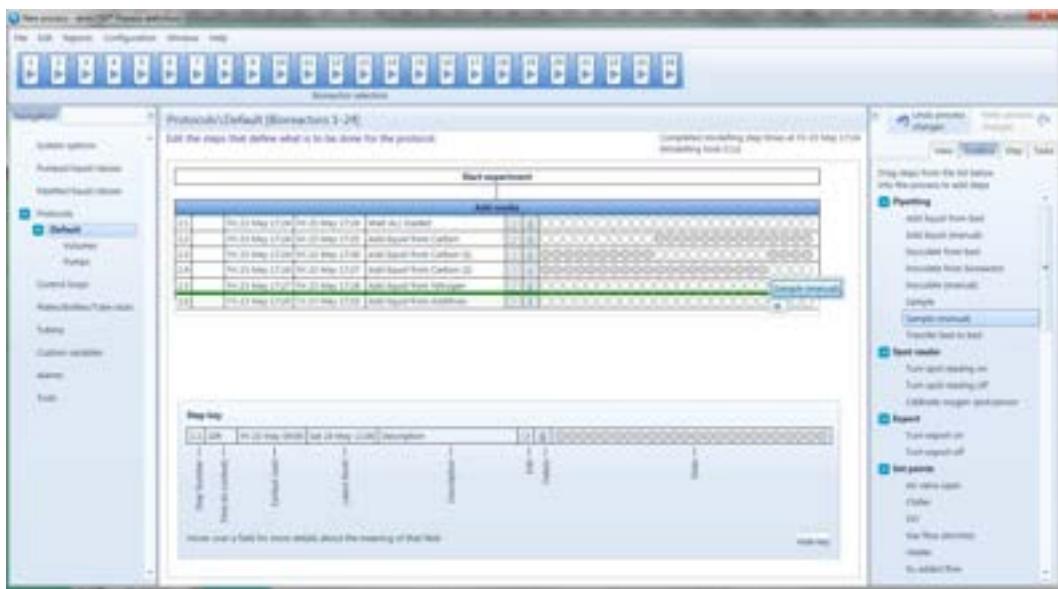


Figure 168 Dragging a step from the toolbox

Steps can be moved by dragging the step within the page.

A context menu is available for steps.



Figure 169 Steps context menu

The context menu may contain the commands below:

- **Edit** – edits step
- **Insert before**, **Insert after** and **Insert into** contain options that will insert before, after or into the selected step. The steps available reflect where the step will be added so will vary depending on the step selected.
- **Cut**, **Copy**, **Paste** and **Delete** cut, copy, paste and delete the selected step.

Editing a step displays a window where the parameters of the step can be edited.

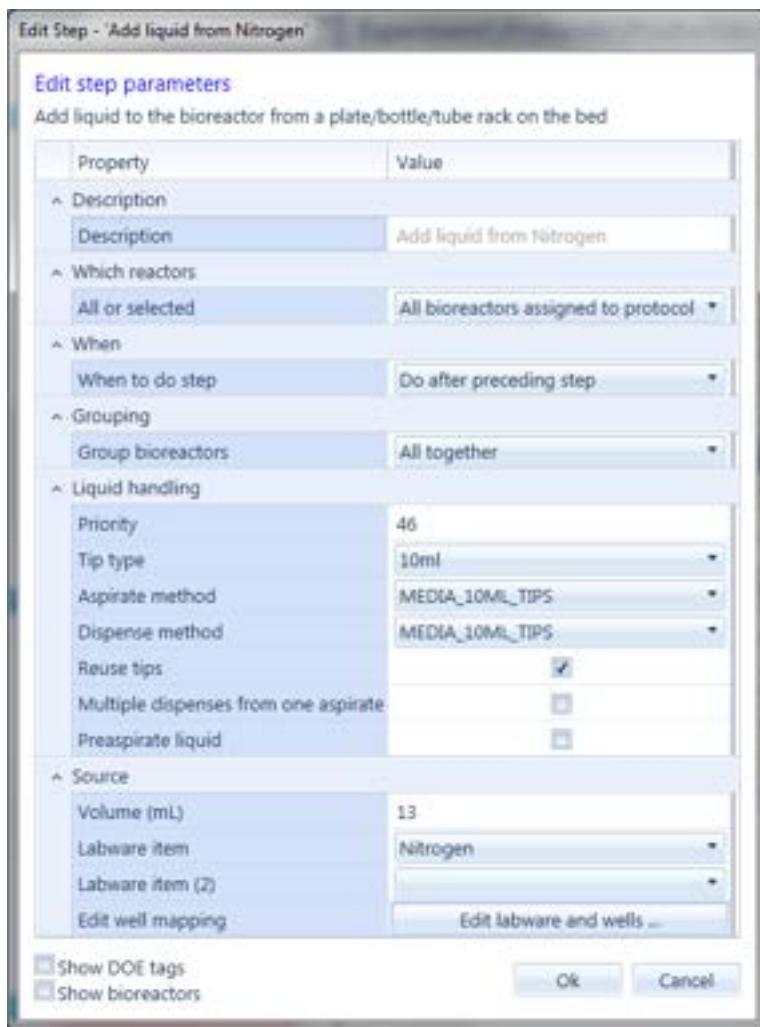


Figure 170 Edit step window

Show DOE tags shows or hides the column with DOE tags for parameters.

Show bioreactors shows or hides the columns with override values for parameters.

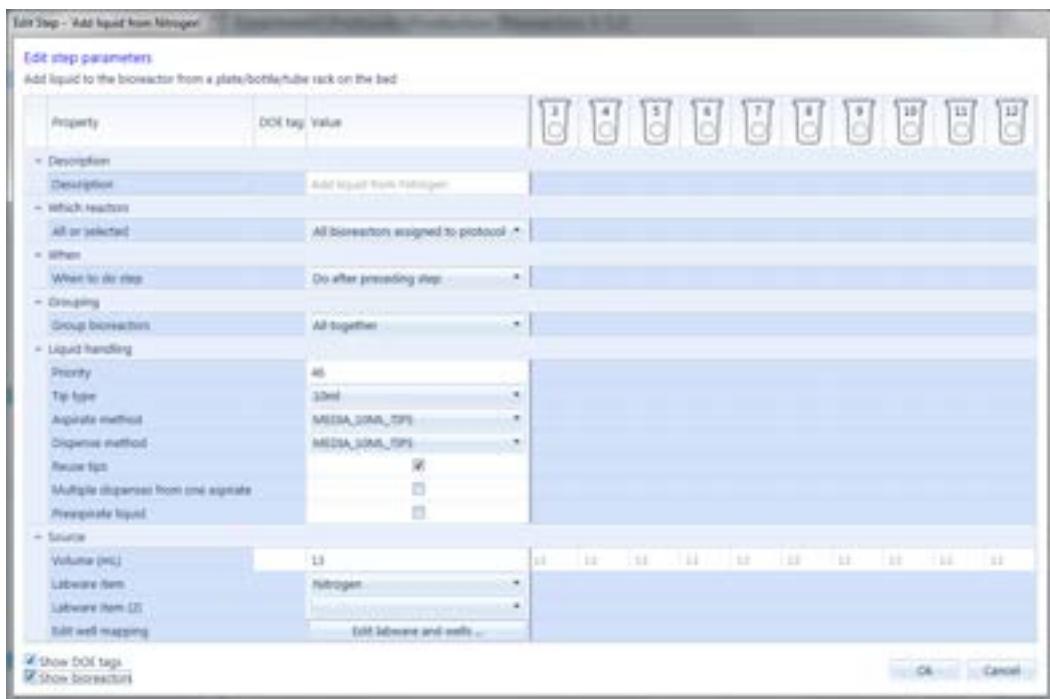


Figure 171 Edit step window with DOE tags and bioreactors displayed

4.5.5.2 Disabling step execution at runtime

Execution of steps can be disabled. When execution of steps is disabled the system will not start new steps or complete conditions. Steps that are already in progress will continue.

This can be used so that steps are not rapidly executed after editing.

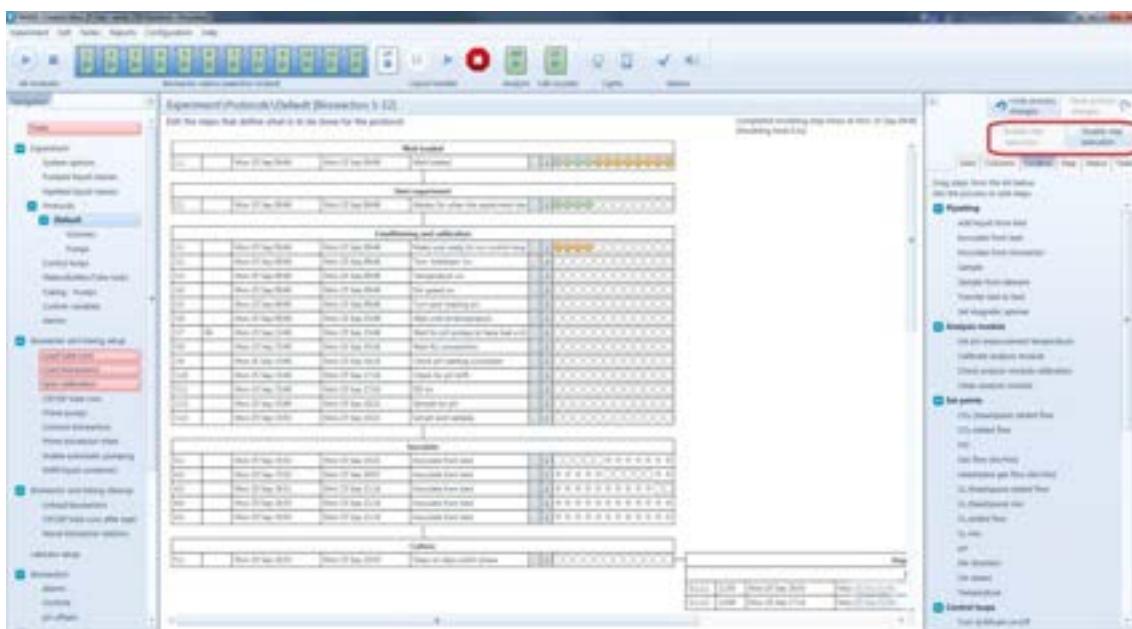


Figure 172 Disable step execution and Enable step execution options

Select **Disable step execution** to stop step execution.

Select **Enable step execution** to resume step execution.

The system will raise a warning if step execution is required and step execution is disabled for more than five minutes.

4.5.5.3 View tab

The **View** tab allows zooming into or out of the step display and provides an overview of the complete protocol.

Drag the blue area within the view area to see different parts of the process.

Use the menu available by right clicking with the mouse or doing a long click on a touch screen to zoom the steps display in or out.

Type text into the box at the top of the **View** tab to highlight steps containing the text either directly or as the name of a liquid that the step references.

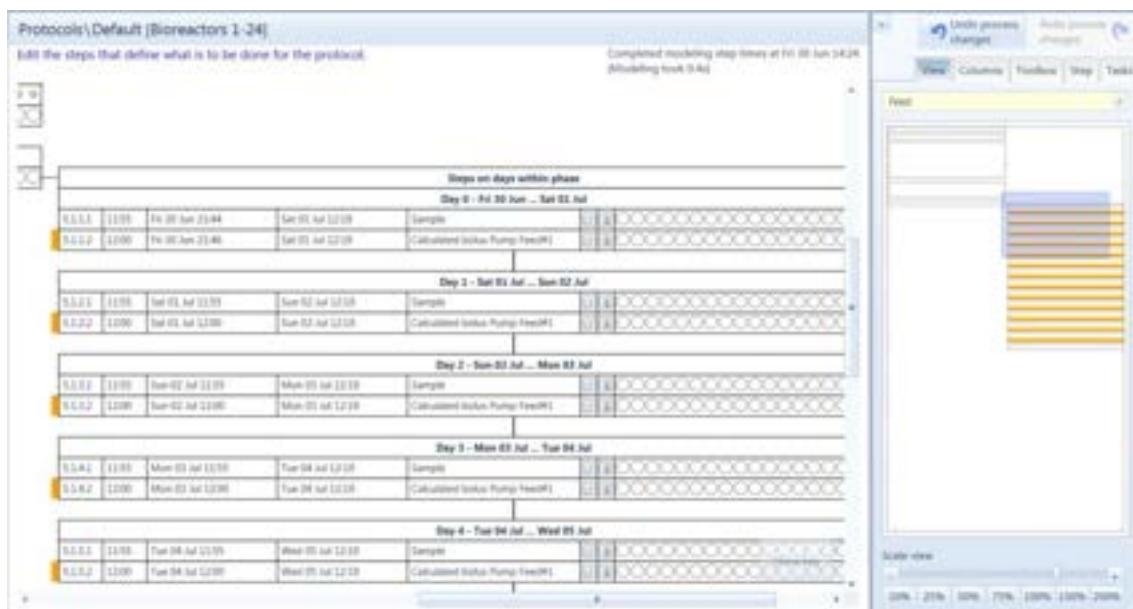


Figure 173 **View** tab with overview of process

4.5.5.4 Choosing what to display

The step can show various pieces of information about the step. The key shows the fields displayed. The **Show key** and **Hide key** buttons can be used to show and hide the key.

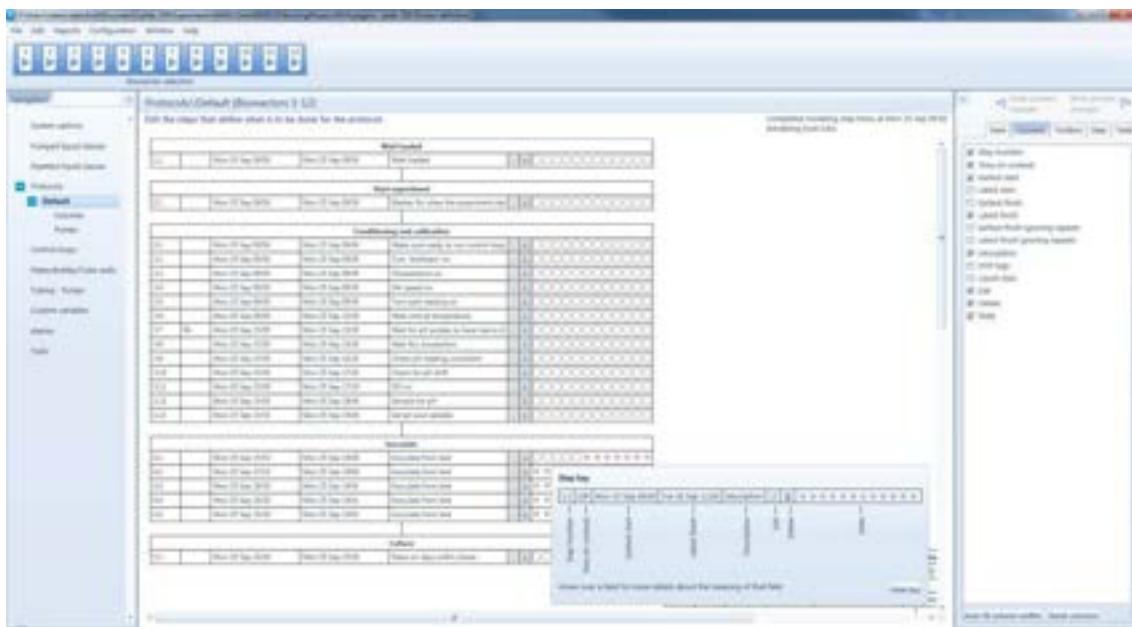


Figure 174 Steps page - View pane

The **Columns** tab lets the user:

- Choose which fields to display for a step
- Reset or fit the column widths

4.5.5.4.1 Field selection

The fields available are:

- **Step number** – a numerical identifier for a step. The step's number will change as steps are inserted before it.
- **Time (in context)** – displays key time parameters for steps that are waiting until a time in a phase or for a time of day
- **Earliest start** – the earliest time the step is expected to (or did) start for the first bioreactor to start the step
- **Latest start** – the latest time the step is expected to (or did) start for the last bioreactor to start the step
- **Earliest finish** – the earliest time the step is expected to (or did) finish for the first bioreactor to finish the step
- **Latest finish** – the latest time the step is expected to (or did) finish for the last bioreactor to finish the step
- **Earliest finish ignoring repeats** – shows the next time the step will start for one of the bioreactors, ignoring previous repetitions of the step,
- **Latest finish ignoring repeats** – shows when the step will finish its next repetition for the last bioreactor, ignoring future repetitions of the step
- **Description** – a description of the step
- **Comments** – Comments on the step
- **DOE Tags** – a display of the any DOE tags attached to parameters in the step

- **Edit** – edit the definition of the step
- **Delete** – delete the step
- **State** – the state of the step for each bioreactor. When defining a protocol just shows which bioreactors the step applies to (white) and which it does not apply to (light grey.)

4.5.5.4.2 Column widths

The width of the columns of text can be adjusted. The altered widths are saved as part of the process and are common to all the protocols within the process.

Click on a column divider and move left or right to adjust a column width.

Double click on a column divider to auto-fit a single column.

Use **Auto fit column widths** to auto-fit all of the column widths.

Use **Reset columns** to reset the displayed columns and their widths to the default values.

4.5.5.4.3 Zooming

Moving the **Scale graphic view** slider or clicking on the **25%, 50%, 75%, 100%, 150%, 200%** buttons will shrink or enlarge the display of the steps.

4.5.5.5 Reviewing step details

A tooltip is displayed with summary details of a step when the mouse hovers over the step. The tooltip shows the values of the more important step parameters and highlights any parameter with invalid values.

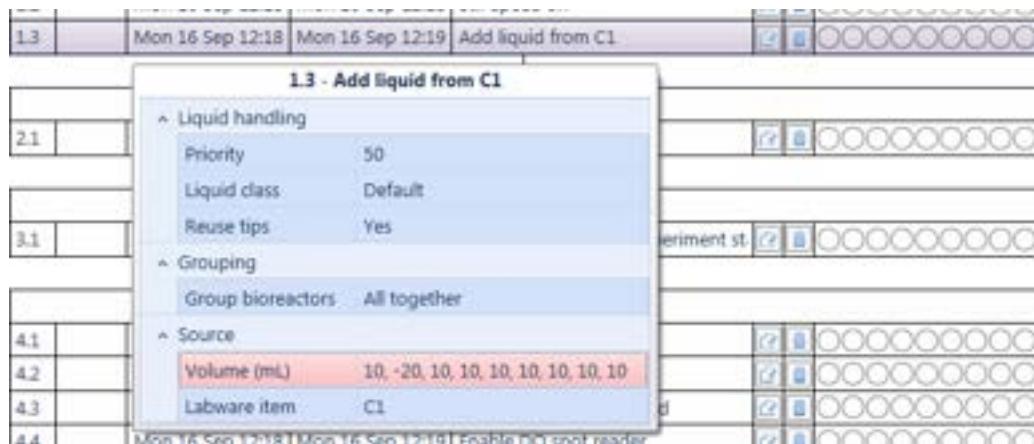


Figure 175 Step tooltip

The same information together with buttons to **Edit** or **Delete** the step is also shown on the **Step** panel.

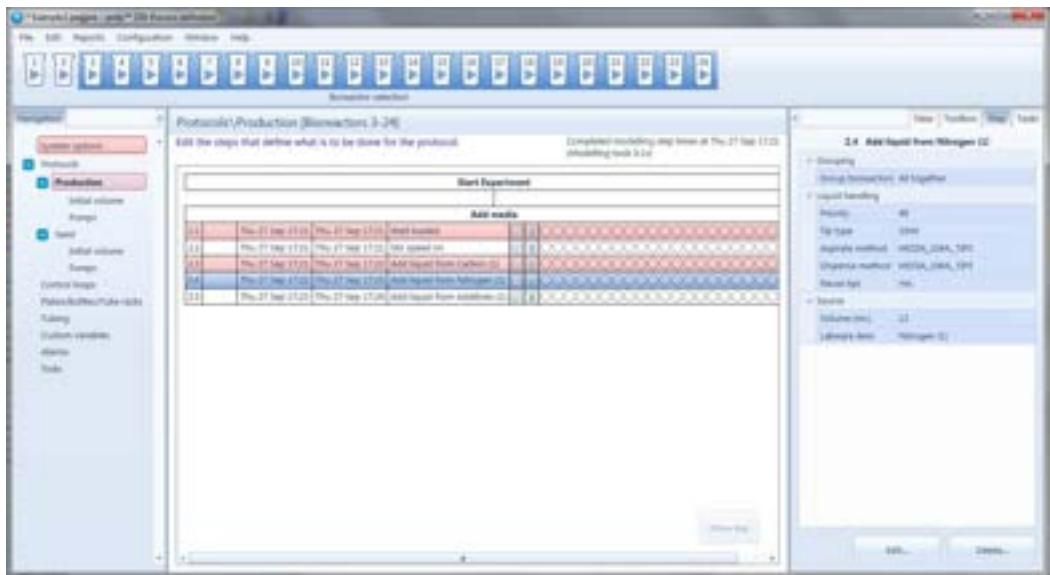


Figure 176 Step panel

4.5.5.6 Conditioning and calibration

The **Conditioning and calibration** wizard is available from the **Tasks** tab on the page showing the steps in the protocol. This wizard is only available within the definition application.

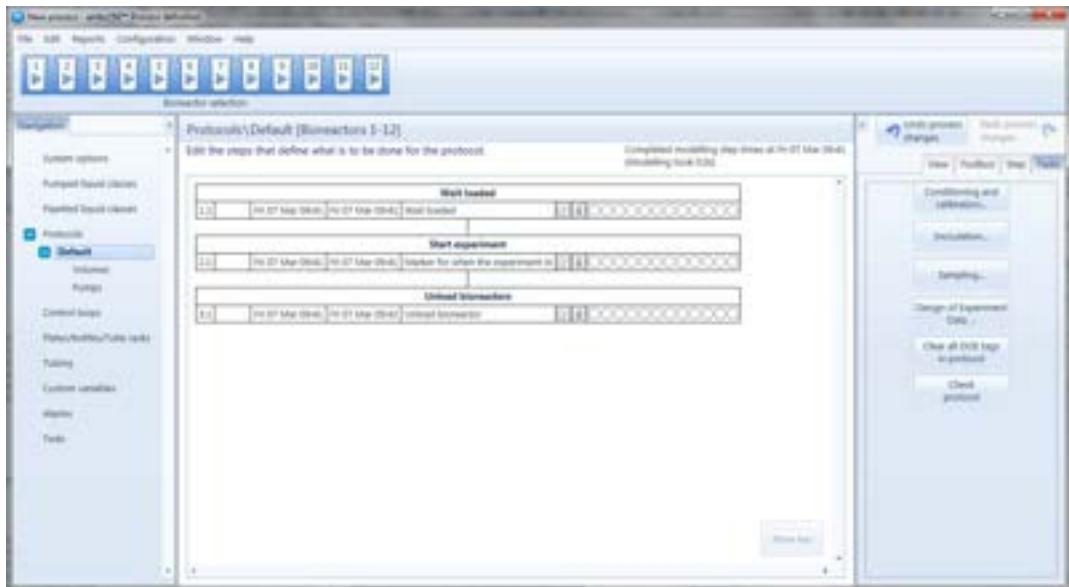


Figure 177 Steps page with **Conditioning and calibration** option

The **Conditioning and calibration** wizard creates or edits a phase called **Conditioning and calibration** in the process. If you have added steps to the phase then note that applying the wizard deletes all the steps within the phase.

The wizard also inserts a pH probe check at the start of the process.

The steps:

- Set the culture temperature

- Wait until 2 and then 6 hours have elapsed from loading the bioreactors and then checks that the initial pH of the bioreactors in the protocol is consistent.
- Check that the pH probe readings are not drifting excessively.
- Enable the DO spot reader
- Enable any foaming control loop
- Optionally control gassing to calibrate the DO spot and/or off-gas oxygen sensor at air saturation/zero OUR conditions
- Optionally enable the CO₂ spot reader
- Optionally control gassing or use an at line or offline value to calibrate the CO₂ spot
- Disable the CO₂ spot reader if enabled.
- Set the DO set point
- Do a 1-point pH calibration
- Set the pH set point
- Confirm that the pH set point is achieved.

Conditioning and calibration wizard

Add or update steps to set the initial conditions for the culture

Property	DOE tag	Value
Temperature		
Temperature (°C)	Initial T	37
Stir speed		
Stir speed	Initial stir speed	500
Dissolved oxygen		
DO (% air sat.)	Initial DO	30
DO control loop		DO
pH		
pH	Initial pH	6.8
Control mode		Acid and base
Upper pH control loop		pH upper
Lower pH control loop		pH lower
Foaming		
Foaming control loop	Do not turn on foaming control	
DO spot and/or oxygen sensor calibration		
DO spot	Use provided calibration data	
pH sample		
How to take pH sample	At-line pH station	
Liquid class	Sample analysis module (1ml) (auto continue)	
Gas hold up (%)	0	
pH checks		
Check that initial pH readings are consistent	<input checked="" type="checkbox"/>	
Check that pH reading is not drifting excessively	<input checked="" type="checkbox"/>	
Check pH control working	<input checked="" type="checkbox"/>	
pH drift check		
Wait and check for drift over	1h	
Maximum drift	0.100	
pH control check		
Maximum time to reach pH	1h	
CO₂ spot calibration		
CO ₂ spot	Recalibrate	
How to perform the CO ₂ spot calibration	At-line Flex2	
Liquid class	Sample (1ml) (auto continue)	
Gas hold up (%)	0	
<input checked="" type="checkbox"/> Show DOE tags <input type="checkbox"/> Show bioreactors		<input type="button" value="Ok"/> <input type="button" value="Cancel"/>

Figure 178 Conditioning and calibration wizard

The **Temperature**, **DO** and **pH** options set the initial values for the process. The parameter for the set point in the steps is given the **DOE tag** specified and so can be edited via the **Design of Experiment Data** option on the **Tasks** tab.

The **Stir speed** is used during the DO spot calibration but is typically superseded by the stir speed in the DO control loop.

Use the **DO control loop**, **Upper pH control loop**, **Lower pH control loop** and **Control mode** options to choose the loops that the system will use to control DO and pH. The properties of the selected DO control loop are also used to set the gassing conditions for any DO spot calibration.

Optionally a **Foaming control loop** can be selected to control foaming.

The **DO spot** option allows choosing between:

- **Use provided calibration data**
- **Recalibrate air saturation point**

The **O₂ sensor** option allows choosing between:

- **Do not recalibrate**
- **Recalibrate air saturation point**

How to take pH sample specifies how the pH calibration is done. The options are:

- **Manual sample, off-line measurement:** the operator takes a sample, measures the pH of the sample and enters the pH into the system.
- **Automatic sample, off-line measurement:** the system takes a sample and the operator must measure the pH of the sample and enter the pH into the system.
- **At-line pH station:** the system takes a sample and measures its pH automatically.

If the liquid handler is being used then the **Liquid class** specifies the set of options for taking the sample.

If the system is taking the pH sample then **Gas hold up** specified the gas hold up to assume when the pH sample is taken.

If an off-line measurement is needed then **Volume** specifies the size of the sample to take.

If required **Labware type** allows the labware that the sample will be placed in to be specified.

Automatic sampling is done using the **Prompt before samples** option so that the operator is prompted for each sample so that they can take a timely measurement of the pH.

The **CO₂ spot** option allows choosing between

- **Use provided calibration data**
- **Recalibrate**

How to perform the CO₂ spot calibration specifies how the CO₂ spot calibration is done. The options are:

- **Manual sample, off-line measurement:** the operator takes a sample, measures the pCO₂ of the sample and enters the pCO₂ into the system.

- **Automatic sample, off-line measurement:** the system takes a sample and the operator must measure the pCO₂ of the sample and enter the pCO₂ into the system.
- **At-line Flex2:** the system takes a sample and measures its pCO₂ automatically.

If the liquid handler is being used then the **Liquid class** specifies the set of options for taking the sample.

If the system is taking the pCO₂ sample then **Gas hold up** specified the gas hold up to assume when the pCO₂ sample is taken.

If an off-line measurement is needed then **Volume** specifies the size of the sample to take.

If required **Labware type** allows the labware that the sample will be placed in to be specified.

Automatic sampling is done using the **Prompt before samples** option so that the operator is prompted for each sample so that they can take a timely measurement of the pCO₂.

4.5.5.6.1 pH checks

The pH checks options allow checks to be made on the operation of the pH probes.

- **Check that the initial pH readings are consistent** creates a check that the initial pH of the bioreactors in the protocol are consistent. Unselect this option if the bioreactors in the protocol will contain different media. To make the check the protocol starts by waiting for all the bioreactors to be ready. Unselect this option if you do not want this.
- **Check that pH reading is not drifting excessively** introduces a delay after the bioreactor reaches its specified temperature where the system checks that the reading from the pH probe does not drift excessively. Unselect this option to avoid the extra delay or if the pH of the media itself is drifting.
- **Check pH control working** introduces a step at the end of the conditioning that waits for the pH to reach its set point value. Unselect this option if the process has to move on to the next step without waiting for the pH to reach its set point.

The **pH drift check** has additional options:

- **Wait and check for drift over** specifies for how long to wait and monitor the pH probe.
- **Maximum drift** specifies the allowed change in pH reading.

The **pH control check** has additional options:

- **Maximum time to reach pH** specifies the time allowed for the pH to reach its set point – to the accuracy defined by the dead bands of the control loops.
- **Maximum volume of acid to pump** – specifies the maximum amount of acid that can be pumped before the pH reaches its set point. This option is present if there is a pump with the role of Acid in the protocol.
- **Maximum volume of base to pump** – specifies the maximum amount of base that can be pumped before the pH reaches its set point. This option is present if there is a pump with the role of Base in the protocol.

If any of the limits is exceeded then pH control is turned off and an error is raised.

4.5.5.7 Inoculation

The **Inoculation wizard** is available from the **Tasks** tab on the page showing the steps in the protocol. This wizard is only available within the definition application.

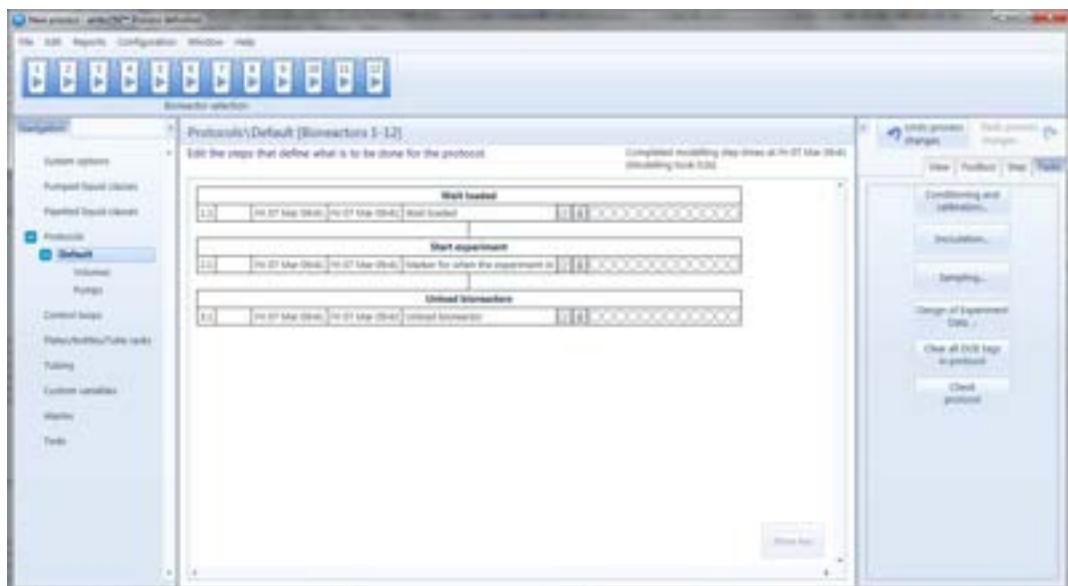


Figure 179 Steps page with **Inoculation** option

The **Inoculation wizard** creates or edits a phase called **Inoculate** in the protocol. If you have added steps to the phase then note that applying the wizard deletes all the steps within the phase. If the wizard is doing inoculation from a seed protocol then it also creates or replaces **Supply inocula** and **Unload bioreactors** phases within the seed protocol.

Inoculation wizard

Add steps to inoculate the bioreactor

Property	Value
Inoculation	
How to do the inoculation	From seed bioreactor
Inoculation volume calculation	Specified volume
Normalise volume with discard	<input type="checkbox"/>
Volume of inocula (mL)	20
Liquid class	Inoculation
Gas hold up (%)	5

Show DOE tags
 Show bioreactors

Figure 180 **Inoculation wizard** for inoculating with a fixed volume from seed bioreactors

How to do the inoculation allows selection of:

- **By hand** to prompt the user to inoculate the bioreactors by hand.
- **From labware on bed** to use the liquid handler to inoculate the bioreactors from bottles on the bed of the system.

- From seed bioreactor to use the liquid handler to inoculate the bioreactors from their designated seed bioreactors.

If **By hand** is selected then no other options are required. The operator enters the amount of inocula added when they inoculate the bioreactor. Otherwise additional options specify the volume of inocula, how to do the inoculation, and whether and how to take any sample of the seed culture before inoculation.

Inoculation volume calculation specifies how the volume of inocula will be calculated. The options are:

- Specified volume** to define the volume as part of the process definition.
- Enter volume at runtime** to prompt the user for the volume when the process is run.
- Calculate from cell density** for the system to calculate the volume of inocula based on the **Seed - Viable cell density** value for the bioreactor and the required cell density after inoculation.

If **Specified volume** is selected then **Volume of inocula** specifies how much inocula to add to the bioreactor.

If the volume of inocula is to be calculated or entered at runtime then **Min. volume of inocula** and **Max. volume of inocula** specify the valid range of volumes. If the volume calculated or entered is outside this range the system will not perform the inoculation.

If the volume is to be calculated then **Target cell density** specifies the required density of cells in the bioreactor just after the inocula has been added to the bioreactor.

Normalise volume with discard instructs the system to discard media from the bioreactor being inoculated so that the volume in the bioreactor after inoculation is the **Nominal volume after inoculation**. For example if the bioreactor contained 105mL of media, 10mL of inocula were to be added and the **Nominal volume after inoculation** required was 110mL then the system would first dispose of 5mL of media and then add the 10mL of inocula. **Discard liquid class** specifies how the discard is done. **Discard labware type** specifies the sort of bottle to place the discarded media in.

4.5.5.7.1 Inoculation from bed

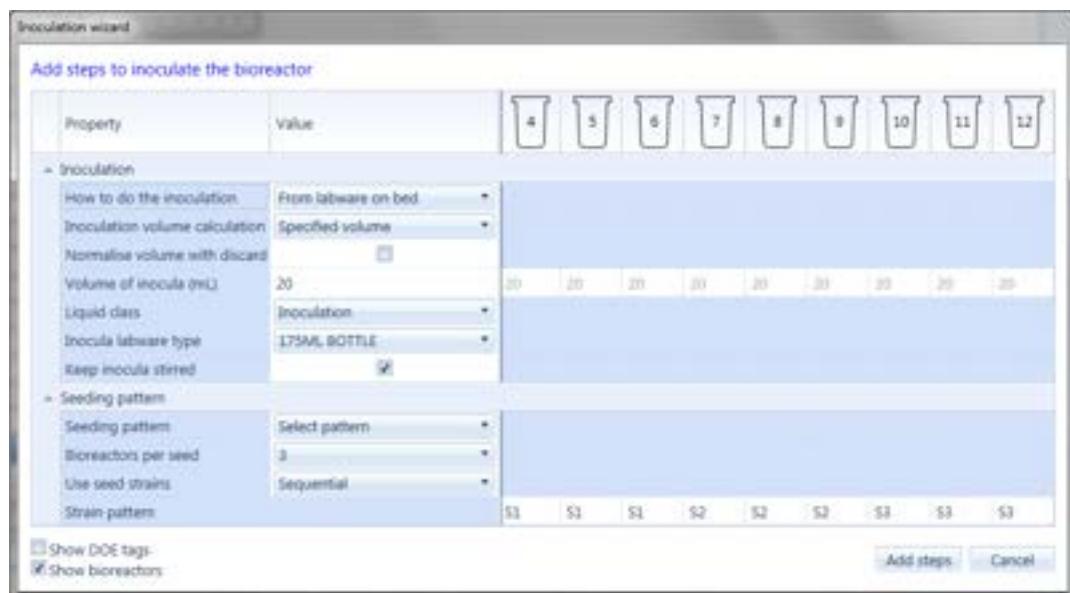


Figure 181 Inoculation wizard used for *From labware on bed*

When **From labware on bed** is selected **Liquid class** specifies how the liquid handler will perform the inoculation; **Inocula labware type** specifies the sort of labware the inocula will be presented in. If the system supports stirred locations then **Keep inocula stirred** specifies that the inocula should be placed on one of the stirred locations.

The options grouped under **Seeding pattern** specify which bioreactors share the same seed and which bioreactors have different seeds.

The **Seeding pattern** option itself can be one of:

- **Use existing strain data** to use the existing strain data
- **Select pattern** to use the options in this wizard to map the bioreactors to distinct seeds.

If **Select pattern** is chosen then **Bioreactors per seed** and **Use seed strains** define the mapping to be used.

Strain pattern shows the resulting mapping of bioreactors to distinct strains in each case.

4.5.5.7.2 Seed cell count

When an integrated cell counter is installed on the system then the **Inoculation wizard** allows the specification of a cell count to be taken to determine the density of the cells in the seed culture whether that is in labware on the bed or in a seed bioreactor.

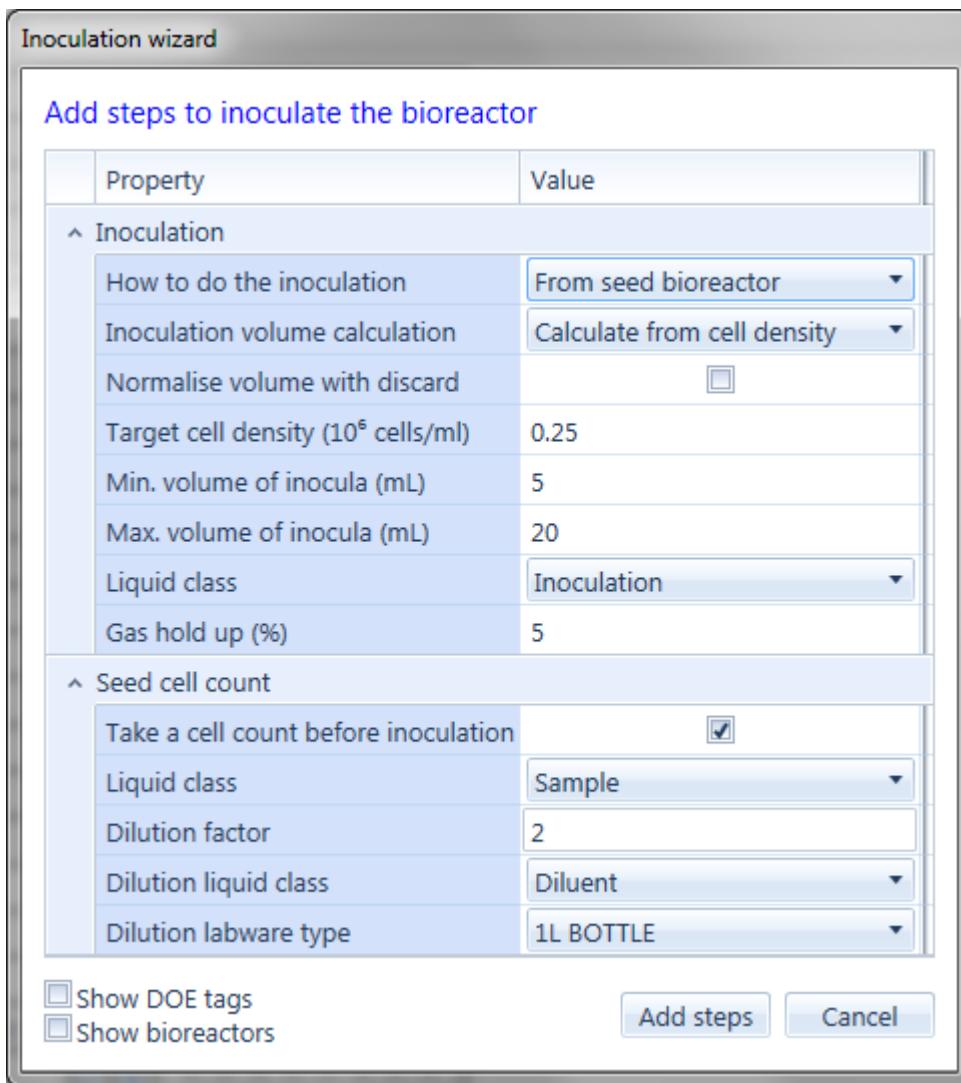


Figure 182 **Inoculation wizard** used for taking a cell count before inoculation

Take a cell count before inoculation makes the selection to take the cell count.

Liquid class specifies how the liquid handler takes the sample.

Dilution factor specifies the factor by which the sample is diluted either to bring the cell density into the range that the cell counter can handle or to use a small amount of culture at the expense of reduced accuracy.

If the **Dilution factor** is greater than 1 then **Dilution liquid class** specifies how the liquid handler performs the dilution and **Dilution labware type** specifies the type of labware in which the diluent is found.

4.5.5.8 Sampling

The **Sampling wizard** is available from the **Tasks** tab on the page showing the steps in the protocol. This wizard is available within both the definition and the runtime applications.



Figure 183 Steps page with **Sampling** option

The **Sampling wizard** creates and edits **Sample** steps within the protocol definition. Other steps and sample steps that the **Sampling wizard** cannot handle are not affected by the use of the **Sampling wizard**.

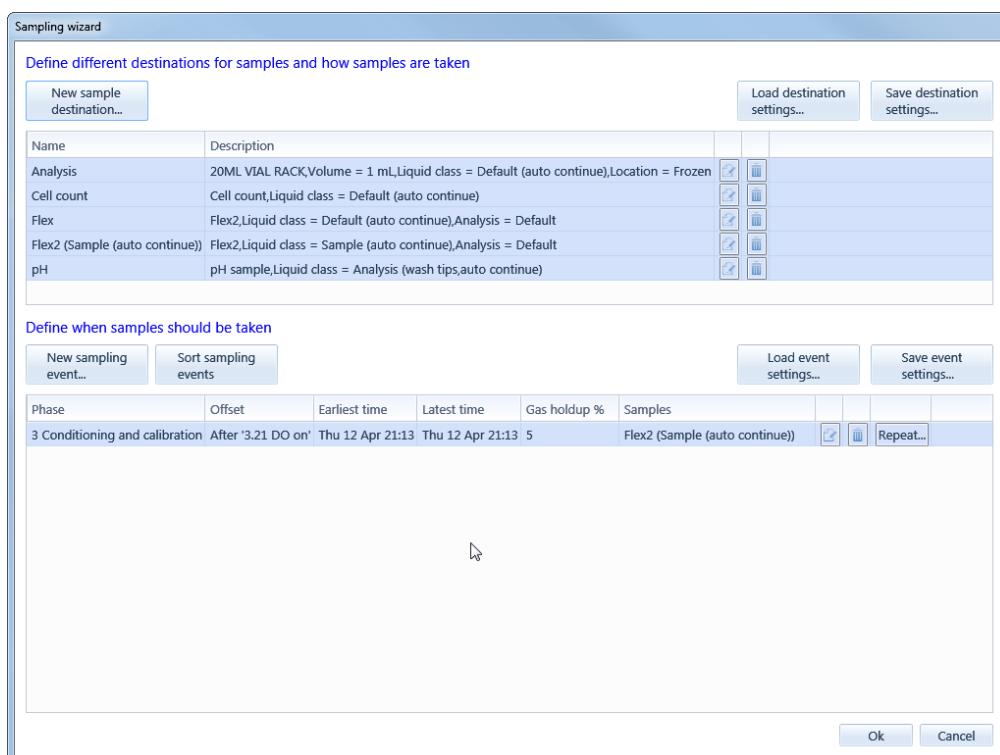


Figure 184 Sampling wizard

The **Sampling wizard** defines samples in two parts:

Sample destinations specify a sample destination including whether the sample is taken to labware or an integrated analyser; the volume of the sample; and what sort of labware the sample is placed in.

Sample events specify what samples are taken at a point in time. The event specifies some destinations; the time; and the expected gas holdup in the samples.

For each sample event the system shows:

- The **Phase** containing the event.
- The **Offset** of the event relative to the phase or the step it is after.
- The **Earliest time** and **Latest time** that the sample is predicted to happen. These times are estimates based on the current modelling of the start times or the phases and steps and do not take into account how those times may be altered when phases and steps wait on sampling happening.
- The **Gas holdup** entered for the event
- The **Samples** included in the event

4.5.5.8.1 Sample destinations

New sample destination creates a new sample destination.

Load destination settings and **Save destination settings** load or save a complete set of destinations as defaults.

4.5.5.8.1.1 Manual

Selecting a **Sample type of Manual** specifies a sample for the operator to take by hand.

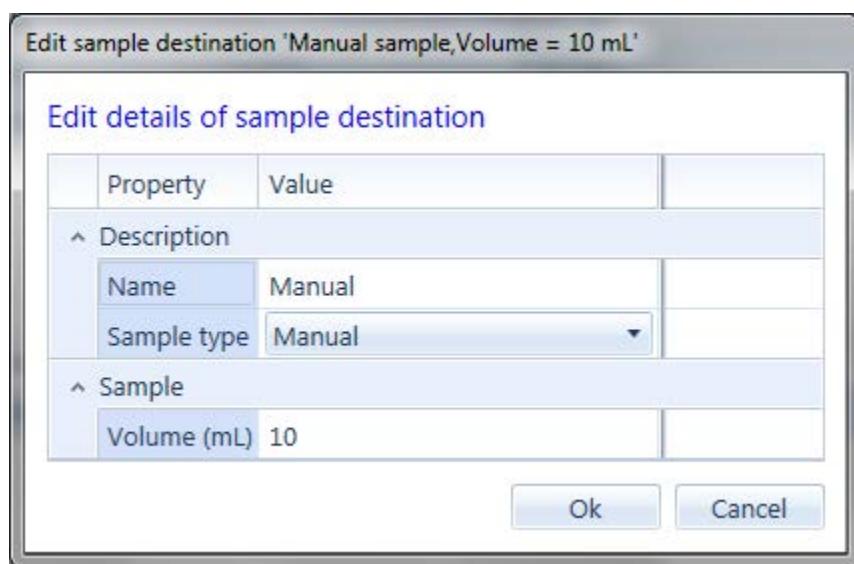


Figure 185 Sample destination for a manual sample

Volume specifies the amount of liquid to remove from the bioreactor.

4.5.5.8.1.2 Labware

Selecting a **Sample type of Labware** specifies a sample taken by the liquid handler and placed in labware on the bed.

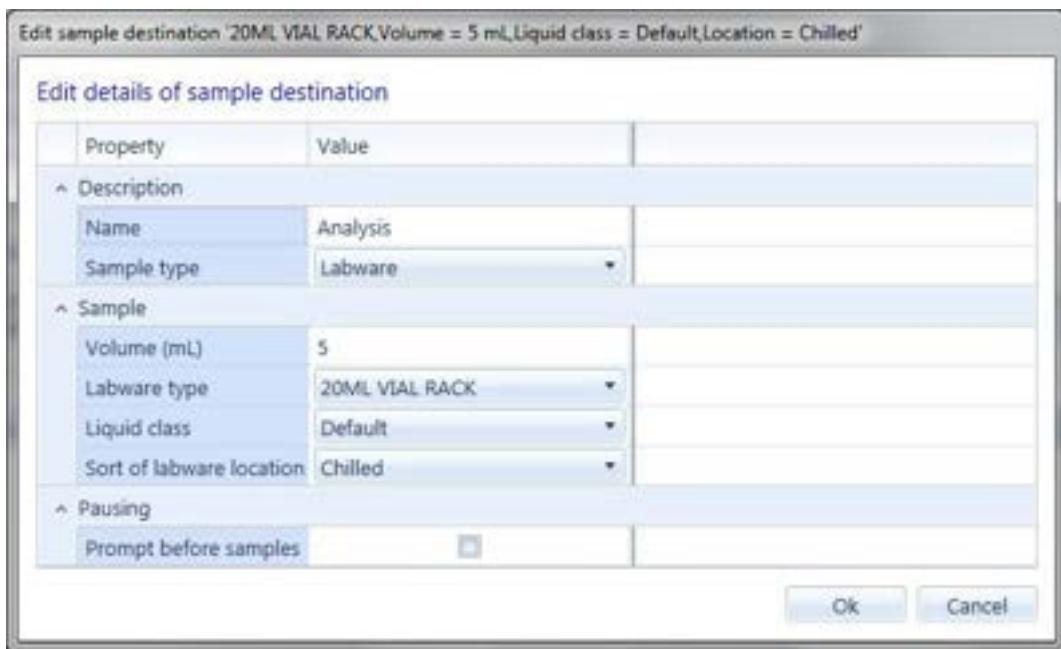


Figure 186 Sample destination for a labware sample

Volume specifies the amount of liquid to remove from the bioreactor.

Labware type specifies the sort of labware to receive the sample.

Liquid class specifies how the liquid handler takes the sample.

Sort of labware location specifies where the labware can be placed.

Prompt before samples allows the liquid handler to prompt the user for each individual sample taken. If selected then **Prompt text** specifies some text for the prompt.

4.5.5.8.1.3 pH

Selecting a **Sample type** of **pH** specifies a sample taken by the liquid handler and measured using an at-line pH station integrated as part of the Ambr® 250 system.

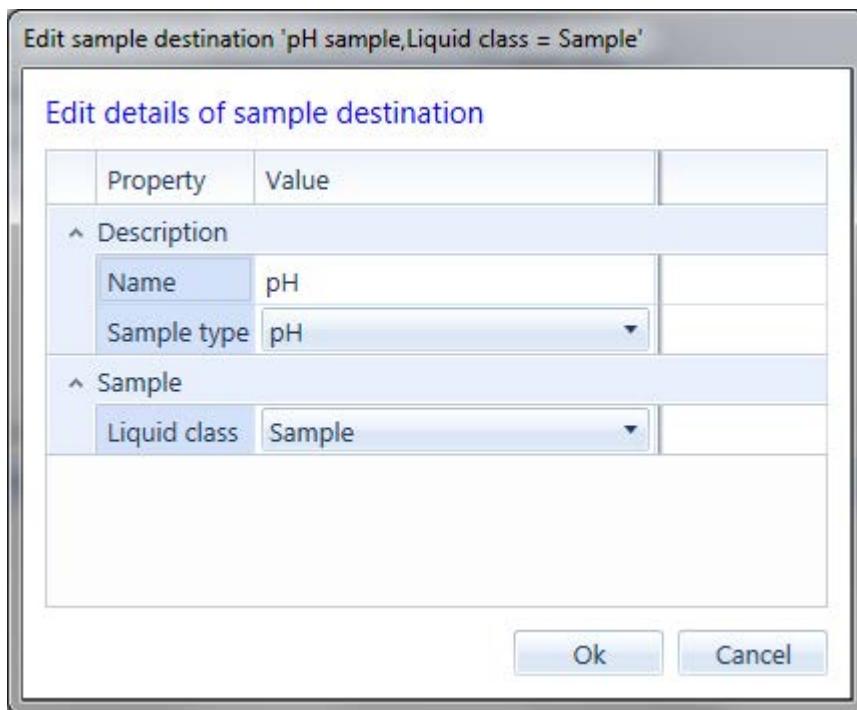


Figure 187 Sample destination for a pH sample

Liquid class specifies how the liquid handler takes the sample.

4.5.5.8.1.4 Cell count

Selecting a **Sample type** of **Cell count** specifies a sample taken by the liquid handler and measured using an at-line cell counter integrated as part of the Ambr® 250 system.

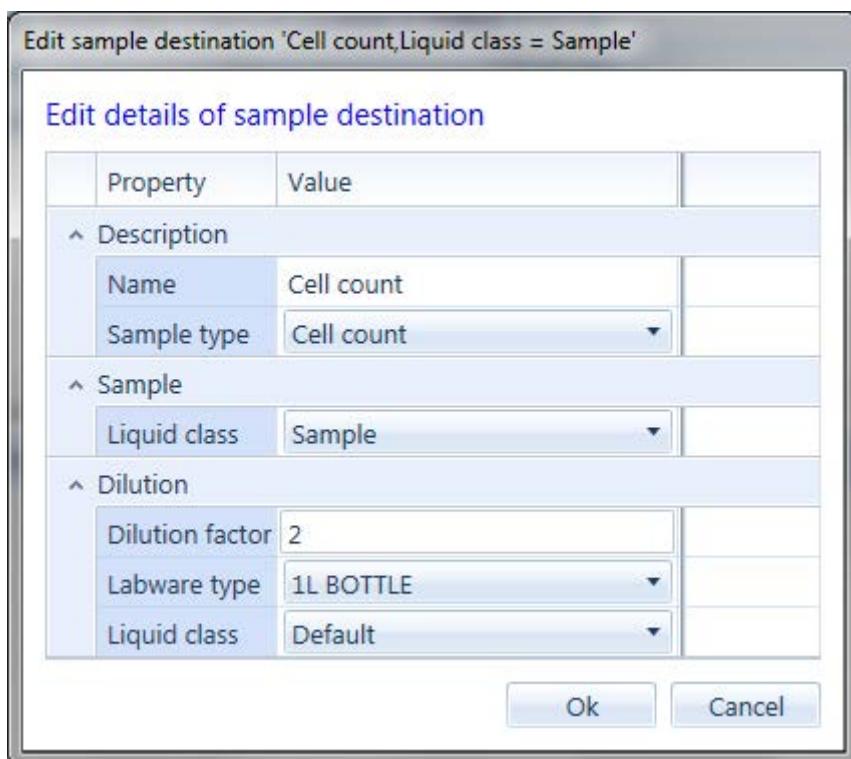


Figure 188 Sample destination for cell count sampling

Liquid class under **Sample** specifies how the liquid handler takes the sample.

Dilution specifies optional dilution of the sample to bring the cell density of the sample into a range the cell counter can handle.

Dilution factor specifies the factor by which to dilute the sample.

Labware type specifies the sort of labware to take the diluent from.

Liquid class specifies how the liquid handler performs the dilution.

4.5.5.8.1.5 Flex2

Selecting a **Sample type** of **Flex2** specifies a sample taken by the liquid handler and measured using an at-line Nova Biomedical Bioprofile Flex2, integrated as part of the Ambr® 250 system.

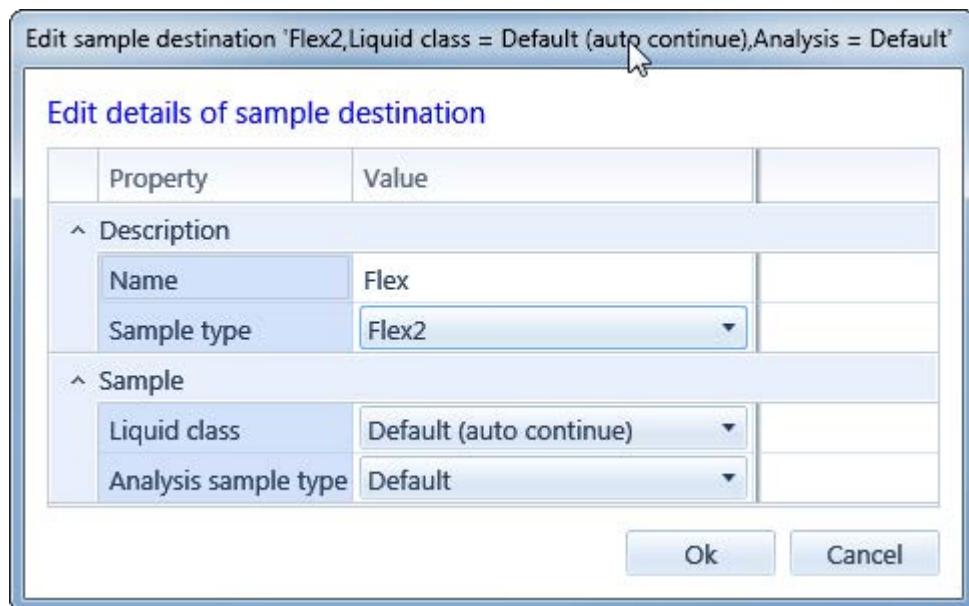


Figure 189 Sample destination for Flex2 sampling

Liquid class under **Sample** specifies how the liquid handler takes the sample.

Analysis sample type under **Sample** specifies one of the Flex2 analysis panels that define the measurements that will be conducted on the sample.

4.5.5.8.2 Sample events

New sampling event creates a new sample event.

Load event settings and **Save event settings** load or save a complete set of events as defaults.

4.5.5.8.2.1 Editing or creating a sample event

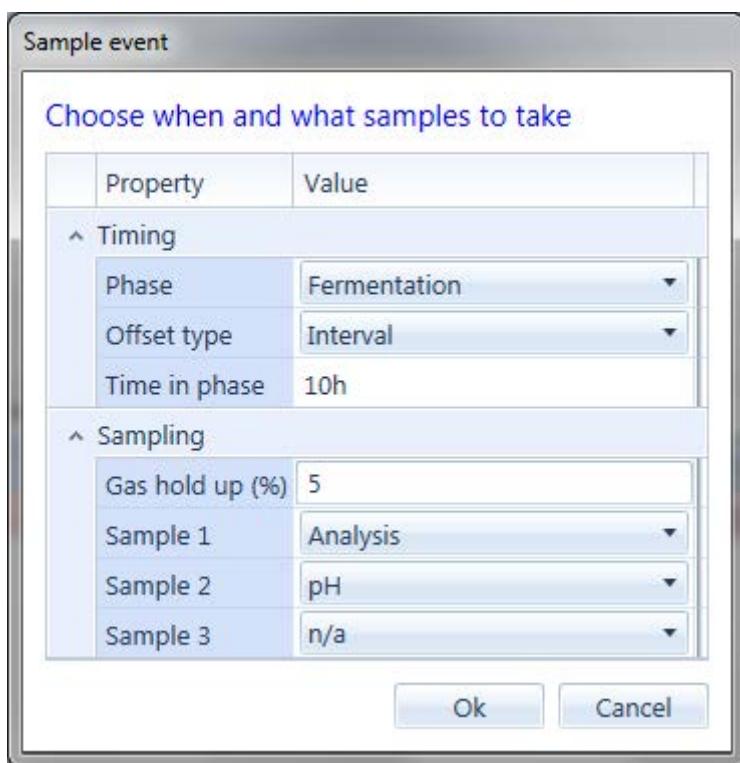


Figure 190 **Sample event** window

The options under **Timing** specify when the sample should be taken.

Phase specifies the phase within which the **Sample** step will be placed either directly or in a **Parallel block** or **Steps on days within phase** step.

Offset type specifies one of:

Interval – the sample should happen at Time in phase after the start of the Phase

Day and time – the sample should happen on the specified logical Day and Time of day after the start of the Phase

After step – the Sample step(s) should be placed following the specified step within the Phase.

The options under **Sampling** specify the samples to be taken.

Gas hold up specifies percentage of the volume in the source bioreactor taken up by gas within the liquid. The amount aspirated by the liquid handler is increased so that the required volume of liquid is aspirated to account for the gas in the liquid.

Sample 1, **Sample 2** and **Sample 3** allow the destinations of the samples taken by this event to be selected.

4.5.5.8.2.2 Repeating an event

Selecting **Repeat** displays a window that can be used to create multiple copies of an event.

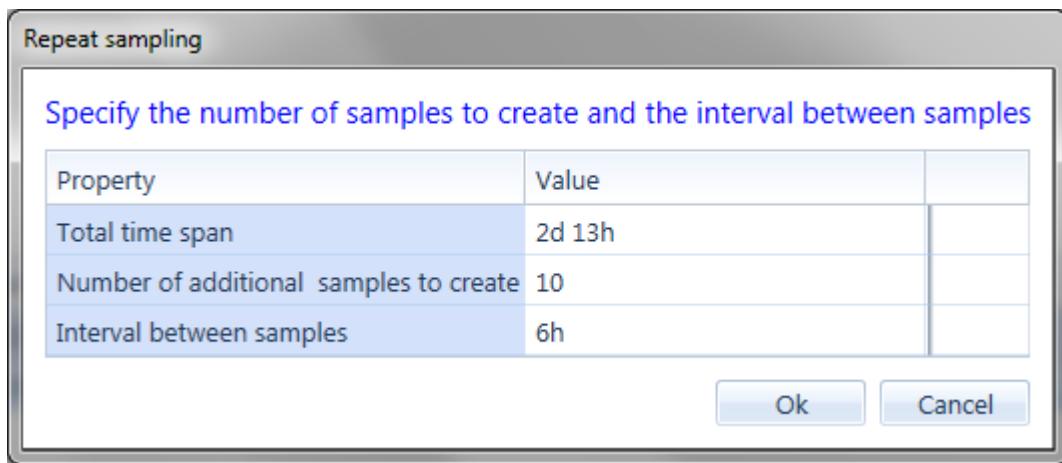


Figure 191 **Repeat sampling** window

Editing the total time covered by the samples (**Total time span**), the **Number of additional samples to create** and the **Interval between samples** updates the remaining fields appropriately.

4.5.5.9 DOE Parameters

DOE parameters are used to collate step parameters that should be varied across different bioreactors – typically because the parameters for the experiment have been defined by a Design of Experiment tool.

Step parameters can be marked as DOE parameters by giving them a **DOE tag**.

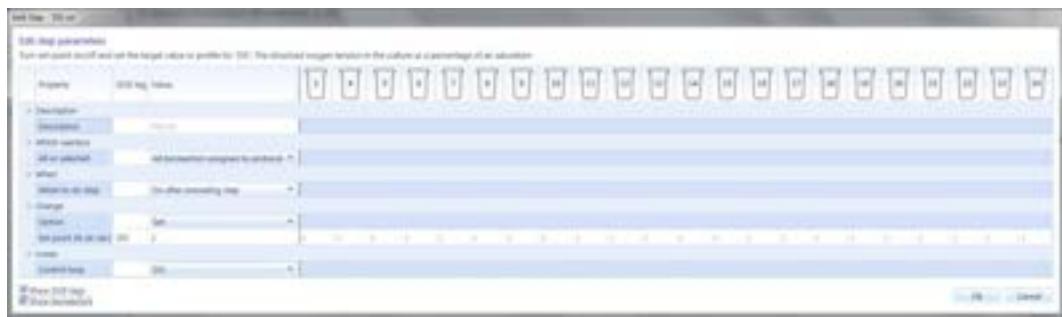


Figure 192 Step window with DOE tag

To give a parameter a **DOE tag**:

- 3) edit the step and select the **Show DOE tags** option
 - 4) type the tag into the **DOE tag** column

Having given some parameters DOE tags the **Design of Experiment Data...** option can be used to edit those parameters.

The **Clear all DOE tags in protocol** option can be used to clear all of the DOE tags in all of the steps in the protocol.

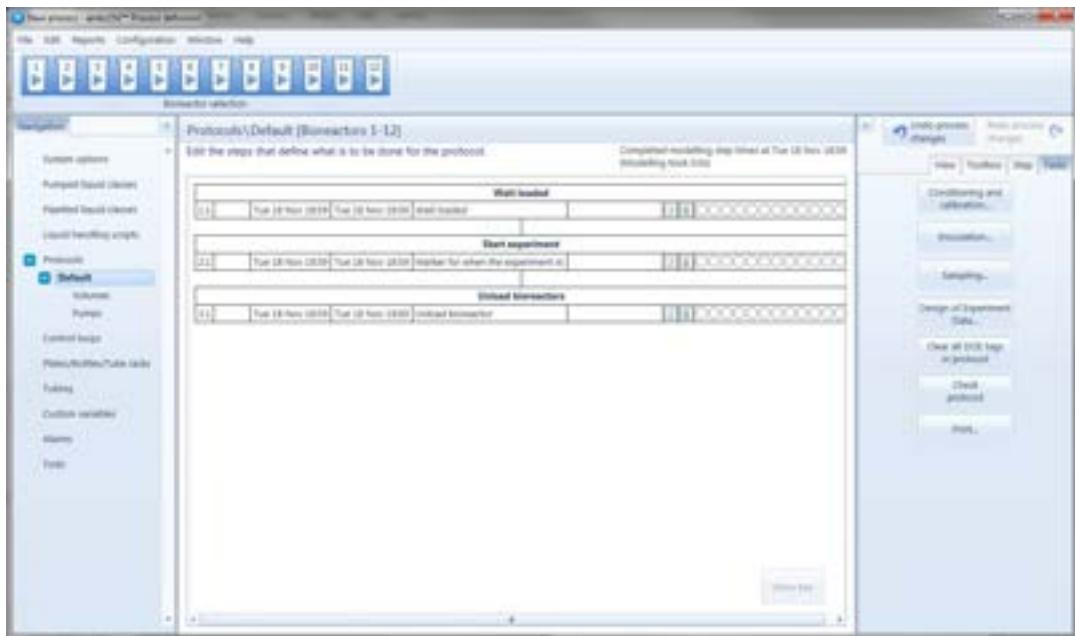


Figure 193 Tasks panel with **Design of Experiment Data...** and **Clear all DOE tags in protocol** options

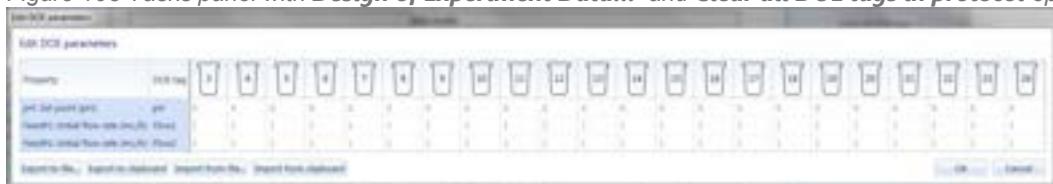


Figure 194 Design of Experiment Data window

The **Design of Experiment Data** window shows the values of the parameters for each of the bioreactors in the protocol and allows those parameters to be edited.

The **Export to file...**, **Export to clipboard**, **Import from file...** and **Import from clipboard** options export and import the contents of the window. Data is matched to the window by the **DOE tag**.

The same DOE tag may be used for the same parameter in multiple steps in the process allowing this dialog to give the same values to a parameter in those steps. If the tag has different values for bioreactors in the different contexts then the **Design of Experiment Data** window will highlight the conflicts.

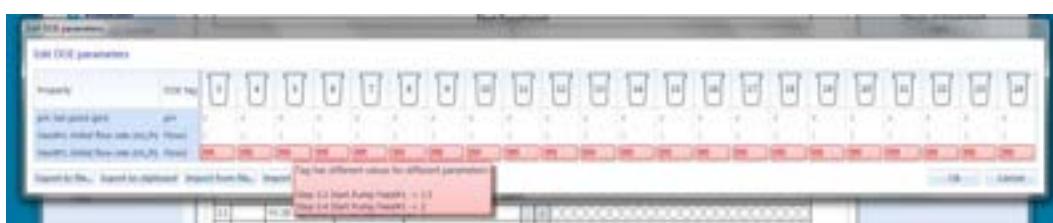


Figure 195 Design of Experiment Data window where parameter has conflicting values defined

The Design of Experiment Data window will also highlight if different parameters have been given the same tag.



Figure 196 **Design of Experiment Data** window where distinct parameters have been given the same tag. The DOE tags in an experiment can also be seen if **DOE tags** is selected in the View panel.

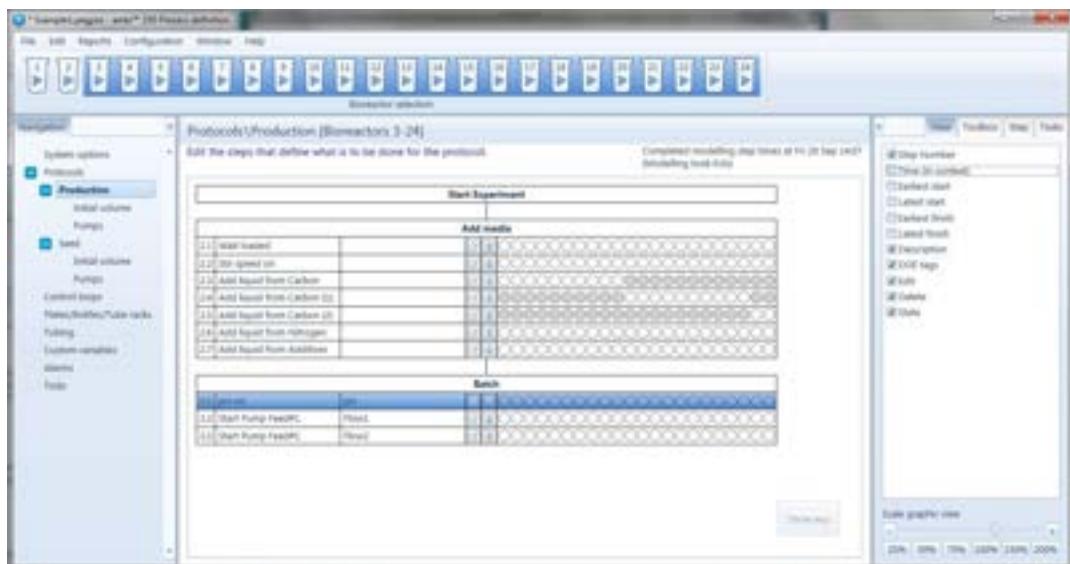


Figure 197 Steps view customised to display **DOE tags**.

4.5.5.10 Check Protocol

The **Check protocol** checks for issues in a protocol that indicate probable errors with the process definition.

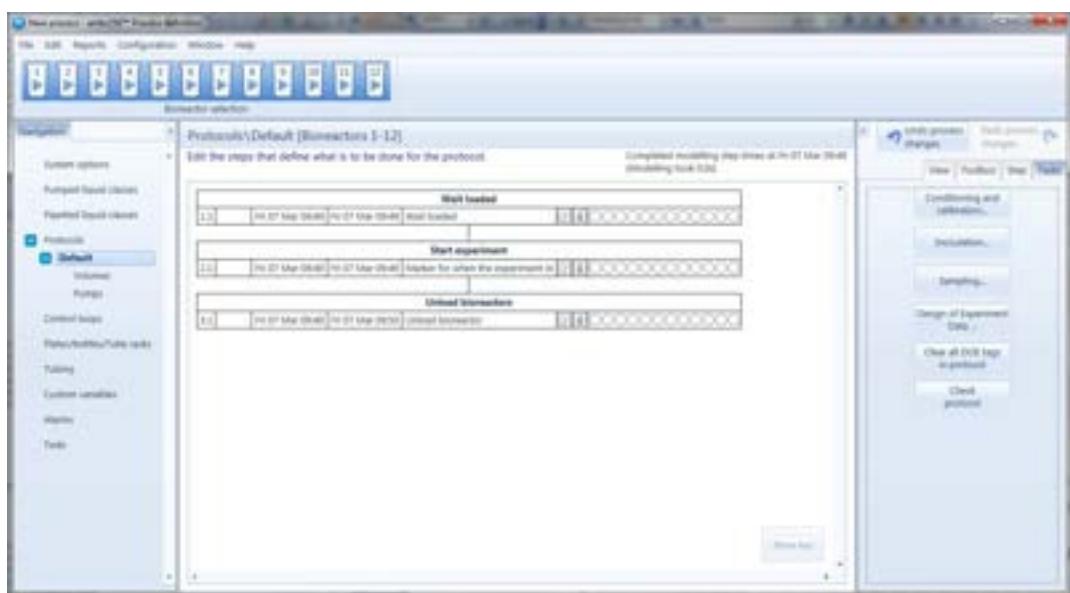


Figure 198 Tasks panel with **Check protocol** option

Selecting **Check protocol** displays a window with the issues found.

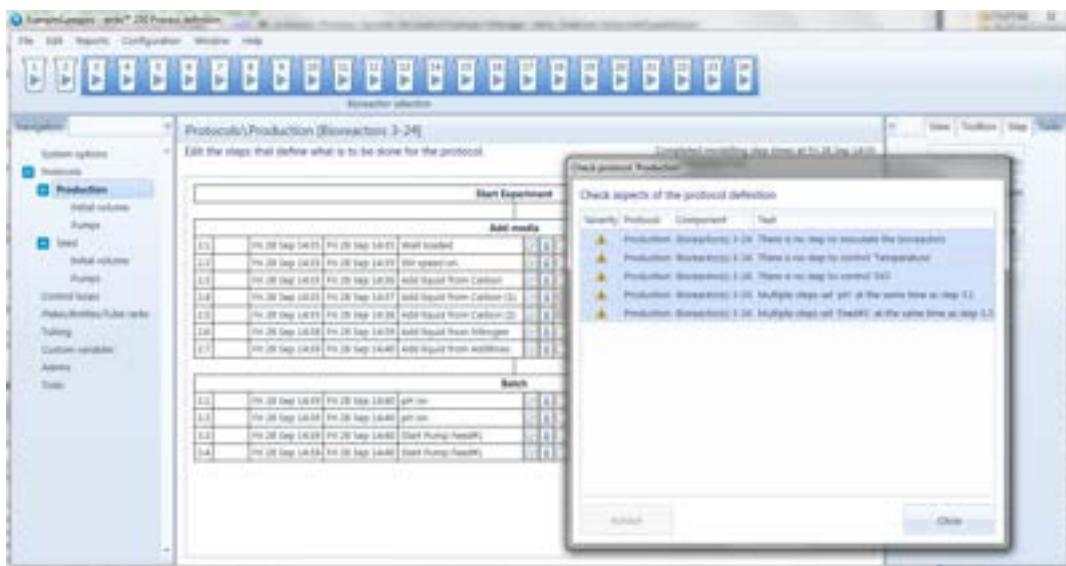


Figure 199 Results of **Check protocol**

Checks include:

- There should be a step that inoculates the bioreactors
- There should be steps to control DO, Temperature and pH
- Control loops set to **Run condition** should be enabled at some point in the process
- There should not be multiple steps that adjust the same set point or control the same pump without a delay between the steps. Even if the control is to ramp the set point to a new value the step completes immediately leaving the value ramping. Another set point at the same time will overwrite the first steps change which is probably not intended.
- Steps that are set to run at a particular time will plausibly wait until that time to run. The system checks if the time the step is set to run at will already have passed when the step is reached.

The **Refresh** button can be used to redo the checks after changes have been made to the process definition.

4.5.5.11 Print protocol

The steps can be printed by selecting the **Print** button in the **Tasks** tab.



Figure 200 Tasks menu

Selecting the Print button brings up the **Printing preferences** dialog

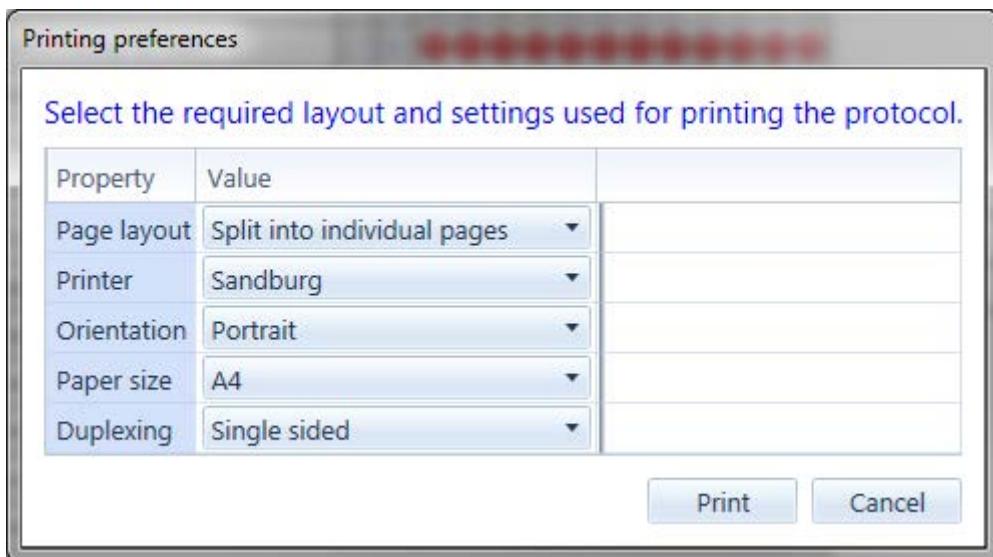


Figure 201 Printing preferences dialog

The **Printing preferences** page allows:

- The **Page layout** to be specified. The print can be **Split into individual pages** or **Scale to fit** onto a single page.
- The **Printer** to be chosen from the list of installed printers on the PC.
- The **Orientation** of the print to be either **Portrait** or **Landscape**.

- The **Paper size** to be chosen from a list of available paper sizes for the selected printer.
- The **Duplexing** to be specified as single or double sided.

Print prints the document.

Pressing **Cancel** on the progress dialog cancels the print.

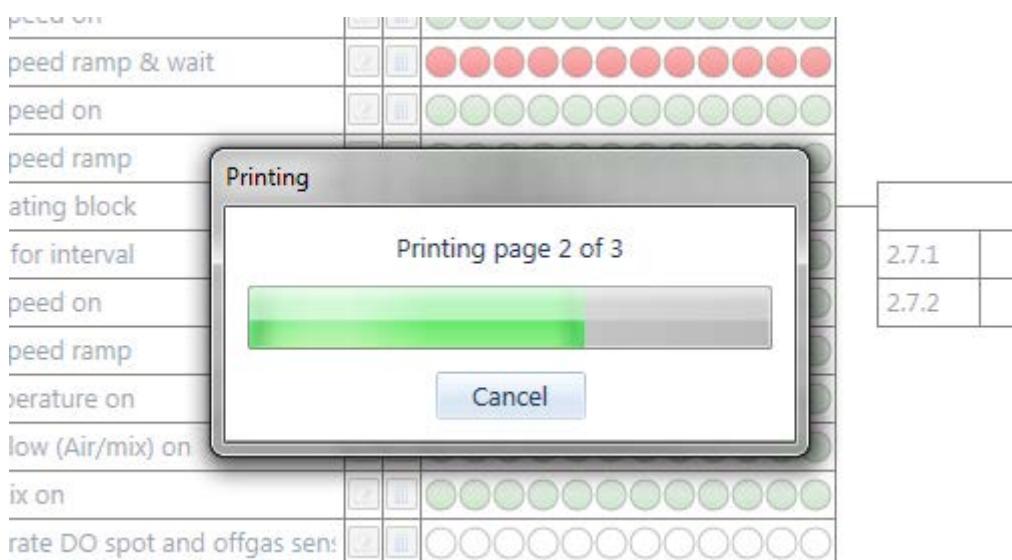


Figure 202 Printing progress dialog

4.5.5.12 Process step view

The process step view dialog provides a view of the labware used by a step.

The dialog is opened from the Tasks tab on the steps editing page.

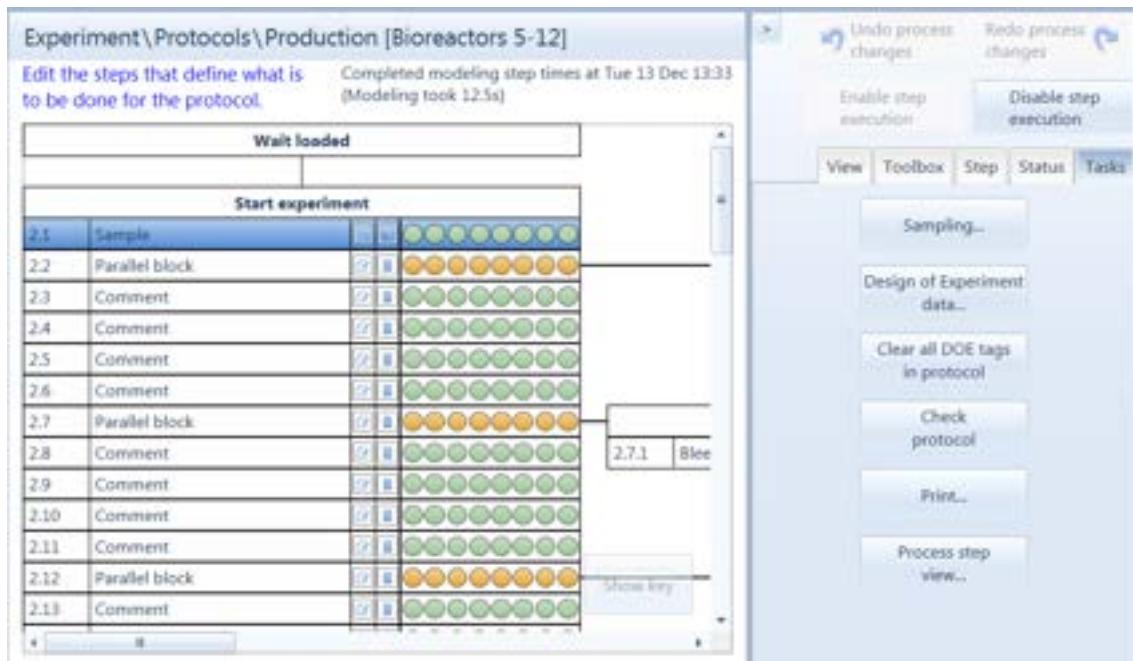


Figure 203 Process step view option

The dialog shows the labware used with related wells and bioreactors indicated by color. Hovering over wells and bioreactors provides more details of the transfers between them.

4.5.6 Options

The protocol Options page shows options defined at the protocol level.



Options page is shown only if the **Allow protocols to have different types of bioreactor** option is selected in the **Advanced features** window.

When the protocol **Options page** is shown the relevant options are offered on this page instead of on the **System options** page.

4.5.6.1 Vessel Type

Bioreactor vessel type selects the type of bioreactor vessel to be used with the system.

Selecting a vessel type that includes external condensing enables the use of external condensing when the system supports this.

Selecting a perfusion vessel type enables perfusion when the system supports this.

4.5.6.2 Perfusion filter priming

If the **Bioreactor vessel type** supports perfusion then the **Perfusion filter priming** options will be available.

Permeate volume specifies the volume of liquid to pump to prime the outside of the hollow filters. **Permeate flow rate** specified the rate at which to pump while priming.

4.5.6.3 Offgas

If the **Bioreactor vessel type** supports external condensing then the **Use external condenser** option will be available. This should be checked if the external condenser is to be used and not checked if the external condenser will be bypassed and internal condensing used instead.

4.5.7 Volumes

The **Volumes** page allows:

- Entry of the initial contents of the bioreactors.
- On systems with a liquid handler generating the steps that will fill the bioreactor with these contents.
- Entry of other key volumes for the bioreactors.

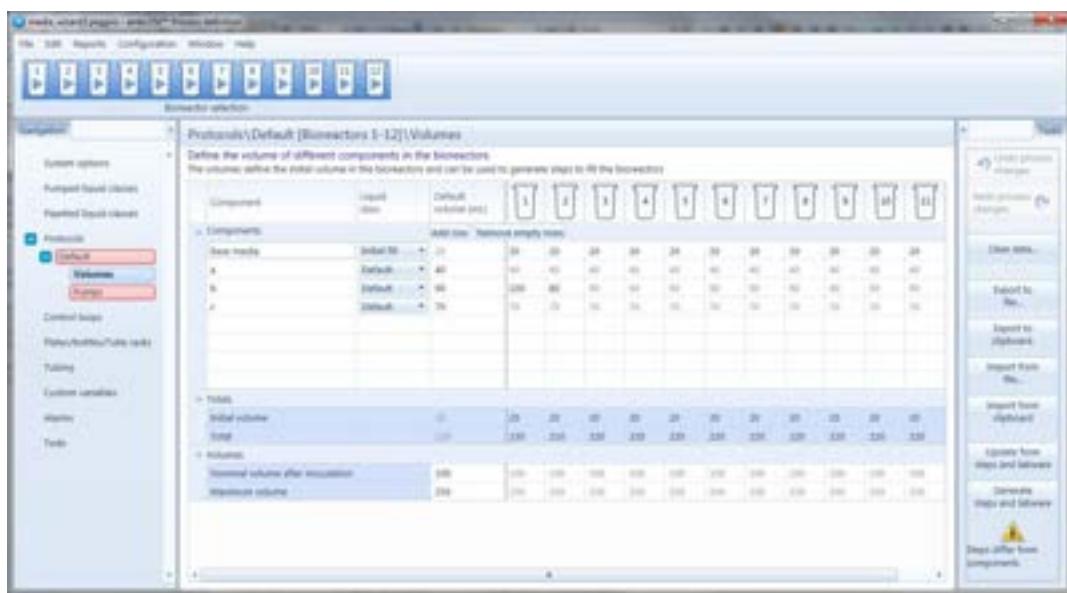


Figure 204 *Volumes* page

Under **Components** is a row for each component to be included in the bioreactor.

In the **Component** column is a description of the component.

On systems with a liquid handler the **Liquid class** column can be used to select between Initial fill for components assumed to have been placed in the bioreactor before it was loaded on the system and one of the liquid classes for components to be added by the liquid handler.

If the Ambr® 250 system is configured to have stirred location then the **Needs Stirring** column is displayed. Check this option if the component needs to be kept stirred. The system will choose stirred locations for the bottles containing the component.

In the **Default** volume column and the individual bioreactor columns are the volume of the component.

The system adds up the components to compute the **Initial volume** in the bioreactor when it is loaded onto the system and the **Total** volume after the liquid handler has added the additional

components. If the **Initial volume** is altered after the bioreactor has been loaded then the volume of liquid in the bioreactor will be adjusted accordingly.

Under **Volumes** some key volumes can be edited:

- **Nominal volume after inoculation** – the nominal volume of liquid to be in the bioreactor after it has just been inoculated.
 - May be used implicitly as a parameter in steps inoculating the bioreactor to a fixed volume by discarding excess liquid from the bioreactor.
 - May be used to normalise OUR and CER results



The Nominal volume after inoculation is only displayed if the **Allow normalising OUR/CER by reference to a nominal inoculation volume** option or the **Show option to discard media during inoculation to achieve the specified final volume** option is selected in the **Advanced features** window.

- **Maximum volume** – the volume of liquid allowed to be in the bioreactor before the system will disable pumping and refuse to add extra liquid to the bioreactor with the liquid handler. The limit before excess volume will cause issues depends on how vigorously the vessel will be stirred and gassed and so it is possible to change the maximum volume from its default value.

The **Export to file...**, **Export to clipboard**, **Import from file...** and **Import from clipboard** options export and import the contents of the window.

The system displays when the definition in this page differs from what the steps in the process definition do.

Press **Update from steps and labware** to update this page from what is defined in the steps. Update looks for an existing **Add media** phase and populates this page with the details of the media composition deduced from the steps in the phase. A warning is shown if there are any **Add liquid from bed** steps with options that the page cannot handle. These steps will be left untouched when new steps are generated.

On systems with a liquid handler press **Generate steps and labware** to generate the steps that will fill the bioreactor.

Generating the steps:

- creates the **Add media** phase if it does not already exist
- removes old **Add liquid from bed** steps from the phase
- adds the required new steps to the phase. If the component will not fit in a single bottle then multiple bottles will be used with one step for each bottle.
- creates or updates the bottles used by the steps
- deletes any bottles that were used by the old steps and are no longer required

4.5.8 Pumps

The Pumps page allows the role of the bioreactor pumps to be defined for the protocol.



Figure 205 Pumps page

For each pump to be used the Role for the pump must be selected. The Role is how pumps are selected in steps and in control loops. The Role also defines the allowed Refill rates.

The available **Roles** are defined by text files in the configuration which can be edited if required. For example if it is preferred to have a role called 'Substrate' instead of 'Feed' then that can be done.

The **Liquid class** specifies a **Pumped liquid class** with definitions for how fast the pump can pump through a bioreactor inlet filter and how fast the pump can be refilled.

The specific **Liquid name** to be used for a role can be entered and can be different for different bioreactors using the same role. In the example shown different feed liquids can to be used for different bioreactors.

Remove inlet filter allows the process designed to require that any pump on the bioreactor inlet filter is removed. This can be necessary if a bioreactor vessel with filters is being used with an antifoam where the filters remove the active components or a viscous liquid that needs to be pumped quickly. The operator is prompted to remove the bioreactor inlet filters when the bioreactor is loaded and connected.

The **Priming** option chooses how priming should occur. The options are:

- **Manual prime** - the bioreactor inlet must be manually primed so that the liquid is at the top of the bioreactor before automatic operation of the bioreactor is started
- **Auto prime** - the system will operate using an assumed pumped volume between connecting the bioreactor and liquid reaching the end of the tube inside the bioreactor.

Manual prime should be chosen if maximum accuracy is required when starting to pump the liquid into the bioreactor. Typically the assumed volume will be adequate and will require less effort priming.

Start pumping back from end by (mL) specifies an offset to apply when moving the liquid to the end of the bioreactor inlet at the start of pumping within a process. The exact position of the

liquid having nominally pumped it to the end of the bioreactor inlet will vary because of variations in how accurately the bioreactor is primed and because of variations in the construction of the bioreactor. Specify a larger value to reduce the frequency with which a little liquid is pumped into the process. Specify a negative value to reduce the frequency with which the liquid is not pumped far enough.

4.6 Control loops

The control loops used by the system to control DO and pH can be defined and edited and control loops can be defined to control other parameters.

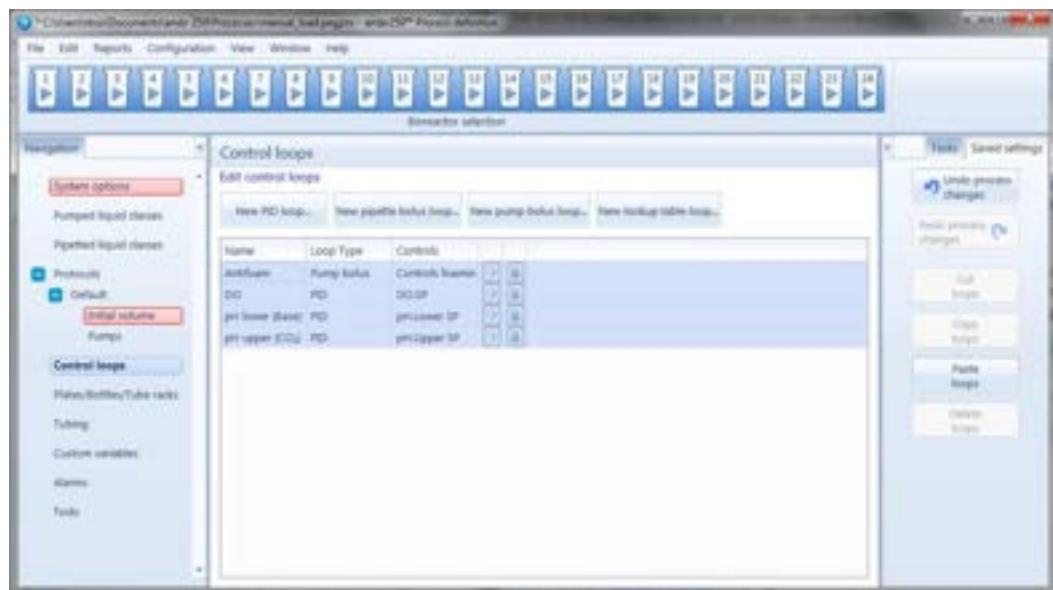


Figure 206 Control loops page

From the Control loops page you can:

- edit and delete existing control loops
- define new control loops
- copy control loops to paste into other processes

4.6.1 Creating a new control loop

To create a new control loop click on the option for creating the desired type of control loop:

- **New PID loop...** shows a window for creating a new PID loop with one or more levels of cascade
- **New pipette bolus loop...** shows a window for creating a new loop that pipettes a bolus when a condition is true
- **New pump bolus loop...** shows a window for creating a new loop that pumps a bolus when a condition is true
- **New lookup table loop...** shows a window for creating a loop that sets an output based on a lookup table

The window for creating a new control loop has the same general form for all of the different control loops.

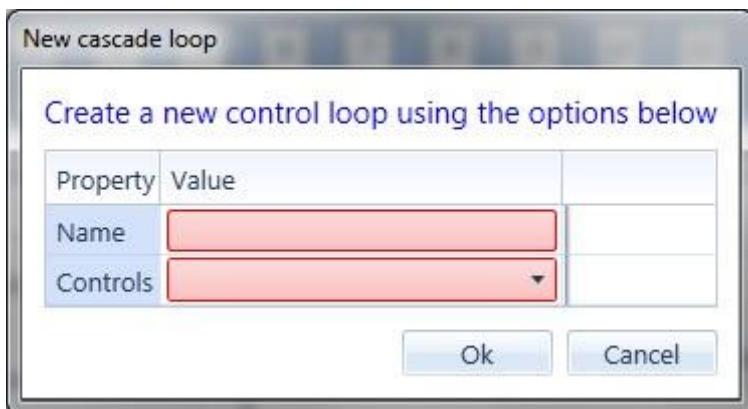


Figure 207 New cascade loop window

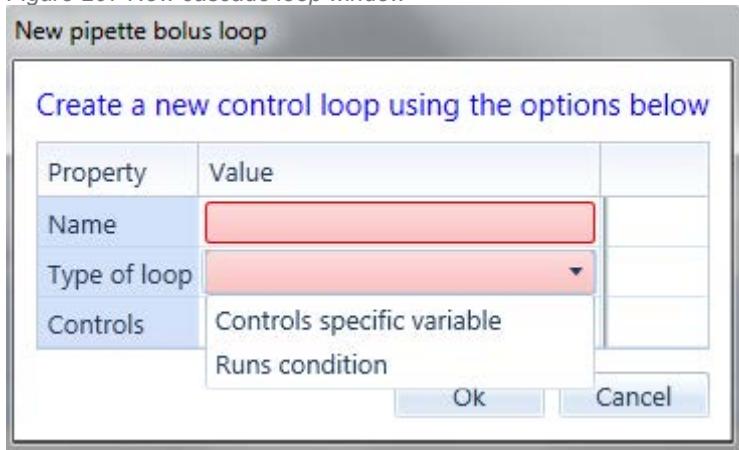


Figure 208 New bolus loop

The **Name** of the new loop should be entered

The bolus loops support two types of loop and so the **Type of loop** must be chosen:

- **Controls specific variable** is used for typical loops to control something to a set point
- **Runs condition** is used where there is no explicit set point to target

Other control loops always control a specific variable.

The variable controlled must be selected using the **Controls** parameter unless the **Runs condition** option has been selected.

Once the variable to be controlled has been selected or Runs condition has been selected then a choice of any defaults defined on the system will be presented. Use the **Defaults** option to choose the initial values to be used for the control loop.

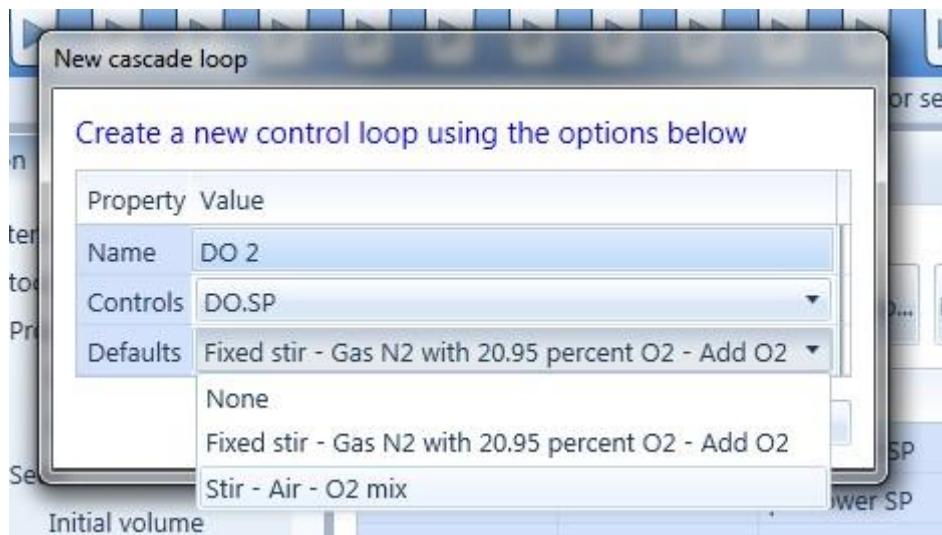


Figure 209 Choice of control loop **Defaults**

Pressing **Ok** will present the dialog to finish creating the control loop.

4.6.2 PID loop

The PID loop option provides a general PID loop with one or more levels of cascade.

There are two typical applications of the PID loop:

- pH control by pumping acid or base or by gassing with CO₂ using a single level of cascade and just a proportional term
- DO control by stirring and gassing using multiple levels of cascade with proportional and integral terms

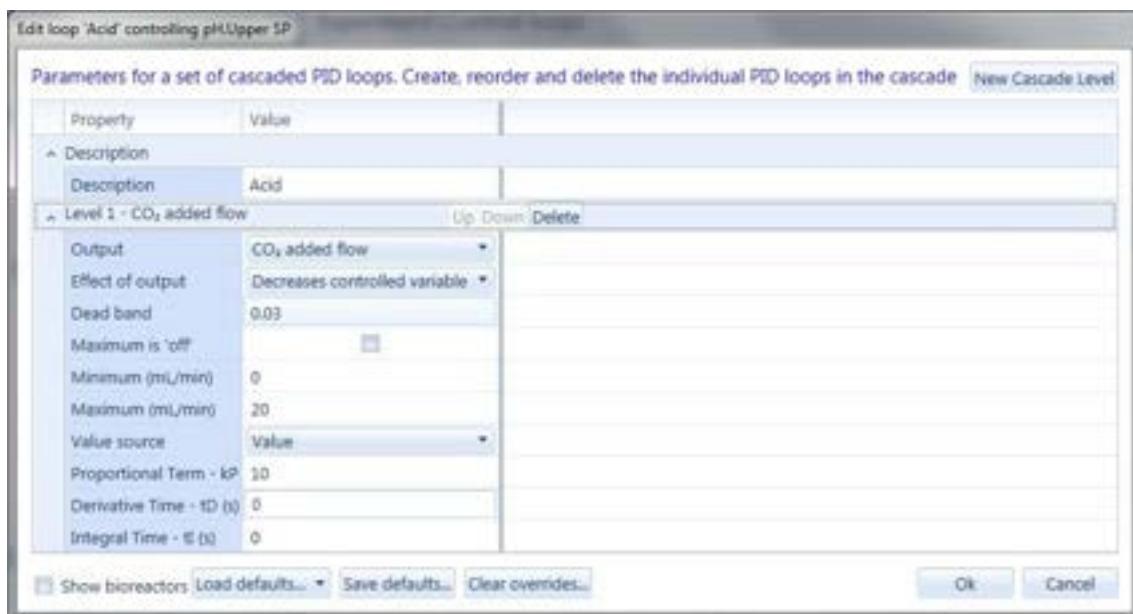


Figure 210 pH control loop using CO₂.

The **Description** field defines the name of the control loop.

For each level there are the options below:

Output defines the output of the level in the cascade.

Effect of output defines whether the output tends to decrease or increase the controlled variable. The sign of the deviation is chosen so that the proportional term is always positive. Adding CO₂ reduces the pH and so Decreases controlled variable has been chosen.

If the output **Increases controlled variable**:

$$\text{Deviation} = +(SP - PV)$$

When controlling DO if the present value is 5% and the set point is 20% then we should stir and gas more.

If the output **Decreases controlled variable**:

$$\text{Deviation} = -(SP - PV)$$

When controlling CO₂ if the present value is 7.1 and the set point is 7 the deviation is -(7-7.1) = 0.1 and we should pump CO₂

A **Dead band** can be defined for the first level when there is no integral term for that level. The output of the loop remains at its minimum value (typically zero) unless the error is larger than the dead band. If a dead band of 0.1 were chosen in this case that the gas flow would be zero unless the pH was more than 0.1 greater than the pH Upper set point. The dead band can be used to allow pH control with a shared upper and lower set point without the acid and base controls continually fighting one another. A dead band cannot be combined with an integral term.

Minimum is the minimum value that this level of the loop should set the output to.

Maximum is the maximum value that this level of the loop should set the output to.

Proportional term – kP is the coefficient of the proportional term in the loop

Derivative Time – tD is the scaling factor for the derivative term. Higher values of tD represent more derivative control

Integral Time – tI is the scaling factor for the integral term. Lower values of tI represent more integral control. Zero represents no integral control.

The output of the level can be represented as:

$$\text{Output} = kP(\text{Deviation} + tD \frac{d\text{Deviation}}{dt} + \frac{1}{tI} \int \text{Deviation} dt) + \text{OffValue}$$

The OffValue is the minimum value unless **Maximum is 'off'** has been chosen.

Note: changing kP changes the magnitude of the integral and derivative terms.

A common alternative formulation of a PID loop is:

$$\text{Output} = kP * \text{Deviation} + kD * \frac{d\text{Deviation}}{dt} + kI * \int \text{Deviation} dt$$

In this format:

$$kD = kP * tD$$

and

$$kI = kP/tI$$

If multiple levels are present in the cascade then when output by one level of the cascade reaches its minimum or maximum level control is passed to the next level of the cascade.

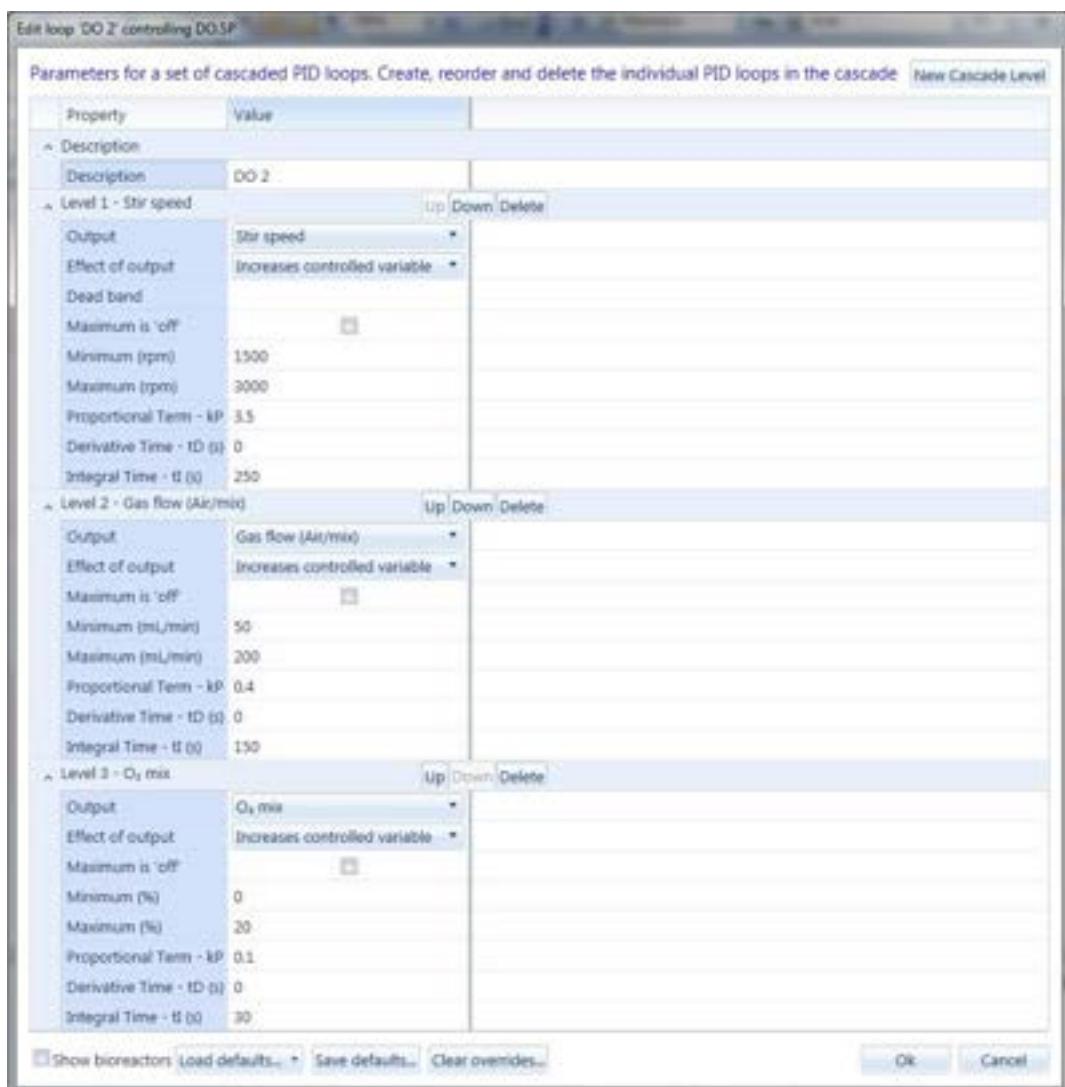


Figure 211 DO cascade with three levels

The figure above shows a cascade with 3 levels. When the loop is first enabled control will start at the bottom of the first level – the stir speed will be 1500 rpm, the gas flow 50 mL/min and the O₂ mix 0%. As the oxygen demand from the culture increases the stir rate will rise to 3000 rpm. The gas flow will rise then to 200 mL/min and then the O₂ mix will increase to 20%.

Levels can be added with the **New** cascade level button.

Levels can be moved up and down using the **Up** and **Down** buttons.

Levels can be deleted using the **Delete** button.

4.6.2.1 Advanced features – maximum output is ‘off’



Maximum is ‘off’ is supported if the **Support cascade level where the maximum output value is the default (no effect) value** option is enabled in the **Advanced features** window.

Maximum is ‘off’ should be selected if the maximum value of the output represents ‘off.’ If the output is a flow rate multiplier with a maximum of 1 and a minimum of 0 then the **Maximum is ‘off’** should be selected because 1 represents having no effect and 0 represents completely turning off the pump.

4.6.2.2 Advanced features – calculated PID terms

The values to be used in PID loops can be set to calculated values by changing the **Value source** option for the relevant level in the cascade from **Value** to **Expression**.



To display the **Value source** option select **Allow terms in PID loops to be specified as expressions** option in the **Advanced features** window.

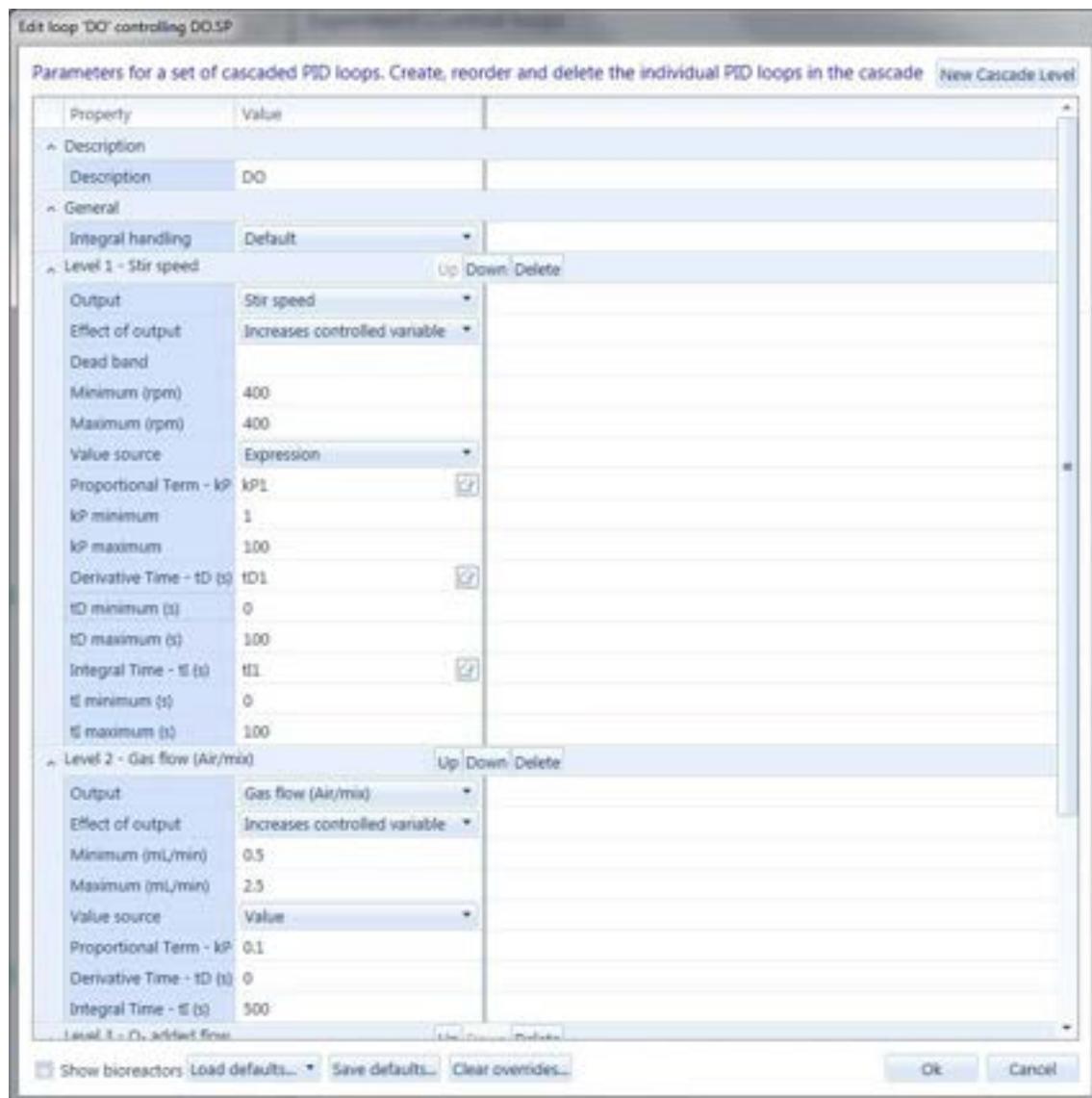


Figure 212 Control loop with calculated PID terms.

When the **Value source** is set to be **Expression** then the **Proportional Term – kP**, **Derivative Time – tD** and **Integral Time – tI** are all specified by expressions.

If the value of the expressions lies outside the range specified by **kP minimum ... kP maximum**, **tD minimum ... tD maximum**, **tI minimum ... tI maximum** then the value is truncated to the specified range.

4.6.3 Pipette bolus loop

The Pipette bolus loop requests the liquid handler to pipette a bolus when a specified condition is true.

The pipette bolus loop could be used to:

- control pH in a culture that occasionally needs some acid or base added

- control foaming using a mammalian antifoam that can be pipetted by the system



Figure 213 Pipette bolus loop

The **Description** field defines the name of the control loop.

4.6.3.1 Controls options

If the loop has been defined as Runs condition then the **Description of what this loop does** option allows a description of what the loop does to be entered.

4.6.3.2 Conditions options

Number of conditions specifies how many conditions to use.

4.6.3.3 Condition options

One or more **Condition** sections define when a bolus is wanted. When the condition is true – e.g. in this example when the Deviation has been greater than 0.01 continuously for 5 minutes.

See section 3.6.2 above for all the options that conditions support.

4.6.3.4 Bolus options

The Bolus options define how large the bolus should be and how long to leave after delivering one bolus before allowing the next bolus to be delivered.

Effect of bolus defines whether a bolus tends to decrease or increase the controlled variable. The sign of the deviation is chosen so that the **Deviation** is a positive number when a bolus would be helpful.

The **Bolus volume** may be a constant or may be a general expression. The expression may refer to the **Deviation**.

The **Dead band** optionally specifies a dead band such that no bolus is delivered unless the **Deviation** is greater than the **Dead band**.

If the bolus is specified by an expression then the **Minimum bolus** and **Maximum bolus** options specify limits on the size of the bolus. If the required bolus is smaller than the **Minimum bolus** then no bolus will be delivered. If the required bolus is greater than the **Maximum bolus** then the size of the bolus will be capped at the **Maximum bolus**.

Maximum total volume optionally specifies a maximum volume that the control loop should deliver during the course of an experiment. When this maximum volume has been delivered the system will raise an error message and the control loop will not deliver any more boluses.

The **Dead time** specifies an interval to wait after delivering one bolus before delivering the next bolus. Unlike the Pump bolus loop only boluses from this loop are taken into account for the purpose of considering the **Dead time** e.g. using a process step to add liquid will NOT trigger the dead time on the control loop.

The **Condition** and **Bolus** options combine to deliver a bolus when:

The whole **Condition** is true – that is the underlying comparison has been true continuously over the **Dead time**

and the bolus volume is greater than or equal to the **Minimum bolus**

and the **Deviation** is greater than or equal to the **Dead band**

and at least the **Dead time** has elapsed since the last bolus was delivered

4.6.3.5 Estimates options

The **Estimates** options provide information on how many tips the control loop will use for each bioreactor. The system will prompt the user to load tips based on the values of these options.

Tips per day specifies how many tips are expected to be used each day on average.

Number of tips to maintain specifies a minimum number of tips to have loaded on the system for this control loop for each bioreactor.

4.6.3.6 Liquid handling options

The Liquid handling options provide the standard options for the liquid handler.

4.6.3.7 Labware options

The Labware options provide the standard options for selecting the labware to use. The labware must have the role of **Liquid source** or **Multiple uses**.

4.6.4 Pump bolus loop

The Pump bolus loop requests the pump to deliver a bolus when a specified condition is true.

The pump bolus loop could be used to:

- control DO in a culture by adding a bolus of feed each time the DO rises
- control foaming using an antifoam that can be pumped by the system

Edit loop 'Antifoam'

Edit parameters for control loop

Property	Value
Description	
Description	Antifoam
Pump	
Pump	Antifoam
Flow rate (mL/h)	
Controls	
Description of what loop does	Controls foaming
Condition	
Expression	'Foam sensor'
Comparison	Greater than
Compare against	Value
Value	200
Delay time	
Bolus	
Bolus volume (mL)	0.1
Dead time	10m

Show bioreactors

Figure 214 Pump bolus loop

The **Description** field defines the name of the control loop.

4.6.4.1 Pump options

The pump specification specifies the **Pump** used to deliver the bolus and optionally the **Flow rate** at which to deliver the bolus. If the **Flow Rate** is not specified then the maximum flow rate allowed for the pump will be used.

4.6.4.2 Controls options

The Pump bolus loop accepts the same **Controls** options as the Pipette bolus loop.

4.6.4.3 Conditions options

The Pump bolus loop accepts the same **Conditions** options as the Pipette bolus loop.

4.6.4.4 Condition options

The Pump bolus loop accepts the same **Condition** options as the Pipette bolus loop.

See section 3.6.2 above for all the options that conditions support.

4.6.4.5 Bolus options

The Pump bolus loop accepts the same **Bolus** options as the Pipette bolus loop.

Note however that all boluses are taken into account for the purpose of considering the **Dead time** and not just those initiated by the control loop e.g. using a process step to pump a one-off bolus will also stop the loop triggering for a period equal to the dead time.

4.6.5 Lookup table loop

The Lookup table loop sets the value of its output based on a lookup table from the value or deviation of what it is controlling.



Creating **Lookup table** loops is available as an option if **Show options to create lookup table control loops** option is enabled in the **Advanced features** window.

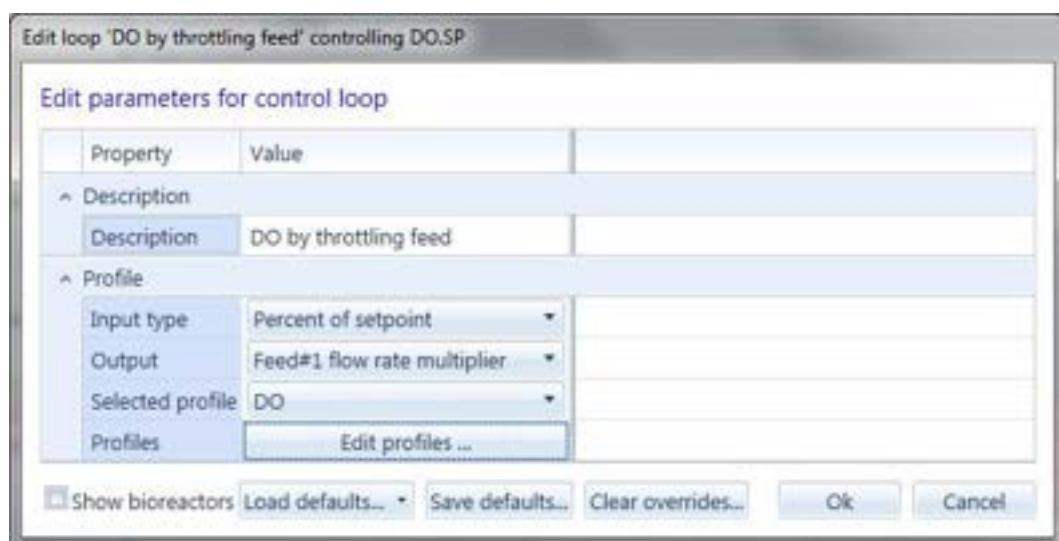


Figure 215 Lookup table loop

The **Lookup table** loop has the following options.

Description gives a name to the loop

Input type selects that the lookup table is based on:

- **Percent of setpoint** – the present value of the controlled variable as a percentage of its set point
- **Deviation** – the difference between the present value and the set point
- **Present value – ignore set point** – the present value itself

When **Deviation** is used then **Effect of output** defines whether the output tends to decrease or increase the controlled variable. The sign of the deviation is chosen so that the proportional term is always positive. Adding CO₂ reduces the pH and so Decreases controlled variable has been chosen.

If the output **Increases controlled variable**:

$$\text{Deviation} = +(SP - PV)$$

When controlling DO if the present value is 5% and the set point is 20% then we should stir and gas more.

If the output **Decreases controlled variable**:

$$\text{Deviation} = -(SP - PV)$$

When controlling CO₂ if the present value is 7.1 and the set point is 7 the deviation is $-(7-7.1) = 0.1$ and we should pump CO₂

Selected profile selects the profile to be used from the profiles that can be edited via the **Profiles** option.



Figure 216 Edit profile for lookup table loop

Pressing the **Edit profiles...** option brings up the standard profile editor window shown above.

In this case if the DO is 100% or more of the DO set point then the flow multiplier is 1.

As the DO reduces to 50% of its set point then the flow multiplier is progressively reduced to 0.75.

Between 50% and 25% of its set point the flow multiplier is progressively reduced to 0.5.

Finally between 25% and 0% of its set point the flow multiplier is progressively reduced to 0.

4.6.6 Overrides for Different Bioreactors

Different bioreactors can have different values of control loop options.

To show or hide these different values select **Show bioreactors**.

Clear all overrides... clears the overrides for all of the bioreactors.

4.6.7 Defaults

Ambr® 250 supports built in and user defined defaults for control loops.

A default set of parameters can be used when creating a new control loop.

Having created a control loop the loop can be reset to use default values or the settings saved as defaults.

The **Load defaults...** option presents a list of applicable defaults. To compare a loop against some defaults and if required reset the loop to use the defaults then select the appropriate default from this option.

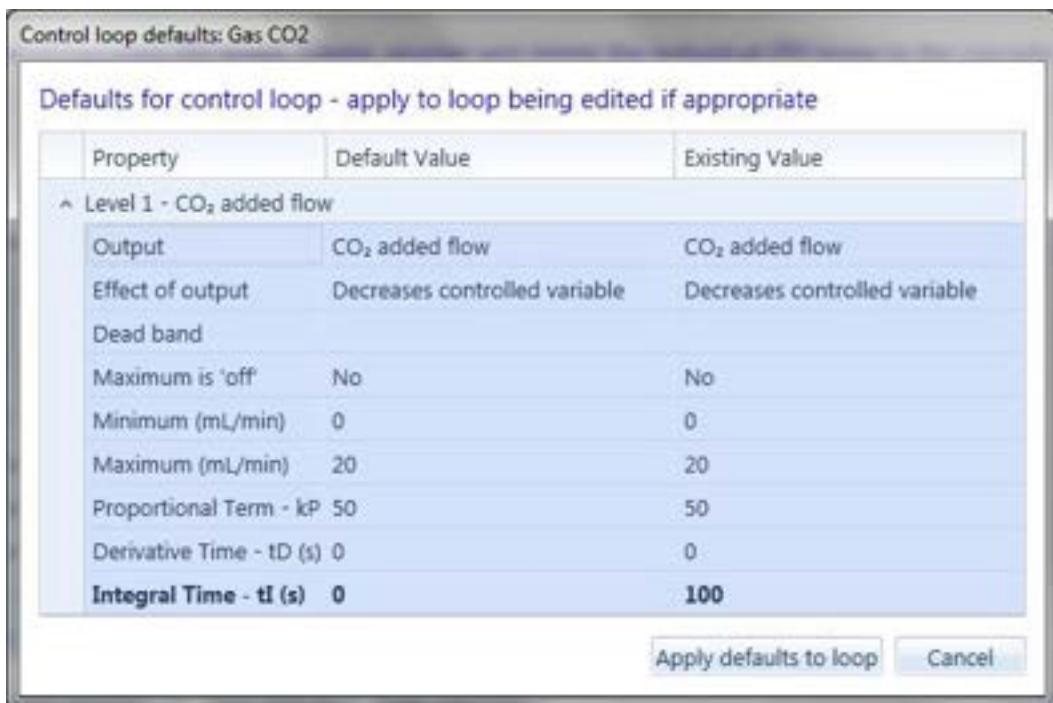


Figure 217 Defaults window

The defaults window shows in bold which default values differ from the existing values.

Selecting **Apply defaults to loop** will update the loop being edited to use the default values.

Save defaults... presents a window to save the current settings of the loop as defaults.

4.7 Perfusion control loops

The following additional control loops are specific to perfusion systems.

4.7.1 Perfusion VVD loop

The Perfusion VVD loop controls the permeate flow rate and feed rate to maintain a constant or specified volume.

Edit loop 'Volume' controlling VVD.SP

Edit parameters for control loop

Property	Value
^ Description	
Description	Volume
^ Inflow	
Pump	Feed#1
^ Volume high	
When volume greater than setpoint	Reduce inflow
Control band (ml)	5.0
Minimum flow rate (%)	50.0
^ Volume low	
When volume less than setpoint	Reduce permeate
Control band (ml)	5.0
Minimum flow rate (%)	50.0

Show DOE tags
 Show bioreactors

Figure 218 Perfusion VVD loop

To turn the loop on set the desired VVD (volume per volume per day) value desired.

If no volume set point has been specified then the loop will request equal inflow and outflow rates so that a constant volume will be maintained in the absence of an issue that affects the inflow and outflow rates differently. If a volume set point has been specified then the loop will adjust the inflow and outflow rates to achieve the requested volume. The volume set point can be set by a control loop or explicitly via a **Set volume SP** step.

When a perfusion VVD loop is active then the pumps involved (the pump selected for the inflow and the permeate pump) are interlocked such that events that stop one pump also stop the other pump. Such events include low level pauses at the firmware level as well as when a pump is stopped to allow a media bag or permeate bag to be changed.

The **Inflow** option **Pump** sets the pump that will be used for the inflow.

The **Volume high** and **Volume low** options describe how the flow rates should be adjusted away from the nominal VVD in order to adjust the volume to the set point.

When volume greater than setpoint allows choosing to **Reduce inflow** or **Increase permeate**.

When volume less than setpoint allows choosing to **Increase inflow** or **Reduce permeate**.

Control band specified the range of volume error over which the adjustment will scale up from zero to its full value.

When a flow rate is being reduced then **Minimum flow rate** specifies the percentage of the nominal flow rate that will be used once the error is greater than the size of the **Control band**.

When a flow rate is being increased then **Maximum flow rate** specifies the percentage of the nominal flow rate that will be used once the error is greater than the size of the **Control band**.

4.7.2 Perfusion cell density loop

The perfusion cell density control loop controls the target volume in the bioreactor and bleeding in order to maintain a target cell density.

Edit loop 'Bleed and volume control' controlling Perfusion target cell density.SP

Edit parameters for control loop

Property	Value
Description	
Description	Bleed and volume control
Strategy	
Bleed method	Bleed
Cell density	
Measured cell density	Cell density
Growth rate	Growth rate (user)
Maximum volume	
Value type	Value
Maximum volume (mL)	220.0
Bleed trigger volume	
Value type	Value
Bleed trigger volume (mL)	210.0
Bleed volume	
Value type	Value
Bleed volume (mL)	8.0
Minimum volume	
Value type	Value
Minimum volume (mL)	200.0

Show DOE tags Show bioreactors

The **Bleed method** can be one of:

- **No automatic bleeds** – the loop will only control the target volume. Steps in the process should be used to control bleeding.
- **Bleed** – the loop will initiate bleeds as required to maintain the cell density.

- **Bleed to level** – the loop will use bleed to level operations to maintain the cell density.

The **Measured cell density** and the **Growth rate** options define the variables that have data for the cell density measurements and an estimate of the growth rate of the cells. The loop uses this data to calculate the present value of the **Perfusion target cell density** variable.

The Maximum volume, Bleed trigger volume, Bleed volume and Minimum volume set the limits on the target volume that will be set and when bleeds will occur.

The loop will calculate the volume in the bioreactor that would make the **Perfusion target cell density** match the value of **Perfusion target cell density.SP** that has been set. If that target volume is greater than the maximum volume then the loop will target the maximum volume. If that target volume is less than the minimum volume then the loop will target the minimum volume.

The loop will initiate a bleed of the specified **Bleed volume** when the volume in the bioreactor is greater than the **Bleed trigger volume** and the **Perfusion target cell density** is greater than the **Perfusion target cell density.SP**.

The normal operation of the loop is shown below with the volume of the bioreactor varying between the bleed trigger volume and the bleed trigger volume less the bleed volume.

Assuming that the estimate cell growth rate is accurate culture will be performed at a constant cell density.

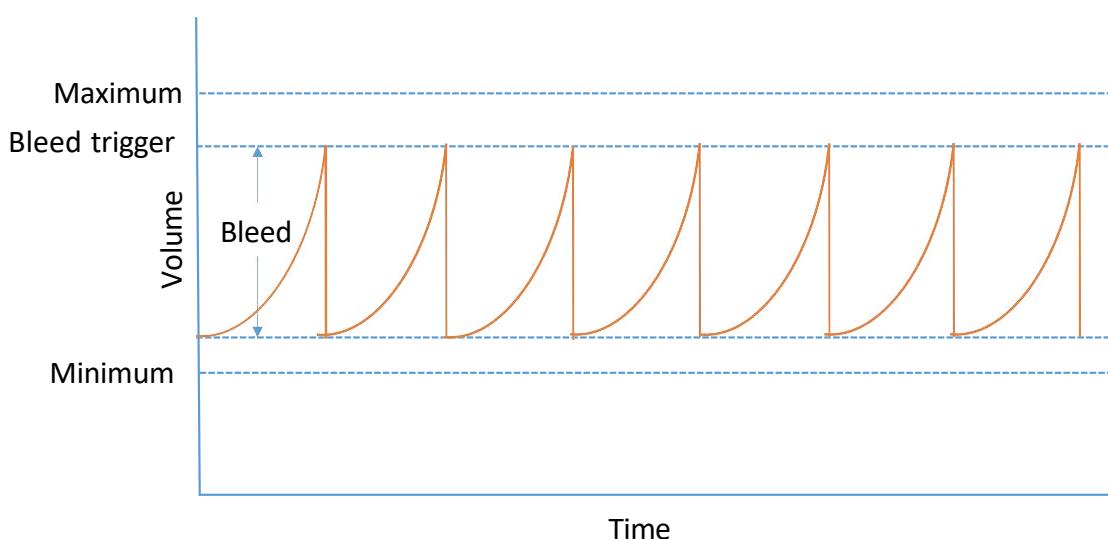


Figure 219 Volume of culture

Control with the perfusion cell density loop is enabled by the **Set target cell density** step. Before enabling control with a perfusion cell density loop volume control must first be enabled via a **Set perfusion VVD** step.

Edit step - 'Perfusion target cell density on'

Edit step parameters

Turn set-point on/off and set the target value or profile for 'Perfusion target cell density': Target for cell density in the culture

Property	Value
Description	
Description	Perfusion target cell density on
Which reactors	
All or selected	All bioreactors assigned to protocol
When	
When to do step	Do after preceding step
Creation time	Tue 20 Aug 13:12
Change	
Option	Set
Set point (10^6 cells/ml)	110
Control loops	
Control loop	Bleed and volume control
How to initialise loop	Reset loop on first use
<input type="checkbox"/> Show DOE tags	
<input type="checkbox"/> Show bioreactors	
<input type="button" value="Ok"/> <input type="button" value="Cancel"/>	

Figure 220 Step to enable cell density control

4.8 Plates/Bottles/Tube racks

The **Plates/Bottles/Tube racks** page shows and allows editing of the plates, bottles and tube racks defined for the process.

Name	Type	Location	Role
Carbon source	3L BOTTLE	Bottle 9	Liquid source
Carbon source (1)	3L BOTTLE	Bottle 8	Liquid source
Carbon source (2)	3L BOTTLE	Bottle 7	Liquid source
Nitrogen	3L BOTTLE	Bottle 6	Liquid source
Sample 1 - 25	10ML CENTRIFUGE TUBE, Chilled 4		Sample

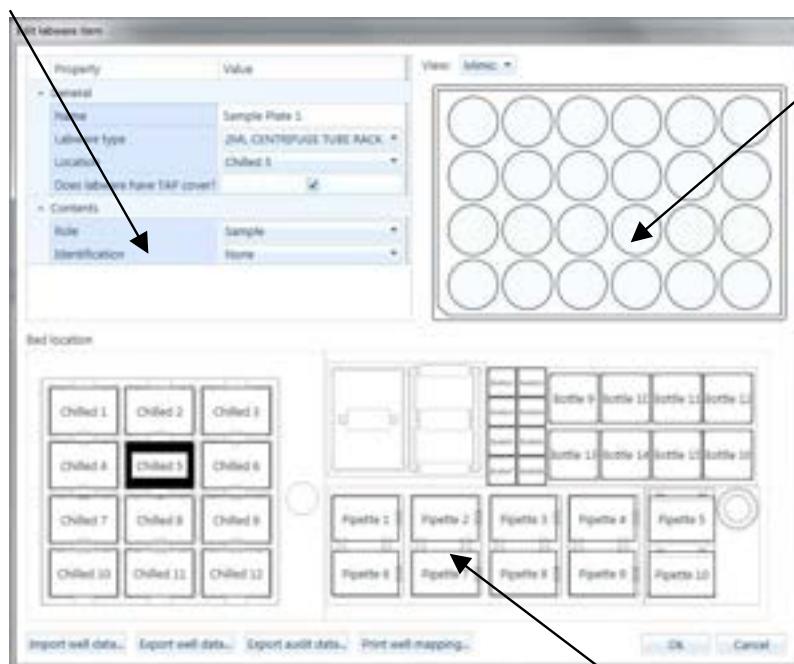
Figure 221 Plate/Bottles/Tube racks page

For each item the page shows the **Name** of the item, the **Type** of the item, the **Location** of the item and the **Role** of the item.

Selecting **New item...** or editing an existing item displays the **Edit labware item** window.

Options

Item view



Bed view

Figure 222 **Edit labware item** window

The Edit labware item window shows three things: the options for the labware item; a graphical item view of the item or its contents; and a view of the bed with an indication of the location of the item.

The options are described below:

Name sets the name by which the plate/bottle or tube rack is known.

Labware type defines what sort of item this is.

Location defines where the item will be loaded. The **Location** can be edited directly or by clicking on locations in the bed view.

Does labware have TAP cover defines whether the item will have a removable cover.

For a bottle the TAP cover is a cap that can be placed on top of the bottle and lifted off the bottle by the liquid handler.

For a plate or tube rack the TAP cover is a lid that rests on top of the plate or rack.

Plates or tube racks stored in frozen or freezable locations do not have a TAP cover. The freezable locations have their own cover and the system will automatically pick up the covers as required.

The **Role** indicates how the item will be used. The roles are:

- **Liquid source** – item is used to provide liquid
- **Sample** – item is used as a destination for samples
- **Waste** – item is used for waste liquid to be disposed of
- **Inocula** – item is used as a source of inocula
- **Multiple uses** – item may be used for multiple purposes. The item is made available for all uses.

When the **Role** is **Liquid source** or **Inocula** then the **Initial available volume per well** and **Initial fill volume per well** options are required to indicate the volume in the bottle, or in each well of a plate or each tube in a tube rack. The **available** and **fill** volumes are related by the **Dead volume** and each is automatically calculated from the other. This volume can be overridden for different wells within a plate using the **Initial available volumes** and **Initial available volumes** views. (The Dead volume can be edited from the **Liquid handler\Maintenance** page as described in section 11.5 below.)

In the runtime software the **Current available volume** and **Current volume** are also displayed.

If the labware is a bottle and the **Role** is **Liquid source** then the **Liquid** option allows the contents of the bottle to be entered.

Identification indicates if the item has any barcodes or lot numbers associated with it. Options are:

- **None**
- **Lot id**
- **Lot id and barcode**
- **Plate/rack barcode**
- **Rack and tube barcodes**
- **Tube barcodes only**

Options to accept the identification will be available depending on the option chosen. These options do not need to be completed until the item is loaded onto the system at runtime.

Lot id will accept a lot id

Barcode will accept a barcode for the item as a whole

For plates and tube racks the liquid in each well can be seen in the Graphical View described below. **Import well data...** and **Export well data...** allow the barcode and liquid for each well to be loaded from an external file or saved to an external file.

In the runtime and experiment viewer applications **Export audit data...** exports a record of the aspirates and dispenses to and from the labware.

For sample plates or tube racks **Print well mapping...** will display a printable report of which bioreactors have placed samples into which well.

4.8.1 Graphical View

The Graphical View shows either a mimic of the labware type, or for labware types with multiple wells or tubes, a grid of the Barcodes, the Liquid or the Initial volume in each well or tube.

4.8.1.1 Mimic

If the labware type is a bottle then a mimic is shown with a view of the vessel type.

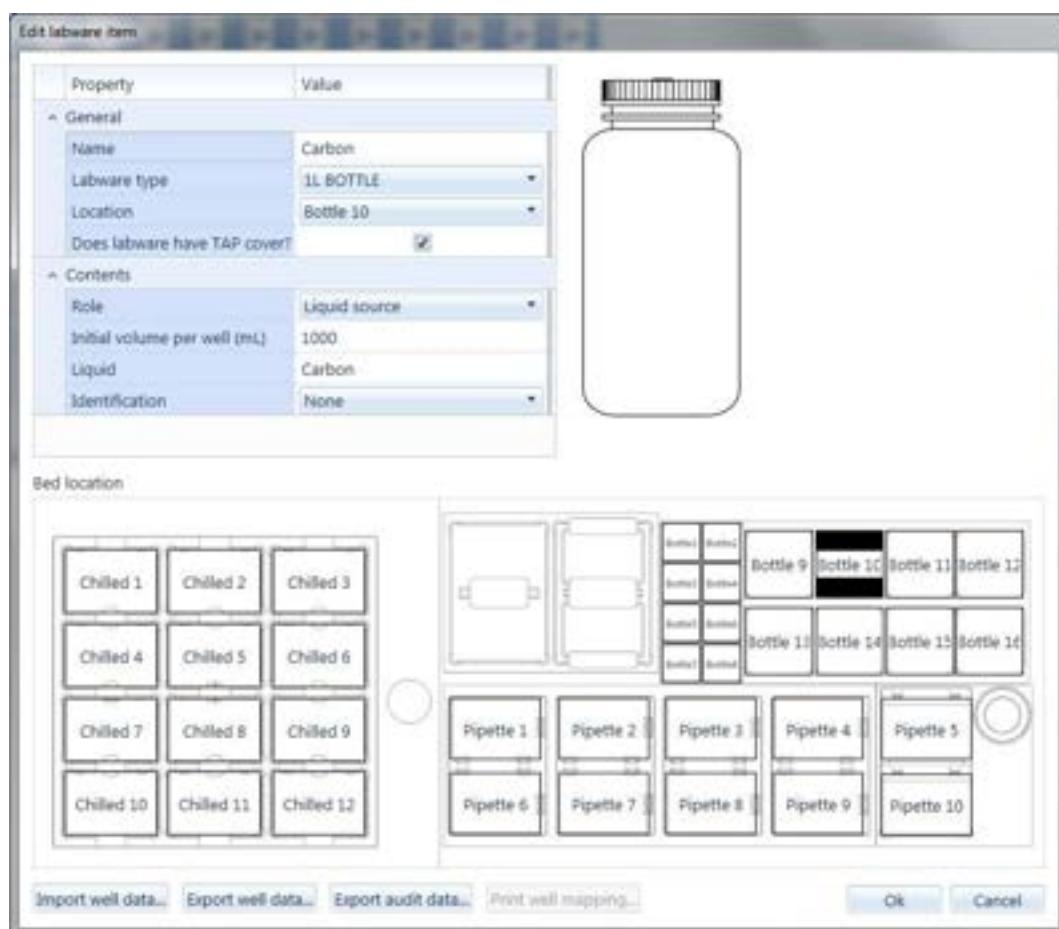


Figure 223 Mimic with view of a bottle

If the labware is a plate or a tube rack then **View:** offers the option to view **Mimic, Barcodes, Liquids or Initial volumes**

If **Mimic** is chosen then a mimic of the labware is displayed.

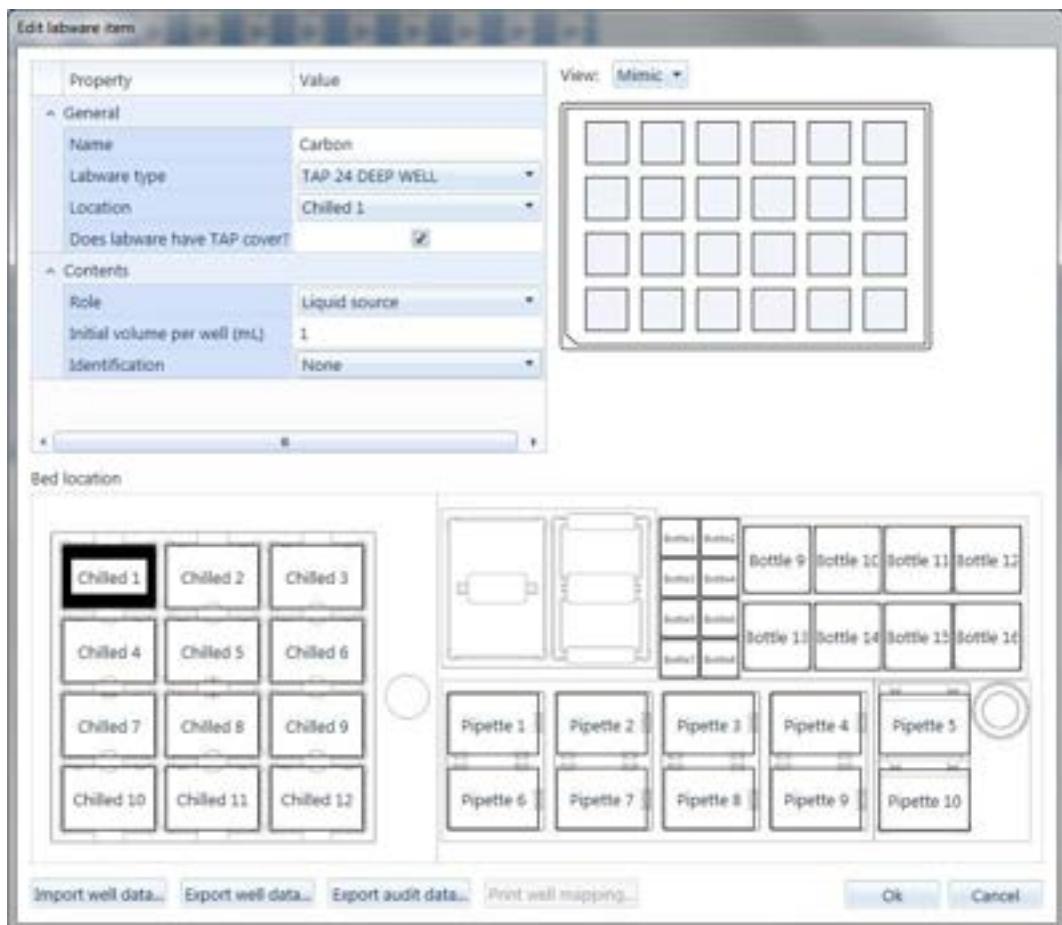


Figure 224 Mimic with view of a plate

4.8.1.2 Liquids

Choosing Liquids for the View: option allows the liquid in each well of a plate or tube rack to be entered if required. This is optional, but can be helpful for keeping track of how wells in a plate or tube rack are being used.

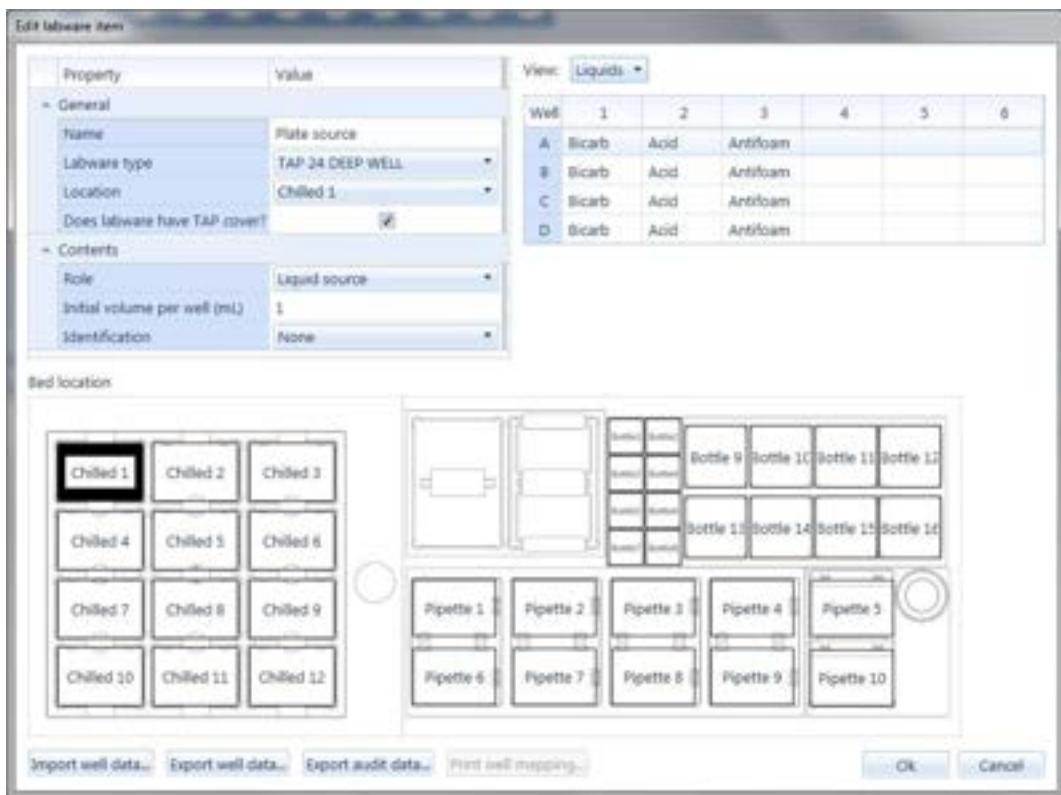


Figure 225 Plate with details of the liquid in different wells

4.8.1.3 Barcodes

Tube barcodes are edited in the graphical view of the item when **Barcodes** is selected for the **View:** option.

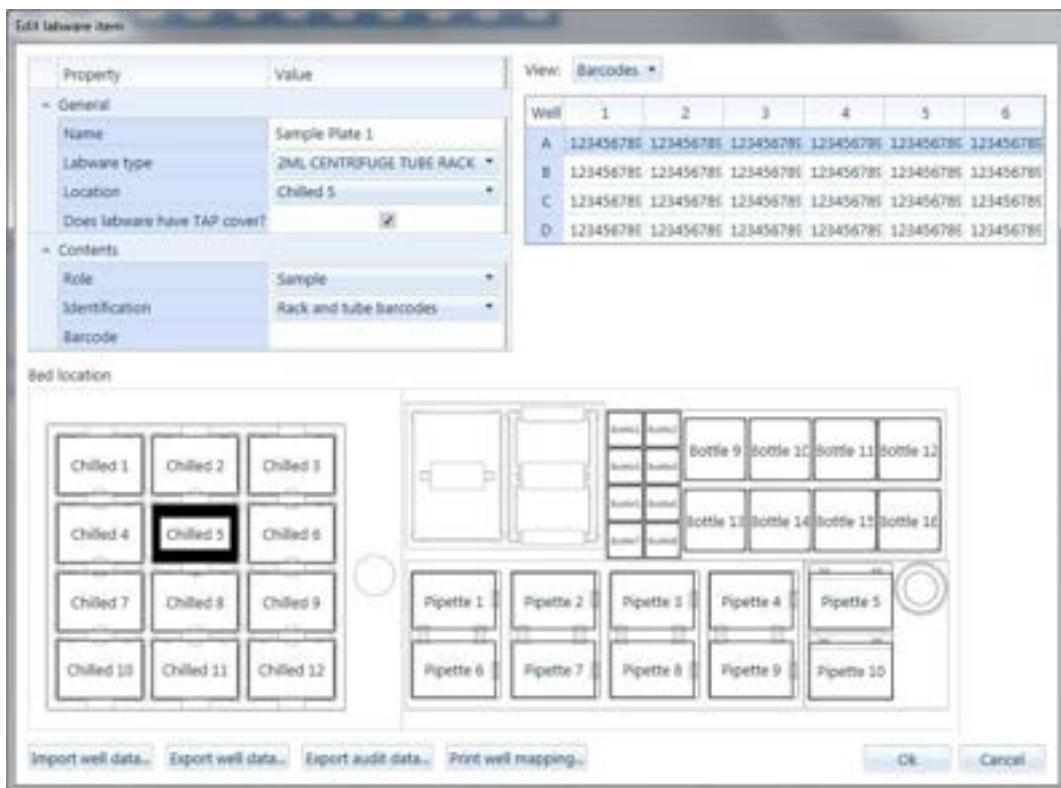


Figure 226 **Edit labware item** window showing tube barcodes

To enter tube barcodes:

- click into the cell for the barcode to be entered
- scan the barcode of the appropriate tube with a keyboard wedge barcode reader (not supplied)
- when the barcode reader sends the return character the window is set up to edit the next cell

4.8.1.4 Initial volumes

If required the initial volume in each well can be set individually. If the volume is not set then the one volume specified as **Initial volume per well** is used for all the wells.



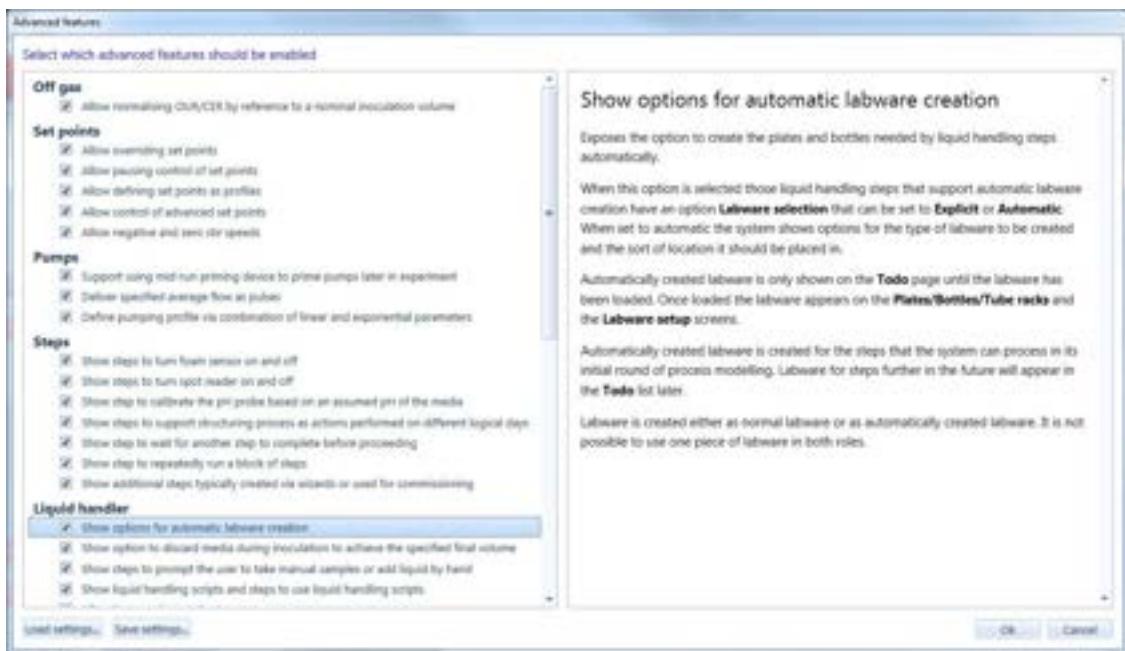
Figure 227 **Edit labware item** window showing initial volumes

4.8.2 Automatic labware creation

Certain liquid handling steps can be defined to have labware created for them automatically. This allows the liquid to be transferred to be a DOE parameter and can simplify sampling scenarios.



The feature is enabled by selecting the **Show options for automatic labware creation** option in the **Advanced features** window.



When the feature is enabled the labware selections where the feature is supported have an option of **Labware selection** which can be **Explicit** or **Automatic**.

When **Automatic** is selected options are shown for the **Labware type**, the **Sort of labware location** and any parameters the step needs to specify the contents of the Labware.

Add step - 'Add liquid from MixA, MixB'

Edit step parameters
Add liquid to the bioreactor from a plate/bottle/tube rack on the bed.

Property	DOE tag	Value										
- Description												
Description			Add liquid from MixA, MixB									
- Which reactors			All bioreactors assigned to protocol									
- All or selected			All bioreactors assigned to protocol									
- When												
When to do step			Do after preceding step									
Creation time			Wed 14 Feb 13:27									
- Error handling												
If the step fails:			Stop									
Maximum time to wait			Never									
- Liquid handling												
Priority			50									
Liquid class			Default									
Selected delivery specification			MEDIA; MEDSA; V >= 0.25									
Reuse tips			<input type="checkbox"/>									
- Source												
Volume type			Specified volume									
Volume (mL)			100									
Labware selection			Automatic									
Labware type			1L BOTTLE									
Sort of labware location			Bottle (Stirred)									
Liquid			Additive									
			MixA	MixB	MixA	MixB	MixA	MixB	MixA	MixB		
<input checked="" type="checkbox"/> Show DOE tags <input checked="" type="checkbox"/> Show bioreactors												
										OK	Cancel	

Figure 228 Add liquid step using automatic labware creation

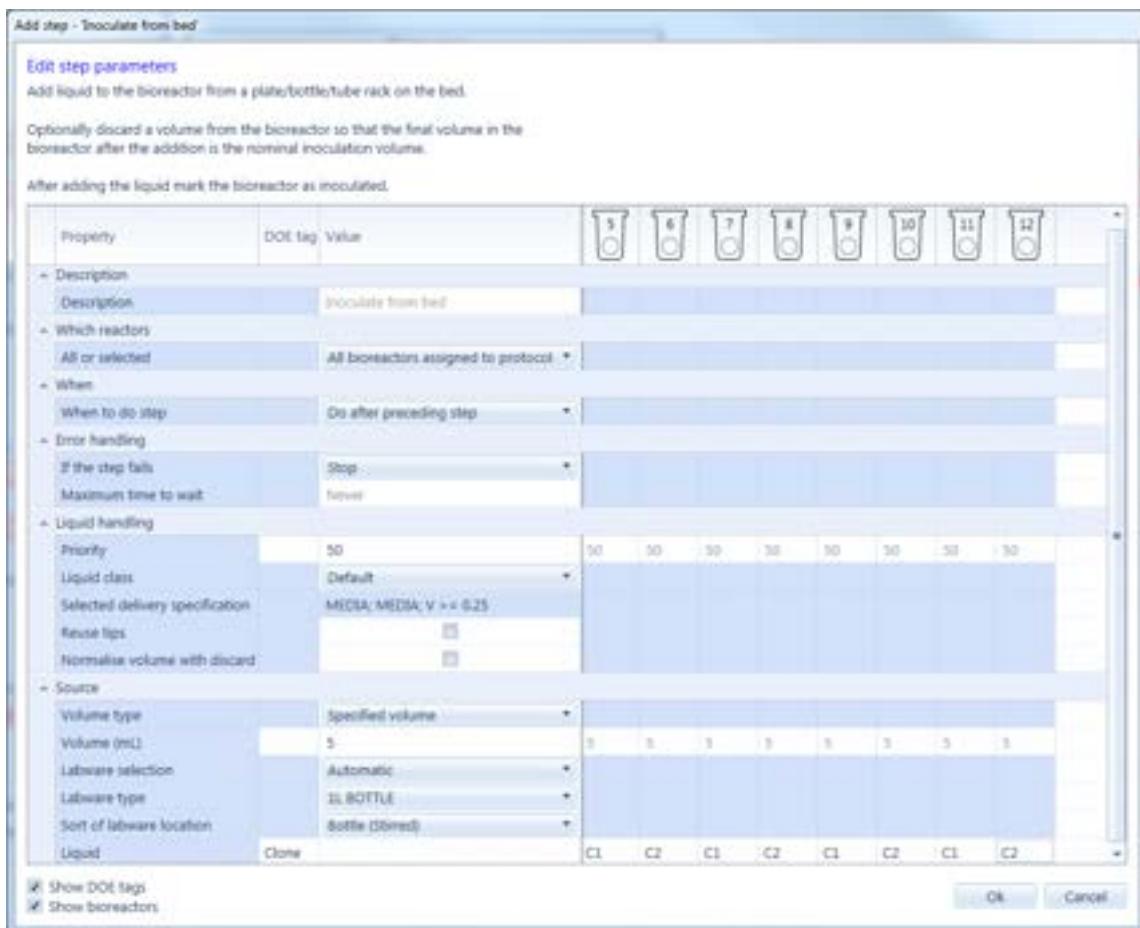


Figure 229 Inoculate from bed step with Liquid specified.

The **Add liquid** and **Inoculate from Bed** steps require the **Liquid** in the labware to be specified.

The **Sample step** does not require any additional parameters for automatic labware.

Automatic labware is created for steps once the steps are close enough to the present for the steps to be fully modelled in the first round of the systems internal modelling.

Once created automatic labware is shown with other labware and can be loaded and unloaded as required.

Plates/Bottles/Tube racks				
Name	Location	Role	Load	
Innocula C1	Bottle 9	Inocula (auto)	Load...	<input type="checkbox"/> <input checked="" type="checkbox"/>
Innocula C2	Bottle 7	Inocula (auto)	Load...	<input type="checkbox"/> <input checked="" type="checkbox"/>
MixA	Bottle 7	Liquid source (auto)	Load...	<input type="checkbox"/> <input checked="" type="checkbox"/>
MixB	Bottle 8	Liquid source (auto)	Load...	<input type="checkbox"/> <input checked="" type="checkbox"/>
MixC1	Bottle 7	Liquid source (auto)	Unload...	<input type="checkbox"/> <input checked="" type="checkbox"/>
Sample	Chilled 1	Sample (auto)	Unload...	<input type="checkbox"/> <input checked="" type="checkbox"/>
Sample (1)	Chilled 2	Sample (auto)	Unload...	<input type="checkbox"/> <input checked="" type="checkbox"/>
Sample 1 - pH sample	Chilled 4	Sample	Load...	<input type="checkbox"/> <input checked="" type="checkbox"/>
Sample 1 - pH sample (1)	Chilled 4	Sample	Load...	<input type="checkbox"/> <input checked="" type="checkbox"/>

Figure 230 Loading controls with mixture of automatically and explicitly created labware.

A Role column has been added to the labware screens that shows the roles of the different labware and whether the labware was automatically created or not.

Explicitly created and automatically created labware cannot be mixed. Steps using explicit labware to not offer automatically created labware items for selection.

4.9 Tubing - Pumps

The Tubing - Pumps page allows the layout of the tubing connecting the bioreactors and their pumps to containers to be designed.

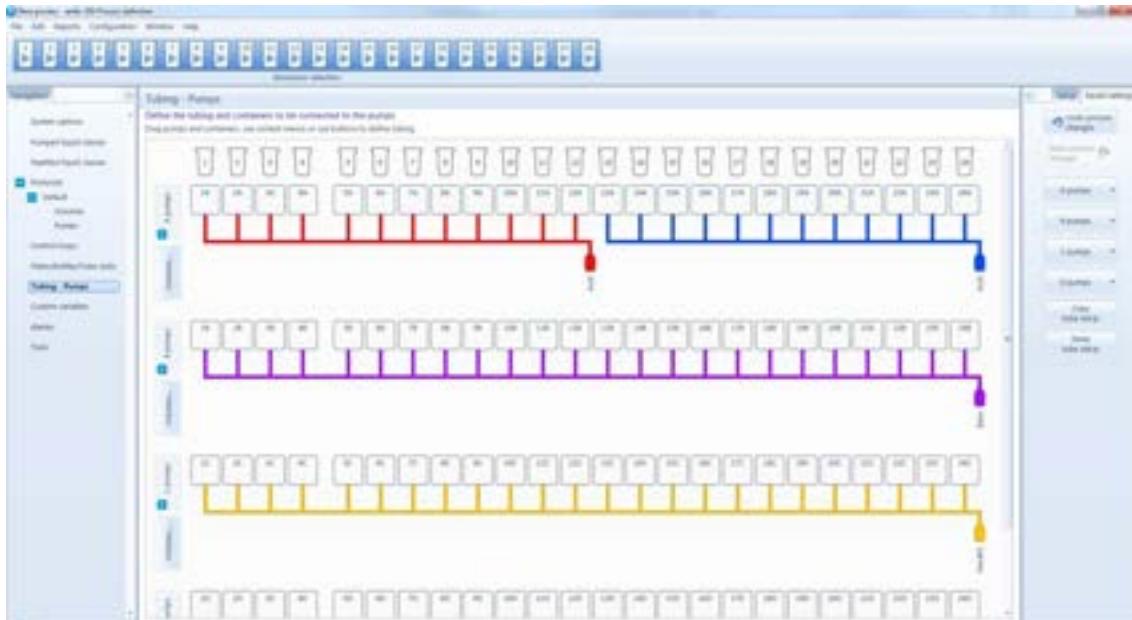


Figure 231 Tubing - Pumps page

The **Tubing - Pumps** page has rows with the **A** pumps for each bioreactor, the **B** pumps for each bioreactor, the **C** pumps for each bioreactor and the **D** pumps for each bioreactor.

The tubing for the pumps is shown below the row of pumps. One run of tubing can serve either one or more **A** pumps, or **B** pumps, or **C** pumps or **D** pumps but cannot serve a mixture of pumps such as some **A** pumps and some **B** pumps.

At the end of the tubing is a representation of the container connected to the tubing.

It is possible to have multiple tubing runs that share the same container. In the example above pumps 17A-20A are connected to one tube set and pumps 21A-24A to a second tube set both sharing the one container. This can be a helpful arrangement using multiple standard tube sets or dealing with viscous liquids.

A context menu on containers allows a colour to be assigned to a container. Containers and tubing runs not required by the current process definition are dimmed out.

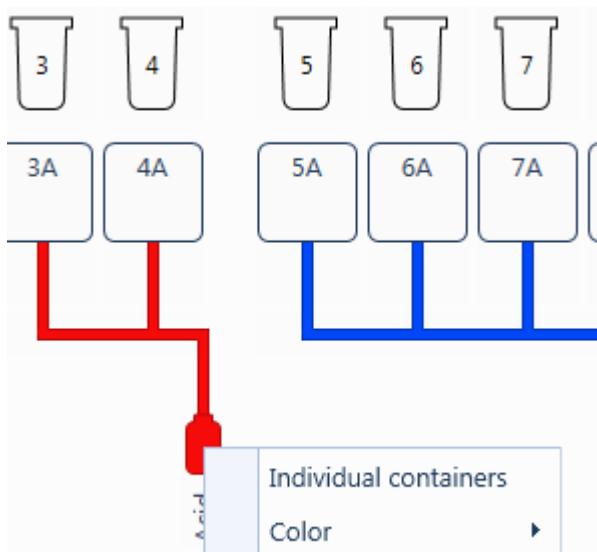


Figure 232 Context menu on containers

If one tube run or container is connected to bioreactors which have different roles or liquids assigned to their pumps then an error is shown on the page.

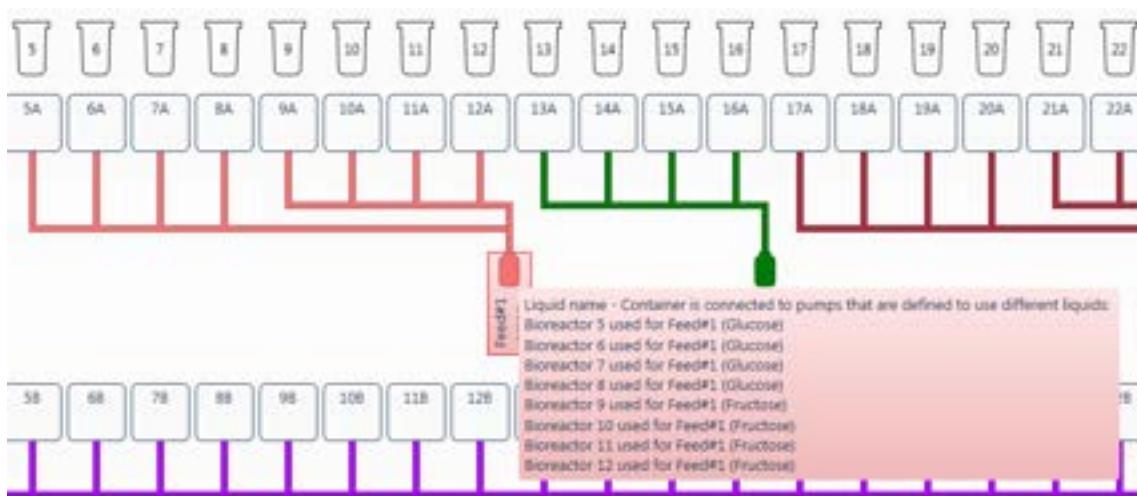


Figure 233 Error shown when container is connected to pumps defined to use different liquids.

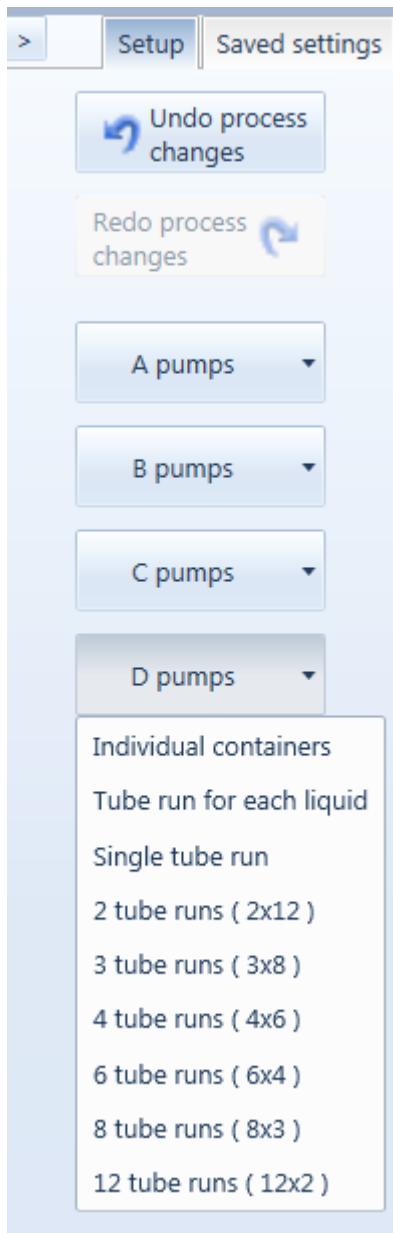
The tubing runs can be edited via context menus on the items in the display, from the controls in the **Tasks** panel and from dragging between items in the display.

Drag one item to another item to connect the two items:

- Drag a pump to a container to connect the pump to a container
- Drag a container to a pump to connect the container to the pump
- Drag a pump to a tube run to connect the pump to the tube run
- Drag a tube run to a pump to connect the tube run to the pump
- Drag a container onto a container to connect two tube runs to a single container
- Drag one tube run onto another tube run to merge them into a single run



Figure 234 Context menus on A pumps, a container, pump 5A and on a tube run



Individual containers

Tube run for each liquid

Single tube run

2 tube runs (2x12)

3 tube runs (3x8)

4 tube runs (4x6)

6 tube runs (6x4)

8 tube runs (8x3)

12 tube runs (12x2)

Figure 235 *Setup* panel with options on **D pumps** shown

Individual containers converts the relevant pumps to use a separate container for each pump.

Tube run for each liquid converts the relevant pumps to use one tube run for each distinct liquid.

The following options convert a complete set of pumps to use a specified pattern of pumps:

- **Single tube run** uses one tube run for all the pumps
- **2 tube runs (2x12)** uses two tube runs, one for pumps 1...12 and one for pumps 13...14.
- **3 tube runs (3x8)** uses three tube runs, one for pumps 1...8 and one for pumps 9...16 and one for 17...14.
- etc.

Split tube run after pump splits the tube run connected to the pump after the pump.

Split tube run before pump splits the tube run connected to the pump before the pump.

Use own container makes the pump use its own container and tube run.

Container for each tube run creates a separate container for each tube run sharing a container.

Merge tube runs creates a single tube run from one or more tube runs sharing a container.

Copy tube setup and **Paste tube setup** lets the tubing setup be transferred between process definitions.

4.10 Tubing – Permeate Collection

When perfusion is selected the **Tubing – Permeate Collection** page allows the tubing for the permeate to be configured.

All the controls work in the same manner as the **Tubing – Pumps** page.

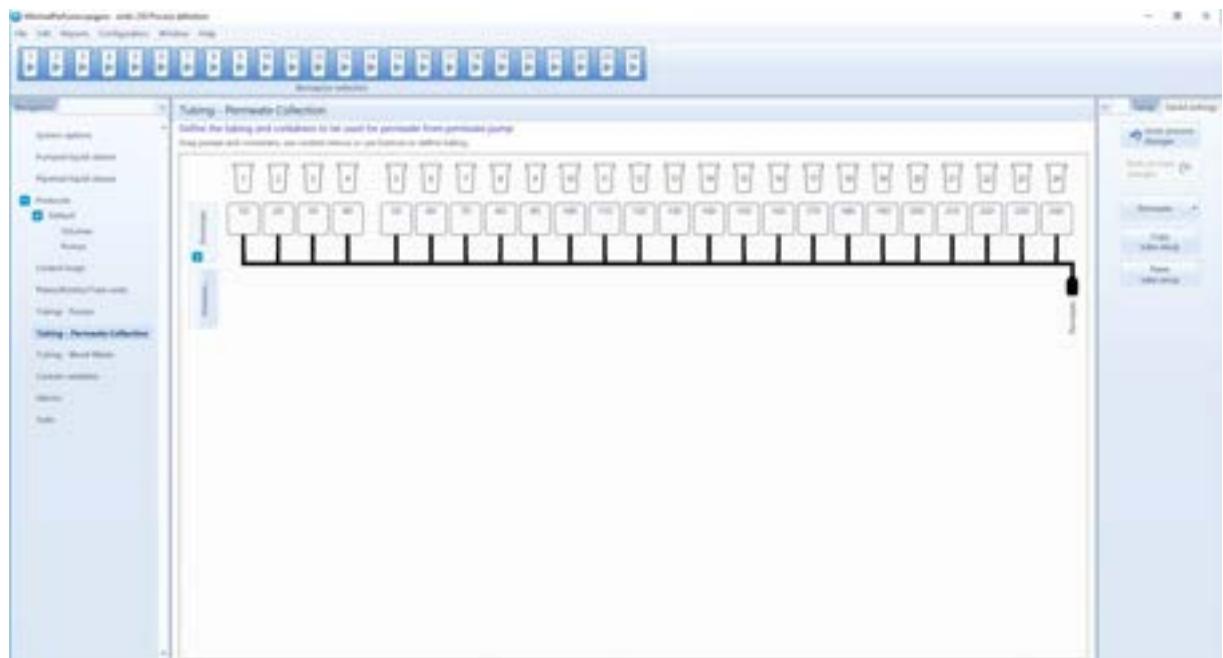


Figure 236 Tubing- Permeate Collection page

4.11 Tubing – Bleed Waste

When perfusion is selected the **Tubing – Bleed Waste** page allows the tubing for the bleed to be configured.

All the controls work in the same manner as the **Tubing – Pumps** page.

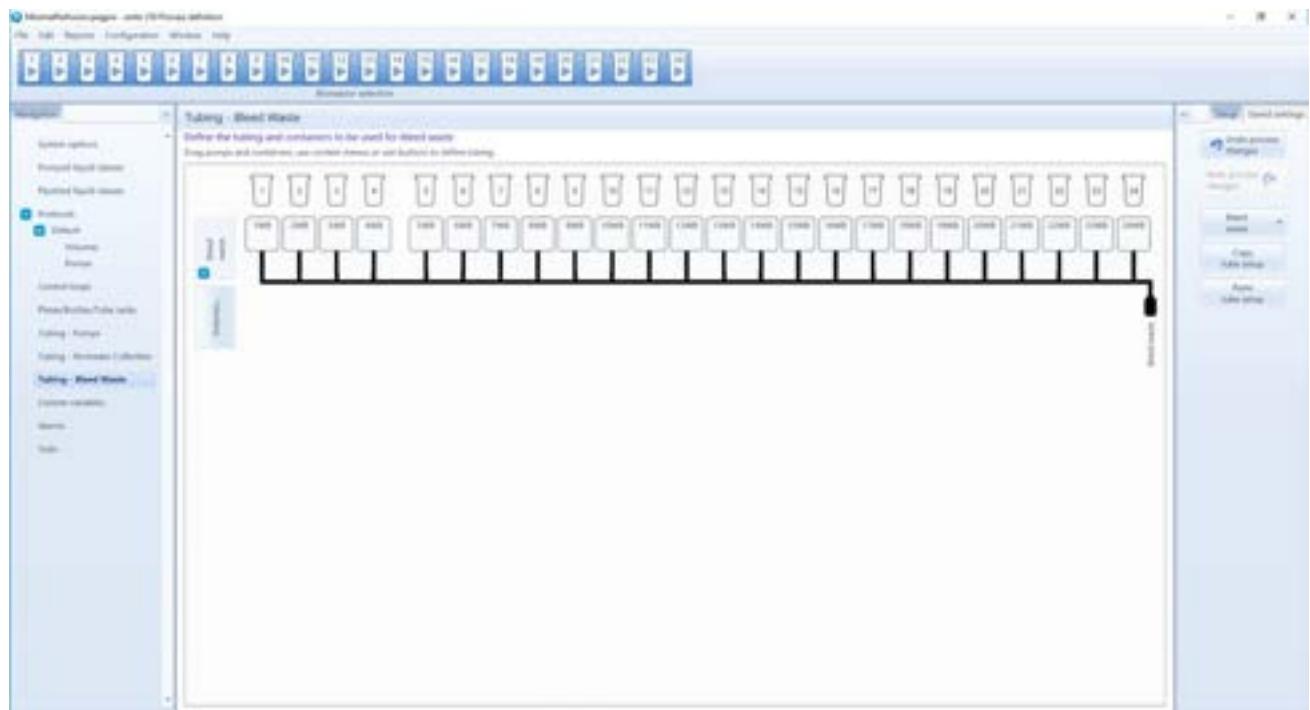


Figure 237 Tubing - Bleed Waste page

4.12 Custom variables

The Custom variables page allows the definition of custom variables which can be used:

- to provide slots for entering user defined data items
- to provide calculated values
- to provide additional things that can be set as set points

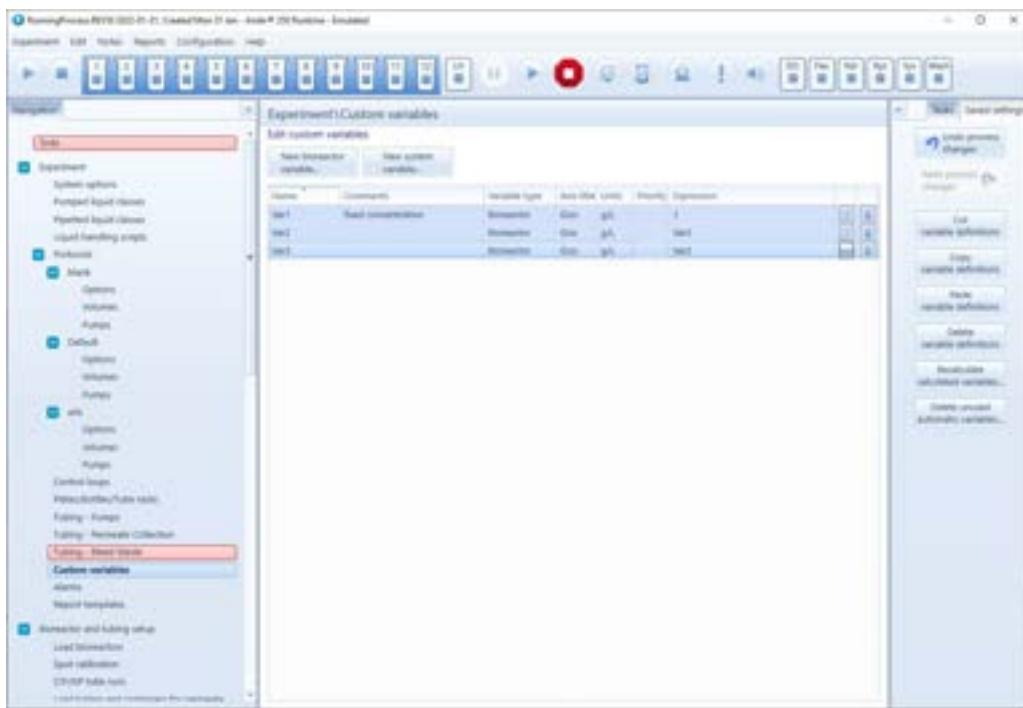


Figure 238 Custom variables page

New bioreactor variable... allows a new variable to be created.

Cut variable definitions, **Copy variable definitions**, **Paste variable definitions** allow variable definitions to be transferred between processes.

Delete variable definitions deletes the selected variable definitions. Variable definitions cannot be deleted once they have data associated with them.

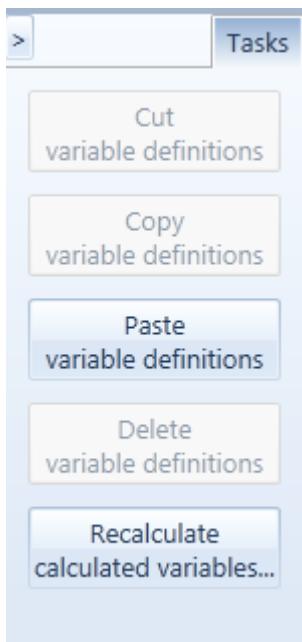


Figure 239 Additional runtime options

At runtime an additional option is present.

Recalculate the system calculates what the value of a custom variable would have been in the past had it been defined then. **Recalculate calculated variables** redoes these calculations.

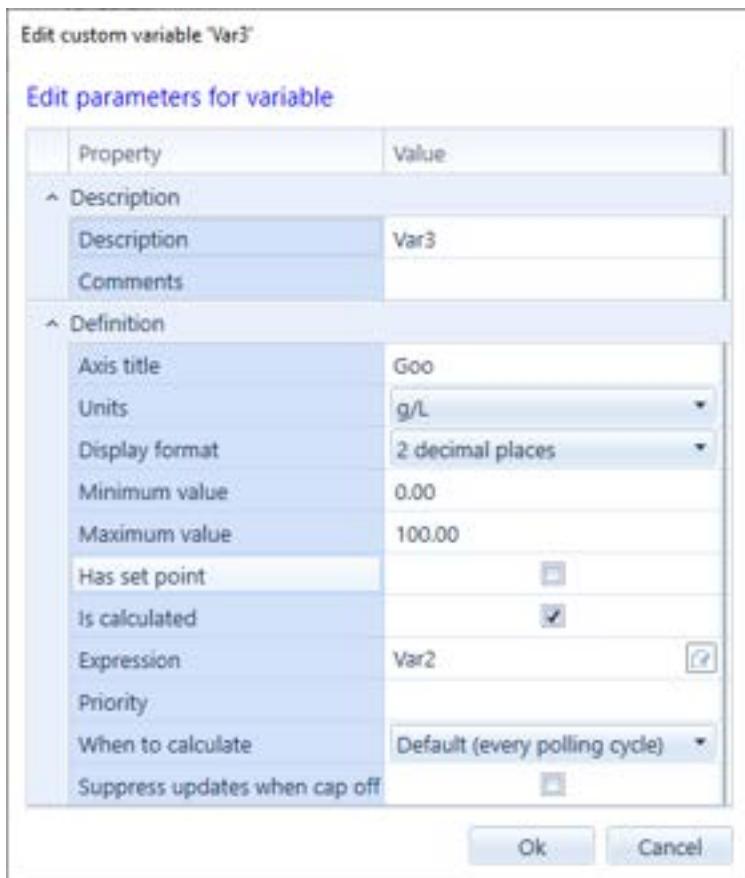


Figure 240 Custom variable definition

A custom variable definition has the options described below.

Description is used as the name of the definition.

Axis title and **Units** define the axis title and units of the variable. The axis title and units together determine which axis the variable will be shown against on graphs. The **Units** field has a list of standard units together with the option **Custom**. When **Custom** is selected then the **Custom units** field allows the user to enter other units.

The **Display format** option defines how the values of custom variables are displayed within the system.

The **Minimum value** and **Maximum value** define the valid values for data entry and the valid values for a set point targeting the variable.

Has set point defines whether a set point is exposed for the variable. If so then the set point can be controlled via steps in the process or directly from the bioreactor controls and a control loop can be used to try to make the system follow the set point.

Is calculated defines whether the present values of the variable accept data entered by the user or if the present values are calculated by the system from the Expression.

When to calculate defines when the custom variable should be recalculated. The variable is considered for recalculation each time values are read from the bioreactor. Depending on the value of this option the variable may be recalculated or not. Possible values are:

- **Default (every polling cycle)** – the value of the variable is recalculated on every polling cycle.
- **Any variable used has new value** – the value of the variable is recalculated if any of the variables that the expression refers to have a more recent value than the variable.
- **All variables used have new value** – the value of the variable is recalculated if all of the variables that the expression refers to have a more recent value than the variable.
- **Specified variable has new value** – the value of the variable is recalculated if the variable specified by **Variable with new value** has a more recent value than the variable. **Variable with new value** offers a selection from the variables the expression refers to.
- **Value older than** – the value of the variable is recalculated if the variable was last calculated more than **Time between calculations**.

Note that “have a more recent value than the variable” refers to the current date of the variable for the bioreactor for which the custom variable is being evaluated. This is true even if the expression gets the value of that variable for other bioreactors or a time in the past.

Priority allows the default order of calculation for calculated variables to be overridden. Variables with a higher priority are evaluated before variables with a lower priority irrespective of whether one variable depends on the value of the other variable.

SUPPRESS UPDATES WHEN CAP OFF can be set to stop the system calculating new values for the variable from when the cap is removed from a bioreactor until an interval after the cap has been replaced. This is the same behaviour as the in-built calculations of OUR, CER etc.

At runtime there are three variables for a calculated definition with an associated set point:

- Name – e.g. **RQ2** – the value calculated as the run proceeded and used as an input to conditions and other custom variables.
- Name (current value) – e.g. **RQ2 (current value)** – the value calculated according to the current definition of the variable. This value is recalculated if the variable definition is edited.
- Name.SP e.g. **RQ2.SP** – the value of the set point

4.12.1 System custom variables

Custom variables can be defined for the system as a whole.



This feature is enabled by the **Allow creation of custom system variables** option on the **Advanced features** window.

Advanced features

Select which advanced features should be enabled:

Control loops	<input type="checkbox"/> Allow items in PDE loops to be specified as expressions. <input type="checkbox"/> Support preserving values when switching to or between control loops. <input type="checkbox"/> Support cascade level where the maximum output value is the default (no effect) value. <input type="checkbox"/> Show options to create lookup table control-loops.
System	<input type="checkbox"/> Allow different protocols to have different types of bioreactor vessel. <input type="checkbox"/> Support exporting data from the system periodically as CSV files. <input type="checkbox"/> Show screens and features for maintenance and commissioning. <input type="checkbox"/> Allow entry of stock data. <input checked="" type="checkbox"/> Allow creation of custom system variables.

Allow creation of custom system variables

This feature exposes the option to create custom system variables. Custom system variables are like custom bioreactor variables except that:

- There is only one value for the variable across the whole system; rather than an individual variable for each bioreactor.
- Calculated custom system variables are not supported.
- Data entry is allowed for the set point of a custom system variable.
- The set point of a custom system variable can be set from a **Set variable** step.

To create a custom system variable press the **New system variable** button on the **Custom variables** page.

[Load settings...](#) [Save settings...](#) [OK](#) [Cancel](#)

Values of these variables can be set using the **Set variable** step or using **Edit value** from the Tables page.

Experiment\Custom variables

Edit custom variables

[New bioreactor variable...](#) [New system variable...](#)

Name	Variable type	Axis title	Units	Priority	Expression		
Sys1	System	Custom property					

Figure 241 **Custom variables** page with creation of system variables enabled.

To create a custom system variable press **New system variable**.

The options for creating system variables are the same as for creating bioreactor variables except that calculated values are not supported.

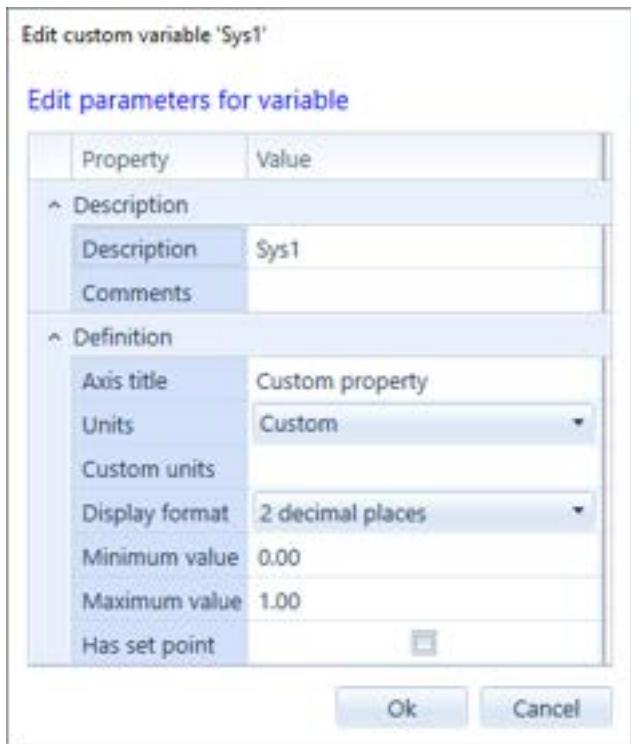


Figure 242 Dialog to create or edit system variables.

4.13 Alarms

The **Alarms** page allows the definition of alarms which test a condition and in response may:

- alert the user
- stop the liquid hander
- stop gassing on a bioreactor
- stop stirring on a bioreactor
- stop liquid handling on a bioreactor
- stop pumping on a bioreactor

There are two sorts of alarms supported: bioreactor alarms which work on individual bioreactors and system alarms which work only on properties of the system as a whole. The two sorts of alarms differ only slightly in the options available and so are described together here.

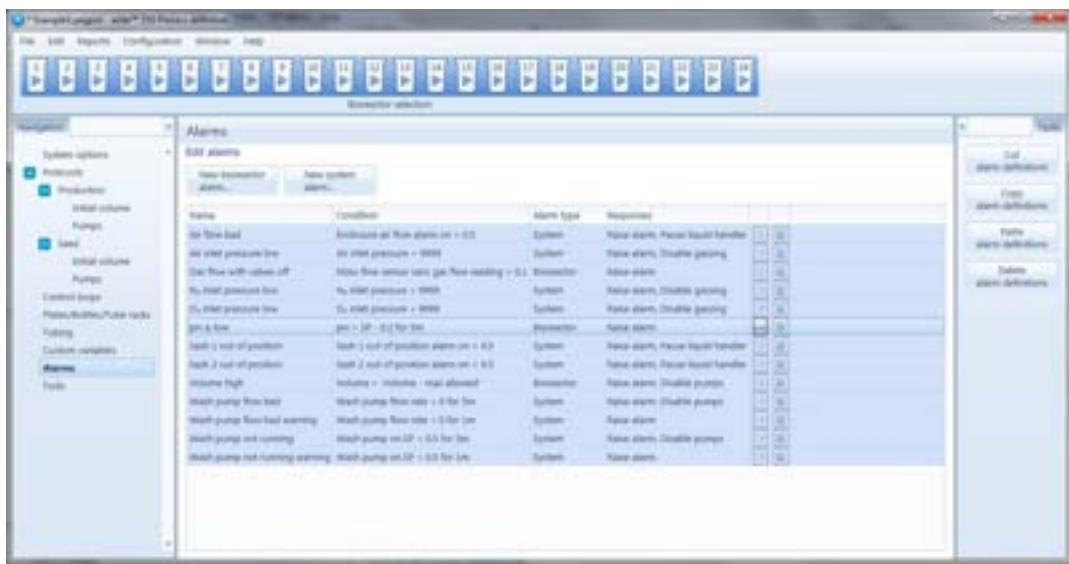


Figure 243 **Alarms** definition page

Some alarms are hard-coded by the system and can be viewed but not edited.

Viewing or editing an alarm definition displays the **Edit alarm** window with the options described below.

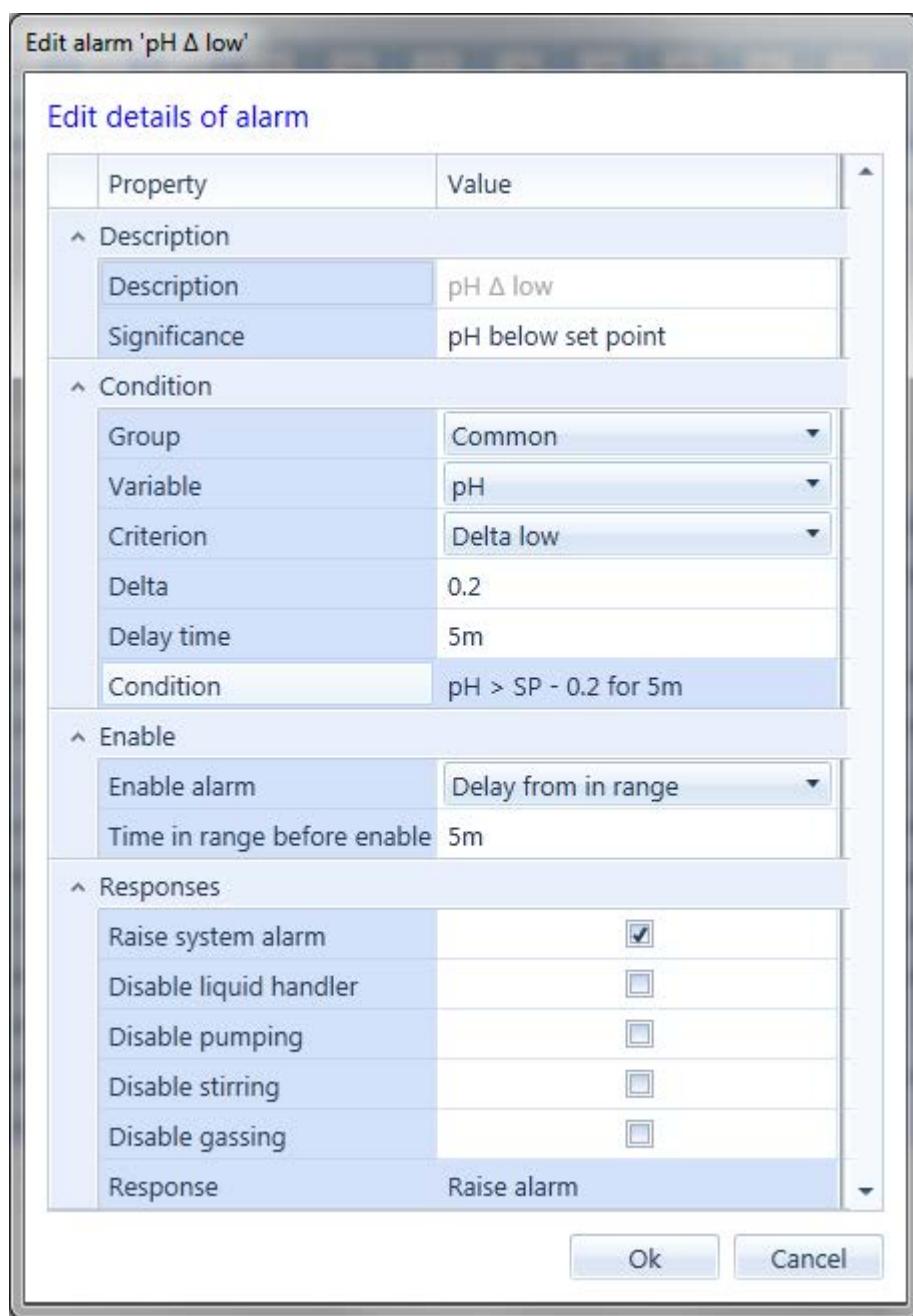


Figure 244 Bioreactor alarm definition

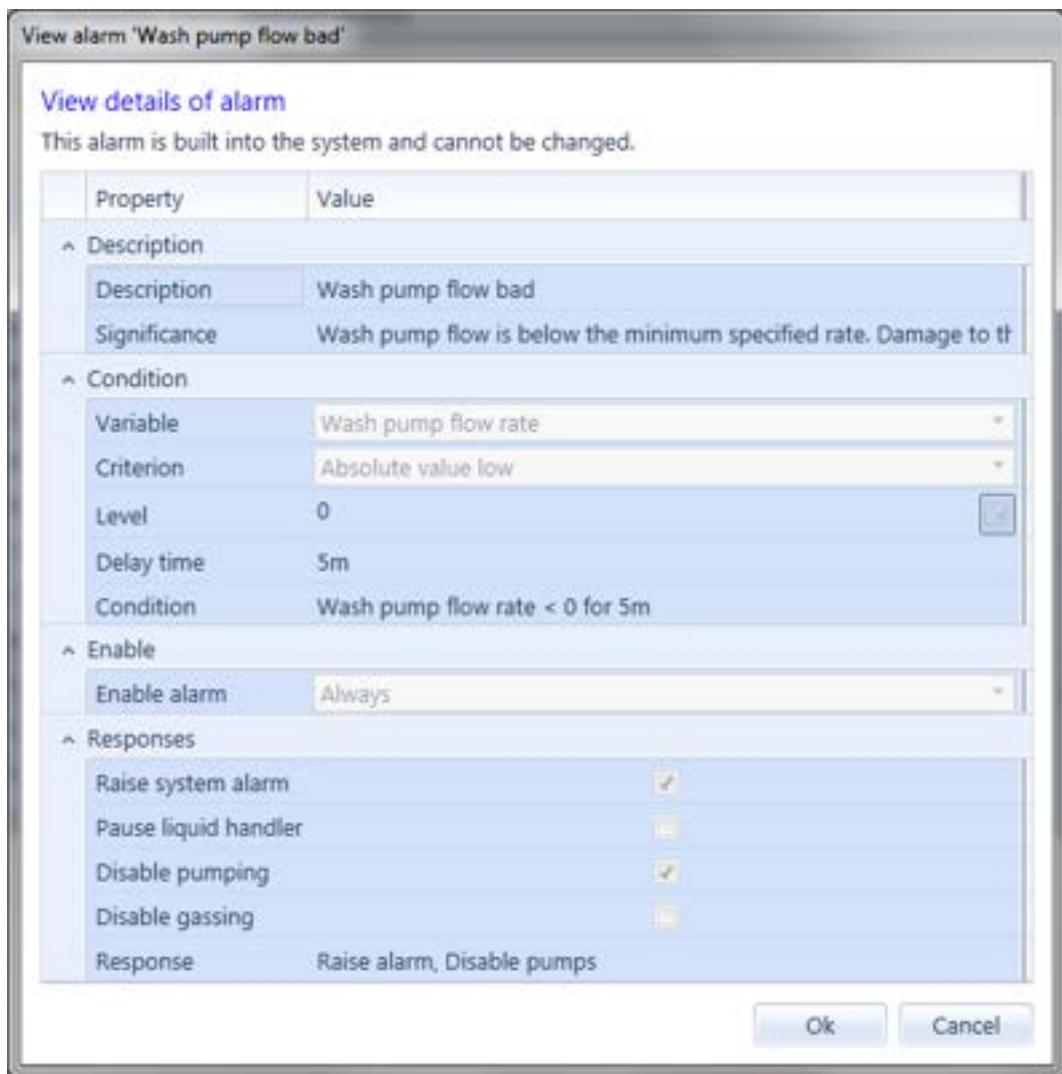


Figure 245 A hard coded system alarm definition

4.13.1 Description Options

Description allows a custom name to be given to the alarm. A default name is provided if no description is entered.

Significance allows the significance of the alarm to be specified. The significance of the Air correction factor being high might be that the sparge tube or out flow filter could possibly be blocked and should be checked.

4.13.2 Condition Options

The **Condition** options specify the condition that triggers the alarm.

For a bioreactor alarm the **Group** and **Variable** options select the variable that is monitored. For a system alarm only the **Variable** option is required.

Criterion specifies what test is made on the value of the variable. The choices are:

- **Absolute value high** – the alarm is triggered if the variable is higher than the specified **Level**

- **Absolute value low** – the alarm is triggered if the variable is lower than the specified **Level**
- **Delta high** – the alarm is triggered if the variable is more than the specified **Delta** above the corresponding set point
- **Delta low** – the alarm is triggered if the variable is more than the specified **Delta** below the corresponding set point
- **Percent high** – the alarm is triggered if the variable is more than the specified **Percentage** of the set point value above the corresponding set point
- **Percent low** – the alarm is triggered if the variable is more than the specified **Percentage** of the set point value below the corresponding set point

Level, Delta or Percentage specify the value of the variable at which the alarm is triggered.

Delay time specifies how long the variable must be outside of the specified criteria before the alarm is triggered.

Condition is a summary describing the **Condition** options.

4.13.3 Enable Options

The **Enable** options control when testing of the alarm is enabled.

Alarms are enabled when the bioreactor is loaded and the conditions here are met.

Enable alarm selects from:

- **Always** – the alarm is enabled so long as the bioreactor is loaded
- **Delay from set point on** – the alarm is not enabled until the set point has been enabled for Time setpoint on before enable.
- **Delay from in range** – the alarm is not enabled until the variable has been in range for Time in range before enable

The Delay options facilitate waiting until the variable should be under control before the alarm is enabled.

4.13.4 Responses Options

The **Responses** options control what happens when an alarm is triggered. **Response** summarises the chosen options.

- **Raise system alarm** – raises the alarm condition within the user interface and may send a message about the alarm.
- **Pause liquid handler** – brings the liquid handler to a controlled stop at the end of whatever action it is doing and puts lids back on tip boxes and plates. The liquid handler will not perform any more work until the user starts the liquid handler again.
- **Disable liquid handler** – requests that the liquid handler does not access the bioreactor. Work already queued for the bioreactor may still happen, but new work will not be queued for this bioreactor. Can be used to avoid accessing a liquid handler where contamination is suspected.
- **Disable pumping** – disables pumping to the bioreactor or for a system alarm to all bioreactors.
- **Disable stirring** – disables stirring on a bioreactor.

- **Disable gassing** – disables gassing to the bioreactor or for a system alarm to all bioreactors.
- **Cool culture** – turns temperature control of the bioreactor off cools the bioreactor as quickly as possible.

On systems with perfusion there are additional options.

- **Disable crossflow** – stops the crossflow (perfusion) pump.
- **Disable permeate pumping** – stops the crossflow (perfusion) pump.
- **Change perfusion filter** – prompts the user to change the perfusion (hollow fibre) filter.

4.13.5 Disabling Alarms

While running an experiment alarms can be disabled and enabled if required. This may be appropriate especially if there is a problem with a sensor used by one of the hard-coded system alarms so that the alarm is triggering spuriously.

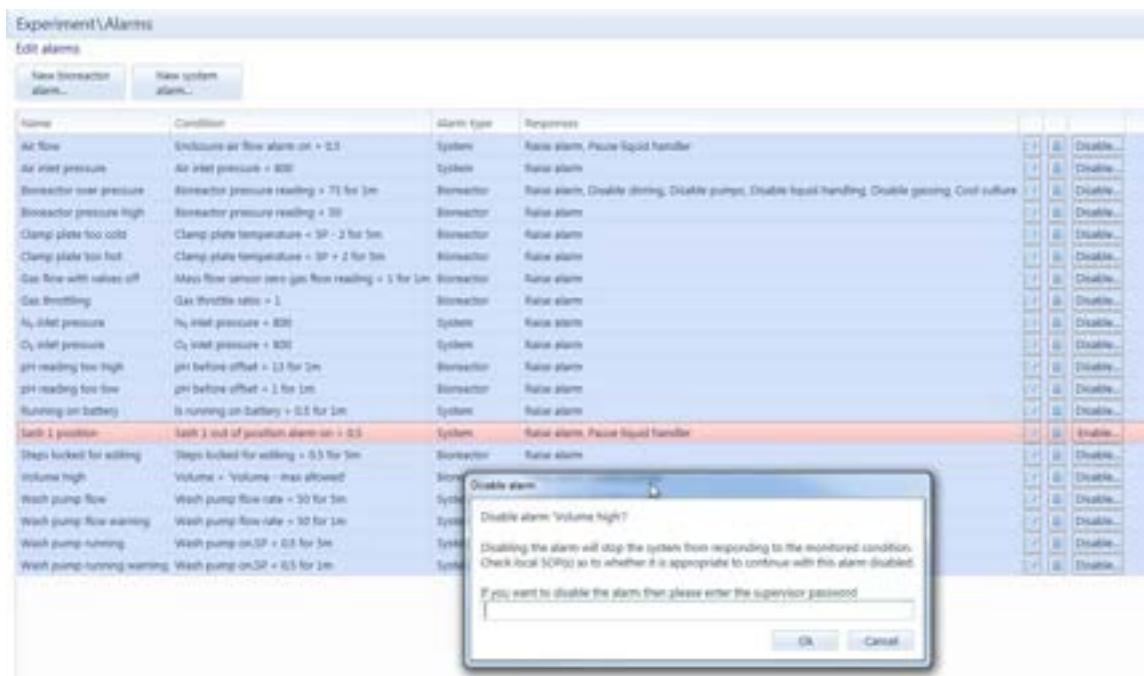


Figure 246 Alarms page while running an experiment

To disable an alarm press the **Disable...** option and then when prompted enter the supervisor password.

To re-enable an alarm press the **Enable...** option.

Attention is drawn to disabled alarms by showing them as invalid.

4.14 Report templates

The **Reports templates** page allows you to create a template for the data variables and layout that will be output if used in an **Export report** step.

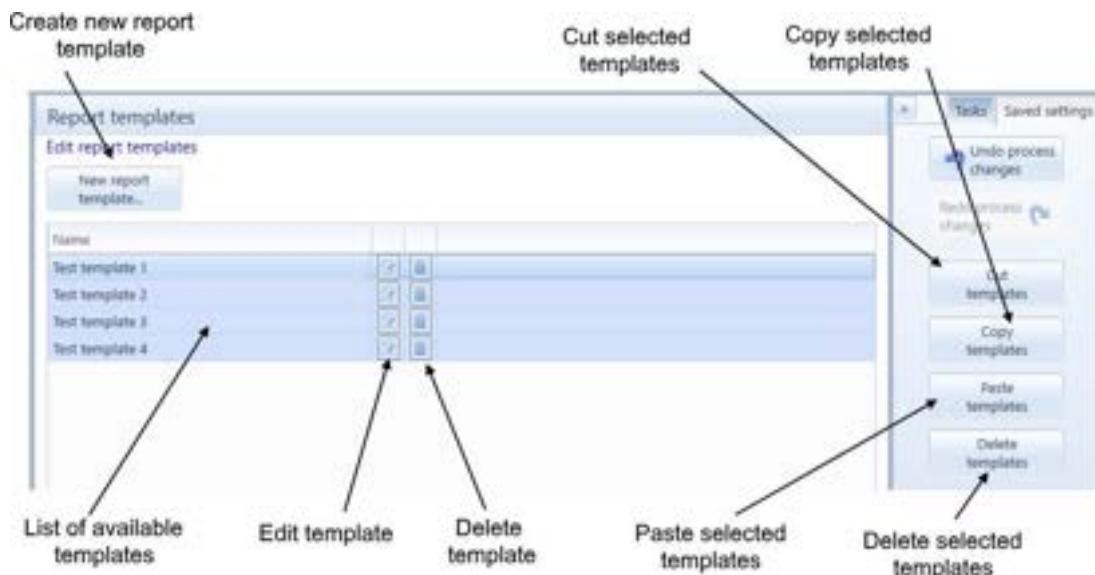


Figure 247 Report templates page

Report templates can be created, edited, copied and deleted from this screen.

- **New report template...** – Shows the edit dialog for creating a new report template.
- **Cut templates** – Cuts the highlighted template.
- **Copy templates** – Copies the highlighted templates.
- **Paste template** – Pastes copied or cut templates.
- **Delete template** – Deletes the highlighted templates.

4.14.1 New report template...

Report templates are defined and edited in the same way as **Tables**. (See section 10.3 Tables for details)

Figure 248 shows the 'Edit report template' dialogue box. The left panel, titled 'Table settings', contains a table of properties and values. The right panel, titled 'Variables', lists variables with checkboxes indicating they are selected. A preview panel in the center shows a table of data.

Property	Value
Name	Deposit
What sort of table do you want?	Values over time
Table type	monotonically increasing by dataset
Y-axis	Dependent time column
Interpolation	interpolated values
Interpolation method	linear
Display times as	Absolute time
Start time	
End time	
How should values be shown?	
Interpolation method	Last value
Number format	Default
Replace null's with	NaN
General format options	
Include units	<input checked="" type="checkbox"/>
Make columns unique	<input checked="" type="checkbox"/>
Header clearing	No clearing
String clearing	No clearing
Labels	
Label 1	Name
Label 2	Name
Label 3	Name
Label 4	Name
How should times be shown?	
Formatting	Date Time
Format	Local time

Variables:

- Audit
- CO₂
- Energy
- pH
- RH (percent)
- Temperature
- Volume

Preview:

Date/Time	Parameter 1 - pH (pH)	Date/Time	Parameter 2 - pH (pH)	Date/Time	Parameter 3 - pH (pH)	Date/Time	
2020-05-03 23:00:00	2.000000	2020-05-03 23:00:00	1.114	2020-05-03 23:00:00	1.179	2020-05-03 23:00:00	1.498
2020-05-04 00:00:00	6.271	2020-05-04 00:00:00	6.305	2020-05-04 00:00:00	6.313	2020-05-04 00:00:00	6.313
2020-05-04 01:00:00	6.393	2020-05-04 01:00:00	7.029	2020-05-04 01:00:00	6.658	2020-05-04 01:00:00	6.658
2020-05-04 02:00:00	7.875	2020-05-04 02:00:00	7.147	2020-05-04 02:00:00	7.194	2020-05-04 02:00:00	7.194
2020-05-04 03:00:00	4.397	2020-05-04 03:00:00	8.484	2020-05-04 03:00:00	8.774	2020-05-04 03:00:00	8.774
2020-05-04 04:00:00	9.171	2020-05-04 04:00:00	9.163	2020-05-04 04:00:00	9.096	2020-05-04 04:00:00	9.096
2020-05-04 05:00:00	8.285	2020-05-04 05:00:00	8.285	2020-05-04 05:00:00	8.274	2020-05-04 05:00:00	8.274
2020-05-04 06:00:00	6.149	2020-05-04 06:00:00	6.149	2020-05-04 06:00:00	6.159	2020-05-04 06:00:00	6.159
2020-05-04 07:00:00	8.284	2020-05-04 07:00:00	8.284	2020-05-04 07:00:00	8.279	2020-05-04 07:00:00	8.279
2020-05-04 08:00:00	9.295	2020-05-04 08:00:00	9.343	2020-05-04 08:00:00	9.294	2020-05-04 08:00:00	9.294
2020-05-04 09:00:00	8.285	2020-05-04 09:00:00	8.285	2020-05-04 09:00:00	8.274	2020-05-04 09:00:00	8.274
2020-05-04 10:00:00	9.093	2020-05-04 10:00:00	9.093	2020-05-04 10:00:00	9.093	2020-05-04 10:00:00	9.093
2020-05-04 11:00:00	7.029	2020-05-04 11:00:00	7.029	2020-05-04 11:00:00	7.029	2020-05-04 11:00:00	7.029
2020-05-04 12:00:00	6.159	2020-05-04 12:00:00	6.159	2020-05-04 12:00:00	6.159	2020-05-04 12:00:00	6.159
2020-05-04 13:00:00	7.194	2020-05-04 13:00:00	7.194	2020-05-04 13:00:00	7.194	2020-05-04 13:00:00	7.194
2020-05-04 14:00:00	8.774	2020-05-04 14:00:00	8.774	2020-05-04 14:00:00	8.774	2020-05-04 14:00:00	8.774
2020-05-04 15:00:00	9.096	2020-05-04 15:00:00	9.096	2020-05-04 15:00:00	9.096	2020-05-04 15:00:00	9.096
2020-05-04 16:00:00	8.274	2020-05-04 16:00:00	8.274	2020-05-04 16:00:00	8.274	2020-05-04 16:00:00	8.274
2020-05-04 17:00:00	7.029	2020-05-04 17:00:00	7.029	2020-05-04 17:00:00	7.029	2020-05-04 17:00:00	7.029
2020-05-04 18:00:00	6.159	2020-05-04 18:00:00	6.159	2020-05-04 18:00:00	6.159	2020-05-04 18:00:00	6.159
2020-05-04 19:00:00	7.029	2020-05-04 19:00:00	7.029	2020-05-04 19:00:00	7.029	2020-05-04 19:00:00	7.029
2020-05-04 20:00:00	6.159	2020-05-04 20:00:00	6.159	2020-05-04 20:00:00	6.159	2020-05-04 20:00:00	6.159
2020-05-04 21:00:00	7.194	2020-05-04 21:00:00	7.194	2020-05-04 21:00:00	7.194	2020-05-04 21:00:00	7.194
2020-05-04 22:00:00	8.774	2020-05-04 22:00:00	8.774	2020-05-04 22:00:00	8.774	2020-05-04 22:00:00	8.774
2020-05-04 23:00:00	9.096	2020-05-04 23:00:00	9.096	2020-05-04 23:00:00	9.096	2020-05-04 23:00:00	9.096
2020-05-05 00:00:00	8.274	2020-05-05 00:00:00	8.274	2020-05-05 00:00:00	8.274	2020-05-05 00:00:00	8.274
2020-05-05 01:00:00	7.029	2020-05-05 01:00:00	7.029	2020-05-05 01:00:00	7.029	2020-05-05 01:00:00	7.029

Figure 248 Edit report template dialogue

The Report templates screen is arranged with the **Table settings** panel on the left and the **Variables** selection panel on the right. If the template is being defined in the Runtime the report layout and data are shown in the **Preview** panels.

4.14.1.1 Variables

The **Variables** panel allows the selection of variables for the template.

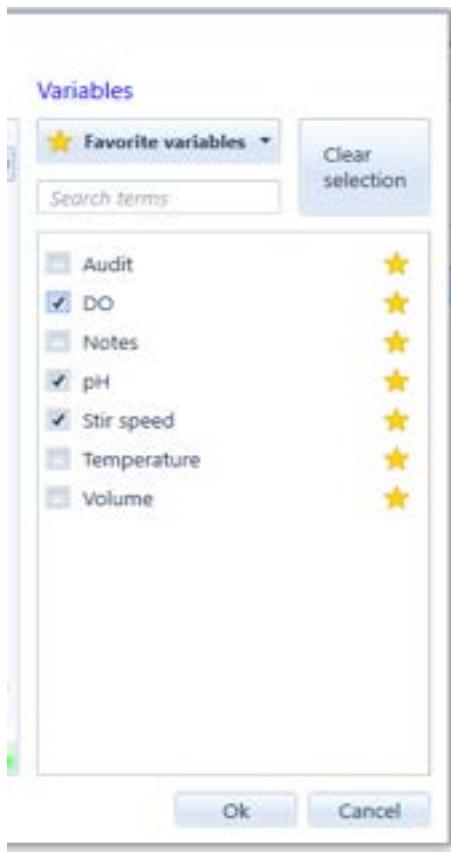


Figure 249 Variables panel

The drop down menu chooses which variables to display in the list. There are three special groups at the top:

- **All variables**, all typically useful variables from all groups are shown. (Additional variables that are not typically useful for end users are shown in the **Diagnostics variables** group.)
- **Favorite variables** the variables that have been marked as favorites. Click the star at the right of the list to mark a variable as a favorite.
- **Recent variables** this group is automatically updated to show recently used variables.

Clear selection de-selects all of the variables.

Type text into the **Search terms** box to filter the list of variables.

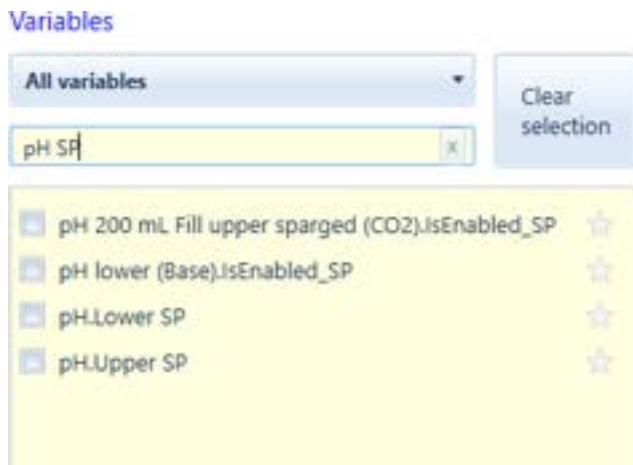


Figure 250: Using search terms to filter the variables

Search terms should be separated with spaces. Text search is case insensitive if only lower case is used (use mixed case to do case sensitive search). The background is coloured yellow to indicate when the variable list is being filtered.

4.14.1.2 Table settings

The **Table settings** allows the layout of the selected variables for the report to be defined.

4.14.1.2.1 Options settings

Name specifies the name of the report that will be produced by the report template.

4.14.1.2.2 What sort of table do you want?

Table type allows the selection or the type of table

- **Values over time**, multiple values over a time period are selected.
- **Values at time** single values at a particular time point are selected.

If **Values at time** is selected then:-

Option allows displaying the data:

- **Now** – shows the latest value of the variables.
- **Specified date and time** – allow a specific date and time to be entered.
 - **Date and time** the date and time for the selection.
- **Reference time** allows the **Report time** option to be specified:-
 - End – the time is specified relative to the end of the experiment.
 - Start – the time is specified relative to the start of the experiment for the bioreactors i.e. when the bioreactor started running.
 - **Phase** – the time is specified relative to the start time of the current phase.
 - **Inoculation** – the time is specified relative to when the bioreactors were inoculated. No data will be shown for bioreactors which have not been inoculated.

- **Sample time** – the time is specified relative to the last sample for the bioreactors, that is when the bioreactor was last sampled.
- **Offset** – allows an optional positive offset to be added to the reference time.

If **Values over time** is selected then:-

- **Layout** allows displaying the data:-
 - **Horizontally grouped by object** – all of the fields for the object are grouped together horizontally in the same row.
 - **Horizontally grouped by variable** – all variables of the same name are grouped together horizontally on the row
 - **Stack object vertically** – all of the fields for the object are grouped vertically for each object in turn.
- **Times** allows the time of the variables to be displayed as:-
 - **Single time column** – a single column containing times for all fields
 - **Separate time columns** – a time column is associated with each variable.
- **Interpolation** allows the time series to be chosen from:-
 - **Interpolated values** – the time series data is interpolated at the **Interpolation interval**
 - **Raw values** – the raw data from the time series.
 - **Plotted values** – the time series data at the times as shown on the graph
 - **Sampled values** – the time series data at the times of samples.
- **Display times as** chooses how to display the times:
 - **Absolute time** – the absolute time of the series data when the data was recorded.
 - **Logical day relative to reference** – the time relative to the **Reference time** as a number of hours from the start of the logical day.
 - **Time relative to reference** – the time relative to the **Reference time** as a number of hours
- **Start offset** – the offset from the start of times when data should start.
- **End offset** – the offset from the start of times when data should end.

4.14.1.2.3 How should values be shown?

The **How the values should be shown?** setting allows the variables values to be interpolated and displayed in different numeric formats.

Interpolation method specifies the method that should be applied to interpolated values

- **Default** – No interpolation is performed
- **Interpolated value**. – Values are interpolated over the interpolated period and calculated at the interpolated time points.
- **Mean value** – The mean value of the points in the interpolated period are used as the value.

- **Last value** – The last value of the variable in the interpolated period is used as the value.
- Number format** specifies how the numeric values in the report will be displayed
- **Default** – the default format for the variable
 - **Integer** – 7
 - **1 decimal place** – 7.5
 - **2 decimal places** – 7.58
 - **3 decimal places** – 7.583
 - **4 decimal places** – 7.5830
 - **5 decimal places** – 7.58300
 - **General, 1 significant figure** – 0.7
 - **General, 2 significant figure** – 1.71
 - **General, 3 significant figure** – 1.312E+1
 - **General, 4 significant figure** – 1.7110
 - **General, 5 significant figure** – 1.31200E+1
 - **Scientific, 1 significant figure** – 7.5E0
 - **Scientific, 2 significant figure** – 7.58E0
 - **Scientific, 3 significant figure** – 1.312E+1
 - **Scientific, 4 significant figure** – 1.3123E+1
 - **Scientific, 5 significant figure** – 7.58300E0

Replace NaN with – replace NaN (not a number) values with optional text.

4.14.1.2.4 General format options

The General format options specifies how the column headings and string field in the report should be handled.

Include units – include the units of the variable in the heading of the column.

Make columns unique – make name in the columns unique. Especially for Date/Time columns.

Header cleaning – choose how to clean the text in the header columns:-

- **No cleaning** – the text is left as is.
- **ASCII only** – only characters in the ASCII character set are displayed. Characters not in the ASCII character set are replaced with a ?.
- **A-Za-z0-9 and space only** – only upper and lowercase characters A-Z, number 0-9 and space are allowed. Unknown characters are removed.

String cleaning – choose how to clean text fields in the columns.

- **No cleaning** – the text is left as is.

- **ASCII only** – only characters in the ASCII character set are displayed. Characters not in the ASCII character set are replaced with a ?.
- **A-Za-z0-9 and space only** – only upper and lowercase characters A-Z, number 0-9 and space are allowed. Unknown characters are removed.

4.14.1.2.5 Labels

Up to 4 labels can be chosen to label the objects in the report **Label1 ... Label4**. At least one label must have a variable assigned.

The data in the labels can be chosen from the following variables

- **None** – no variable.
- **Name** – the name of the bioreactor.
- **Position** – the numeric position of the bioreactor.
- **Experiment** – the name of the experiment.
- **Protocol** – the name of the protocol in which the bioreactor is included.
- **Batch** – the bioreactors batch.
- **Strain** – the bioreactors strain.
- **User label** – the bioreactors user label.

4.14.1.3 Loading and saving report templates

The **Load...** button allows previously saved report templates to be loaded

The **Save...** allows the report template to be named and saved

5 STEPS AND STEP PARAMETERS

This section provides details of the specific steps that can form part of an Ambr® 250 process and their parameters.

5.1 Common options

All steps have some common options.

5.1.1 Description options

Description – optionally override the default description of the step.

5.1.2 Which reactors options

The **Which reactors** options control which bioreactors the step will apply to. By default new steps will apply to all of the bioreactors assigned to the protocol, but the step can be edited to apply only to selected bioreactors. Steps created when retrying steps or by wizards may also apply only to selected bioreactors.

All or selected – Choose option for which reactors this step should be applied to from:

- **All bioreactors assigned to protocol**
- **Selected bioreactors**

Reactors to apply to – If **All or selected** is **Selected bioreactors** then this option selects which reactors the step should be applied to. Other bioreactors will skip over the step.

5.1.3 When options

The **When** options control when the step will be executed.

When to do step – selects the option for when to do step. Different options are available depending on the context that the step is in.

- **Do after preceding step** – the step is executed once the preceding step has finished.
- **Do after preceding step AND time in phase** – the step is executed once the preceding step has finished and the bioreactor has been in the phase containing the step for the specified interval.
- **Do after preceding step and at specified time** – the step is executed once the preceding step has finished and the specified time of day has been reached. The option is available for steps contained within a **Steps on Day** step.

Time in phase – Wait until this interval from the start of the phase the step is contained in before performing the step.

Time of day – The time of day at which the step should happen.

Creation time – Displays when the step was added to the process. Shown when the process has been run or is running and the time that the step would have started earlier but it was not created until this time.

5.1.4 Grouping options

Some steps allow bioreactors to be grouped together so that the step is performed for all bioreactors at the same time. Examples include liquid handling steps where tips can be reused between bioreactors; conditions that can wait until a condition is true of all bioreactors and prompts that can accept one response for multiple bioreactors.

The **Group bioreactors** option selects whether the action is done for multiple bioreactors together or independently for different bioreactors. The default when the option is not available is to do the action independently for different bioreactors.

- **One at a time** – the action is performed independently for different bioreactors.
- **All together** – the step waits until all the bioreactors are ready to perform the action before doing the action.
- **Group by source** – the step waits until all the bioreactors using the same source are ready to perform the action before doing the action.

Note that **Disabled** bioreactors are ignored when waiting for bioreactors to be ready.

The **Maximum time to wait for group** option defines a timeout after which the step will proceed with those bioreactors that are ready. Later bioreactors will be grouped together when they reach the step.

5.2 Pipetting

The Pipetting steps deal with adding or removing liquid from the bioreactors using either the liquid handler or a manual pipette.

The steps involving the liquid handler are only present when the configuration includes a liquid handler.

Common options for the liquid handling steps are described in sections 3.6.2.1, 3.6.4 and 3.6.5 above.

5.2.1 Add liquid from bed

The **Add liquid from bed step** transfers liquid from a plate or bottle or tube rack on the bed of the system to the bioreactor.

5.2.1.1 Source options

The **Source** options present the standard labware options for the vessel to transfer liquid from.

The volume can be calculated.

5.2.2 Add liquid (manual)



On systems that have a liquid handler the manual pipetting steps are only shown if the **Show steps to prompt the user to take manual samples or add liquid by hand** option is selected in the **Advanced features** window.

The **Add liquid (manual)** step prompts the user to add some liquid to the bioreactor.

5.2.2.1 Prompt options

Respond within – how long to allow for the user to respond to the prompt when planning when interactions should happen.

5.2.2.2 Details options

Volume – the volume to be added by the user.

Liquid – what should be added by the user.

5.2.3 Add liquid from bed to maintain concentration

The Add liquid from bed to maintain concentration step can be used to maintain the concentration of a selected analyte in the culture.

If when the step is run there is a new measured value for the analyte then the step adds the required volume (if any) to achieve a target concentration. If there is no new measured value, or if the no liquid needs to be added, then the step completes successfully and does not add any liquid.

Measured concentration specifies the variable that contains the measured value of the concentration.

Measured concentration units shows the units of that variable.

Target concentration units specifies the units in which the target, trigger and source concentrations will be specified. If the measured and target concentrations are not the same then **Conversion factor** is the number to multiple the measured concentration by to covert it into the target units.

Target concentration is the desired concentration to achieve.

Trigger concentration is a threshold to avoid making too many small additions. If the measured concentration is greater than the trigger concentration no addition will be made.

Source concentration is the concentration in the liquid source that will be added.

Maximum volume specifies the maximum volume to add and **What to do if volume is too large** specifies the behaviour if the calculated volume is larger than this limit.

5.2.4 Inoculate from bed

The **Inoculate from bed** step:

- 1) Optionally discards liquid from the bioreactor such that the volume in the bioreactor at the end of the step is the **Nominal inoculation volume**.



The option to discard liquid is hidden unless the **Show option to discard media during inoculation to achieve the specified final volume** option is selected in the **Advanced features** window.

- 2) Transfers liquid to the bioreactor from the bed.
- 3) Marks the bioreactor as being inoculated. Marking the bioreactor as inoculated sets the reference time for graphing and enables calculation of integrated OUR and CER totals when applicable.

5.2.4.1 Liquid handling options

The **Inoculate from bed** step has the following options in addition to the standard liquid handling options.

Normalise volume with discard – selects the option to discard volume before doing inoculation so that the final volume is the nominal inoculation volume.

Discard liquid class – the liquid class specifying how to dispose of the excess volume.

Share tip for discard and transfer – optionally share the same tip for the discard and the transfer.

5.2.4.2 Waste Container options

The **Waste container** options present the standard labware options for the vessel to receive the discarded liquid from the target bioreactor.

5.2.4.3 Source options

The **Source** options present the standard labware options for the vessel to transfer liquid from.

The volume can be calculated.

5.2.5 Inoculate from bioreactor

The **Inoculate from bioreactor** step:

- 1) Optionally discards liquid from the target bioreactor such that the volume in the bioreactor at the end of the step is the Nominal inoculation volume.



The option to discard liquid is hidden unless the **Show option to discard media during inoculation to achieve the specified final volume** option is selected in the **Advanced features** window.

- 2) Transfers liquid to the target bioreactor from the bioreactor designated as its Seed source in the Assign protocols to bioreactors window.
- 3) Marks the bioreactor as being inoculated. Marking the bioreactor as inoculated sets the reference time for graphing and enables calculation of integrated OUR and CER totals when applicable.

5.2.5.1 Liquid handling options

The **Inoculate from bioreactor** step has the following options in addition to the standard liquid handling options.

Normalise volume with discard – selects the option to discard volume before doing inoculation so that the final volume is the nominal inoculation volume.

Discard dispense method – the method to be used to dispense liquid to be discarded.

Share tip for discard and transfer – optionally share the same tip for the discard and the transfer.

5.2.5.2 Waste Container options

The **Waste container** options present the standard labware options for the vessel to receive the discarded liquid from the target bioreactor.

5.2.5.3 Source options

The Source options specify how much inocula to transfer.

The volume can be calculated.

5.2.6 Inoculate (manual)



On systems that have a liquid handler the manual pipetting steps are only shown if the **Show steps to prompt the user to take manual samples or add liquid by hand** option is selected in the **Advanced features** window.

The **Inoculate (manual)** step prompts the user to manually inoculate a bioreactor.

5.2.6.1 Prompt options

Respond within – how long to allow for the user to respond to the prompt when planning when interactions should happen.

5.2.7 Sample

The Sample step aspirates liquid from a bioreactor and delivers it to one or more destinations.

5.2.7.1 Liquid handling options

In addition to the standard Liquid handling options the Sample step has the options below. The options are only shown then the system has the appropriate hardware integrated so not all of these options may be shown on a particular system.

Number of samples – How many destinations to deliver a labware sample to with this step.

- None,
- One
- Two
- Three

Take AM pH sample – selects taking a sample for at-line pH measurement using an integrated pH station or ambrAM.

Take cell count – selects taking a sample for an at-line cell count using an integrated cell counter. The results of the cell count are recorded as values for the bioreactor being sampled and as “Seed – “ values for the bioreactors for which at the time of sampling the bioreactor being sampled was assigned as the **Seed source**.

Prompt before samples – option for the liquid handler to pause and display a prompt before each sample so that the user can be ready for the sample the moment it is taken.

Prompt text – the prompt to display when the liquid handler is paused.

5.2.7.2 Destination 1 options

The **Destination 1** options contain the standard labware options for the vessel to receive the first sample from this step.

5.2.7.3 Destination 2 options

The **Destination 2** options contain the standard labware options for the vessel to receive the second sample from this step.

5.2.7.4 Destination 3 options

The **Destination 3** options contain the standard labware options for the vessel to receive the third sample from this step.

5.2.7.5 pH sample options

The **pH sample** options specify the options for the pH sample.

Ignore offset changes below – if set and the change to the offset would be less than this amount then do not adjust the offset.

Warn if change offset above – if set then raise a message if the change to the offset is greater than this amount.

Max. allowed change to offset – fail the sample if the change to the offset is greater than this amount.

Warn if temp. differs by (°C) – raise a message if a sample is taken and the pH check station temperature differs from the culture temperature by more than this much. This option is not included if the pH check station temperature is under automatic control.

Response – Options for what to do in response to the pH reading.

- **Continue so long as got an offset** – the system continues so long as a pH offset has been determined even if the step was unable to get a reading and update the offset.
- **Fail if do not get reading** – the system fails the step if it does not get a successful reading.
- **Read pH but do not adjust offset** – the system just takes a reading and does not adjust the offset.

Required quality -- the system will only accept readings that meet the required quality indication returned from the analysis module.

- **Pass** – only accept readings that have a pass indication
- **Pass with caution** – accept reading that have pass or pass with caution indication.

The criteria used by the analysis module are summarised in the table below:

Reading	Measurement	Normal cell density	High cell density
Pass	Reading slope	<= 0.03 mV/s	<= 0.05 mV/s
	Overall drift	<= 1 mV	<= 2 mV
	Slope stability	Reading start to mid-point delta <= ±0.01 Reading mid-point delta to end <= ±0.01 Reading start to end delta <= ±0.015	

		Total reading time is 20 seconds. pH is evaluated in the last 5 seconds.	
Pass with caution	Reading slope	<= 0.03 mV/s	> 0.05 mV/s and <= 0.08 mV/s
	Overall drift	<= 1 mV	<= 2 mV
	Slope stability	Reading start point to mid-point delta > ±0.01 Reading midpoint delta to end > ±0.01 Reading start to end delta > ±0.015 Total reading time is 20 seconds. pH is evaluated in the last 5 seconds	
Fail	Reading slope	> 0.03 mV/s	> 0.08 mV/s
	Overall drift	> 1 mV	> 2 mV
	Slope stability	Not applicable	

5.2.7.5.1 pH sample options with pH check station

The following options are used when the pH sample is handled by the pH check station.

These options are not shown when an ambrAM is used.

The **Volume** to transfer is fixed.

Check calibration after samples – if selected then do a re-calibration when the pH station has the opportunity.

5.2.7.5.2 pH sample options with Flex2

The following options are used when the pH sample is handled by the Flex2.

pH response – Options for what to do in response to the pH reading.

- **Adjust offset** – the system takes a reading and adjust the offset.
- **Read but do not adjust offset** – the system just takes a reading and does not adjust the offset.

The **Warn if temp. differs by (°C)** option is not shown when a Flex2 is used. The Flex2 uses the temperature of the bioreactor when the sample was taken to compensate for the difference in between the temperature of its pH raw measurement and the bioreactor.

5.2.7.6 Analysis module options

The **Analysis module** options specify the options for the sample passed to an integrated ambrAM module. The sample is analysed as specified by the options in the step.

The **Volume** to transfer is fixed.

5.2.7.7 Cell counter sample options

The **Cell counter sample options** specify the options for the cell counter sample.

The **Volume** to transfer is fixed.

Dilution factor – Factor by which to dilute the sample to bring the cell density into a suitable range for the counter. A factor of 3 would be 3-fold dilution with 1 part cells and 2 parts diluent.

The sample and diluent volumes are automatically calculated to give the specified **Dilution factor**. Depending on the system configuration, either the sample volume is fixed and the diluent volume is additional, or the total volume delivered to the sample cup is fixed.

Response – Options for what to do in response to the cell count.

- **Continue even if cell reading failed**
- **Fail if do not get reading**

If the Dilution factor is greater than 1 then **Diluent** options specify the source for the diluent.

5.2.7.8 Flex2 sample options

The **Flex2** options specify the options for the Flex2 sample.

The **Analysis sample type** specifies the set of analyses that will be performed by the Flex2.

Response – Options for what to do in response to the analyses.

- **Continue even if reading failed**
- **Continue so long as got a pH offset**
- **Fail if do not get reading**

The **Volume** to transfer is fixed

Cell counter sample options only appear if the **Analysis sample type** only specifies a cell count only. The Dilution factor can only be set to 1 (no dilution) or 2. If the dilution factor is set to 2 then **Diluent** options specify the source for the diluent.

5.2.7.9 Flex2 conditional panel selection

Flex2 samples can be set to use one of two sample types depending on the result of an expression. The **Alternative sample type** must be compatible with the main sample type:

- If one sample type measures pH then so must the other.
- If dilution is being done by the liquid handler then both sample types must be compatible with the use of dilution.

Edit step - 'Sample'

Edit step parameters

Take a sample from the bioreactor and deliver it to one or more destinations.

Property	Value
When to do step	Do after preceding step
^ Error handling	
If the step fails	Stop
Maximum time to wait	Never
^ Liquid handling	
Priority	50
Liquid class	Sample (auto continue)
Selected delivery specification	SAMPLE; SAMPLE_SLOW
Gas hold up (%)	5
Reuse tips	<input type="checkbox"/>
Number of labware samples	None
Take Flex2 sample	<input checked="" type="checkbox"/>
Take AM pH sample	<input type="checkbox"/>
Take cell count	<input type="checkbox"/>
Retry analysis	2 retries
Prompt before samples	<input type="checkbox"/>
^ Flex2	
Analysis sample type	Cell count
Response	Continue even if reading failed
Volume (mL)	0.675
Dilution factor	1
^ Flex2 (alternative)	
Alternative analysis sample type	Cell count 6x
Expression	'Cell density'
Comparison	Greater than
Compare against	Value
Value	50

Show DOE tags Show bioreactors

Ok **Cancel**

Figure 251 Sample step with choice of Alternative analysis sample type selected

5.2.7.10 Flex2 custom sample id

The Flex2 integration can be configured to send a custom sample id to the Flex2 by editing the External sample id option on the System options page.

^ Flex2	
External sample id	Explicit
Use temperature corrected pH	<input type="checkbox"/>
Offset to add to pH	0 <input type="button" value="P"/>
Minimum value of offset	0
Maximum value of offset	0

Figure 252 Flex2 section on the System options page

When this option is selected a sample id must be entered as part of the sample steps. This option can be given different values for different bioreactors. It is the user's responsibility to ensure that the values chosen satisfy any uniqueness or other requirements they have. The sample id is passed to the Flex2 and can be used to identify samples within the Flex2.

Edit step - 'Sample'

Edit step parameters

Take a sample from the bioreactor and deliver it to one or more destinations.

Property	DOE tag	Value
if the step fails	Stop	
Maximum time to wait	Never	
Liquid handling		
Priority	50	50
Liquid class	Sample (auto continue)	
Selected delivery specification	SAMPLE; SAMPLE_SLOW	
Gas hold up (%)	5	
Reuse tips		
Number of labware samples	None	
Take Flex2 sample	<input checked="" type="checkbox"/>	
Take AM pH sample		
Take cell count		
Retry analysis	2 retries	
Prompt before samples		
Flex2		
Analysis sample type	Default	
Response	Continue even if reading failed	
Volume (mL)	0.675	
External sample id	Sample1	XEVIG
Flex2 (alternative)		
Alternative analysis sample type	None	
pH sample		
pH response	Adjust offset	
Ignore offset changes below		
Warn if change offset above		
<input checked="" type="checkbox"/> Show DOE tags		Ok
<input checked="" type="checkbox"/> Show bioreactors		Cancel

Figure 253 Sample step with External sample id

5.2.8 Sample from labware

The **Sample from labware** step takes a sample from labware on the bed and takes a cell count reading.

The step is optimised so that only one cell count is taken from each labware well regardless of how many bioreactors refer to the labware.

The results of the cell count are recorded as the “Seed” cell counts for the bioreactors.

5.2.8.1 Source options

The Source options specify the source of the sample.

5.2.8.2 Cell counter sample options

The **Cell counter sample options** specify the options for the cell counter sample.

The **Volume** to transfer is fixed.

Dilution factor – Factor by which to dilute the sample to bring the cell density into a suitable range for the counter. A factor of 3 would be 3-fold dilution with 1 part cells and 2 parts diluent.

The sample and diluent volumes are automatically calculated to give the specified **Dilution factor**. Depending on the system configuration, either the sample volume is fixed and the diluent volume is additional, or the total volume delivered to the sample cup is fixed.

Response – Options for what to do in response to the cell count.

- **Continue even if cell reading failed**
- **Fail if do not get reading**

If the Dilution factor is greater than 1 then **Diluent** options specify the source for the diluent.

5.2.8.3 Flex2 cell count options

The **Flex2** options specify the options for the Flex2 sample.

The **Volume** to transfer is fixed.

The **Analysis sample type** specifies the cell count analysis that will be performed by the Flex2. Only analyses that perform a cell count only are shown in the drop down.

Response – Options for what to do in response to the cell count.

- **Continue even if cell reading failed**
- **Fail if do not get reading**

Dilution factor – Factor by which to dilute the sample to bring the cell density into a suitable range for the counter. A factor of 2 would be 2-fold dilution with 1 part cells and 1 parts diluent. Dilution factors can only be 1 (no dilution) or 2

The sample and diluent volumes are automatically calculated to give the specified **Dilution factor**.

5.2.9 Sample (manual)



On systems that have a liquid handler the manual pipetting steps are only shown if the **Show steps to prompt the user to take manual samples or add liquid by hand** option is selected in the **Advanced features** window.

The **Sample (manual)** step prompts the user to take a manual sample.

5.2.9.1 Prompt options

Respond within – how long to allow for the user to respond to the prompt when planning when interactions should happen.

5.2.9.2 Details options

Volume type – chooses between **Specified volume** if the volume is to be specified explicitly or **Calculated volume** if the volume is to be the result of an expression.

Volume – the volume to be removed by the user.

If the Volume type is **Calculated volume** then additional options deal with the limits to the allowed volume.

Minimum volume specifies the minimum volume to present as a requested sample volume. Lower volumes will be treated as an error or rounded to zero depending on the setting of **What to do if the volume is too low**.

Maximum volume specifies the maximum volume to present as a requested sample volume. Larger volumes will be treated as an error.

What to do if the volume is too low specifies what to do if the calculated volume is a small or negative value.

- **Volume less than min. is error** treats the volume as an error.
- **Round volume less than min to zero** rounds the volume to zero.
- **Round volume less than min to zero, error if negative** treats negative volumes as an error but rounds small positive volumes to zero.

5.2.10 Transfer bed to bed

The **Transfer bed to bed** step transfers liquid between labware on the bed.

Mapping type selects between:

- **Link source and destination** to bioreactors to specify the transfers by associated a source well and a destination well with each bioreactor.
- **List transfers** to specify a list of the transfers to be done without any particular reference to a particular bioreactor.

Source options specify the source of the transfer.

Destination options specify the destination of the transfer and, if the **Mapping type** is **Link source and destination**, the volume of liquid to be transferred.

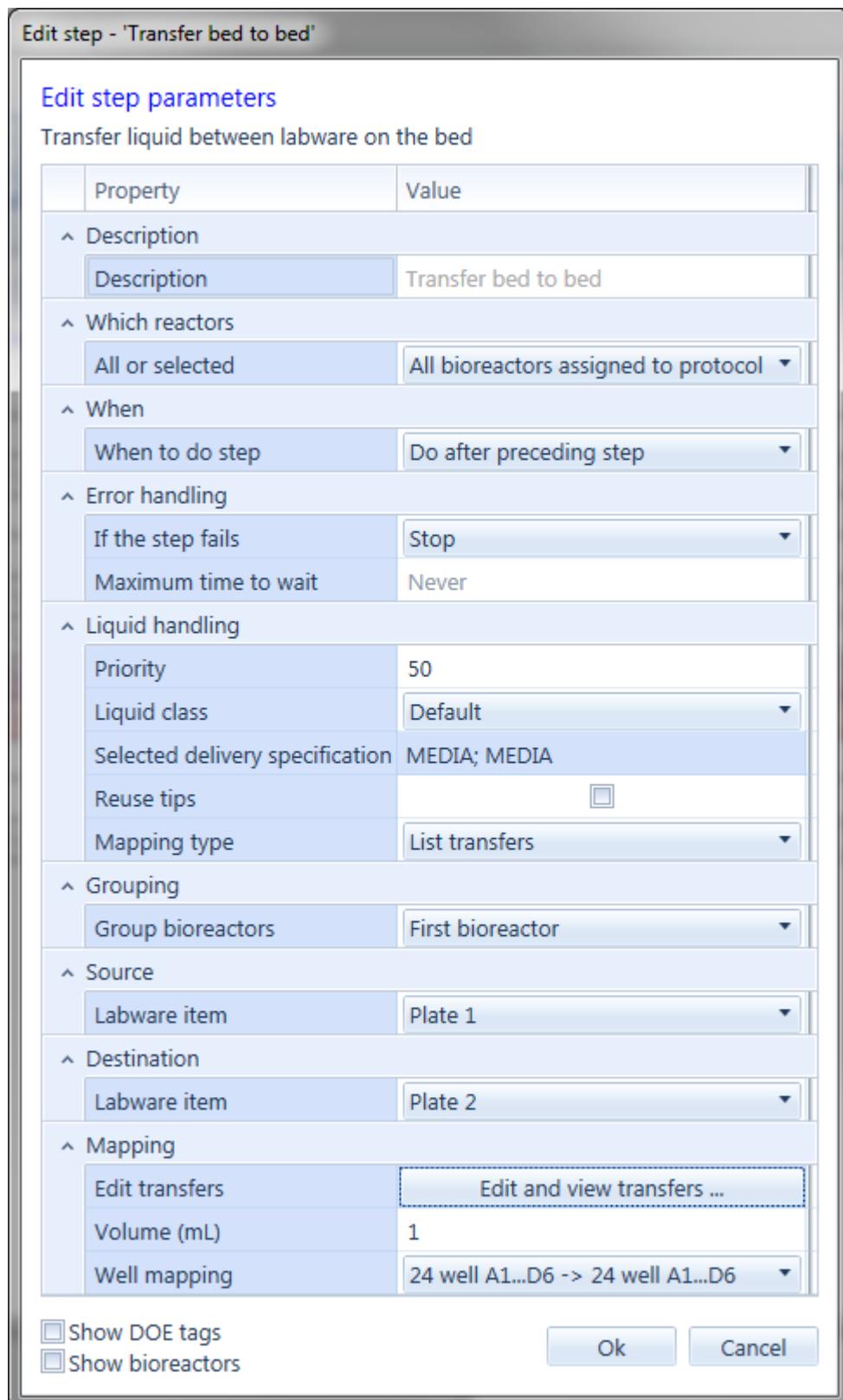


Figure 254 Transfer bed to bed step using **List transfers**

With **List transfers selected** the following **Mapping** options are available.

Edit transfers provides access to a window to edit the mappings.

Volume specifies the default volume to transfer.

Well mapping allows choosing between standard mappings and User defined.

If **User defined** is selected then **Transfers** shows a summary of the transfers.

Clicking **Edit and view transfers** displays the **Edit transfers for Transfer bed to bed** window.



Figure 255 *Edit transfers for Transfer bed to bed* window

The **Edit transfers for Transfer bed to bed** window shows the **Volume** and **Well mapping** options.

The labware for the transfer is shown with wells coloured to highlight the mapping between the labware.

The list of transfers is shown on the right of the window.

Add new transfer adds a new transfer to the list.

Source specifies the source of the transfer.

Volume specifies the volume of the transfer. By default the same volume is used for all the transfers, but this can be overridden for each transfer.

Destination specifies the destination of the transfer.

Import well mappings allows the list of transfers to be imported.

Export well mappings allows the list of transfers to be saved.

Transfers can also be edited by dragging and dropping within the mimic.

Transfers can be created by dragging from the source well to the destination well.



Figure 256 Creating a transfer by dragging between wells.

Existing transfers can be edited by dragging the source and destination of the transfer within the source or destination plate.

Selecting a well selects all the wells connected to it by transfers.

5.2.11 Run liquid handling script



Liquid handling scripts are enabled by the **Show liquid handling scripts and steps to use liquid handling steps** option in the **Advanced features** window.

The **Run liquid handling script** step runs liquid handling based on the specified liquid handling script for each bioreactor to which the step applies.



Figure 257 Run liquid handling script step

The **Liquid handling script** option specifies the script to run.

The system checks the selected script and adds options for the volume to transfer and the labware to use that are required by the selected script.

The volume in each case can either be specified explicitly – **Volume type of Specified volume** – or can be calculated – **Volume type of Calculated volume**.

For **Specified volume** the volume is specified by the **Volume** option.

For **Calculated** volume the volume is specified by a **Volume** option which has the expression for the volume.

What to do if volume is too low specifies how low and negative volumes should be handled.

Minimum volume specifies a minimum valid volume.

Maximum volume specifies a maximum valid volume. Volumes larger than this always represent a problem.

5.2.11.1 Overlapping liquid handling scripts

Some liquid handling scripts support overlapping pausing for later bioreactors with liquid handling work for previous bioreactors.

To make use of interleaving then in the **Run liquid handling script** step:

- 1) choose for **Group bioreactors** the option **All together**
- 2) Select the **Overlap operations** option

Enter into **Time to allow to run script** the total time to allow for the script to run including both the pause part of the script and the liquid handling part. The time should be large enough to allow for the largest volumes that will be used in the step, and the use of the bioreactors furthest from the other labware.

5.2.12 Set magnetic spinner

The Set magnetic spinner step turns the optional magnetic spinner under the liquid handling bed on or off and sets the speed of the magnetic spinner.

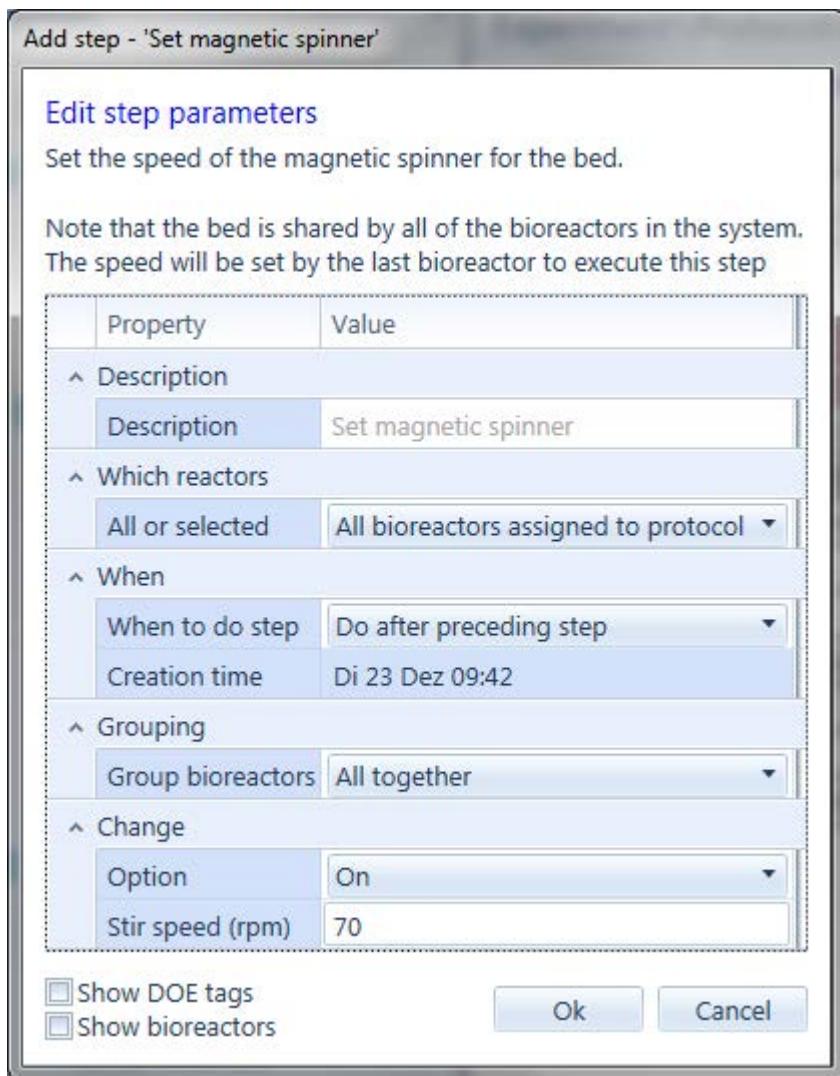


Figure 258 Set magnetic spinner step

Group bioreactors chooses between **All together** when the step will wait for the last bioreactor to arrive and then apply the changes and **One at a time** when the step will apply the changes as each individual bioreactor arrives.

Option chooses whether to turn the magnetic spinner on or off.

Stir speed sets the desired speed for the magnetic spinner.

5.2.13 Set freezer

The **Set freezer** step turns the temperature control of the optional frozen locations on the liquid handling bed on or off and sets the desired temperature.

N.B. It may be necessary to manually switch the chiller valve on/off depending on the temperatures required.

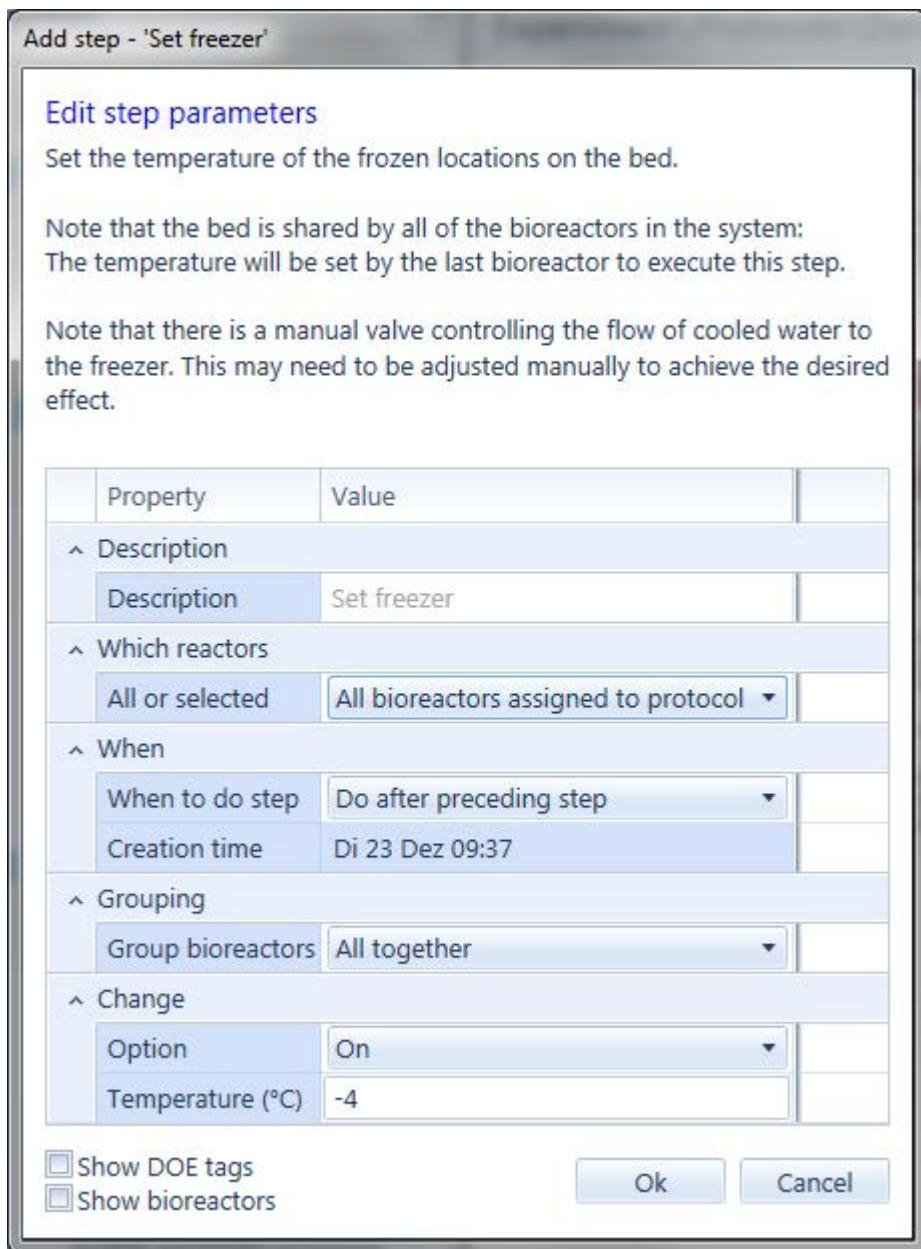


Figure 259 Set freezer step

Group bioreactors chooses between **All together** then the step will wait for the last bioreactor to arrive and then apply the changes and **One at a time** when the step will apply the changes as each individual bioreactor arrives.

Option chooses whether to turn the freezing on or off.

Temperature sets the desired temperature for the freezer.

5.3 Spot reader

The Spot reader steps allow control of the reader that scans the spots on the base of the bioreactor vessels.

DO spot reading is automatically turned on when valid calibration data is entered so these steps are not normally required.



Steps to turn DO spot reading on and off are enabled by the **Show steps to turn spot reader on and off** option in the **Advanced features** window.

5.3.1 Turn DO spot reading on

The **Turn DO spot reading on** step starts reading of the DO spots for the bioreactor.

The step raises an error if there is no calibration data for the spots when the step is executed.

5.3.2 Turn DO spot reading off

The **Turning DO spot reading off** step stops reading of the DO spots for the bioreactor.

5.3.3 Calibrate oxygen spot/sensor

The **Calibrate oxygen spot/sensor** step allows calibration of the off-gas oxygen sensor and/or the DO spot on a bioreactor.

This calibration is done with liquid in the bioreactor at the appropriate temperature maximising the accuracy of the off-gas readings when used to calculate OUR.

The step:

- 1) waits until all of the measurements required for the calibration are stable
- 2) updates the calibration figures for the spot or for the oxygen sensor

Before the step is executed the required gasses must have been turned on and – if the spot is being calibrated – the spot reader must have been enabled.

Calibration can be done for the low oxygen response at 0% DO and for the high oxygen response at either 100% DO (Air saturation) or at a custom air saturation.

For many processes the default calibration of the DO spots is adequate, especially at 0% DO.

To perform a calibration at 100% DO the process should:

- 1) Enable the spot reader
- 2) Enable gassing with air and turn off all other gasses
- 3) Wait for an interval to allow the system to equilibrate
- 4) Execute a Calibrate oxygen spot/sensor step

To perform a calibration at a custom air saturation the process should:

- 1) Enable the spot reader
- 2) Enable gassing with the required mixture of gasses
- 3) Wait for an interval to allow the system to equilibrate
- 4) Execute a Calibrate oxygen spot/sensor step

To perform a calibration at 0% DO the process should:

- 1) Enable the spot reader

- 2) Enable gassing with nitrogen and turn off all other gasses
- 3) Wait for an interval to allow the system to equilibrate
- 4) Execute a Calibrate oxygen spot/sensor step

Gassing in order to perform the calibration may strip carbon dioxide or other gasses from the media and so may not be appropriate for all cultures.

5.3.3.1 Timing options

The **Timing** options specify how long the step is expected to take to execute and what to do if the step does not complete in the expected time.

Timeout behaviour – What to do if the condition is not true after the step has been executing for the maximum wait time.

- **Stop** – the step should fail
- **Do next steps** – the protocol should proceed automatically with the next step
- **Wait forever** – the step should continue to wait forever

Minimum expected wait time – the minimum time that the step will wait for.

Maximum wait time – the maximum time that it is expected to take waiting for this step. Used when estimating when subsequent steps will be performed and depending on the timeout behaviour to decide what to do after the maximum time has expired.

5.3.3.2 Calibrate options

The **Calibrate** options specify what is to be calibrated.

What to calibrate – what to calibrate

- Just DO spot
- Just offgas sensor
- DO spot and offgas sensor

Which point – which end of the response is being calibrated and the expected gas concentration

- Air saturation
- Zero oxygen
- Custom upper oxygen concentration

Delay time – the time over which readings must all be steady before calibration is done.

5.3.3.3 Maximum parameter change options

The **Maximum parameter change** options specify for each reading involved in the calibration the maximum amount that it can vary by in the interval before the calibration completes.

For each parameter calibration waits until parameter changes by no more than this amount in one **Delay time** before doing the calibration.

- **Max. change oxygen reading (mV)**

- **Max. change CO₂ reading (%)**
- **Max. change in expected air saturation (%)**
- **Max. change spot phase (°)**
- **Max. change culture temperature (°C)**
- **Max. change off-gas temperature (°C)**

5.3.4 Turn pCO₂ spot reading on

The **Turn pCO₂ spot reading on** step starts reading of the CO₂ spots for the bioreactor.

The step raises an error if there is no calibration data for the spots when the step is executed.

5.3.5 Turn pCO₂ spot reading off

The **Turning pCO₂ spot reading off** step stops reading of the CO₂ spots for the bioreactor.

5.3.6 Calibrate pCO₂ spot (gassing)

The **Calibrate pCO₂ spot/sensor** step allows calibration of the CO₂ spot on a bioreactor.

The step

- 1) Waits until all the measurements required for the calibration are stable
- 2) Updates the calibration figures for the CO₂ spot

Before the step is executed the required gasses must have been turned on and the the spot reader must have been enabled.

To perform a calibration the process should.

- 1) Enable the spot reader.
- 2) Execute a **Turn pCO₂ spot reading on** step.
- 3) Enable gassing with **Gas flow (Air/Mix)** step and a **CO₂ mix** step with a CO₂ mix between 4 and 15% and turn off the other gasses.
- 4) Wait for an interval to allow the system to equilibrate
- 5) Execute a Calibrate pCO₂ spot step

Gassing to perform the calibration may affect the pH and the media and so may not be appropriate for all cultures.

5.3.6.1 Timing options

The **Timing** options specify how long the step is expected to take to execute and what to do if the step does not complete in the expected time.

Timeout behaviour – What to do if the condition is not true after the step has been executing for the maximum wait time.

- **Stop** – the step should fail
- **Do next steps** – the protocol should proceed automatically with the next step
- **Wait forever** – the step should continue to wait forever

Minimum expected wait time – the minimum time that the step will wait for.

Maximum wait time – the maximum time that it is expected to take waiting for this step. Used when estimating when subsequent steps will be performed and depending on the timeout behaviour to decide what to do after the maximum time has expired.

5.3.6.2 Calibrate options

Delay time – the time over which readings must all be steady before calibration is done.

5.3.6.3 Maximum parameter change options

The **Maximum parameter change** options specify for each reading involved in the calibration the maximum amount that it can vary in the interval before the calibration completes.

For each parameter calibration waits until parameter changes by no more than the specified amount in one **Delay time** before doing the calibration.

- **Max. change in expected CO₂ inflow (%)**
- **Max. change spot phase (°)**
- **Max. change in expected CO₂ saturation (%)**
- **Max. change culture temperature (°C)**
- **Max. change in atmospheric pressure**
- **Atmospheric pressure units**

5.4 Export

The system supports the periodic export of data to files. The **Export** steps turn that export on and off.



Steps to turn the export of data on and off are enabled by the **Support exporting data from the system periodically as CSV files** option in the **Advanced features** window.

5.4.1 Turn export on

The **Turn export on** step starts the periodic export of data for the bioreactor.

5.4.2 Turn export off

The **Turn export off** step stops the periodic export of data for the bioreactor.

5.5 Analysers

The steps below set the options for the analysers that may be connected to the system.

5.5.1 Set cell counter options

The **Set cell counter options** step allows the options for the cell counter readings to be set.

The **Cell Type** specifies the cell type profile to be used when analysing a cell count. The drop down list is populated from the configured cell types in the cell counter.

Use invalid cell counter readings specifies if the cell count readings are to be used and if the cell counter flags the readings as invalid.

5.5.2 Set Flex2 depro period

If the system is fitted with Flex2 analyser the **Set Flex2 depro period** step enables periodic automatic deproteinization of the Flex2 wells and External Sample Module (ESM) after sampling.

Option chooses whether to turn the depro period on or off.

Periodicity sets the desired time after the last flex sample when the deproteinization should be run.

When enabled a timer is started after a Flex sample for the specified period. If during this period another Flex sample is taken then the timer is reset. Once the period has elapsed the software schedules deproteinizations of the well and the ESM on the Flex2.



NOTE: The volume of deproteinization fluid in the Flex2 chemistry pack is dependent on the pack type and the ambr250 software does not monitor the fluid levels in the pack. Care should be taken not to schedule too many deproteinizations that will exhaust the fluid in the pack.

5.5.3 Set spectrometer options

The **Set spectrometer options** step sets the capture options for the spectrometer.

Capture settings selects the capture settings for the spectrometer that will be used from the list of capture setting profiles configured on the spectrometer server

Prediction settings selects the data model that will be used to predict analysis from the captures from the list of models configured on the spectroscopy server.

5.5.4 Calibrate pCO₂ spot (one point)

The **Calibrate pCO₂ spot (one point)** step calibrates the CO₂ spot from a measured value.

Typically this step will follow a step that measures or imports a pCO₂ reading.

5.5.4.1 Error handling

Depending on what has been chosen for **If the step fails** then either the protocol will move on and **Do next steps** or **Stop** and wait for the user to deal with the error.

5.5.4.2 Reading

The **Measured value** specifies a simple expression for the measured pCO₂ based on the variable with the pCO₂ measurement.

5.5.4.3 pCO₂ offset change parameter options

The **pCO₂ offset** options specify the options for the CO₂ spot calibration.

Ignore offset changes below – if set and the change to the offset would be less than this amount then do not adjust the offset.

Warn if change offset above – if set then raise a message if the change to the offset is greater than this amount.

Max. allowed change to offset – if set fail the step if the change to the offset is greater than this amount.

Max. age of measured reading – fail the step if the measured value is older than the specified value.

5.5.4.4 Correction to reading

Offset to add to raw pCO₂ allows and expression to define an offset that is added to the raw result. Typically used to make an allowance for out gassing from samples to improve the correlation between the pCO₂ measured using an analyzer and the pCO₂ measured using other procedures.

5.6 Analysis module

The steps below support the operation of an integrated ambrAM that can measure pH and other parameters.

5.6.1 Set pH measurement temperature

This step sets the temperature at which the pH measurement is performed within the ambrAM.

Group bioreactors selects between:

- **One at a time** – to set the temperature for each bioreactor executing the step
- **All together** – to wait until all the bioreactors for the step execute the step and then set the temperature once.

Temperature specifies the temperature to set.

5.6.2 Calibrate analysis module

This step can be used to make the analysis module do a calibration.

This step is not normally required in a process: the analysis module calibrates itself automatically when required.

Group bioreactors selects between:

- **First bioreactor** – to do the calibration once when the first bioreactor executes the step.
- **Last bioreactor** – to do the calibration once when the last bioreactor executes the step.

Calibration selects the calibration to perform:

- **pH** does a 2-point pH calibration
- **pH quick** does a 1-point pH calibration

5.6.3 Check analysis module calibration

This step can be used to make the analysis module run a check on its calibration by measuring one of its reference liquids and if that reading is too far out according to the modules internal criteria performing a full calibration.

This step is not normally required in a process: the analysis module calibrates itself automatically when required.

Group bioreactors selects between:

- **First bioreactor** – to do the calibration once when the first bioreactor executes the step.
- **Last bioreactor** – to do the calibration once when the last bioreactor executes the step.

Calibration selects the calibration to perform:

- **pH** does a 2-point pH calibration

5.6.4 Clean analysis module

This step runs a cleaning cycle on the analysis module.

This step is not normally required in a process: the analysis module cleans itself automatically after each sample.

Group bioreactors selects between:

- **First bioreactor** – to do the calibration once when the first bioreactor executes the step.
- **Last bioreactor** – to do the calibration once when the last bioreactor executes the step.

5.6.5 Set analysis module options

This step enables the Measurement mode for the analysis module to be set for the bioreactors. By default the analysis module will run in normal cell density mode.

Mode selects between:

- **Normal cell density** – up to 60×10^6 mammalian cells/ml
- **High cell density** – up to 100×10^6 mammalian cells/ml

5.7 Foam sensor

The system allows the foam sensor to be turned on and off.

By default the foam sensor is turned on for the bioreactor. It only needs to be turned off if the culture is particularly light sensitive.



Steps to turn the foam sensor on and off are enabled by the **Show steps to turn foam sensor on and off** option in the **Advanced features** window.

5.7.1 Turn foam sensor on

The **Turn foam sensor on** step enables the foam sensor for the bioreactor.

5.7.2 Turn foam sensor off

The **Turn foam sensor off** step disables the foam sensor for the bioreactor.

5.8 Set points

The **Set points** steps allow the values of individual set points to be set to a constant or changing value and for the control loops used to effect the set points to be selected where applicable.

As soon as the step has stored the instructions for the values the set point will follow control passes to the next step in the protocol. The system does not wait for any ramp or profile to complete before moving on to the next step in the protocol.

The Set points steps all share a set of common options described below.

5.8.1 Change options

Option – specifies how to set the set point.

The options available depend on which advanced features have been enabled – profiles, pausing and overrides are all controlled as advanced features.

- **Off** – the set point is turned off and any control loops controlling the set point are turned off
- **Set** – the set point is set to a fixed value
- **Ramp over time** – the set point is set to ramp from its current value when the step is executed to a new value over a specified interval
- **Ramp at rate** – the set point is set to ramp from its current value when the step is executed to a new value over at a specified rate
- **Profile** – the values of the set point are set to follow a specified profile
- **Calculated** – the set point is set to a calculated value
- **Change control loops** - the control loops used to effect the set point are changed. The value of the set point is not changed.
- **OPC** – the set point is exposed for writing over the OPC interface. Values set over that interface control the set point. This option is only available on systems where the OPC interface has been licensed.
- **Pause control** – pauses the control loops maintaining the set point for a specified duration.
- **Clear pause** – un-pauses the control loops maintaining the set point.
- **Override value** – assigns a temporary override value for the set point for a specified duration.
- **Clear override** – clears any temporary override of the set point.

Use upper and lower setpoint – allows both upper and lower setpoints if the control loop supports them.

If the **Option** is **Set**, **Ramp over time** or **Ramp at rate** then:

Set point – specifies the new value for the set point.

For the ramping options

Rate of change – specifies the rate at which to change from the current value of the set point to the new set point.

Time to change – specifies the time over which to change from the current value of the set point to the new set point.



Profiles are an advanced feature enabled by the **Allow defining set points as profiles** option in the **Advanced features** window.

If the **Option** is **Profile** then:

Selected profile – specifies the profile to use for each bioreactor from the profiles stored under the Profiles option.

Profiles – stores a set of profiles for use in this step

If the **Option** is **Calculated** then the value of the set point is defined by **Expression**. **Calculate expression** specifies when the expression should be evaluated:

- **Once when set point changed** – the expression is evaluated once when setting the set point.
- **Continuously** – the expression is continuously with the set point being updated accordingly.

See section 3.6.6 above for more details of profiles.

5.8.1.1 Overrides and pauses



Overriding and pausing are advanced features enabled by the **Allow overriding set points** and the **Allow pausing control of set points** options in the **Advanced features** window.

Overriding a value gives a new temporary value to a set point for the duration of the override. The override replaces temporarily both any value given directly to the set point and any value given to the set point by a control loop.

Pausing a value turns off the control loops giving effect to that value. Pausing can be applied to those set points controlled by control loops in the process – pH, DO – and to Temperature.

If **Override value** is selected then **Override time** allows the duration of the override to be specified.

If **Pause control** is specified then **How long to pause** for allows the duration of the pause to be specified. Where the control loops being paused are part of the process definition then there are additional options to specify how the outputs of the control loop are handled.

Outputs while paused has options **Default**, **Off** and **Average**. **Off** turns the outputs off for the duration of the pause. **Average** sets the outputs to their average interval over the last **Averaging time**. **Default** turns pump outputs off and sets other outputs to their average value.

5.8.2 Set point steps – When to finish step

Set point steps – e.g. pH, Temperature – that have a natural end have an option **When to finish step** that selects between:

- **Step finishes immediately**

And one of the options below as appropriate to the selected option.

- **Wait for ramp to complete** or
- **Wait for profile to complete** or
- **Wait until set point is not overridden** or
- **Wait until set point is not paused**

Wait for ramp to complete waits until the ramp has reached its final value.

Wait for profile to complete waits until the profile has reached its final point before completing.

Wait until flow rate is not overridden waits until the set point is no longer overridden. This may be because the override applied by the step has timed out or may be because some other action on the system has cleared the override.

Wait until flow rate is not paused waits until the set point is no longer paused. This may be because the pause applied by the step has timed out or may be because some other action on the system has cleared the pause.

5.8.3 pH change options

For pH there are additional change options because pH has separate upper and lower set points.

By default the upper and lower set points are both given the same value, and the dead bands of the control loops controlling pH prevent conflicting additions of acid and base.

Use upper and lower set points – select the use of separate lower and upper setpoints.

If **Use upper and lower set points** is selected then the new value of the set point is specified by **Upper set point** and **Lower set point**.

5.8.4 Control loops options

Where control of the set point is not built into the system the control loop to affect the set point must be specified.

Control loop specifies the control loop to use to keep the output at the setpoint.

5.8.5 pH Control loops options

pH control typically uses two loops – one to add acid and one to add base.

Control mode – selects which control loops are required. The options are:

- Acid and base
- Acid only
- Base only

Upper set point control loop specifies the control loop to use to keep the pH below the upper setpoint, typically by adding acid.

Lower set point control loop – The control loop to use to keep the pH above the lower setpoint, typically by adding base.

5.8.6 pCO₂ Control loop options

pCO₂ control typically uses two loops – one to control the upper setpoint and one to control the lower setpoint.

Control mode – selects which control loops are required. The options are:

- Control to upper and lower setpoints
- Control to upper setpoint only
- Control to lower setpoint only

Upper set point control loop specifies the control loop to use to keep the pCO₂ below the upper setpoint.

Lower set point control loop – The control loop to use to keep the pCO₂ above the lower setpoint.

5.8.7 Changing and initialising control loops

The options **How to initialise loop**, **How to initialise lower loop** and **How to initialise upper loop** control how the corresponding cascade control loop is initialised. These options are not shown for other types of control loop.



The options **How to initialise loop**, **How to initialise lower loop** and **How to initialise upper loop** are only shown if the **Support preserving values when switching to or between control loops** option is selected in the **Advanced features** window.

- **Reset loop on first use** resets the loop the first time it is used.
- **Reset loop** explicitly resets the loop
- **Match from bottom** sets the start of the loop by working through each level of the cascade from the bottom. When a level is found that can be matched to the current output of the system then that output is used to set the level and output of the cascade.
- **Match from top** sets the start of the loop by working through each level of the cascade from the top. When a level is found that can be matched to the current output of the system then that output is used to set the level and output of the cascade.
- **Match specified output** sets the start of the loop by matching the specified output of the system. The corresponding option of **Output from which to initialise loop**, **Output from which to initialise lower loop** or **Output from which to initialise upper loop** specified which of the cascade control loop's outputs is matched.
- **Set output** sets the state of the loop by setting the value of **Output from which to initialise loop**, **Output from which to initialise lower loop** or **Output from which to initialise upper loop** to the value specified as **Initial value for loop output**, **Initial value for lower loop output** or **Initial value for upper loop output**.
- **Set calculated output** sets the state of the loop by setting the value of **Output from which to initialise loop**, **Output from which to initialise lower loop** or **Output from which to initialise upper loop** to the value or the expression specified as **Initial value for loop output**, **Initial value for lower loop output** or **Initial value for upper loop output**.

5.8.8 Available set points

The built in set points that can be set by steps are described below. In addition custom variables with a set point can have the value of that set point set.

Temperature controls the temperature of the liquid in the bioreactor.

pH controls the acidity of the liquid in the bioreactor.

DO controls the dissolved oxygen content of the liquid in the bioreactor.

Stir speed and **Stir direction** control the stirring of the bioreactor. Depending on the configuration of the bioreactor vessel the **Stir direction** can be **ACW** or **CW** for anti-clockwise and clockwise respectively, or can be **Up** or **Down**.

Gas flow (GAS/mix), **GAS mix** and **GAS added flow** control the flow of gas GAS into the bioreactor. The set points available depend on the configuration of the system and the gassing option chosen.

More direct control of certain outputs may be available:

Air valve open, **O₂ valve open**, **N₂ valve open**, **CO₂ valve open** provide direct control of the gassing valves.

Chiller and **Heater** provide direct control of the chilling and heating of the bioreactor if the temperature is not being controlled directly.



Direct control of gas valves, chilling and heating is enabled by the **Allow control of advanced set points** option in the **Advanced features** window.

Other set points in the system such as the pump flow multipliers are only usefully set by control loops and cannot be set by steps.

Turning on control of a set point via a control loop stops direct control of the set point value and when the control loop is disabled the set point remains off until a new value is set.

5.8.8.1 Example 1

This example describes the effect of enabling and disabling a control loop.

We assume there is a loop DO_Stir_Gas that sets the **Stir speed** and **Gas flow (Air/mix)** outputs.

- 1) **Gas flow (Air/mix)** is set to 100mL/min
- 2) **Stir speed** is set to 150 rpm
- 3) Sometime later **DO** is set to 20% with control by the DO_Stir_Gas control loop. The values of **Gas flow (Air/mix)** and **Stir speed** are set by the control loop.
- 4) **DO** is set to be **Off**. **Gas flow (Air/mix)** and **Stir speed** are both **Off** also.

5.8.8.2 Example 2

This example describes the effect of enabling and disabling higher level control of a parameter where a lower level control is also available.

- 1) **Chiller** is set to 50%
- 2) Sometime later **Temperature** is set to 37 °C. The control built into the system is now controlling the **Chiller**.
- 3) **Temperature** is set to be **Off**. **Chiller** is also **Off**.

5.9 Pumps

The **Pumps** steps provide control of the pumps connected to the bioreactors.

All the Pumps steps have a **Pump** option that specifies the pump to use by its role e.g. Feed#1 or Antifoam or Acid.

The default operation of pump steps is to set the pump doing some pumping whether continuously or as a bolus and then to complete as soon as the pump is able to take that instruction. The steps do not by default wait for boluses to finish. The option **When to finish step** can be used when there is a natural end to the pumping and selects between:

- **Step finishes immediately** – the default behaviour

And one of the options below as appropriate to the selected pumping.

- **Wait for bolus to complete** or
- **Wait for flow rate to reach final value** or
- **Wait for profile to complete** or
- **Wait until flow rate is not overridden**

Step finishes immediately is the behaviour in older versions of the software where the pump is given its instructions and the step completes immediately.

Wait for bolus to complete waits for a bolus to be delivered.

Wait for flow rate to reach final value waits for an exponential or linear feed to reach its limiting value.

Wait for profile to complete waits until the profile has reached its final point before completing.

Wait until flow rate is not overridden waits until the pump is no longer overridden. This may be because the override applied by the pumping step has timed out or may be because some other action on the system has cleared the override.

5.9.1 Start pumping

The Start pumping step starts pumping on a step. As soon as the instructions for pumping have been passed to the pump control passes to the next step in the protocol. The step does not wait for any bolus or profile to be complete.

The **Change** options specify how fast to pump for and how long to pump for.

Option specifies the sort of profile to follow.

- **Constant** – pump pumps indefinitely at a constant rate

- **Linear** – pump pumps at flow rate increasing or decreasing linearly with time
- **Exponential** – pump pumps at a flow rate increasing or decreasing exponentially with time
- **Bolus** – pump pumps a fixed volume at a specified flow rate and then stops
- **Generic** – a combination exposing the parameters for Linear and Exponential. Only one of the Linear term and Exponential term can be non-zero for each bioreactor.



The **Generic** option is an advanced feature enabled by the **Deliver pumping profile by combination of linear and exponential parameters** option in the **Advanced features** window.

- **Profile** – pumps following a specified profile.
- **Calculated** – the set point is set to a calculated value
- **OPC** – the set points for the flow rate and for the pump volume at which to stop pumping are exposed for writing over the OPC interface. Values set over that interface control the set point. This option is only available on systems where the OPC interface has been licensed.

Initial flow rate (mL/h) specifies the initial flow rate a

Linear term (mL/h/h) specifies the linear term b in the flow rate

Exponential term (/h) specified the exponential term c in the flow rate

If t is the time since the pumping instruction was given then the flow rate F is given by:

$$F = ae^{ct} + bt$$

Final flow rate (mL/h) specifies the final flow rate. Once a **Linear**, **Exponential** or **Generic** flow reaches the **Final flow rate** then the flow rate is capped at the **Final flow rate**.

When telling a pump to use a linear or exponential profile the **Final flow rate** can be left null (empty). In this case the flow rate will increase to the maximum rate supported by the pump.

This limit is applied after any corrections to the flow rate from the **Adjust for sampling** feature.

When a **Final flow rate** is specified then that rate takes effect before the **Adjust for sampling** feature and so the actual flow rate from the pump can be limited to a lower value than desired.

Expression (mL/hour) specifies the expression for the flow rate when **Calculated** is selected.

Within the expression ‘**Time**’ refers to the time in hours since the pumping instruction was given.

Total volume (mL) specifies the total volume to pump as a bolus.

Adjust for sampling specifies what adjustments if any are made to take account of the effect of sampling on the number of cells remaining relative to the number of cells that would remain in a large bioreactor where sampling is completely negligible. The options are:

- **None** – no adjustments for samples are made.
- **Flow rate and volume (sampling since inoculation)** – pumping is reduced in proportion to the cells lost to sampling since the bioreactor was inoculated.
- **Flow rate and volume (sampling after step)** – pumping is reduced in proportion to the cells lost in sampling after the step has started the profile.

If the **Option** is **Profile** then:

Selected profile – specifies the profile to use for each bioreactor from the profiles stored under the Profiles option.

Profiles – stores a set of profiles for use in this step

The **Move liquid to end** option specifies what should happen if the liquid has not already been pumped beyond the end of the bioreactor inlet. The options are:

- **First move liquid to end**
- **Pump from current position**

Select **Pump from current position** if you do not want the system to automatically move liquid to the end of the bioreactor inlet.

The **Move liquid to end** option is not shown when controlling a pump directly and the pump has already pumped liquid into the bioreactor.

5.9.1.1 Permeate pump

When the Permeate pump is selected then steps to start pumping have an additional option **Perfusion filter** to control priming of the outside of the hollow fibre filter. The options are:

- **Prime if required**
- **Pump from current position**

Select **Pump from current position** if you do not want the system to automatically prime the outside of the hollow fibre filter.

5.9.1.2 Pulse Flow options



Pulse flow is an advanced feature enabled by the **Deliver specified flow as pulses** option in the **Advanced features** window.

Depending on the configuration of the system and the advanced features selected then the **Start pumping** step may have **Pulse flow** options.

Use pulse flow selects option to deliver the flow is a sequence of pulses

Pulse on time the time that flow should be on within a cycle. Flow rate while pulse is on is adjusted to give the correct overall flow rate.

Pulse off time the time that flow is off within a cycle.

5.9.2 Calculated bolus

The **Calculated bolus** step sets a pump pumping a bolus where the volume is specified by an expression. Like other pumping steps the step does not wait for the bolus to complete.

The **Calculated bolus** step has the following **Change** options.

Flow rate (mL/h) the rate at which to pump the bolus – if not specified the bolus is pumped in as fast as possible

Volume (mL) an expression for the volume to pump.

Adjust flow for sampling selected between **None** and **Flow rate and volume**. If **Flow rate and volume** is selected then the total volume pumped and the flow rate are reduced to allow for the effect of sampling on the number of cells remaining relative to the number of cells that would remain in a large bioreactor where sampling is completely negligible.

Maximum volume (mL) the maximum allowed value for the amount to pump. If the expression evaluates to a larger value then an error is raised.

The **Calculated bolus** step always moves liquid to the end of the bioreactor inlet before starting.

The **Calculated bolus** step records the volume of the bolus to be pumped in the variable '[Role] calc. bolus volume' where **[Role]** is the role of the selected pump e.g. Feed#1.

What to do if the volume is too small controls what happens if the calculated bolus volume is negative:

- **Do not do transfer** and
- **Error if negative**.

What to do if volume is too large controls what happens if the calculated bolus volume is greater than the maximum:

- **Error if volume too large** and
- **Transfer maximum volume**

5.9.3 Start calculated pumping

The **Start calculated pumping** step sets a pump pumping with the parameters of the pumping profile calculated as expressions.

When the system comes to run the step it evaluates the expressions. If the resulting parameters are valid then the system starts pumping with the specified parameters. If the parameters are invalid then an error is raised and the step waits until the situation is resolved.

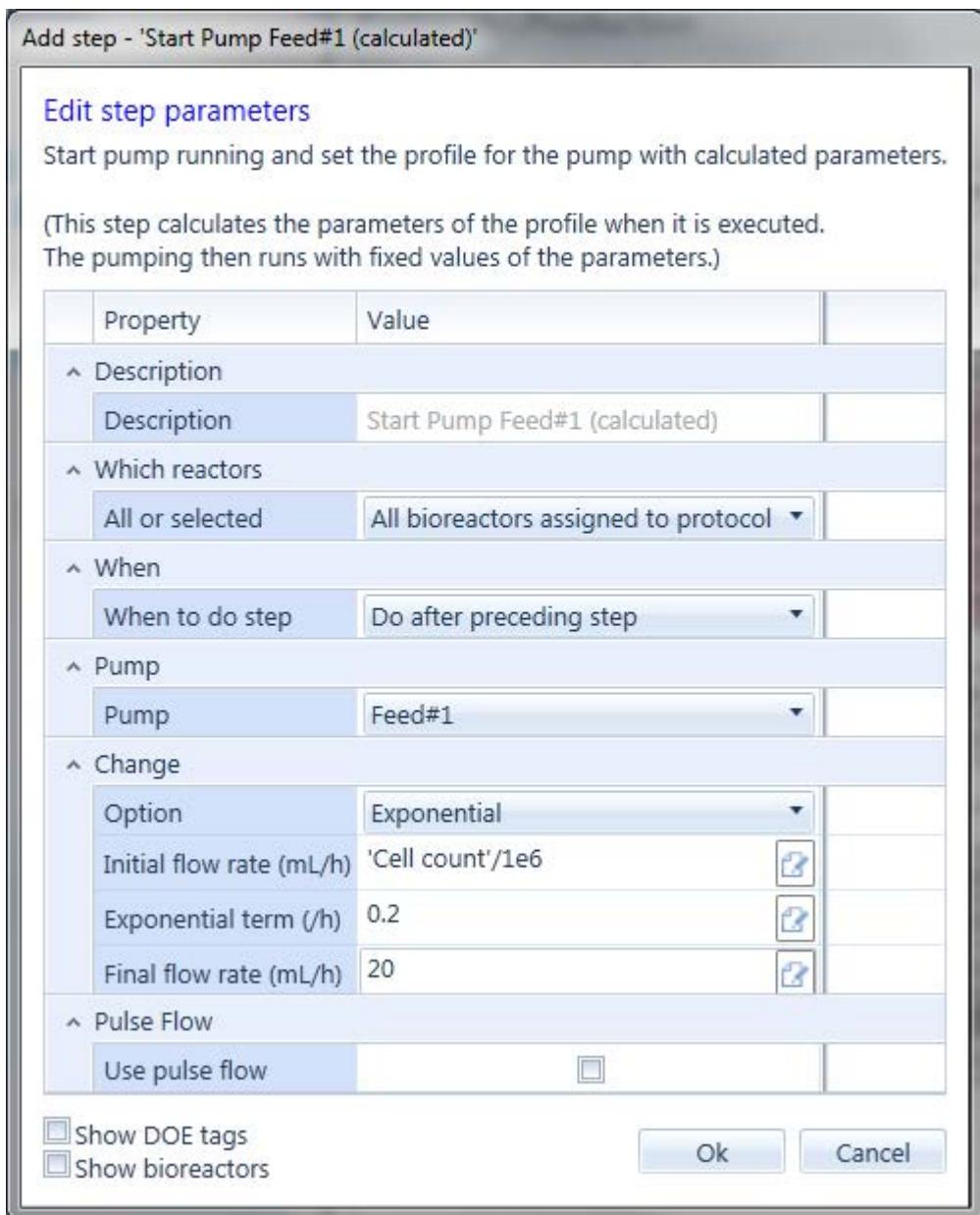


Figure 260 *Start calculated pumping* step window

The **Change** options specify how fast to pump for and how long to pump for.

Option specifies the sort of profile to follow.

- **Constant** – pump pumps indefinitely at a constant rate
- **Linear** – pump pumps at flow rate increasing or decreasing linearly with time
- **Exponential** – pump pumps at a flow rate increasing or decreasing exponentially with time

Initial flow rate (mL/h) specifies an expression which is evaluated to give the initial flow rate a

Linear term (mL/h/h) specifies an expression which is evaluated to give the linear term b in the flow rate

Exponential term (/h) specified an expression which is evaluated to give the exponential term c in the flow rate

If t is the time since the pumping instruction was given then the flow rate F is given by:

$$F = ae^{ct} + bt$$

Final flow rate (mL/h) specifies an expression which is evaluated to give the final flow rate.

Once a **Linear** or **Exponential** flow reaches the **Final flow rate** then the flow rate is capped at the **Final flow rate**.

Adjust flow for sampling selected between **None** and **Flow rate and volume**. If **Flow rate and volume** is selected then the total volume pumped and the flow rate are reduced to allow for the effect of sampling on the number of cells remaining relative to the number of cells that would remain in a large bioreactor where sampling is completely negligible.

Pulse flow options if present work as for the **Start pumping** step.

The **Start calculated pumping** step always moves liquid to the end of the bioreactor inlet before starting.

5.9.4 Stop pumping

The **Stop pumping** step stops the specified pump.

5.9.5 Reset pumped volume

The **Reset pumped volume** step resets the pumped volume for the specified pump. The volume pumped after the reset is recorded in the variable **xxx volume since reset** where xxx is the specified pump.

5.10 Sequencing

The **Sequencing** steps allow grouping steps together so that they can be run at particular times or run conditionally.

5.10.1 If condition

The **If condition** step allows conditionally doing or skipping steps. When executed the step does the steps in the **When TRUE** or **When FALSE** section as appropriate. The steps in the other section are marked as skipped

2.9		Tue 20 Aug 14:50	Tue 20 Aug 15:15	If condition	<input checked="" type="checkbox"/> <input type="checkbox"/> <input type="radio"/>
When TRUE					
2.9.1.1		Tue 20 Aug 14:50	Tue 20 Aug 15:02	Sample with cell counter	<input checked="" type="checkbox"/> <input type="checkbox"/> <input type="radio"/>
When FALSE					
2.9.2.1		Tue 20 Aug 15:02	Tue 20 Aug 15:15	Sample with Flex2	<input checked="" type="checkbox"/> <input type="checkbox"/> <input type="radio"/>

Figure 261 If condition with contained steps

The condition in the **If condition** step works in the same way as the condition in the **Wait condition** step, but is restricted to conditions that can be evaluated at an instant in time and are not looking for changes to values.

Edit step - 'If condition'

Edit step parameters

Executed one or other of the sets of steps contained in the block depending on whether the condition is true or not.

Property	Value
Description	
Description	If condition
Which reactors	
All or selected	All bioreactors assigned to protocol
Clauses	
Number of clauses	1
Applies to	This bioreactor
Condition 1	
Expression	Cell density
Comparison	Greater than
Compare against	Value
Value	50

Show DOE tags Show bioreactors

Figure 262 If condition step

(When simulating the process the steps in both branches are simulated. As a result the simulation may differ from what will actually happen by for example over estimating how many tips the liquid handler will use.)

5.10.2 One bioreactor at a time step

The **One bioreactor at a time** step allows one or more steps to be grouped so that the system only allows the step or steps to be executing for one bioreactor at a time.

The step is intended for the case where one wants the system to perform work in the order:

- Step 1 for Bioreactor A
- Step 2 for Bioreactor A
- Step 1 for Bioreactor C
- Step 2 for Bioreactor C
- ...

The step can also enforce that the bioreactors are processed from left to right instead of in arrival order. Where left to right ordering is wanted this can provide a more robust ordering than setting the priorities for different bioreactors in the liquid handling step.

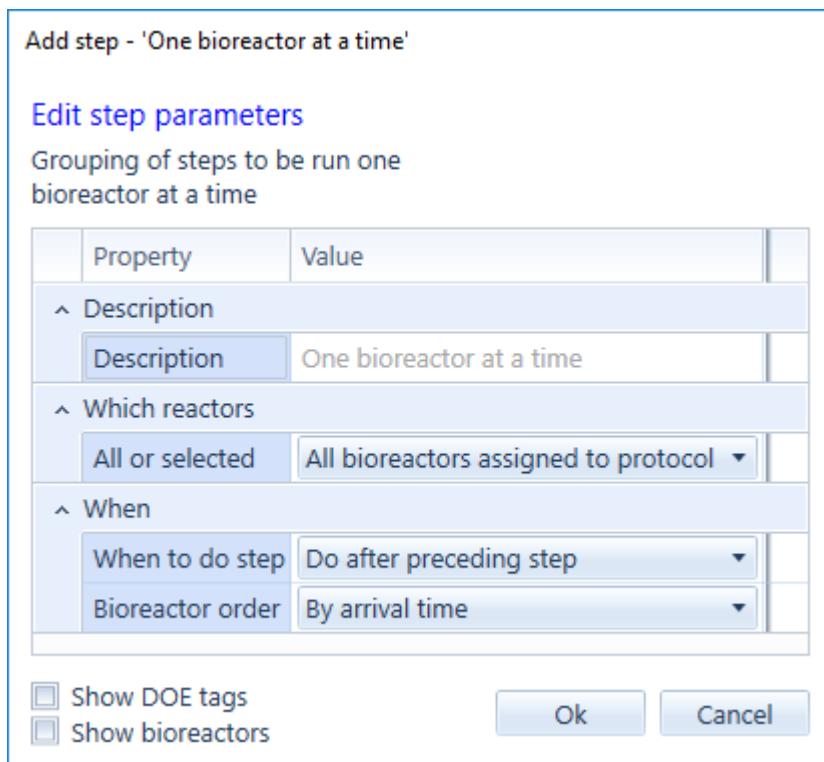


Figure 263 One bioreactor at a time step

The Bioreactor order option allows choosing between:

- **By arrival time** to have the first bioreactor to reach the step be processed first and
- **Left to right** to have bioreactors wait and proceed in left to right order.
- **Expression (lowest first)** to have bioreactors processed in order of the value of the given expression.

When the order is not **By arrival time** then **Maximum time to wait for predecessors** can be set to allow bioreactors to proceed out of order if there is a problem.

Steps within the **One bioreactor at a time** step are shown indented.

Phase				
1.1	Wed 03 Apr 15:27	Wed 03 Apr 15:27	Comment:	<input checked="" type="checkbox"/> <input type="checkbox"/>
1.2	Wed 03 Apr 15:27	Wed 03 Apr 17:47	One bioreactor at a time	<input checked="" type="checkbox"/> <input type="checkbox"/>
1.2.1	Wed 03 Apr 15:27	Wed 03 Apr 17:42	Sample first	<input checked="" type="checkbox"/> <input type="checkbox"/>
1.2.2	Wed 03 Apr 15:33	Wed 03 Apr 17:47	Sample as soon as possible after pre	<input checked="" type="checkbox"/> <input type="checkbox"/>
1.3	Wed 03 Apr 15:39	Wed 03 Apr 17:47	Comment:	<input checked="" type="checkbox"/> <input type="checkbox"/>

Figure 264 One bioreactor at a time step with two samples

5.10.3 Parallel block

The **Parallel block** provides a container for steps to be executed in parallel with the steps in the Phase containing the Parallel block.

This can be useful for example if there are Sample steps which should happen at specified times independent of the progress of the rest of the experiment.

5.10.3.1 Stop Condition options

The **Stop condition** options specify what should happen when the phase containing the **Parallel block** completes its other steps.

Stop activities selects when system should stop doing the steps within the **Parallel block**.

- **After performing steps in block** – the steps in the **Parallel block** are completed even if the enclosing phase is otherwise complete.
- **When containing phase completes** – once the enclosing phase is otherwise complete remaining steps in the **Parallel block** are skipped.
- **When specified phase completes** – once the specified phase is otherwise complete remaining steps in the **Parallel block** are skipped.
- **When specified day completes** – the block stops when the specified day completes. Note that the day does not complete until all the steps in the day have been completed and midnight is reached so there is no possibility of more steps being added to the day.
- **When specified step completes** – the block stops when the specified step completes.

If **When specified phase completes** is selected then Phase selects the phase at the end of which to stop steps within this **Parallel block**.

5.10.4 Phase

Phase steps provide:

- a grouping for steps to help make the process structure clearer
- a time reference for when steps within the Phase can happen
- control of when steps in Parallel blocks are skipped

5.10.5 Repeating block

The **Repeating block** step allows a set of steps to be executed repeatedly as part of a process.



The **Repeating block** step is hidden unless the **Show step to repeatedly run a block of steps** option is selected in the **Advanced features** window.

Add step - 'Repeating block'

Edit step parameters

Repeat the enclosed steps

Property	Value
Description	
Description	Repeating block
Comments	
Which reactors	
All or selected	All bioreactors assigned to protocol
When	
Creation time	Mon 11 Oct 17:11
Frequency	
Repetition basis	Minimum repeat time
Minimum loop time	5m
Stop Condition	
Stop activities	Stop after duration
When stopping	Stop as soon as possible
Maximum duration	1d
<input type="checkbox"/> Show DOE tags	<input type="button" value="Ok"/>
<input type="checkbox"/> Show bioreactors	<input type="button" value="Cancel"/>

Figure 265 Repeating block step

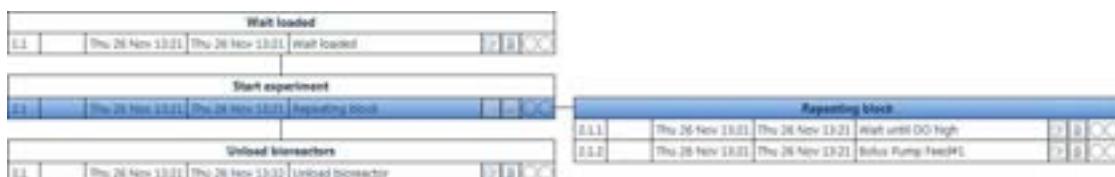


Figure 266 Minimal process containing Repeating block

Note that because the steps in the Repeating block are executed repeatedly the display only shows the current state of the block.

- Any errors are cleared each time the block repeats.
- Steps in the block can be edited even if they have completed. Only the latest parameters of the step are visible.
- A full history of the steps run by the system is shown in the Audit trail.

5.10.5.1 Options

The **Repeating block** step has options described below.

Repetition basis controls how to specify the frequency with which steps are repeated.

- If **Minimum repeat time** is selected then the **Minimum loop time** sets a minimum time between starting one pass through the steps and starting the next pass through the steps.
- If **Dead time before repeat** is selected the **Dead time** sets the time between ending one pass through the steps and starting the next pass through the steps.
- If **Periodic from start of steps** is selected then the steps are repeated periodically as specified by **Period**. Should the steps not complete before the next period is due then that period is skipped.

5.10.5.2 Stop Condition options

The **Stop condition** options specify when the **Repeating block** should stop repeating its steps.

- **When containing phase completes** – once the enclosing phase is otherwise complete remaining steps in the **Repeating block** are skipped.
- **When specified phase completes** – once the specified phase is otherwise complete remaining steps in the **Repeating block** are skipped.
- **When specified day completes** – the block stops when the specified day completes. Note that the day does not complete until all the steps in the day have been completed and midnight is reached so there is no possibility of more steps being added to the day.
- **When specified step completes** – the block stops when the specified step completes.
- **Stop after duration** – the block stops after the specified **Maximum duration**.

If **When specified phase completes** is selected then Phase selects the phase at the end of which to stop steps within this **Repeating block**.

When a block is stopping the **When stopping** option allows a choice between **Stop as soon as possible** and **Stop at end of block**.

5.10.6 Steps on day

The **Steps on day** step provides a grouping for steps to be run on a particular day within a **Phase**.



The **Steps on day** and **Steps on days within phase** steps are hidden unless the **Show steps to support structuring process as actions performed on different logical days** option is selected in the **Advanced features** window.

Steps within a **Steps on day** step can have a time of day specified at which the step will run.

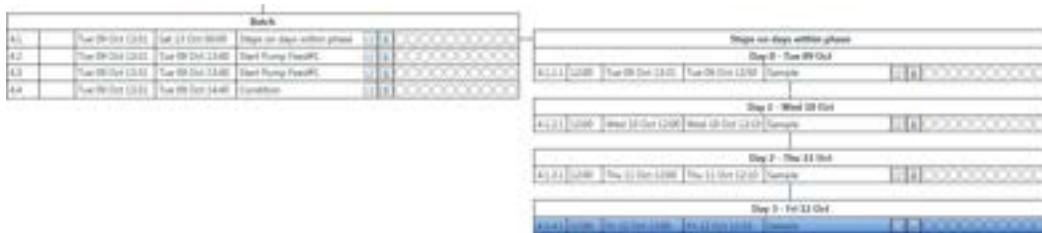


Figure 267 **Steps on day** steps within **Steps on days within phase** step. Each **Steps on day** step has a **Sample** step scheduled to occur at 12:00

5.10.7 Steps on days within phase

The **Steps on days** within phase provides a container for **Steps on day** steps that allow steps to be run at logical days and times within a **Phase**.



The **Steps on day** and **Steps on days within phase** steps are hidden unless the **Show steps to support structuring process as actions performed on different logical days** option is selected in the **Advanced features** window.

Typically tThe **Steps on days within phase** should step must be the first step within the phase. Unexpected results will occur if there is a significant delay between starting the phase and starting the **Steps on days within phase** step.

5.10.7.1 Stop Condition options

The **Stop condition** options specify what should happen when the phase containing the **Steps on days within phase** completes its other steps.

Stop activities selects when system should stop doing the steps within the **Steps on days within phase** step.

- **After performing steps in block** – the steps in the **Steps on days within phase** step are completed even if the enclosing phase is otherwise complete.
- **When containing phase completes** – once the enclosing phase is otherwise complete remaining steps in the **Steps on days within phase** step are skipped.
- **When specified phase completes** – once the specified phase is otherwise complete remaining steps in the **Steps on days within phase** step are skipped.

If **When specified phase completes** is selected then Phase selects the phase at the end of which to stop steps within this **Steps on days within phase** step.

5.11 Waiting

The **Waiting** steps allow waiting for various conditions to occur.

5.11.1 Action and timing options

The steps that wait for some sort of condition have a common set of **Action** and **Timing** options.

The **Action** options specify what the step does and what to do if the step does not complete in its specified interval.

The **Action** option selects between:

- **Wait until condition** is met for a normal condition that waits for something to be true.
- **Fail if condition** is met for a check that should fail if something is true.

If fail or timeout – What to do if the condition is not true after the step has been executing for the maximum wait time.

- **Stop** – the step should fail
- **Do next steps** – the protocol should proceed automatically with the next step
- **Wait forever** – the step should continue to wait forever

Significance of failure can contain a message with instructions about what failure of this step means.

The **Timing** options which specify how long the step is expected to take to execute and what to do if the step does not complete in the expected time.

Depending on the step the option **Expected timing** can be present to select how the expected timing of the condition is modelled.

- **Wait for duration** – the step is modelled as expecting to wait for a duration from starting
- **Wait until time** – the step is modelled as expecting to wait until a specified date and time

Minimum wait time – the minimum time that it is expected to take waiting for this step. Used when estimating when subsequent steps will be performed. If the condition is true before the minimum wait time then the option **If condition satisfied early** allows choosing between **Continue immediately** and **Wait until minimum time**

Maximum wait time – the maximum time that it is expected to take waiting for this step. Used when estimating when subsequent steps will be performed, and depending on the timeout behaviour to decide what to do after the maximum time has expired.

When **Wait until time** has been selected then **Expected completion** specifies the date/time when the condition is expected to complete. Used when estimating when subsequent steps will be performed, and depending on the timeout behaviour to decide what to do after the date/time

5.11.2 Wait condition

The **Wait condition** step waits for a specified condition to be true.

5.11.2.1 Action and Timing options

The **Wait condition** step has the common Action and Timing options described in section 5.11.1 above.

5.11.2.2 Clauses options

Number of clauses selects the number of individual conditions to include in the step.

Applies to select whether the condition applies to the properties of the bioreactor itself or of its seed bioreactor.

- **This bioreactor** – the condition applies to the bioreactor
- **Seed bioreactor** – the condition applies to the bioreactor designated as the waiting bioreactor's **Seed source**

The **Applies to** option is only available on systems with an integrated liquid handler.

5.11.2.3 Condition options

Condition 1 options and if applicable Condition 2 options specify the generic conditions that the step waits for.

The options are described in section 3.6.2 above.

5.11.3 Wait for interval

The **Wait for interval** step waits for a specified **Wait duration** from starting this step to completing this step.

Add step - 'Wait for interval'

[Edit step parameters](#)

Wait for a specified interval

Property	Value
Description	
Description	Wait for interval
Comments	
Which reactors	
All or selected	All bioreactors assigned to protocol
When	
When to do step	Do after preceding step
Wait	
Duration type	Calculated duration (min)
Wait duration (min)	IF(pH>7,1,2)
Minimum duration (min)	0
Maximum duration (min)	5

Show DOE tags Show bioreactors

[Ok](#) [Cancel](#)

Figure 268 Wait for interval step with calculated duration

Duration type can be **Specified duration**, a fixed time interval, or a **Calculated duration** in minutes or .hours.

Wait duration can be either a constant duration or a calculated field.

If a **Calculated duration** is specified then the step waits for the **Minimum duration** even if the result of the calculation is less than that.

The step completes after the **Maximum duration** even if the result of the calculation is more than that.

5.11.4 Wait date/time

The **Wait date/time** waits until a specified **Date and time**.

5.11.5 Wait other step

The **Wait other step** step waits until a specified step completes.



The **Wait other step** step is hidden unless the **Show step to wait for another step to complete before proceeding** option is selected in the **Advanced features** window.

The relevant **Action** and **Timing** options described in section 5.11.1 above are incorporated in the **Error handling** section.

In addition:

Applies to specifies whether the step to wait for applies to the bioreactor the **Wait other step** step is being run for or whether it applies to that bioreactor's seed bioreactor.

Step to wait on specifies which step this step should wait for.

Delay after step completes specifies an optional delay between the step that is waited on completing and this step completing.

5.11.6 Wait loaded

The **Wait loaded** steps waits until the bioreactor is loaded.

The step has the standard **Action** and **Timing** options described in section 5.11.1 above.

5.11.7 Wait connected

The **Wait connected** steps waits until the bioreactor is connected.

The step has the standard **Action** and **Timing** options described in section 5.11.1 above.

5.11.8 Wait pumps and loops enabled

The **Wait pumps and loops enabled** steps waits until full automatic control of the bioreactor has been enabled.

The step has the standard **Action** and **Timing** options described in section 5.11.1 above.

5.11.9 Wait new value

The **Wait new value** step waits until a variable has been given a new value more recently than the last occurrence of an event. If the event has never occurred then the wait completes as soon as the variable has been given a value.

The typical use for the step would be to wait for something like the glucose concentration to have been entered after a sampling step and before pumping a bolus based on that concentration.

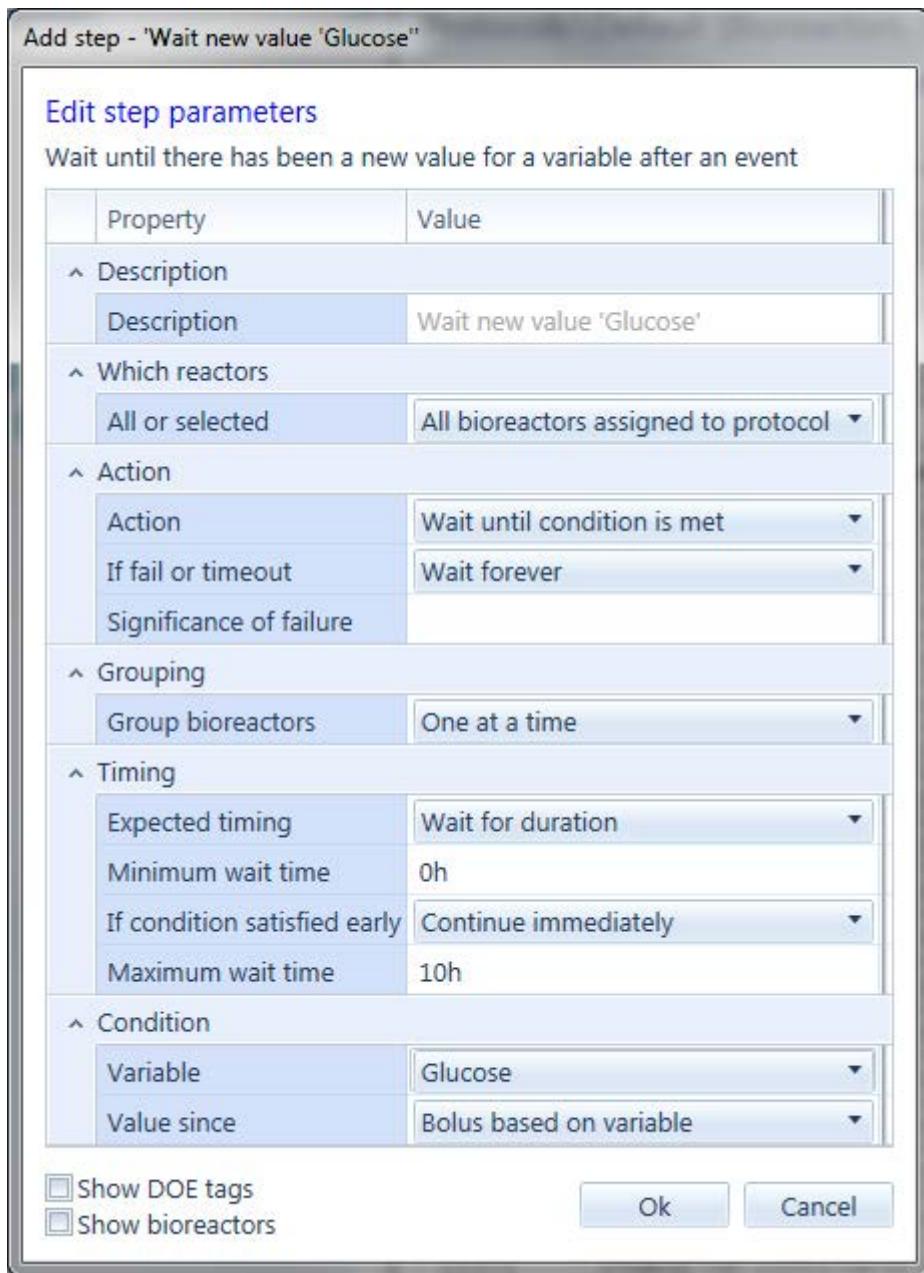


Figure 269 **Wait new value** step

Figure 269 above shows a sample **Wait new value** step.

The step has the standard **Action** and **Timing** options described in section 5.11.1 above.

Variable specifies the variable that must have a new value.

Value since specifies the event that the value must be more recent than. Options include:

- **Bolus based on variable** – the value must be more recent than the last calculated pumped bolus based on the value of the variable.

- **Calculated bolus** – the value must be more recent than the last calculated pumped bolus.
- **Bolus [Role]** – the value must be more recent than the last pumped bolus of the given role. The bolus may or may not have been a calculated bolus,
- **New value for other variable** – the value must be more recent than the value of some other value. For example one might look for the value of **Cell density** to be more recent than another variable that will be derived from it in a **Set variable** step.

Other variable specifies the variable that the value must be more recent than.

5.12 Prompts

The Prompts steps wait for the user to respond either to confirm that they have done something, or to respond by entering data/

5.12.1 Prompt user

The **Prompt user** step presents a prompt to the user and proceeds once the user has responded to the prompt.

5.12.1.1 Grouping options

The **Grouping options** allow a separate prompt to be presented for each bioreactor or for one prompt to be presented for all of the bioreactors.

Group prompts choose whether to prompt for each bioreactor individually or to wait until all the bioreactors reach the step and then prompt once.

- **One at a time**
- **All together**

5.12.1.2 Error handling

The **Maximum time to wait** option when set the prompt step will fail if the user has not responded within the specified time. Depending on what has been chosen for **If the step fails** then either the protocol will move on and **Do next steps** or **Stop** and wait for the user to deal with the error.

5.12.1.3 Prompt options

The **Prompt options** customise the prompt.

Prompt text specifies the prompt to display

Respond within specifies how long to allow for the user to respond to the prompt before raising the prompt as an issue needing immediate attention.

5.12.2 Enter data

The **Enter data** step prompts the user to enter values for a specified list of variables.

In addition to the standard parameters of a Prompt user step the options **Variable 1, Variable 2, ...** allow the variables to be specified.

Add step - 'Enter data for Ammonia, Calcium'

Edit step parameters

Prompt the user to enter data.

Property	Value
^ Description	
Description	Enter data for Ammonia, Calcium
Comments	
^ Which reactors	
All or selected	All bioreactors assigned to protocol
^ When	
When to do step	Do after preceding step
^ Grouping	
Group prompts	One at a time
^ Error handling	
If the step fails	Do next steps
Maximum time to wait	Forever
^ Prompt	
Prompt text	
Ask user to respond within	
^ Variables	
Variable 1	Ammonia
Variable 2	
Variable 3	
Variable 4	
Variable 5	Calcium
Variable 6	
Variable 7	
Variable 8	
Variable 9	
Variable 10	
<input type="checkbox"/> Show DOE tags <input type="checkbox"/> Show bioreactors	
<input type="button" value="Ok"/> <input type="button" value="Cancel"/>	

Figure 270 Enter data step with two variables chosen.

5.12.3 Calibrate pH

The **Calibrate pH** step prompts the user to enter measured pH values in order to do a 1-point calibration of the pH.

5.12.4 Calibrate DO

The **Calibrate DO** step prompts the user to enter measured DO values in order to do a 1-point offset of the DO.

5.12.5 Calibrate reflectance

The **Calibrate reflectance** step prompts the user to enter measured biomass values in order to do a 1-point offset of the reflectance.

5.12.6 Calibrate pCO₂

The **Calibrate pCO₂** step has been added which prompts the user to enter measured pCO₂ values in order to do a 1-point calibration of the CO₂ spot.

5.13 Perfusion

The **Perfusion** steps group contains the steps specific to perfusion.

Note that pumping of permeate is done via the standard **Start pumping** step.

5.13.1 Bleed

The **Bleed** step requests a bleed using the normal bleed path taking liquid from a low level in the bioreactor.

Value source chooses between setting the variable from a specified **Value**; from an **Expression** or from a bioreactor or system **Parameter**

When to finish step has options:

- **Step finishes immediately** – the step starts adds the bleed to the requested bleed total and completes immediately.
- **Wait until bleed to complete** – the step waits for the bleed to be complete before finishing.

When **Wait until bleed to complete** is selected then setting **Maximum time to wait** sets the maximum time that the step will wait for.

Volume specified the amount to add to the requested bleed volume. **Volume** is an expression or a fixed value depending on the option used for **Value source**.

Min. volume specifies a minimum volume to bleed. If the calculated value is less than this volume the step will report an error.

Max. volume specifies a maximum volume to bleed. If the calculated value is greater than this volume the step will report an error.

Max. pending volume specifies a maximum bleed volume that can be waiting to happen. The step will not progress if it would make the pending bleed volume larger than this value.

5.13.2 Bleed to level

The **Bleed to level** step requests a bleed using the bleed to level path. Excess volume can be bled in order to reset the volume of liquid in the bioreactor to a standard level.

The totals for **Bleed** and for **Bleed to level** are independent.

5.13.3 Cancel bleed

The **Cancel bleed** step cancels all requested bleeding.

5.13.4 Crossflow rate

The **Crossflow rate** step is a set point step that sets the crossflow rate (the rate of pumping liquid tangentially through the hollow fibre filter).

When to finish step offers options:

- **Step finishes immediately** – the step sets the requested crossflow rate.
- **Wait for pumping to stop** – when turning the crossflow off the step waits until the pump stops.
- **Wait for normal pumping** – when turning the crossflow on the step waits until normal crossflow is established.

When stopping crossflow an additional choice of **Filter state** is presented with options:

- **Empty filter of liquid** – if all the liquid in the filter should be returned to the bioreactor.
- **Leave filter filled** – if liquid should remain in the filter once perfusion has stopped.

5.13.5 Prime filter/permeate

The **Prime filter/permeate** step primes the outside of the hollow fibres with permeate.

When to finish step has options:

- **Step finishes immediately** – the step starts the priming and then completes immediately.
- **Wait until priming is complete** – the step waits for the priming to be complete before finishing.

Initial flow rate specifies the flow rate to use for the priming.

Total volume specified the volume to pump to prime the filter.

5.13.6 Set perfusion VVD

The **Set perfusion VVD** step sets the flow rate to be used by a perfusion VVD control loop as described in section 4.7.1 above.

5.13.1 Set target cell density

The **Set target cell density** step sets the cell density to be used by a perfusion cell density control loop as described in section 4.7.2 above.

5.13.1 Set volume SP

The **Set volume SP** step sets the target volume to be used by a perfusion VVD control loop as described in section 4.7.1 above.

5.13.2 Stop priming filter/permeate

The **Stop priming filter/permeate** step stops any priming of the priming filter/permeate that is in progress.

5.13.3 Perfusion side heater offset

The **Perfusion side heater offset** step is a set point step that allows setting the offset between the culture temperature set point and the set point for the perfusion side heater.

5.13.4 Sample permeate

The **Sample permeate** step prompts the user to take a manual sample from the permeate line.

The **Grouping options** allow a separate prompt to be presented for each bioreactor or for one prompt to be presented for all of the bioreactors.

Group prompts choose whether to prompt for each bioreactor individually or to wait until all the bioreactors reach the step and then prompt once.

- **One at a time**
- **All together**

5.13.4.1 Prompt options

The **Prompt options** customise the prompt.

Respond within specifies how long to allow for the user to respond to the prompt before raising the prompt as an issue needing immediate attention.

5.13.4.2 Details options

Volume type – chooses between **Specified volume** if the volume is to be specified explicitly or **Calculated volume** if the volume is to be the result of an expression.

Volume – the volume to be removed by the user.

If the Volume type is **Calculated volume** then additional options deal with the limits to the allowed volume.

Minimum volume specifies the minimum volume to present as a requested sample volume. Lower volumes will be treated as an error or rounded to zero depending on the setting of **What to do if the volume is too low**.

Maximum volume specifies the maximum volume to present as a requested sample volume. Larger volumes will be treated as an error.

What to do if the volume is too low specifies what to do if the calculated volume is a small or negative value.

- **Volume less than min. is error** treats the volume as an error.
- **Round volume less than min to zero** rounds the volume to zero.
- **Round volume less than min to zero, error if negative** treats negative volumes as an error but rounds small positive volumes to zero.

5.13.5 Change perfusion filter

The **Change perfusion filter** step prompts the user to change the perfusion filter.

5.13.5.1 Grouping options

The **Grouping options** allow a separate prompt to be presented for each bioreactor or for one prompt to be presented for all of the bioreactors.

Group prompts choose whether to prompt for each bioreactor individually or to wait until all the bioreactors reach the step and then prompt once.

- **One at a time**
- **All together**

5.13.5.2 Prompt options

The **Prompt options** customise the prompt.

Prompt text specifies the prompt to display

Respond within specifies how long to allow for the user to respond to the prompt before raising the prompt as an issue needing immediate attention.

5.14 Biomass monitoring

The Biomass monitoring steps allow control of the biomass monitors that reads the reflectance of laser light in the bioreactor through a clear window on the base of the bioreactor vessels.

5.14.1 Baseline biomass reading

The **Baseline biomass reading** step monitors the reflectance over a period (default 3 minutes). At the end of this period if the readings are stable the baseline value is recorded and used to offset the reflectance reading to remove background reflectance of the media. The step will fail bioreactors that have unstable readings.

5.14.2 Turn biomass monitoring on

The **Turn biomass monitoring on** step starts the monitoring of biomass data for the bioreactor. Monitoring should normally start after the bioreactor has been inoculated.

5.14.2.1 Timing options

The timing options specify how the biomass values are to be averaged for measurements and growth rate calculations.

- **Measurement averaging time constant (s)** – the time over which, in seconds, the data of the raw reflectance value is averaged. Values can be one of 0, 30, 60, 120, 240, 480
- **Growth rate time (s)** – the time window, in seconds, used for calculation of the growth rate. Values can be one of 60, 120, 240, 480, 960, 1920.

5.14.2.2 Output options

The output options specify the calibration curve that will be used, from the list of installed calibrations, to transform the biomass reflectance readings into different measures.

- **Dry cell weight** – the calibration curve for converting reflectance to dry cell weight.
- **Wet cell weight** – the calibration curve for converting reflectance to wet cell weight.
- **Optical density** – the calibration curve for converting reflectance to optical density.

5.14.2.3 Optical density calculation options

The calculation options specify how the biomass values should be calculated when reflectance values are outside the range of the reflectance values that were used to calculate the calibration curve. **Below low range** and **Above high range** can be specified

- **Extrapolate** – biomass value is always calculated using the curve.
- **Clip** – biomass value is clipped at the low or high range value and is recorded as either the maximum or minimum biomass value used to obtain the calibration curve.
- **Do not calculate** – No calculation is performed. The biomass value is recorded as a NaN.

5.14.3 Turn biomass monitoring off

The **Turn biomass monitoring off step** stops the monitoring of biomass data for the bioreactor.

5.15 Miscellaneous

The **Miscellaneous** steps group together some additional capabilities.

5.15.1 Set variable step

The **Set variable** step allows the value of a variable to be set to either a specified value (possibly varying between bioreactors) or to the result of an expression.

The variables that can be set are those variables that support manual data entry including the present values of custom variables that are not calculated.

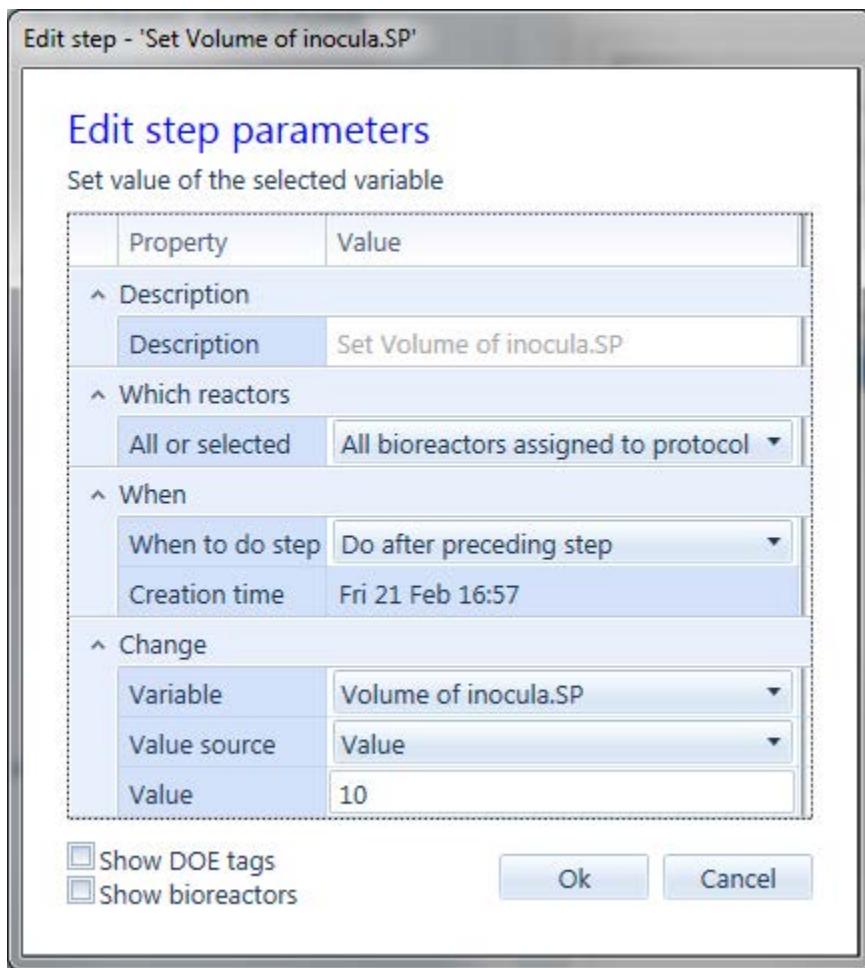


Figure 271 Set variable step

Variable specifies which variable to set.

Value source chooses between setting the variable from a specified **Value**; from an **Expression** or from a bioreactor or system **Parameter**

Value specifies the value to give the variable.

Expression specifies an expression from which to calculate the value to give to the variable.

5.15.2 Unload bioreactor

The **Unload bioreactor** step prompts the user to unload the bioreactor and waits until the bioreactor has been unloaded.

5.15.2.1 Grouping options

The **Grouping options** allow a separate prompt to be presented for each bioreactor or for one prompt to be presented for all of the bioreactors.

Group prompts choose whether to prompt for each bioreactor individually or to wait until all the bioreactors reach the step and then prompt once.

- **One at a time**

- **All together**

5.15.2.2 Prompt options

The **Prompt options** customise the prompt.

Prompt text specifies the prompt to display

Respond within specifies how long to allow for the user to respond to the prompt before raising the prompt as an issue needing immediate attention.

5.15.3 Comment

The **Comment** step does nothing, but can be used to add additional descriptions into the process.

5.15.4 Export report

The **Export report** step creates a report for the selected report template.

Group bioreactors allows reports to be produced as a **Single report** for all bioreactors or as a **Report per bioreactor**.

Template specifies the report template that will be used for the export.

The **File naming** option controls how reports are named

- **Include report name in directory name only** – includes the report name in just the directory.
- **Include report name in file and directory names** – includes the report name in the file name as well as in the directory name

Exported reports are saved as .csv files in the “**Documents\ambr 250\Reports\<experiment name>\<report name>**” folder - e.g. Documents\ambr 250\Reports\Run 43Test report\ - where <report name> is the **Name** as defined in the template.

For example if the **Include report name in directory name only** option was selected the exported report files in this folder follow the naming convention YYYYMMDDHHmm-Bnn[-nn].csv where YYYYMMDDHHmm is the time when the report was produced, and Bnn[-nn] is the bioreactors included in the report. E.g. 20204211037-B3.csv, 202004231319-B8-19.csv.

5.15.5 Enable alarm

The **Enable alarm** step enables the alarm for all bioreactors. The **Group bioreactors** option controls whether this happens once all the bioreactors have got to the step, or whether the alarm is updated each time a bioreactor reaches the step.

The **Alarm** option selects which alarm is enabled.

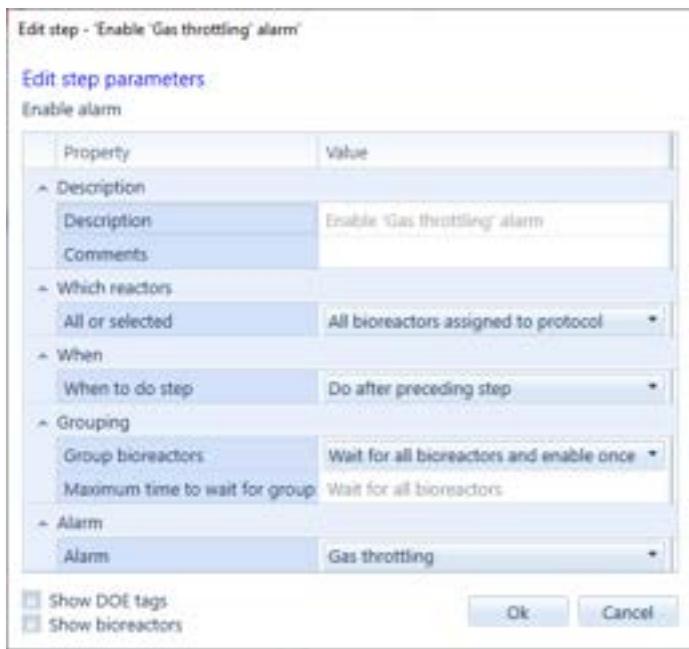


Figure 272 Step to enable alarm

5.15.6 Disable alarm

The **Disable alarm** step disables the alarm for all bioreactors. The **Group bioreactors** option controls whether this happens once all the bioreactors have got to the step, or whether the alarm is updated each time a bioreactor reaches the step.

The **Alarm** option selects which alarm is disabled.

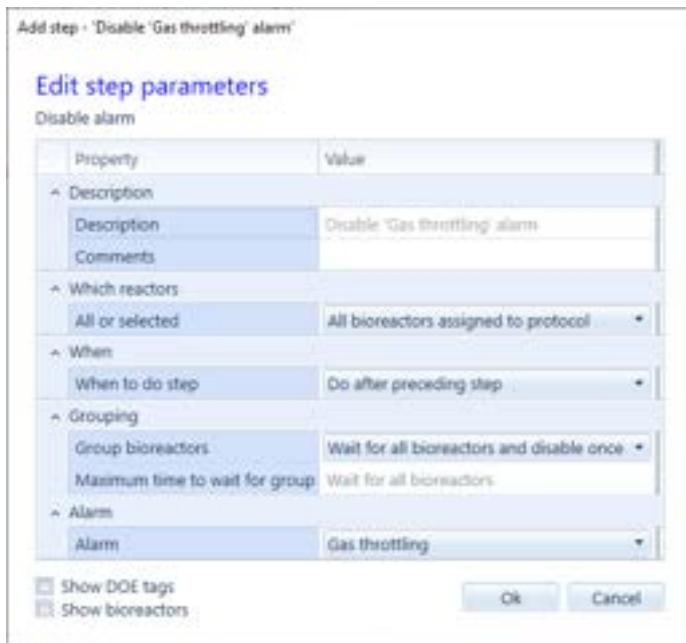


Figure 273 Disable alarm step

5.15.7 Mark as inoculated

The **Mark as inoculated** step marks the bioreactor as having been inoculated.

It is not needed in typical processes where an explicit step is used to inoculate the bioreactor.



The **Mark as inoculated** step is hidden unless the **Show additional steps typically created via wizards or used for commissioning** option is selected in the **Advanced features** window.

5.15.8 Set pH and validate

The **Set pH and validate** step operates as a standard set point step for pH but having set the pH set point waits until the pH reaches its set point. Reach is defined as:

- pH >= pH Lower Set point – dead band

AND

- pH <= pH Upper Set point + dead band

where the values of dead band are extracted from the appropriate control loops.



The **Set pH and validate** step is hidden unless the **Show additional steps typically created via wizards or used for commissioning** option is selected in the **Advanced features** window.

The **Set pH and validate** step has options:

- **Maximum time to reach pH** specifies the time allowed for the pH to reach its set point – to the accuracy defined by the dead bands of the control loops.
- **Maximum volume of acid to pump** – specifies the maximum amount of acid that can be pumped before the pH reaches its set point. This option is present if there is a pump with the role of Acid in the protocol.
- **Maximum volume of base to pump** – specifies the maximum amount of base that can be pumped before the pH reaches its set point. This option is present if there is a pump with the role of Base in the protocol.
- **If fail to reach pH** allows choosing what to do if the pH is not reached between **Turn off pH control** to turn pH control off and **Leave pH control on** to raise an error but leave the pH control on.

5.15.9 Check pH reading consistent

A **Check pH reading consistent** step has been added to support checking that the pH readings for the bioreactors in a protocol are consistent.

The step is a condition step that makes a specific check on the pH readings of the bioreactors in the protocol.



The **Check pH reading consistent** step is hidden unless the **Show additional steps typically created via wizards or used for commissioning** option is selected in the **Advanced features** window.

5.15.10 Calibrate assumed pH

The **Calibrate assumed pH** step allows an approximate calibration of the pH to be done on the basis that the pH of the bioreactor contents is independently known. This may be an adequate assumption with some well buffered medias where tight control and measurement of pH is not required.

The step waits until the required readings are constant and then calibrates the pH by calculating an offset between the raw pH reading and the assumed pH.

The step has the standard **Timing** options described in section 5.11.1 above.



The **Calibrate assumed pH** step is hidden unless the **Show step to calibrate the pH probe based on an assumed pH of the media** option is selected in the **Advanced features** window.

5.15.10.1 Assumed Values options

Assumed pH specifies the assumed pH of the culture

5.15.10.2 Calibrate options

The **Calibrate** options specify details of the calibration.

Delay time – the time over which readings must all be steady before calibration is done.

5.15.10.3 Maximum parameter change options

The **Maximum parameter change** options specify for each reading involved in the calibration the maximum amount that it can vary by in the interval before the calibration completes.

For each parameter calibration waits until parameter changes by no more than this amount in one **Delay time** before doing the calibration.

- **Max. change raw pH reading**
- **Max. change culture temperature**

5.15.11 Set from loop properties step

The **Set from loop properties** step sets the outputs of a cascade control loop to values based on the properties of the loop. It is intended in particular to facilitate calibration of the off-gas sensors with the gas flows that will be used by the DO loop at the start of culture.



The **Set from loop properties** step is hidden unless the **Show additional steps typically created via wizards or used for commissioning** option is selected in the **Advanced features** window.



Figure 274 *Set from loop properties* step

Variable controlled by loop selects the variable that the control loop controls. This defines the set of control loops available for selection.

Action to take can be:

- **Set to values when loop at minimum** which sets the outputs of the loop to the values that the loop would have given them initially.
- **Turn outputs off** which turns off all the outputs of the loop.

Control loop is the loop that defines the outputs set by the step and the values given to those outputs.

5.15.12 Write bioreactor parameters

The **Write bioreactor parameters** step writes the values of one or more expressions to the calibration parameters stored inside the bioreactor.

This step is intended only for internal use in calibration purposes and should not be used in normal customer processes.



The **Write bioreactor parameters** step is hidden unless the **Show additional steps typically created via wizards or used for commissioning** option is selected in the **Advanced features** window.

5.15.13 Write system parameters

The **Write system parameters** step writes the values of one or more expressions to the calibration parameters stored inside the system control board.

This step is intended only for internal use in calibration purposes and should not be used in normal customer processes.

When using this step only one bioreactor should be used in the process to avoid repeated writes to the system board of the same parameter.



The **Write system parameters** step is hidden unless the **Show additional steps typically created via wizards or used for commissioning** option is selected in the **Advanced features** window.

5.15.14 Write perfusion parameters

The **Write perfusion parameters** step writes the values of one or more expressions to the calibration parameters stored inside the perfusion pump control board.

This step is intended only for internal use in calibration purposes and should not be used in normal customer processes.

When using this step only one bioreactor should be used in the process to avoid repeated writes to the perfusion pump control board of the same parameter.



The **Write perfusion parameters** step is hidden unless the **Show additional steps typically created via wizards or used for commissioning** option is selected in the **Advanced features** window.

5.15.15 Write biomass monitor parameters

The **Write biomass monitor parameters** step writes the values of one or more expressions to the calibration parameters stored inside the biomass monitor control board.

This step is intended only for internal use in calibration purposes and should not be used in normal customer processes.

When using this step only one bioreactor should be used in the process to avoid repeated writes to the biomass monitor control board of the same parameter.



The **Write biomass monitor parameters** step is hidden unless the **Show additional steps typically created via wizards or used for commissioning** option is selected in the **Advanced features** window.

5.15.16 Enable automatic write to board parameters

The **Enable automatic write to board parameters** step enables the **Write bioreactor parameters**, **Write system parameters**, **Write perfusion parameters** and **Write biomass parameter** steps to proceed without prompting the user for confirmation.

This step is intended only for internal use in calibration purposes and should not be used in normal customer processes.

This step should only be included in well validated processes. Writing incorrect values to the parameters can cause damage to the system and incorrect operation.



The **Enable automatic write to board parameters** step is hidden unless the **Show additional steps typically created via wizards or used for commissioning** option is selected in the **Advanced features** window.

5.15.17 Disable automatic write to board parameters

The **Disable automatic write to board parameters** step disables the automatic writing of board parameters. The **Write bioreactor parameters**, **Write system parameters**, **Write perfusion parameters** and **Write biomass parameter** steps will prompt the user for confirmation.

This step is intended only for internal use in calibration purposes and should not be used in normal customer processes.



The **Disable automatic write to board parameters** step is hidden unless the **Show additional steps typically created via wizards or used for commissioning** option is selected in the **Advanced features** window.

6 SETTING UP A RUN

The Ambr® 250 software guides the user through the tasks required to set up bioreactors and tubing for a run.

Setting up plates/bottles/tube racks and pipette tips is dealt with separately. See the **Labware setup** page.

The next actions to be performed can be seen:

- on the **Todo** page
- on the **Bioreactor and tubing setup, and Bioreactor and tubing cleanup** pages
- by looking at which of the setup pages are highlighted indicating that the action for that page is ready to be done for one or more bioreactors

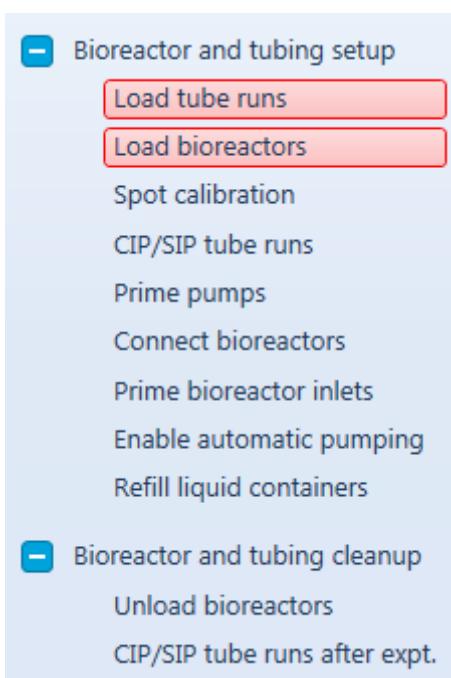


Figure 275 **Bioreactor and tubing setup** navigation showing that **Load tube runs** and **Load bioreactors** are ready to be done.

6.1 Tasks required

The tasks in setting up a run are:

- Load the tube runs to be used by the run. If existing tube runs are to be used then confirm that the tube runs are correct.
- Load the bioreactors, that is: place the bioreactors into the system; clamp the bioreactors in position; connect any pH lead from the bioreactor to the system; but **do not** connect the liquid manifold yet,
- Spot calibration, enter details of the calibration of the spots in the bioreactors.

- CIP/SIP tube runs, pump liquids and/or air through the tube runs to clean and sterilize the tube run and the pumps it is attached to
- Prime pumps, pump liquid through the pumps so that there is no air left in the pump or in the tubing connected to the pumps and liquid is flowing through the fixed liquid manifold
- Connect bioreactors, connect the liquid manifold on the bioreactor to the liquid manifold on the pump so that a) liquid can flow to the bioreactor b) the bioreactor is sealed and can be gassed
- Prime bioreactor inlets, optionally pump the liquid on until it is at the top of the bioreactor. (If done this reduces the variation in pumped volume due to variations in the volume of the tubing and filter between the liquid manifold and the inside of the bioreactor.)
- Enable automatic pumping, allow the system to run using control loops and using steps to control pumping.

Finally when processing on bioreactors is complete:

- Unload bioreactors.

And if a new run is not going to be set up soon using all the pumps:

- CIP/SIP tube runs after run, pump liquids and/or air through the tube so that where pumps are not going to be used for some time they and the waste lines are not left full of liquid that may dry, crystallize and cause problems.

Note it is implicit in starting a new experiment that the bioreactors from the previous experiment have been unloaded. It is only necessary to explicitly unload bioreactors if you want to remove some bioreactors from the system before the end of the experiment or you want to reuse the bioreactors for a new protocol.

If some but not all of the bioreactors have been unloaded:

- Reuse bioreactors stations, export data for unloaded bioreactors and reset the bioreactors so that they can be used for another run while keeping the existing bioreactors running their current protocol.

Perfusion runs have additional steps described in section 7 below.

6.2 Typical ways to set up a run

There are two typical ways to set up a run:

- i) load, clean and prime the tubing; ii) load and connect bioreactors; iii) finish priming
- i) load tubing and start cleaning; ii) load bioreactors and start liquid handler operating to add media to bioreactors; iii) connect bioreactors iv) finish priming

The figures below show the two ways of performing the setup. If a lengthy clean in place process is required then it can be advantageous to allow the liquid handler to fill bioreactors with media at the same time as the clean in place is happening.

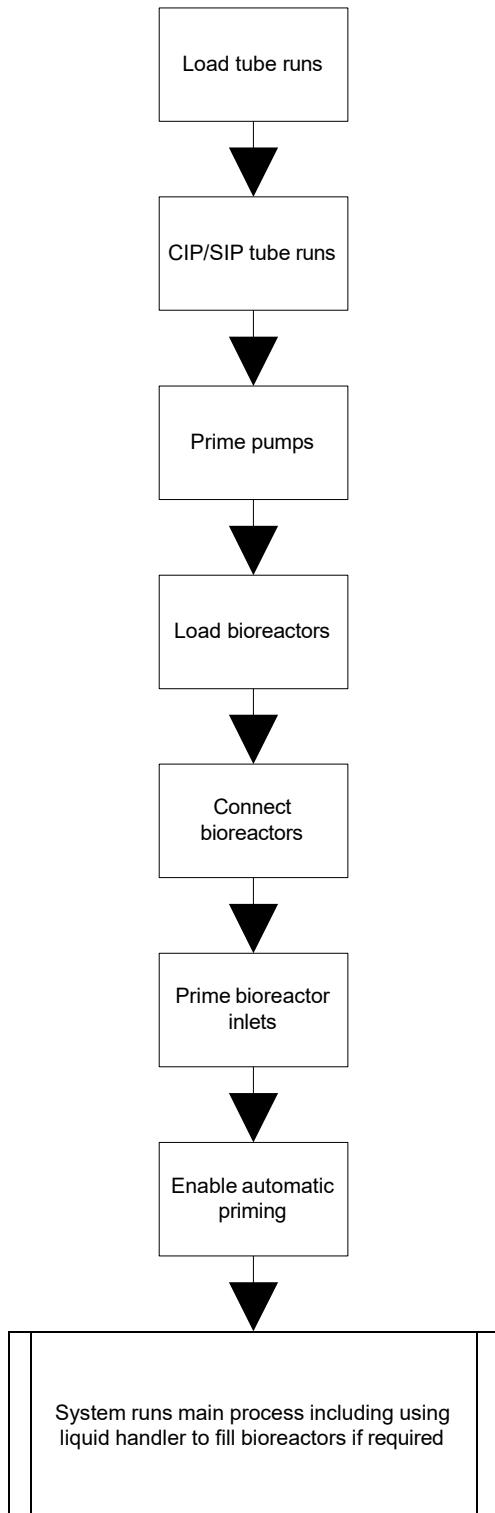


Figure 276 Simple setup

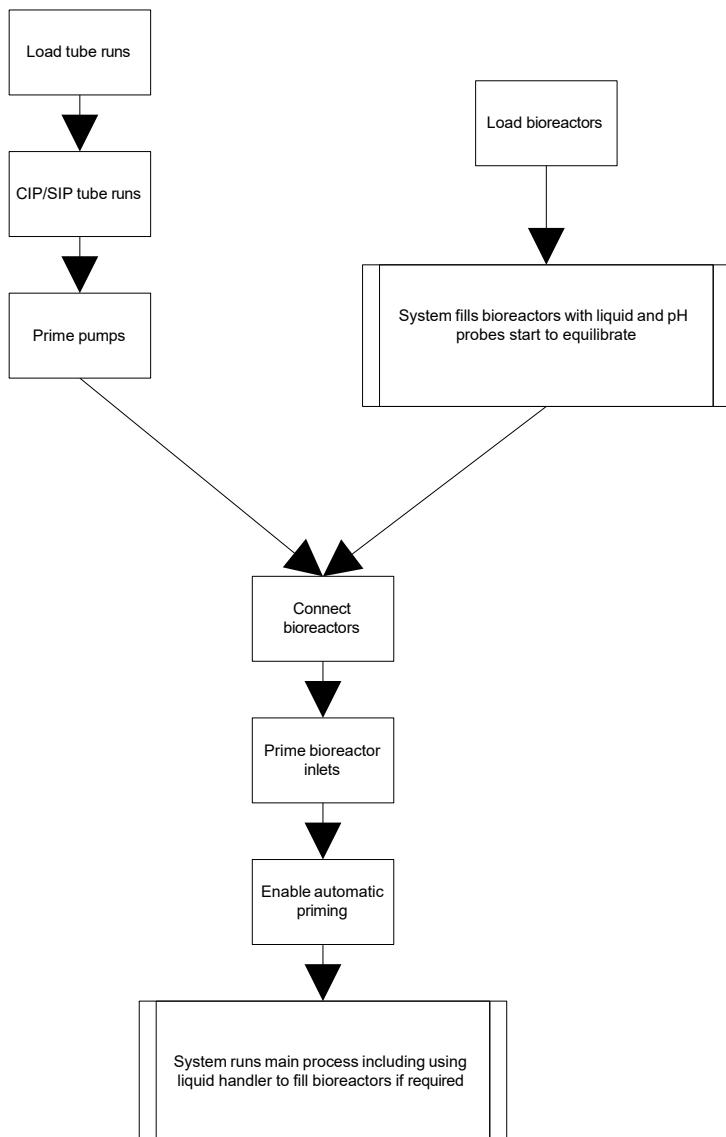


Figure 277 Parallel setup

6.3 Bioreactor and tubing setup

The bioreactor and tubing setup page displays an overview of the setup.

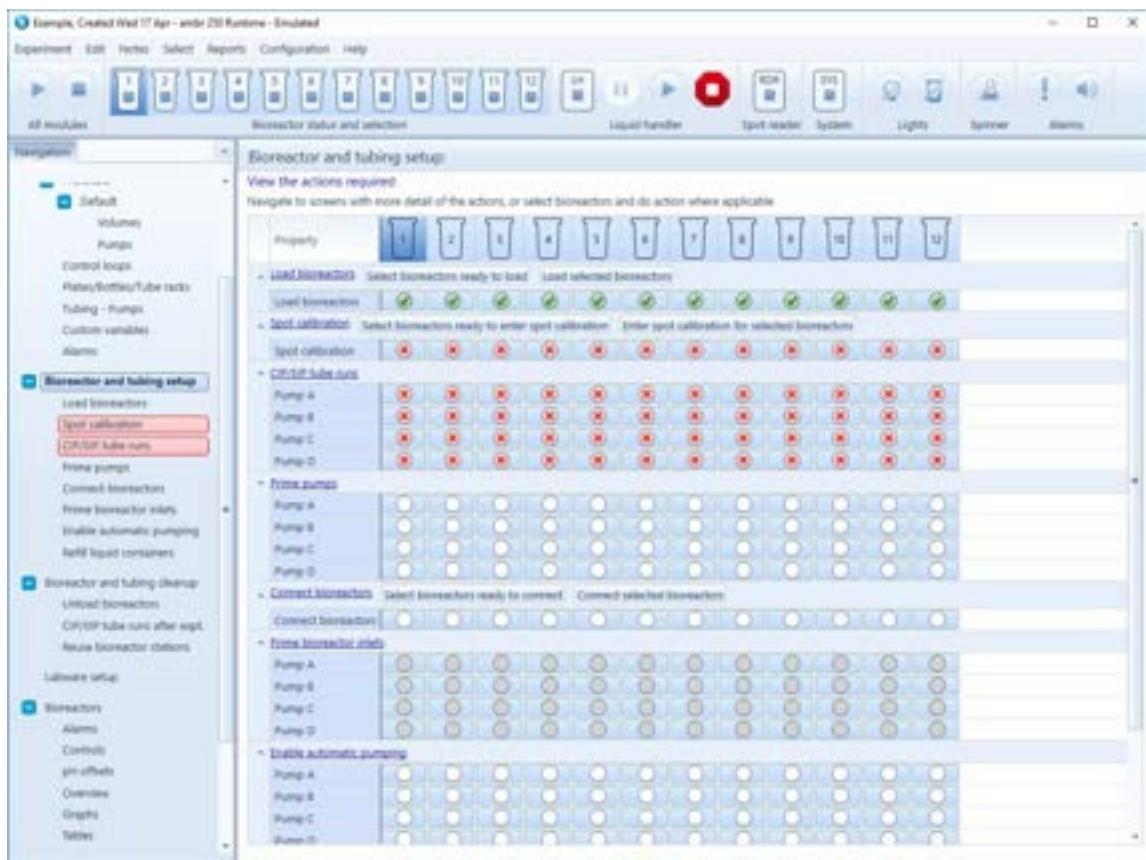


Figure 278 Bioreactor and tubing setup

The page shows an indicator for each action indicating whether the action has been done for a bioreactor. For actions that apply to individual pumps an indicator is shown for each pump.

The indicator shows:

- Completed action
- Action needs to be done and could be done next
- Action does not need to be done for this process
- Action will need to be done, but is not appropriate yet

Clicking the link describing an action displays the page for performing the action.

Actions that only require a selection of bioreactors can be performed from this page. For example:

- **Select bioreactors ready to load** selects the bioreactors ready to be loaded.
- **Load selected bioreactors...** displays the window for loading bioreactors.

6.4 Operation pages

The operation pages for loading and unloading all work in the same way.

- 1) Select the item or items to work on
- 2) Display window to perform the action or confirm the action

Items indicate whether the action has been performed or not.

- Completed action
- Action needs to be done and could be done next
- Action does not need to be done for this process
- Action will need to be done, but is not appropriate yet

Items can be selected by clicking on the items.

Clicking on a bioreactor selects items relating to that bioreactor and selecting an item selects the bioreactors relating to that item.

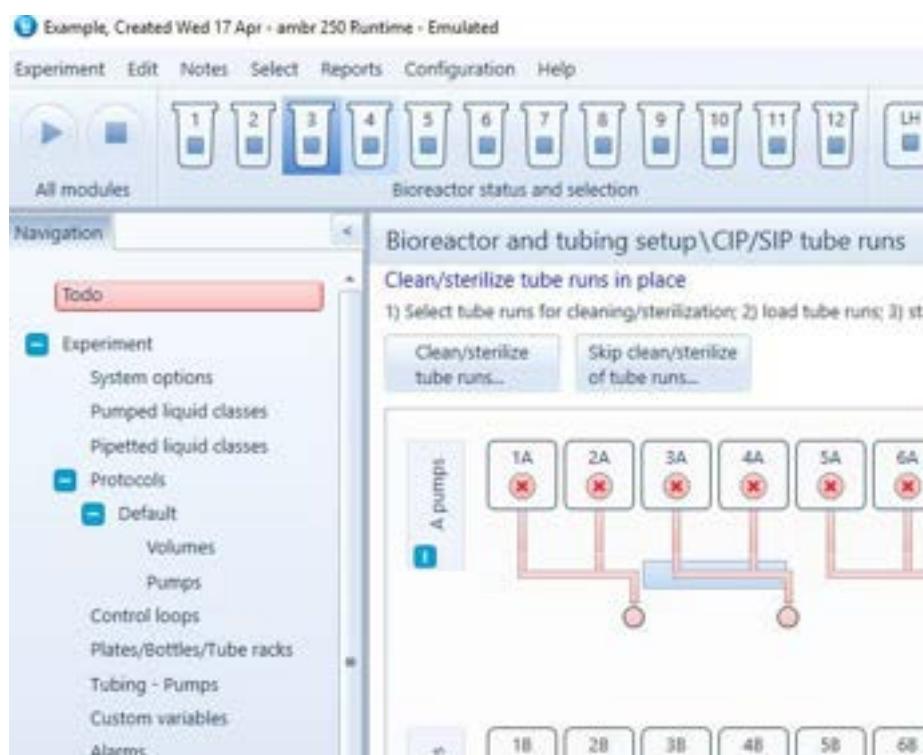


Figure 279 Clicking on the tube run to pumps 3A and 4A has selected bioreactors 3 and 4
The **Select** panel shows controls for selecting items.

Note also that the shortcut Ctrl-A for selecting all items can be used.

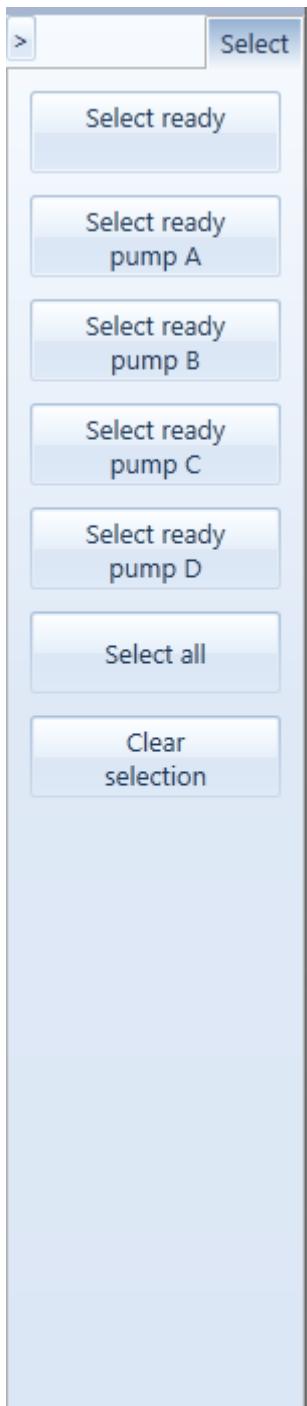


Figure 280 *Select* panel for **Load tube runs** page

Select ready selects all the items that are ready to perform the action on the page.

Select ready for pump A selects all the items pertaining to the A pumps that are ready to perform the action on the page.

Select ready for pump B selects all the items pertaining to the B pumps that are ready to perform the action on the page.

Select ready for pump C selects all the items pertaining to the C pumps that are ready to perform the action on the page.

Select ready for pump D selects all the items pertaining to the D pumps that are ready to perform the action on the page.

Select all selects all the selectable items on the page. Unlike **Select ready**, this may include items where the action has been performed but could be done further.

Clear selection clears the selection.

6.5 Load bioreactors

The **Load bioreactors** page allows the operator to load bioreactors onto the system.

Loading a bioreactor comprises:

- Unwrapping the bioreactor and placing the bioreactor in its station
- Connecting the pH lead to the system
- Placing the clamp plate in position on top of the bioreactor

DO NOT CONNECT THE LIQUID MANIFOLD AT THIS TIME.



Figure 281 **Load bioreactors** page. Bioreactors 1 and 3 have already been loaded. Bioreactors 13...24 are not assigned to a protocol. The remaining bioreactors are ready to be loaded.

- 1) Select the bioreactors to load by clicking on items as appropriate.
- 2) **Load bioreactors...** displays the window to confirm that the bioreactors are loaded correctly. The system will switch the lights on the bioreactors to be loaded on.



- 3) Load the indicated bioreactors.

- 4) Should you not have loaded a bioreactor – for example you have dropped the bioreactor on the floor – then deselect the **Do for** option for that bioreactor.



Figure 283 **Load bioreactors** window with bioreactor 8 not being loaded

- 5) Press **Loaded bioreactors** to confirm that the bioreactors are loaded

6.6 Spot calibration

The **Spot calibration** page allows the operator to enter the calibration data for the spots in the bioreactors.



Figure 284 **Spot calibration** page

- 1) Select the bioreactors to enter data for. Typically you should select all the bioreactors that come from the same batch.
- 2) **Edit calibration...** displays the window to enter the calibration data.

Edit calibration

Enter calibration data for the marked bioreactors

Property	Value	1	2	3	4	5	6	7	8	9	10	11	12
+ Bioreactors													
Do for		90	90	90	90	90	90	90	90	90	90	90	90
+ DO Spot													
Upper calibration percentage (%)	100	100	100	100	100	100	100	100	100	100	100	100	100
Phase (100% DO) (°C)	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6
Temperature (100% DO) (°C)	37	37	37	37	37	37	37	37	37	37	37	37	37
Phase (5% DO) (°C)	27.8	27.8	27.8	27.8	27.8	27.8	27.8	27.8	27.8	27.8	27.8	27.8	27.8
Temperature (5% DO) (°C)	37	37	37	37	37	37	37	37	37	37	37	37	37
Pressure (mbar)	1013	1013	1013	1013	1013	1013	1013	1013	1013	1013	1013	1013	1013
+ pCO ₂ Spot													
Lower phase limit	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1
Upper phase limit	30	30	30	30	30	30	30	30	30	30	30	30	30
Scale	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4
Sensitivity	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Temperature (°C)	36.7	36.7	36.7	36.7	36.7	36.7	36.7	36.7	36.7	36.7	36.7	36.7	36.7
Pressure (mbar)	1006.8	1006.8	1006.8	1006.8	1006.8	1006.8	1006.8	1006.8	1006.8	1006.8	1006.8	1006.8	1006.8
+ Batch data													
Vessel batch number	221301257	221301	221301	221301	221301	221301	221301	221301	221301	221301	221301	221301	221301
Vessel part number	0011-N01102	0011-N0											
Vessel expiry date	Sat 12 Jul 2024 01:00	Sat 12											
DO spot batch number	2048-P1-1003-02	2048-P1											
pCO ₂ spot batch number	2032-02	2032-02	2032-02	2032-02	2032-02	2032-02	2032-02	2032-02	2032-02	2032-02	2032-02	2032-02	2032-02
<input type="button" value="Reuse last default values"/>													
<input type="button" value="OK"/> <input type="button" value="Cancel"/>													

Figure 285 Enter spot calibration data window

- 3) If the bioreactors are from the same batch as the last set of bioreactors for which data was entered press **Reuse last default values** and review the values displayed to confirm that they match the calibration data for these bioreactors.

Note that the **Vessel batch number**, **Vessel part number**, **Vessel expiry date**, **DO spot batch number** and **pCO₂ spot batch number** fields are optional.

- 4) Press the Scan calibration barcode button or enter the values from the calibration data supplied with the bioreactors. If all the bioreactors for which you are entering data are from the same batch you can enter the data once in the **Value** column.
- 5) If scanning a barcode when the barcode is scanned the data will be entered into the form.

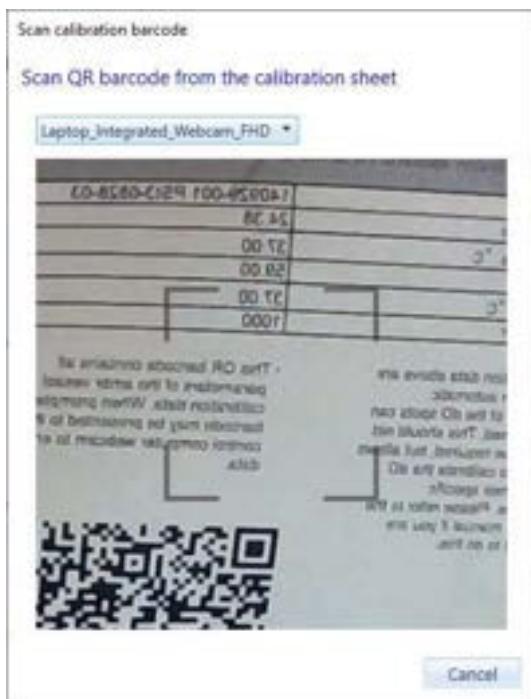


Figure 286 Scan calibration barcode dialogue

- 6) Press **Ok** to save the data

6.6.1 DO Offset

A single point offset can be applied to the DO readings from the system.



This feature is enabled by the **Support DO offset** option on the **Advanced features** window.

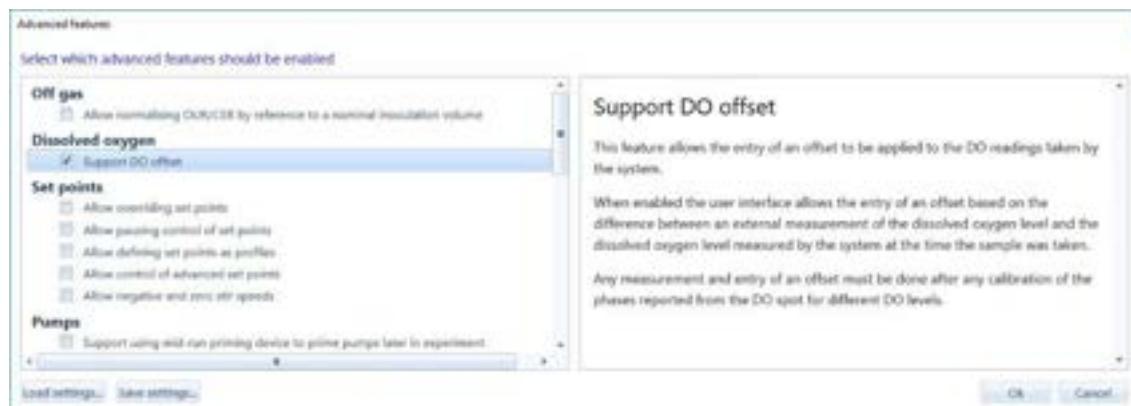


Figure 287 Advanced feature to support DO offset

When the option is enabled the Spot calibration page has an **Edit DO offset...** button and displays the details of any DO offsets.

Enter the calibration settings for the spots on the bioreactor vessels.

	DO	CO ₂	pCO ₂	DO/pCO ₂
Property				
Current value				
DO	11.1	11.1	11.1	11.1
pCO ₂	38.1	38.1	38.1	38.1
DO spot				
Upper calibration percentage (%)	100	100	100	100
Phase (0000-200 (%)	22.0	22.0	22.0	22.0
Temperature (0000-300 (°C))	27	27	27	27
Phase (00-200 (%)	22.0	22.0	22.0	22.0
Temperature (00-300 (°C))	27	27	27	27
Pressure (kPa)	1013	1013	1013	1013
CO₂ spot				
CO ₂	0.1	-0.1	-0.1	-0.1
Measured (m)	12	12	12	12
Last change offset	0.1	-0.1	-0.1	-0.1
Age of offset (h)	0.0	0.0	0.0	0.0
pCO₂ spot				
CO ₂	0.2	-0.2	-0.2	-0.2
Measured (pCO ₂)	28.2	28.2	28.2	28.2
Last change offset	0.2	-0.2	-0.2	-0.2
Age of offset (h)	0.0	0.0	0.0	0.0
DO/pCO₂ spot				
Lower phase limit	11.1	11.1	11.1	11.1
Upper phase limit	38.0	38.0	38.0	38.0
Rate	1.4	1.4	1.4	1.4
Sensitivity	0.4	0.4	0.4	0.4
Temperature (°C)	36.7	36.7	36.7	36.7
Pressure (kPa)	1000.0	1000.0	1000.0	1000.0

Figure 288 Spot calibration page with offset enabled

6.6.2 pCO₂ Offset

If configured for CO₂ spots an **Edit pCO₂ offset...** button is available and the details of any pCO₂ offsets are shown.

6.6.3 Editing Offsets

Pressing the edit offset button displays a dialog where measured values of DO/pCO₂ can be entered.

Enter DO data

Enter externally measured DO and when the DO sample was taken:

Use current date
 Use named dates
 Use current date and time

Biomarker	When sample taken (exact)	External DO / Recorded system DO: DO offset: Change to offset:
Biomarker 1	Mon 06 Oct 11:00:41 - Started edit	20.0 17.4 2.8
Biomarker 2		
Biomarker 3		
Biomarker 4		
Biomarker 5		
Biomarker 6		
Biomarker 7		
Biomarker 8		
Biomarker 9		
Biomarker 10		
Biomarker 11		
Biomarker 12		
Biomarker 13		
Biomarker 14		
Biomarker 15		
Biomarker 16		
Biomarker 17		
Biomarker 18		
Biomarker 19		
Biomarker 20		

[Export to file...](#) [Export to clipboard](#) [Import from file...](#) [Import from clipboard](#) [OK](#) [Cancel](#)

Figure 289 Dialog for entering measured DO values.

6.7 CIP/SIP tube runs

The **CIP/SIP tube runs** page allows the operator to either clean/sterilize the tubing and pumps or to indicate that cleaning is not required, typically because the same tubing and liquid is being used as in the previous run.

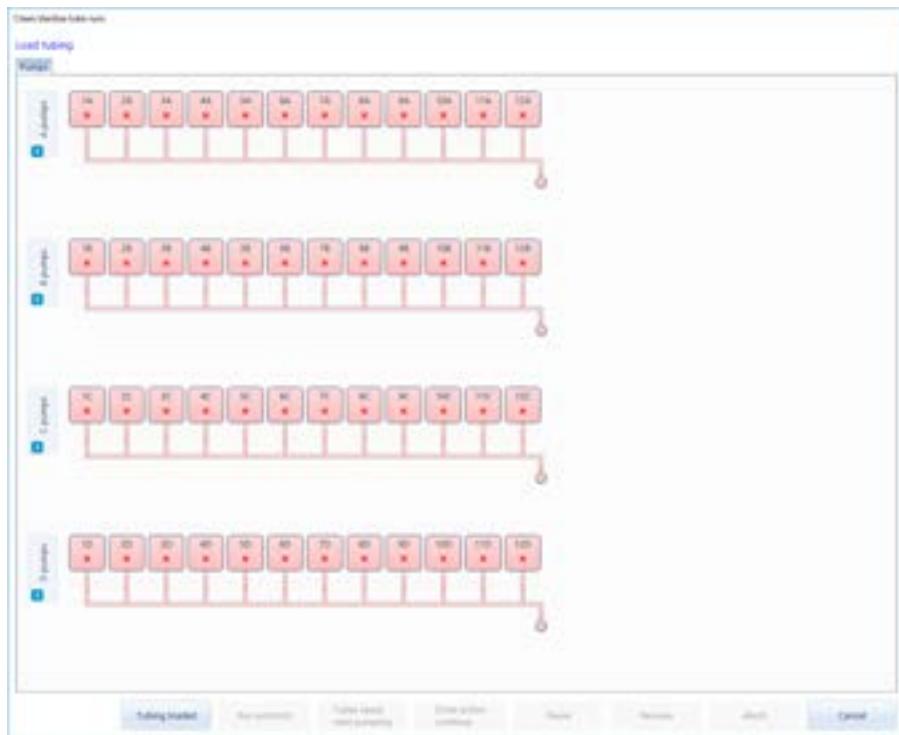


Figure 290 CIP/SIP tube runs page

6.7.1 Doing CIP/SIP

To clean tube runs and the pumps they are connected to:

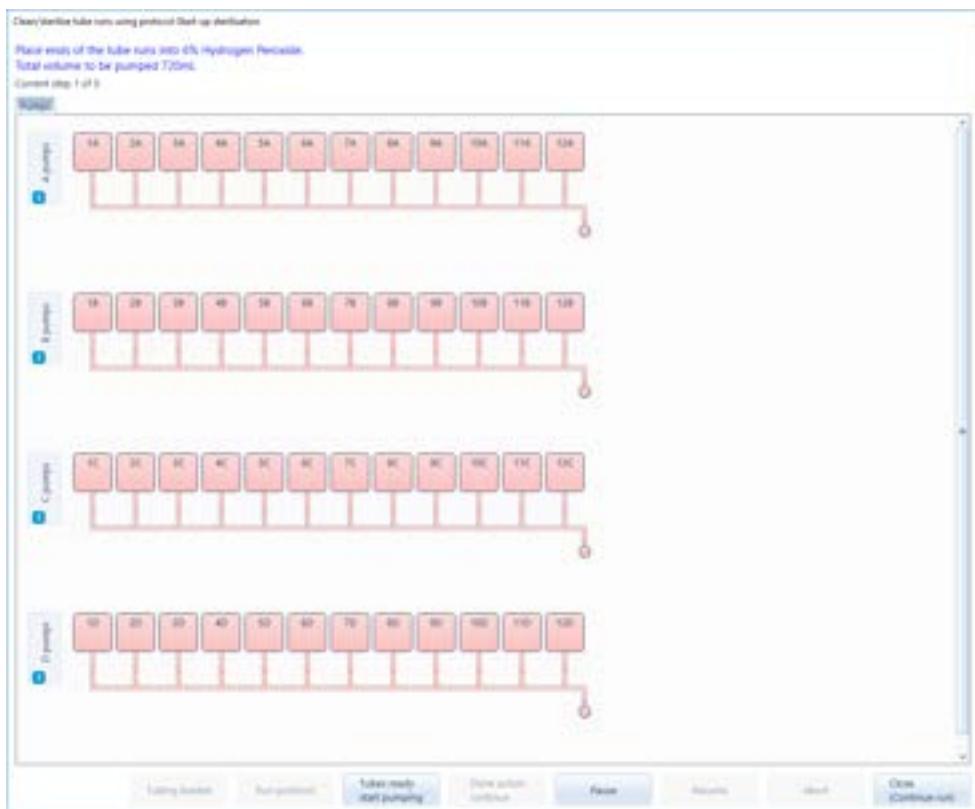
- 1) Select the tube runs to clean by clicking on items as appropriate.
- 2) **Clear/sterilize tube runs...** displays the **Clean/sterilize tube runs** window. (If the bioreactors are not running then the system will first prompt for the bioreactors to be started.)



- 3) If the bioreactor tubing has not been confirmed as loaded the CIP screen will show the option to load the tubing. Once the tubing is loaded press **Tubing loaded** and the options to select the CIP/SIP protocol will be shown.



- 4) Select the protocol to run to clean the tubes. See here for details of how to configure the Clean/Sterilize in Place protocols.
- 5) Press **Run protocol** to start the protocol. The system will display a prompt with the required liquid to use for the next step of the protocol.



- 6) Place the ends of the tube runs being cleaned into a beaker or bottle with the required liquid.
- 7) Press **Tubes ready start pumping**. The system will pump the liquid and then wait for the specified hold time.



- 8) Wait until the hold is complete. If required repeat steps 5 – 7. When the protocol has completed the window will show the status below.



- 9) Press Close (Clear run) to close the window

Steps can require the user to take some specified action. If a step requires an action then do the action and press **Done action continue** to move onto and hold and then the next step in the protocol.

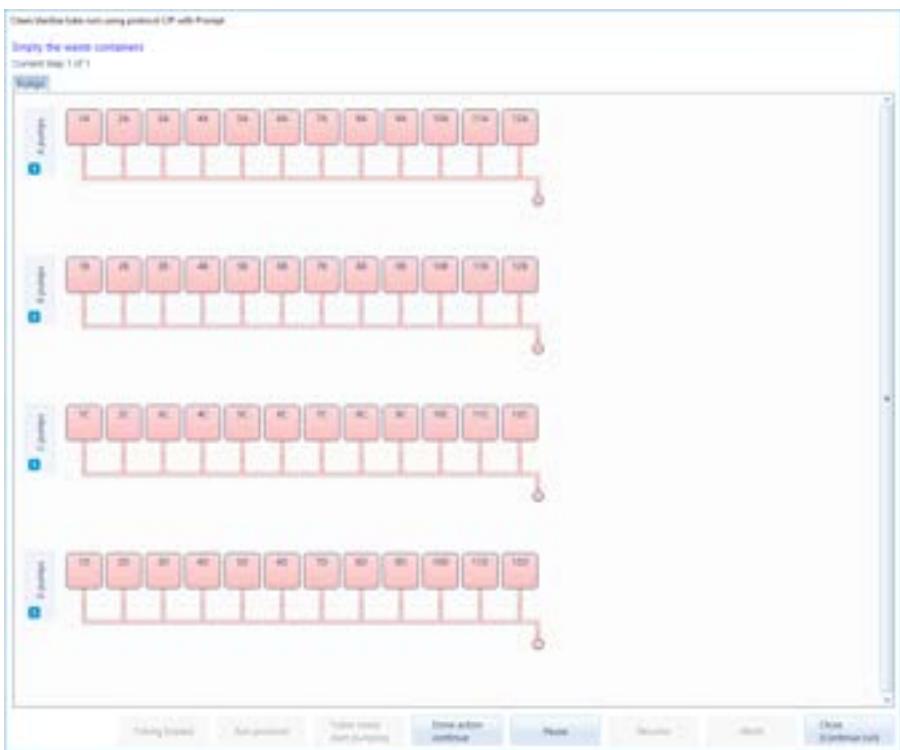


Figure 291 Clean/sterilize in place dialog prompting the user to take some action

While the protocol is running press **Pause** to pause the run if required. Once the run is paused press **Resume** to continue the run or **Abort** to abort the run.

The **Abort** button also has the option **Abort step** to abort the current step of the run and move on to the next step. This can be useful if there is a problem with one of the pumps during a run.

The Clean/sterilize tube runs window can be closed while the protocol continues to run. To close the window press **Close (Continue run)**.

In progress runs can be opened from the list on the CIP/SIP tube runs page.

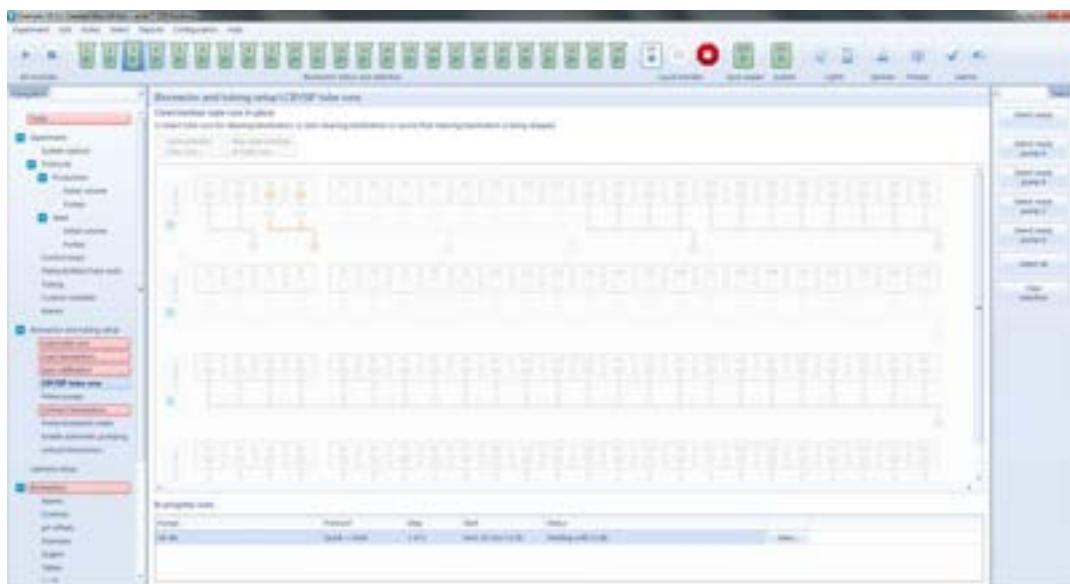


Figure 292 **CIP/SIP tube runs** window with an **In progress** run

In progress runs are also displayed on the Todo page.

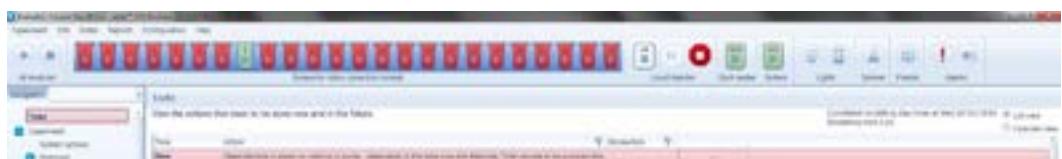


Figure 293 Todo page with CIP/SIP run needing attention

6.7.2 CIP/SIP with automated liquid changes

An optional hardware module is available to support automatic switching between liquids in a clean in place protocol.

When the module is installed the clean in place screen presents an option for how to change liquids in the protocol.

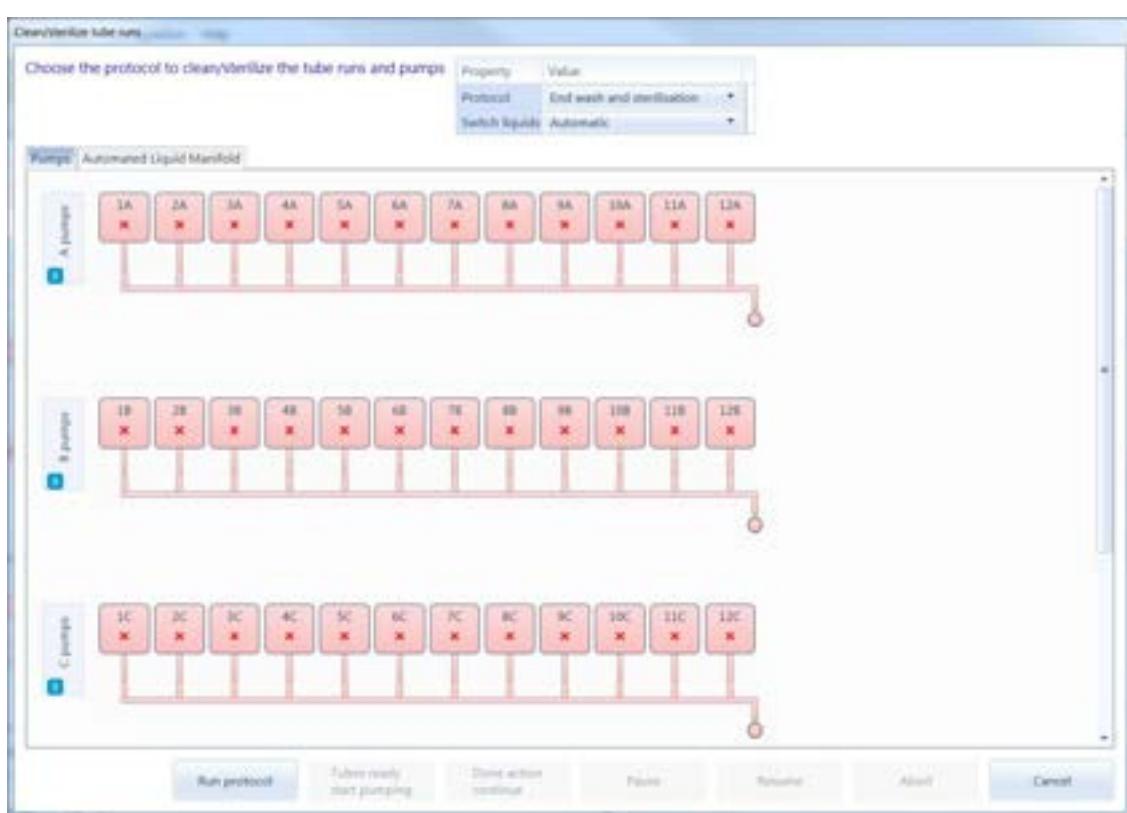


Figure 294 Clean/sterilize tube runs screen with **Switch liquids** option

Select **Manual** for the **Switch liquids** option to change liquids manually.

Select **Automatic** for the **Switch liquids** option to change liquids automatically.

Once you have selected a protocol and the automatic option press **Run protocol**. The system will initialize the automated CIP/SIP module.

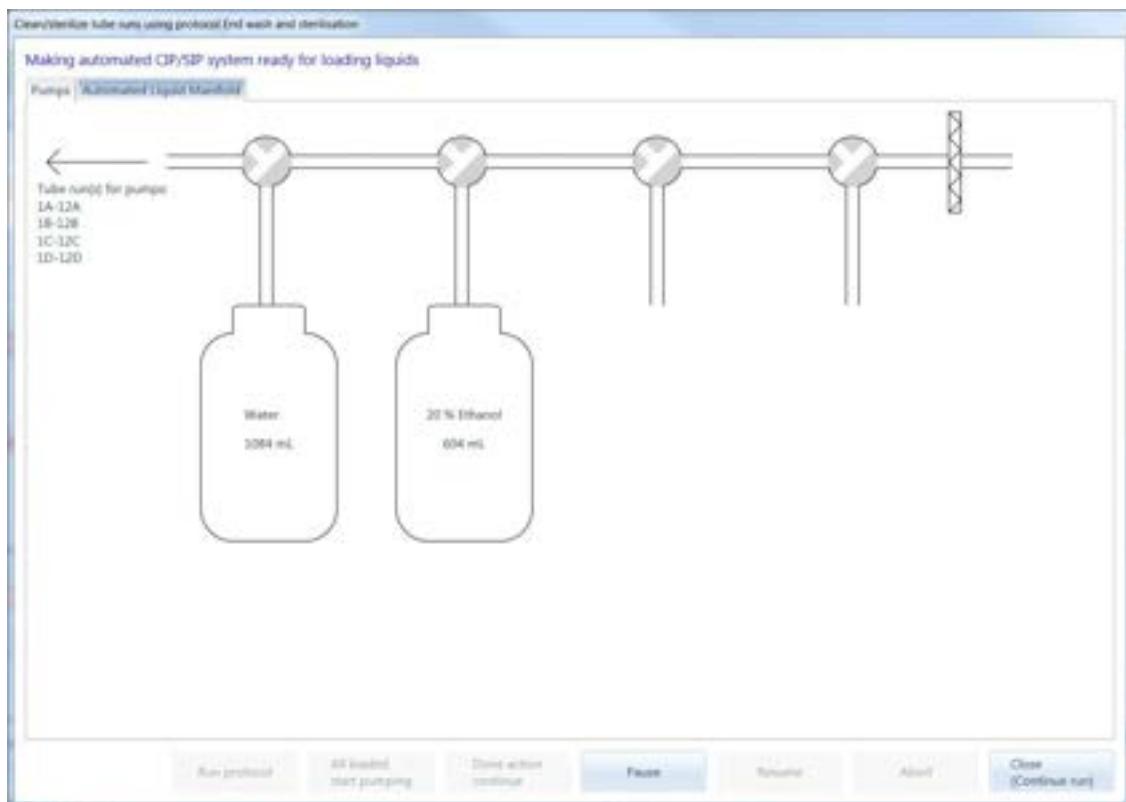


Figure 295 System initialising automated CIP/SIP system

Once the system is initialised the screen will show the liquids to load.

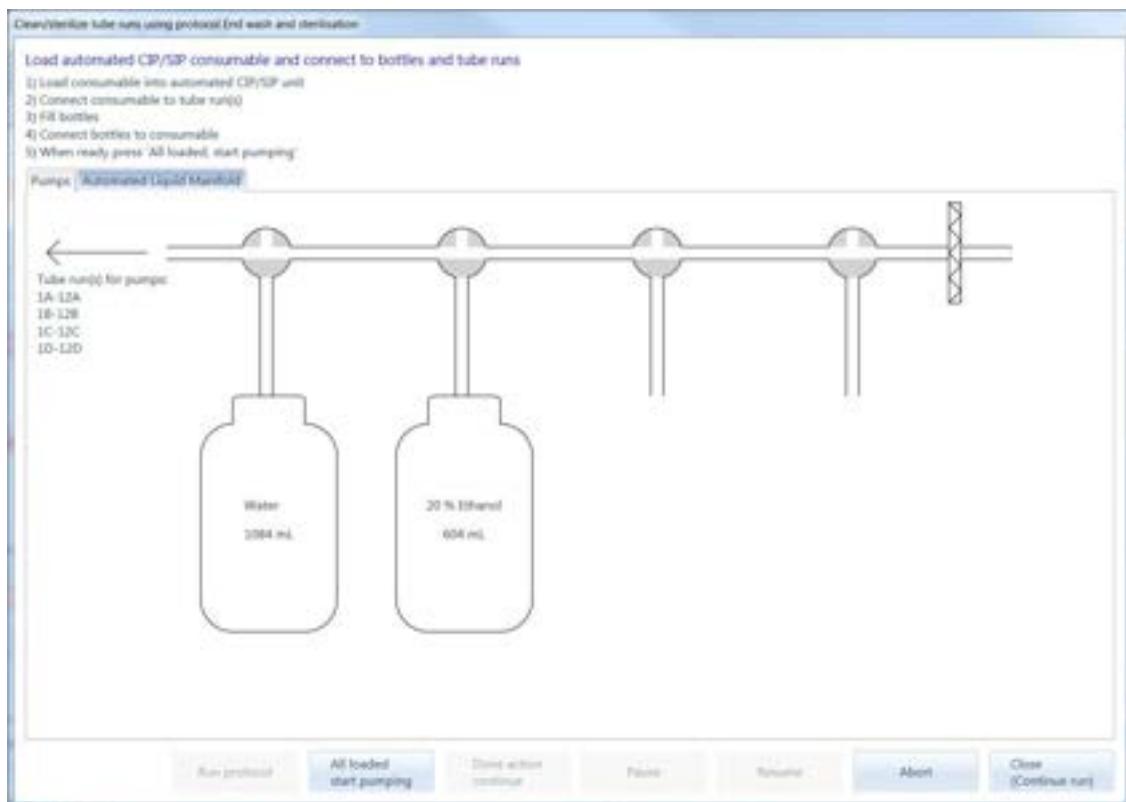


Figure 296 System showing liquids to load for CIP/SIP

Once all the liquids are loaded press **All loaded start pumping** to run the protocol. The system will then run the CIP protocol automatically.

Note:

- The system adds additional air gaps and volumes of liquid to the protocol to allow for the volumes in the automated CIP/SIP tubing and manifold. If non-standard tubing arrangements are used then review the volumes of liquid used in the base protocol.
- The protocol runs automatically. Do not run as a single protocol liquids that you do not want to mix together in the waste from the system.

6.7.3 Skipping CIP/SIP

To skip cleaning tube runs and the pumps they are connected to:

- 1) Press **Skip clean/sterilize of tube runs...** The system will display the **Skip clean/sterilize tube runs** window.

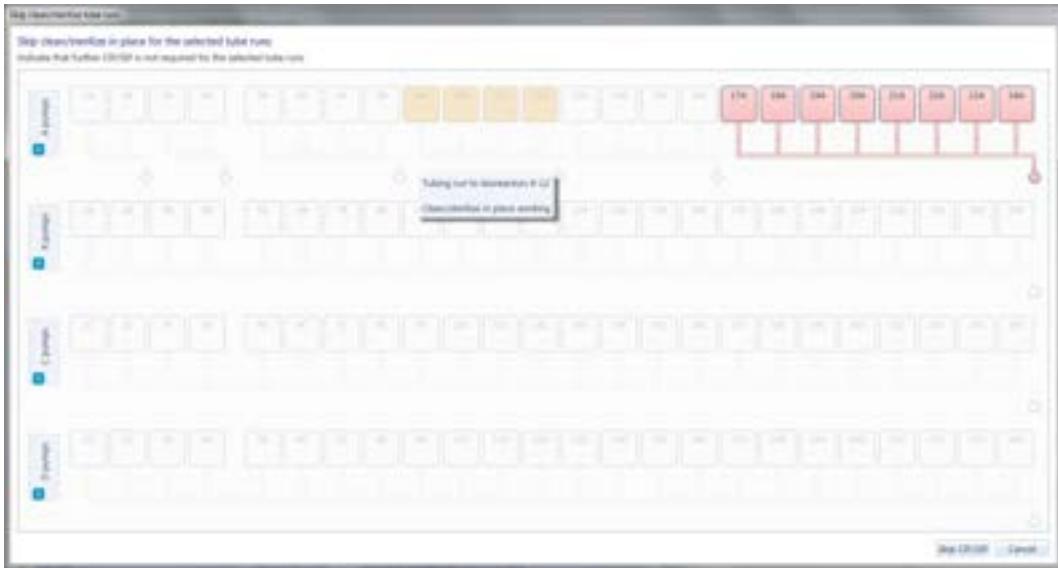
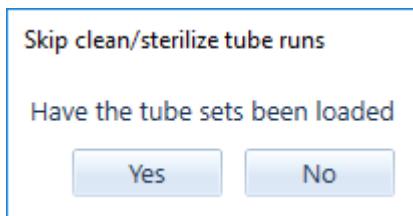


Figure 297 **Skip clean/sterilize tube runs** window

- 2) Press **Skip CIP/SIP** to indicate that the tube runs do not need cleaning.
- 3) If required then confirm that the tubing is loaded.



6.8 Prime pumps

The **Prime pumps** page allows the operator to prime pumps by pumping liquid through the tubing attached to the pump, through the pump and out though the fixed liquid manifold.



Figure 298 **Prime pumps** page

- 1) Select the pumps to prime by clicking on items as appropriate.
- 2) **Pump liquid...** displays the **Prime pumps** window.



Figure 299 Prime pumps window

- 3) Select a **Volume** to pump and if required change the **Flow** rate. The available flow rates will depend on the number of pumps selected and on the **Refill rate** specified for those pumps in the **Pumps** definition.
- 4) Press **Start pumping** to pump the required pumping.
- 5) If required press **Stop pumping** to stop the pumps.
- 6) When you are satisfied that the pumps are primed press **Mark done and close**, otherwise repeat steps 3...5.

Whilst the pumps are pumping the window can be closed.



Figure 300 Options while pumping

Press **Close (Continue pumping)** to close the window.

Once pumping is complete the window can be closed either discarding the run – **Clear run and close** – or keeping the run in progress – **Close (Save selection)**.

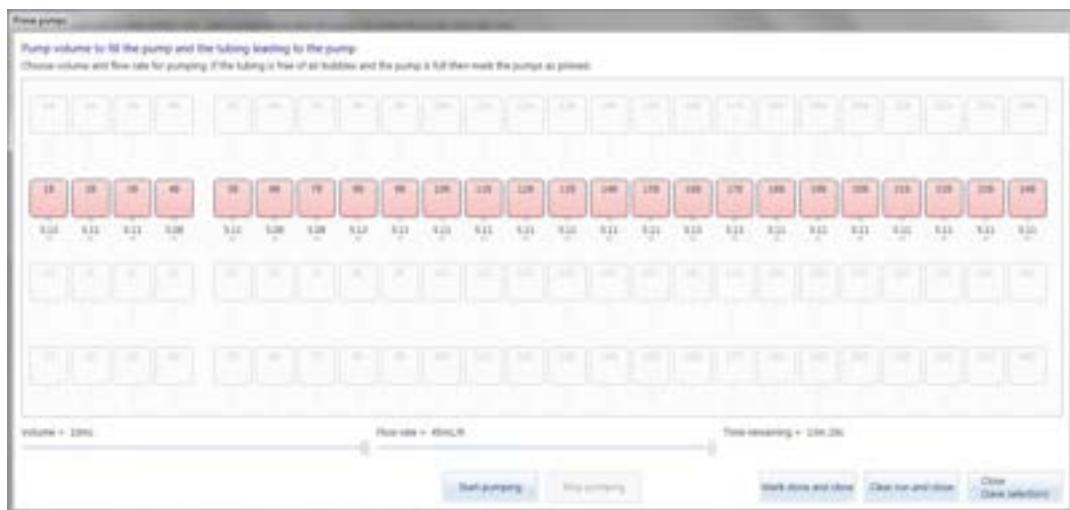


Figure 301 **Prime pumps** window after stopping priming.

In progress runs are shown on the **Prime pumps** page and on the **Todo** page.



Figure 302 **Prime pumps** page with **In progress** run

6.9 Connect bioreactors

The **Connect bioreactors** page allows the operator to connect the bioreactors.

Connecting a bioreactor comprises connecting the bioreactor's liquid handling manifold to the fixed liquid handling manifold on the system.

Once the bioreactor is connected the system will allow gassing of the bioreactor.

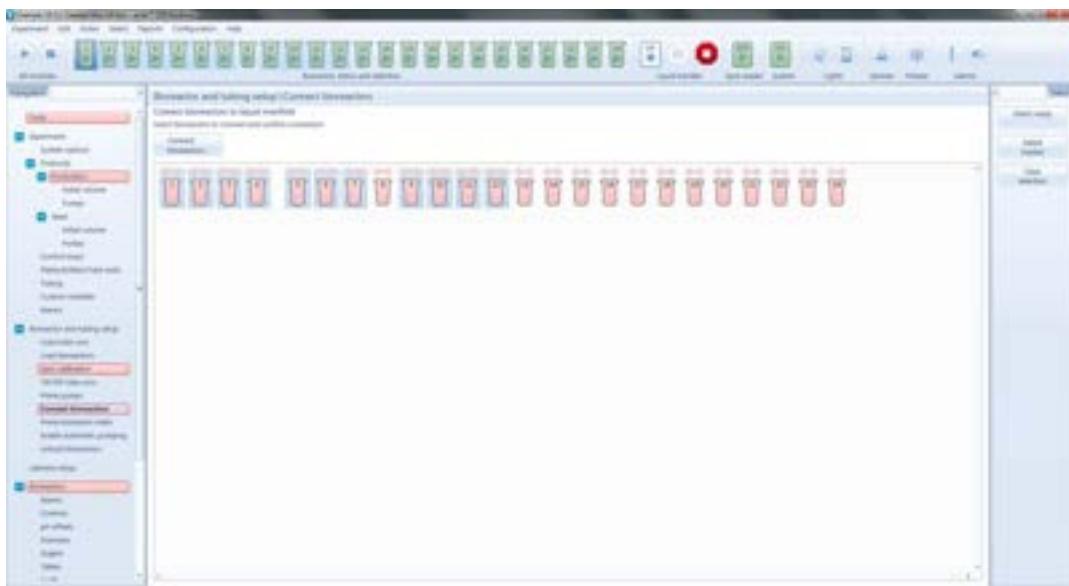


Figure 303 **Connect bioreactors** page. Select the bioreactors to connect by clicking on items as appropriate.

- 1) **Connect bioreactors...** displays the window to confirm that the bioreactors are connected. The system will switch the lights on the bioreactors to be connected on.



Figure 304 **Connect bioreactors** window

- 2) Connect the indicated bioreactors.
- 3) Should you not have connected a bioreactor – for example you have dropped the bioreactor on the floor – then deselect the **Do for** option for that bioreactor.
- 4) Press **Connected bioreactors** to confirm that the bioreactors are connected.

6.10 Prime bioreactor inlets

The **Prime bioreactor inlets** page allows the operator to prime the tubing leading to the bioreactor for the greatest accuracy when pumping.

To prime the bioreactor inlets the operator moves the liquid until it is at the top of the bioreactor and then tells the system the liquid is at that reference position.



Figure 305 Prime bioreactor inlets page

6.10.1 Remote Priming Application

The bioreactor inlets can be primed using the remote priming application.

Press **Enable remote priming** to allow the remote application to connect. A window is displayed indicating whether the remote application is connected.

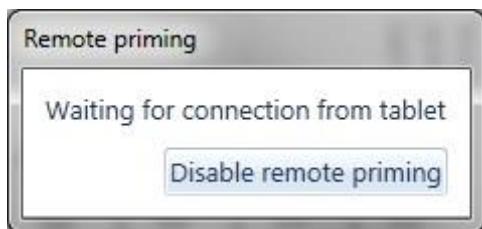


Figure 306 **Remote priming** window shown while remote priming application is allowed to connect
Press **Disable remote priming** to stop remote priming.

To start remote priming on the Android device select the **ambr 250 Bioreactor Primer** application from the applications menu.

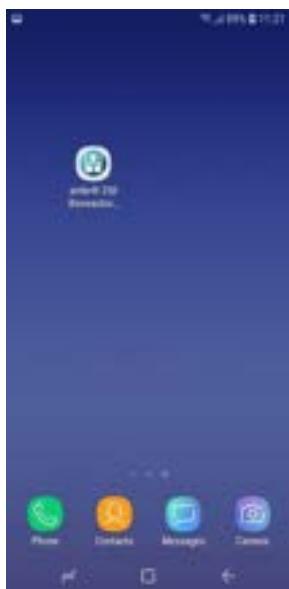


Figure 307 Android application menu showing the ambr 250 Bioreactor Primer application

Once the application has started the following screen will be shown.



Figure 308 Bioreactor Primer connect screen

Touch **CONNECT** at the top of the screen to connect to the main application.

On successful connection the main priming control screen will appear.

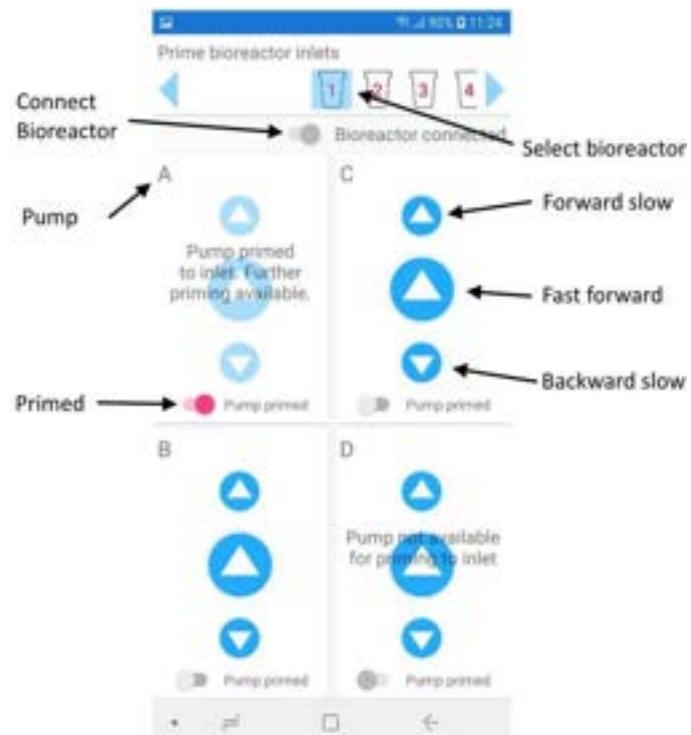


Figure 309 Bioreactor primer priming control screen

The scroll wheel at the top of the screen allows the bioreactor of interest to be selected. When selected the bioreactor light is illuminated allowing the operator to easily identify the bioreactor. The number of the bioreactor in the scroll bar indicates if action is required for the bioreactor. The number is shown in red if action is required.

Forward and backward controls allow the liquid level for the pump inlet to be adjusted. Pumps whose inlets do not require priming are marked with "Prime not needed". Although it is not necessary to prime the inlet, the pump can still be controlled and the liquid level adjusted if desired.

Pressing a pump control changes the state of the pump to running and pumps a small volume of liquid. The state of the pump is reflected on the main application.

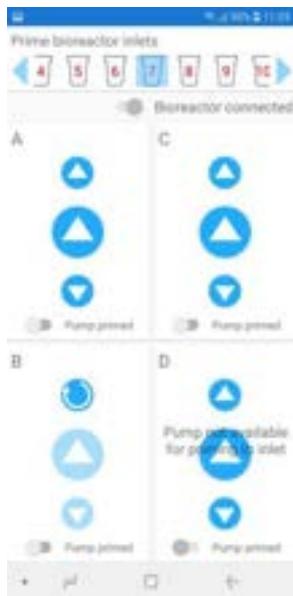


Figure 310 Bioreactor primer showing pump B inlet slow forward priming running

Once the inlet has been primed, touching **Pumped primed** marks the pump for the selected bioreactor as primed.

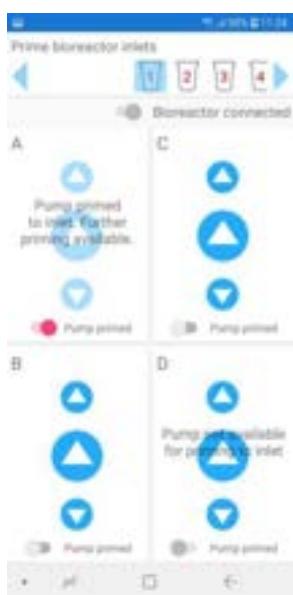


Figure 311 Bioreactor primer showing Pump A inlet primed

If the inlet for a previously primed pump needs to be subsequently re-adjusted then touching the **Pumped primed** again displays a confirmation dialog that allows the pump to be marked as not having been primed and the forward and backward controls re-enabled.

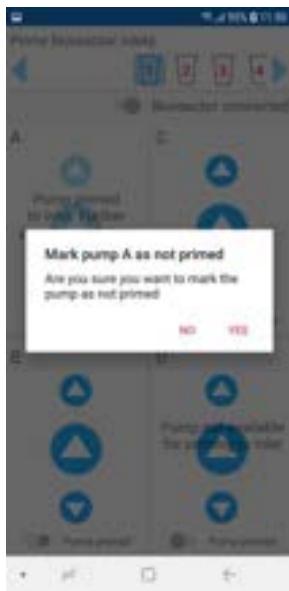


Figure 312 Confirmation dialog for marking a pump as not primed

It is also possible to connect a bioreactor by touching on the **Bioreactor connected** if required...
The status of the bioreactor is reflected on the main application.



Figure 313 Dialog showing a disconnected bioreactor

Once all inlet priming has been finished touching the back button or home disconnects the Bioreactor primer application from the main application and returns it to the connect screen.

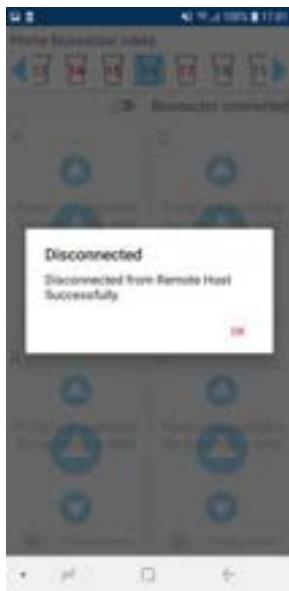


Figure 314 Dialog showing disconnection from Ambr® 250

Touch **SETTINGS** shows you the current settings for the device. These can be changed if the device is to be operated via a corporate wifi network.



Figure 315 remote priming device network settings

6.10.2 Priming bioreactor inlets via Desktop

The bioreactor inlets can be primed using a similar interface to **Prime pumps**.

Positive and negative volumes can be chosen to move the liquid to the required position.



Figure 316 **Prime bioreactor inlets** window

6.11 Enable automatic pumping

The **Enable automatic pumping** page allows the operator to enable automatic control of pumps and other aspects of the bioreactor. This step avoids pumps starting into action as soon as the bioreactor inlets have been primed.

Automatic pumping allows the operation of pumps from steps in the protocol and the operation of the control loops in the protocol.



Figure 317 **Enable automatic pumping** page. Select the pumps to enable by clicking on items as appropriate.

- 1) **Enable automatic pumping...** displays the window to confirm that pumps are to be set to pump automatically. The system will switch the lights on the relevant bioreactors on.

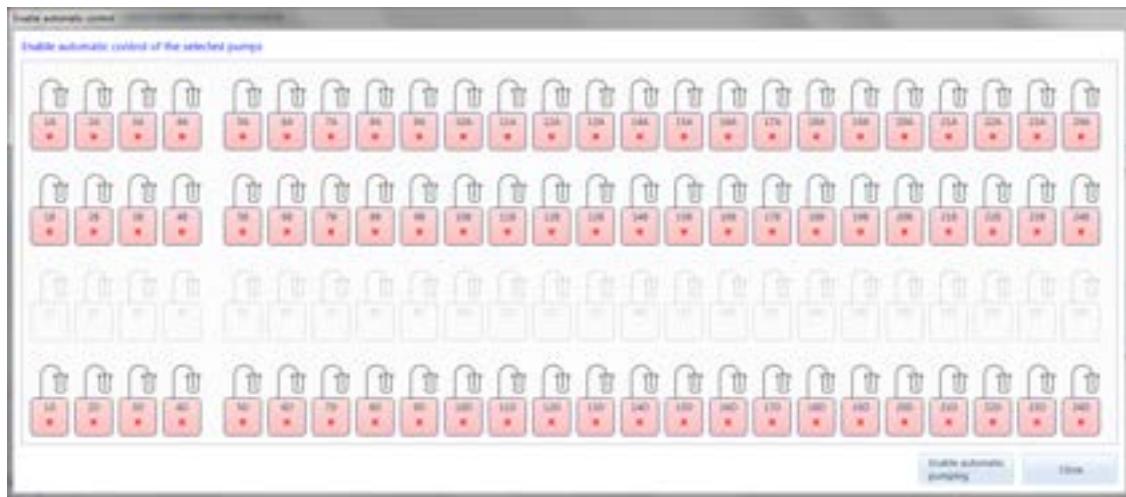


Figure 318 **Enable automatic pumping** window

- 2) Press **Enable** to enable automatic pumping on the selected pumps

6.12 Bioreactor and tubing cleanup

The **Bioreactor and tubing cleanup** page displays an overview tasks required at the end of an experiment.

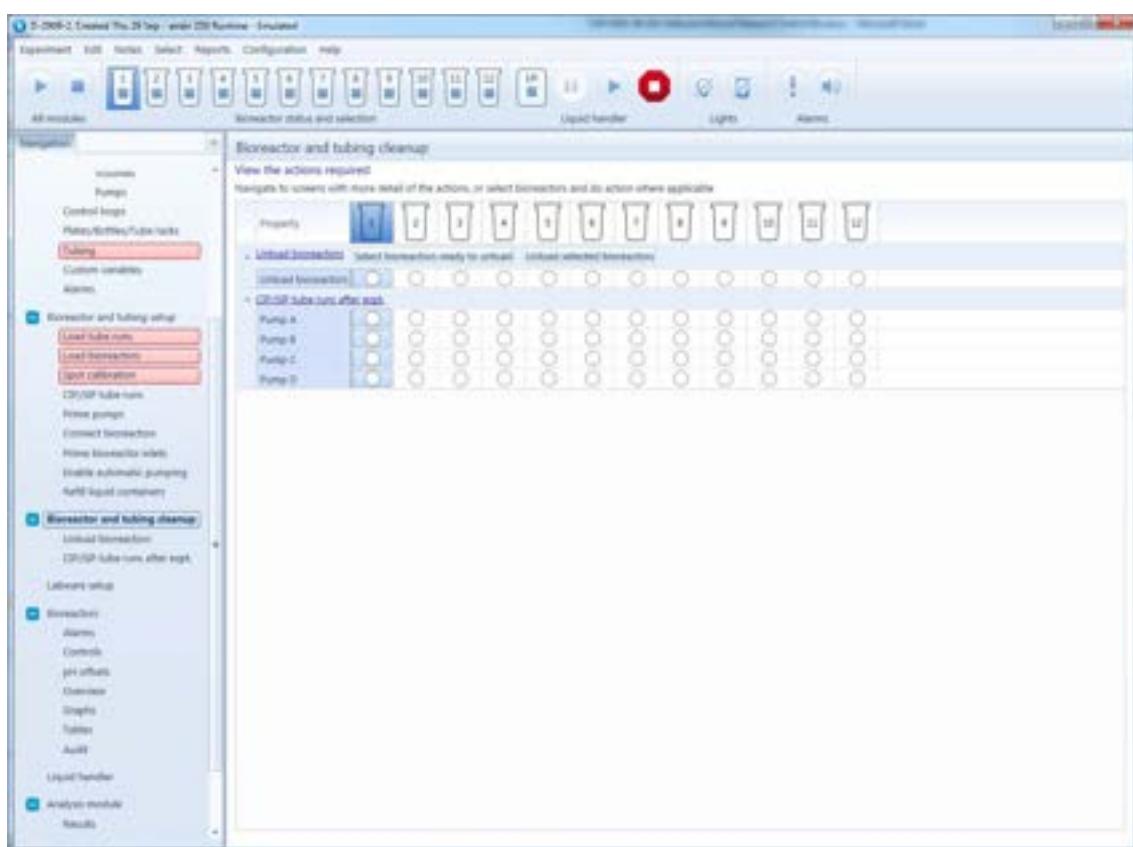


Figure 319 Bioreactor and tubing cleanup

The page shows an indicator for each action indicating whether the action has been done for a bioreactor. For actions that apply to individual pumps an indicator is shown for each pump.

The indicator shows:

- Completed action
- Action needs to be done and could be done next
- Action does not need to be done for this process
- Action will need to be done, but is not appropriate yet

Clicking the link describing an action displays the page for performing the action.

Actions that only require a selection of bioreactors can be performed from this page. For example:

- **Select bioreactors ready to unload** selects the bioreactors ready to be unloaded.
- **Unload selected bioreactors...** displays the window for unloading bioreactors.

6.13 Unload bioreactors

The **Unload bioreactors** page allows the operator to unload bioreactors while the system continues to run.

If all the bioreactors are unloaded together at the end of an experiment then this page can be skipped. New experiments start assuming that all of the bioreactors have been unloaded.

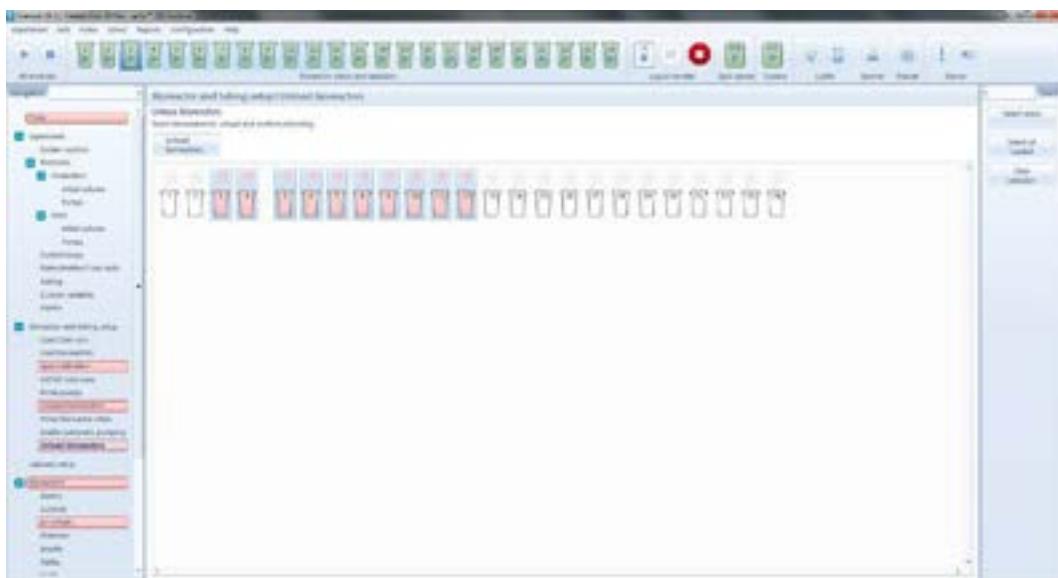


Figure 320 **Unload bioreactors** page. Select the bioreactors to unload by clicking on items as appropriate.

1) Press **Unload bioreactors...** The system:

- a) Stops temporarily gassing, stirring and pumping of the selected bioreactors.
- b) Turns the lights on the bioreactors to be unloaded on.

- c) Displays the window to confirm that the bioreactors are unloaded.



Figure 321 Unload bioreactors window

- 2) Unload the indicated bioreactors.
- 3) Should you not have unloaded a bioreactor then deselect the **Do for** option for that bioreactor.
- 4) Press **Unloaded bioreactors** to confirm that the bioreactors have been unloaded.

If you press **Cancel** or you have deselected the **Do for** option for a bioreactor then gassing, stirring and pumping will resume on the appropriate bioreactors when the **Unload bioreactors** window closes.

Once you have unloaded the bioreactor the system turns off all the set points and control loops for the bioreactor and stops running steps for the bioreactor.

6.14 CIP/SIP tube runs after expt.

The CIP/SIP tube runs page shown below works in the same way as the CIP/SIP tube runs page described in section 6.7 above.

The page provides a prompt and facility for cleaning pumps after an experiment. If the pumps are going to be used soon for another experiment then CIP/SIP can be done as part of that next experiment and does not need to be done here.



Figure 322 CIP/SIP tube runs after expt. page

6.15 Reuse bioreactor stations

Once bioreactors have been unloaded from the system before the bioreactor stations can be reused.

The reuse process:

- Gets the user to export the data prior to reusing the bioreactors.
- Clears data associated with the reused bioreactors.
- Removes the reused bioreactors from their protocol.
- Deletes labware definitions and protocols that are no longer referenced because of the reuse of the bioreactors.
- Clears the state of tubing runs loaded on the reused bioreactors and not shared by other bioreactors.

Bioreactor stations cannot be reused if removing the protocol from the bioreactor would disturb the definitions for other bioreactors in the protocol. In particular the system checks for the use of the **By relative position** option in liquid handling steps.

On high throughput machines the **Reuse bioreactors page** is displayed within the **Bioreactor and tubing setup** section.

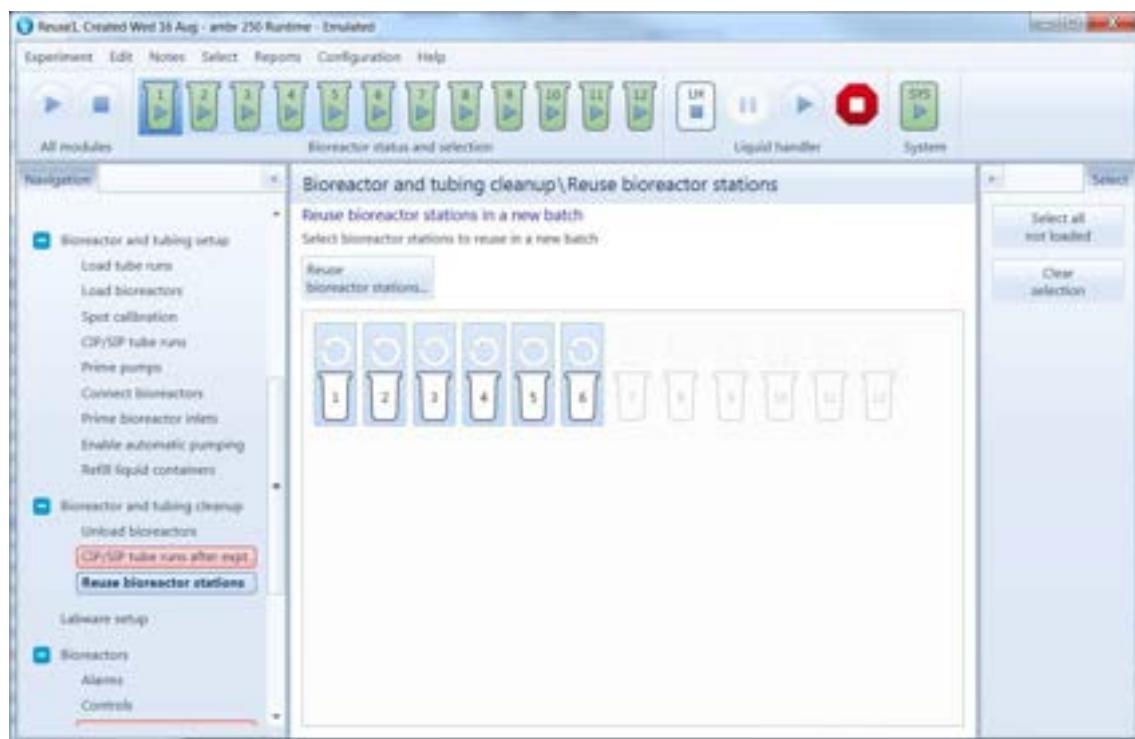


Figure 323 **Reuse bioreactors stations** page

Select the bioreactors to be reused and press **Reuse bioreactor stations**. The system will display the **Reuse bioreactors stations** window with the ability to confirm which bioreactors to reuse.

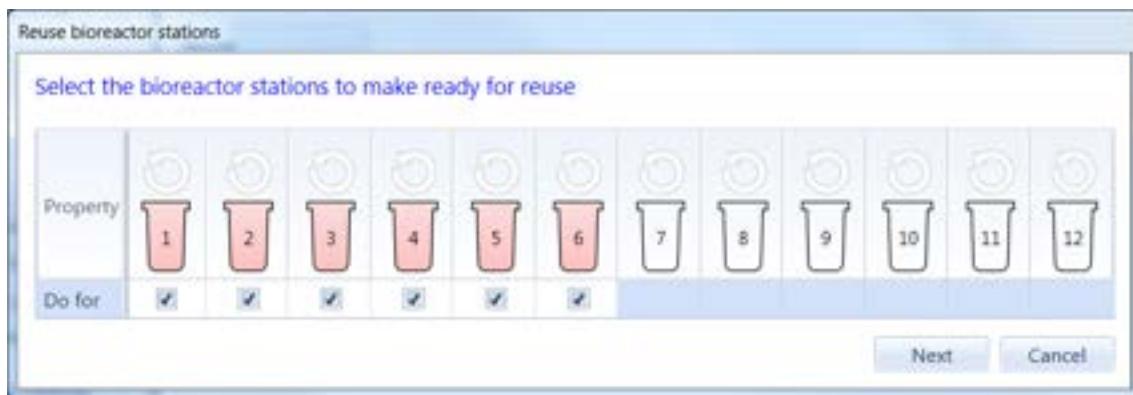


Figure 324 **Reuse bioreactors stations** dialog with options to select bioreactors.

Update the selection of bioreactors as required and press **Next**. The system will show options for exporting the current experiment data.

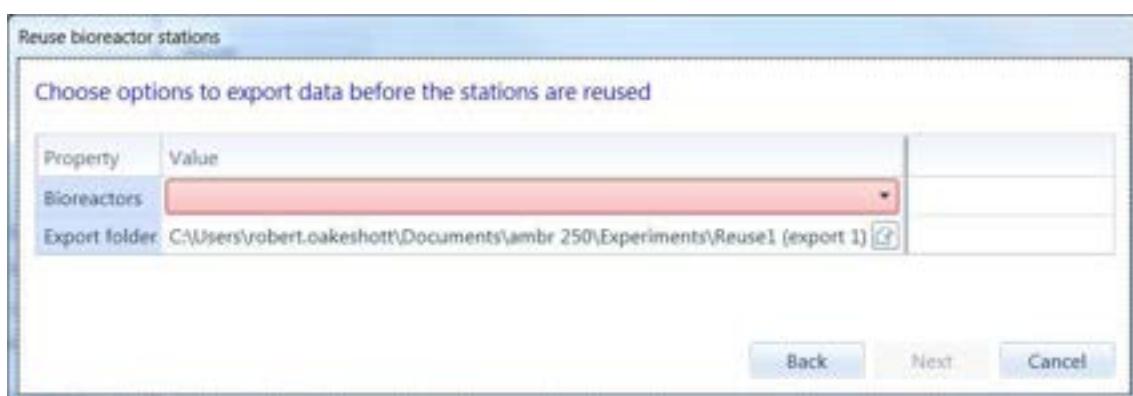


Figure 325 **Reuse bioreactors stations** dialog with for exporting data.

Bioreactors offers the options to export data for **All bioreactors** or just for **Bioreactors to be reused**.

Press **Next** and the system will display details of what will be done.

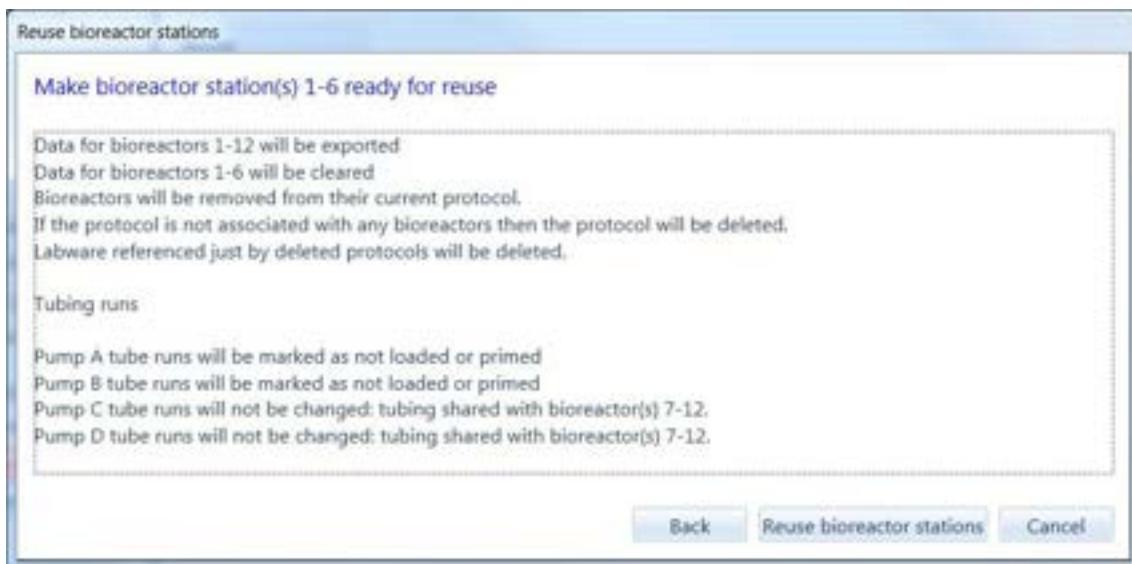


Figure 326 **Reuse bioreactors stations** dialog with details of the data that will be exported.

Press **Reuse bioreactor stations** to get the system to export the existing data and clear the data for the bioreactors to be reused.

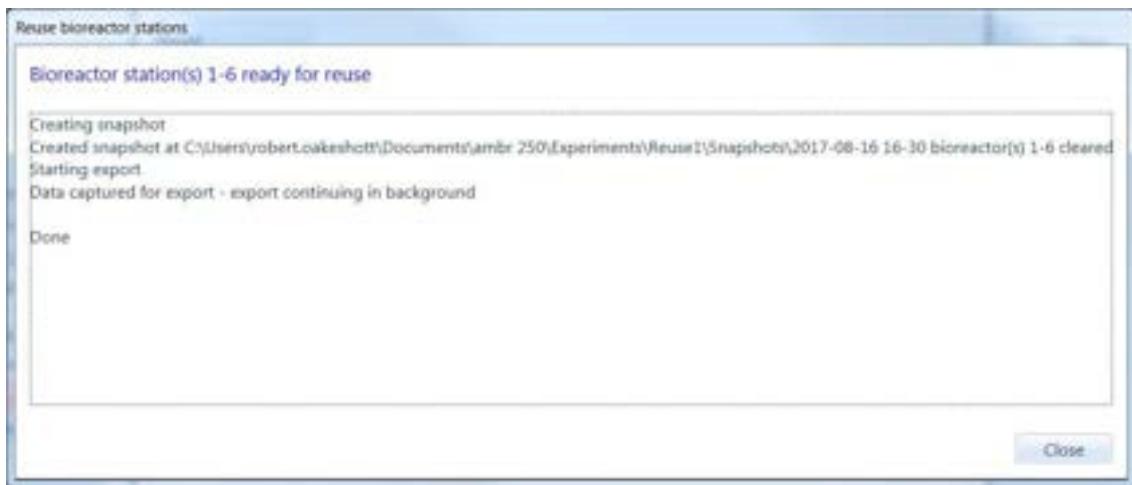


Figure 327 **Reuse bioreactors stations** dialog with details of progress.

Once the process of reusing the bioreactors is complete press **Close**, then define the protocols and other details of the process that the reused bioreactors are to follow.

6.15.1 Data storage

All the data for the bioreactors is stored in one experiment directory until a new experiment is created. The system notes which data applies to the bioreactors as loaded.

In addition to the exported experiment the system creates an internal record of the state of the system when the bioreactors were reused. These snapshots can be opened from the **ambr250 Experiment Viewer**.

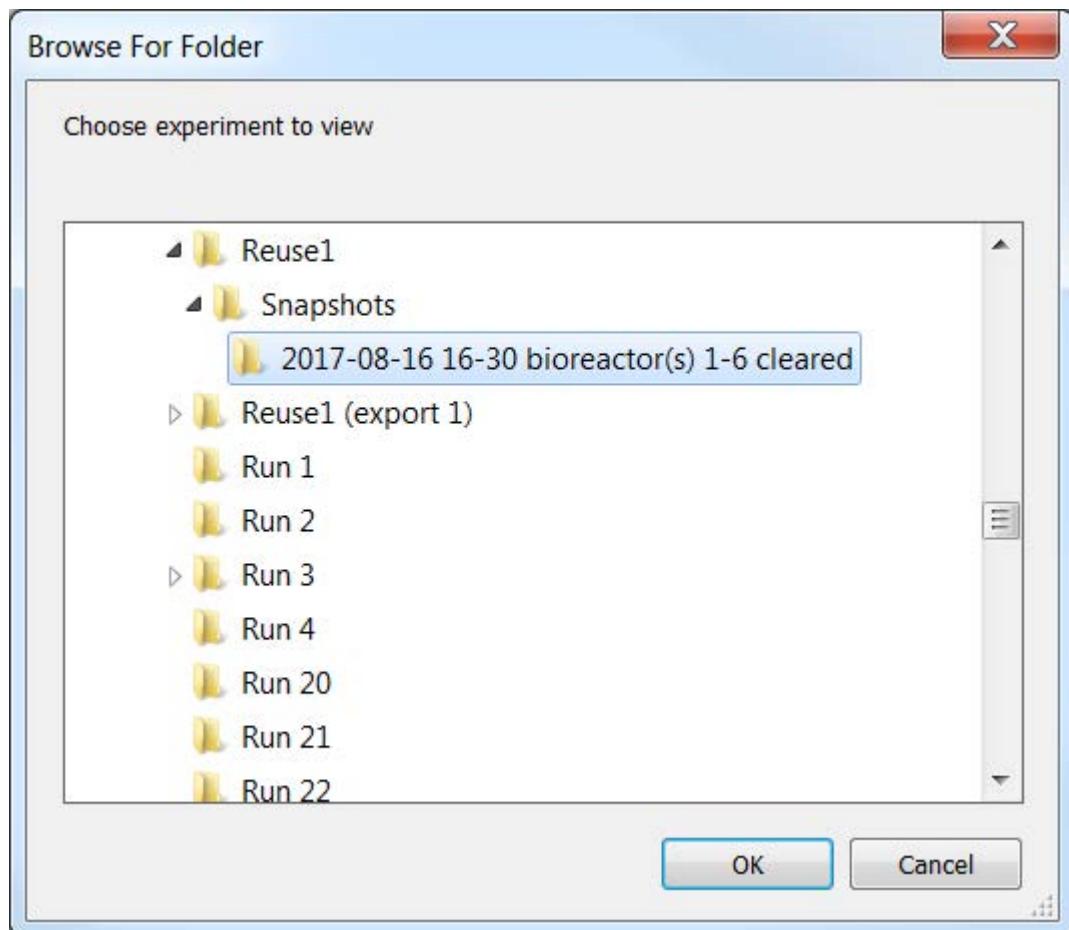


Figure 328 Choosing a snapshot from experiment with the data (just before) bioreactors 1-6 were reused.

7 PERFUSION SET UP PAGES

Systems running perfusion have additional set up pages.

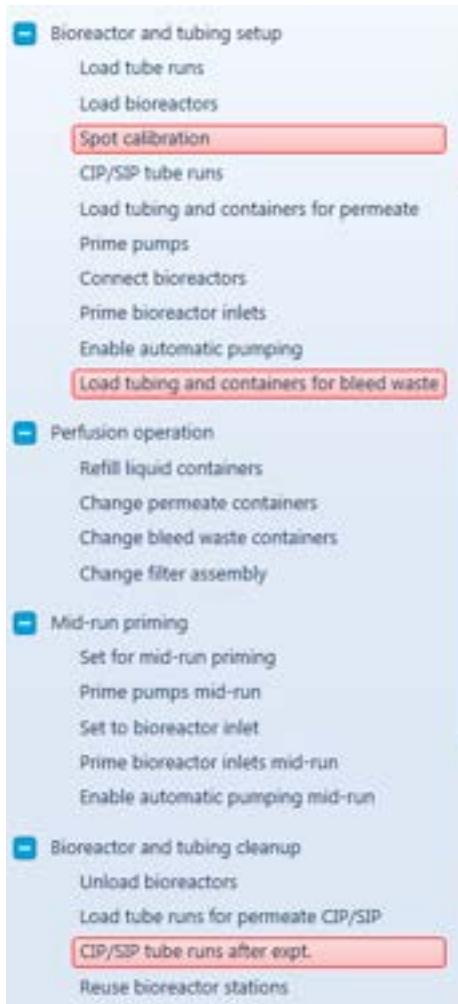


Figure 329 Set up pages in a perfusion run

Stopping and starting perfusion operations can take some time. While the system is getting the perfusion system into the right state for the selected operation the white bioreactor lights and the mimic for the relevant bioreactors will flash. Once the system is in the correct state the white bioreactor light will show the selected bioreactors.

7.1 Additional tasks required

The additions tasks required while setting up the run are:

- **Load tubing and containers for permeate.** Once CIP/SIP of the pumps is complete the lines used to feed cleaning fluid to the permeate pump must be removed and replaced with the lines that will take the permeate coming out of the system.
- **Load tubing and containers for bleed waste.** Before bleeding is done the tubing to receive the bleed waste must be connected.

During the run additional **Perfusion operation** tasks are relevant.

- **Refill liquid containers** supports changing media or other bags temporarily stopping pumping using the relevant pumps.
- **Change permeate containers** supports changing the containers used to receive the permeate. Permeate pumping is stopped while the containers are changed.
- **Change bleed containers** supports changing the containers used to receive the bleed output. Bleeding is stopped while the containers are changed.
- **Change filter assembly** allows the hollow fibre to be exchanged and allows issues with the labware to be diagnosed.

Note that stopping a pump may indirectly stop other pumps via the interlocks described in section **Error! Reference source not found..**

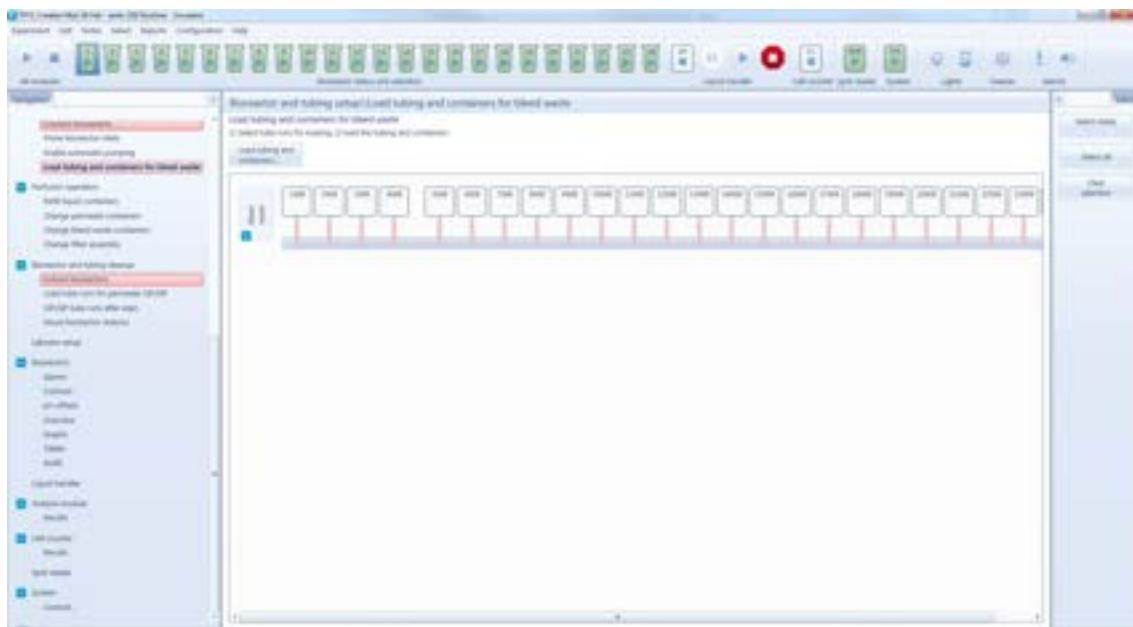
7.2 Load tubing and containers for permeate

The **Load tubing and containers for permeate** page works in the same way as the Load tube runs page described in section **Error! Reference source not found..**

Figure 330 Load tubing and containers for permeate page

7.3 Load tubing and containers for bleed waste

The **Load tubing and containers for bleed waste** page works in the same way as the Load tube runs page described in section **Error! Reference source not found..**



7.4 Refill liquid containers

The **Refill liquid containers** page allows refilling containers during a run.



Figure 331 Refill liquid containers page

Select the containers to be refilled and press **Refill containers**. Only containers belonging to a single pump row can be selected together.

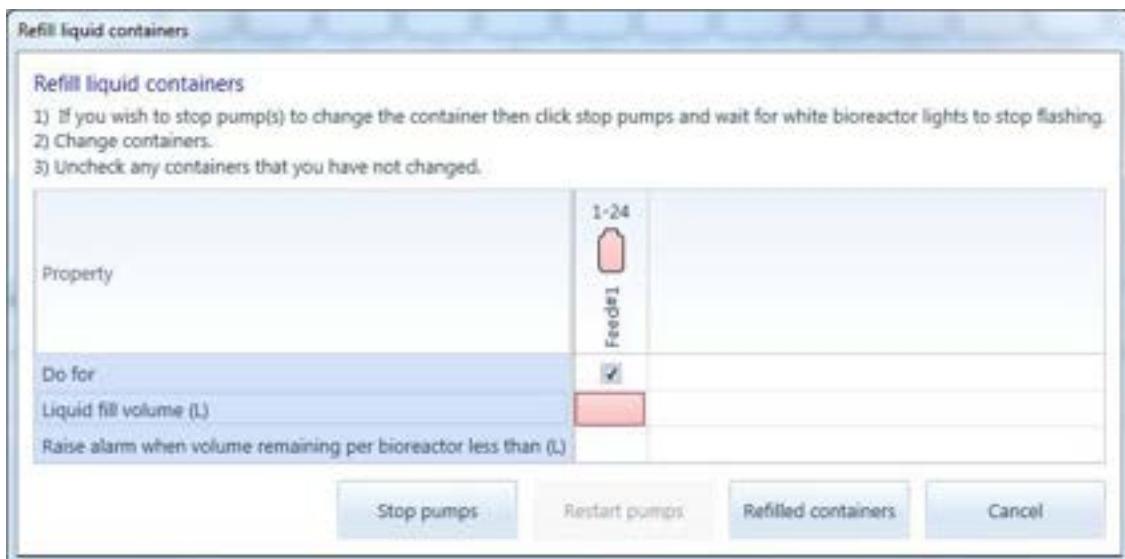


Figure 332 Refill liquid containers dialog

In the dialog that appears press **Stop pumps** and **Restart pumps** to stop and start the pumps as required.

Enter in **Liquid fill volume (L)** the new volume in the containers.

Enter in **Raise alarm when volume remaining per bioreactor less than (L)** alarm volume. System will alert the user to refill the container when the remaining volume is less than this value.

When you have refilled the containers press **Refilled containers**. If you have refilled some but not all of the containers use the **Do for** option to tell the system which containers have been changed.

If the dialog is closed with pumping stopped then a section will appear at the bottom of the page allowing the interaction to be resumed.

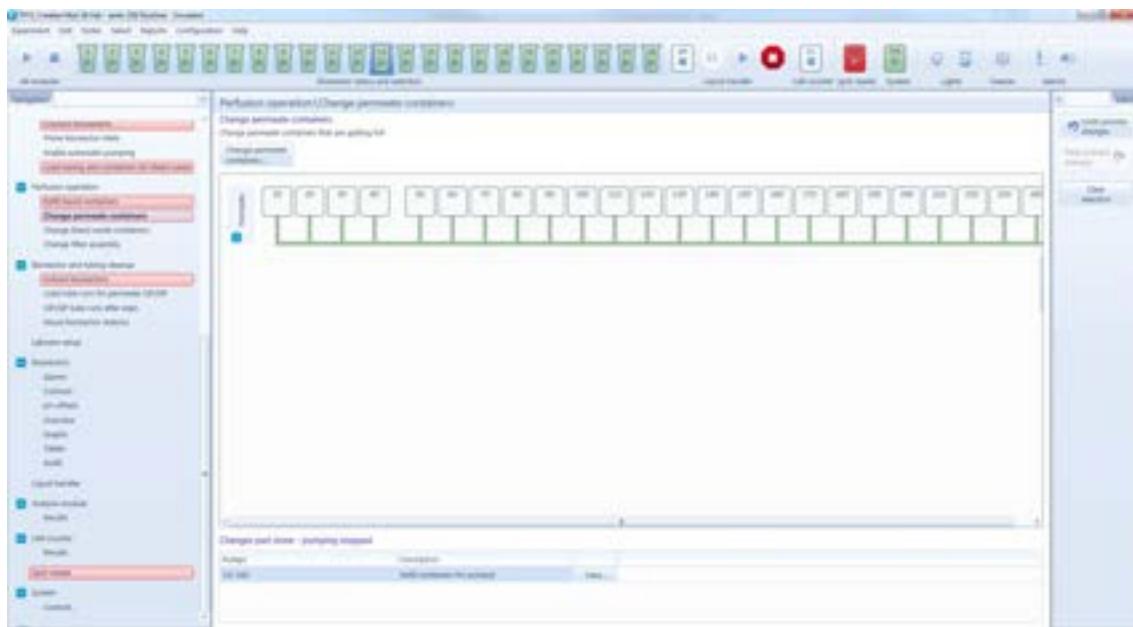


Figure 333 Refill liquid containers with paused interaction

7.5 Change permeate containers

The **Change permeate containers** page allows changing permeate containers during a run.

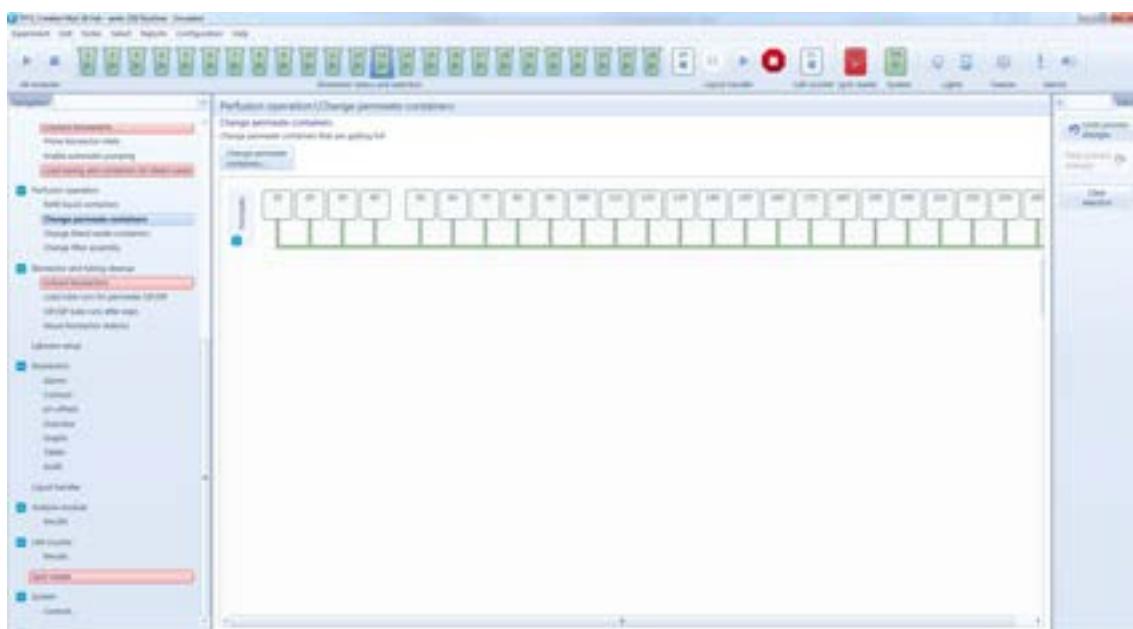


Figure 334 Change permeate containers page

Select the containers to be refilled and press **Change permeate containers**.

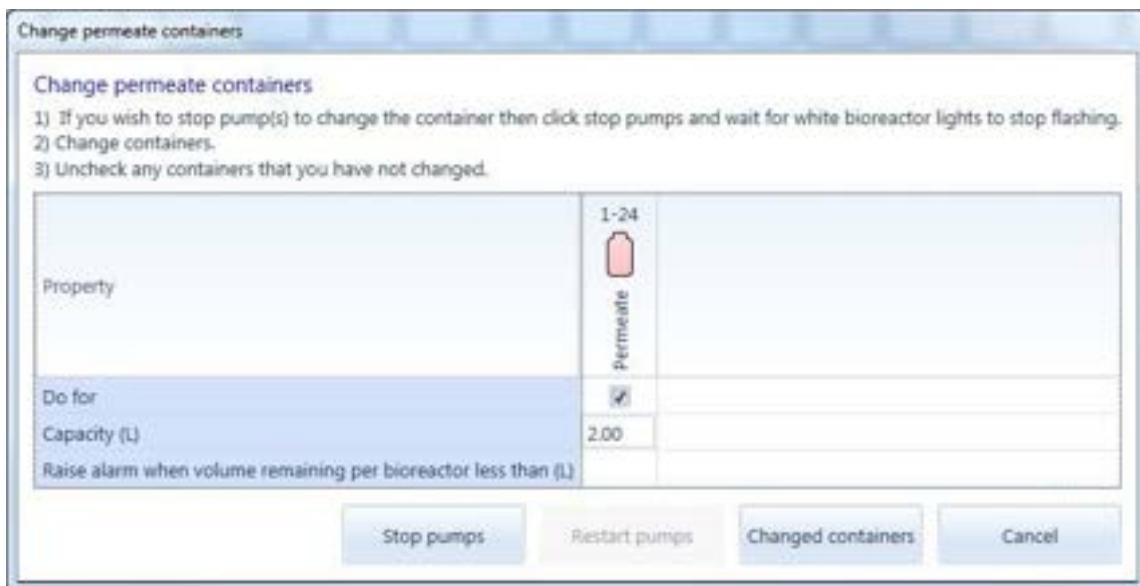


Figure 335 Change permeate containers dialog

In the dialog that appears press **Stop pumps** and **Restart pumps** to stop and start the pumps as required.

Enter in **Capacity (L)** the volume available in the new containers.

Enter in **Raise alarm when volume remaining per bioreactor less than (L)** alarm volume. System will alert the user to change the container when the remaining volume is less than this value.

When you have changed the containers press **Changed containers**. If you have refilled some but not all of the containers use the **Do for** option to tell the system which containers have been changed.

If the dialog is closed with pumping stopped then a section will appear at the bottom of the page allowing the interaction to be resumed.

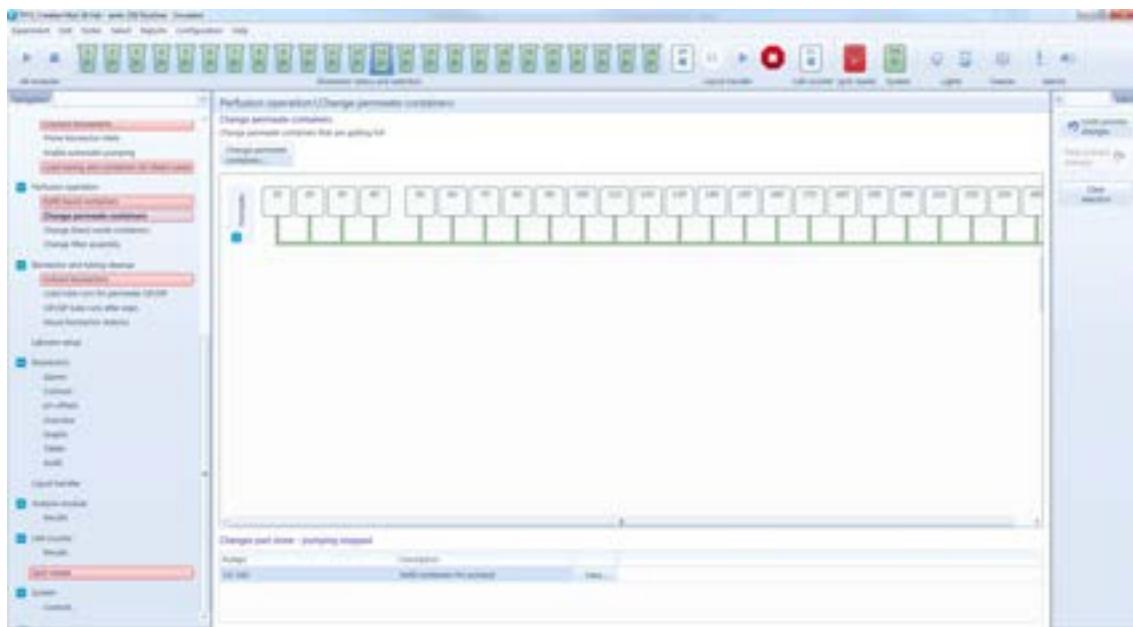


Figure 336 Change permeate containers with paused interaction

7.6 Change bleed containers

The **Change bleed containers** page allows changing bleed containers during a run.

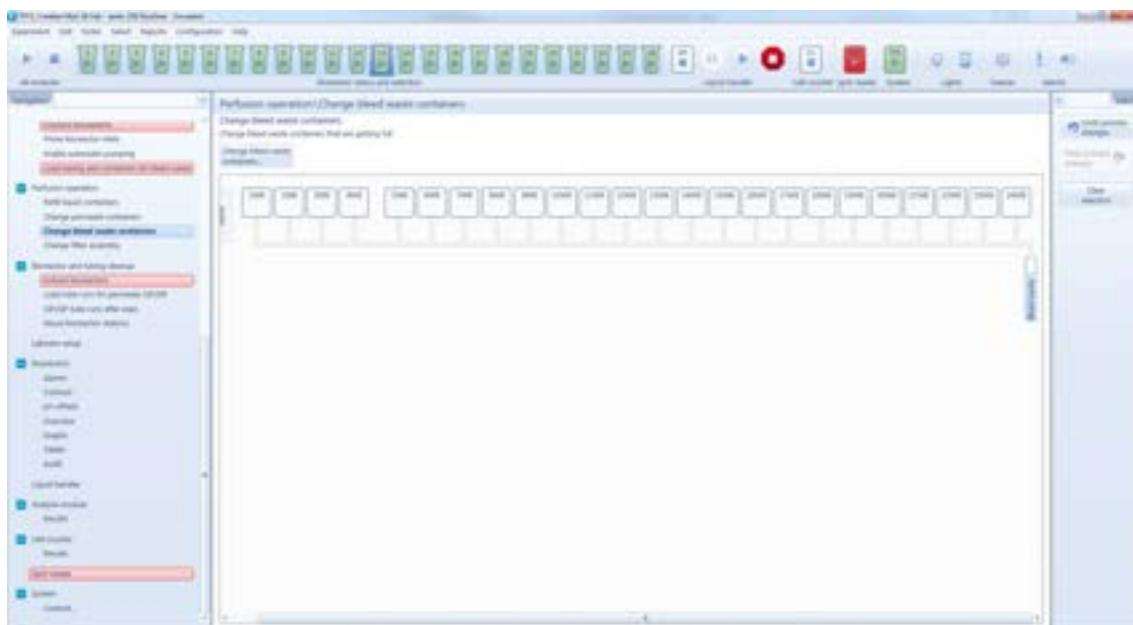


Figure 337 Change bleed containers page

Select the containers to be refilled and press **Change bleed waste containers**.



Figure 338 Change bleed waste containers dialog

In the dialog that appears press **Stop bleeding** and **Restart bleeding** to stop and start bleeding as required.

Enter in **Capacity (L)** the volume available in the new containers.

Enter in **Raise alarm when volume remaining per bioreactor less than (L)** alarm volume. System will alert the user to change the container when the remaining volume is less than this value.

When you have changed the containers press **Changed containers**. If you have refilled some but not all of the containers use the **Do for** option to tell the system which containers have been changed.

If the dialog is closed with bleeding stopped then a section will appear at the bottom of the page allowing the interaction to be resumed.

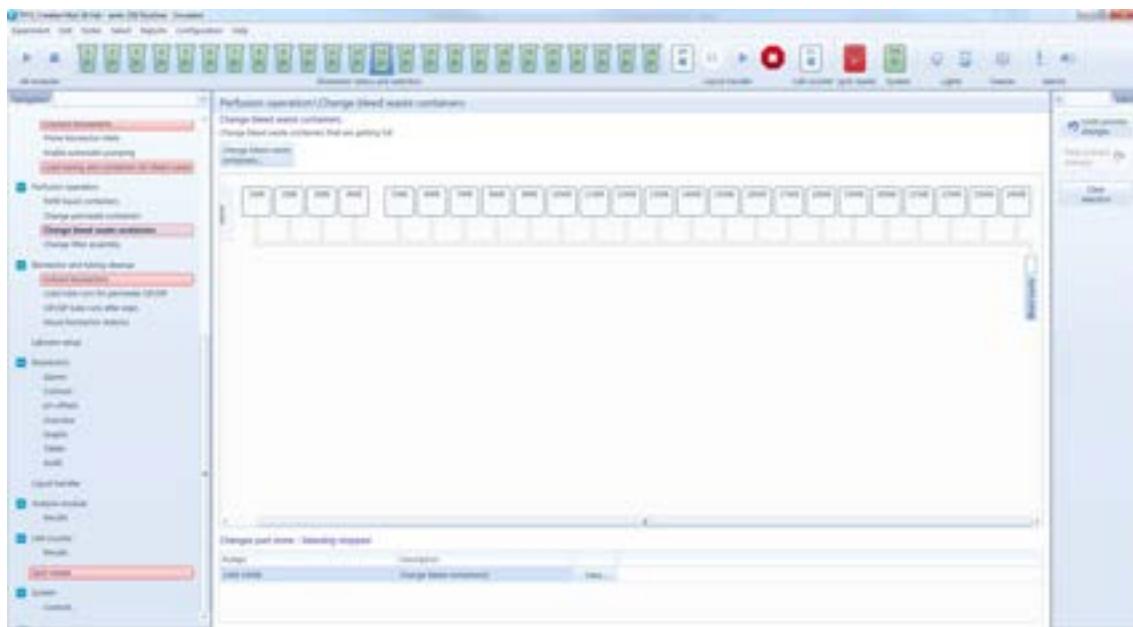


Figure 339 Change bleed waste containers with paused interaction

7.7 Change filter assembly

The Change filter assembly page allows:

- **Change perfusion assembly** – to change the perfusion assembly.
- **Change perfusion filters** – to change the perfusion filters.
- **Clear flag from alarm** – to clear the flag that can be set up alarms to alert the user to change filters.
- **Run pressurization test** – to run a test of the labware.
- **Unclamp tubing cassettes** – to unclamp the tubing cassette against which the pinch clamp operates to allow checking the cassette is properly engaged and check for any issues.

An indicator under each filter shows the pressurization test status for that filter.

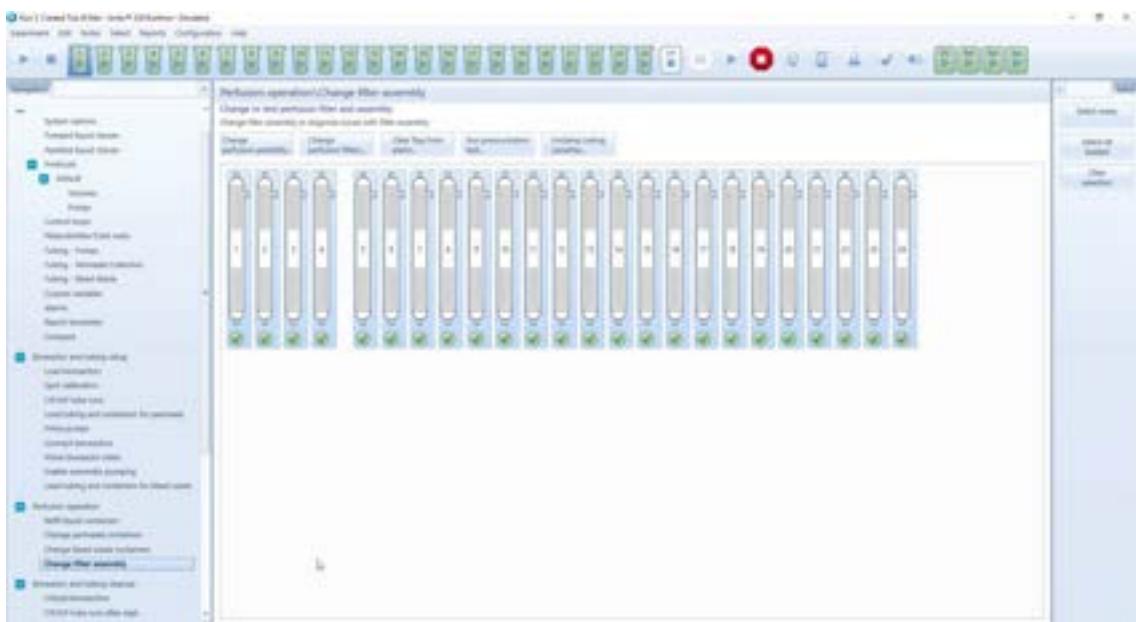


Figure 340 Change filter assembly page

7.7.1 Change perfusion assembly

To change assemblies select the assemblies to change and press **Change perfusion assembly**.

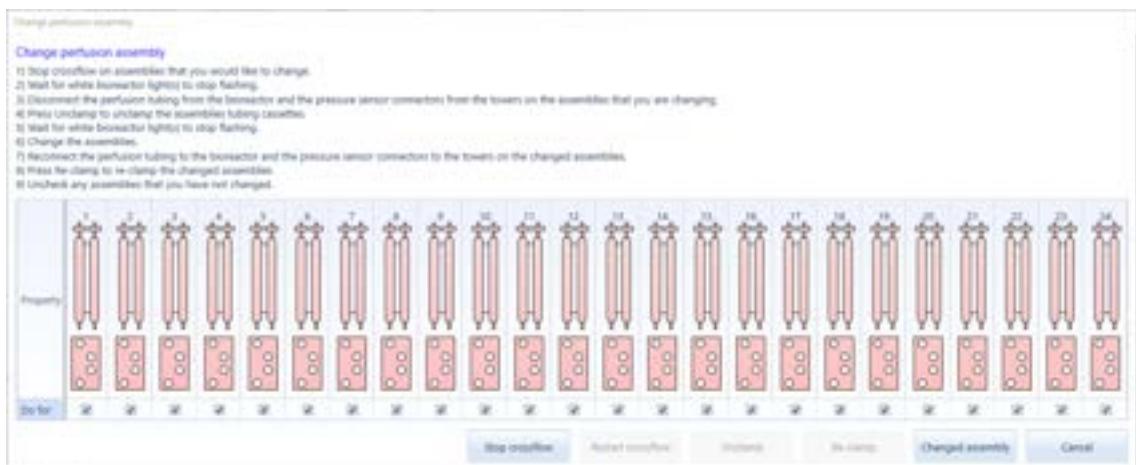


Figure 341 Change perfusion assembly dialog

In the dialog that appears press **Stop crossflow** and **Restart crossflow** to stop and start crossflow pumping as required.

When crossflow has stopped press **Unclamp** to release the pinch valves. Press **Re-clamp** if you wish to re-clamp the pinch valves.

When you have changed the assembly press **Changed assembly**. If you have changed some but not all of the assemblies use the **Do for** option to tell the system which assemblies have been changed.

After changing the assemblies, assemblies will be clamped and pressurization tests run. A dialog will appear to **Prime filters**.



Figure 342 Prime filters dialog

If the dialog is closed with assemblies unclamped, crossflow stopped or filters not primed then a section will appear at the bottom of the page allowing the interaction to be resumed.

7.7.2 Change perfusion filters

To change filters select the filters to change and press **Change perfusion filters**.

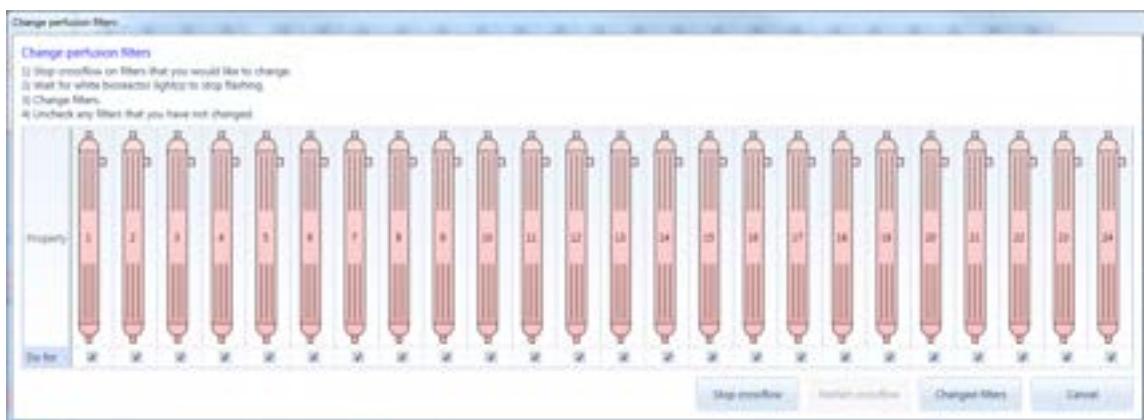


Figure 343 Change perfusion filters dialog

In the dialog that appears press **Stop crossflow** and **Restart crossflow** to stop and start crossflow pumping as required.

When you have changed the filters press **Changed filters**. If you have changed some but not all of the filters use the **Do for** option to tell the system which filters have been changed.

If the dialog is closed with crossflow stopped then a section will appear at the bottom of the page allowing the interaction to be resumed.

7.7.3 Clear flag from alarm

To clear the flag select the filters to change and press **Clear flag from alarm**.

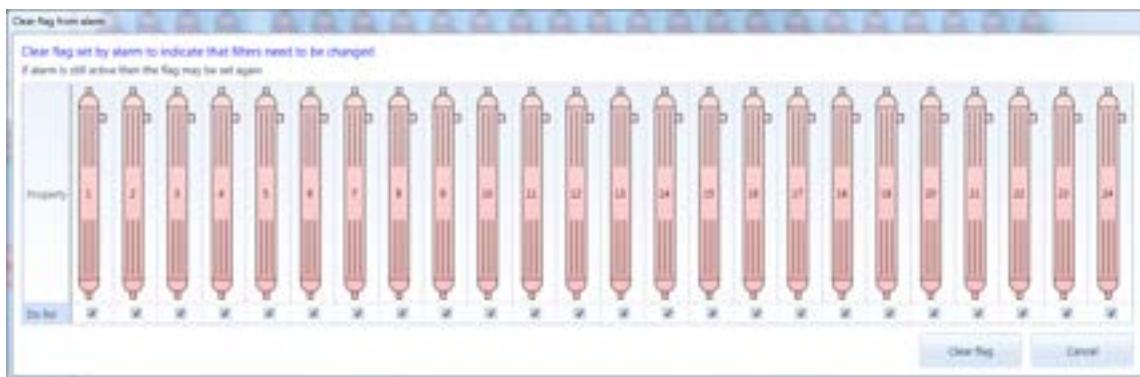


Figure 344 Clear flag from alarm dialog

Press **Clear flag** to clear the flag. To clear the flag for some but not all of the filters selected use the **Do for** option to tell the system which filters should have their flag cleared.

7.7.4 Run pressurization test

To run a pressurization test select the filters to test and press **Run pressurization test**.

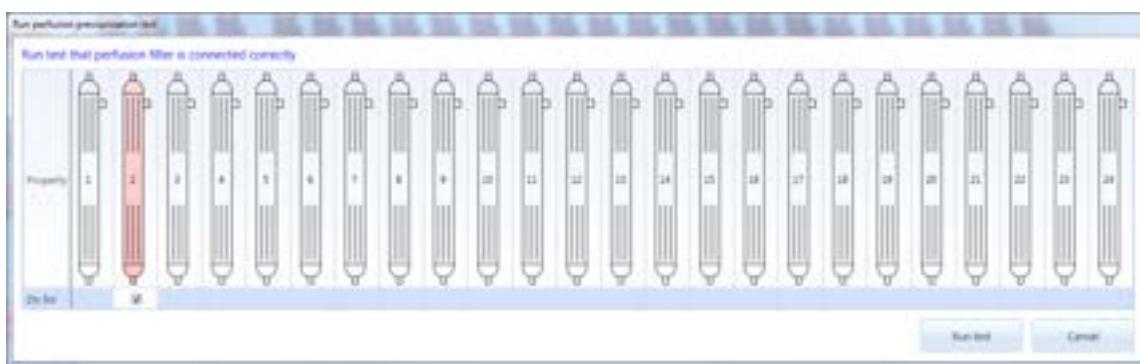


Figure 345 Run perfusion pressurization test dialog

Press **Run test** to start the test. To clear the flag for some but not all of the filters selected use the **Do for** option to tell the system which filters should have a test run.

7.7.5 Unclamp tubing cassettes

To unclamp the tubing cassettes select the filters to unclamp are press **Unclamp tubing cassettes**.

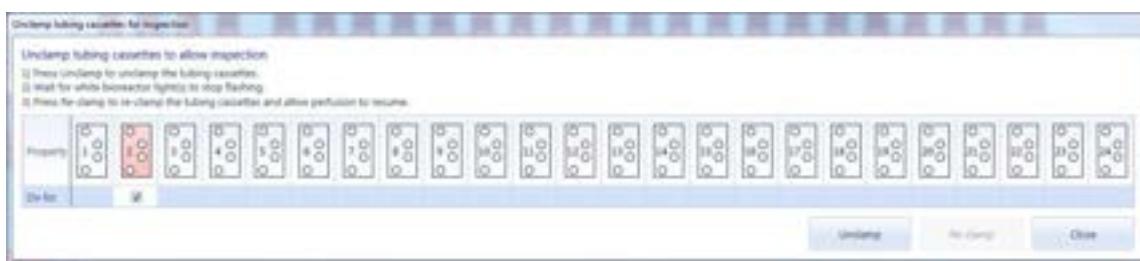


Figure 346 Unclamp tubing cassettes for inspection dialog

In the dialog that appears press **Unclamp** and **Re-clamp** to unclamp and re-clamp the cassettes as required.

If the dialog is closed with cassettes unclamped stopped then a section will appear at the bottom of the page allowing the interaction to be resumed.

8 LABWARE SETUP

The **Labware setup** page shows the plates, tube racks, bottles and pipette racks that the system has been told are on loaded.

At the start of an experiment the system assumes that:

- There are no bottles, plates or tube racks loaded.
 - The same racks of pipette tips are still loaded on the system as previously.



Figure 347 Labware setup page

Labware can be loaded and unloaded from this page. Alternatively labware can be loaded and unloaded in response to the actions listed on the **Todo** page. Those actions display the same windows for loading and unloading items described below.

The Labware setup page also allows editing and deleting labware definitions.

Todo			
View the actions that need to be done now and in the future.			
Time	Action	↳ Bioreactors	↳
Before experiment	Load bioreactors.	8	Xxxx
Before experiment	Calibrate pH	1-2	Xxxx
Before experiment	Enter spot calibration data	5-12	Xxxx
Before experiment	Clear/sterilize tube runs.	1-24	
Before experiment	Connect bioreactors.	1-2,13-24	Xxxx
Before experiment	Connect bioreactors.	8	
Before experiment	Prime bioreactor inlets	8	
Before experiment	Enable automatic pumping	1-2,8,13-24	
Before experiment	Calibrate pH	3-12	
Wed 10 Oct 16:02	Start liquid handler		Start...
Thu 11 Oct 11:47	Load 'Carbon' at 'Bottle 10'		Load 'Carbon'...
Thu 11 Oct 11:47	Load 'Carbon II' at 'Bottle 16'		Load 'Carbon II'...

Figure 348 **Todo** page including actions to load labware

The Magnetic spinner and the Freezer (if fitted) can also be controlled from this page.



Figure 349 Freezer and Magnetic stirrer controls

A check box to enable the liquid handler to run without tip box lids can be selected from this page.

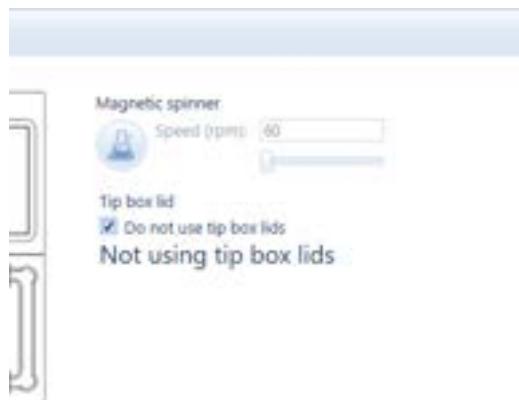


Figure 350 Not using tip box lids option



NOTE: Running the system with no tip box lids allows marginally faster operation of the system when pipetting and facilitates use of the system should there be reliability issues with the tip box lid handling. Running the system without tip box lids may increase the risk of contamination.

A check box to enable the liquid handler to run without freezer lids can be selected from this page.

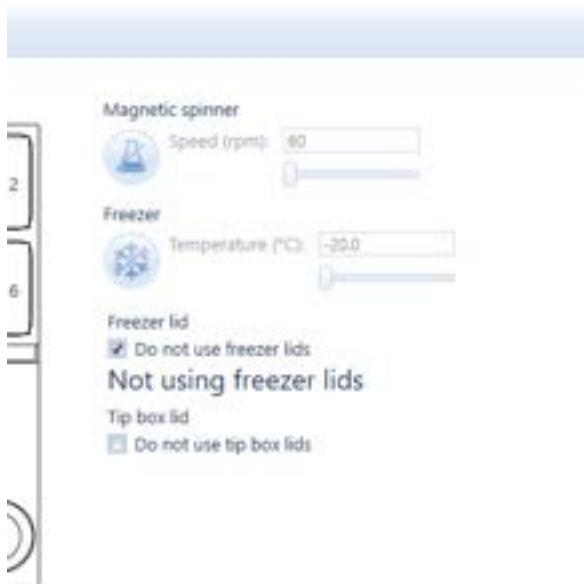


Figure 351 Not using freezer lid option



NOTE: Running the system with no freezer lids should not be used when freezing is required, since condensation will build up and freeze.

8.1 Loading a plate/bottle/tube rack

- 1) On the Labware setup page press **Load...** to load a plate/bottle/tube rack or on the Todo page press **Load 'N'...** where N is the name of the item to load.

The Load labware window is displayed with details of the labware to load and where the labware is to be loaded.

A warning is displayed if there are steps that are expected to take more liquid from the labware than is set to be available. Note that the system deals only with the nominal amount of liquid in the labware – additional liquid should be loaded to allow for the dead volume in the labware.

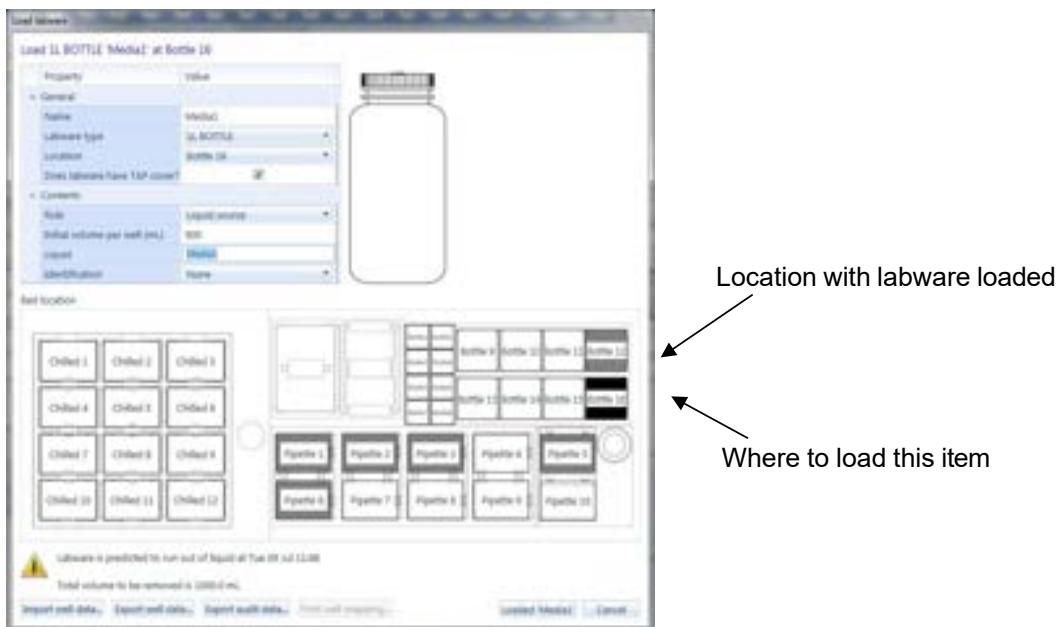


Figure 352 Load labware window

- 2) Amend details of the labware if required. If a barcode or lot number is required enter those details.
- 3) Place the item onto the bed of the system in the indicated position. If another item is there already then remove that item from the system.
- 4) Press **Loaded 'N'** to indicate that the item has been loaded.

8.1.1 Reloading an item

Where an item has previously been loaded onto the system then when loading the item again the **Refill?** option must be entered to choose whether:

- **Refilled** – the labware item has been refilled with its initial volume. (For a sample or waste item that is the labware has been emptied.)
- **Reloaded – not refilled** – the contents of the labware item are as they were when the labware was unloaded from the system.

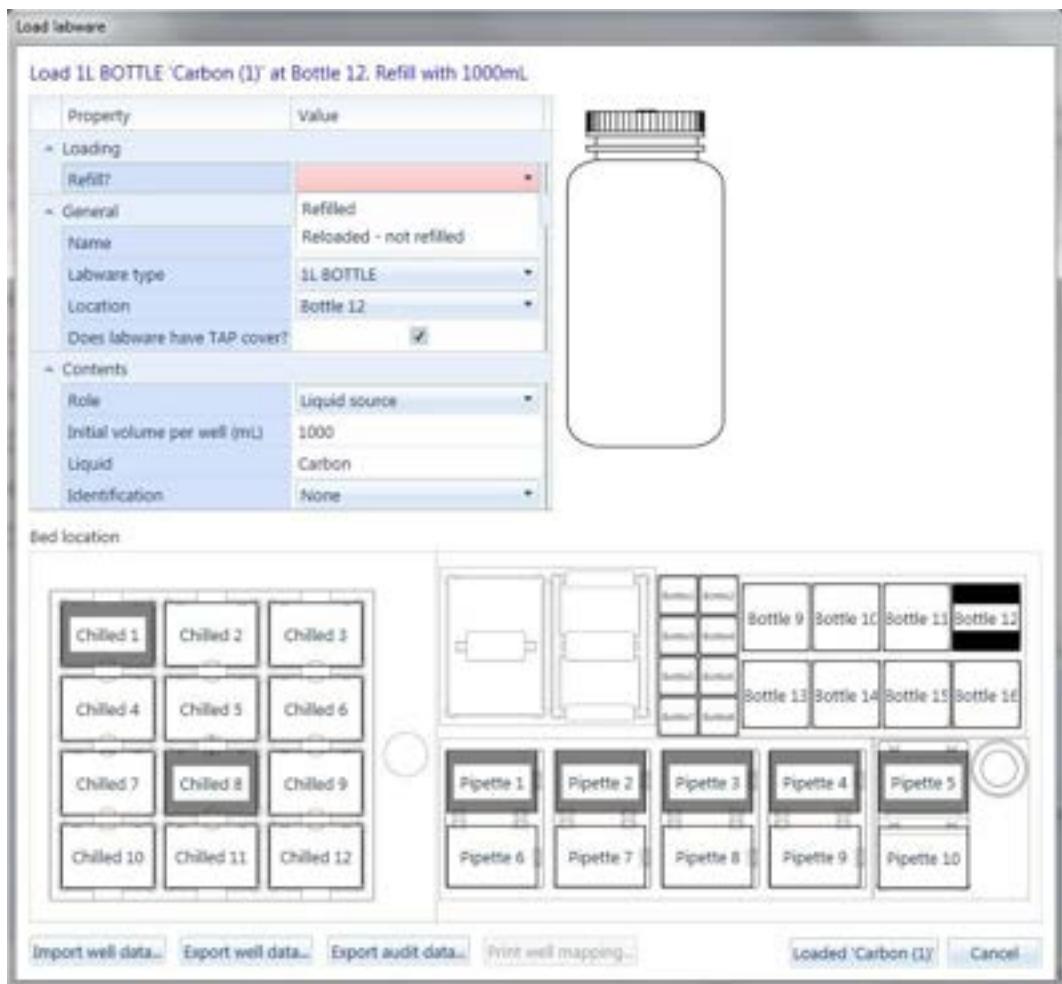


Figure 353 Reloading a labware item

8.2 Unloading a plate/bottle/tube rack

- 1) On the Labware setup page press **Unload...** to load a plate/bottle/tube rack or on the Todo page press **Unload 'N'...** where N is the name of the item to load.

The Unload labware window is displayed with details of the labware to unload and where the labware is to be unloaded from.

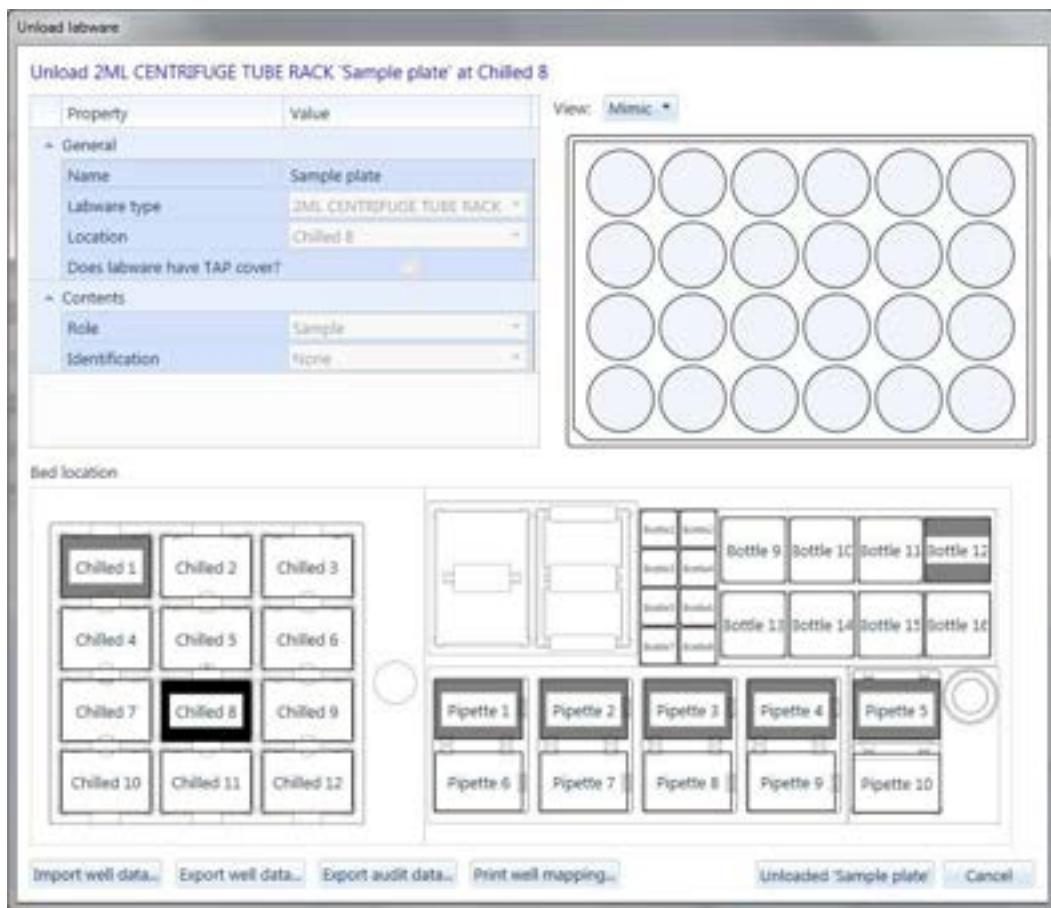


Figure 354 Unload labware window

- 2) Remove the item from the bad of the system.
- 3) Press **Unloaded 'N'** to indicate that the item has been loaded.

For a sample plate **Print well mapping...** will provide a printable view of which wells contain samples from which bioreactors. The view can be displayed later by editing the labware item.

8.3 Loading pipette tips

Pipette tips can be loaded either from the **Labware setup** page or from actions on the **Todo** page.

Pipette tips							
Location	Type	Number Left	300µL	10mL	10mL wide-bore	Unload...	
Pipette 1	10ml	1		Load 10mL...	Load 10ml wide...	Unload	
Pipette 2	10ml	10		Load 10mL...	Load 10ml wide...	Unload	
Pipette 3	10ml	1		Load 10mL...	Load 10ml wide...	Unload	
Pipette 4	10ml	1		Load 10mL...	Load 10ml wide...	Unload	
Pipette 5	300µl	59	Load 300µL...			Unload	
Pipette 6				Load 10mL...	Load 10ml wide...	Unload	
Pipette 7				Load 10mL...	Load 10ml wide...	Unload	
Pipette 8				Load 10mL...	Load 10ml wide...	Unload	
Pipette 9				Load 10mL...	Load 10ml wide...	Unload	
Pipette 10			Load 300µL...			Unload	

Figure 355 Pipette tips section on the **Labware setup** page

Any tip box location can be used for any tip type when the system is configured for Sartorius 1ml and 10ml automation tips.

- 1) Press the Load button for the tip type and location to load e.g. press **Load 300µl...** to load a set of 300µl tips.

The system will display the **Load pipette tip box** window.

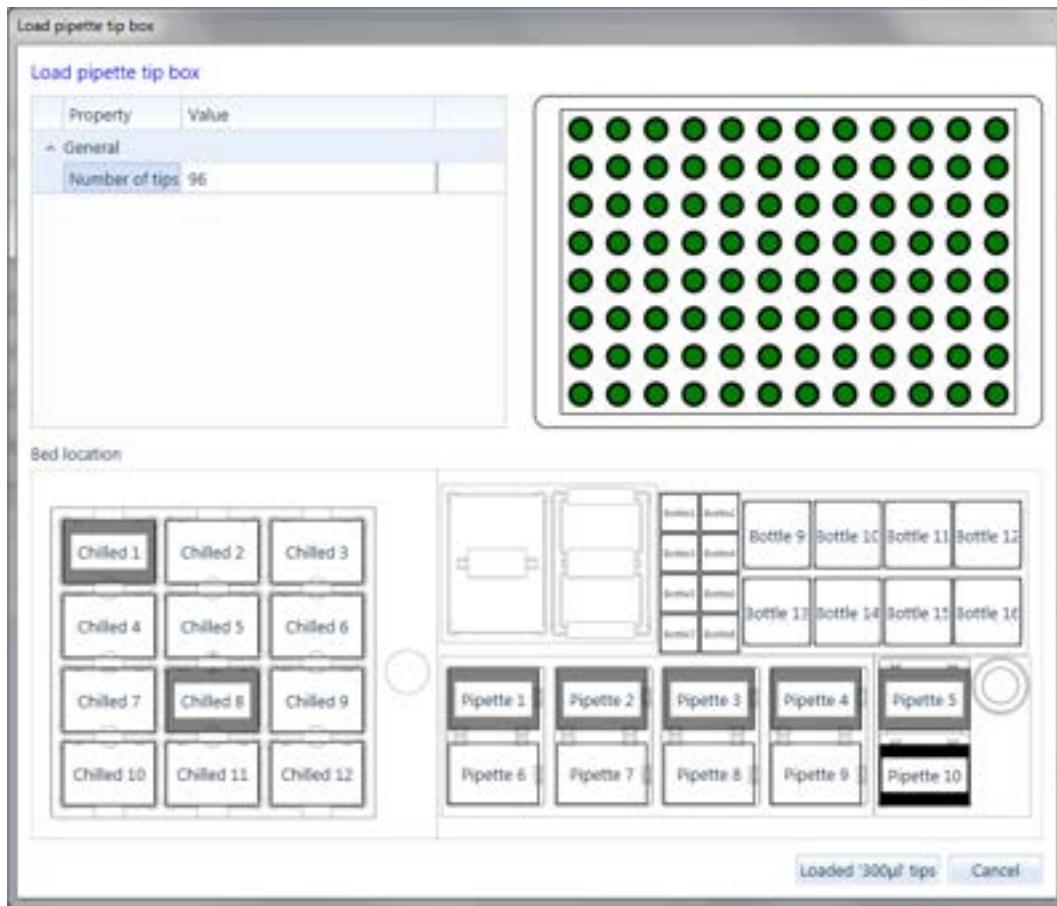


Figure 356 **Load pipette tip box** window

- 2) If the tip box being loaded has been used previously and only contains a partial set of tips then enter the number of tips remaining in the **Number of tips** option.
- 3) Place the tip box onto the bed of the system in the indicated position. If another tip box is there already then remove that tip box from the system.
- 4) Press **Loaded 'N' tips** to indicate that the tip box has been loaded.

8.4 Unloading pipette tips

Pipette tips can be unloaded either from the **Labware setup** page or from actions on the **Todo** page.

Pipette tips							
Location	Type	Number Left	300µL	10mL	10mL wide-bore	Unload...	
Pipette 1	10ml	1		Load 10mL...	Load 10ml wide...	Unload	
Pipette 2	10ml	10		Load 10mL...	Load 10ml wide...	Unload	
Pipette 3	10ml	1		Load 10mL...	Load 10ml wide...	Unload	
Pipette 4	10ml	1		Load 10mL...	Load 10ml wide...	Unload	
Pipette 5	300µL	59	Load 300µL...			Unload	
Pipette 6				Load 10mL...	Load 10ml wide...	Unload	
Pipette 7				Load 10mL...	Load 10ml wide...	Unload	
Pipette 8				Load 10mL...	Load 10ml wide...	Unload	
Pipette 9				Load 10mL...	Load 10ml wide...	Unload	
Pipette 10			Load 300µL...			Unload	

Figure 357 Pipette tips section on the **Labware setup** page

- 1) Press the **Unload** button for the location to unload tips from.

The system will display the **Unload pipette tip box** window.

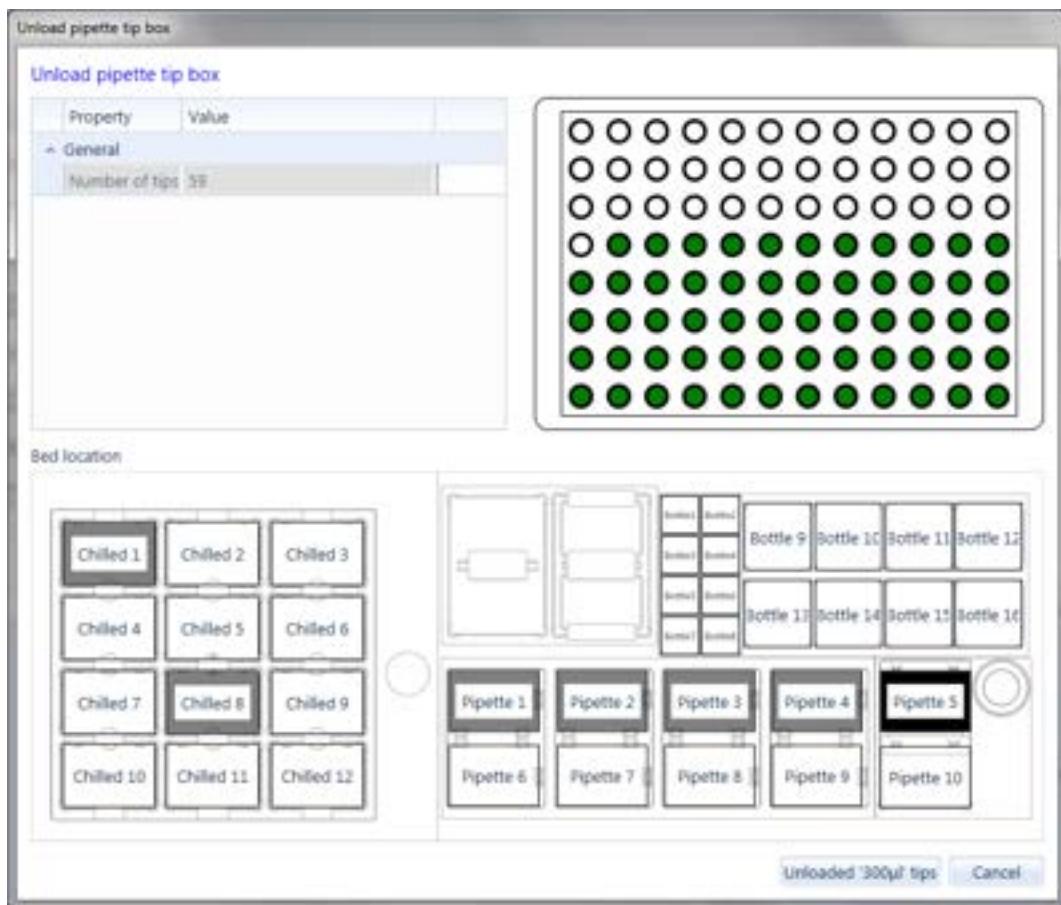


Figure 358 **Unload pipette tip box** window

- 2) Press **Unloaded 'N' tips** to indicate that the tip box has been unloaded.

9 BIOREACTORS

The Ambr® 250 software lets the user monitor the status of the bioreactors and allows direct control of the bioreactors. This section describes facilities for:

- monitoring details of the status of individual bioreactors
- monitoring the status of alarms for different bioreactors
- directly controlling the set points on a bioreactor
- manually entering off-line pH results
- performing low level adjustments to the bioreactors for maintenance

9.1 Bioreactors

The **Bioreactors** page shows an overview of the status of each bioreactor.



Figure 359 *Bioreactors* page

Faults displays an indication of whether there have been any faults on the bioreactor. Hovering over an indicator displays more details about the faults.

The statuses are:

- OK (green)
- There has been a problem that has not been acknowledged (yellow, flashing)
- There is an on-going problem (red)
- There is an on-going problem that has not been acknowledged (red, flashing)

- The bioreactor is disabled



Acknowledge all alarms and **Acknowledge selected alarms** can be used to acknowledge faults.

Start and **Stop** can be used to start and stop an individual bioreactor.

Enabled can be used to disable and re-enable a bioreactor. A disabled bioreactor is not started automatically when other bioreactors are started. Other bioreactors do not wait for a disabled bioreactor when they come to a step where bioreactors are grouped together.

Figure 360 Disabled bioreactor

Individual aspects of bioreactor function can be disabled or enabled. In particular this page can be used to re-enable aspects of bioreactor function that have been disabled by alarms that have subsequently cleared.

- Liquid handling** – requests that the liquid handler does not access the bioreactor. Work already queued for the bioreactor may still happen, but new work will not be queued for this bioreactor. Can be used to avoid accessing bioreactor where contamination is suspected.

- **Pumping** – disables pumping to the bioreactor or for a system alarm to all bioreactors.
- **Gassing** – disables gassing to the bioreactor or for a system alarm to all bioreactors.
- **Stirring** – disables stirring on a bioreactor.
- **Culture cooling** – forcibly cools a culture as quickly as possible.

Additional aspects may be present on systems with additional options. Where a part of the underlying bioreactor functionality stops an aspect that is normally hidden may appear allowing that aspect of the functionality to be restarted.

Show trace displays a polling report with details of the last polling run on the bioreactor. This can be used by the operator or more typically by Sartorius staff to understand the internal operation of the system.

The options in the Tasks tab can be used to re-enable normal functioning across all the bioreactors:

- **Enable liquid handling** – re-enables liquid handling
- **Enable pumping** – re-enables pumping
- **Enable gassing** – re-enabled gassing
- **Enable stirring** – re-enables stirring
- **Stop culture cooling** – re-enables normal temperature control

Bioreactor2 Polling Report

Bioreactor 2 Polling Report

Polling started at Thu 11 Oct 12:35:31

Read state after stopping

Read data from 1:Bioreactor

Temperature

Temperature= 6.2 °C
Camp plate temperature= 12.3 °C
Block temperature= 6.2 °C
Bock temperature.SP= 0.0 °C
Heater= 0.0 %
Chiller = 0.0 %
Camp plate heater= .0 %
Clamp plate chiller= 0.0 %

pH

pH= 13.212 pH

pH before offset= 13.212 pH
pH offset = 0 pH

pH.Prade.mV = -3,2.7 mV
pH.Probe.Temperature= 6.2 °C

Stir Rate

Stirs-speed= 0.0 rpm
Stir direction= Up

Pumping

Pump A

E'ump A flow rate = 0.0 mL/h
Pump A volume pumped= 0 mL

Details

Bioreactor is not connected to plllllp

Pump B

E'ump B flow rate= 0.0 mL/h
E'ump B volume pumped= 0 mL

Details

Bioreactor is not connected to plllllp

Pump C

[cancel]

Figure 361 Polling report

The messages section of the page shows messages for the selected bioreactors.

Time	Component	Module	V	Severity	V	Status	V	Test	V	Time	V
Wed 23 Dec 13:58:55 2010	Monolithic	2	0.000	Warning	0.000	Waiting for "last reader" to initialize	0.000		0.000		
Wed 23 Dec 13:58:56 2010	Monolithic	2	0.000	Warning	0.000	Waiting for "last reader" to initialize	0.000		0.000		
Wed 23 Dec 13:58:57 2010	Monolithic	2	0.000	Warning	0.000	Initialization counter = 0	0.000		0.000		
Wed 23 Dec 13:58:58 2010	Monolithic	2	0.000	Warning	0.000	Initialization counter = 0	0.000		0.000		
Wed 23 Dec 13:58:59 2010	Monolithic	2	0.000	Warning	0.000	Initialization counter = 0	0.000		0.000		
Wed 23 Dec 13:59:00 2010	Monolithic	2	0.000	Warning	0.000	Found 4 flows with v<0 - 44.43994 volumes 20.0000 Mc	0.000		0.000		
Wed 23 Dec 13:59:01 2010	Monolithic	2	0.000	Warning	0.000	Found 4 flows with v>0 - 44.43994 volumes 20.0000 Mc	0.000		0.000		
Wed 23 Dec 13:59:02 2010	Monolithic	2	0.000	Warning	0.000	Found 4 flows with v>0 (Dropped)	0.000		0.000		
Wed 23 Dec 13:59:03 2010	Monolithic	2	0.000	Warning	0.000	Found 4 flows with v>0 (Dropped)	0.000		0.000		
Thu 24 Dec 00:00:00 2011	Monolithic	2	0.000	Warning	0.000	System initialized	0.000		0.000		

Figure 362 Messages for selected bioreactors

9.2 Alarms

The **Alarms** page shows the status of the bioreactor alarms.

Figure 363 Alarms page

Acknowledge all alarms and **Acknowledge selected alarms** can be used to acknowledge alarms.

For each alarm and bioreactor the system shows the status of the alarm.

- The status is OK

 - The alarm is not active – typically the bioreactor is not loaded or the set point monitored has not been set

 - The alarm is disabled

 - The alarm has been triggered
 (flashing)

- The alarm has been triggered and acknowledged
- The alarm is now OK, but it was triggered and has not been acknowledged
- The definition of the alarm is invalid. The system is not monitoring the alarm.

9.3 Controls

The **Controls** page allows direct control of each bioreactor.

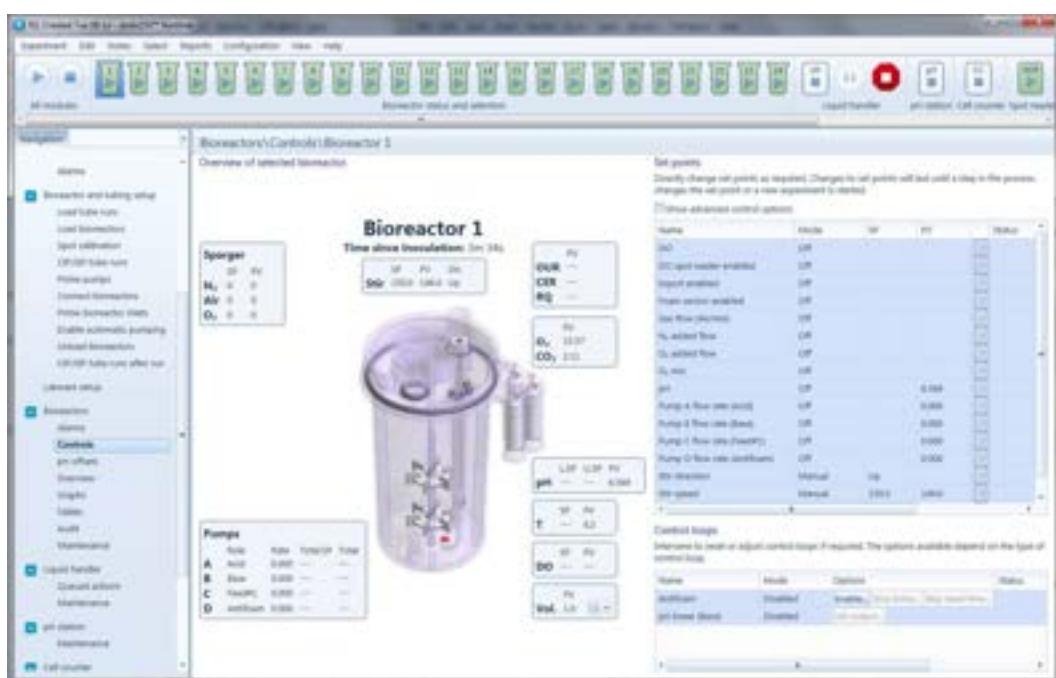


Figure 364 **Controls** page

The Control page contains:

- Overview of selected bioreactor** – a view of the key properties of the selected bioreactor.
- Set points** – the value and status of the set points for the bioreactor. Show advanced control options shows more detailed set points that are hidden by default.
- Control loop** – the status of the control loops for the bioreactor.

9.3.1 Set points

Set points

Directly change set points as required. Changes to set points will last until a step in the process changes the set point or a new experiment is started.

Show advanced control options

Name	Mode	SP	PV	Status
DO	Manual	20.0	18.4	
DO spot header enabled	Manual	enable		
Export enabled	Off			
Gas flow (Air/min)	Auto	8.1		
N ₂ added flow	Off			
O ₂ mix	Auto	20.0		
pH	Off		6.424	
Pump A flow rate (Feed%)	Off	0.0		
Pump B flow rate (L/sec)	Bolus	5.0	0.0	
Pump C flow rate	Off		0.0	
Pump D flow rate	Off		0.0	
Stir direction	Manual	Up		
Stir speed	Auto	360.0	337.7	
Temperature	Off		5.6	

Figure 365 Set points

For each set point the display shows:

- The **Name** of the set point
- The **Mode** of the set point
 - **Off** – the set point is off
 - **Manual** – the set point has been given a specified value. That may be constant or may be a ramp or profile
 - **Auto** – the set point is being given its value by a control point
 - **Bolus** – the set point (a pump flow rate) is pumping a bolus
 - **Bolus override** – the set point (a pump flow rate) was under Auto control and was then set to pump a bolus. Auto control will resume when the bolus has completed

It is also possible in some circumstances to get the two modes

- **Manual (Ineffective)** – the set point has been given a specified value but there is no control loop to make the system follow the set point.
- **Auto (Ineffective)** – the set point has been given an auto value but there is no control loop to make the system follow the set point.

On systems where the OPC interface is licensed there are also the following modes:

- **OPC** – values for the set point are being set via the OPC interface
- **OPC (Ineffective)** – values for the set point are being set via the OPC interface but there is no control loop to make the system follow the set point.
- **Liquid to end**: a pump has been set to be controlled by OPC. Before external control is enabled the system is moving the liquid to the nominal end of the bioreactor inlet.
- The value **SP** of the set point
- The present value **PV** corresponding to the set point. Note that for some set points there is no present value directly corresponding to the set point.
- An **Edit** button to edit the set point

- The **Status** of the set point and in particular if the set point is not applicable given whether the bioreactor is loaded, connected, ...

The Edit button displays the **Change set point** window if the set point can be set. An error message is displayed if the set point cannot be controlled at the moment for some reason.

The set point is changed when **Ok** is pressed on the **Change set point** window. The present values will change once the system has sent the new instructions to the bioreactor and the bioreactor has reported new values back in turn.

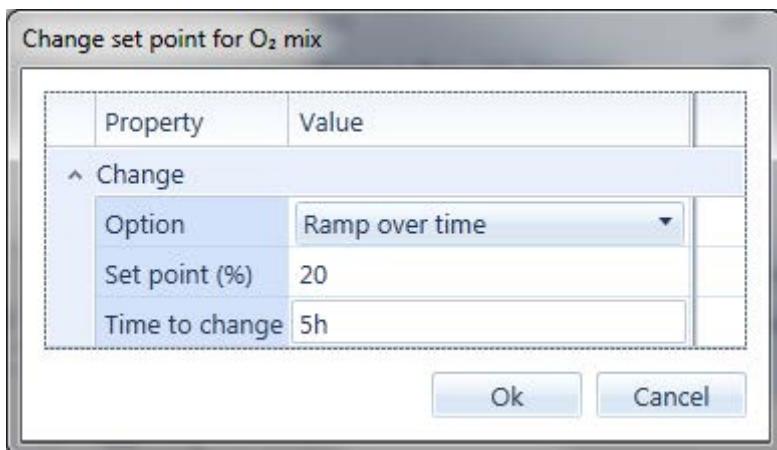


Figure 366 Change set point window

The options available in the **Change set point window** are the **Change** options described in sections 5.8.1 and 5.9.1 above.

There is one additional option. If the set point is following a profile then it is possible to amend the current profile or to start a new profile.

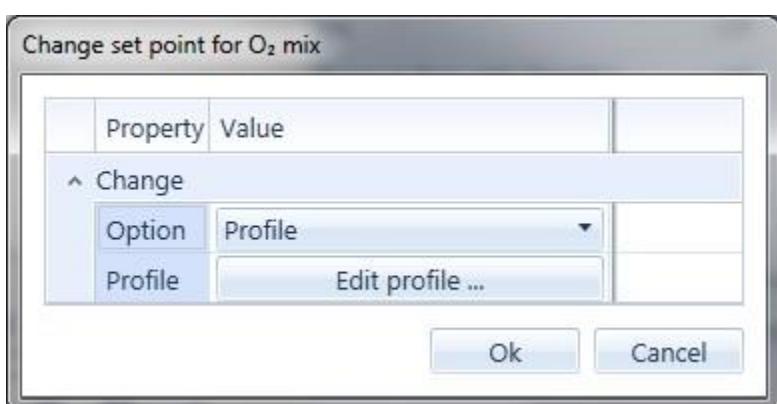


Figure 367 Setting a set point to a profile

When setting a set point to a profile initially the **Profile** option displays the window to edit the profile.

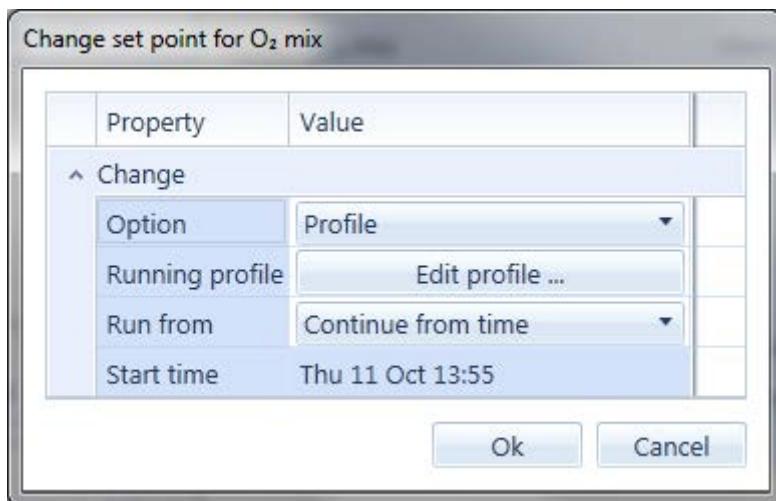


Figure 368 Amending a running profile

When editing a set point which is following a profile the Run from option allows the choice of:

- **Continue from time** to amend the running profile
- **Now** to create a new profile



Figure 369 Starting a new profile

If **Continue from time** is chosen then Running profile displays a window to edit the running profile. Points in the past are read-only and an extra point is added when the window is opened so that only future values of the curve can be edited.

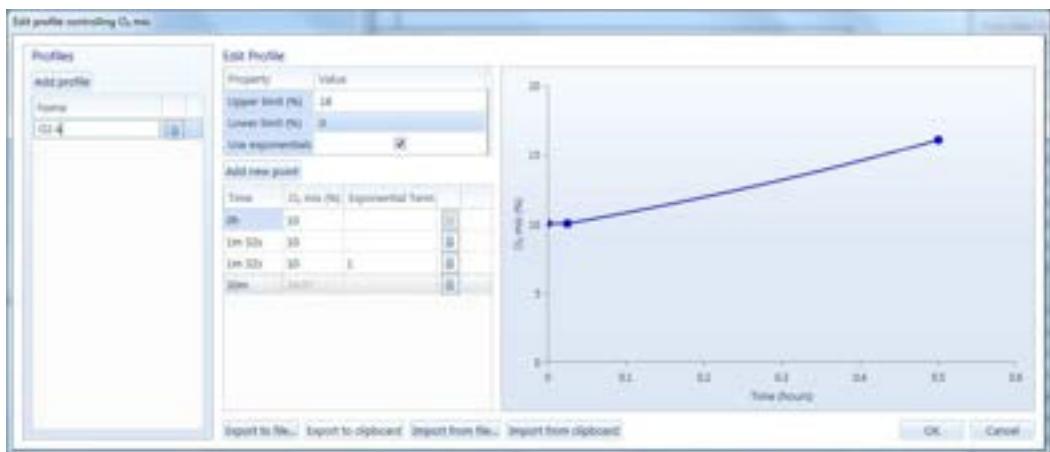


Figure 370 Profile editor amending a running profile

9.3.2 Bleeding

In perfusion runs additional entries appear allowing bleeding to be controlled directly from the **Controls** page.



Figure 371 Controls page with bleed and other perfusion set points shown

Press **Bleed** to show the dialog for setting the amount that should be bled. The amount set replaces any previous setting for the bleed.

Press **Bleed to level** to show the corresponding dialog for the amount that should be bled via the Bleed to level route.

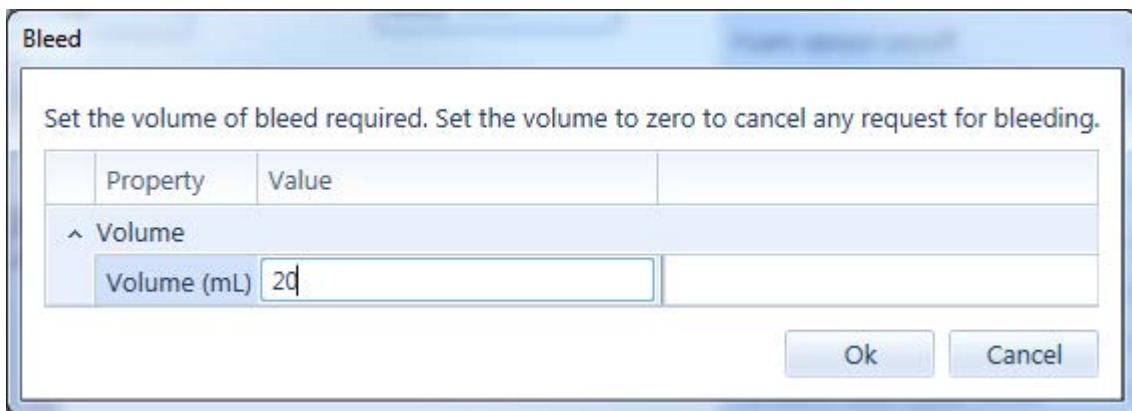


Figure 372 Bleed dialog with option for Volume to bleed

9.3.3 Control loops

The **Control loops** section of the page provides a view of the status of the different control loops and allows some intervention with the operation of the control loops.

Control loops			
Intervene to reset or adjust control loops if required. The options available depend on the type of control loop.			
Name	Mode	Options	Status
Acid	Disabled	Set output... Enable/Disable... Stop batch... Skip dead time...	
Antifoam			
Base	Disabled	Set output...	
DO	Enabled	Set output...	Active level 2 - Gas flow (Air/min)
Pipette acid	Disabled	Set output... Skip dead time...	

Figure 373 Control loops

For each control loop the display shows:

- The **Name** of the control loop
- The **Mode** of the control loop
 - **Off** – the control loop is not running
 - **On** – the control loop is running
- **Options** for the control loop depending on the type of the control loop
- The **Status** of the control loop

9.3.3.1 PID loop options

PID loops have a **Set output...** option that allows the output of the PID loop to be forced to a specified value. **Set output...** can only be applied to loops with integral terms.

Pressing **Set output...** presents the **Force cascade control loop** window.

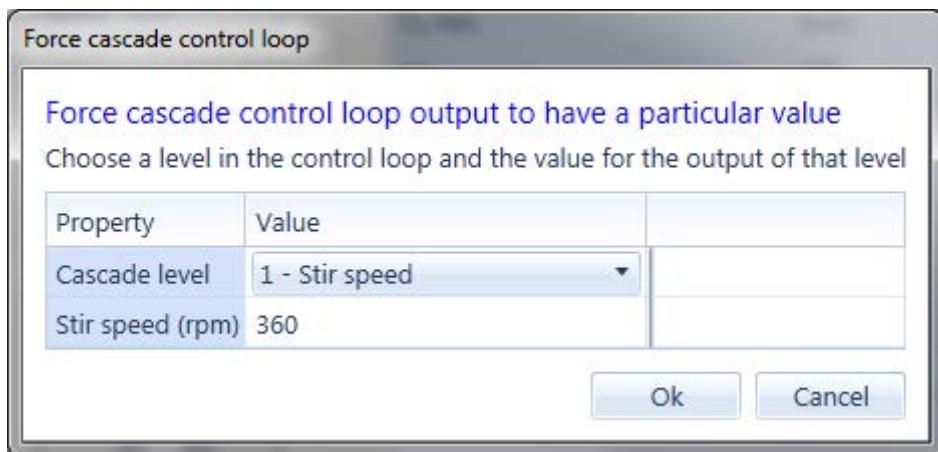


Figure 374 Force cascade control loop window

Cascade level selects the new active level of the control loop and the option for the property – in this case **Stir speed** – selects the value of the output from that level of the cascade. The output from the other levels in the cascade is implied by the choice of active level.

After forcing the output value the output will continue to evolve under control of the PID loop.

9.3.3.2 Pipette bolus loop options

The Pipette bolus control loop has the option **Skip dead time...** to stop the loop waiting for its dead time to elapse. The loop will do the next bolus when its condition is true again.

If the loop is a Runs condition loop then the **On/Off...** option can be used to enable or disable the loop.

9.3.3.3 Pump bolus loop options

The Pump bolus control loop has the option **Skip dead time...** to stop the loop waiting for its dead time to elapse. The loop will do the next bolus when its condition is true again.

The Pump bolus control loop has the option **Stop bolus...** to stop the bolus the loop is pumping. The loop will then consider when to do the next bolus.

If the loop is a Runs condition loop then the **On/Off...** option can be used to enable or disable the loop.

9.3.4 Manual liquid handling options

The manual liquid handling options are accessed through the **Edit** button in the Volume status.

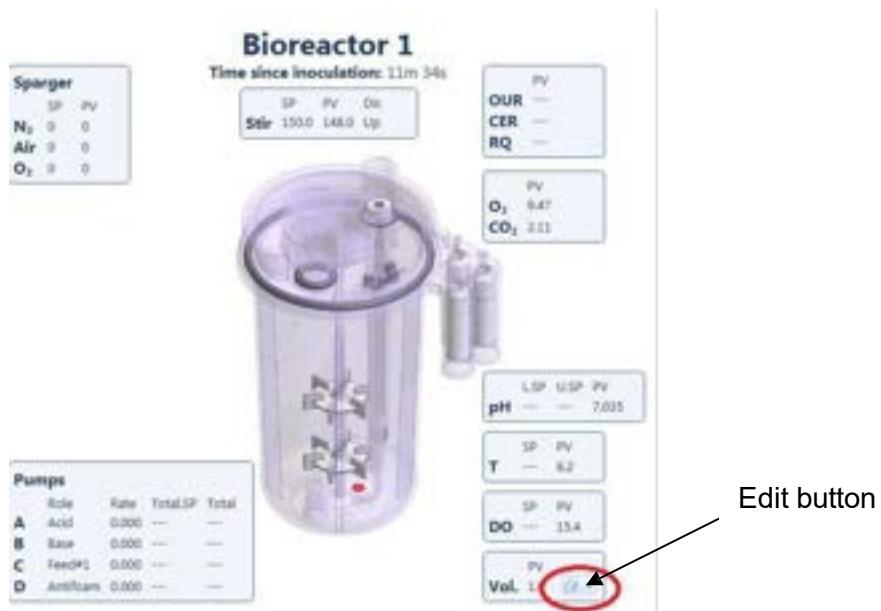


Figure 375 Controls page overview section- identifying Edit button for Manual liquid handling.

The following options are available.

9.3.4.1 Edit volume

The **Edit volume...** option allows the user to specify the volume that is in the bioreactor.

9.3.4.1.1 Volume changes and perfusion cell density control

The estimated cell density is affected by the volume in the bioreactor, for example if the cells have been diluted by adding more media then the estimated cell density will be reduced.

In perfusion experiments it is sometimes necessary to correct the system's model of the volume in the bioreactor.

The model – that a volume change represents diluting or concentrating the culture – is applied when the volume in the bioreactor is edited. Depending when the cell density was last measured, the change to the estimate may or may not be helpful to the control of the system. If the measurement is recent then the volume at the time of the measurement may well already have been the volume entered into the system.

To help deal with this then when the cell density control loop is active and the change in volume will affect its operation a dialog is presented allowing the value of the variable used for control to be cleared.

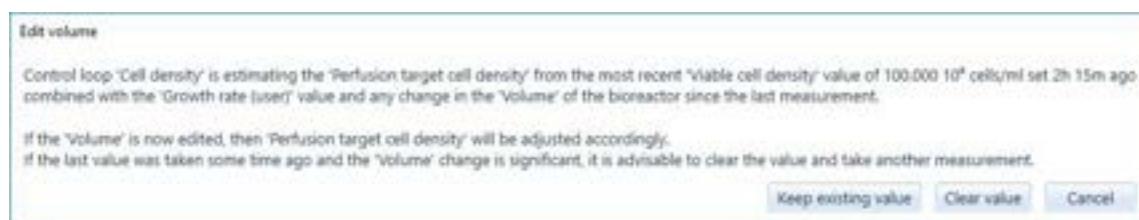


Figure 376 Message about effect of changing volumes

The ideal order for checking volumes is then:

- 1) Check volume
- 2) Edit volume where required and clear cell density reading
- 3) Take a new cell density reading

9.3.4.2 Add liquid

The **Add liquid...** option prompts the user to manually add a specified volume of a defined liquid to the bioreactor.

9.3.4.3 Sample liquid

The **Sample liquid...** option prompts the user to manually remove a specified volume of liquid from the bioreactor.

If the system has been configured to enable them, then the user can associate a barcode with the sample.

9.3.4.4 Sample permeate

The **Sample permeate...** option prompts the user to manually remove a specified volume of liquid from the permeate line.

9.3.4.5 Inoculate

The **Inoculate...** option prompts the user to manually inoculate the bioreactor. If the bioreactor has previously been inoculated, a warning will be provided to this effect.

9.3.4.6 Unscheduled action handling

For each option the system checks if there is a corresponding planned action defined as part of the process. If there are planned actions then a dialog is shown with the planned actions. If there are no planned actions the dialog to perform the action is shown immediately.

Either click on the button for a planned action to perform that action or click on **Do as unscheduled action** to perform the action without updating the action from the process definition.

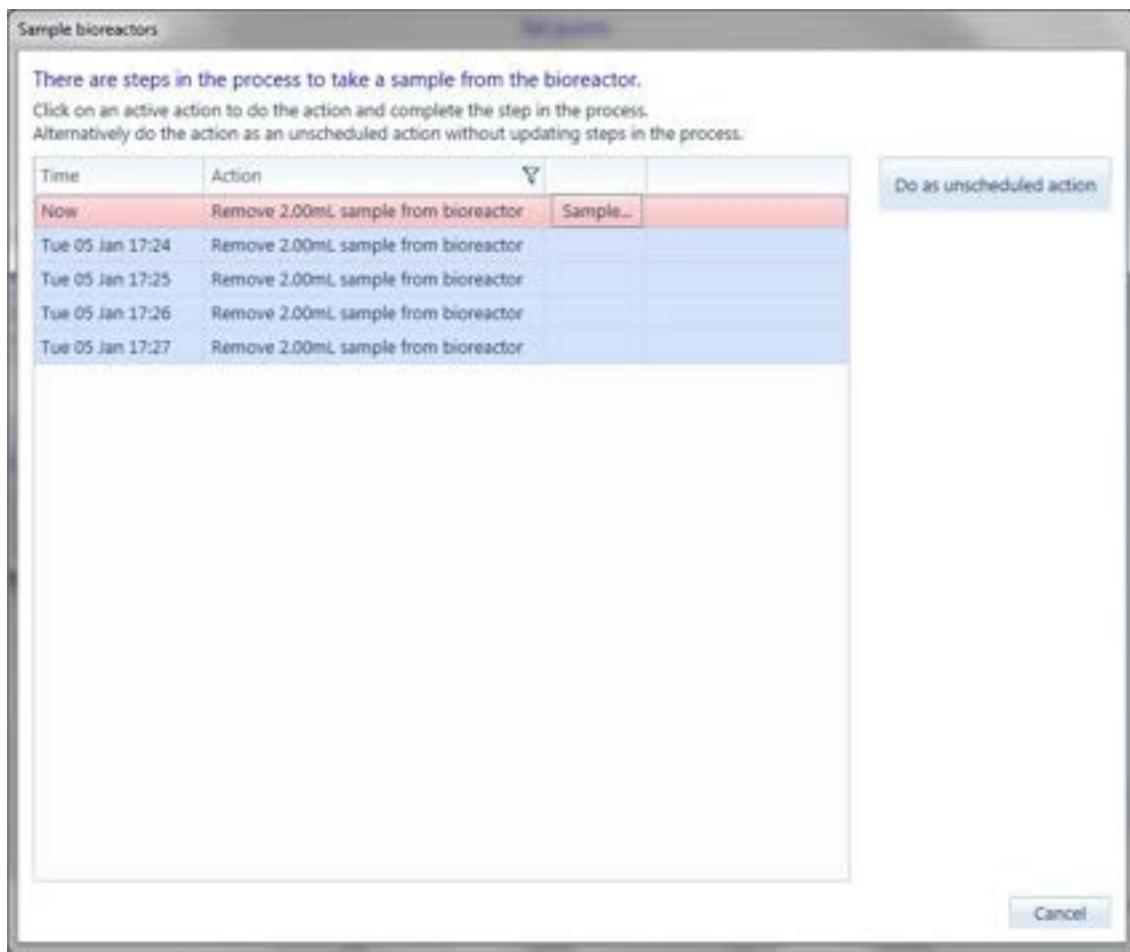


Figure 377 Dialog with planned actions.

9.4 pH offsets

The **pH offsets** page allows calibration of the pH of a bioreactor via manual entry of an externally measured pH.

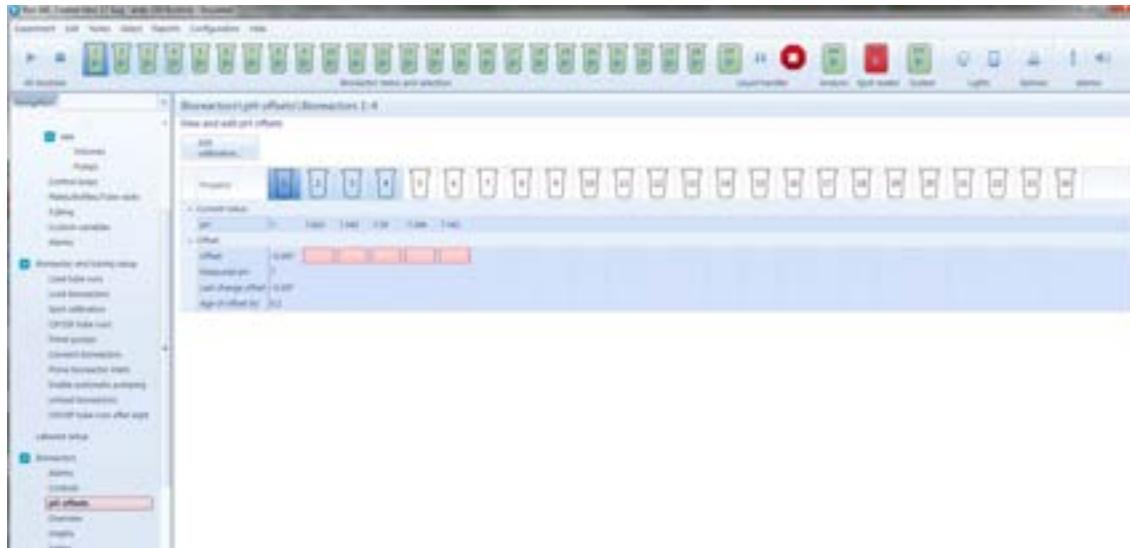


Figure 378 pH offsets page

The pH offsets page shows for each bioreactor:

- **pH** – the current value of the pH
- **Offset** – the offset currently being used to calculate the pH
- **Measured pH** – the last measured pH used to calculate the **Offset**. The **Measured pH** can be used to see how far the pH has changed since the **Offset** was last calculated and therefore whether recalibration might be required.
- **Last change offset** – how much the offset changed by the last time it was updated
- **Age of offset** – how many hours have elapsed since the **Offset** was updated. The **Age of offset** can be used to see how long it is since the **Offset** was last calculated and therefore whether recalibration might be required.
- **Edit calibration** – displays the **Enter pH data** window.

The screenshot shows a software dialog titled "Enter pH data". The main area contains a table with columns: Bioreactor, When sample taken (select), External pH, Recorded system pH, pH offset, and Change to offset. The table lists four entries for Bioreactor 1, 2, 3, and 4. Row 1: Bioreactor 1, Thu 20 Aug 16:45:04 - Sample 1.00 mL to rack/A1, 7.000, 7.000, 0.000, 0.000. Row 2: Bioreactor 2, Thu 20 Aug 16:45:14 - Sample 1.00 mL to rack/A2, 6.900, 7.432, -0.532, 0.000. Row 3: Bioreactor 3, Thu 20 Aug 16:45:21 - Sample 1.00 mL to rack/A3, 7.342, 7.342, 0.000, 0.000. Row 4: Bioreactor 4, Thu 20 Aug 16:45:28 - Sample 1.00 mL to rack/A4, 7.390, 7.390, 0.000, 0.000. Below the table is a dropdown menu for selecting the date type: Use custom dates (radio button), Use named dates (radio button, selected), and Use current date and time (radio button). At the bottom are buttons for Export to file, Export to clipboard, Import from file, Import from clipboard, Ok, and Cancel.

Bioreactor	When sample taken (select)	External pH	Recorded system pH	pH offset	Change to offset
Bioreactor 1	Thu 20 Aug 16:45:04 - Sample 1.00 mL to rack/A1	7.000	7.000	0.000	0.000
Bioreactor 2	Thu 20 Aug 16:45:14 - Sample 1.00 mL to rack/A2	6.900	7.432	-0.532	0.000
Bioreactor 3	Thu 20 Aug 16:45:21 - Sample 1.00 mL to rack/A3	7.342	7.342	0.000	0.000
Bioreactor 4	Thu 20 Aug 16:45:28 - Sample 1.00 mL to rack/A4	7.390	7.390	0.000	0.000
Bioreactor 5					
Bioreactor 6					
Bioreactor 7					
Bioreactor 8					
Bioreactor 9					
Bioreactor 10					
Bioreactor 11					
Bioreactor 12					
Bioreactor 13					
Bioreactor 14					
Bioreactor 15					
Bioreactor 16					
Bioreactor 17					
Bioreactor 18					
Bioreactor 19					
Bioreactor 20					
Bioreactor 21					
Bioreactor 22					
Bioreactor 23					
Bioreactor 24					
Bioreactor 25					
Bioreactor 26					
Bioreactor 27					

Figure 379 Enter pH data window

The **Enter pH data** window enables entry of the **External pH** i.e. the externally measured pH and the time for **When sample taken (select)**.

The system calculates **pH offset** using the values it was reading for the pH at the time **When sample taken**.

Recorded system pH was the pH reading at the selected time after any offset was applied.

The **pH offset** is the offset that will be applied after entering the new data.

The **Change to offset** is the difference between the new offset and any previous offset that had been applied.

When sample taken (select) can be taken as the **current date and time**, chosen from a list of times when samples were taken from the bioreactor or entered manually if **Use custom time** is selected.

If required, data may be exported to or imported from the clipboard or a .csv file

9.4.1 Manually calibrating a bioreactor

To manually calibrate a bioreactor on a system with no liquid handler:

- 1) Open the Edit pH offset window for the bioreactor
- 2) Take a sample
- 3) Press the **Note current time** button
- 4) Measure the pH of the sample offline
- 5) Enter the pH into **Measured pH**

9.5 Maintenance

The bioreactors Maintenance page provides access to the parameters configuring individual bioreactors and allows commands to be sent directly to bioreactors.

Incorrect use of this page can cause mechanical damage to the Ambr® 250 system. Access to the pages is protected by a password and the pages should only be used by competent maintenance staff.



Figure 380 Maintenance page

9.5.1 Perfusion tower loading

The Perfusion towers page allows the operator to tell the system whether the perfusion towers are loaded on the system or not. When the towers are not loaded the system will not talk to the perfusion board and therefore will not raise error messages if there is a problem with the board.

To see the screen the advanced option **Show screens and features for maintenance and commissioning** must be selected. After loading towers consider running the appropriate tests on the **Perfusion tests** page.

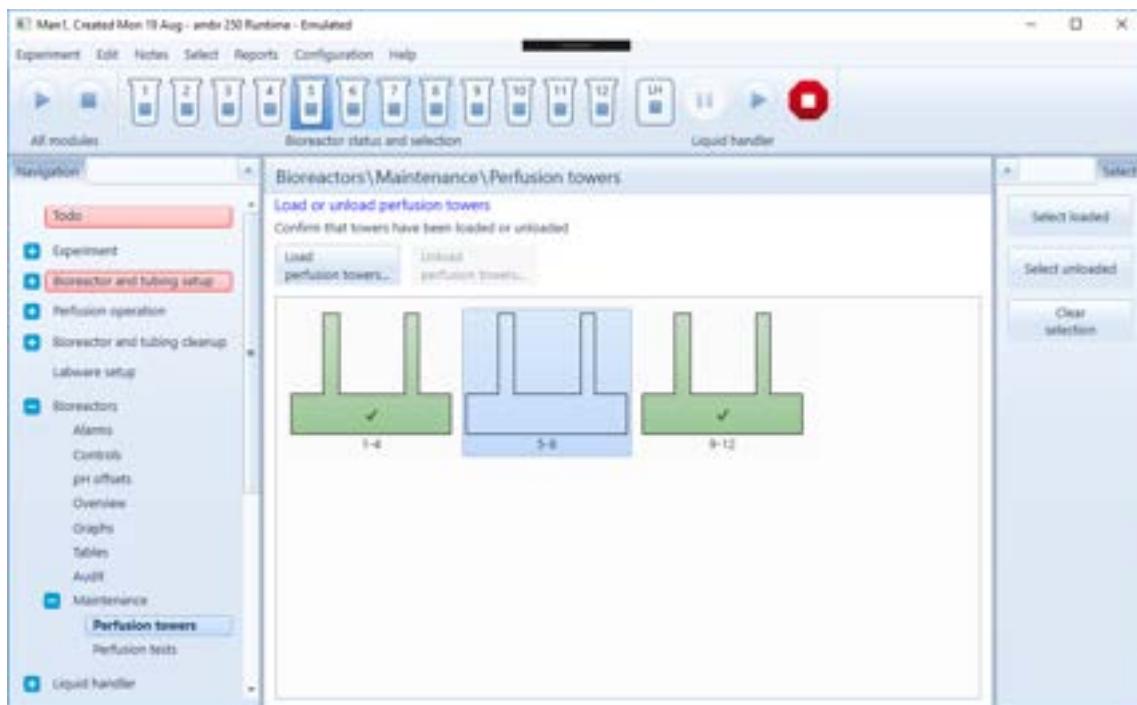


Figure 381 Perfusion towers page with towers for bioreactors 5-8 unloaded.

10 RESULTS

The Ambr® 250 software keeps extensive records of the parameters measured by the system and the set points requested of the system. This section describes the facilities for:

- viewing the recorded data
- exporting the recorded data
- importing externally measured results
- importing reference data

10.1 Graphs

The **Graphs** page allows data to be plotted for multiple bioreactors.

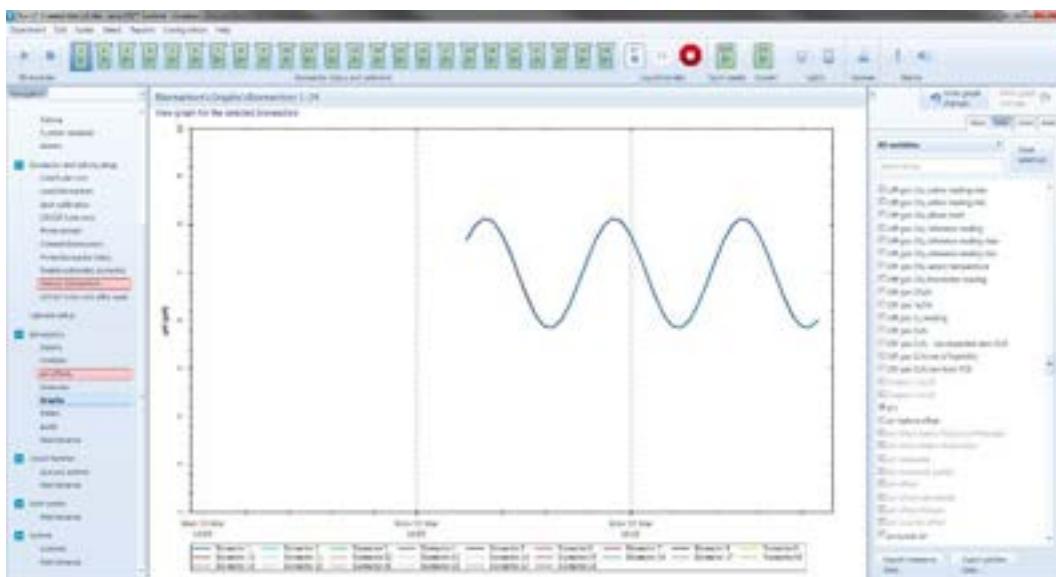


Figure 382 *Graphs* page

Zooming and panning of the graph are enabled when **Show real time** mode is not selected.

Select an area of the graph to zoom into the graph.

Press Ctrl and the left mouse button to pan the graph by moving the mouse.

Click on an individual axis to pan (click and drag) or zoom (click to select, then use the scroll wheel) the axis.

Double click on an axis to auto scale it. To auto scale all axes, double click on the graph itself.

Within the **Experiment Viewer** application the graphs screen is presented as **Results**

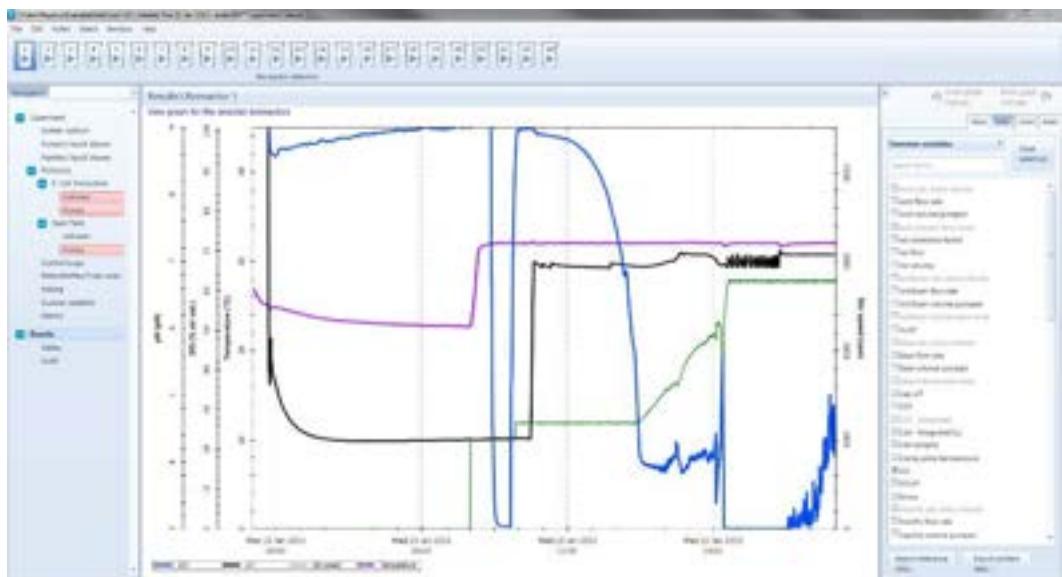


Figure 383 Results page in the Experiment Viewer

Undo graph changes and **Redo graph changes** undo and redo changes made to the definition of the graph. Undo and redo do not change which bioreactors are selected.

10.1.1 Graph settings

Graph settings store the variables selected, the axis limits, the colour of lines and other graph settings. Graph settings do not store which bioreactors are selected.

Load settings... opens a dialog that allows one or more saved settings to be opened.



Figure 384 Load settings for graphs dialog

Clicking on one of the settings in the list of settings switches the graph to use the current values of the settings.

Save settings dropdown allows the current loaded settings to be saved

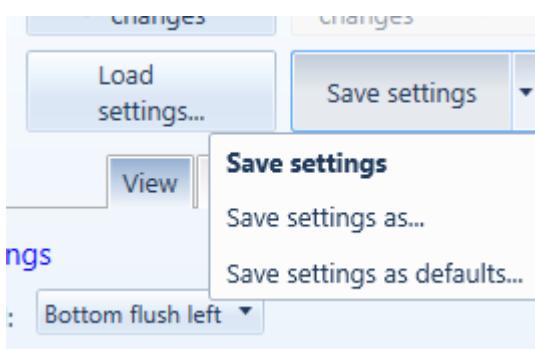


Figure 385 Save settings dropdown

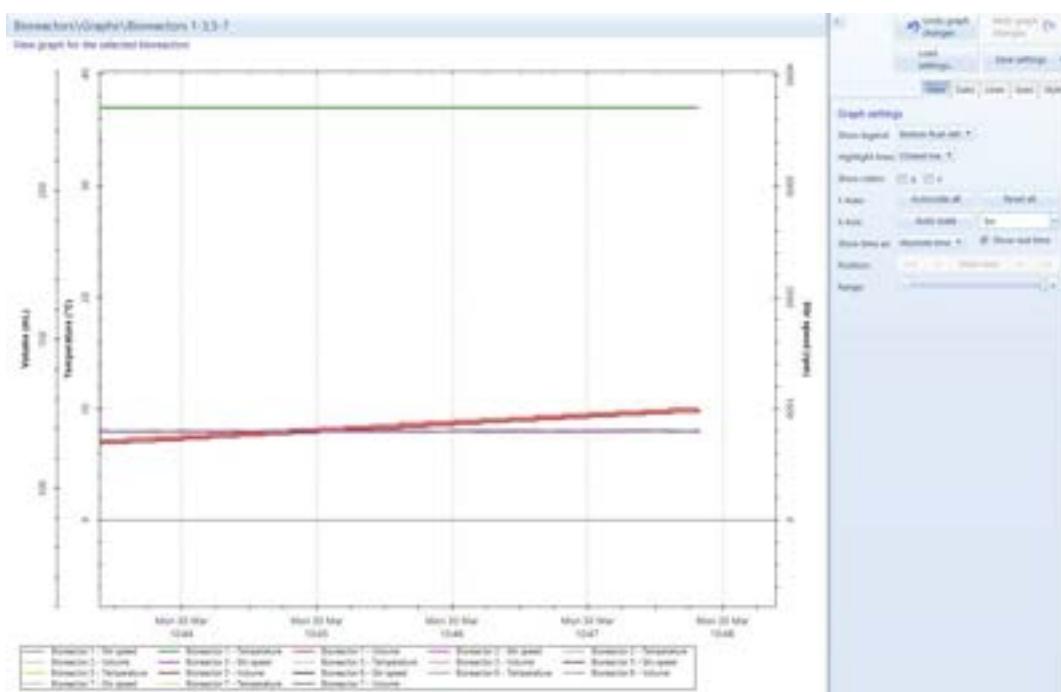
Save settings saves the current settings.

Save settings as... saves another copy of the current settings.

Save settings as defaults... saves the settings so that they are used for the initial graph the next time the software is started.

10.1.2 View

The View panel provides controls for changing options on the graph.



Zooming and panning of the graph are enabled when **Show real time** mode is not selected.

10.1.2.1 View options

The view options set properties of the graph.

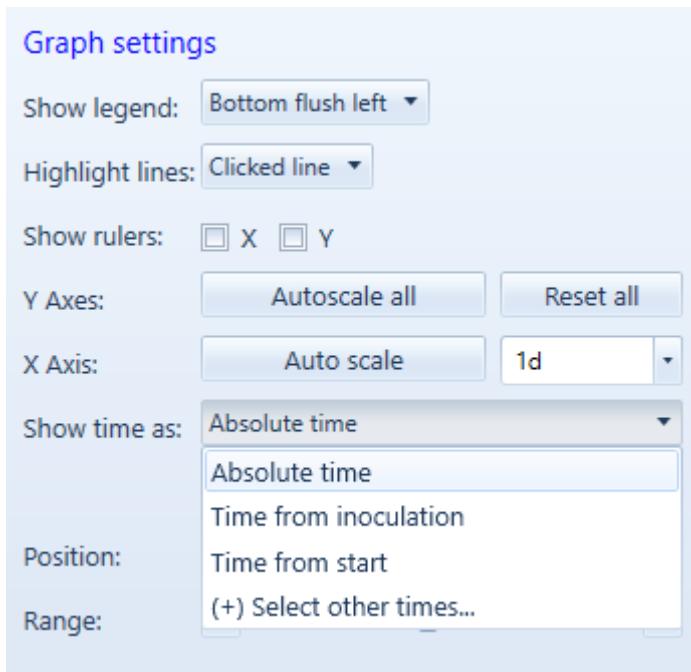


Figure 386 View options

Show legend selects the position of the legend.

- **None** – no legend is shown
- **Top** – legend is aligned horizontally at the top in line with the left edge of the graph panel
- **Left** – legend is aligned vertically at the top to the left of all axes
- **Right** – legend is aligned vertically at the top to the right of all axes
- **Bottom** – legend is aligned horizontally in line with the left edge of the graph panel
- **Inside top left** – legend is aligned horizontally in the top left corner inside the graph panel
- **Inside top right** – legend is aligned horizontally in the top right corner inside the graph panel
- **Inside bottom left** – legend is aligned horizontally in the bottom left corner inside the graph panel
- **Inside bottom right** – legend is aligned horizontally in the bottom right corner inside the graph panel
- **Top center** – legend is aligned horizontally at the top center of the graph panel
- **Bottom center** – legend is aligned horizontally at the bottom center of the graph panel
- **Top flush left** – legend is aligned horizontally at the top to the left of all axes
- **Bottom flush left** – legend is aligned horizontally at the bottom to the left of all axes

Highlight lines offers options for highlighting lines:



- **None** – no line is highlighted
- **Line under pointer** – the line if any under the pointer is highlighted
- **Lines(s) for main selected object** – the lines for the main (primary) selected object are highlighted
- **Clicked line** – clicking on a line highlights the line

Show rulers offers the options to display an **X** and/or a **Y** ruler. The X ruler shows its position and the value of each variable at its position. The Y ruler just displays its position.

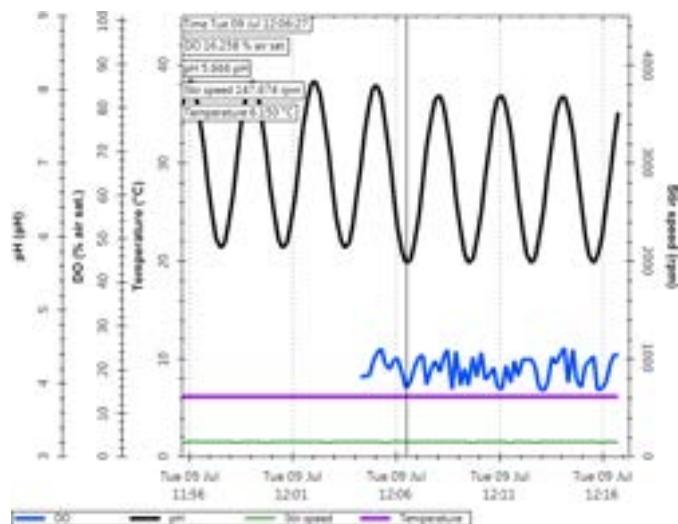


Figure 387 X ruler. The ruler can be moved with the mouse.

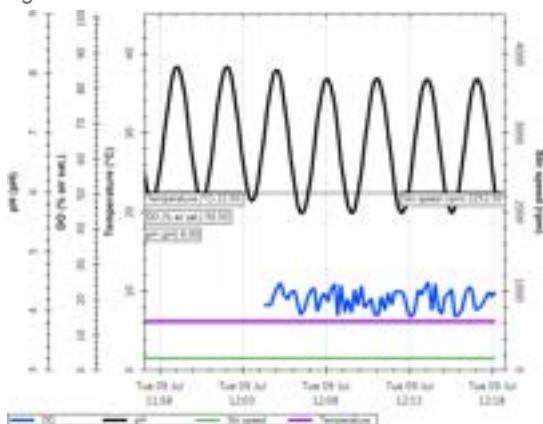


Figure 388 Y ruler. The ruler can be moved with the mouse.

Autoscale all autoscales all of the Y axes and **Reset all** resets all of the Y axes to their default limits.

Auto scale autoscales the X axis. The time field displays and allows editing of the range of the X axis.

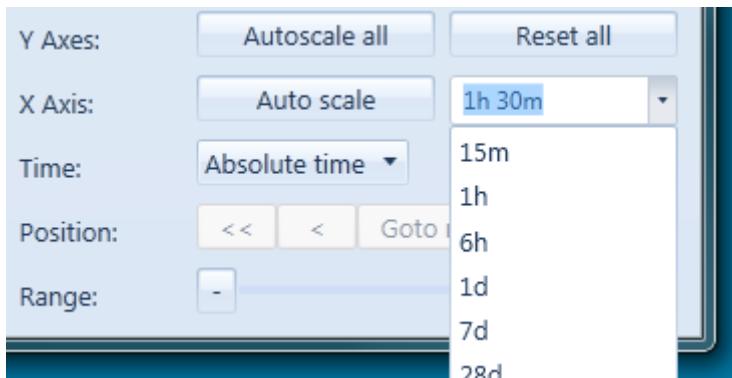


Figure 389 Time field showing custom time and choice of standard time ranges

The **Show time as...** option allows a choice of plotting data as:

- **Absolute time** – data is plotted against a date/time e.g. 1 Jan 2011.
- **Time from start** – data is plotted against the time from when the bioreactor was started for the experiment
- **Time from inoculation** – data is plotted against the time from when the bioreactor was inoculated

(+) **Select other times...** -- Choosing this option brings up a dialog that allows other times to be selected as options.

The dialog allows selecting from times of:

- Start of phase
- Start of step
- End of step
- Time of first value of variable
- Time of last value of variable

Select an item in the list on the left and use >>> to move the item to the right and include the item in the reference times available for the graph.

Select an item in the list on the right and use <<< to remove the item from the reference times that can be used in the graph.

Type in the filter page to see just reference times that match the filter text.

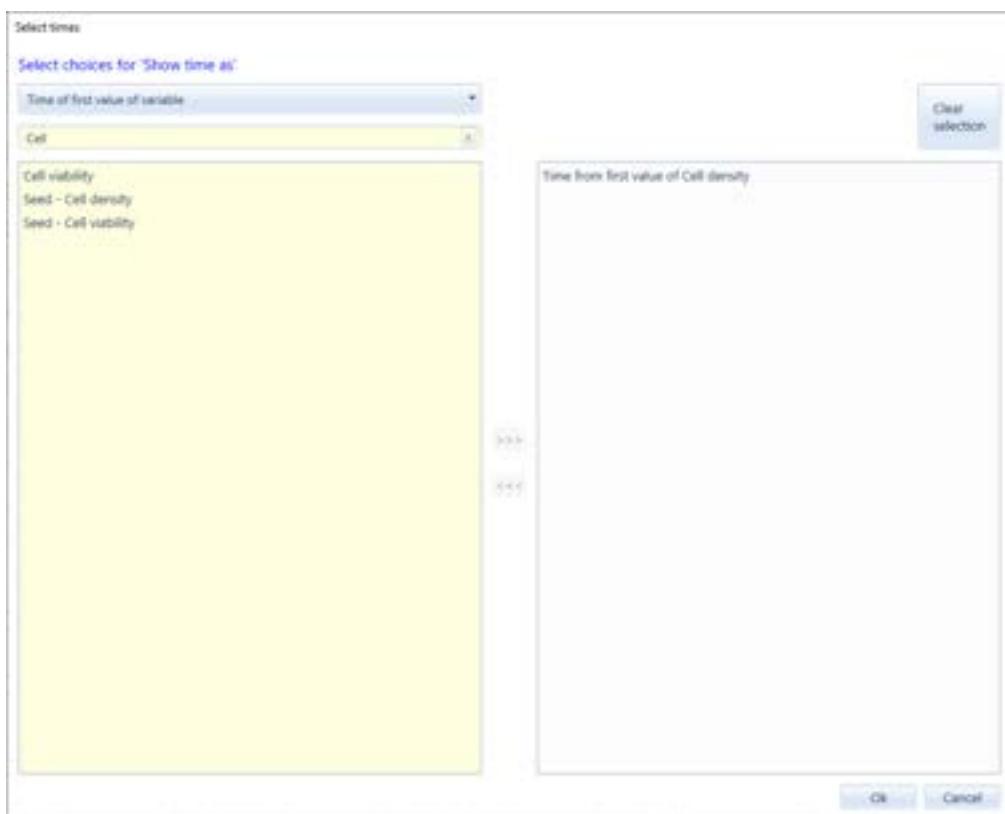


Figure 390 Select times dialog showing variables for adding Time from first value references for Cell variables.

The last variable added in the dialog will be automatically chosen as the reference time in the graph.

Show real time selects between a static display of the data and an auto-updating display always showing the current time.

When **Show real time** is not selected **Position** can be used to move the X axis. << moves the displayed range to the start and >> moves the displayed range to the end. < and > move the displayed range left and right. **Goto now** moves the range to show the current time.

Range can be used to control the range of time covered by the X axis. + and – zoom in and out and the slider can be used to vary the range continuously.

Easy access to a selection of options is also available from the context menus on the graph and on the X-axis.

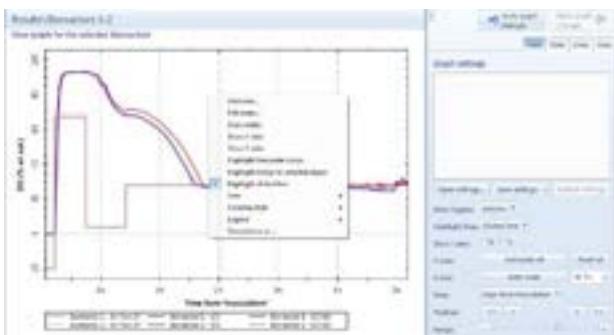


Figure 391 Graph context menu

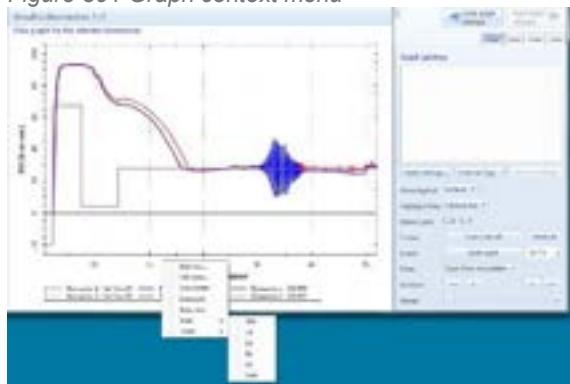


Figure 392 X-axis context menu

10.1.2.2 Saving pictures

The context menu on the graphs/results page has a **Copy picture** option that copies the graph to the clipboard. The picture can then be pasted into presentations.

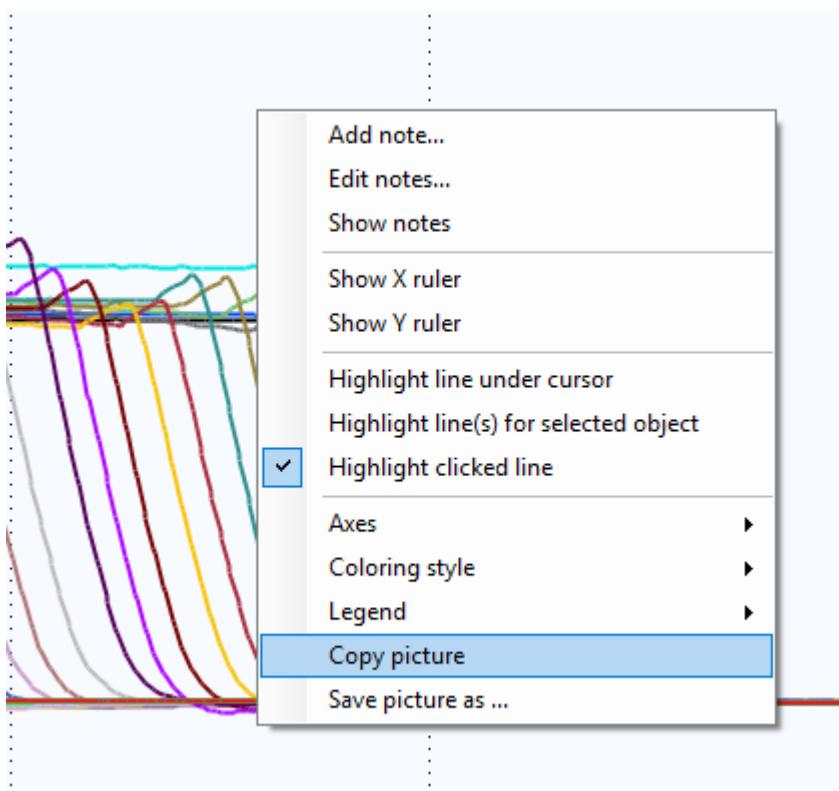


Figure 393 Graph context menu with **Copy picture** option

Alternatively the **Save picture as** option saves the picture to a file.

10.1.3 Data

The **Data** panel allows the displayed variables to be selected.

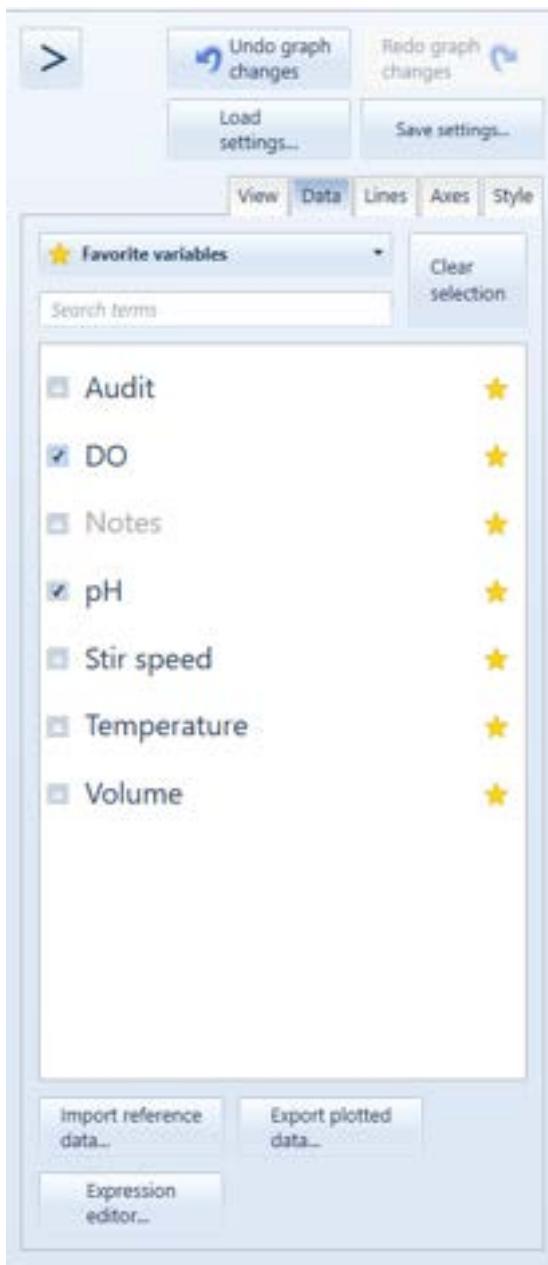


Figure 394 Data panel

The drop down menu chooses which variables to display in the list. There are three special groups at the top:

- **All variables**, all typically useful variables from all groups are shown. (Additional variables that are not typically useful for end users are shown in the **Diagnostics variables** group.)
- **Favorite variables** the variables that have been marked as favorites. Click the star at the right of the list to mark a variable as a favorite.
- **Recent variables** this group is automatically updated to show recently used variables.

Clear selection de-selects all of the variables.

Type text into the **Search terms** box to filter the list of variables.

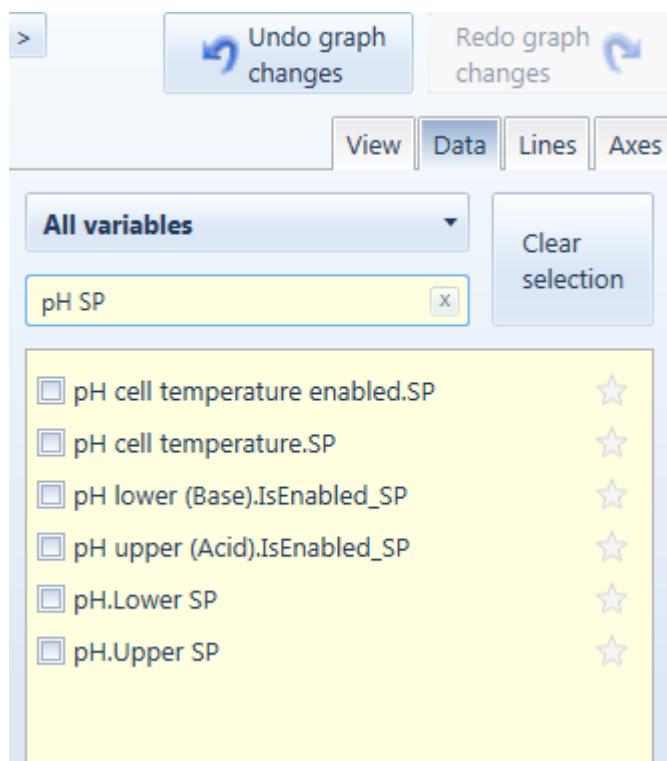


Figure 395: Using search terms to filter the variables

Search terms should be separated with spaces. Text search is case insensitive if only lower case is used (use mixed case to do case sensitive search). The background is coloured yellow to indicate when the variable list is being filtered.

Import reference data... allows importing reference data that can be plotted against the results of a running culture.

Export plotted data... allows exporting plotted data for further analysis in Excel or other applications.

Expression editor... displays the expression editor described in section 3.6.1 above.

10.1.3.1 Exporting plotted data

Export plotted data... displays the **Export plotted data** window.

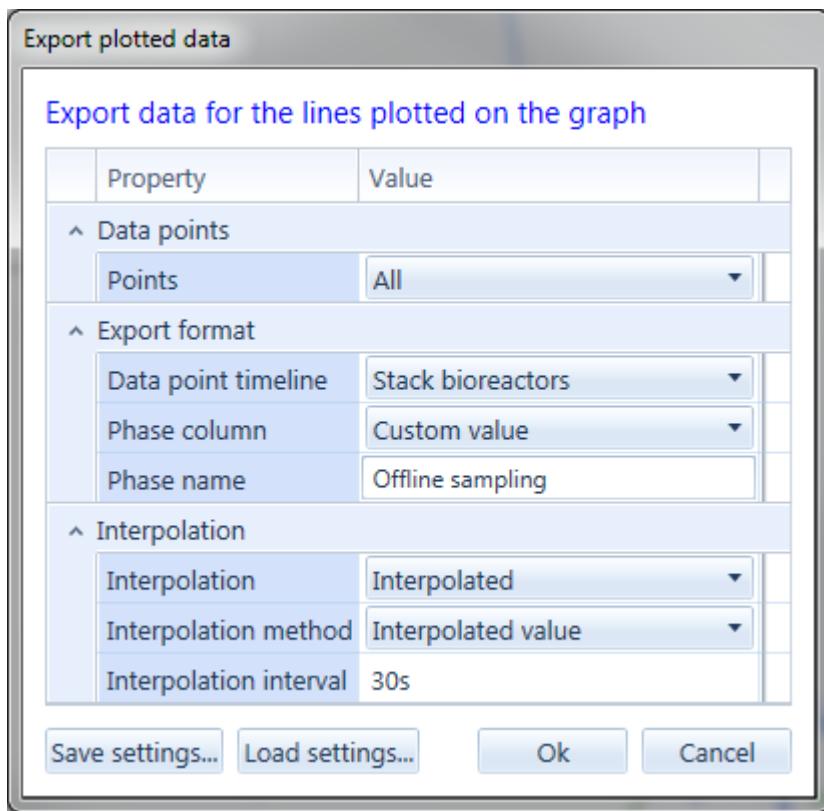


Figure 396 Export plotted data window

Points selects between:

- **All** - exporting points for the complete range of times in the experiment
- **Plotted data** – exporting points matching the range of the X-axis of the graph
- **Last value** – exporting just the last value of each variable

Data point timeline selects between:

- **Single** – there is one date/time column in the export file
- **Separate** – there is an individual date/time column for each exported line
- **Stack bioreactors** – data is exported in bioreactor order. There is one date/time column in the export file. A **Phase column** can be added to the export. Options are: **None** – no column; **Sampled** – the phase in which the sampled data was recorded; **Custom** – the **Phase name** is used as the phase for all rows in the export
- **Stack bioreactors for SIMCA** – the same as **Stack bioreactors** but with a range of characters removed from column headings.

Interpolation selects between:

- **Plotted points** – exports the points shown on the graph. These are typically a subset of the underlying data chosen to show a reasonable number of points on the screen.
- **Raw data points** – exports the raw data retained by the system. An element of data reduction is performed by discarding duplicate values when variables are not varying, but otherwise there are all the values read by the system.

- **Interpolated** – samples the data at points spaced the **Interpolation interval** apart. The same X value is used for all variables making the data easier to plot in Excel. **Interpolation method** chooses how the interpolation is done between **Interpolated value**, **Mean value** and **Last value**.

Points are exported as dates/times or as a time in hours depending on what is being displayed on the X-axis.

When exporting times the Date format option allows choosing between:

- **Local times** – the default. As in previous versions of the software the data is exported according to the time zone of the computer where the export is being done.
- **UTC times with time zone** – dates are exported as UTC times with an explicit time zone. Tools that support the format can safely import the data on other computers that may have different time zone settings.

Save settings... enables the current export settings to be named and saved.

Load settings... enables saved settings to update and replace the current export settings.

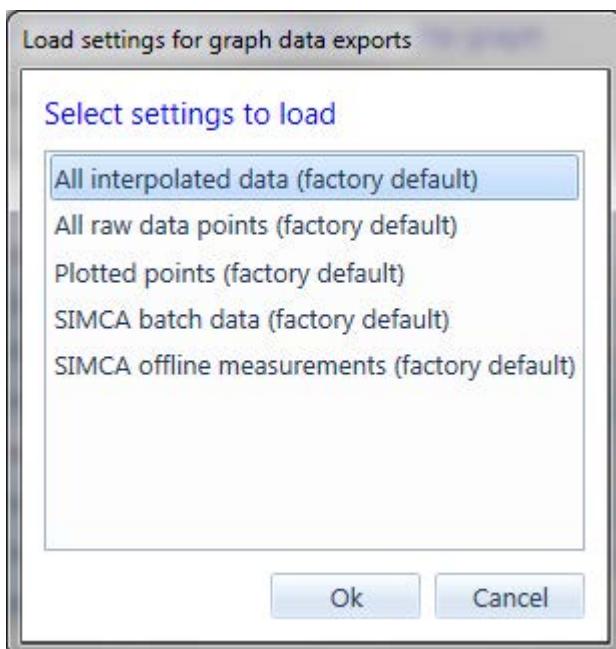
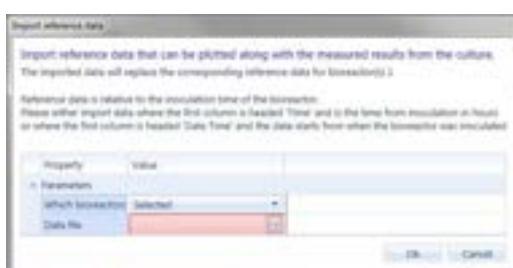


Figure 397 Load graph export settings

10.1.3.2 Importing reference data

Import reference data... displays the Import reference data window.



Which bioreactors chooses which bioreactors the reference data will apply to:

- **Selected** applies the reference data to the selected bioreactors
- **All** applies the reference data to all the bioreactors

Data file chooses the file to import data from.

Some suitable sample data as exported by the Export plotting data function is shown below.

The “Bioreactor 1 –“ prefix is generated for data from Ambr® 250 to indicate which bioreactor the data is for. The prefix is not required when importing reference data – the column could as well have been headed “DO”

```
"Time","Bioreactor 1 - DO"
0,86.3505850934649
0.1666666666666667,76.3694346209503
0.3333333333333333,69.5752230677668
0.5,67.8201773751714
0.6666666666666667,66.9184096491681
0.8333333333333333,63.9034548535745
1,59.6757746300904
1.1666666666666667,53.2402826133282
1.3333333333333333,44.071525057427
1.5,34.0936876073342
1.6666666666666667,27.324476124427
1.8333333333333333,26.4666458739938
2,26.350754329496
2.1666666666666667,27.0151296259231
2.3333333333333333,27.3933634246194
2.5,28.4224924293046
2.6666666666666667,27.782521962907
2.8333333333333333,28.3127592736978
3,28.8877458190925
3.1666666666666667,25.8326177949363
3.3333333333333333,10.325182881708
3.5,17.812493887912
3.6666666666666667,32.2679320249001
3.8333333333333333,26.4081974169738
4,26.2312276400074
4.1666666666666667,26.4467714934515
4.3333333333333333,26.5310230184773
4.5,26.1729426993899
4.6666666666666667,25.2267732446489
4.8333333333333333,24.5086696056457
```

The system creates from this data a DO.REF variable that can be plotted and is grouped in the **Reference data** group.

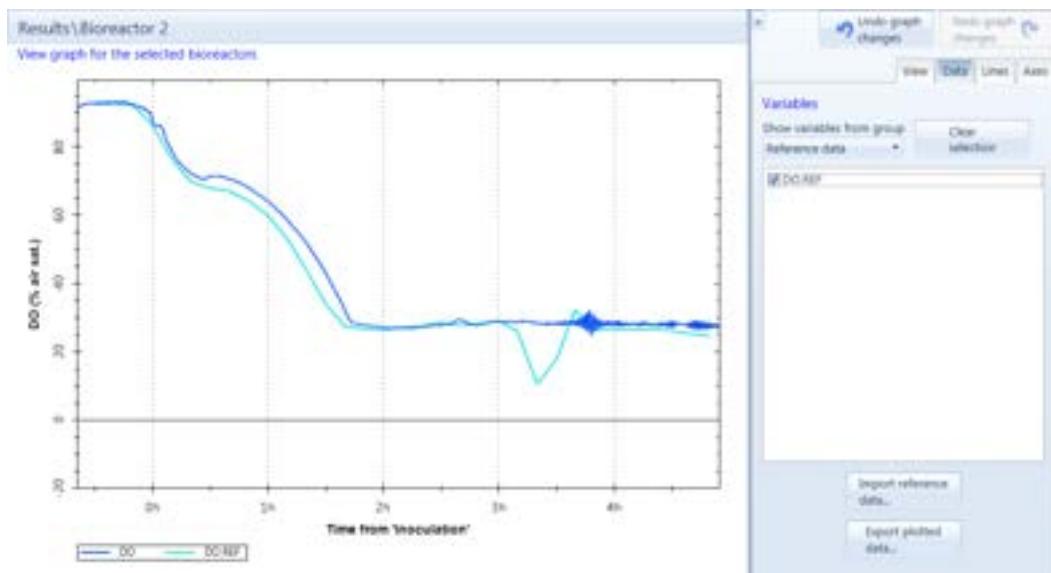


Figure 398 Plot of actual and reference data

10.1.4 Lines

The Lines panel allows the style of the lines on the graph to be changed.



Figure 399 *Lines* panel

Coloring style selects the overall strategy for line colours and styles:

- **Automatic** – chooses automatically from the styles below
- **By bioreactor** – lines for the same bioreactor have the same style
- **By variable** – lines for the same variable have the same style
- **By bioreactor and variable** – every line has an individual style

For each style the **Bioreactor** and **Variable** that the style applies to are listed.

Style shows a sample of the style.

Color allows the color of the line to be chosen.

Width allows the width of the line to be chosen.

Symbol allows data point symbols to be chosen.

Dashes allows the dash type to be chosen

Reset resets a modified style to its default.

Save as default saves a style as a default.

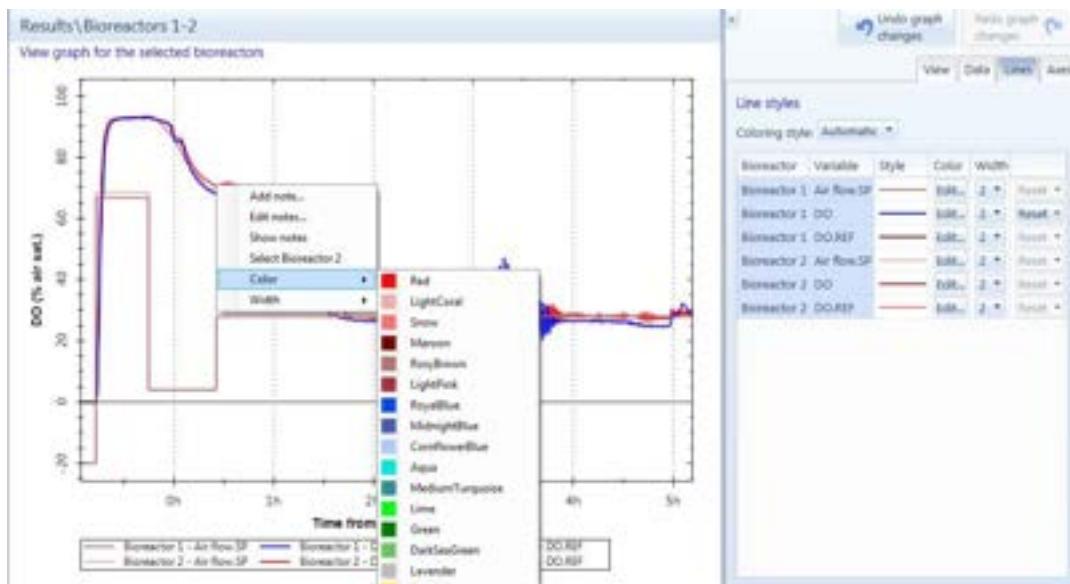


Figure 400 Line context menu

The **Color** and **Width** options can also be edited using the context menu on individual lines.

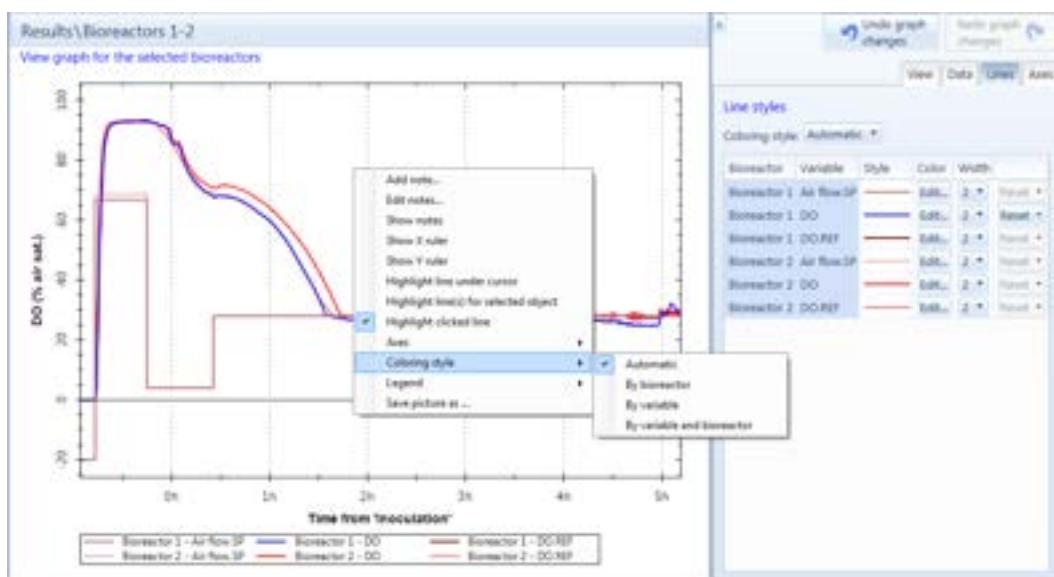


Figure 401 Graph context menu

The overall **Coloring style** can also be edited using the main graph context menu.

10.1.5 Axes

The **Axes** panel allows the axis limits and positions to be adjusted.



Figure 402 **Axes** panel

For the X-axis

Time units choose how relative times are displayed from:

- **d h m s** – the system uses a flexible format depending on the range of data displayed
- **Decimal days** – the axis is labelled with the time as a number of days
- **Decimal hours** – the axis is labelled with the time as a number of hours

Anchor axis selects how times are scaled to show the current time on the graph.

- **Current time on right** places the current time at the right of the graph. Data slides to the left as time progresses.
 - **Start on left** places zero relative time at the left of the graph. In real time mode scale of the graph is adjusted as required to keep all the data in range.

For each Y-axis

Min sets the minimum value of the axis.

Max sets the maximum value of the axis.

Position chooses from a selection of positions for the axis including hiding the axis.

Autoscale auto-scales the axis.

Reset resets the axis to its defaults.

Save as default saves the axis settings as a default.

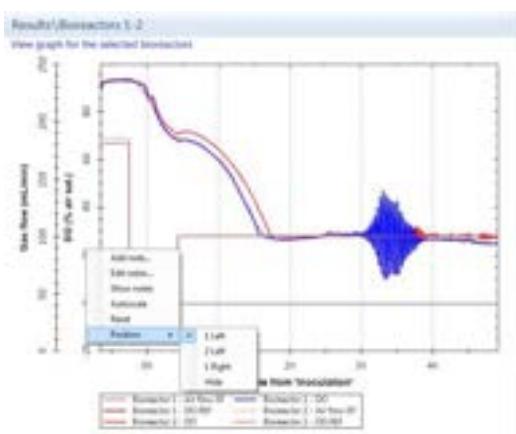


Figure 403 Axis context menu

The **Autoscale**, **Reset** and **Position** options are also available on the context menu for axes.

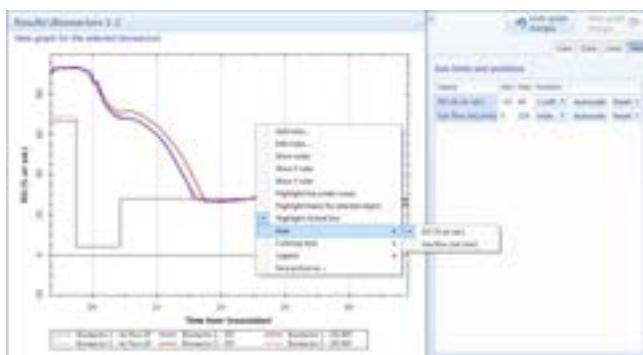


Figure 404 Graph context menu

Axes can also be hidden or displayed from the main graph context menu.

10.1.6 Style

The **Style** panel allows the appearance of the graph to be customised.

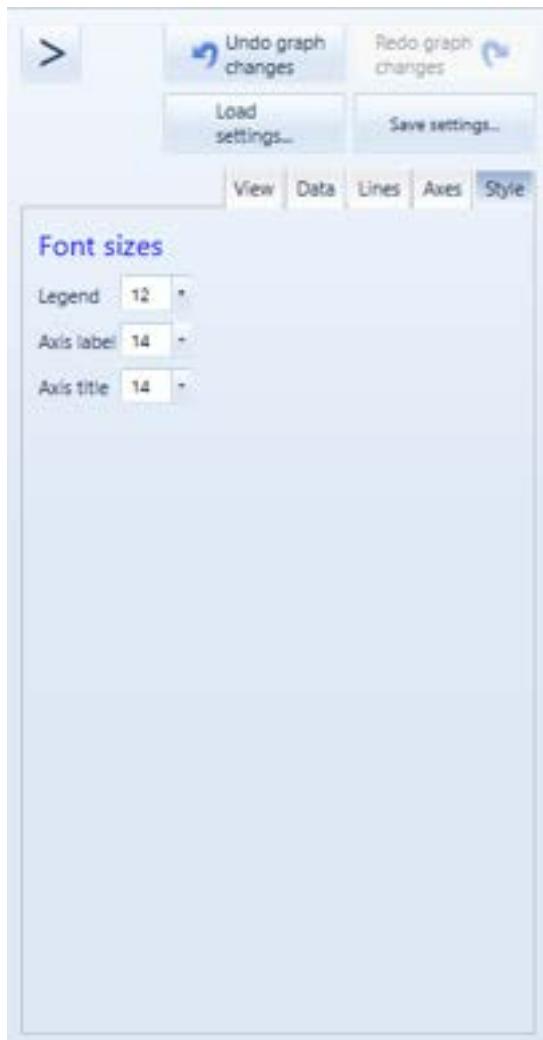


Figure 405 Style panel

Legend changes the size of the font used in the legend.

Axis label changes the size of the font used to label the axes with values.

Axis title changes the size of the font used for axis titles.

10.2 Overview

The Overview page shows a pair of graphs for a single bioreactor together with a view of the current properties of the bioreactor.

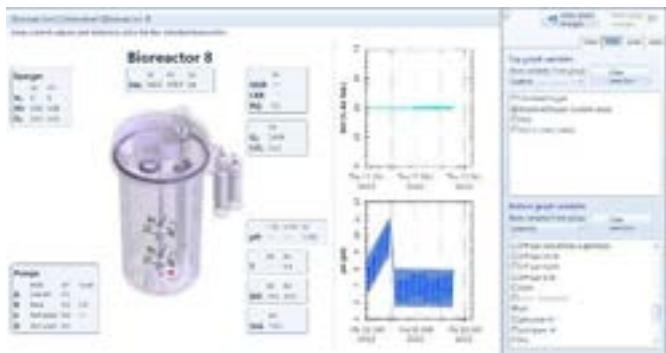


Figure 406 Overview page

The Overview page has the same controls at the Graphs or Results page with some minor differences.

- Both graphs share the same X-axis.
 - The data, line styles and Y-axes can be chosen independently for the two graphs
 - Styles apply to the pair of graphs

10.3 Tables

The Tables page displays the current values of variables and allows entering external data.

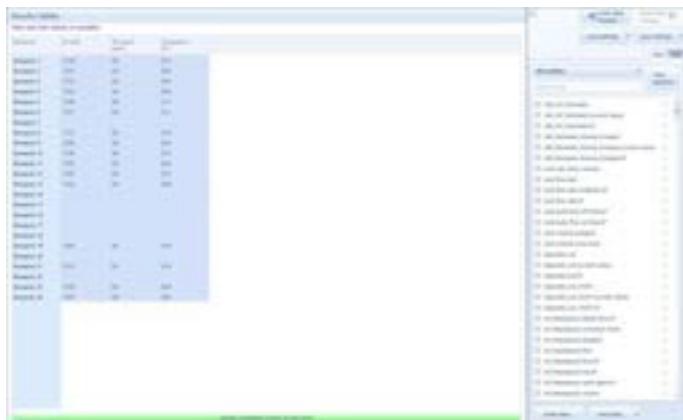


Figure 407 Tables page

Double-clicking on a cell will display the **Edit data** window allowing viewing and appropriate editing of the values of that variable.

Data in the table is automatically updated. Unchecking the Auto update checkAutomatic updates can beautomatic updates to be disabled. Disabling automatic updates avoids issues from the scrolling being reset when the data is updated.

The **Update** button can be used to update the table.



Figure 408 Edit data window

Press **New point** to add a new point.

The **Note** column can be used to annotate the stored data with extra information about the value entered.

The option **Save data** can be used to save the data to a file.

10.3.1 Frozen column

The bioreactor column in the table view is frozen so that the bioreactor column remains visible when the view is scrolled horizontally.

Bioreactors\Tables

View and edit values of variables

Bioreactor	pH (pH)
Bioreactor 1	7.112
Bioreactor 2	
Bioreactor 3	
Bioreactor 4	
Bioreactor 5	
Bioreactor 6	
Bioreactor 7	
Bioreactor 8	7.049
Bioreactor 9	7.115
Bioreactor 10	7.128
Bioreactor 11	7.103
Bioreactor 12	7.096
Bioreactor 13	7.053
Bioreactor 14	7.047
Bioreactor 15	7.042
Bioreactor 16	7.061
Bioreactor 17	7.120
Bioreactor 18	7.099
Bioreactor 19	7.036
Bioreactor 20	7.046
Bioreactor 21	7.081
Bioreactor 22	7.121
Bioreactor 23	7.030
Bioreactor 24	7.088

Auto update Update completed at Tue 12 Oct 08:35



Figure 409 Table view with bioreactor column frozen.

10.3.2 Loading and saving settings

The **Load settings** dropdown allows the table settings or just the table layout to be loaded.

Load settings... opens a dialog that allows saved settings to be opened.

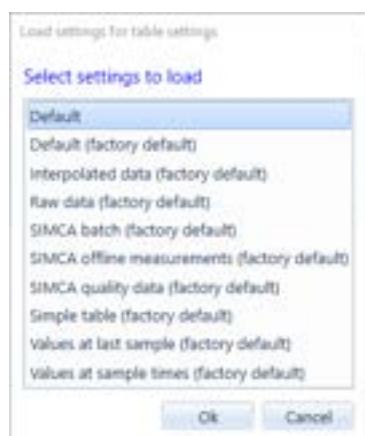


Figure 410 Load table settings dialog

Clicking on one of the settings in the list of settings switches the table to use the current layout and values of the settings.

Load layout... opens a dialogue that allows a saved layout to be loaded. The layout is applied to the current fields in the table.

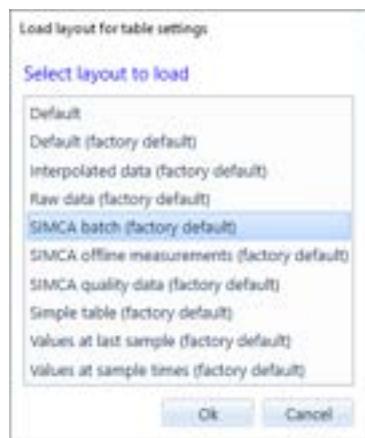


Figure 411 Load layout settings dialog

The **Save settings** dropdown allows table settings to be saved in the same manner as for graphs.

10.3.3 View

The View panel allows the set of bioreactors, the time of period, the format and layout of the displayed data to be selected.



Figure 412 Table **View** panel

10.3.3.1 What should table be shown for?

The **Show for** option allows displaying the table for **All bioreactors** or just for the **Selected bioreactors**.

10.3.3.2 What sort of table do you want?

The Table type option allows displaying the data for **Values at time** or **Values over time**

If **Values at time** is selected then:-

Option allows displaying the data:

- **Now** – shows the latest value of the variables.
- **Specified date and time** – shows the values as of a date and time that can be explicitly edited.
- **Graph X ruler position** – shows the values as of the date and time that the X ruler is displayed on the graph page.

Reference time chooses **Report time** between:-

- **End** – the time is specified relative to the end of the experiment.
- **Start** – the time is specified relative to the start of the experiment for the bioreactors, that is when the bioreactor started running.
- **Phase** – the time is specified relative to the start time of the current phase.
- **Inoculation** – the time is specified relative to when the bioreactors were inoculated. No data will be shown for bioreactors which have not been inoculated.
- **Sample time** – the time is specified relative to the last sample for the bioreactors, that is when the bioreactor was last sampled.

Offset – allows an optional positive offset to be added the reference time.

If **Values over time** is selected then:-

Layout allows displaying the data:-

- **Horizontally grouped by object** – all of the fields for the object are grouped together horizontally in the same row.
- **Horizontally grouped by variable** – all variables of the same name are grouped together horizontally on the row
- **Stack object vertically** – all of the fields for the object are grouped vertically for each object in turn.

Times allows the time of the variables to be displayed as:-

- **Single time column** – a single column with containing data and time for all fields
- **Separate time columns** -- a time column is associated with each variable.

Interpolation allows the time series to be chosen from:-

- **Interpolated values** – the time series data is interpolated at the **Interpolation interval**
- **Raw values** – the raw data from the time series.
- **Plotted values** – the time series data at the times as shown on the graph
- **Sampled values** – the time series data at the times of a samples.

Display times as chooses how to display the times:-

- **Absolute time** – the absolute time of the series data when the data was recorded.
- **Logical day relative to reference** – the time relative to the **Reference time** as a number of hours from the start of the logical day.
- **Time relative to reference** -- the time relative to the **Reference time** as a number of hours

Start offset -- the offset from the start of times when data should start.

End offset -- the offset from the start of times when data should end.

10.3.3.3 How should values be shown?

Number format – choose the format for the numbers:-

- **Default** – the default format for the variable
- **Integer** – 7
- **1 decimal place** – 7.5
- **2 decimal places** – 7.58
- **3 decimal places** – 7.583
- **4 decimal places** – 7.5830
- **5 decimal places** – 7.58300
- **General, 1 significant figure** – 0.7
- **General, 2 significant figure** – 1.71
- **General, 3 significant figure** – 1.312E+1
- **General, 4 significant figure** – 1.7110
- **General, 5 significant figure** – 1.31200E+1
- **Scientific, 1 significant figure** – 7.5E0
- **Scientific, 2 significant figure** – 7.58E0
- **Scientific, 3 significant figure** – 1.312E+1
- **Scientific, 4 significant figure** – 1.3123E+1
- **Scientific, 5 significant figure** – 7.58300E0

Replace NaN with – replace NaN (not a number) values with optional text.

10.3.3.4 General format options

Include units – include the units of the variable in the heading of the column

Make columns unique – make name in the columns unique. Especially for Date/Time columns.

Header cleaning – choose how to clean the text in the header columns:-

- **No cleaning** – the text is left as is.
- **ASCII only** – only characters in the ASCII character set are displayed. Characters not in the ASCII character set are replaced with a ?.
- **A-Za-z0-9 and space only** – only upper and lowercase characters A-Z, number 0-9 and space are allowed. Unknown characters are removed.

String cleaning – choose how to clean text fields in the columns. Options are the same as header cleaning.

10.3.3.5 Labels

Up to 4 labels can be chosen to label the objects in the report **Label1..Label4**. At least one label must have a variable assigned.

The data in the labels can be chosen from the following variables

- **None** – no variable.
- **Name** – the name of the bioreactor.
- **Position** – the numeric position of the bioreactor.
- **Experiment** – the name of the experiment.
- **Protocol** – the name of the protocol in which the bioreactor is included.
- **Batch** – the bioreactors batch.
- **Strain** – the bioreactors strain.
- **User label** – the bioreactors user label.

See section 4.5.3 Assign bioreactors to protocols.

10.3.4 Data

The Data panel allows the selection of the variables to be shown and entering externally measured data.

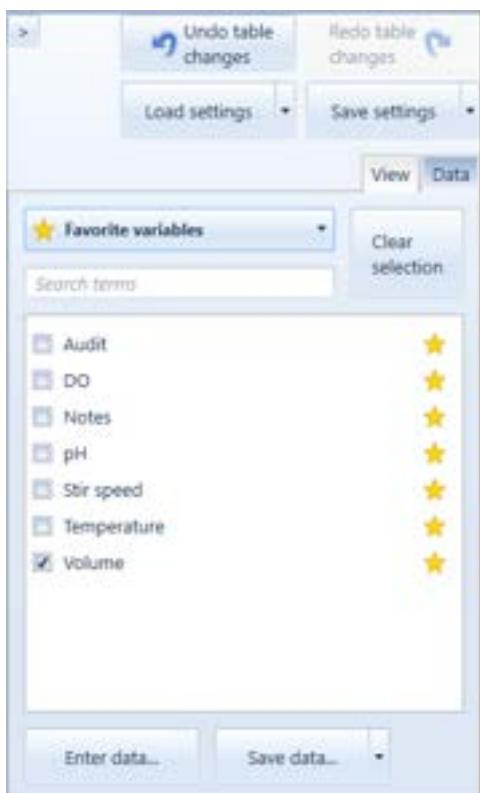


Figure 413 Data panel

Press **Enter data** to enter or import data as described further below.

Save data provides access to options to export data.

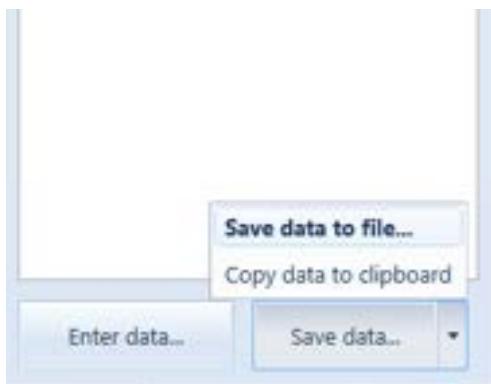


Figure 414 Save data options on Tables page

Select **Save data to file** to save the data displayed to a file.

Select **Copy data to clipboard** to copy the data displayed to the clipboard.

10.3.4.1 Enter data options

Pressing **Enter data** displays options for how to enter data, and how to undo recent data entry.



Figure 415 Enter data options

Enter values for data shows the enter data page present in previous versions of the software.

Load standard file prompts the user to select a file that has been pre-formatted for Ambr® 250 and then shows that in the same enter data page present in previous versions of the software.

Import data using a template presents a screen letting the user mark which parts of their file correspond to data, which to dates, which to bioreactor identifiers, ...

Undo data import presents a screen letting the user undo recent data entry. Note that undo is only supported when data has been entered while the current program is running.

10.3.4.2 Entering values for data

Use the **Enter data** page to enter values manually.

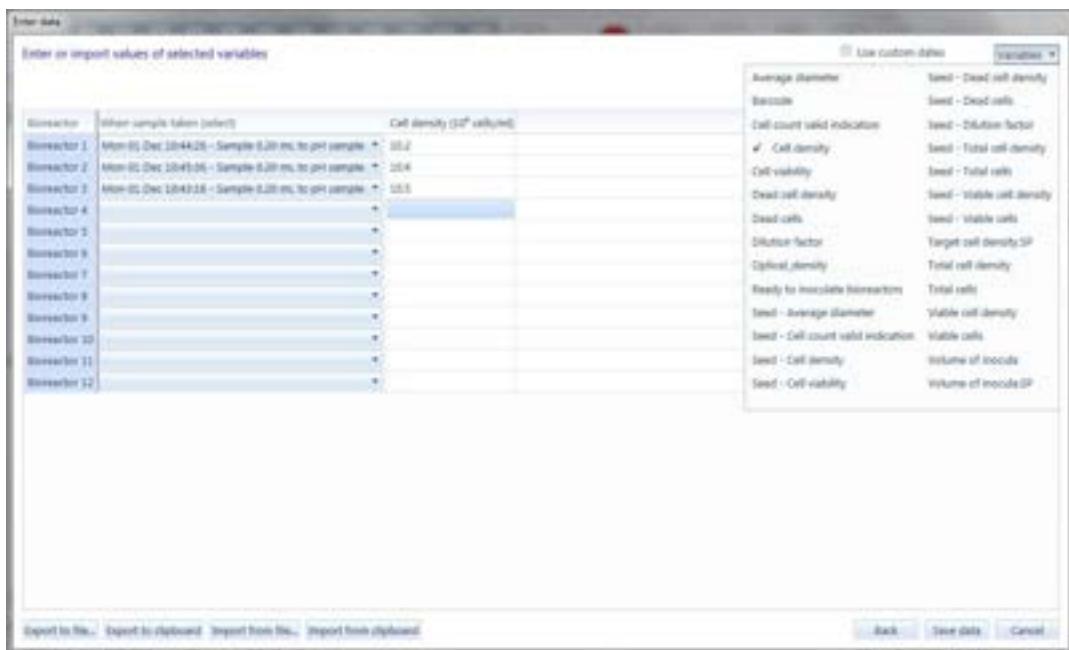


Figure 416 Enter data window

The window allows importing or exporting data using the **Export to file...**, **Export to clipboard**, **Import from file...** and **Import from clipboard** options.

Importing data will automatically select the imported variables for display and forces the selection of the **Use custom dates** option.

Variables can be used to select the variables displayed. The current value of the variable is displayed for reference.

Use custom dates, **Use named dates** and **Use current date and time** select the option for the date and time to be associated with the data.

Use named dates offers a selection of dates including when the window was opened and the times of samples taken from the bioreactor.

Use current date and time uses the current date and time when the window is applied.

Use custom dates allows an arbitrary date and time in the past to be entered.

10.3.4.3 Load standard file

Load standard file provides a simple route to import data generated on an external system and in the standard format used in the **Enter data** window.

- 1) Press **Load standard file** and choose the file from which to import data
- 2) Press **Ok** or edit the loaded data if required.

10.3.4.4 Import data using a template

Import data using a template allows the import of data in different formats.

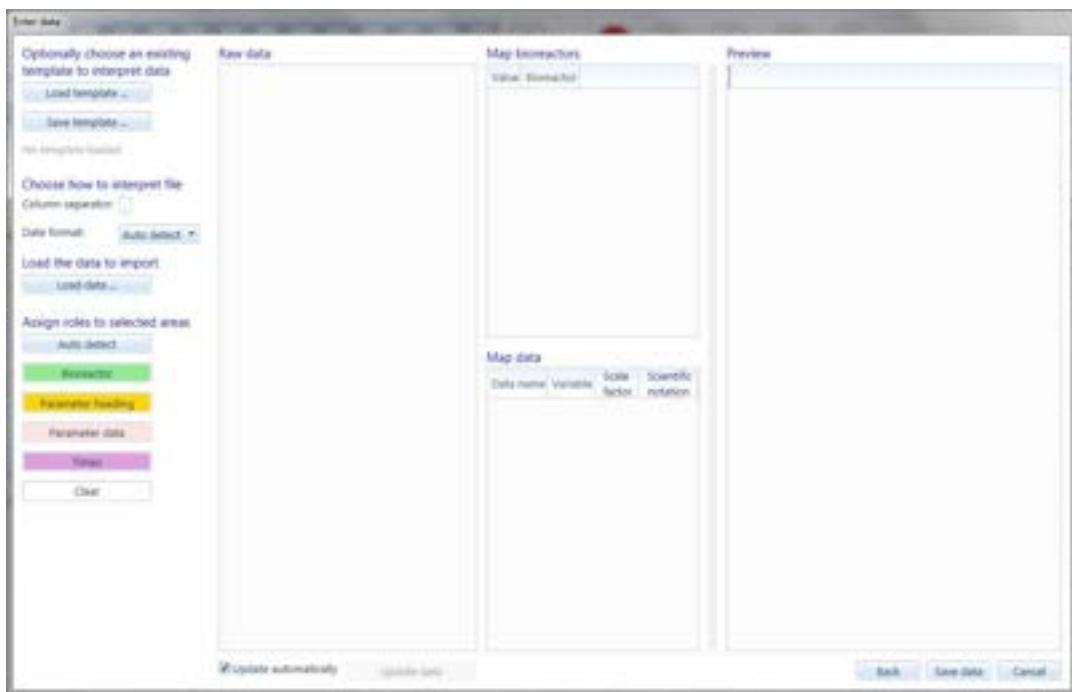


Figure 417 Import data using a template

To import data using this page:

- 1) If you have a template for the data then load that template using the **Load template** button.
- 2) Update the options for interpreting the data file as required:
 - a) **Column separator** can be edited to change the character used to split up the data file into columns.
 - b) **Date format** specifies how dates in the file should be interpreted. If the file contains dates whose interpretation is ambiguous – e.g. 05-04-2013 – then one of the options Force day then month or Force month then day should be chosen. If the date format is unambiguous then the Auto detect option may be used.
- 3) Load the file of data using the **Load data** button. The system will display the loaded data in the **Raw data** panel. If a template was loaded then Map bioreactors, Map data and Preview may show details of the imported data.
- 4) Mark up the cells in the **Raw data** panel that contain dates, data, headings and times. To mark up cells:
 - a) Select the cells to update
 - b) Choose from:
 - i) **Auto detect** to let the system try and assign a role to the cells
 - ii) **Bioreactor** to mark the cells as containing data that identifies the bioreactor the data applies to.

iii) **Parameter heading** where the cells can be used as labels for what variable the data represents.

iv) **Parameter data** where the cells contain the values to load.

v) **Times** where the cells contain the date/time the data applies to.

vi) **Clear** to clear the role assigned to the cells.

The values to be imported pick up their parameter heading by looking first directly up the data and if no heading is found there looking for a heading directly to the left of the data.

The values to be imported pick up their bioreactor by looking first directly up the data and if no Bioreactor cell is found there looking for a Bioreactor cell to the left of the data.

The values to be imported pick up their date/time as follows:

- If there is no date/time field then the current date/time is used
- If there is a single date/time field then that field is used wherever it is
- If there are multiple date/time fields then the values to be imported pick up their date/time by looking first directly up the data and if no date/time cell is found there looking for the a date/time cell directly the left of the data.

- 5) For each bioreactor heading choose in the **Map bioreactors** section which bioreactor it applies to.
- 6) For each parameter heading choose in the **Map data** section which variable inside Ambr® 250 it maps to and optionally introduce a **Scaling factor** between the values in the file and the values to be stored.
- 7) Review the values in the **Preview** pane to make sure that they are as expected.
- 8) If you are going to import a similar file in the future save the settings in the dialog as a template using the **Save template** button.
- 9) Choose **Save data** to import the data

The picture below shows the page with marked up data.

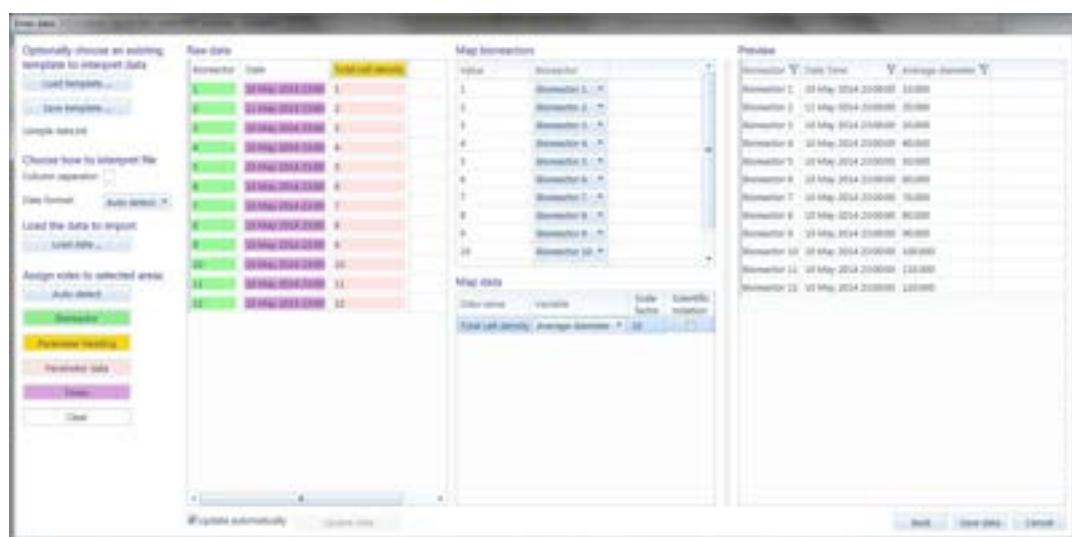


Figure 418 Formatted data import with marked up data

When large data sets are being imported the system will disable automatic updating of the interface. Press **Update data** to update the data or select **Update automatically** to reenable automatic updates.

If the data does not contain the required header information or dates then you can insert additional rows or columns using the context menu (right mouse click) within the raw data grid. Data within the raw data grid can be edited to provide the required data.

The division of the page between preview and other data can be adjusted using the grey bar to the left of the preview area. Drag the bar to one side or another to adjust the dialog.

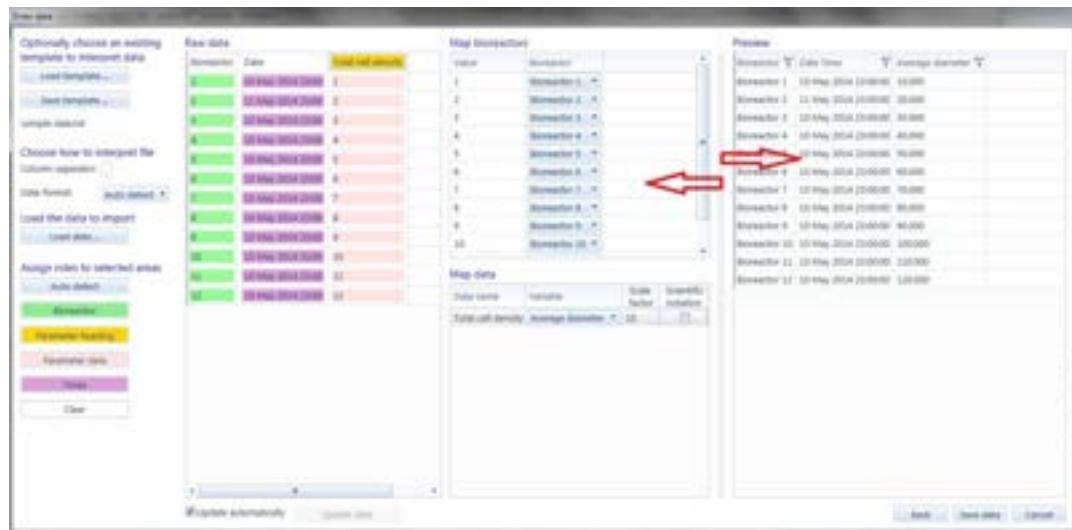


Figure 419 Divider bar can be moved to adjust space given to Preview

10.3.4.5 Undo data import

The Undo data import page shows data that has been imported into the application.

Undo data entry						
Imported	Bioreactor	Variable	Time	Value	Remove	
10th-23 Nov 2014 17:05:49	Bioreactor 0	Average diameter	Sat 23 May 2014 23:00:00	30.00	<input type="checkbox"/>	
10th-23 Nov 2014 17:05:49	Bioreactor 2	Average diameter	Sun 24 May 2014 00:00:00	30.00	<input type="checkbox"/>	
10th-23 Nov 2014 17:05:49	Bioreactor 0	Average diameter	Sat 23 May 2014 23:00:00	30.00	<input type="checkbox"/>	
10th-23 Nov 2014 17:05:49	Bioreactor 4	Average diameter	Sat 23 May 2014 23:00:00	40.00	<input type="checkbox"/>	
10th-23 Nov 2014 17:05:49	Bioreactor 9	Average diameter	Sat 23 May 2014 23:00:00	30.00	<input type="checkbox"/>	
10th-23 Nov 2014 17:05:49	Bioreactor 1	Average diameter	Sat 23 May 2014 23:00:00	30.00	<input type="checkbox"/>	
10th-23 Nov 2014 17:05:49	Bioreactor 10	Average diameter	Sat 23 May 2014 23:00:00	30.00	<input type="checkbox"/>	
10th-23 Nov 2014 17:05:49	Bioreactor 7	Average diameter	Sat 23 May 2014 23:00:00	30.00	<input type="checkbox"/>	
10th-23 Nov 2014 17:05:49	Bioreactor 8	Average diameter	Sat 23 May 2014 23:00:00	30.00	<input type="checkbox"/>	
10th-23 Nov 2014 17:05:49	Bioreactor 9	Average diameter	Sat 23 May 2014 23:00:00	30.00	<input type="checkbox"/>	
10th-23 Nov 2014 17:05:49	Bioreactor 11	Average diameter	Sat 23 May 2014 23:00:00	30.00	<input type="checkbox"/>	
10th-23 Nov 2014 17:05:49	Bioreactor 12	Average diameter	Sat 23 May 2014 23:00:00	30.00	<input type="checkbox"/>	
10th-23 Nov 2014 17:05:59	Bioreactor 1	Cell density	Wed 01 Oct 2014 00:00:00	1,300	<input type="checkbox"/>	
10th-23 Nov 2014 17:05:59	Bioreactor 2	Cell density	Wed 01 Oct 2014 00:00:00	1,200	<input type="checkbox"/>	
10th-23 Nov 2014 17:05:59	Bioreactor 0	Cell density	Wed 01 Oct 2014 00:00:00	1,400	<input type="checkbox"/>	
10th-23 Nov 2014 17:05:59	Bioreactor 4	Cell density	Wed 01 Oct 2014 00:00:00	1,300	<input type="checkbox"/>	
10th-23 Nov 2014 17:05:59	Bioreactor 5	Cell density	Wed 01 Oct 2014 00:00:00	1,400	<input type="checkbox"/>	
10th-23 Nov 2014 17:05:59	Bioreactor 6	Cell density	Wed 01 Oct 2014 00:00:00	1,300	<input type="checkbox"/>	
10th-23 Nov 2014 17:05:59	Bioreactor 7	Cell density	Wed 01 Oct 2014 00:00:00	1,300	<input type="checkbox"/>	

Figure 420 Undo data import page

To use the page:

- 1) **Mark the Remove checkbox** on the data to be deleted.

- 2) Click **Remove data** to delete the data from the system.

To aid in marking the data to be removed you can select one or more rows using the **Select all**, **Select most recent** and **Clear all** buttons. **Select most recent** selects all the rows from the most recent import. Once you have selected some rows choose Mark **selected data for removal** or **Unmark selected data** for removal to mark or unmark the data.

10.4 Audit

The Audit page shows a record of key events affecting the selected bioreactors.

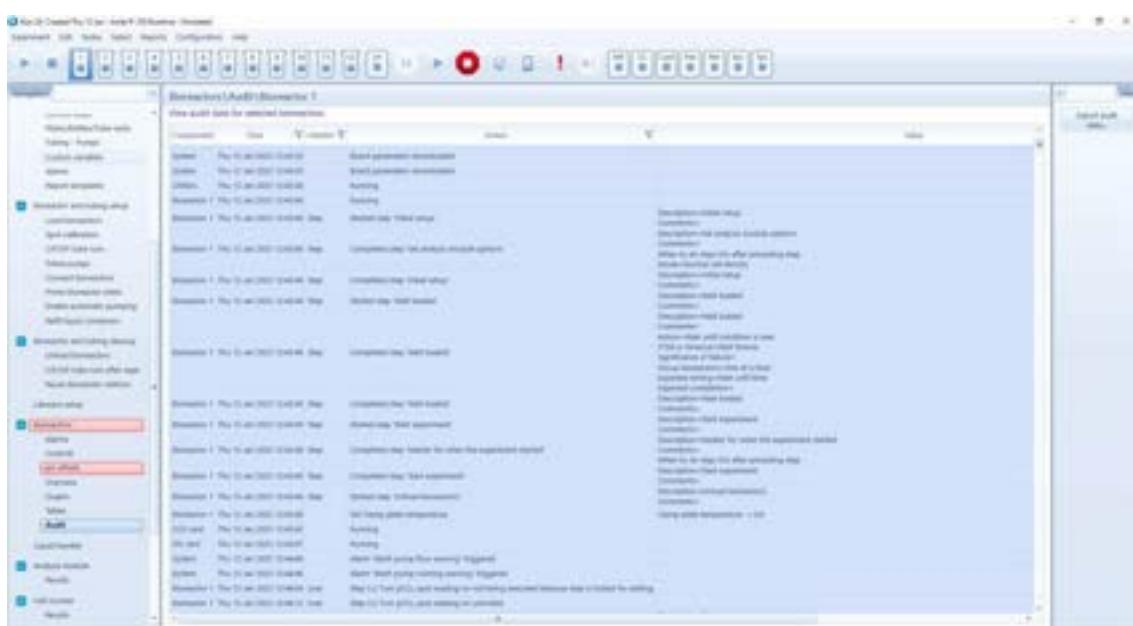


Figure 421 Audit page

For each event the system shows:

- **Component** – the part of the system the event pertains to.
 - **Time** – when the event occurred.
 - **Initiator** – what caused the event. Options include User for actions initiated by the user; Step for events initiated by a step in the process definition; CIP and Priming for events as part of a clean/sterilize in place protocol or priming.
 - **Action** – a basic description of the event.
 - **Values** – parameters for the event.
 - **Source, Source location, Source well** – the source of a liquid handling transfer.
 - **Target, Target location, Target well** – the destination of a liquid handling transfer
 - **Extra info.** – other details.

The actions displayed can be filtered by pressing the filter symbol () which displays a window for filtering what is shown.

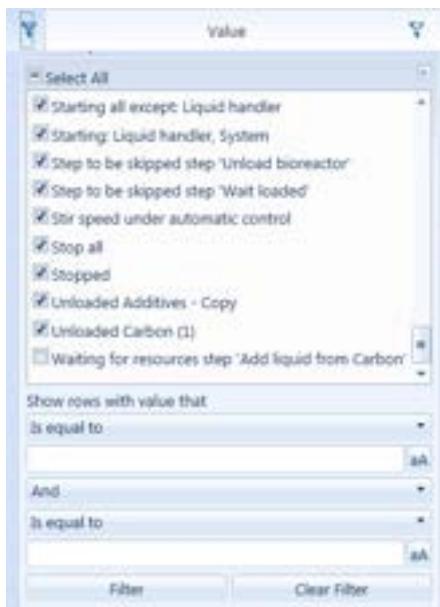


Figure 422 Filter window

Export audit data... exports all the audit records pertaining to the selected bioreactors.

11 LIQUID HANDER

This section describes the options for the direct control and maintenance of the liquid handler.

11.1 Liquid Handler

The Liquid handler page provides controls for starting and stopping the liquid handler; viewing messages, queued actions; acknowledging faults and for routine cleaning.

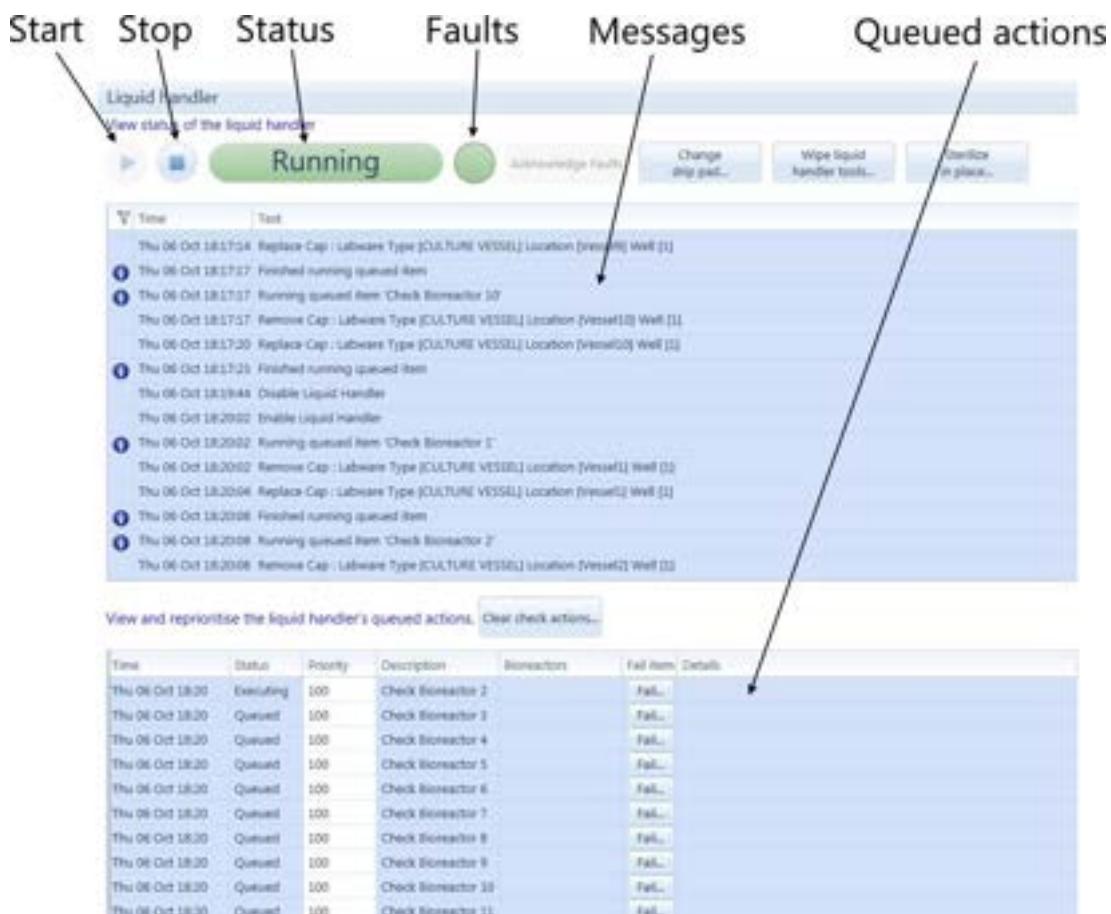


Figure 423 Liquid handler page

The **Start** button starts the liquid handler and the **Stop** button stops the liquid handler.

The **Status** indicator shows the status of the liquid handler and the **Faults** indicator shows if there have been any faults.

Acknowledge faults acknowledges any faults.

The **Messages** area shows messages about what the liquid handler is doing.

The **Queued action** area shows the liquid handler operations that are queued and awaiting action.

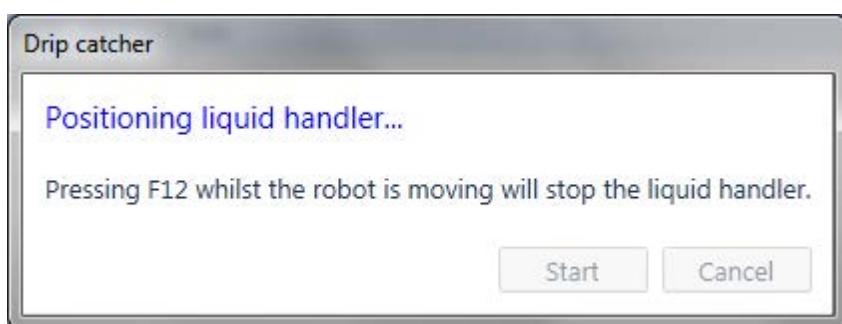
11.1.1 Change drip pad

Change drip pad... presents a window to confirm that the system should present the drip pad for changing.

- 1) If the liquid handler is running then pause or stop the liquid handler
- 2) Press **Change drip pad...** The system displays the **Drip catcher** window.



- Figure 424 Drip catcher window*
- 3) Press **Start**. The robot will move and present the drip catcher.



- Figure 425 Window while robot moves*
- 4) Wait for the liquid handler to present the drip catcher tray
 - 5) Replace the pad in the drip catcher



- Figure 426 Window while drip catcher tray presented*
- 6) Press **Drip pad changed** when you have changed the drip pad or **Cancel** if for some reason you have not changed the drip pad.

The system offers the options **Send liquid handler to safe position** to move the liquid handler back to a safe position or **Close** to leave the liquid handler where it is.



Figure 427 Close window

- 7) Press **Send liquid handler to safe position**. The robot will move to a safe position.



Figure 428 Window while robot moves to a safe position

- 8) Wait for the liquid handler to move the robot to the safe position.

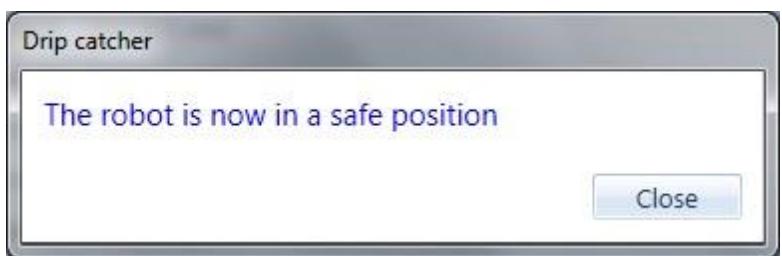


Figure 429 Window when robot is in the safe position

- 9) Press **Close**. The dialog will be closed.

11.1.2 Wipe liquid handler tools

Wipe liquid handler tools ... presents the labware manipulation tools of the liquid handler so that they can be wiped down.

- 1) If the liquid handler is running then pause or stop the liquid handler.
- 2) Press **Wipe liquid handler tools...** The system displays the **Wipe liquid handler tools** window.

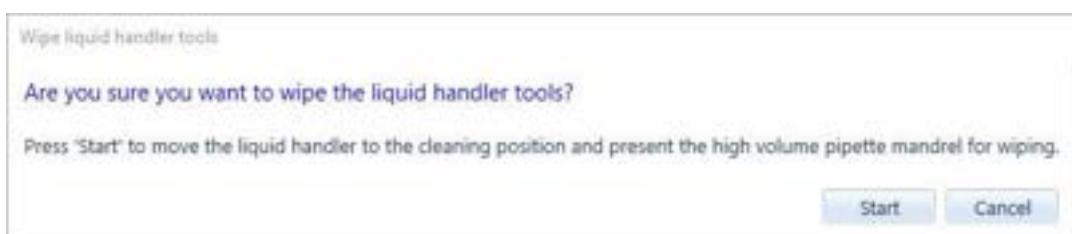
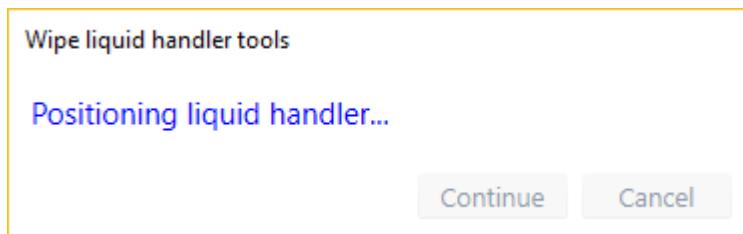
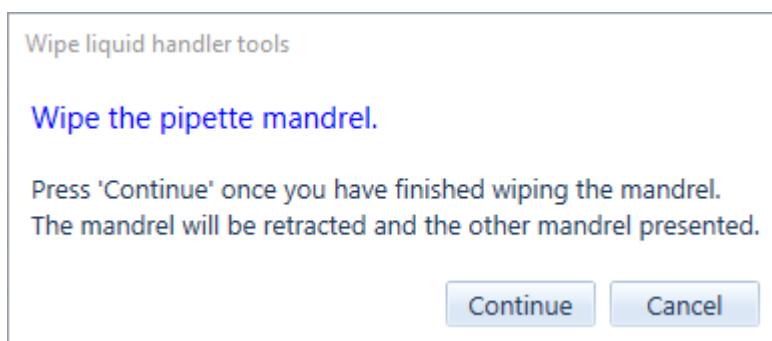


Figure 430 Wipe liquid handler tools window

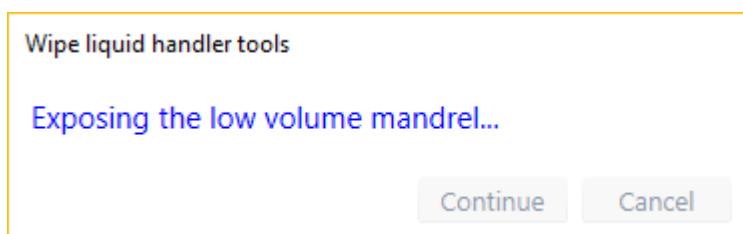
- 3) Press **Start**. The robot will move and present the high volume pipette mandrel.



- Figure 431 Window while robot moves*
4) Wait for the liquid handler to present the mandrel.
5) Wipe the 10ml mandrel following the procedure in the Operator manual.



- Figure 432 Window while 10ml mandrel is presented*
6) Press **Continue**. The robot will retract the high volume pipette mandrel and present the low volume pipette mandrel.
7) Wait for the liquid handler to present the low volume pipette mandrel.



- Figure 433 Window while 300µl mandrel being presented*
8) Wipe the 300µl mandrel following the procedure in the Operator manual

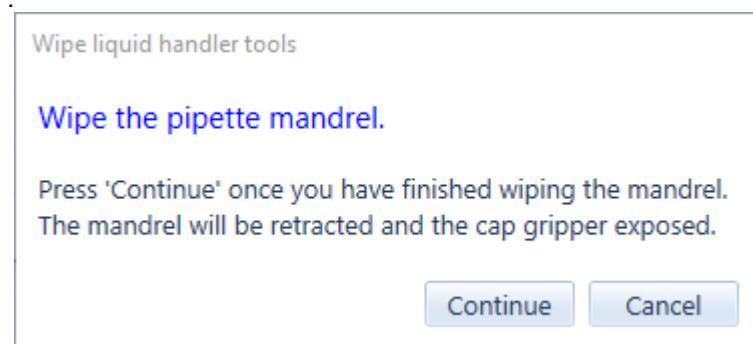


Figure 434 Window while 300µl mandrel is presented

- 9) Press **Continue**. The robot will retract the low volume pipette mandrel and present the cap gripper.
- 10) Wait for the liquid handler to present the cap gripper.

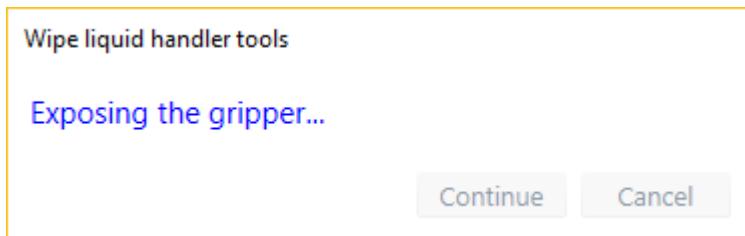


Figure 435 Window while the gripper is being presented
11) Wipe the gripper following the procedure in the Operator manual.

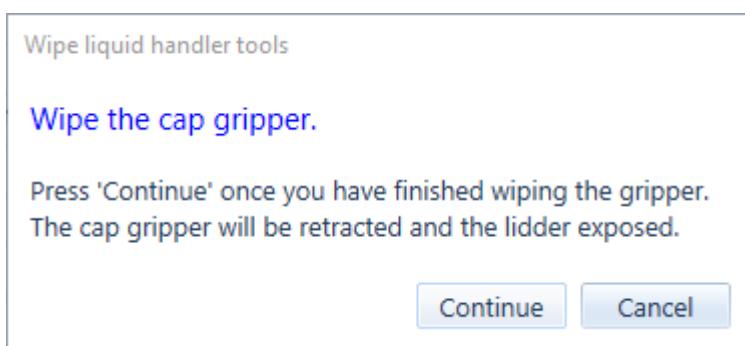


Figure 436 Window while the gripper is presented
12) Press **Continue**. The robot will retract the gripper and present the lidder for wiping.
13) Wait for the liquid handler to present the lidder

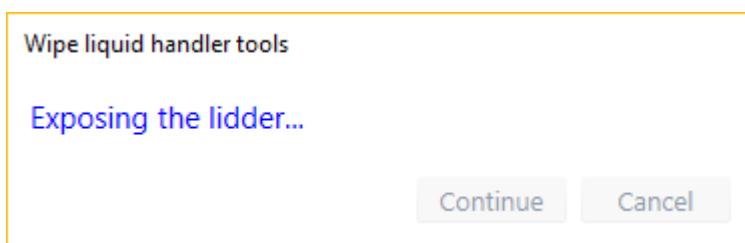


Figure 437 Wait for the liquid handler to present the lidder
14) Wipe the lidder following the procedure in the Operator manual.

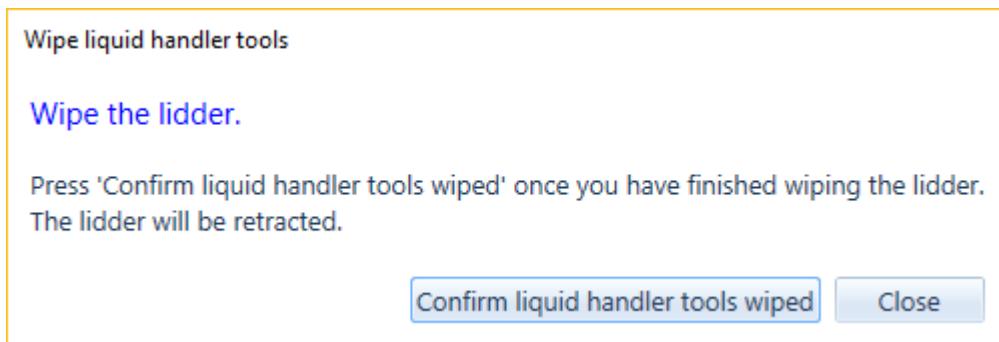


Figure 438 Window while lidder presented

- 15) Press **Confirm liquid handler tools wiped** to acknowledge that you have wiped the tools.
- 16) Wait for the lidder to be retracted

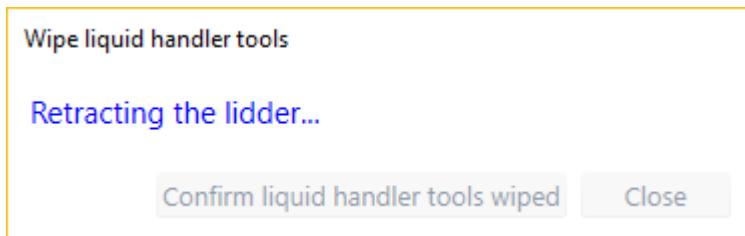


Figure 439 Window while lidder is retracted

- 17) Press **Send the liquid handler to the safe position**.

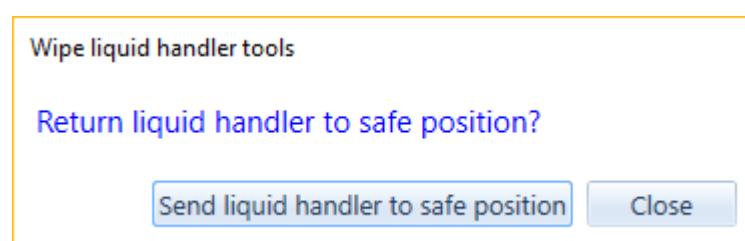


Figure 440 Send liquid handler to safe position dialog

- 18) The robot will move to a safe position

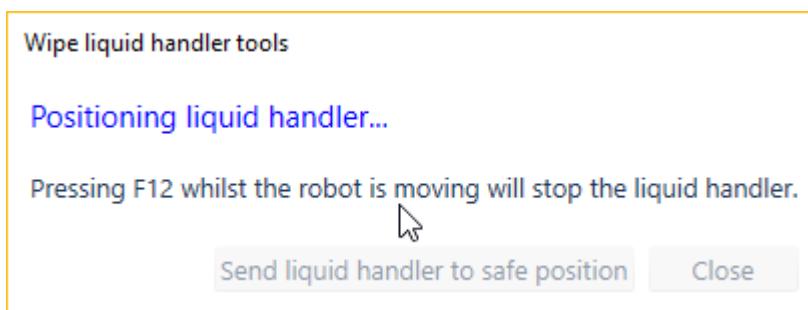


Figure 441 Window while robot is moving to the safe position

- 19) Wait for the liquid handler to move the robot to the safe position.

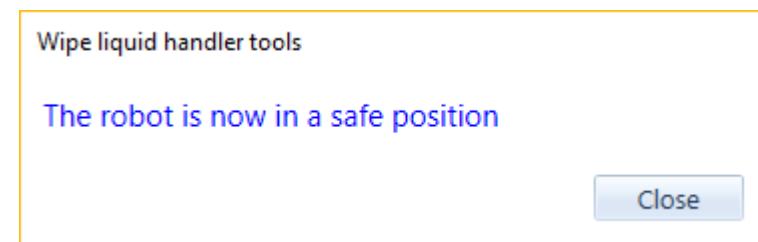


Figure 442 Window when robot is in safe position

- 20) Press **Close**. The dialog will be closed.
- 21) Pressing **Close**. at any point in the sequence will retract the mandrel, gripper or lidder and present the send liquid handler to safe position dialog.

11.1.3 Sterilize in place

Sterilize in place... loads the liquid handling mechanism with hydrogen peroxide to sterilise the system.

- 1) If the liquid handler is running then pause or stop the liquid handler
- 2) Press **Sterilize in place ...** The system displays the **Sterilize in place** window.

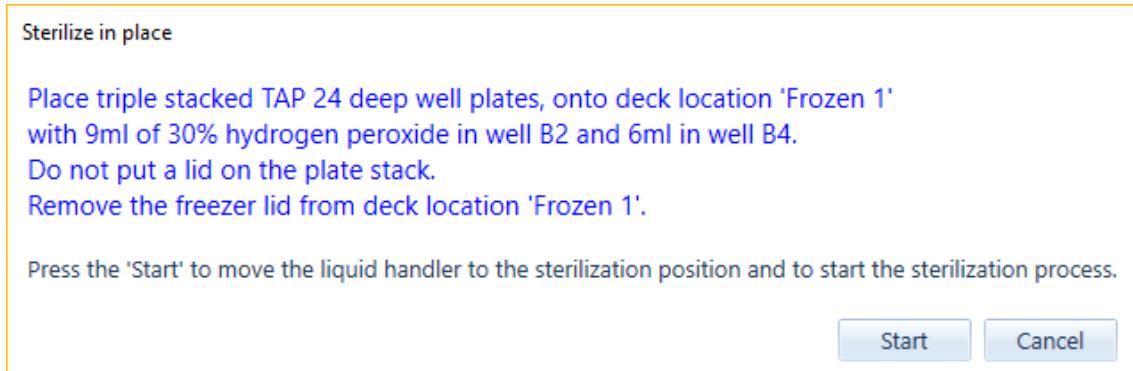
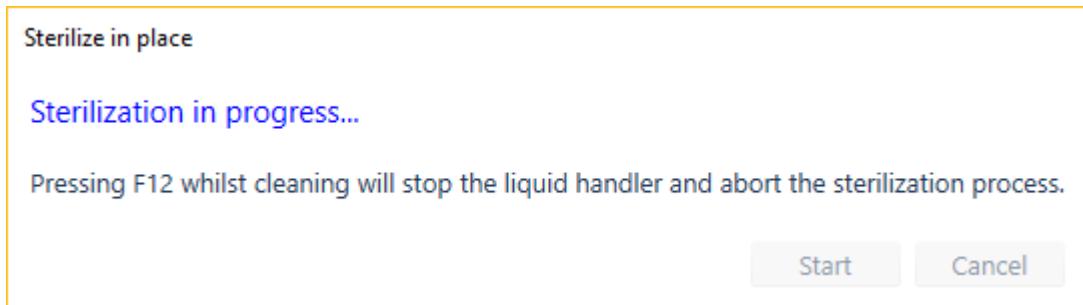


Figure 443 **Sterilize in place** window

- 3) Fill the top plate with hydrogen peroxide and place them into the position specified.
- 4) Press **Start**. The robot will perform the sterilization process.



- 5) Wait for the process to complete.

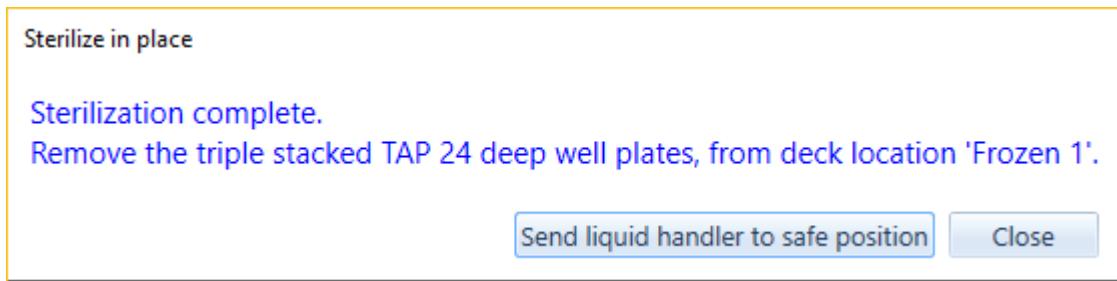


Figure 445 Window while mandrels presented

- 6) Press **Send the liquid handler to the safe position.**



- Figure 446 Window while robot is moving to the safe position
- 7) Wait for the liquid handler to move the robot to the safe position.



- Figure 447 Window when robot is in safe position
- 8) Press **Close**. The dialog will close.

11.2 Pre-run checks

On starting the liquid handler the system will prompt to check newly loaded labware.

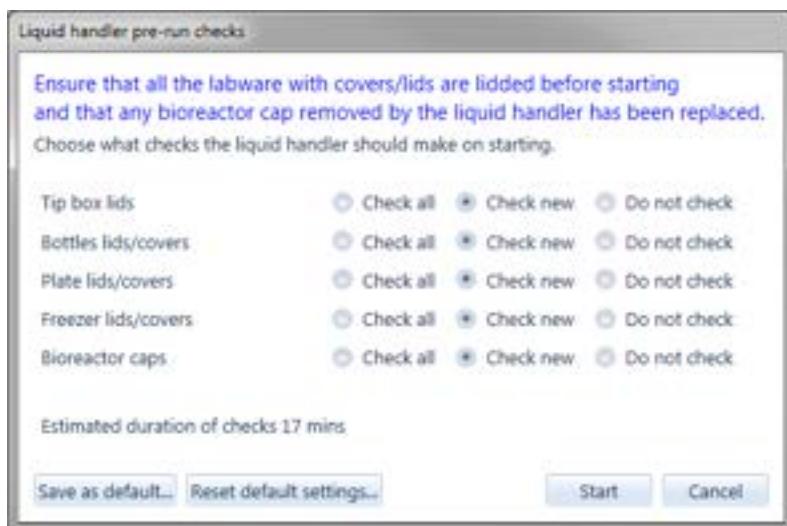


Figure 448 Pre-run checks dialogue

The radio buttons on the dialog allow the user to select the labware items to be checked.

- **Check new** – newly loaded or refilled labware items are checked
- **Check all** – all labware items are checked even if they have previously been checked
- **Do not check** – labware items are not checked

An estimation of the duration the checks will take is displayed. This will vary depending on the number of items to check and if the robot needs initialization.

Save as default... – saves the current selection as the default.

Reset default settings... – restores the current selection to the default selection

Cancel – exits the dialogue without starting the liquid handler.

Start – starts the liquid handler, or if items require checking displays a summary dialogue of the items that will be checked by the liquid handler.

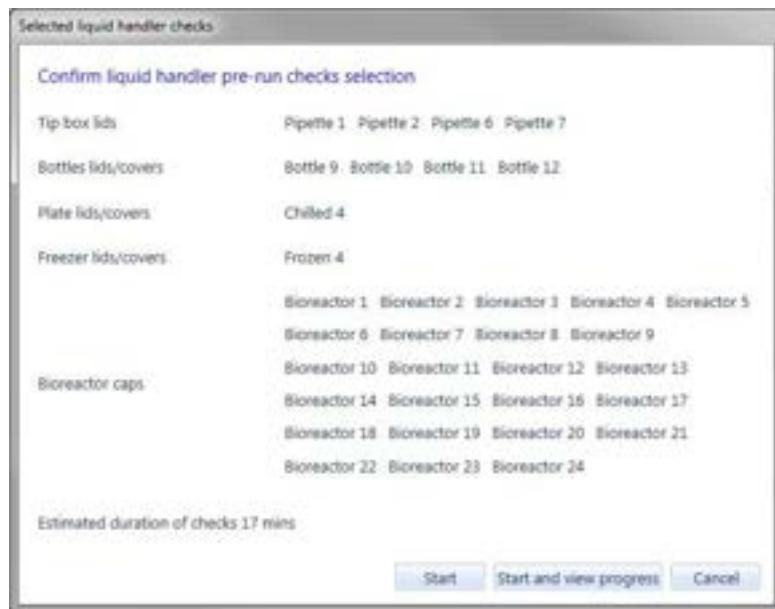


Figure 449 Selected liquid handler checks summary dialogue

Start – starts the liquid handler and adds to the liquid handler queues the labware items that require checks.

Start and view progress – starts the liquid handler and adds to the liquid handler queues the labware items that require checks and displays the **Liquid handler** page so that the queued actions can be viewed.

Cancel – exits the dialogue and returns to the check selection dialogue.

When all checks have been completed, a confirmation dialogue is displayed.

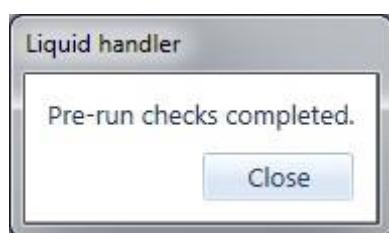


Figure 450 Pre-run checks completed confirmation dialogue.

If e-mails alerts are configured the subscribed user will receive an email that the checks have been successful.

11.2.1 Clearing checked actions

Queued check actions can be cleared from the liquid handler by selecting the **Clear check actions...** button on the Liquid handler page see section 11.3. A confirmation dialogue is displayed.

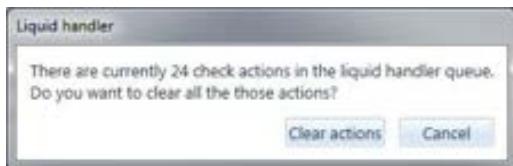


Figure 451 Clear check actions confirmation dialogue

Clear actions – removes all pending check actions from the liquid handler queue

Cancel – closes the dialogue.

11.3 Queued actions

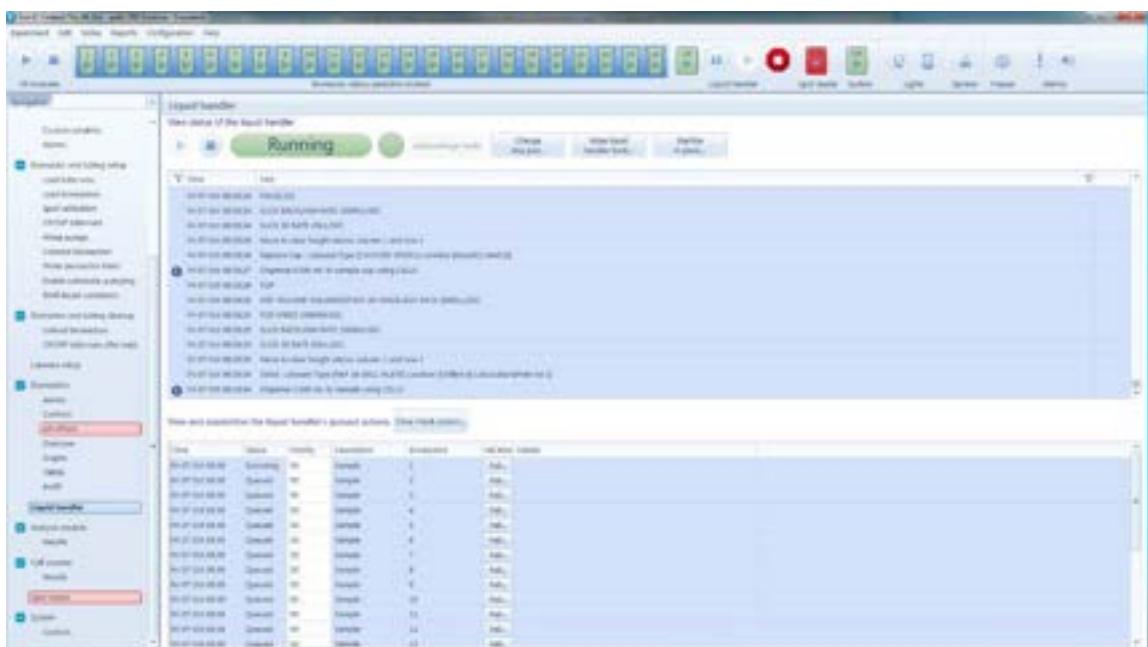


Figure 452 Queued actions page

The page shows each individual action queued for the liquid handler in the order they will be executed. In the example above the first action was given a higher priority after the executing action started.

Time shows when the action was queued.

Status shows:

- **Queued** – the action is in the queue.
- **Executing** – the liquid handler is working on this action
- **Missing resources** – the action cannot be executed because of some condition.

Priority shows the priority of the action. To move an action up or down in the queue edit its **Priority**.

Description shows a brief description of the action.

Bioreactors shows the bioreactors that the action applies to.

Fail item presents the **Fail...** button that fails the action. If the action is associated with a step then the step will be failed for the bioreactors associated with the action. If the action is associated with a control loop the action will queue another action when required.

Details shows more details of why the action cannot be executed.

Clear check actions... button enables pre-run check actions that have not been started to be removed from the liquid handler queue.

11.4 Maintenance

The Maintenance page provides facilities for testing and teaching the liquid handler.

Incorrect use of this page can cause mechanical damage to the Ambr® 250 system. Access to the pages is protected by a password and the pages should only be used by competent maintenance staff.



Figure 453 Maintenance page

11.5 Dead volumes

The dead volume in a piece of labware is the volume of liquid that cannot be removed from a bottle or from a well in the plate because the pipette tips cannot reach the absolute bottom of any labware.

11.5.1 Default dead volumes

New systems shipping with R7 or above have dead volumes defined for the standard bottle types.

To change an existing system to take account of the dead volumes when aspirating from labware enter appropriate values for the dead volumes using the geometry wizard.

The default values recommended are:

1Litre bottle with flea = 115ml

1Litre bottle no flea = 40ml

175ml bottle with flea = 27ml

175ml bottle no flea = 11ml

Defining the dead volume:

- Affects the bottles created by the wizards that populate bottles with the dead volume in addition to the volume that will be used by the process.
- Changes the threshold where the system will consider that there is not enough liquid left in a bottle.

11.5.2 Editing dead volumes

Dead volumes for labware are edited from the **Geometry** tab in the **Liquid handler\Maintenance** page.

Selecting the edit button  on the labware summary table allows the user to edit the dead volume.

For custom labware additional pages are displayed. For standard labware only the dead volume can be edited.

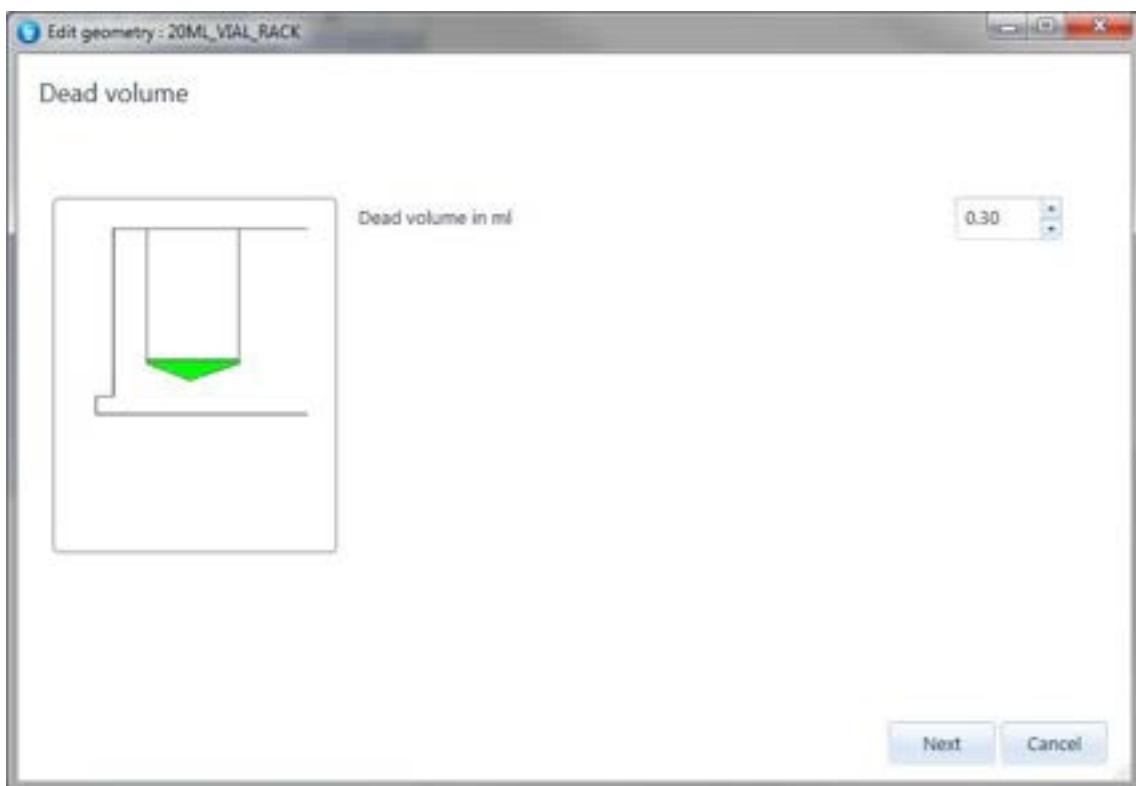


Figure 454 Dead volume page

Edit the dead volume and then press **Next** to review the geometry that will be saved.

11.6 Liquid handler errors

When in operation the liquid handler detects an error condition, if for example the labware is missing a lid, one or more dialogs will be displayed.

If the liquid handler has an automatic recovery behaviour for the problem then a dialog like the one below will be displayed.

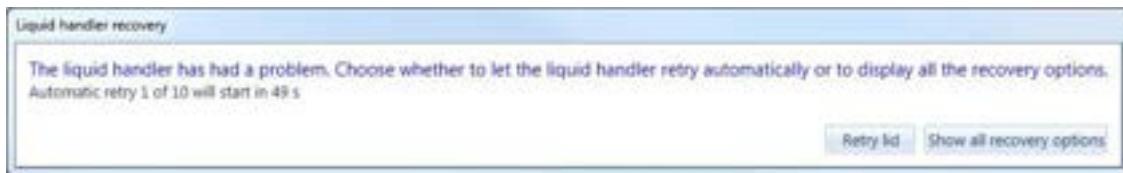


Figure 455 Automatic retry dialog

If no automatic option is applicable or the user has chosen **Show all recovery options** then the **Liquid handler error** dialog will be displayed.



Figure 456 Liquid handler error dialog

The information box at the top of the dialog explains the fault. Depending on the nature of the error one or more of the following actions will be available:

- 1) Manually perform the failed operation. In this case the user performs the operation that would have been done by the robot.
- 2) Let the liquid handler robot retry the operation. If selected the robot will attempt to perform the failed operation again.
- 3) Pause the liquid handling. Pauses the liquid handler operation at the next appropriate point. The problem has not been solved as user intervention is still required to correct the issue.
- 4) Abandon the liquid handling. The current operation is aborted. Manual handing will be required. When restarted the liquid handler will perform a full initialisation.



Figure 457 Abandon liquid handler operation confirmation dialog

The dialogs buttons and text change appropriately dependant on the operation being performed.

In cases where the robot cannot retry the operation, e.g. a tip has not been detected on the mandrel only the pause and abandon liquid handler options are available.



Figure 458 Liquid handler error showing only the pause and abandon options.

12 ANALYSIS MODULE

12.1 Analysis module page

The **Analysis module** page is used to tell the analysis module (ambrAM) when reagents are replaced and to monitor the status of the ambrAM.

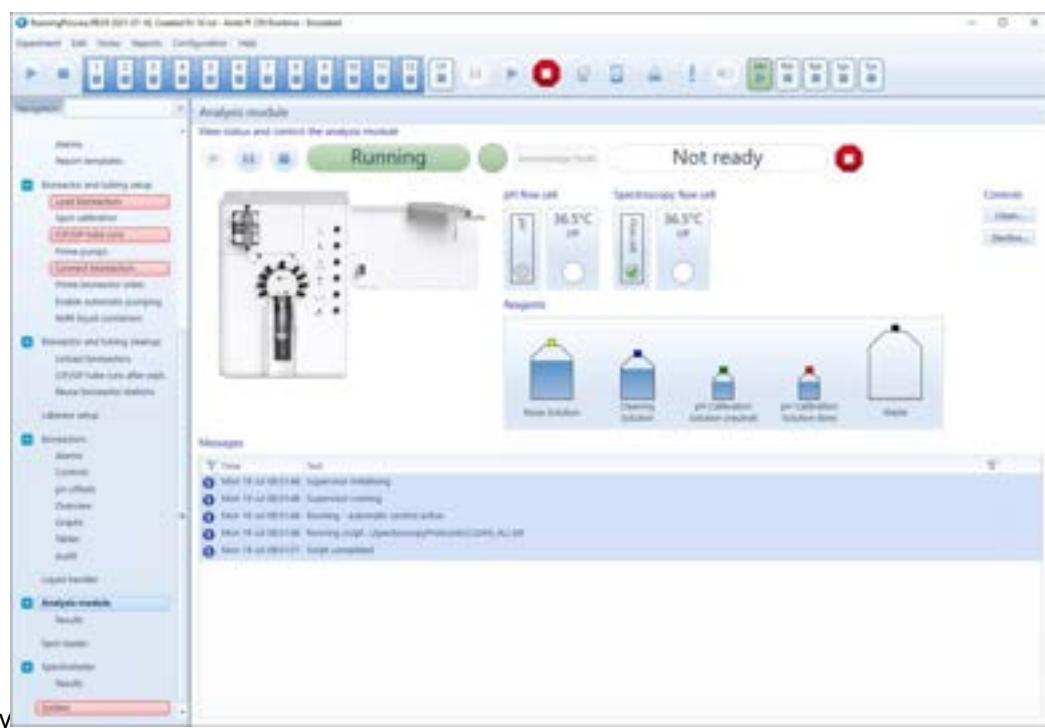


Figure 459 Analysis module page

The controls on the top row are described below.

	Starts the module running. The module is then free to calibrate itself as required and to analyse samples.
	Pauses the module so that reagents or sensors can be changed.
	Stops the module.
	Display of the overall status of the module
	Indication of any faults that have occurred with the module and control to acknowledge those faults.
	Display of the state of the module and what it is doing. Indicates when the module is calibrating, reading ...
	The quick stop button provides a way to interrupt the full pH calibration process.

12.1.1 Cleaning and sterilisation

The **Clean** button runs a cleaning cycle on the analysis module.

The **Sterilise** button can be used to perform additional sterilising or cleaning of the analysis module.

A prompt is displayed to place the appropriate liquid in the sample cup. Refer to the user manual and other documentation for appropriate cleaning liquids.

12.1.2 Temperature



Figure 460 Temperature indication

The Temperature indication shows the desired and actual temperature of the pH sensor.

The status indicates how the temperature is being controlled:

	The system is not controlling the temperature of the pH sensor at this time.
	The system is waiting for the pH sensor to reach the requested temperature.
	The analysis module is at the requested temperature.
	There is a problem with the temperature control.

Click on the indication to change the temperature of the pH sensor. The **Set analysis module temperature** dialog shown below is displayed.

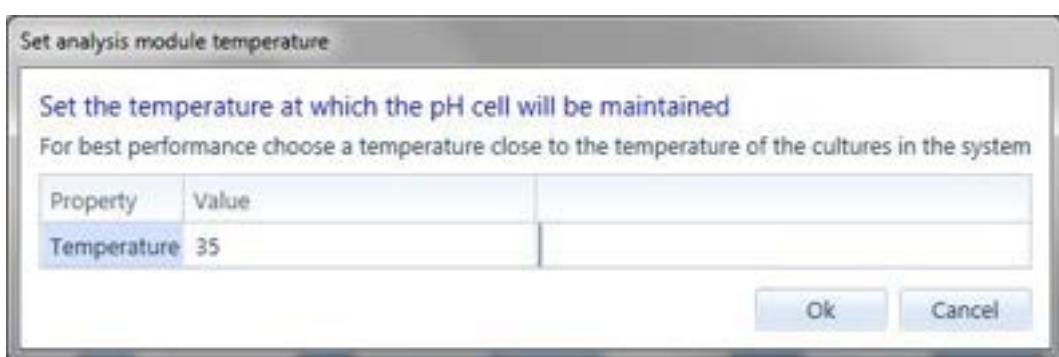


Figure 461 Set analysis module temperature dialog

If a spectrometer is fitted then a second temperature control is displayed. Click on the indication to change the temperature of the spectroscopy flow cell. The **Set spectrometer flow cell temperature** dialog is shown.

12.1.3 Sensors

There is an indicator with the state of each of the analysis sensors in the module.



Figure 462 pH and spectroscopy flow cell sensor status indicators

The status indication shows:

	The sensor needs to be replaced.
	Calibration of the sensor has failed.
	The sensor has not been calibrated.
	The sensor has been successfully calibrated.
	The sensor status is not known.

Click on the indicator to see more details.



Figure 463 pH sensor status

Sensor changed indicates when the sensor was last replaced.

Replacement due indicates when replacement of the sensor is due.

Click on **Replace** to replace the sensor.

Calibrated and **Quick calibrated** indicate when the sensor was last calibrated.

Calibrate performs a full calibration of the sensor.

Check performs a check calibration of the sensor reading a known liquid and calibrating the sensor if the reading for that liquid is out of range.

Quick calibrate performs a quick calibration of the sensor.

The Quick calibration options only apply to sensors where the full calibration is particularly lengthy.

12.1.4 Reagents

The reagents section shows an indication of the reagents loaded.



Figure 464 Reagents section

Below each reagent the system indicates when the reagent needs to be replaced either because the system is predicted to run out of the reagent or because the reagent is reaching its expiry date.

Click on the reagents section to see replace bottles as required.

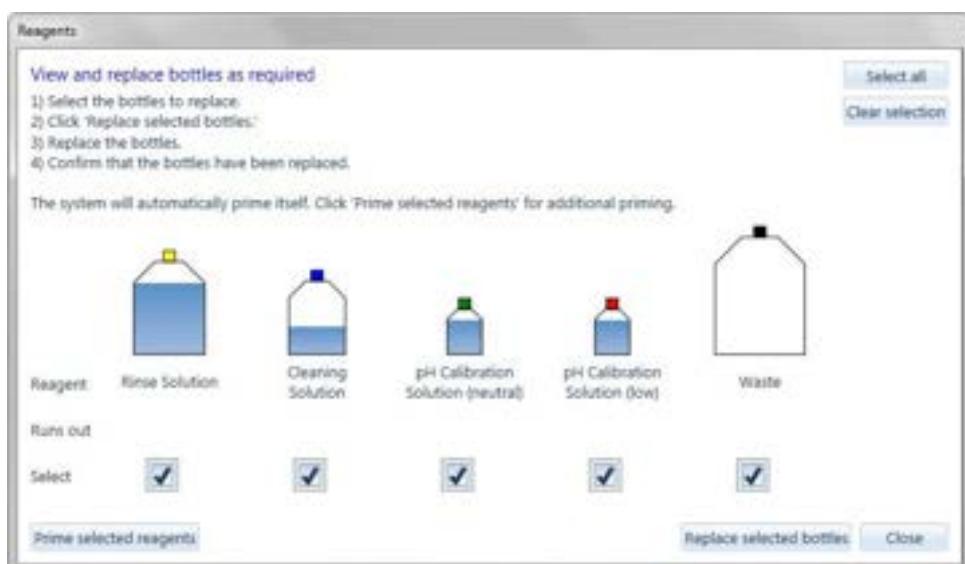


Figure 465 Reagents dialog

Select using the checkboxes which bottles will be replaced.

Select all selects all the bottles.

Clear selection clears all the checkboxes.

Having selected the right bottles, Click **Replace selected bottles**

The system displays a dialog to confirm which bottles are being replaced.

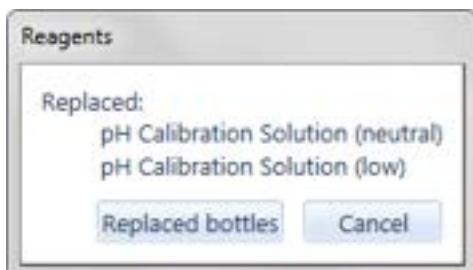


Figure 466 Confirmation of which bottles are being replaced.

Once the bottles have been replaced click **Replaced bottles**.

The system will automatically prime the reagents lines as required. **Select Prime selected reagents** to perform additional priming.

12.2 Analysis module results page

The analysis module results page shows the results from the analysis module.

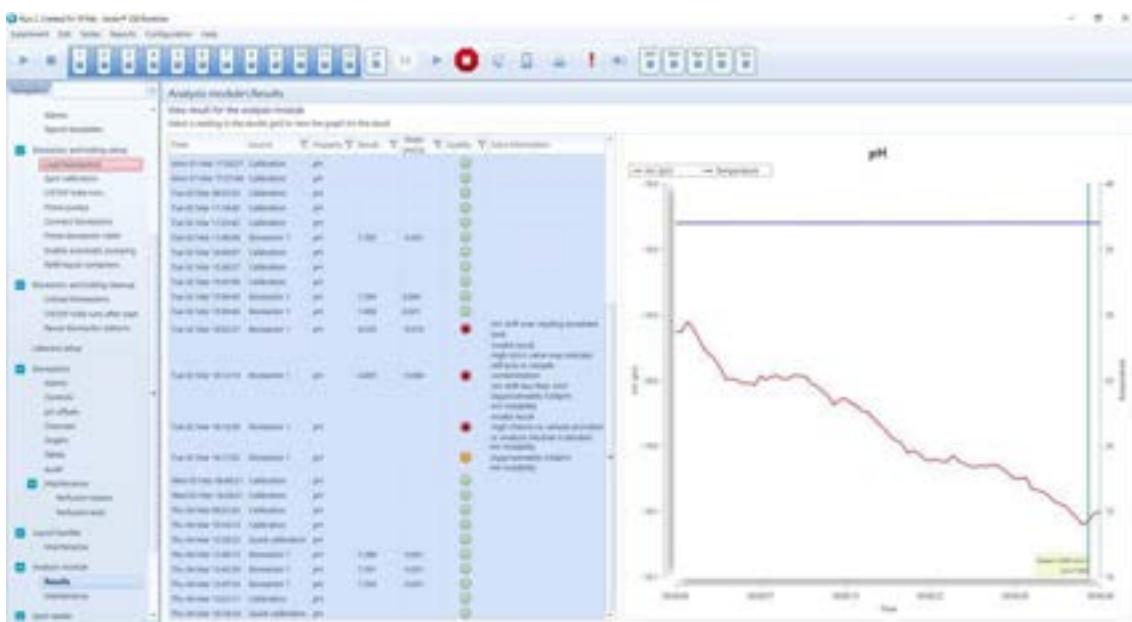


Figure 467 Analysis module results

Click on a result to see the traces associated with that result.

For each result the page shows:

- The **Time** the result was taken.
- The **Source** of the sample for the result.

- The **Property** measured. If multiple properties are measured from the same sample then one line is shown for each property.
- The **Result** of the measurement.
- The Slope in (mV/s) of the measurement
- A **Quality** traffic light for the the measurement.
- The **Measurement mode** that the reading was taken with.
- Any **Extra information** available.

12.3 Maintenance

The Maintenance page provides facilities for testing the analysis module.

Incorrect use of this page can cause damage to the Ambr® 250 system. Access to the pages is protected by a password and the pages should only be used by competent maintenance staff.

See the Maintenance manual for details of how to use the Maintenance page.

13 PH STATION

The **pH station** page allows direct control of the pH station as well as monitoring the status of the station.

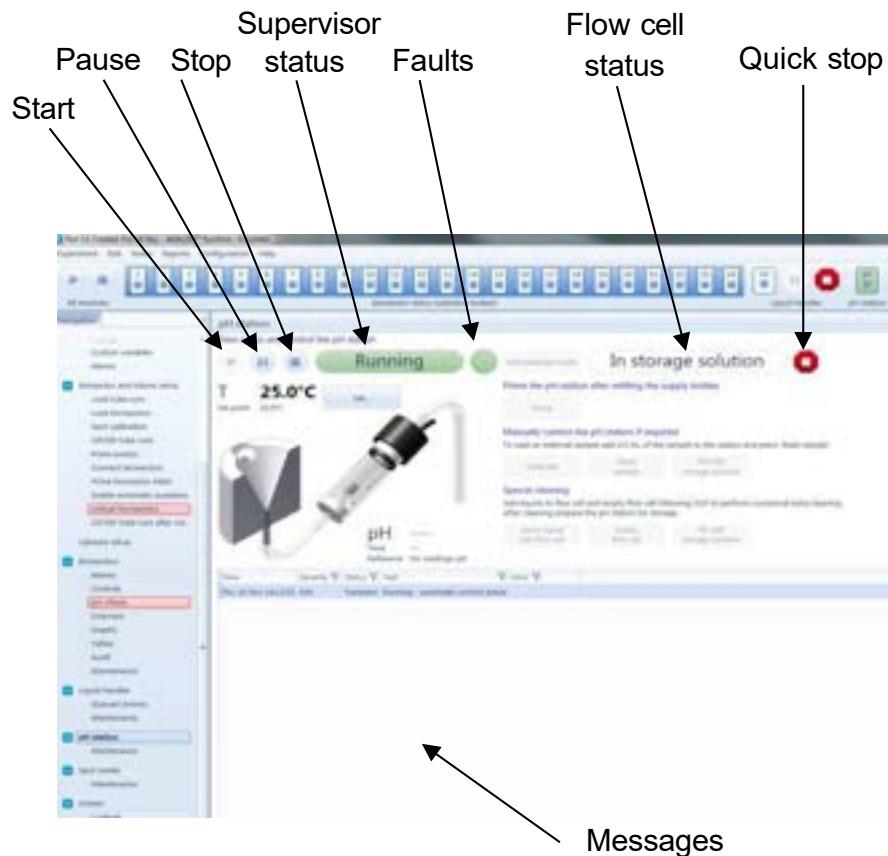


Figure 468 **pH station** page

The main statuses of the pH station are:

- In storage solution – the flow cell is filled with storage solution. The flow cell should be left in this state when it is not being used.
- Calibrated – the flow cell has been calibrated ready for use.
- Not in storage solution – for some reason the flow cell has ended up neither in storage solution nor calibrated. The flow cell should be placed in storage solution as soon as convenient.

When the station is running the system will automatically calibrate the station when a sample step is coming up, and will automatically fill the flow cell with storage solution when no sample steps are coming up or if the station has not been used for some time.

Calibration and readings are only performed when the temperature of the flow cell is at or above the set point.

13.1 Starting and stopping station

Start starts automatic control of the pH station.

Stop stops automatic control of the pH station and fills the flow cell with storage solution so that the flow cell can be left for an extended interval.

Pause stops automatic control of the pH station without filling the flow cell with storage solution. If the station was calibrated it can be used to measure an external sample.

Quick stop stops the flow cell operating as quickly as possible. The quick stop feature provides a method to stop the pumps inside the station should there be a leak.

13.2 Priming

After replenishing the buffers and other solutions in the bottles attached to the pH station press **Prime** to prime the lines from the bottles.

13.3 Manual control

Calibrate runs the calibration procedure for the station.

Read sample can be used to take a reading of an external sample.

Fill with storage solution fills the station with storage solution ready to be left idle.

13.4 Special cleaning

Options **Move liquid into flow cell** and **Empty flow cell** can be used to perform occasional cleaning of the flow cell using a suitable SOP.

Fill with storage solution fills the station with storage solution ready to be left idle.

14 CELL COUNTER

The **cell counter** page provides controls for starting, stopping and pausing the cell counter; viewing messages and acknowledging faults.

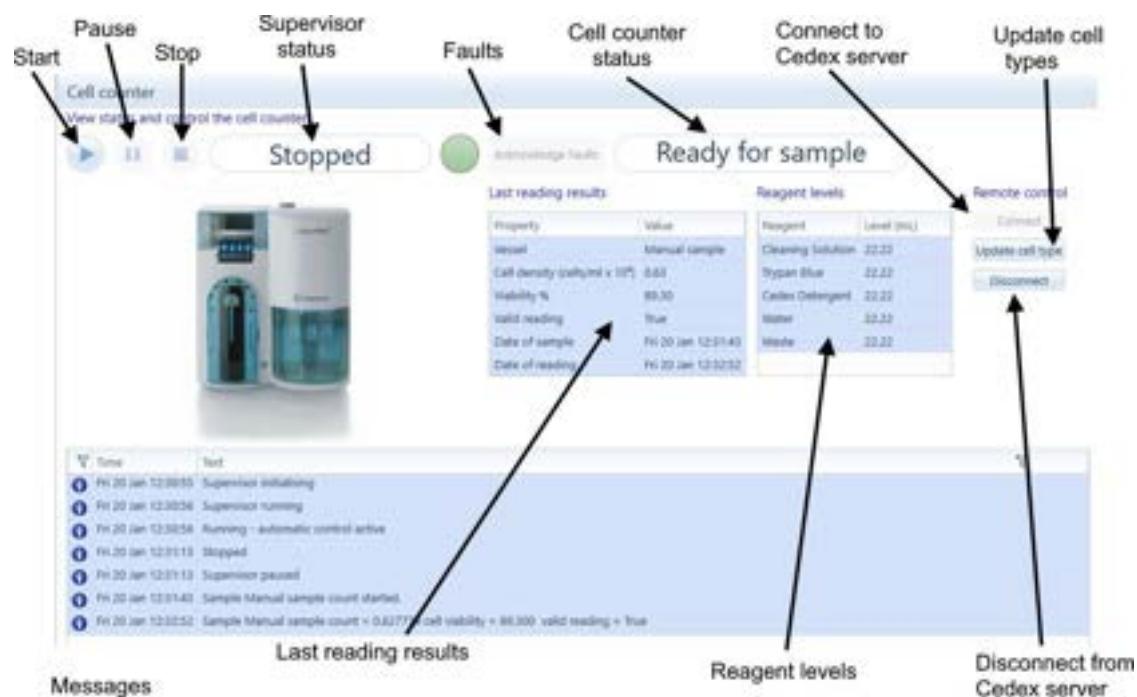


Figure 469 cell counter page for Cedex RC2

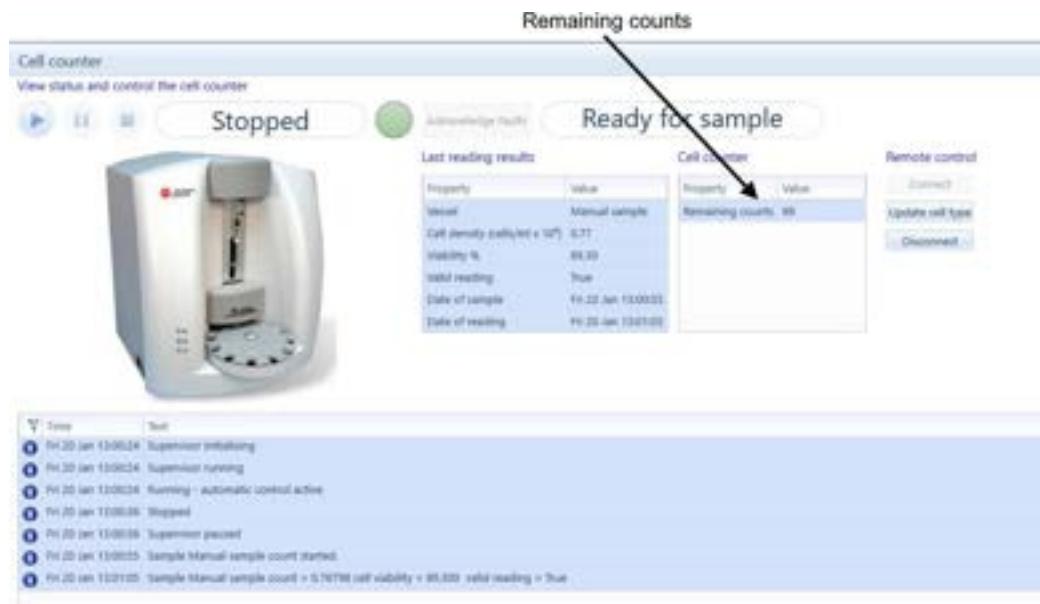


Figure 470 cell counter page for Vicell

14.1 Starting and stopping cell counter

Start starts automatic control of the cell counter.

Stop stops automatic control of the cell counter and performs a liquid management shutdown to allow the counter to be left for an extended period of time.

Pause stops automatic control of the cell counter. Allows the user to control the cell counter from the maintenance screen to perform maintenance operations or a manual cell count.

Acknowledge faults acknowledges any faults.

The **Messages** area shows messages about what the cell counter is doing.

Connect manually connect to the cell counter

Disconnect manually disconnects from cell counter.

Update cell type updates the cell type definitions from the cell counter.

14.2 Cell counter results

The **Cell counter\Results** page summarises the results for the cell counts performed by the system.

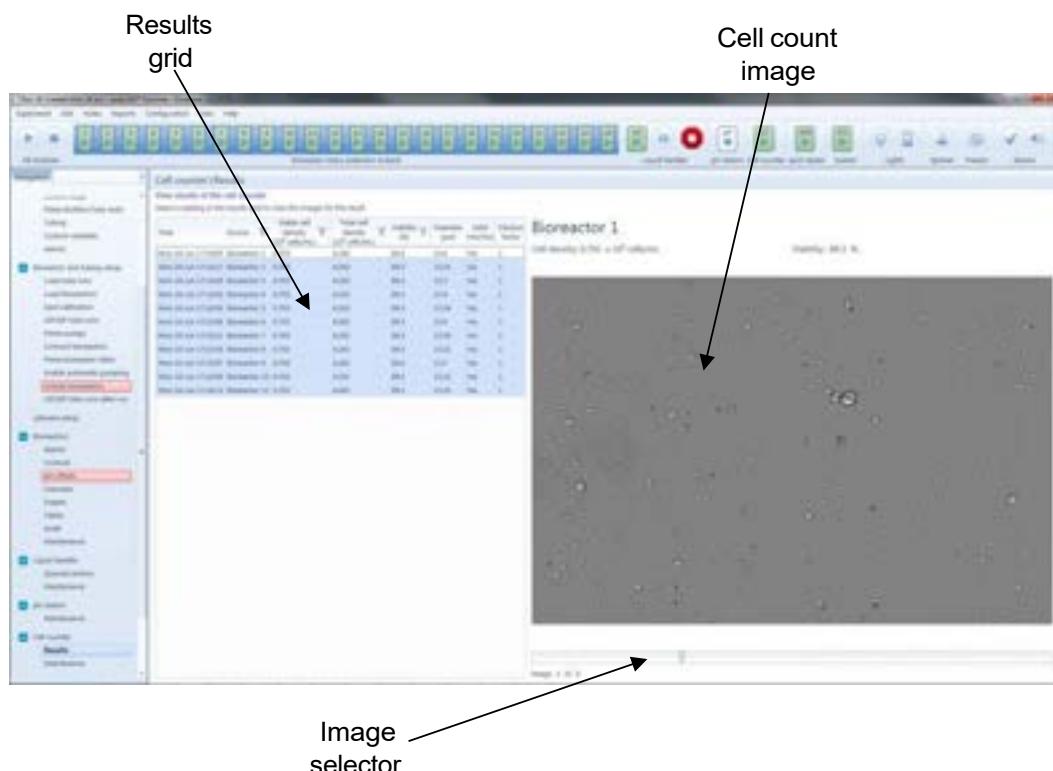


Figure 471 cell counter results

Each row of the results grid summarises the result of a cell count displaying the Cell density, Total cell density, Viability and Diameter of the cell in the sample and the dilution factor and validity of the sample as determined by the cell counter.

Selecting an individual row from the grid displays the images used to determine the readings. These images can be browsed by using the image slider at the bottom of the screen or the left and right arrow keys.

14.3 Maintenance

The **Cell counter\Maintenance** page provides facilities for testing and maintaining the cell counter.

The cell counter supervisor must be paused or stopped in order to do maintenance or a manual cell count.

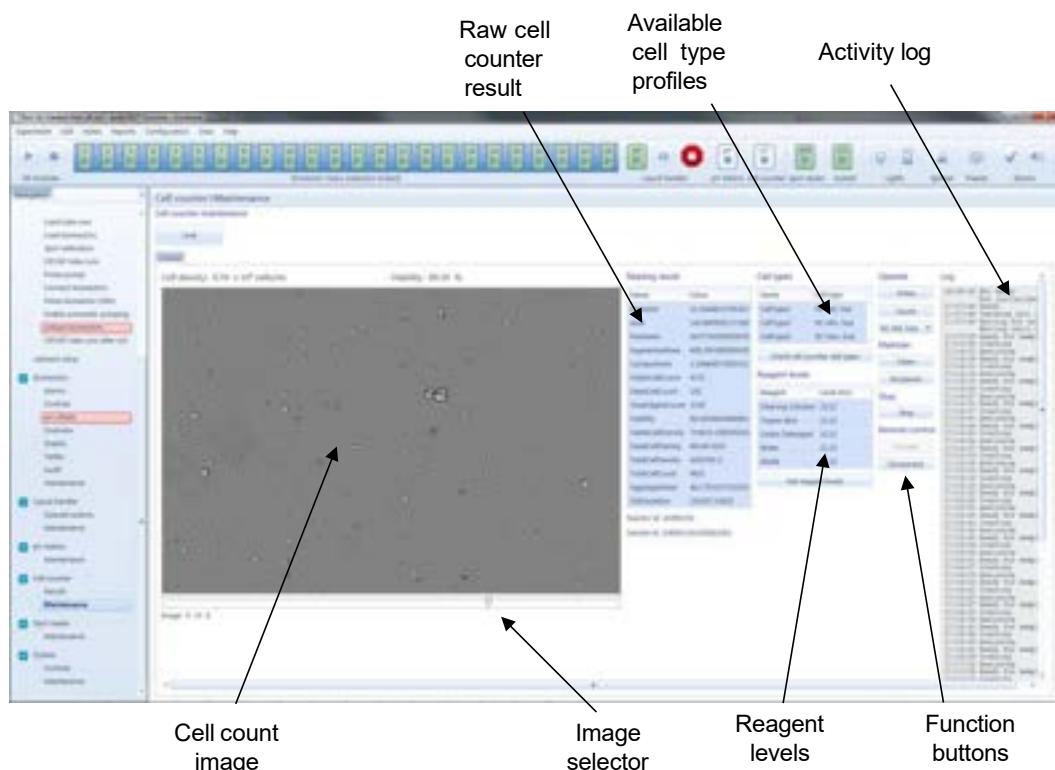


Figure 472 cell counter maintenance Cedex RC2

The page displays the results of last cell count and provides a number of maintenance functions.

Check cell counter cell types checks that the cell counter and the systems cell type configurations match. Allows the system configuration to be updated.

Get reagent levels interrogates the cell counter for its current liquid levels (Cedex only).

Get remaining counts interrogates the cell counter for the remaining number of counts (Vicell only).

Prime prepares the cell counter for a cell count, if necessary by priming the reagents.

Count performs a manual cell count.

Cell type selector select the cell type profile when doing a manual cell count.

Clean performs a clean cycle on the cell counter.

Shutdown performs a liquid management shutdown on the cell counter.

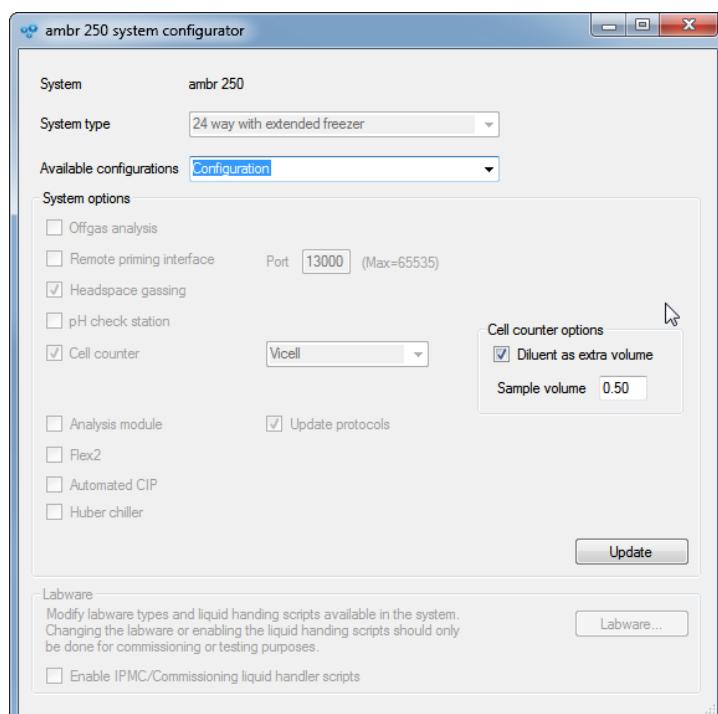
Stop aborts the current cell count as soon as possible.

Connect allows remote control of the cell counter.

Disconnect disconnects the Ambr® 250 software from the cell counter to allow the counter to be controlled directly from its control PC.

14.4 Configuration options

Cell counter options can be configured using the **ambr250 System Configurator** application. This application can be found on the **ambr 250 utilities** folder on the desktop.



The cell counter sample volume and diluent options can be changed. For systems with Vicell cell counters the default volume is 0.5 ml with the diluent added as extra volume. For Cedex cell counters the Sample volume should be 0.3 ml with the diluent not being added as extra volume. Cedex cell counters require a total sample volume 0.3 ml for accuracy as they do not cut the sample to the required volume.

15 FLEX2

The **Flex2** page provides controls for starting, stopping and pausing the Flex2 supervisor; viewing messages and acknowledging faults.

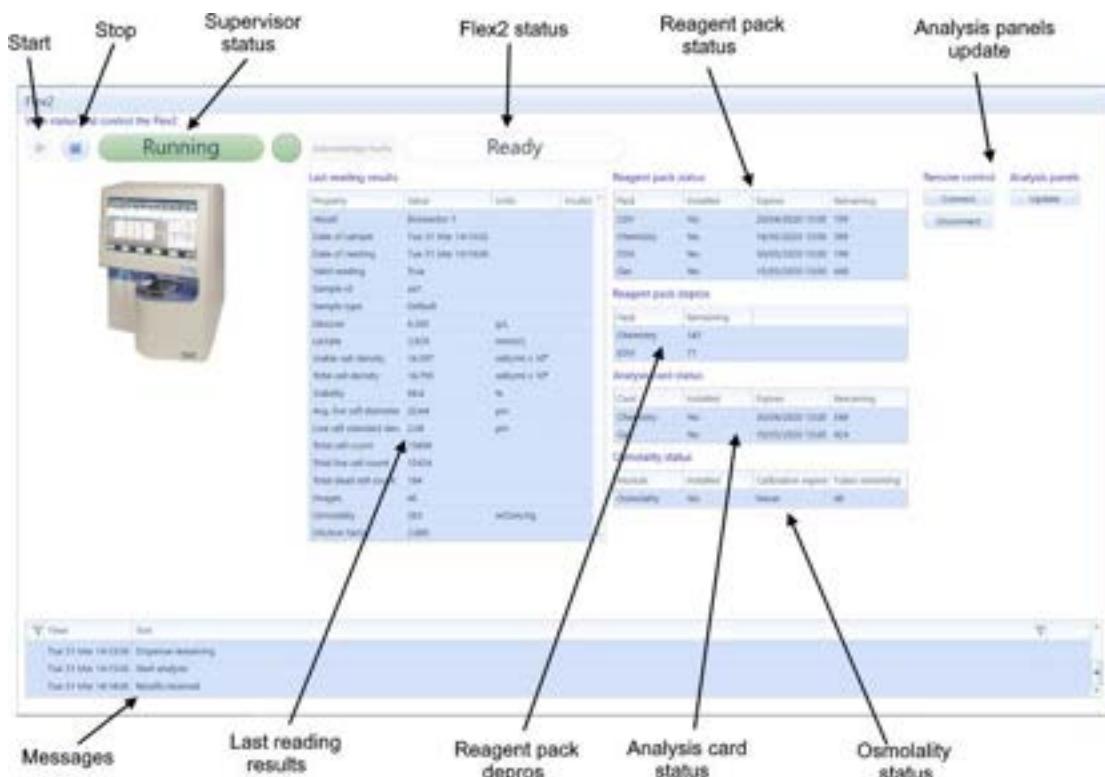


Figure 473 Flex2 page

15.1 Starting and stopping the Flex2

Start starts automatic control of the Flex2.

Stop stops automatic control of the Flex2. If the Flex2 is analysing a sample, stopping automatic control will attempt to abort the analysis.

Acknowledge faults acknowledges any faults.

The **Messages** area shows messages about what the Flex2 is doing.

15.2 Analysis panel configuration

The Flex2 supervisor automatically updates the analysis panels when the supervisor is started.

The analysis panel and status of the Flex2 can be updated manually when the supervisor is not running by using the buttons on the right hand side of the Flex2 page. These are also used when first installing the Flex2 to check the communications and that analysis panels suitable for the Ambr® 250 sampling have been configured within the Flex2.

Remote control Analysis panels

Connect

Update

Disconnect

Figure 474 Flex2 page remote control buttons

- The **Connect** button establishes communication to the Flex2 and fetches the current status of the reagent packs and cards.
- The **Disconnect** button stops communications to the Flex2.
- The **Update** button updates the Ambr® 250 configuration with the analysis panels defined on the Flex2 that are compatible with the Ambr® 250 sampling.

15.3 Flex2 results

The **Flex2\Results** page summarises the results for the analyses performed by the Flex2.

The screenshot shows the 'Flex2\Results' page. On the left is a navigation sidebar with various tabs and sections like 'Reagent and tubing setup', 'Biosafety and tubing cleaning', 'Assays', 'Liquid handling', 'Fuels', and 'Help'. The main area has a title 'Flex2\Results' and a sub-instruction 'Select the Result - reading off the result grid to view the full chart/images on the result'. Below this is a table with columns 'Time', 'Source', 'Y', 'Measure', 'Value', 'Unit', and 'Y scale Y scale'. The table lists numerous rows of data. To the right of the table is a large rectangular area labeled 'Bioreactor 1' with a sub-label 'Health and Activity'. This area contains the text 'No image available' and a progress bar at the bottom labeled 'Image 1 of 1' and '0 images for use / 0 images available'.

Figure 475 Flex2\Results page

For each result the page shows:

- The **Time** the result was taken.
- The **Source** of the sample for the result.

- The **Property** measured. If multiple properties are measured from the same sample then one line is shown for each property.
- The **Result** of the measurement.
- The **Units** of the measurement.
- An indication if the measurement was deemed to be **Invalid**.
- Any **Extra information** available.

Selecting a result table row that contains the **Property Images** will display the image results for the associated sample

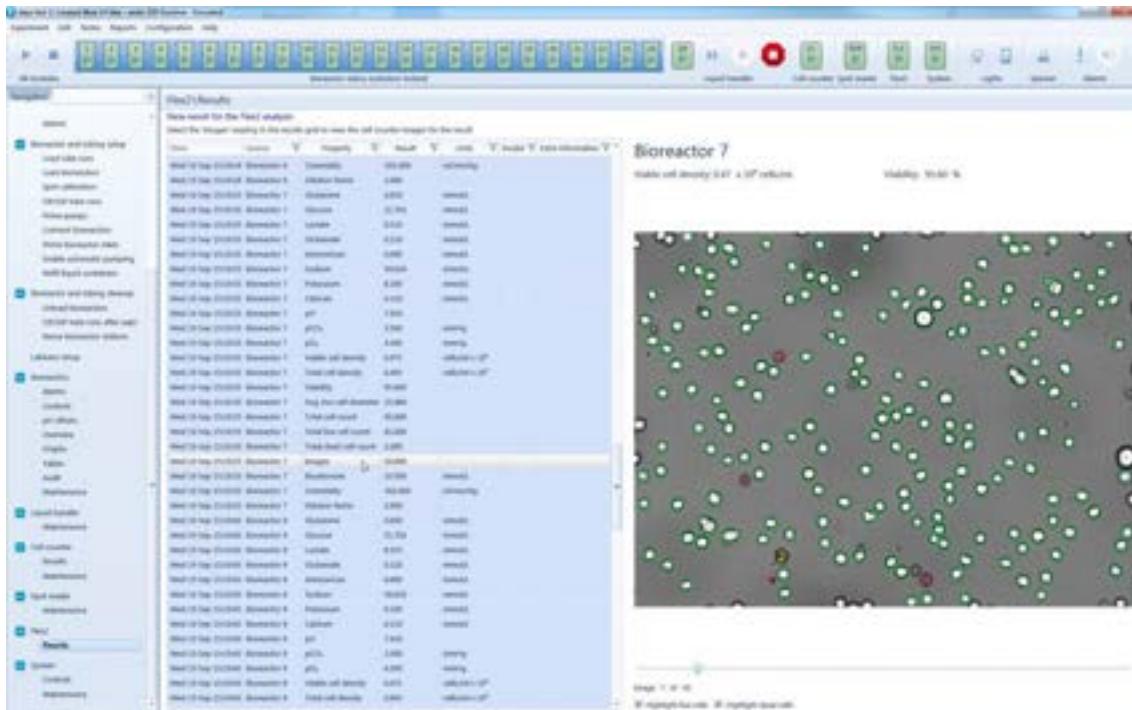


Figure 476 Flex2\Results images

Note. Images are only available in the runtime software and are not exported with experiment summaries.

- Images can be scrolled by using the arrow keys or the image selector slider.
- Live cells can be highlighted by checking the **Highlight live cells** checkbox. Live cells are annotated with a green circle
- Dead cells can be highlighted by checking the **Highlight dead cells** checkbox. Dead cells are annotated with a red cross . Multiple dead cell clusters are annotated in yellow with the number of cells

15.4 Results display units configuration

The units for the results of Flex 2 analysis recorded in the Ambr® 250 data can be configured from ambr250 configuration tool. If values returned from the Flex 2 analysis are not in the same units as have been configured they are converted into in the configured units before being recorded.

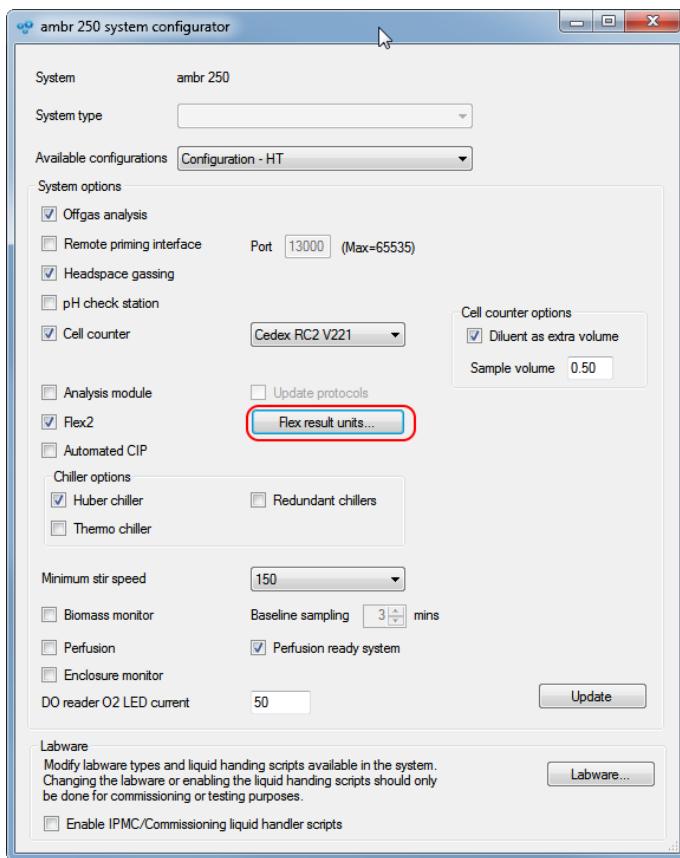


Figure 477 Configuration application units button

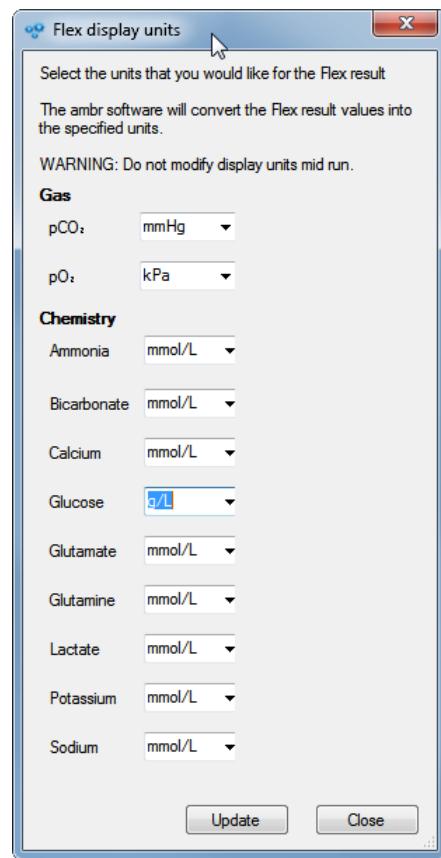


Figure 431 Flex units selection screen

- Units for the Gas results, pCO₂ and pO₂, can be either mmHg or kPa.
- Units for chemistry results can be either mmol/L or g/L/

16 SPECTROMETER

The **Spectrometer** page provides controls for starting, and stopping the Spectrometer supervisor; viewing messages and acknowledging faults.

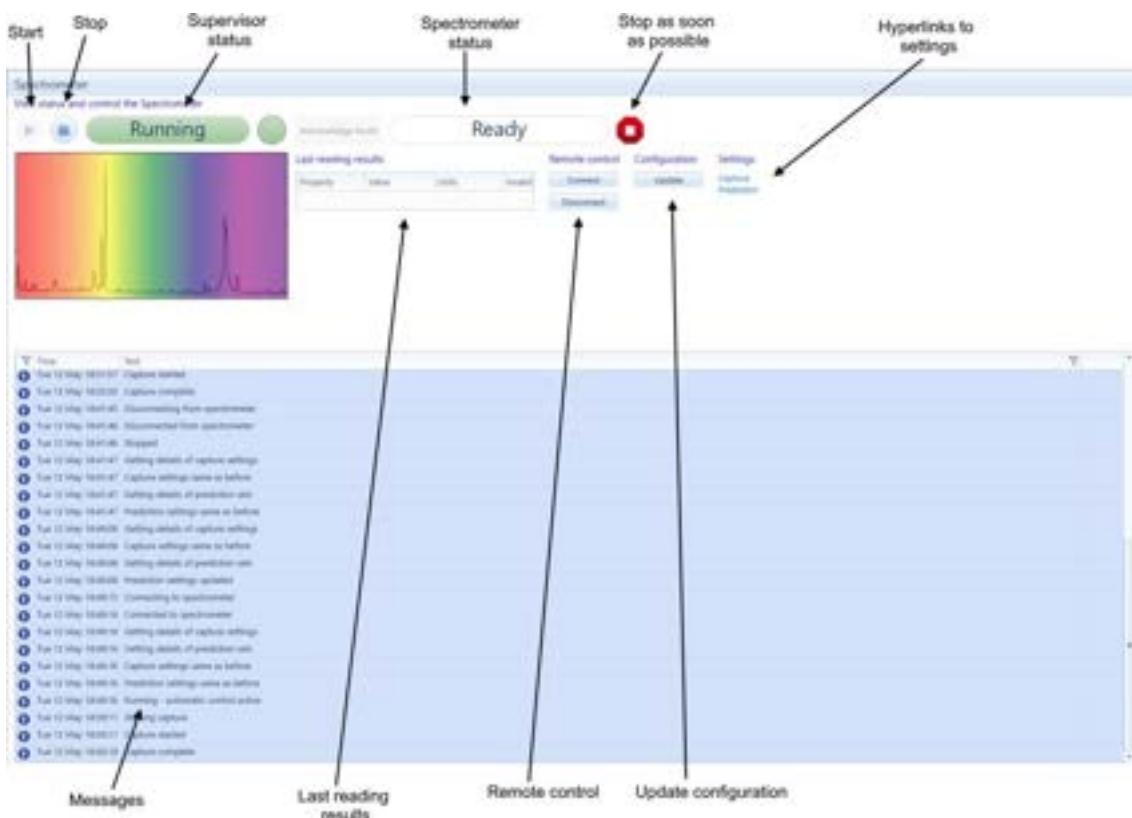


Figure 478 Spectrometer page

16.1 Starting and stopping the Spectrometer

Start starts automatic control of the Spectrometer.

Stop stops automatic control of the Spectrometer

Acknowledge faults acknowledges any faults.

The **Messages** area shows messages about what the Spectrometer is doing.

 **Stop as soon as possible** button posts as confirmation dialog and if confirmed stops whatever the spectrometer is doing as soon as possible.

16.2 Spectrometer configuration

The Spectrometer supervisor automatically updates the configuration of the capture and prediction settings when the supervisor is started.

The capture and prediction settings of the spectrometer can be updated manually when the supervisor is not running by using the buttons on the right hand side of the spectrometer page. These are also used when first installing the spectrometer to check the communications and that some capture settings have been configured on the spectrometer server.

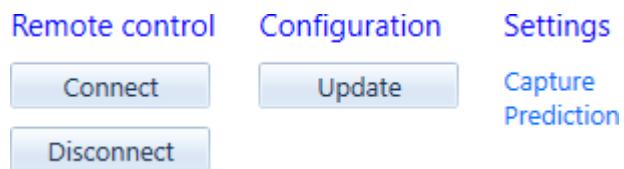


Figure 479 Spectrometer page remote control buttons

- The **Connect** button establishes communication to the spectrometer server.
- The **Disconnect** button stops communications to the spectrometer server.
- The **Update** button updates the Ambr® 250 configuration with the capture and prediction settings that have been defined on the Spectrometer server.
- The **Capture** hyperlink opens the capture settings webpage of the spectrometer server.
- The **Prediction** hyperlink opens the prediction settings webpage of the spectrometer server

16.3 Spectrometer results

The **Spectrometer\Results** page summarises the results for the captures performed by the spectrometer.

Spectrometer\Results									
View results from the spectrometer									
Time	Source	Property	Value	Result	Result Device	Result Category	Comments	Result	Result
Tue 12 Aug 16:00:00	Biosensor 1	Urea	653.10	Result	Result Device value = 653.1 Result Device reading = 12 - 36 Result Device Biosensor 1 = 12 - 36	Result		Result Device value = 653.1 Result Device reading = 12 - 36 Result Device Biosensor 1 = 12 - 36	Result
Tue 12 Aug 16:00:00	Biosensor 1	Carboxy	4021.40	Result	Result Device value = 4021.4 Result Device reading = 12 - 36 Result Device Biosensor 1 = 12 - 36	Result		Result Device value = 4021.4 Result Device reading = 12 - 36 Result Device Biosensor 1 = 12 - 36	Result
Tue 12 Aug 16:00:00	Biosensor 1	Urea	175.00	Result	Result Device value = 175.0 Result Device reading = 12 - 36 Result Device Biosensor 1 = 12 - 36	Result		Result Device value = 175.0 Result Device reading = 12 - 36 Result Device Biosensor 1 = 12 - 36	Result
Tue 12 Aug 16:00:00	Biosensor 1	Carboxy	19106.40	Result	Result Device value = 19106.4 Result Device reading = 12 - 36 Result Device Biosensor 1 = 12 - 36	Result		Result Device value = 19106.4 Result Device reading = 12 - 36 Result Device Biosensor 1 = 12 - 36	Result

Figure 480 Spectrometer\Results page

For each result the page shows:

- The **Time** the result was taken.
- The **Source** of the sample for the result.

- The **Property** measured. If multiple properties are measured from the same sample then one line is shown for each property.
- The **Result** the predicted value of the measurement.
- The **Result (lower)** the predicted value lower limit of the measurement.
- The **Result (upper)** the predicted value upper limit of the measurement.
- The **Units** of the measurement.
- An indication if the measurement was deemed to be **Invalid**.
- Any **Extra information** available.

Hovering over a result in the table shows a summary of the result

Tue 12 May 18:50:19	Bioreactor 1	Lactate	59599.430
	Source=Bioreactor 1		
	ReadingType=Glucose		
	isValidReading=N		
	ExtraInfo=Result invalid: Value < 0.00		
	CaptureId=30		
	ModelSet=testbig		
	Panel=test		
	ProbabilityThreshold=0.9500		
	PredictedValue=-210.45		
	PredictedValueLowerLimit=NaN		
	PredictedValueUpperLimit=NaN		
	HotellingsT2=7.1E+04		
	HotellingsT2Criterion=1E+05		
	DModXPSCombined=4.1E+02		
	DModXPSCombinedCriterion=5E+04		

Figure 481 Hover over summary for spectroscopy result

17 BIOMASS MONITOR

The **Biomass monitor** page provides controls for starting, stopping and pausing the biomass monitor supervisor; viewing messages and acknowledging faults.

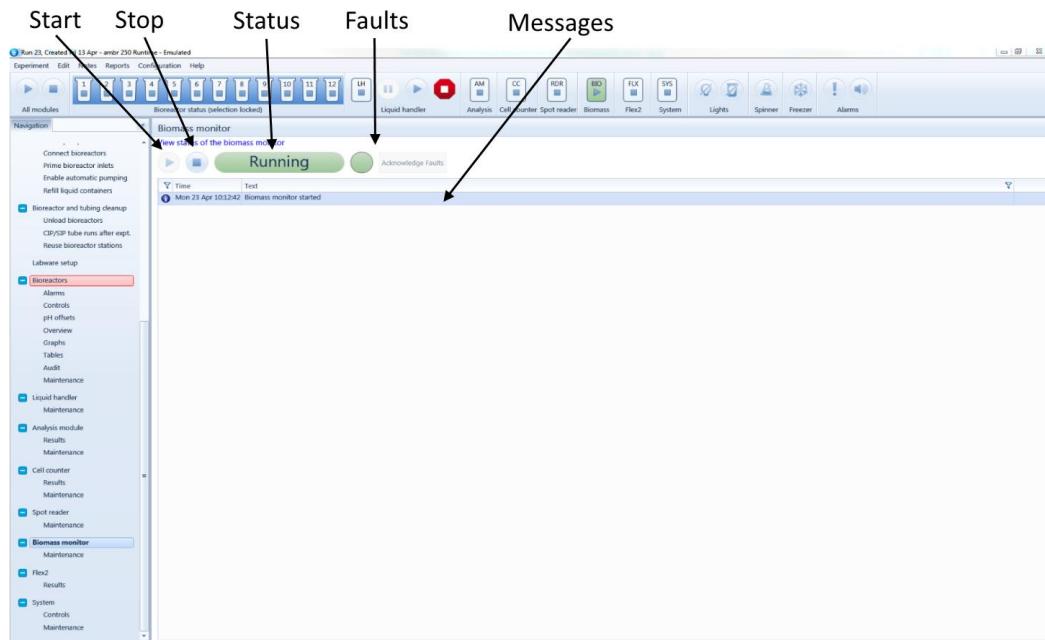


Figure 482 Biomass monitor page

17.1 Starting and stopping the biomass monitor

Start starts automatic control of the biomass monitor.

Stop stops automatic control of the biomass monitor

Acknowledge faults acknowledges any faults.

The **Messages** area shows messages about what the biomass monitor is doing.

17.2 Offsets

The **Offsets** page provides the ability to view biomass readings and to apply an offset to the reflectance to bring the computed biomass in line with external measurements.

Values are shown for the measurement selected on the **System options** page.



Figure 483 Biomass monitor Offsets page

Press **Edit offsets** to display a dialog where externally measured data can be entered.

Figure 484 Dialog to enter external measured data

17.3 Maintenance

The Maintenance page provides facilities for testing the biomass monitor.

17.3.1 Monitor tab

The monitor tab allows the biomass of the vessels to be monitored.

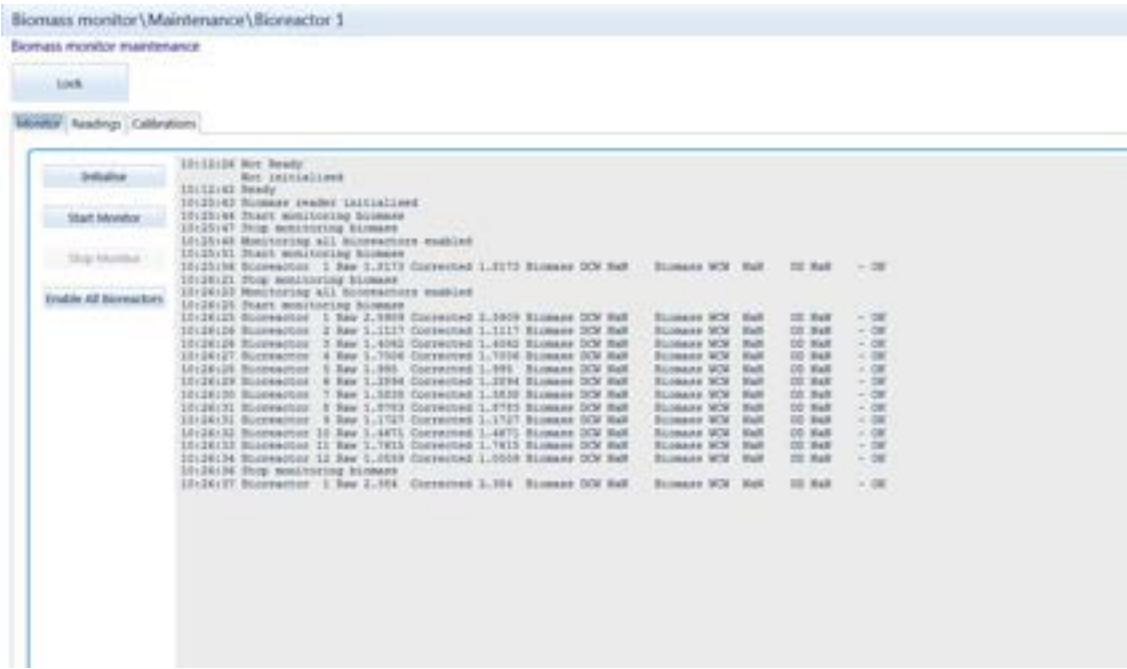


Figure 485 Biomass monitor\Maintenance Monitor tab

Initialise – initialises the biomass monitor.

Start monitor – starts the monitoring of biomass readings

Stop monitor – stops the monitoring of biomass readings

Enable All Bioreactors – enables the biomass readings of all the bioreactors to be monitored

17.3.2 Readings tab

The readings tab allows the parameters for the biomass readings of an individual vessel to be set and monitored. The bioreactor to be monitored can be selected from the drop down box.

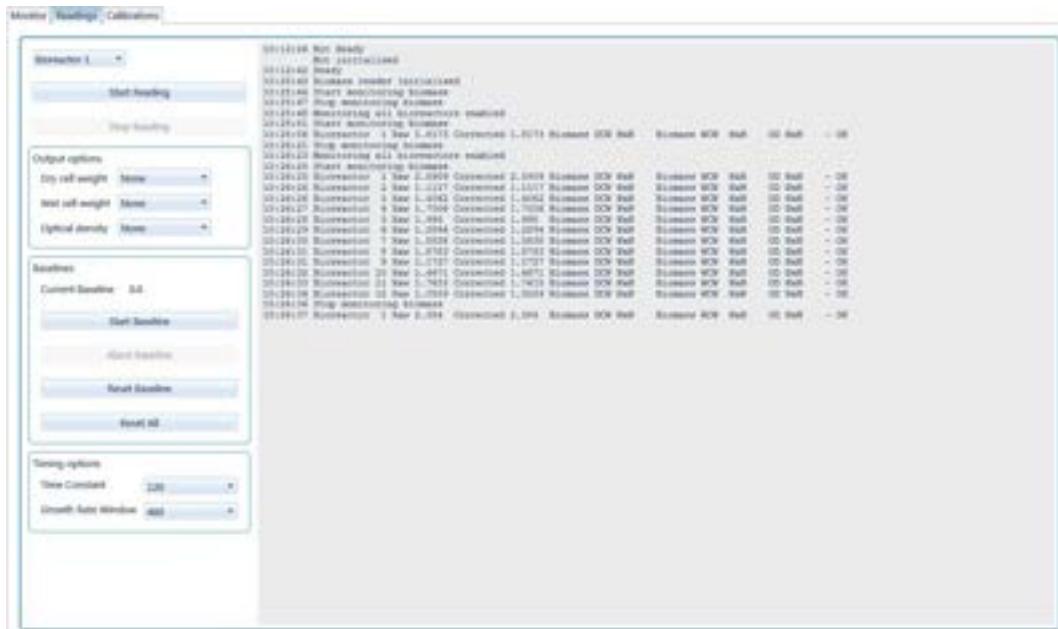


Figure 486 Biomass monitor\Maintenance Readings tab

Start reading – starts the biomass monitor readings

Stop reading – stops the biomass monitor readings

Output options allows the conversion between reflectance and the measure of interest to be specified. Options are chosen from the list of installed conversions for:-

- **Dry cell weight** – convert from reflectance to dry cell weight.
- **Wet cell weight** – convert from reflectance to wet cell weight.
- **Optical density** – convert from reflectance to optical density.

Baselines allows control of the baseline value for the current bioreactor. The current baseline value is displayed and will be subtracted from the raw value of the reflectance. The corrected value for the reflectance will be used in the conversion calculations.

- **Start baseline** – Starts a baseline process.
- **Abort baseline** – Cancels a baseline process.
- **Reset baseline** – Resets the baseline value to zero
- **Reset All** – Resets all baselines for all bioreactors

Timing options allows the timing options for the biomass readings to be set.

- **Time constant** – Sets the period over which biomass reading will be averaged.
- **Growth rate window** – Sets the period over which the biomass readings will be used to calculate the growth rate.

17.3.3 Calibrations tab

Shows the biomass calibrations currently installed.

Biomass monitor\Maintenance\Bioreactor 1													
Biomass monitor maintenance													
Work													
Monitor / Readings / Calibration													
Biomass calibrations													
This screen shows the biomass calibrations currently installed.													
Calibration curve coefficients													
Name	Description	T ⁰	Type	T ¹	r ⁰	r ¹	r ²	r ³	r ⁴	Offset	min	max	User defined
Salin.Dew.DOW	Salinometer Conversion dry cell weight DOW	0	-0.25-00	0.0002	-0.00004099	2.000000	-0.00200279	0	0	0.0001112	0	0.0001112	No
Salin.Dew.COD.WB	Salinometer Conversion optical density COD	0	0	0	-0.00000099	2.000000	-0.00200279	0	0	0.0001112	0	0.0001112	No

Figure 487 Biomass monitor\Maintenance Calibrations tab

18 SPOT READER

This section describes the options for the direct control and maintenance of the spot reader.

18.1 Spot Reader

The Spot reader page provides controls for starting and stopping the spot reader robot and individual spot readers; viewing messages and acknowledging faults.

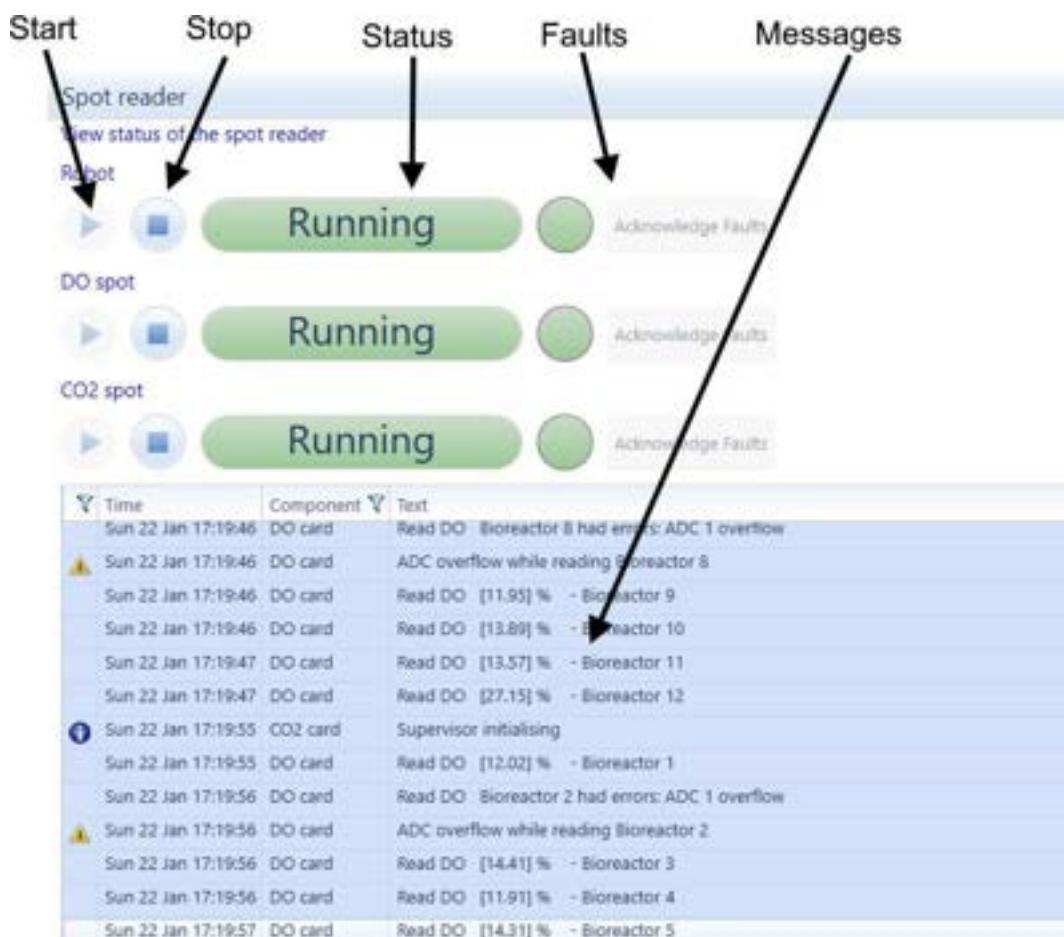


Figure 488 Spot reader page

The **Start** button starts the spot reader and the **Stop** button stops the spot reader.

The **Status** indicator shows the status of the spot reader and the **Faults** indicator shows if there have been any faults.

Acknowledge faults acknowledges any faults.

The **Messages** area shows messages about what the spot reader is doing.

18.2 Maintenance

The Maintenance page provides facilities for testing and teaching the spot reader.

Incorrect use of this page can cause mechanical damage to the Ambr® 250 system. Access to the pages is protected by a password and the pages should only be used by competent maintenance staff.

See the Maintenance manual for details of how to use the Maintenance page.

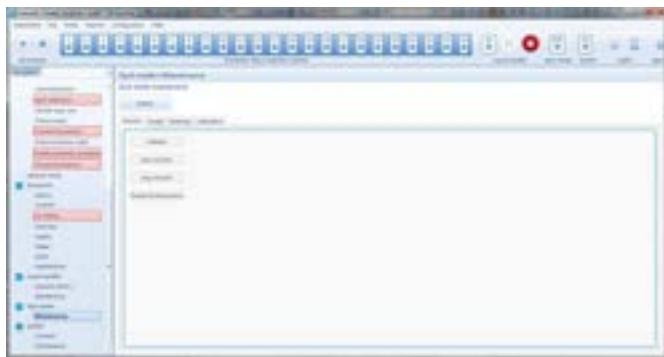


Figure 489 Maintenance page

19 SYSTEM

This section describes the options for the direct control and maintenance of the system supervisor that monitors and controls a number of global aspects of the system.

19.1 System

The System page provides controls for starting and stopping the system supervisor; viewing messages and acknowledging faults.

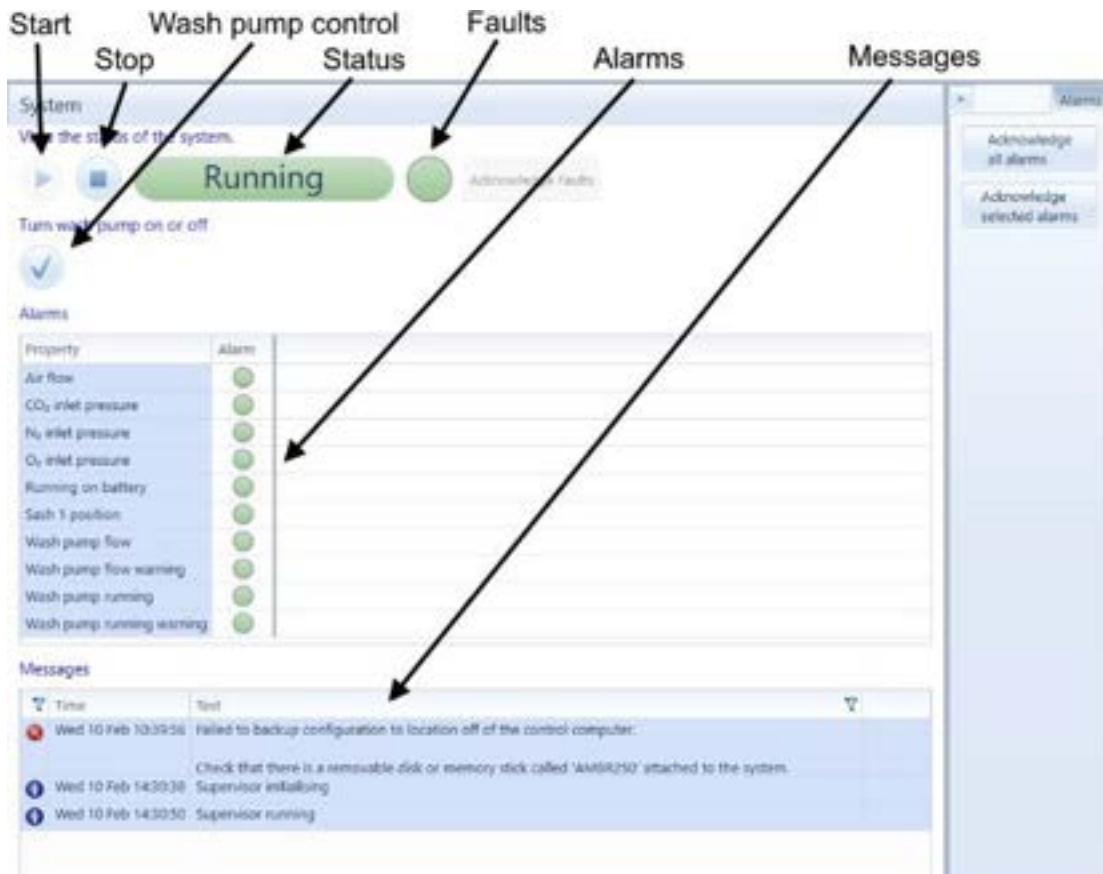


Figure 490 System page

The **Start** button starts the supervisor and the **Stop** button stops the supervisor.

The **Status** indicator shows the status of the supervisor and the **Faults** indicator shows if there have been any faults.

The **Wash pump** button switches the pump that pumps wash fluid around the bioreactors on and off. In normal operation the pump must be running. The pump only needs to be turned off when changing the liquid in the pump circuit or replacing the pump itself.

Acknowledge faults acknowledges any faults.

The **Alarms** area shows the status of the system alarms.

- The status is OK



- The alarm is not active

 - The alarm is disabled

 - The alarm has been triggered
 (flashing)
 - The alarm has been triggered and acknowledged

 - The alarm is now OK, but it was triggered and has not been acknowledged
 (flashing)
 - The definition of the alarm is invalid. The system is not monitoring the alarm.


Acknowledge all alarms and **Acknowledge selected alarms** can be used to acknowledge alarms.

The **Messages** area shows messages about what the liquid handler is doing.

19.2 Maintenance

The Maintenance page provides facilities for testing and configuring the system.

Incorrect use of this page can cause mechanical damage to the Ambr® 250 system. Access to the pages is protected by a password and the pages should only be used by competent maintenance staff.

See the Maintenance manual for details of how to use the Maintenance page.

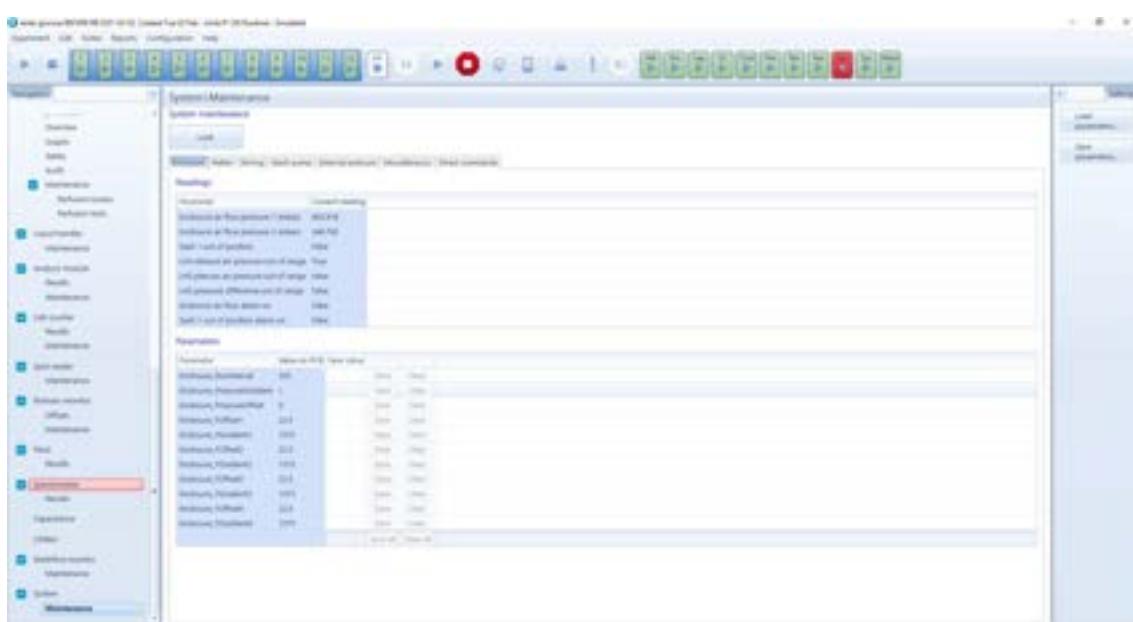


Figure 491 Supervisor maintenance page

20 CHILLERS

This section describes the options for the control of the chillers and monitoring of the chillers attached to the system.

The Chillers page provides controls for starting and stopping the Chillers monitoring supervisor; controlling the chillers; viewing messages and acknowledging faults.

20.1 Single chiller monitoring

For systems configured with a single Huber or Thermo chiller the chiller status is also displayed.

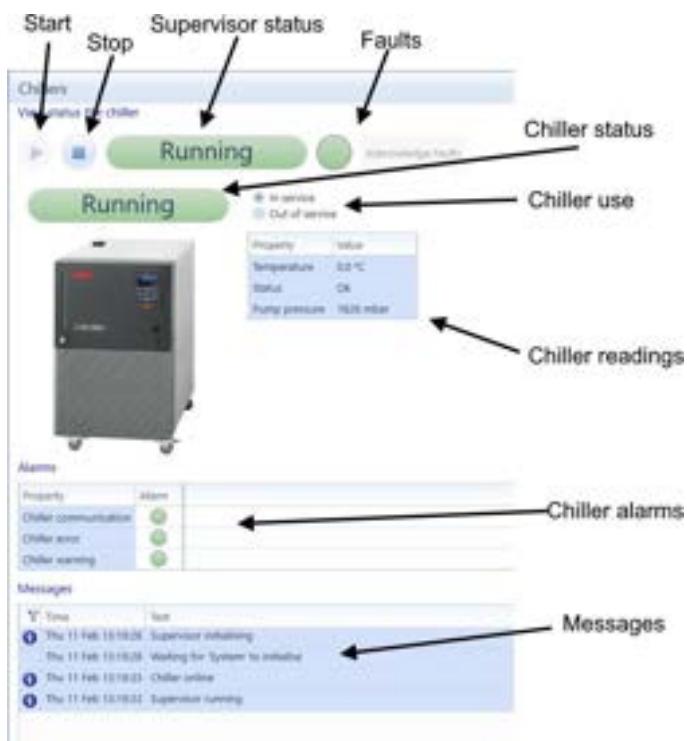


Figure 492 Controls page with a Huber chiller

The **Start** button starts the supervisor and the **Stop** button stops the supervisor.

The **Status** indicator shows the status of the supervisor and the **Faults** indicator shows if there have been any faults.

Acknowledge faults acknowledges any faults.

The **Chiller status** indicator shows the status of the chiller.

The **In service/Out of service** chiller radio buttons allow the monitoring of the chiller to be disabled. In normal operation the chiller should be **In service** and be monitored. The chiller only needs to be taken **Out of service** when maintaining or commissioning the chiller.

The **Readings table** shows the current values of the chiller.

The **Alarms** area shows the status of the chiller alarms. (see System alarms for definitions of alarm states)

Acknowledge all alarms and **Acknowledge selected alarms** can be used to acknowledge alarms.

The **Messages** area shows messages about what the chiller is doing.

Chillers

View status the chiller

Running

Acknowledge Faults

Running

In service
Out of service

Property	Value
Temperature	0.0 °C
Status	OK

Alarms

Property	Alarm
Chiller communication	(green circle)
Chiller error	(green circle)
Chiller warning	(green circle)

Messages

Time	Text
Thu 11 Feb 13:59:40	Supervisor initialising
Thu 11 Feb 13:59:40	Waiting for 'System' to initialise
Thu 11 Feb 13:59:45	Chiller online
Thu 11 Feb 13:59:47	Supervisor running

Figure 493 Controls page with a Thermo chiller

20.2 Dual chiller monitoring

For systems configured with dual chillers the status of the chillers and the dual chiller controller is displayed.

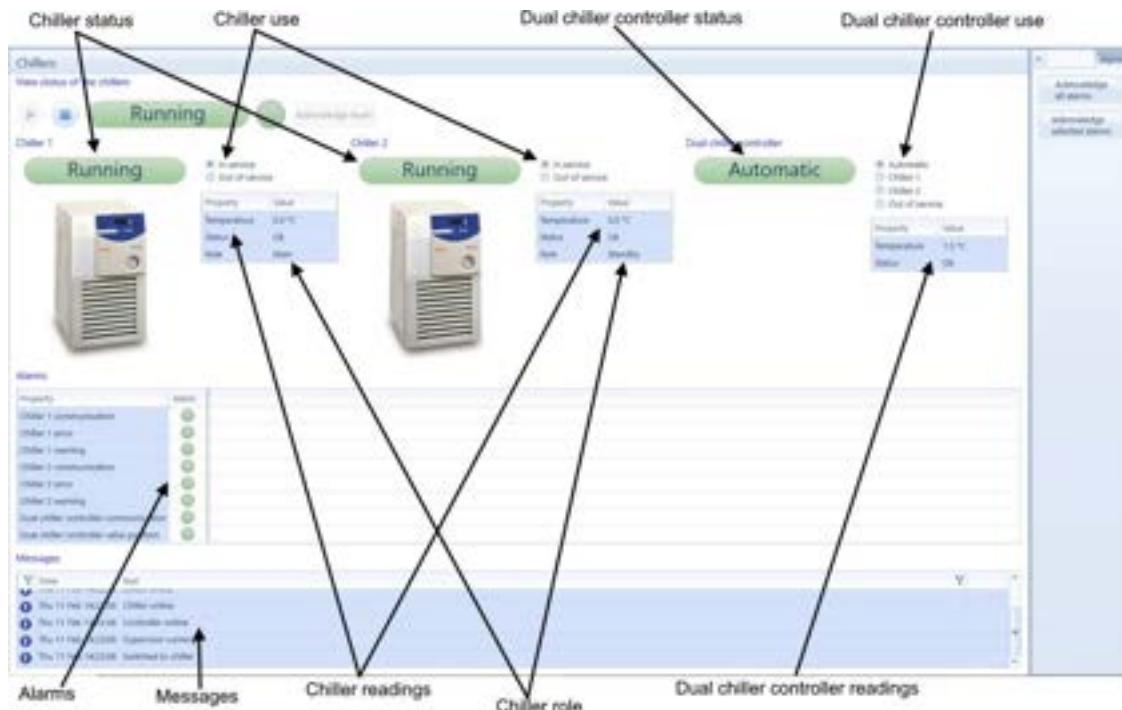


Figure 494 Controls page with Dual chiller controller

The **Status** indicator shows the state of the individual chillers and the dual chiller controller.

The **In service/Out of service** chiller radio buttons allow the monitoring of the chiller to be disabled.

The chillers **Readings** table contains an additional row for the **Role** of the chiller

- **Main** – the chiller that is currently being used by the system.
- **Standby** – the chiller that will be used if there is a fault with the **Main** chiller.

The dual chiller controller radio buttons determine the mode of operation

- **Automatic** – The dual chiller controller monitors the status and temperature of the chiller as well as the temperature of the liquid going into the system. It will switch automatically to the **Standby** chiller if a fault occurs with the **Main** chiller or the temperature of the liquid going into the system getting too warm. The dual chiller controller will also select the Main chiller on a duty cycle of 1 day on one day off for the chiller with switching taking place at midday
- **Chiller 1** – Manual mode with chiller 1 selected as the main chiller. No automatic switching of the chillers on faults.
- **Chiller 2** – Manual mode with chiller 2 selected as the main chiller. No automatic switching of the chillers on faults.

- **Out of service** – Enables the dual chiller controller to be taken out of service. The dual chiller controller should only be taken out of service for maintenance or commissioning.



Figure 495 Controls page with dual chiller controller running in manual mode

The **Alarms** area shows the status of the chiller alarms. (see System alarms for definitions of alarm states)

Acknowledge all alarms and **Acknowledge selected alarms** can be used to acknowledge alarms.

The **Messages** area shows messages from the chillers and the dual chiller controller, the module column indicating the module that the message relates to.

21 WASHFLOW MONITOR

This section describes the options for the direct control and maintenance of the wash flow supervisor that monitors and the washflow around the liquid pumps.

21.1 Washflow monitoring

The Washflow monitoring page provides controls for starting and stopping the washflow monitoring supervisor; viewing messages and acknowledging faults.

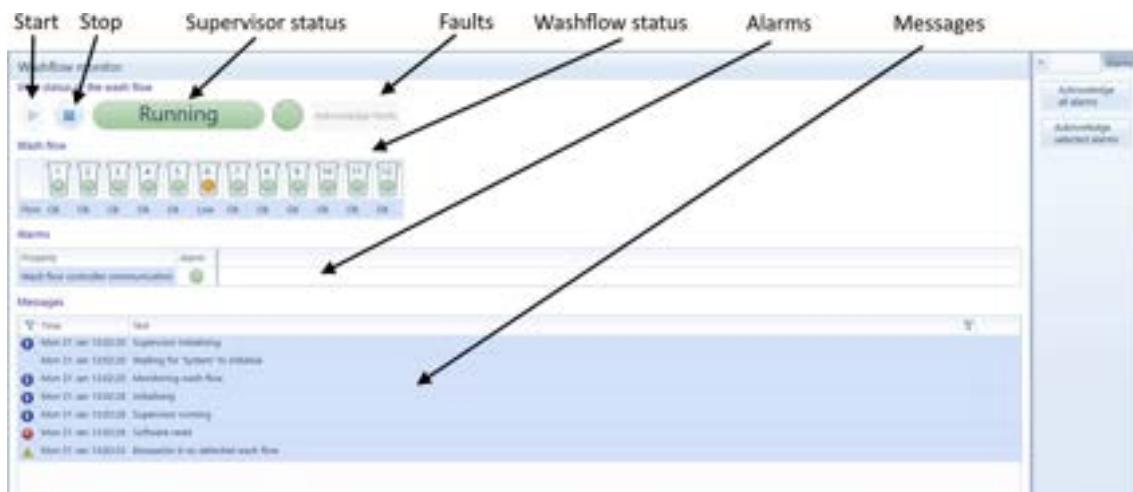


Figure 496 Washflow monitor status page

The **Start** button starts the supervisor and the **Stop** button stops the supervisor.

The **Status** indicator shows the status of the supervisor and the **Faults** indicator shows if there have been any faults.

Acknowledge faults acknowledges any faults.

The **Washflow status** indicators show the status of the washflow around the individual bioreactors

- The status is OK
●
- The washflow monitor is initialising
○ (flashing)
- The washflow monitor is disconnected or the heater is over temperature. The hover text on the status describes the fault.
✖
- The washflow flow is low
○ (solid)

The **Alarms** area shows the status of the washflow alarms. (see System alarms for definitions of alarm states).

Acknowledge all alarms and **Acknowledge selected alarms** can be used to acknowledge alarms.

The **Messages** area shows messages from the washflow monitors

Additional alarms for the individual washflow monitors are included in the **Bioreactor\Alarms** page.

Property	1	2	3	4	5	6	7	8	9	10	11	12
Bioreactor over pressure	●	●	●	●	●	●	●	●	●	●	●	●
Bioreactor pressure high	●	●	●	●	●	●	●	●	●	●	●	●
Clamp plate too cold	○	○	○	○	○	○	○	○	○	○	○	○
Clamp plate too hot	○	○	○	○	○	○	○	○	○	○	○	○
Gas flow with valves off	●	●	●	●	●	●	●	●	●	●	●	●
Gas throttling	●	●	●	●	●	●	●	●	●	●	●	●
pH reading too high	●	●	●	●	●	●	●	●	●	●	●	●
pH reading too low	●	●	●	●	●	●	●	●	●	●	●	●
Steps locked for editing	●	●	●	●	●	●	●	●	●	●	●	●
Volume high	●	●	●	●	●	●	●	●	●	●	●	●
Wash flow monitor heater over temperature	●	●	●	●	●	●	●	●	●	●	●	●
Wash flow no detected flow	●	●	●	●	●	●	●	●	●	●	●	●

Figure 497 Additional bioreactor washflow alarms

21.2 Maintenance

The Maintenance page provides facilities for testing and configuring the washflow monitoring.

Incorrect use of this page can cause mechanical damage to the Ambr® 250 system. Access to the pages is protected by a password and the pages should only be used by competent maintenance staff.

See the Maintenance manual for details of how to use the Maintenance page.

The screenshot shows a software interface for maintenance. On the left is a navigation tree with categories like Bioreactor, Alarms, Control, Gas, Clamps, Sensors, Wash, Audit, and Maintenance. Under Maintenance, 'Washflow monitor' is selected. The main window title is 'Washflow monitor\Maintenance'. It contains a table titled 'Parameters' with two columns: 'Parameter' and 'Value on HMI'. The table lists various parameters with their corresponding values. A red box highlights the last three rows of the table.

Parameter	Value on HMI
Wash_JetVolume	10000-0-0
Wash_JetPressure	0
Wash_JetAngleYaw	0
Wash_JetAnglePitch	0
Wash_JetAngleRoll	0
Wash_JetVelocity_X	0
Wash_JetVelocity_Y	0
Wash_JetVelocity_Z	0
Wash_JetDiameter	0
Wash_JetDiameter_X	0
Wash_JetDiameter_Y	0
Wash_JetDiameter_Z	0
Wash_JetFlowRate_X	0
Wash_JetFlowRate_Y	0
Wash_JetFlowRate_Z	0
Wash_JetFlowRate_Total	0
Wash_JetFlowRate_Total_X	0
Wash_JetFlowRate_Total_Y	0
Wash_JetFlowRate_Total_Z	0
Wash_JetFlowRate_Total_0	0
Wash_JetFlowRate_Total_1	0
Wash_JetFlowRate_Total_2	0
Wash_JetFlowRate_Total_3	0
Wash_JetFlowRate_Total_4	0
Wash_JetFlowRate_Total_5	0
Wash_JetFlowRate_Total_6	0
Wash_JetFlowRate_Total_7	0
Wash_JetFlowRate_Total_8	0
Wash_JetFlowRate_Total_9	0
Wash_JetFlowRate_Total_10	0
Wash_JetFlowRate_Total_11	0
Wash_JetFlowRate_Total_12	0
Wash_JetFlowRate_Total_13	0
Wash_JetFlowRate_Total_14	0
Wash_JetFlowRate_Total_15	0
Wash_JetFlowRate_Total_16	0
Wash_JetFlowRate_Total_17	0
Wash_JetFlowRate_Total_18	0
Wash_JetFlowRate_Total_19	0
Wash_JetFlowRate_Total_20	0
Wash_JetFlowRate_Total_21	0
Wash_JetFlowRate_Total_22	0
Wash_JetFlowRate_Total_23	0
Wash_JetFlowRate_Total_24	0
Wash_JetFlowRate_Total_25	0
Wash_JetFlowRate_Total_26	0
Wash_JetFlowRate_Total_27	0
Wash_JetFlowRate_Total_28	0
Wash_JetFlowRate_Total_29	0
Wash_JetFlowRate_Total_30	0
Wash_JetFlowRate_Total_31	0
Wash_JetFlowRate_Total_32	0
Wash_JetFlowRate_Total_33	0
Wash_JetFlowRate_Total_34	0
Wash_JetFlowRate_Total_35	0
Wash_JetFlowRate_Total_36	0
Wash_JetFlowRate_Total_37	0
Wash_JetFlowRate_Total_38	0
Wash_JetFlowRate_Total_39	0
Wash_JetFlowRate_Total_40	0
Wash_JetFlowRate_Total_41	0
Wash_JetFlowRate_Total_42	0
Wash_JetFlowRate_Total_43	0
Wash_JetFlowRate_Total_44	0
Wash_JetFlowRate_Total_45	0
Wash_JetFlowRate_Total_46	0
Wash_JetFlowRate_Total_47	0
Wash_JetFlowRate_Total_48	0
Wash_JetFlowRate_Total_49	0
Wash_JetFlowRate_Total_50	0
Wash_JetFlowRate_Total_51	0
Wash_JetFlowRate_Total_52	0
Wash_JetFlowRate_Total_53	0
Wash_JetFlowRate_Total_54	0
Wash_JetFlowRate_Total_55	0
Wash_JetFlowRate_Total_56	0
Wash_JetFlowRate_Total_57	0
Wash_JetFlowRate_Total_58	0
Wash_JetFlowRate_Total_59	0
Wash_JetFlowRate_Total_60	0
Wash_JetFlowRate_Total_61	0
Wash_JetFlowRate_Total_62	0
Wash_JetFlowRate_Total_63	0
Wash_JetFlowRate_Total_64	0
Wash_JetFlowRate_Total_65	0
Wash_JetFlowRate_Total_66	0
Wash_JetFlowRate_Total_67	0
Wash_JetFlowRate_Total_68	0
Wash_JetFlowRate_Total_69	0
Wash_JetFlowRate_Total_70	0
Wash_JetFlowRate_Total_71	0
Wash_JetFlowRate_Total_72	0
Wash_JetFlowRate_Total_73	0
Wash_JetFlowRate_Total_74	0
Wash_JetFlowRate_Total_75	0
Wash_JetFlowRate_Total_76	0
Wash_JetFlowRate_Total_77	0
Wash_JetFlowRate_Total_78	0
Wash_JetFlowRate_Total_79	0
Wash_JetFlowRate_Total_80	0
Wash_JetFlowRate_Total_81	0
Wash_JetFlowRate_Total_82	0
Wash_JetFlowRate_Total_83	0
Wash_JetFlowRate_Total_84	0
Wash_JetFlowRate_Total_85	0
Wash_JetFlowRate_Total_86	0
Wash_JetFlowRate_Total_87	0
Wash_JetFlowRate_Total_88	0
Wash_JetFlowRate_Total_89	0
Wash_JetFlowRate_Total_90	0
Wash_JetFlowRate_Total_91	0
Wash_JetFlowRate_Total_92	0
Wash_JetFlowRate_Total_93	0
Wash_JetFlowRate_Total_94	0
Wash_JetFlowRate_Total_95	0
Wash_JetFlowRate_Total_96	0
Wash_JetFlowRate_Total_97	0
Wash_JetFlowRate_Total_98	0
Wash_JetFlowRate_Total_99	0
Wash_JetFlowRate_Total_100	0
Wash_JetFlowRate_Total_101	0
Wash_JetFlowRate_Total_102	0
Wash_JetFlowRate_Total_103	0
Wash_JetFlowRate_Total_104	0
Wash_JetFlowRate_Total_105	0
Wash_JetFlowRate_Total_106	0
Wash_JetFlowRate_Total_107	0
Wash_JetFlowRate_Total_108	0
Wash_JetFlowRate_Total_109	0
Wash_JetFlowRate_Total_110	0
Wash_JetFlowRate_Total_111	0
Wash_JetFlowRate_Total_112	0
Wash_JetFlowRate_Total_113	0
Wash_JetFlowRate_Total_114	0
Wash_JetFlowRate_Total_115	0
Wash_JetFlowRate_Total_116	0
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Wash_JetFlowRate_Total_121	0
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Wash_JetFlowRate_Total_123	0
Wash_JetFlowRate_Total_124	0
Wash_JetFlowRate_Total_125	0
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Wash_JetFlowRate_Total_128	0
Wash_JetFlowRate_Total_129	0
Wash_JetFlowRate_Total_130	0
Wash_JetFlowRate_Total_131	0
Wash_JetFlowRate_Total_132	0
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Wash_JetFlowRate_Total_140	0
Wash_JetFlowRate_Total_141	0
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Wash_JetFlowRate_Total_143	0
Wash_JetFlowRate_Total_144	0
Wash_JetFlowRate_Total_145	0
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Wash_JetFlowRate_Total_162	0
Wash_JetFlowRate_Total_163	0
Wash_JetFlowRate_Total_164	0
Wash_JetFlowRate_Total_165	0
Wash_JetFlowRate_Total_166	0
Wash_JetFlowRate_Total_167	0
Wash_JetFlowRate_Total_168	0
Wash_JetFlowRate_Total_169	0
Wash_JetFlowRate_Total_170	0
Wash_JetFlowRate_Total_171	0
Wash_JetFlowRate_Total_172	0
Wash_JetFlowRate_Total_173	0
Wash_JetFlowRate_Total_174	0
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Wash_JetFlowRate_Total_176	0
Wash_JetFlowRate_Total_177	0
Wash_JetFlowRate_Total_178	0
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Wash_JetFlowRate_Total_180	0
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Wash_JetFlowRate_Total_184	0
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Wash_JetFlowRate_Total_187	0
Wash_JetFlowRate_Total_188	0
Wash_JetFlowRate_Total_189	0
Wash_JetFlowRate_Total_190	0
Wash_JetFlowRate_Total_191	0
Wash_JetFlowRate_Total_192	0
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Wash_JetFlowRate_Total_203	0
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Wash_JetFlowRate_Total_205	0
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Wash_JetFlowRate_Total_227	0
Wash_JetFlowRate_Total_228	0
Wash_JetFlowRate_Total_229	0
Wash_JetFlowRate_Total_230	0
Wash_JetFlowRate_Total_231	0
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Wash_JetFlowRate_Total_301	0
Wash_JetFlowRate_Total_302	0
Wash_JetFlowRate_Total_303	0
Wash_JetFlowRate_Total_304	0
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Wash_JetFlowRate_Total_335	0
Wash_JetFlowRate_Total_336	0
Wash_JetFlowRate_Total_337	0
Wash_JetFlowRate_Total_338	0
Wash_JetFlowRate_Total_339	0
Wash_JetFlowRate_Total_340	0
Wash_JetFlowRate_Total_341	0
Wash	

22 NOTES

Notes can be entered to record events relating to the culture.

Notes can be added and edited from the menu bar or from the context menu on graphs.

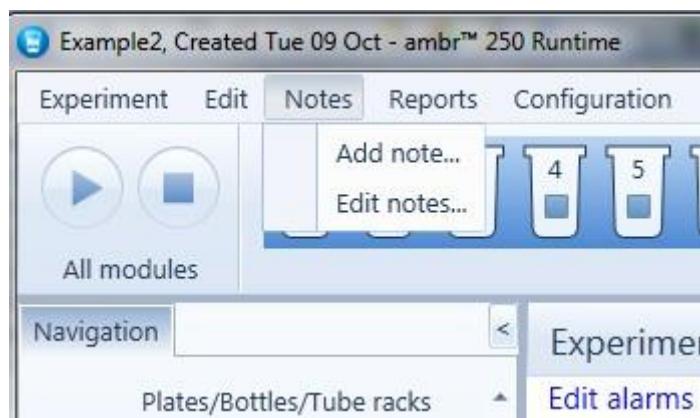


Figure 499 Notes menu

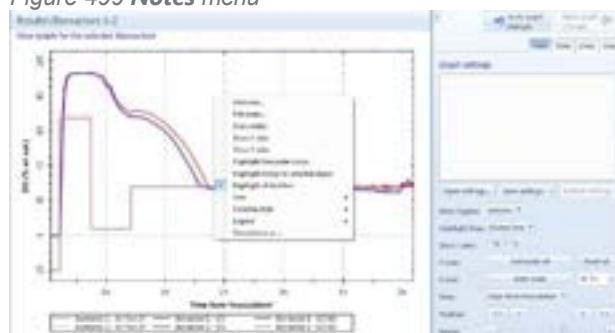


Figure 500 Notes options on graph context menu

Add note... displays the **Add note** window.

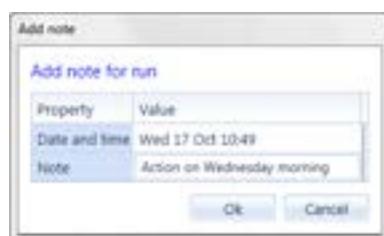


Figure 501 Add note window

Date and time is the time that the note applied to. This defaults to the current time when the window is displayed from the menu and to the time where the mouse was positioned if the window is displayed from the graph context menu.

Hint: Zoom into the graph to get an accurate time for the note.

Note is the text for the note.

Edit notes... displays the **Edit notes** window for editing, adding and deleting notes.

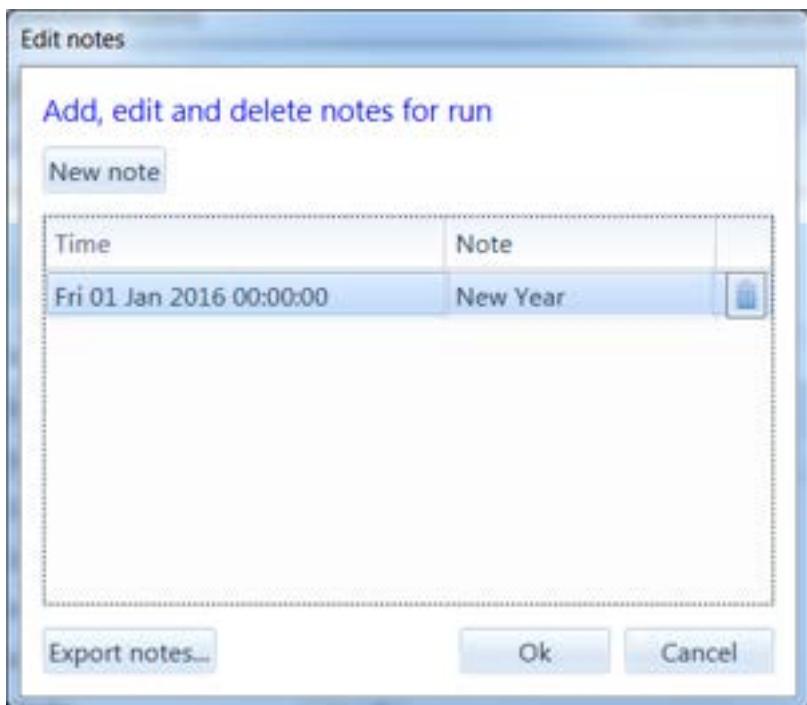


Figure 502 Edit notes window

New note adds a new note.

Export notes opens a dialog to save the notes to a text file.

Notes can be displayed on graphs. Choose the **Notes** variable or select **Show notes** from the graph context menu.

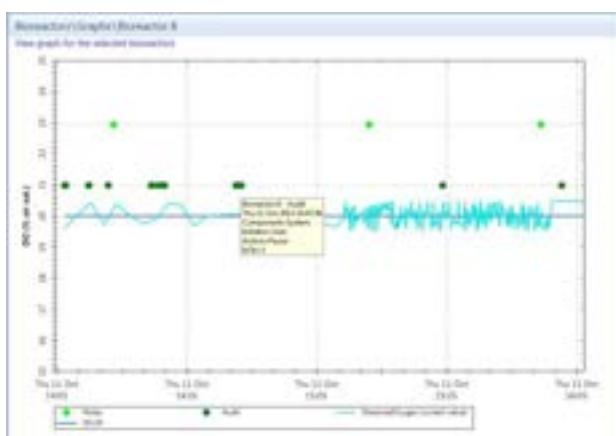


Figure 503 Graph showing notes and audit events

23 REPORTS

The system provides summarised reports. These can be used to help in preparing labware, tubing and other elements for a run.

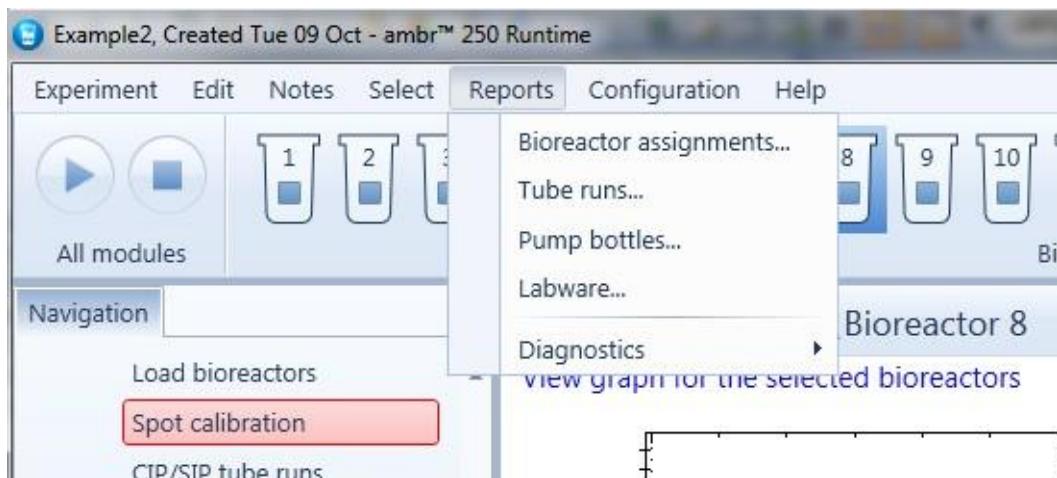


Figure 504 Reports menu

Bioreactor assignments displays a report of the bioreactors used in a process.



Figure 505 Bioreactor assignments report

Tube runs displays a report of the tube runs required.



Figure 506 Tube runs report

Pump containers displays a report of the containers required for the pumps.



Figure 507 Pump bottles report

Labware displays a report of the labware used in a process and when it is likely to be required.



Figure 508 Labware report

The reports can be printed if required.

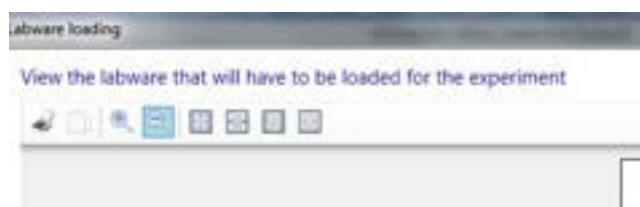


Figure 509 Print and zooming controls

Diagnostics contains additional reports with internal data.

24 USER CONFIGURATION

This section describes the options for configuring the system via the main runtime application. Changes made to these settings are stored as part of the configuration of the system and are used by all experiments and processes until they are changed again.

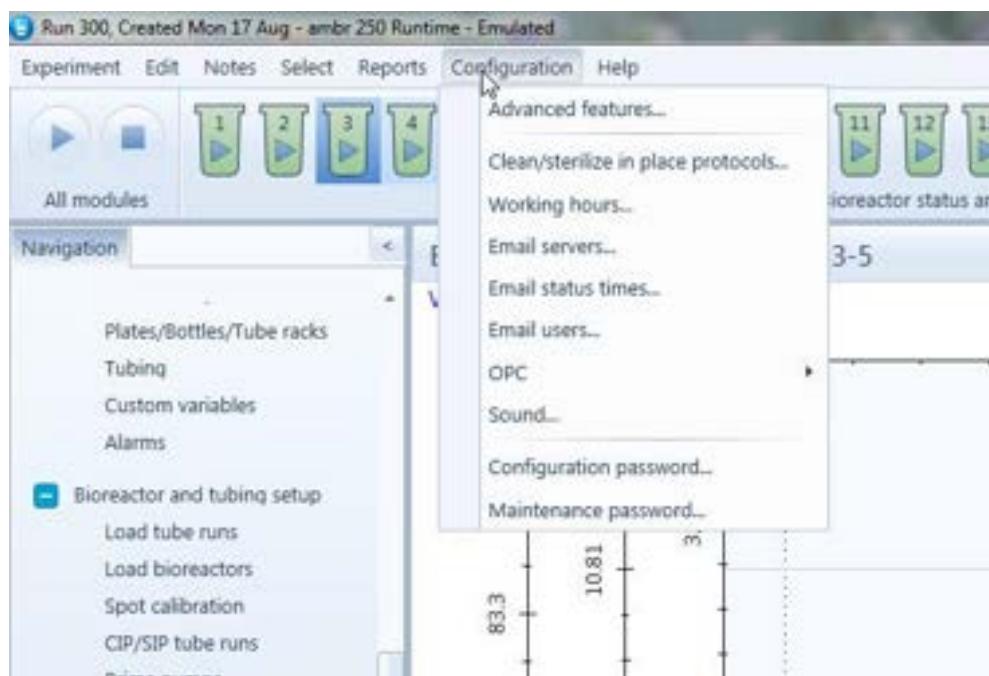


Figure 510 Configuration menu

The various configuration windows can be opened from the **Configuration** menu.

24.1 Advanced features

The **Advanced features** window switches features of the system on and off and provides an explanation of each feature, as described in section 3.2.13

24.2 Clean/Sterilize in Place Protocols

Clean/sterilize in place protocols opens the **Edit clear/sterilize in place protocols** window.

Each protocol comprises a number of steps. Each step can:

- Pump a volume of liquid and then hold for a specified interval.
- Prompt the user to take some action

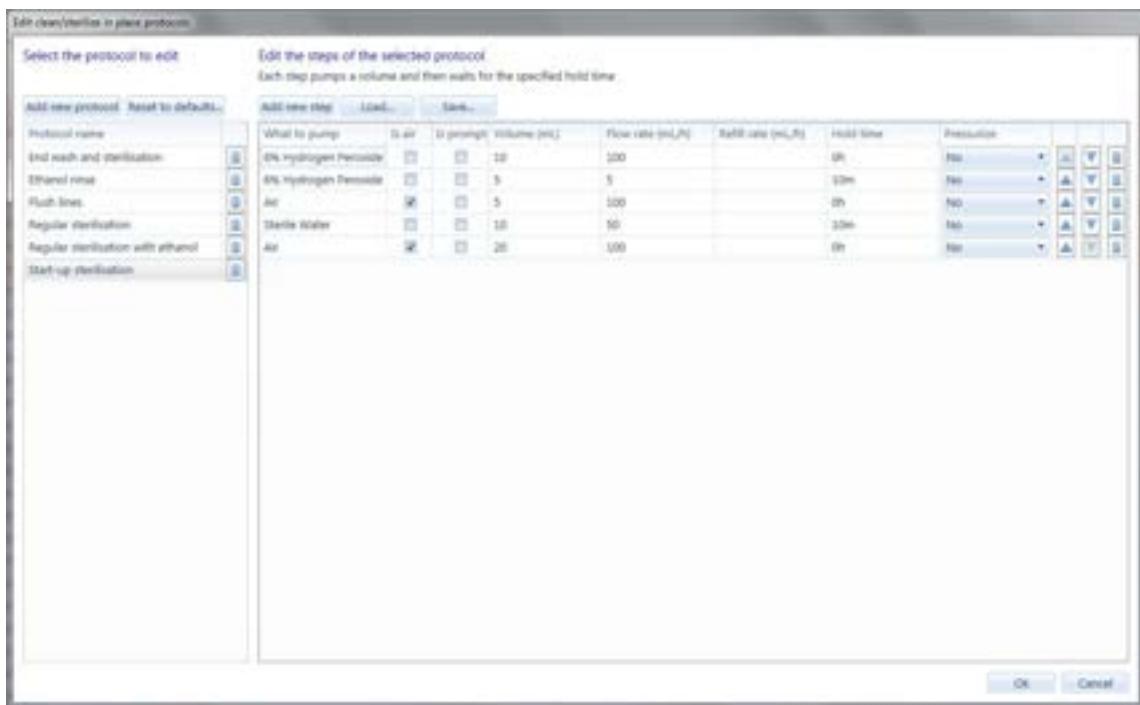


Figure 511 *Edit clear/sterilize in place protocols* window.

Add new protocol adds a new protocol.

Reset to defaults resets the protocols with the factory defaults built into the software plus any defaults saved by the user.

Protocol name edits the name of the protocol.

Add new step adds a new step to the selected protocol.

Load presents a dialog to reset the selected protocol with the contents of a factory or user default.

Save saves the protocol as a user default.

For each step:

What to pump says what is to be pumped by the step. This is the description presented to the operator of the liquid to use. If the step is a prompt to the user then this text is that prompt.

Is air indicates if the step is pumping air. This modifies the description presented to the operator.

Is prompt indicates that the step is a prompt to the user to take some action.

Volume is the volume to pump for each pump.

Flow rate is the average rate at which to pump liquid through the pump.

Refill rate is the rate to pump into the pump. The Refill rate is optional and if not specified the pump will be refilled at its maximum speed. A lower Refill rate may be required when initially cleaning tubing that has contained a viscous liquid.

Pressurize allows the pumps to be pressurized at the start of the hold time.

Hold time is how long to wait after the pumping is complete before either doing the next step in the protocol or completing the protocol.

Use the arrow buttons to move steps up and down within a protocol.



Figure 512 Up, down and delete buttons

24.3 Data interface

The Ambr® 250 software can – if the feature is licensed – automatically export details of samples to a shared directory for consumption by an external process. External process data, associated with the samples, placed in a shared directory, can be automatically imported into the system.

See TAP-9351-06-036 Data Interface manual for more details.

24.4 Email servers

The Ambr® 250 software can send emails with status and error reports and can look for emails requesting a status report.

The **Edit email configuration** window specifies the servers and accounts used to send and receive emails.

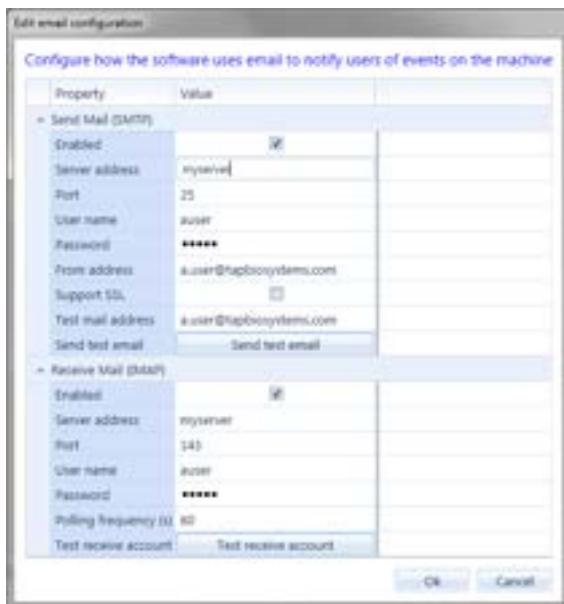


Figure 513 Edit email configuration window

24.4.1 Send Mail (SMTP) options

Send Mail (SMTP) options specify the server and account used to send emails using SMTP.

Your IT department should be able to provide you with the required details.

Enabled enables the sending of emails.

Server address is the address of the email server.

Port is the port to connect to on the server. Typically this is 25 for SMTP.

User name and **Password** are the credentials to be used with the server in order to be allowed to send emails using the server.

From address is the address to say that the emails came from. This is the address that someone replying to the emails will reply to.

Support SSL selects whether the Secure Sockets Layer should be used to connect to the email server. Your IT department can tell you if this option should be selected or not.

Test mail address is an address to which an attempt is made to send an email when **Send test email** is pressed.

24.4.1.1 Receive Mail (IMAP) options

Receive Mail (IMAP) options specify the server and account to be used to receive emails requesting a status email.

Enabled enables receiving emails.

Server address is the address of the email server. This may or may not be the same server used to send emails.

Port is the port to connect to on the server. Typically this is 143 for IMAP.

User name is the name of the email account and **Password** is the password used to access the account

Polling frequency (s) is how often to check to see if new emails have arrived.

Test receive account tests to see if a connection can be made to receive emails.

24.5 Email status times

Email status times displays the **Email status time schedule** window with a list of times when status reports will be sent.



Figure 514 **Email status time schedule** window

Press **New time** to add a new entry to the list of times.

24.5.1 Requesting a status report by email

To request a status report from Ambr® 250 send an email with the subject **#FULL SYSTEM STATUS#** to the IMAP account.

To request a more condensed report use the subjects:

- **#FULL SYSTEM STATUS: MOBILE#** - report optimised for mobiles
- **#SHORT SYSTEM STATUS: TEXT#** – short text report
- **#FULL SYSTEM STATUS: TEXT#** – long text report

24.6 Email users

Email users displays a window with a list of the user profiles configured on the system.



Figure 515 **Email user profiles** window

New profile adds a new profile.

Editing a profile displays the **Email User Settings** window.

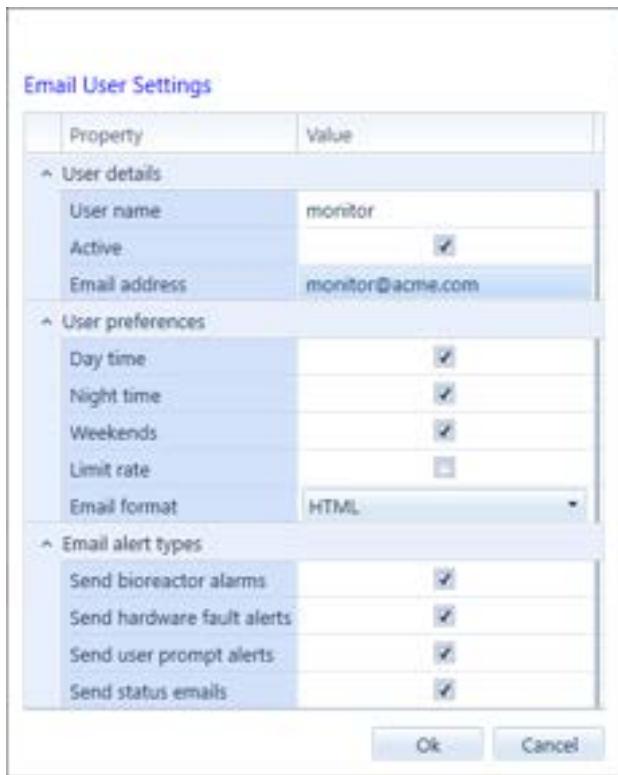


Figure 516 *Edit email user profile* window

User name is a name for this profile entry. It can be especially useful if organisational emails are things like uy56578.

Active indicates if the profile is to be used. Active offers a quick way to temporarily stop sending emails to a particular user.

Email address is the address to send the emails to.

Day time, **Night time** and **Weekends** specify when emails should be sent to this profile.

- **Day time** is working hours on Monday to Friday
- **Night time** is Monday, Tuesday, Wednesday or Thursday night
- **Weekend** lasts from the end of working hours on Friday until the start of working hours on the following Monday

Limit rate option can be used to disable limiting the rate at which messages can be sent.

The default behaviour where the system will stop sending email messages for a while if many messages are raised within an interval can be disabled on a user by user basis. This can be appropriate if the email messages are being handled by an automated system.

Email format chooses a format for emails:

- **HTML** allows rich emails with graphs and other graphics
- **HTML for mobile phones** is a variation of HTML adapted for narrower displays
- **Text only** sends text messages
- **Short form** sends short text messages only

Email alert types offers options for when to send emails:

- **Send bioreactor alarms** sends emails in response to bioreactor alarms.
- **Send hardware fault alerts** sends emails in response to hardware faults.
- **Send user prompt alerts** sends emails when attention is required such as loading labware.
- **Send status emails** sends status reports at the configured Email status times that fall within the times when emails should be sent to this profile.

24.7 Lights

Lights... shows a window to change the default amount of time the bioreactor and enclosure lights will stay on before being automatically shut off.

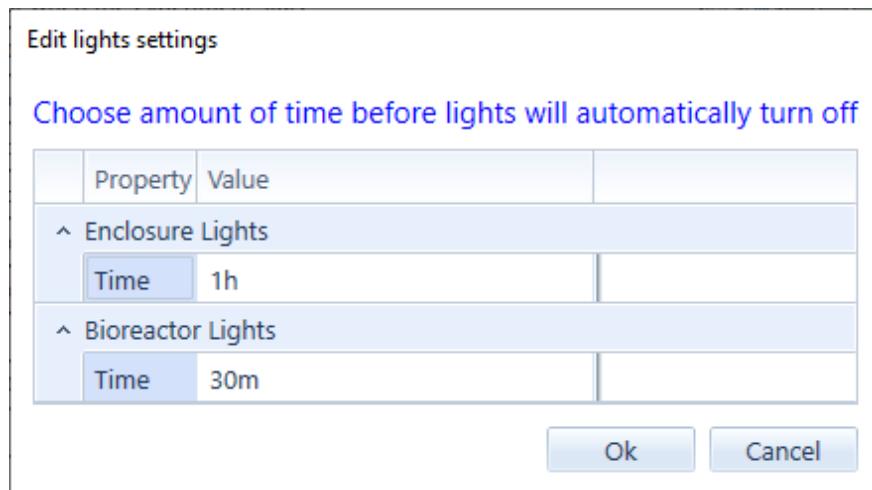


Figure 517 Edit lights settings dialog

24.8 Manual samples

Manual samples... shows a window that allows configuring whether barcodes should be associated with manual samples:

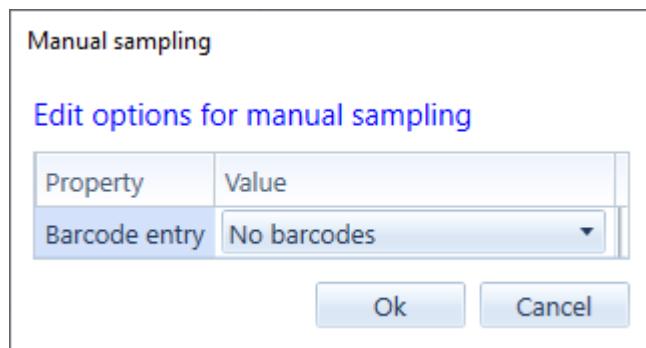


Figure 518 Manual sampling dialog

Barcode entry offers options for barcode entry:

- **No barcodes** – barcode entry field is not displayed on the sample liquid dialog.

- **Barcodes optional** – barcode entry field is displayed on the sample liquid dialog but is not marked as invalid if no barcode is entered.
- **Barcode required** – barcode entry field is displayed on the sample liquid dialog as mandatory. Data can only be entered without a barcode via an additional confirmation dialog.

24.9 Periodic export



Periodic export is available as an option if Support exporting data from the system periodically as CSV files option is enabled in the **Advanced features** window.

Periodic export displays the configuration of the Periodic export function which periodically writes a text file with details of the bioreactor states.

The text file is written:

- When the periodic export function is enabled AND
- Periodic export is been enabled for a bioreactor

The text file contains data for those bioreactors for which periodic export is enabled.

Edit periodic file export configuration	
Configure how the software will periodically export experiment data	
Property	Value
Periodic export enabled	<input checked="" type="checkbox"/>
Export folder	C:\Users\ambr\Documents\ambr 250\Export [?]
Export template	{bioreactor},{datetime},{variable},{value}
Periodicity	30s

Ok **Cancel**

Figure 519 Edit periodic file export configuration dialog

The **Edit periodic file export configuration** window presents the following options.

Periodic export enabled enables or disables the function.

Export folder selects the folder where the files are written. The external software reading the files must read and then delete the files from this folder.

Export template defines a template for the data lines in the files. Export template contains placeholders for values as well as literal text. In the example above the commas are literal text and **{bioreactor}**, **{datetime}**, **{variable}**, and **{value}** are placeholders. The allowed placeholders are:

- **bioreactor** – the number of the bioreactor the data applies to
- **datetime** – the time of the reading encoded as the number of seconds since 1970 in UTC.

- **variable** – the tag for the variable
- **value** – the value of the variable
- **batch** (optional) – the batch value for the bioreactor

Periodicity specifies how often to perform the export.

The sample below indicates how the file might look with a template of
{batch}_{bioreactor}_{tag}_{datetime}_{value}

```

Batch_BioreactorId_Name_Date_Value
BTY-AMER-CAM-1234_1_Active_12345678_1
BTY-AMER-CAM-1234_1_Name_12345678_CVP1-234
BTY-AMER-CAM-1234_1_Batch_12345678_BTY-AMER-CAM-1234
BTY-AMER-CAM-1234_1_StrainName_12345678_FAST-HT1
BTY-AMER-CAM-1234_1_StirRate-PV_12345679_2034
BTY-AMER-CAM-1234_1_StirRate-SP_12345679_2000
BTY-AMER-CAM-1234_1_AirFlow-PV_12345672_235
BTY-AMER-CAM-1234_1_AirFlow-SP_12345672_235
BTY-AMER-CAM-1234_1_Barcode_123456721_C11112222
BTY-AMER-CAM-1234_1_CapOpen_ 12345672_1
BTY-AMER-CAM-1234_1_CO2Flow-PV_ 12345672_200
BTY-AMER-CAM-1234_1_CO2Flow-SP_ 12345672_200
BTY-AMER-CAM-1234_1_CER_12345678_12.3
BTY-AMER-CAM-1234_1_Volume_12345678_231.24
BTY-AMER-CAM-1234_1_DO-PV_12345678_NaN
BTY-AMER-CAM-1234_1_DO-SP_12345678_20
BTY-AMER-CAM-1234_1_Innoculation-Volume_12345678_140
BTY-AMER-CAM-1234_1_Maximum-Volume_12345678_280
BTY-AMER-CAM-1234_1_N2Flow-PV_ 12345672_200
BTY-AMER-CAM-1234_1_N2Flow-SP_ 12345672_200
BTY-AMER-CAM-1234_1_No-10mL-Tips_ 12345672_200
BTY-AMER-CAM-1234_1_No-300uL-Tips_ 12345672_200
BTY-AMER-CAM-1234_1_OUT-PV_12345678_12.3
BTY-AMER-CAM-1234_1_OUT-SP_12345678_12.3
BTY-AMER-CAM-1234_1_OUT-SUPPRESSED_12345678_1
BTY-AMER-CAM-1234_1_Outflow-CO2%_12345678_5
BTY-AMER-CAM-1234_1_Outflow-CO2%_12345678_18.45
BTY-AMER-CAM-1234_1_O2Flow-PV_ 12345672_200
BTY-AMER-CAM-1234_1_O2Flow-SP_ 12345672_200
BTY-AMER-CAM-1234_1_pH-PV_12345679_6.7
BTY-AMER-CAM-1234_1_pH-SP_12345679_6.8
BTY-AMER-CAM-1234_1_RQ_1234569786_1.1
BTY-AMER-CAM-1234_1_T-PV_123456789_37
BTY-AMER-CAM-1234_1_T-SP_123456789_37
BTY-AMER-CAM-1234_1_Volume-Feed#1.123456789_24
BTY-AMER-CAM-1234_1_Volume-Feed#2.123456789_24
BTY-AMER-CAM-1234_1_Volume-Base.123456789_24
BTY-AMER-CAM-1234_1_Volume-Antifoam.123456789_24
ANOTHERBATCHID_2, ..
STILLANOTHERBATCHID_3, ..
...

```

24.10 Sound

24.11 OPC

The **OPC** sub-menu has options for controlling the OPC interface to the system.

This sub-menu is only shown when the OPC option is licensed.

See document **TAP-9351-06-100 OPC Interface Manual** for more details about these options and the OPC interface.

Start starts communications between the Ambr® 250 software and the Kepware server

Stop stops communications between the Ambr® 250 software and the Kepware server

Reconfigure allows regenerating the files which describe which variables are exported from the Ambr® 250 system. To reconfigure the system first stop communications between the Ambr® 250 software and the Kepware server.

Export for BioPAT® MFCS supports exporting descriptions of its OPC configuration that can be imported into the BioPAT MFCS SCADA software to link MFCS and Ambr® 250.

24.12 Sound

Sound shows a window to configure whether and how the system makes sounds.

Note that problems with the laminar air flow and sash positions will cause an audible warning independent of these settings.

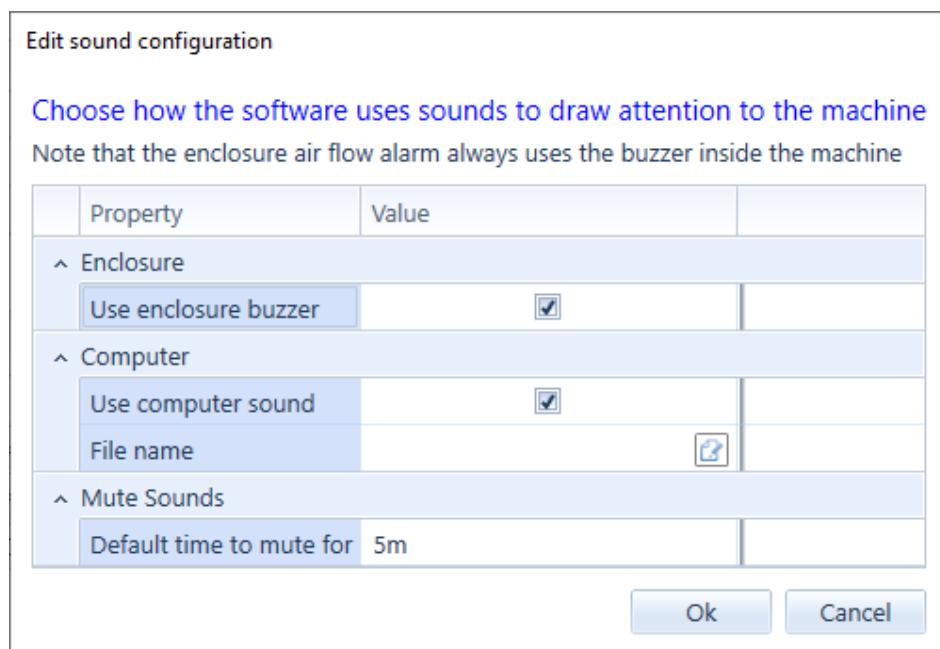


Figure 520 **Edit sound configuration** window

The **Edit sound configuration** window has options:

Use enclosure buzzer selects whether the Ambr® 250 uses the enclosure buzzer to make a sound when the attention window is displayed.

Use computer sound selects whether the Ambr® 250 uses the computer to make a sound when the attention window is displayed.

File name selects a .wav file to be played repeatedly while the attention window is displayed. If no **File name** is specified then the computer beep function is used.

Default time to mute selects how long the mute button will disable sounds.

24.13 Toggle status display

This option allows the user to configure the items that are displayed on the status bar.



Figure 521 The status bar

The visibility of items can be changed by selecting the **Toggle status display...** menu option from the **Configuration** menu.

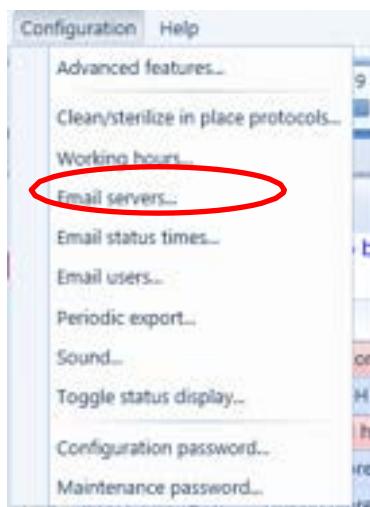


Figure 522 Configuration menu

The **Toggle status display** displays all of the configured status bar items.

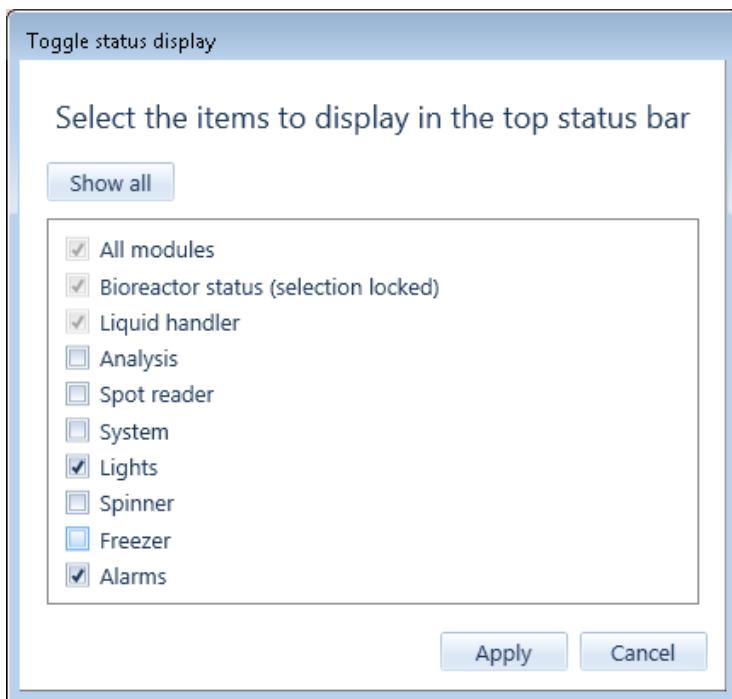


Figure 523 Toggle status display

The **All modules**, **Bioreactor status** and **Liquid handler** items cannot be toggled and are always selected. Individual items can be selected/ de-selected by toggling the checkbox for that item. Pressing the **Show all** button selects all items. **Apply** applies the selection to the status bar.



Figure 524 Status bar with selections applied

24.14 Working Hours

The **Edit working hours** window specifies when to assume that an operator can readily be present.

The working hours are used:

- When deciding whether and to whom to send emails
- When planning when labware should be loaded

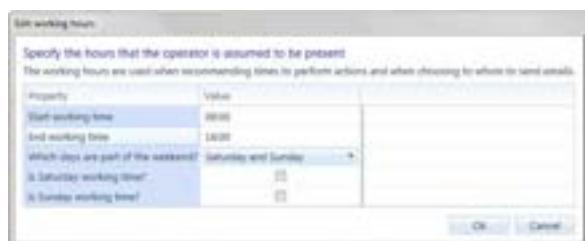


Figure 525 Edit working hours

Start working time and **End working time** specify the start and end of working time on a day.

Which days are part of the weekend? specifies which days should be considered as the weekend when choosing who to send emails to and when to schedule actions.

Is Saturday working time and **Is Sunday working time** specify if Saturday and/or Sunday, or other days specified as the weekend, are to be considered as working days.

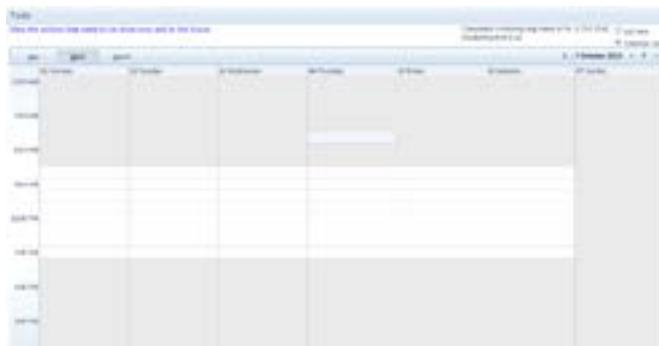


Figure 526 Calendar view showing working hours when **Is Saturday working time** has been selected.

24.15 Passwords

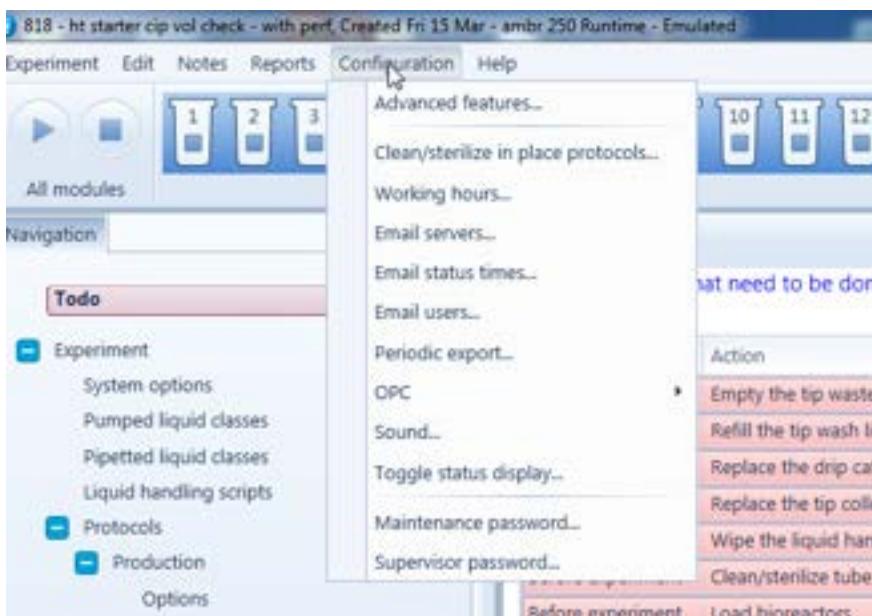


Figure 527 Menu options for editing passwords

Selecting **Maintenance password...** or **Supervisor password...** displays the Edit password window.

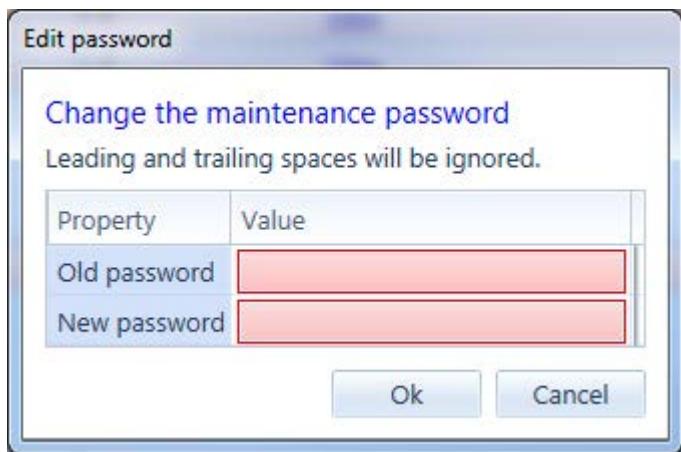


Figure 528 **Edit password** window

Enter the **Old password** and **New password** to change the password.

The passwords are stored in encrypted form in files in a **Passwords** folder within the configuration. Should it be necessary to reset the passwords delete the files in the Passwords folder and restart the software.

The default password for both Configuration and Maintenance is **tap**.

25 TROUBLESHOOTING

25.1 Export log files

The Ambr® 250 software generates log files with details about what the software is doing. In the event of a problem these files may be required to work out what has happened.

The space taken on the disk by these files is limited and old files can be deleted after a small number of days. If a problem occurs it is important to capture the log files before they are deleted.

Log files can be exported using the **Export log files** option on the Help menu.

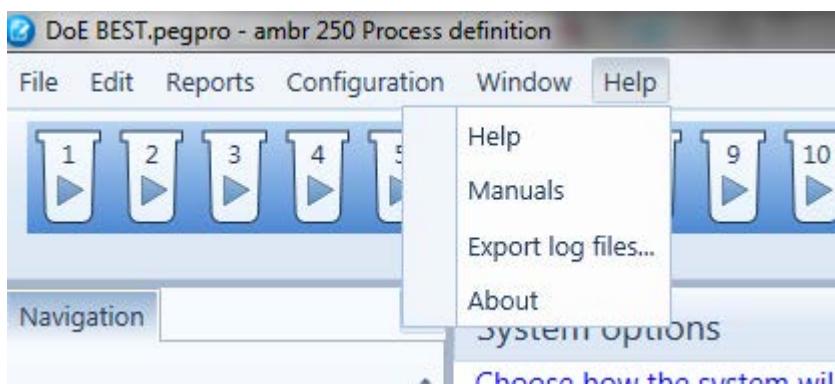


Figure 529 **Export log files** option in definition application.

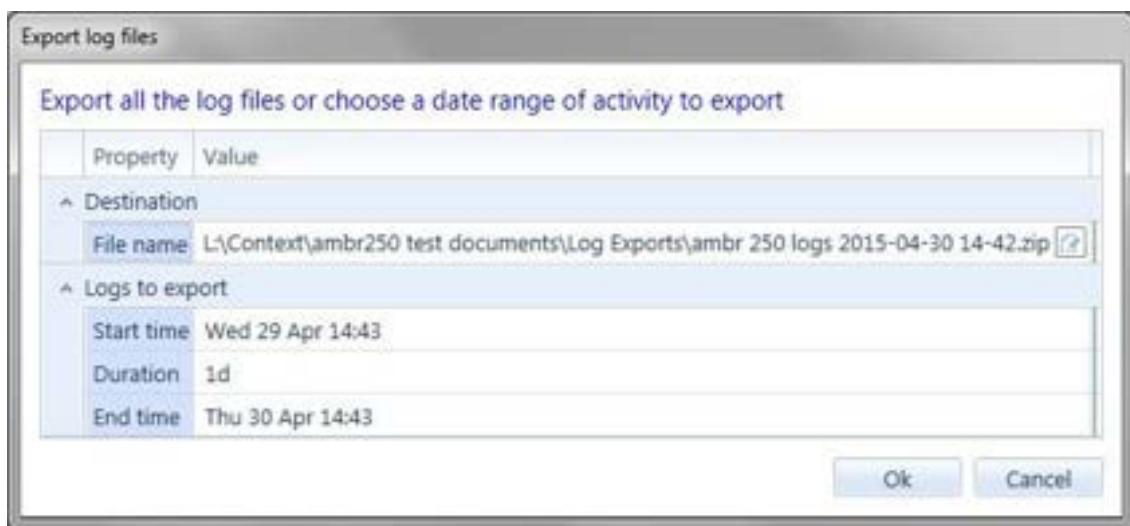


Figure 530 **Export log files** window

File name selects where the log files will be saved.

Start time sets the start of the time for which data will be exported. (Updating the Start time changes the Duration accordingly.)

Duration sets the interval of time over which data will be exported. (Updating Duration changes the Start time accordingly.)

End time sets the end of the time over which data will be exported.

26 MID-RUN PRIMING

Mid-run priming allows pumps to be primed after the bioreactor has been loaded.

Priming consists of filling the tubing leading to the pump and the pump itself with liquid by pumping an excess of liquid through the pump and to waste. The mid-run priming option comprises:

- i) Options in the software to describe switching the liquid from the pump between the bioreactor and waste
- ii) An assembly – the mid-run priming device - that can be inserted into a bioreactor to make the switch



The options for mid-run priming are hidden unless the **Support using mid-run priming device to prime pumps later in the experiment** option is selected in the **Advanced features** window.

Pumps can be set by the **When to prime pump** option on the **Pumps** page to be primed either:

- a) **At start**
- b) **Later**

When **At start** is chosen the pump must be primed before the bioreactor is connected. It is not possible to prime the pump later.

When **Later** is chosen the pump must be primed after the bioreactor is connected. It is not possible to prime the pump earlier.

When a pump has been selected with **When to prime pump** option of **Later** then the following steps are required to use the pump.

- 1) For lines where mid-run priming is to be used insert the mid-run priming device between the liquid manifold and the tubing to the bioreactor.
- 2) Load and connect bioreactor
- 3) Before priming the pump use the **Set for mid-run priming** to make which pumps are set for mid-run priming and make sure that the mid-run priming device is set to direct liquid to waste and not to the bioreactor.
- 4) Use the **Prime pumps** screen to prime the pumps as normal.
- 5) Use the **Set to bioreactor inlet** screen to tell the system that the mid-run priming device has been switched to direct liquid to the bioreactor.
- 6) If **Manual prime** has been selected for the **Priming** option of the pump then use the **Prime bioreactor inlets** page to prime the bioreactor inlet.

Typically steps 3) onwards are triggered in response to a **Prime pump** step included in the process. The **Set for mid-run priming**, **Prime pumps**, **Set to bioreactor inlet** sequence can however be done at any point after the bioreactor has been connected. The sequence can be repeated if additional priming should be required in order to replace the liquid in the tubing or to get rid of bubbles that may have developed in the tubing.

26.1 Mid-run priming device

The mid-run priming device allows liquid to be directed either to the bioreactor or to waste.

26.2 Pump options for mid-run priming

The option that must be selected to use mid-run priming is shown below.

Protocols\Production [Bioreactors 4-12]\Pumps

Define the setup for each pump to be used in the selected protocol.

The protocol will refer to the pump by its role. Optionally the setup can contain an additional description of the liquid attached to each pump for each bioreactor.

The system is configured for 001-5Gox bioreactor vessels. If other bioreactor vessel types are to be used then prime the bioreactor inlets manually.

Property	Value	4	5	6	7	8	9	10	11	12
Pump A										
Role	Feed#1									
Liquid class	Generic low viscosity									
Liquid name										
Remove inlet filter										
When to prime pump	Mid-run									
Priming	Auto prime									
Start pumping back from end by (ml)	0.01									

Figure 531 Pumps screen with option to prime pump mid-run selected

26.3 Prime pump step

The Prime pump step flags that the bioreactor pump should be primed. In response the system indicates that actions are required on the **Set for mid-run priming** page, and once the actions there have been done on subsequent pages.

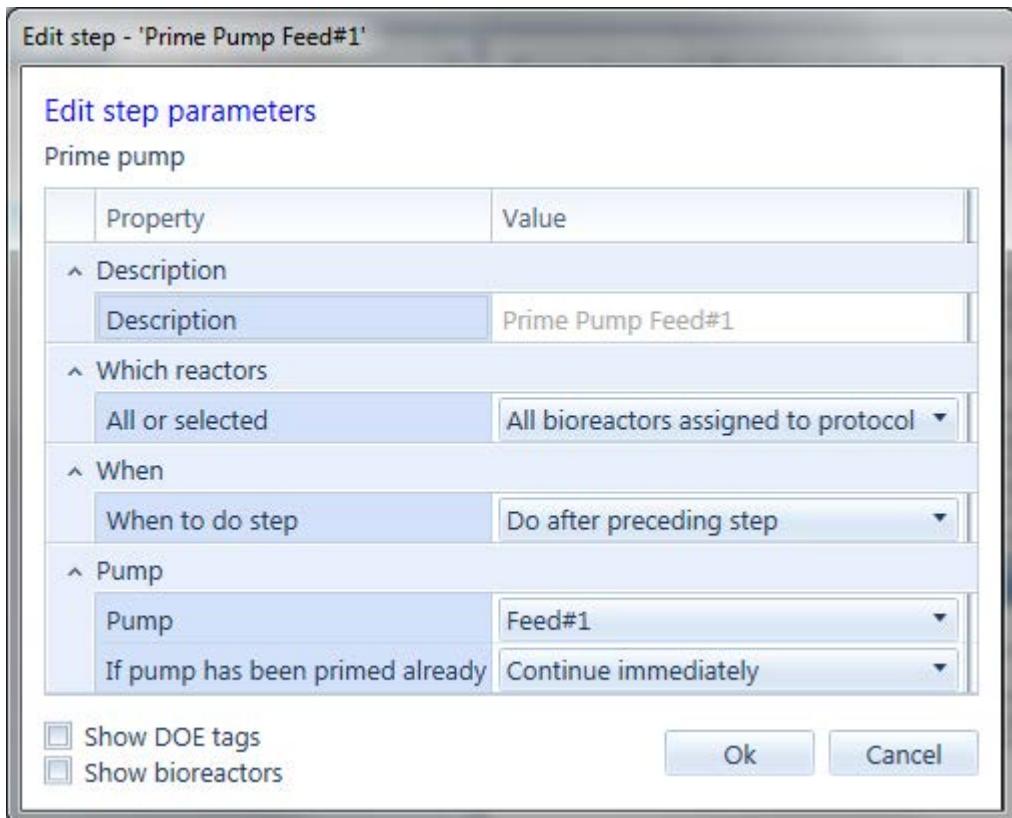


Figure 532 Prime pump step

The **Pump option** selects which pump is to be primed.

If **pump has been primed already** has options:

- **Continue immediately** to continue without doing more priming if the pump has previously been primed.
- **Force re-priming** to force the pump to be re-primed.

26.4 Set for mid-run priming page

The **Set for mid-run priming** page is used to tell the system which pumps have been switched so that liquid goes to waste.

- 1) Select the pumps that are going to be primed.
- 2) Click **Set pumps for mid-run priming**. A confirmation screen is displayed.
- 3) Set the valves for the selected pumps to direct liquid to waste
- 4) Click **Pumps set for mid-run priming** on the confirmation screen to confirm that the action has been done.

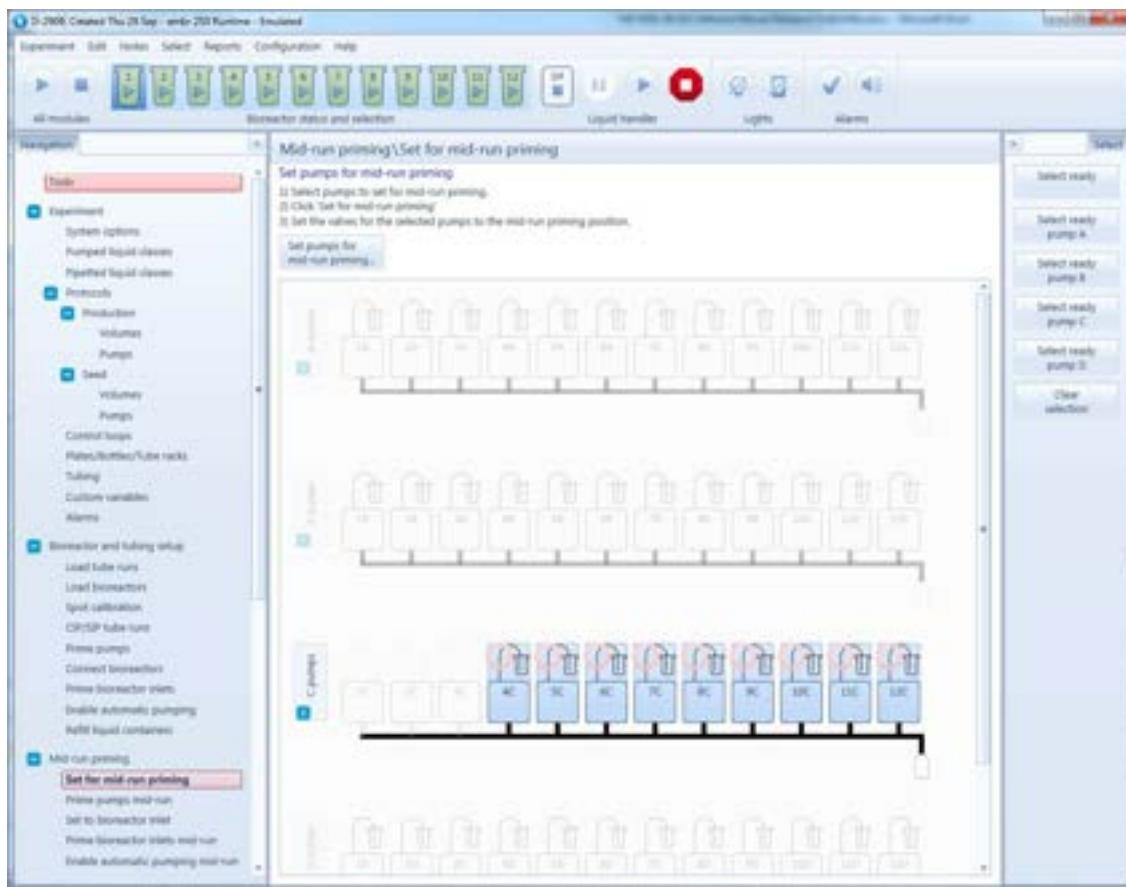


Figure 533 **Set for mid-run priming** page

26.5 Set to bioreactor inlet page

The **Set to bioreactor inlet** page is used to tell the system which pumps have been switched so that liquid goes to the bioreactor.

- 1) Select the pumps that are going to be set so that liquid goes to the bioreactor.
- 2) Click **Set pumps to bioreactor inlet**. A confirmation screen is displayed.
- 3) Set the valves for the selected pumps to direct liquid to the bioreactor
- 4) Click **Pumps set to bioreactor inlet** on the confirmation screen to confirm that the action has been done.

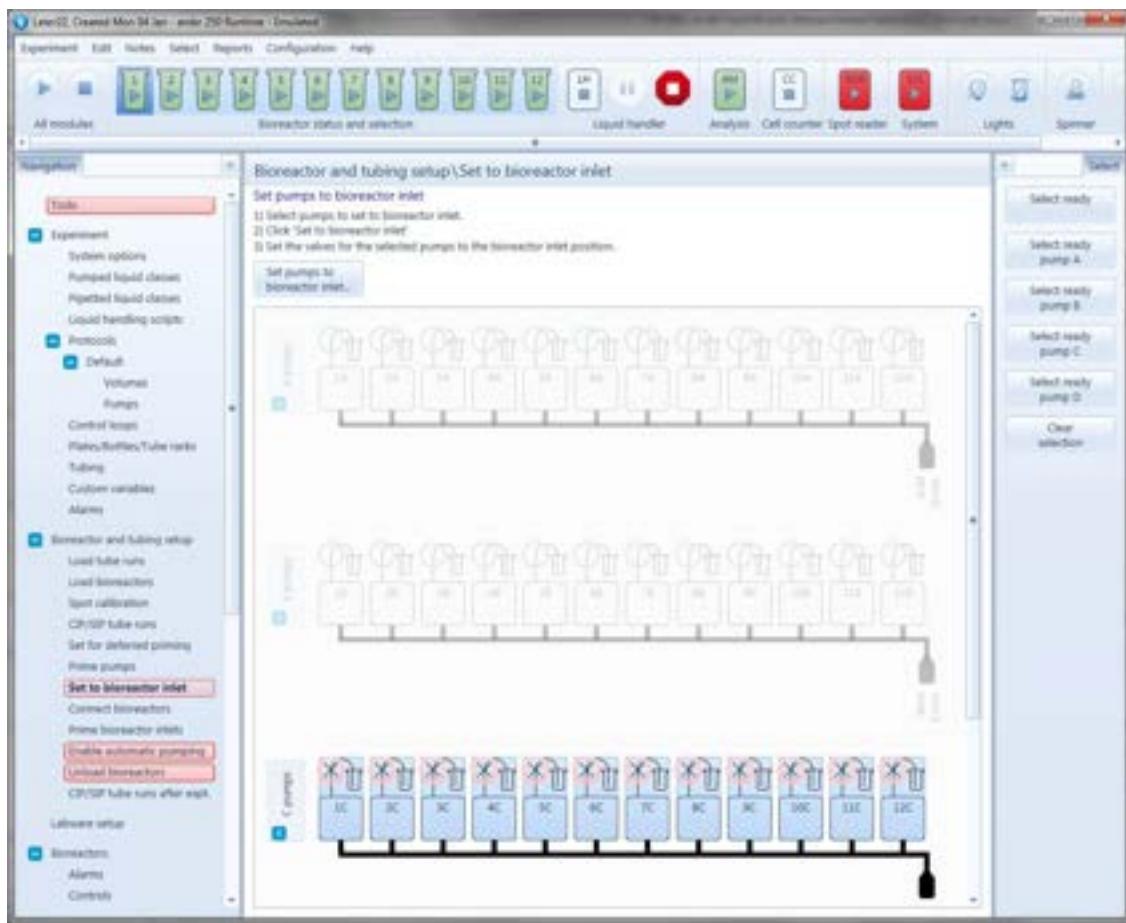


Figure 534 Set to bioreactor inlet page

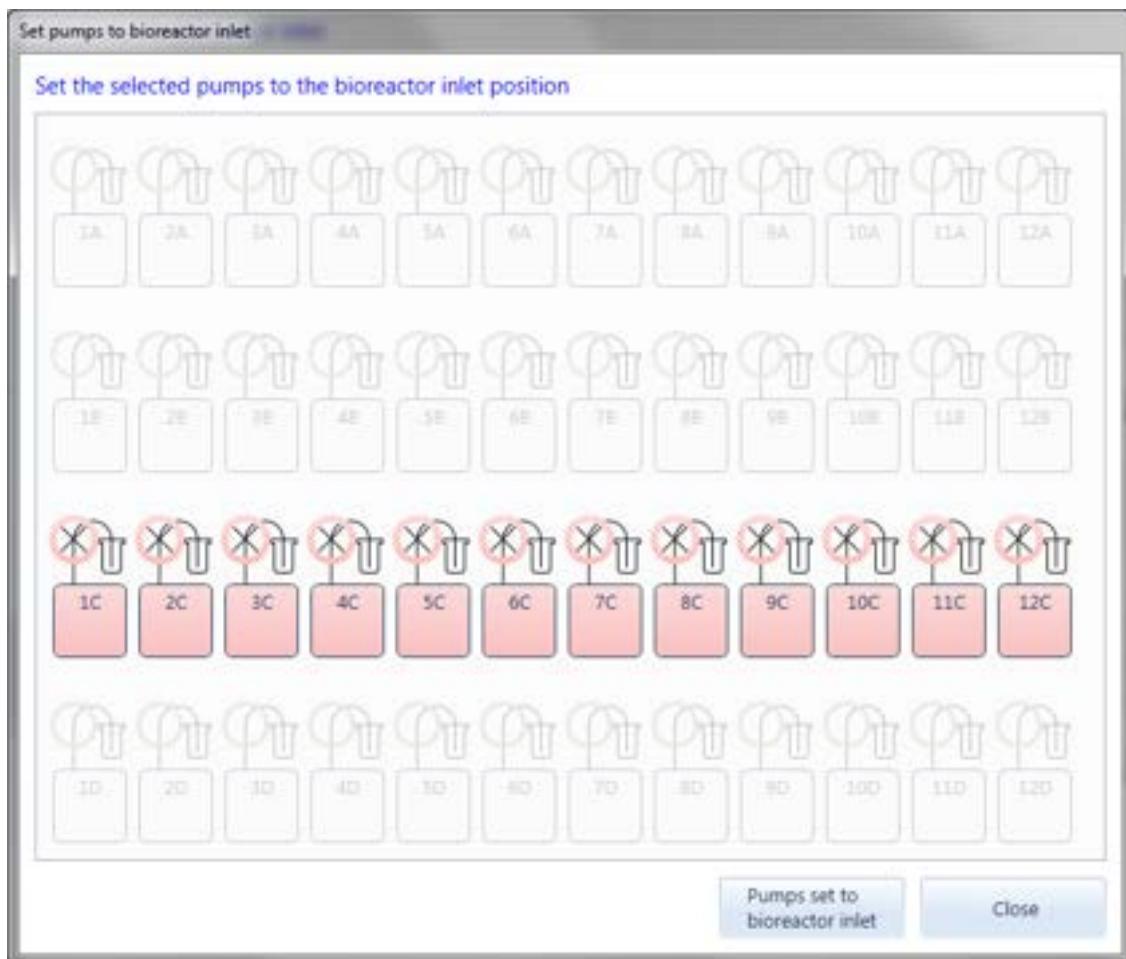


Figure 535 Confirmation page

27 MOCK DATA

Mock data can be injected into the system when it is running in emulation mode. The values of the mock data are then reported by the system as the values measured by the bioreactors or by selected external systems.



To display the mock data options select **Allow entry of mock data** option in the **Advanced features** window.

This feature exposes controls that allow the import or direct entry of mock data in the emulated runtime program. This mock data is then reported back to the runtime. By entering mock data it is possible to test in emulation the responses of control loops, alarms, conditions and other features of the software.

Allowing entry of mock data adds options at the bottom of the **Graphs->Data** tab for:

- **Mock present values**
- **Import mock present values**
- **Clear mock present values**

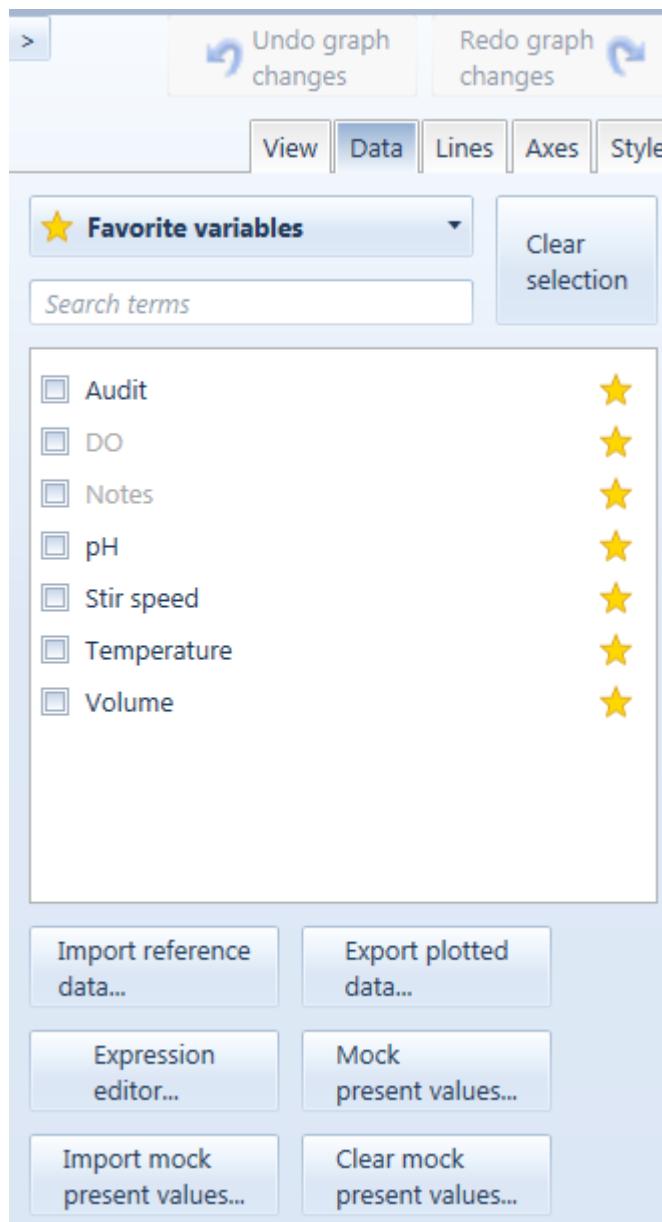


Figure 536 Options for mock data

27.1 Mock present values

Mock present values shows a dialog with the key mockable variables for the currently selected bioreactor. To enter mock values click the checkbox for the relevant value and adjust the current value using the middle text box or the slider.

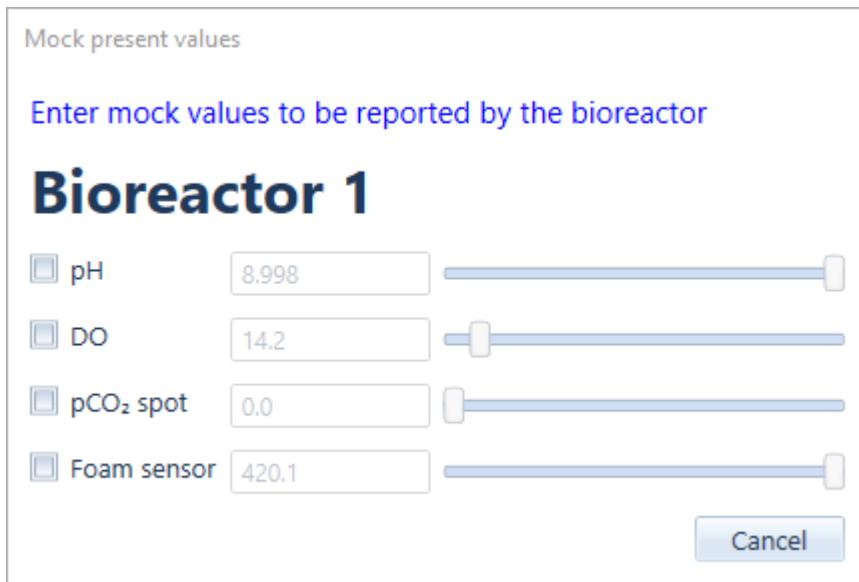


Figure 537 **Mock present values** dialog

Mocking of values ceases when the checkbox is unselected for the relevant variable and bioreactor or when the dialog is closed.

27.2 Import mock present values

Import mock present values displays a dialog that allows importing data to be used as mock values.

The first column in the data must be headed 'Time' or 'Date Time'. The values in the column are adjusted to start from the present time.

The remaining columns should contain the mock data with the name of the variable in the top row of the data.

If both imported mock present values and the **Mock present values** dialog are used then the **Mock present values** dialog values are used and override the imported values.

27.3 Mockable variables report

A report is available that shows the specific variables for which mock data is supported. Specific code in the software checks to see if mock data is present and reports the mock values instead of any other emulated value. To display the report select **Reports->Diagnostics->Mockable variables**.

Note that the data is only reported if the software is looking for the value – for example mock values for DO are only picked up when the spot reader is running.

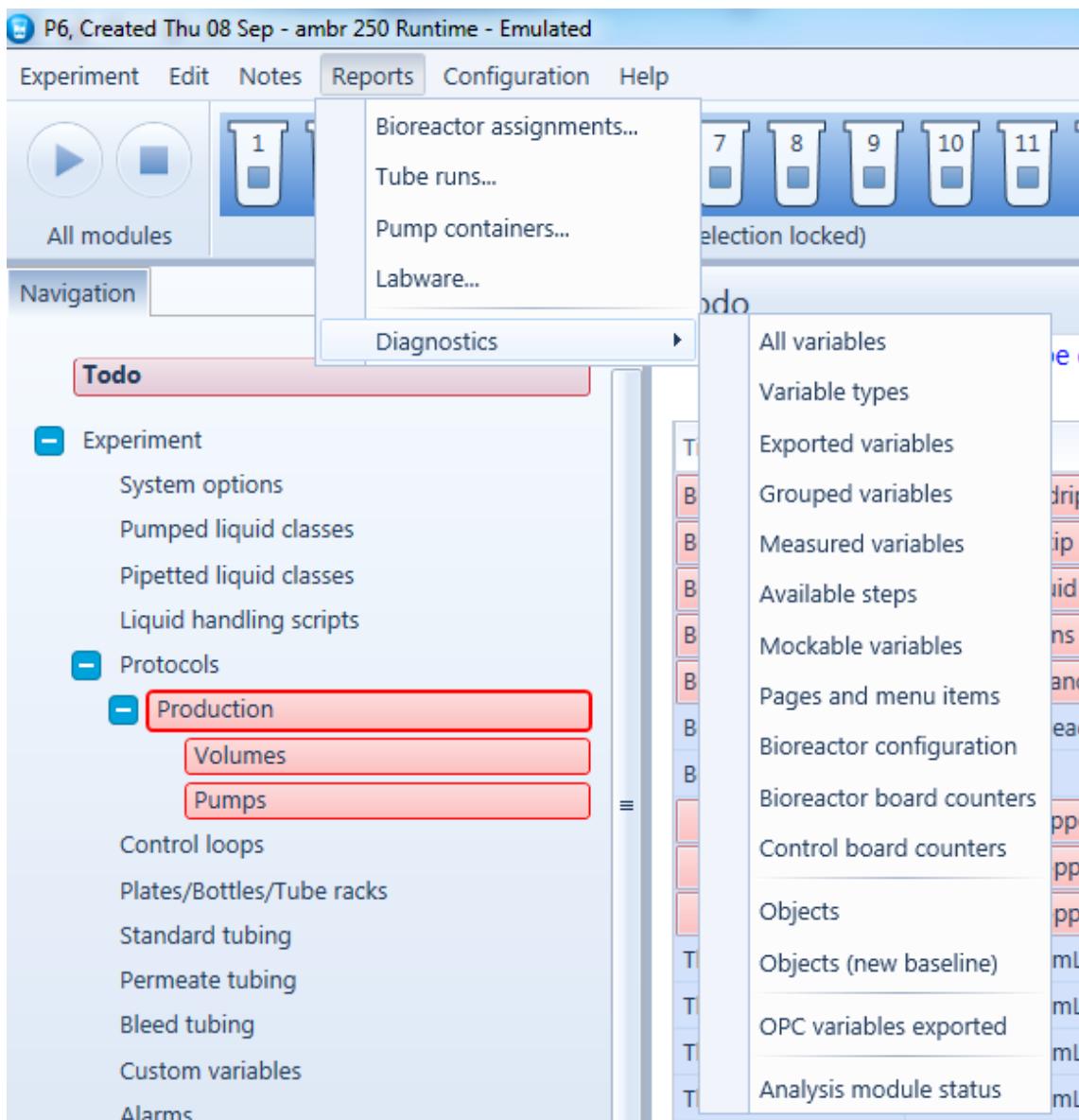


Figure 538 Mockable variables report

In release 8 of the software the following values are mockable.

- Air (headspace) flow
- Air flow
- Average diameter
- Cell density
- Cell viability
- CER
- Clamp plate temperature
- CO₂ (headspace) flow

- CO₂ flow
- Dead cell density
- Dead cells
- DO
- Foam sensor
- N₂ (headspace) flow
- N₂ flow
- O₂ (headspace) flow
- O₂ flow
- Off-gas CO₂%
- Off-gas O₂%
- OUR
- pH
- pH measured
- pH measured (raw)
- Seed - Average diameter
- Seed - Cell density
- Seed - Cell viability
- Seed - Dead cell density
- Seed - Dead cells
- Seed - Total cell density
- Seed - Total cells
- Seed - Viable cell density
- Seed - Viable cells
- Stir speed
- Temperature
- Total cell density
- Total cells
- Viable cell density
- Viable cells
- Volume

28 APPENDIX – COMPENSATION FOR SAMPLING VOLUME

The system now includes an optional compensation that can be applied to reduce pump rates and volumes to more closely match processes in larger bioreactors.

The variable **Fraction of cells remaining** tracks the number of cells remaining in the culture relative to the number of cells that would have been present in the absence of sampling. For example if **Fraction of cells remaining** had a value of 0.5 and a sample of 10% of the culture was taken then the new value would be $0.5 * 90/100 = 0.45$.

Once the bioreactor has been inoculated **Fraction of cells remaining** is updated each time a sample is taken either automatically or manually.

The steps to do pumping can tell the pumps to adjust the volume and rate of pumping by this factor.



Figure 539 Start pump step with **Adjust for sampling** option

Adjust for sampling can have the values:

- **None** – no adjustments for samples are made.
- **Flow rate and volume (sampling since inoculation)** – pumping is reduced in proportion to the cells lost to sampling since the bioreactor was inoculated.
- **Flow rate and volume (sampling after step)** – pumping is reduced in proportion to the cells lost in sampling after the step has started the profile.

29 APPENDIX - TIP WASH BEFORE DISPOSAL OPTION

Systems can optionally be configured so that tips are washed before disposal. This requires additional hardware to be installed into the system and is a chargeable option.

When this option is configured on a system pipetted liquid classes and liquid handling scripts have an option as to whether the system should wash used pipette tips before disposing of them.

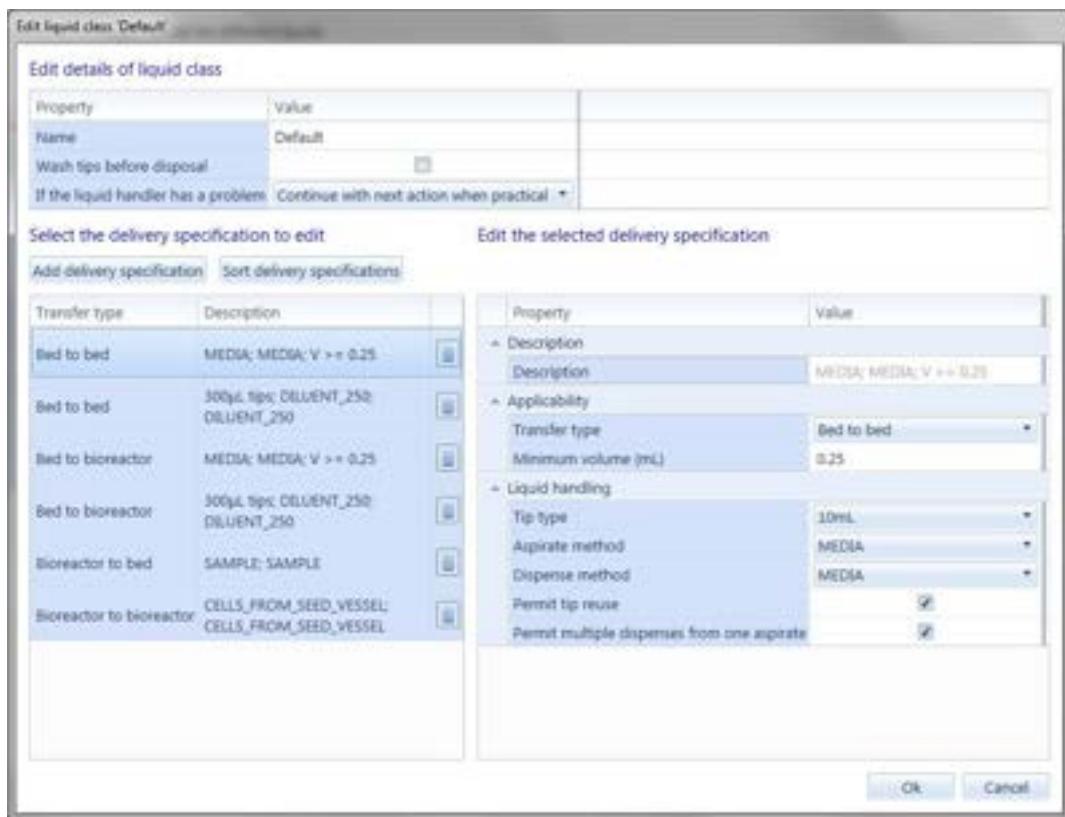


Figure 540 **Wash tips before disposal** option on liquid class

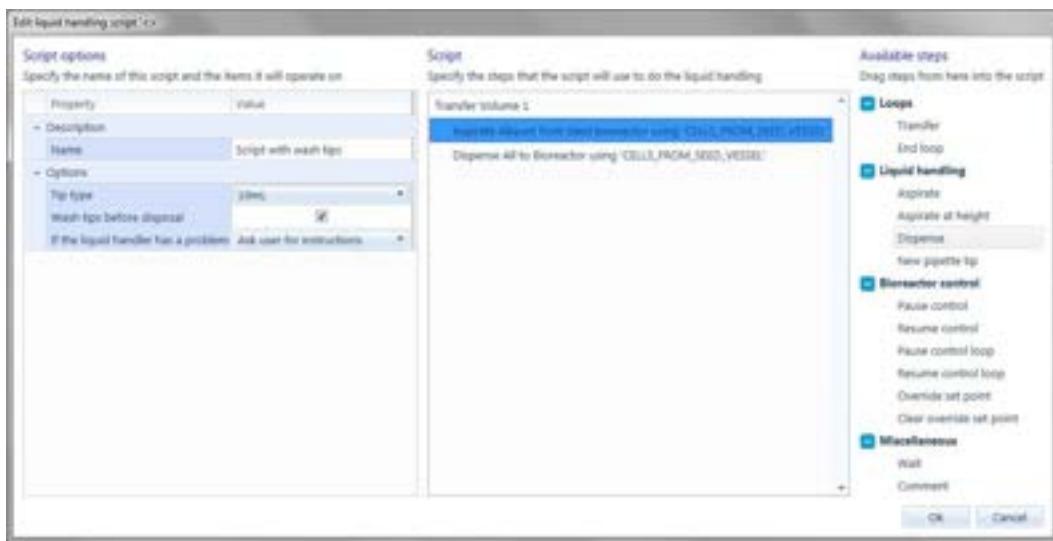


Figure 541 **Wash tips before disposal** option on liquid handling script

When the **Wash tips before disposal** is selected on the pipetted liquid class or liquid handling class being used for a step then before ejecting a tip the liquid handler will:

- Dispense any liquid remaining in the tip into a waste container
- Aspirate and dispense liquid from a wash container.

The liquid handler will also follow this process when disposing of a tip outside of the context of a step.

The system will prompt the user at the start of the process to empty the waste container and refill the wash container.

30 APPENDIX – LIQUID HANDLING METHODS

Modifying the liquid handling methods is considered to be an advanced function and should only be attempted with the support and knowledge of a suitably trained Sartorius staff member.

Methods defined in files with the “.LHScript” file extension are used to control how the liquid handler transfers liquids between bioreactors and plates. This section presents an overview of the methods to facilitate minor changes by the operator; it is not intended as an exhaustive tutorial.

The files reside in the LHSCRIPT directory under the configuration directory.

This directory can have multiple LHSCRIPT files. Each Liquid Handling Script file must have the “.LHScript” file extension.

The contents of each liquid handling script file are case-insensitive and must start with

LHScript

and end with

EndLHScript

Each Liquid Handling Script File can contain multiple Liquid Handling Method Entries.

Each Liquid Handling Method entry must start with

LHMethod

and end with

EndLHMethod

The Liquid Handling method Entry needs to have a number of parameters on the same line, in the correct order.

Type of Method (Dispense or Aspirate)

Then the entry Standard

Then the name of the Method, e.g., Sample

And then finally an optional description entry that starts with / and has no spaces, e.g.

/This_is_a_sample_dispense_it_used_to_transfer_liquid_from_a_vessel_to_a_plate.

This is displayed to the user when they are selecting the method from a list (the _ are replaced by spaces when displayed to the user)

Within the description some special codes can be used:

- [TIPCAPACITY300ONLY] can be used to specify that this method is only for 300 µL tips.
- [TIPCAPACITY10000ONLY] can be used to specify that this method is only for 10 mL tips.
- [EXTRAVOLUMENNN] can be used to specify that the method uses some of the tip capacity internally. NNN should be the volume in uL that is not available for general use in the tip. The liquid handler will use more tips as required to transfer the overall volume requested.

- [OVERASPIRATENNN] can be used to indicate that the method aspirates more liquid than it is asked to. NNN should be the additional volume of liquid in uL that ends up in the tip. This volume is used to adjust updates to the volume of liquids in bioreactors and labware.

Any text after the comment character ';' is ignored.

A method may therefore begin

```
LHMethod Aspirate Standard My_Name /Display_Text ; some comments
```

Comment lines beginning with a semicolon (;) may appear anywhere in the file (including within a method); the entire line is ignored by the system

Each line in the Liquid Handling method (between LHMethod and EndLHMethod) is a command used to control either the movement of the XYZ Robot or the syringe pump. Commands are executed sequentially from the top of the method to the bottom. They begin with a command word and may be followed by one or more parameters depending on the command. Multiple parameters may be specified (in any order) per command. Where units are supported they are optional but are encouraged to aid readability.

30.1 Move commands

- Top – Move the Pipette tip to the top and centre of the plate/bioreactor.
- Move to Top – Same as Top. “Move To” is not needed but adds clarity to the method.
- Bottom – Move the Pipette tip to the bottom and centre of the plate / bioreactor.
- Move to Bottom – Same as Bottom. “Move To” is not needed but adds clarity to the method.
- Height – Move the Pipette tip to the height specified by the calling context above the bottom and centre of the plate / bioreactor. If no height was specified then behaves as Bottom
- LiquidLevel – Move the Pipette tip to the nominal level of the liquid in the vessel. Can only be used with labware where the geometry defines the shape of the vessel otherwise the software will report an error during the liquid handling.
- Clear – Move the Pipette tip to a clear height above the top and centre of the plate / bioreactor.
- Move to Clear – Same as Clear. “Move To” is not needed but adds clarity to the method.

The move commands take the following parameters which may be used in any order.

ZOFFSET nnmm Used in conjunction with Top, Bottom and Clear. To move the pipette tip up or down a number of mm. (positive is up, negative is down). Substitute the desired value for **nn**. Units are mm.

XOFFSET nnmm Used in conjunction with Top, Bottom and Clear. To move the pipette tip left or right a number of mm. (positive is right, negative is left). Substitute the desired value for **nn**. Units are mm.

YOFFSET nnmm Used in conjunction with Top, Bottom and Clear. To move the pipette tip forward or backwards a number of mm. (positive is forward, negative is backwards). Substitute the desired value for **nn**. Units are mm.

Speed ***nn***mm/sec Used in conjunction with Top, Bottom and Clear. To control how fast the pipette tip moves. Substitute the desired value for ***nn***. Units are mm/sec.

30.2 Syringe commands

- Spit – Has a parameter that represents the number of micro litres to dispense
- Suck – Has a parameter that represents the number of micro litres to aspirate
- SpitAll – Dispenses all the liquid in the tip

The syringe commands take the following parameters.

Rate ***nn***ul/sec Used in conjunction with Spit, Suck and SpitAll to control how fast the dispense or aspirate is performed. Substitute the desired value for ***nn***. Units are ul/sec.

VOLUME Used in conjunction with Spit and Suck, this is the volume to dispense or Aspirate. Units are ul. No value is supplied here, since the value is retrieved from the higher-level software.

nnul Used in conjunction with Spit and Suck, this is the explicit volume to dispense or Aspirate in ul. Units are **pl**.

VOLUMEOFFSET Used in conjunction with Spit VOLUME and Suck VOLUME to alter the volume to dispense or aspirate from the value passed in from the higher-level software.

30.3 Miscellaneous commands

- Pause – Has a parameter that represents the number of seconds to pause e.g.
Pause 23seconds

30.4 Stir commands

The stir commands modify the normal stir speed of the bioreactor until the next stir command or the end of the method.

- Stop_Stirring – Stops or reduces stirring within the bioreactor. If the command is followed by the maximum stir speed then the stir speed in the bioreactor will be limited to the specified value, otherwise stirring in the bioreactor will be stopped.

e.g.

Stop_Stirring 150

- Set_Stirring NNN – Sets the stirring speed within the bioreactor.
- Resume_Stirring – Resumes normal stirring within the bioreactor.

30.5 Bed stirrer commands

The spinner commands modify the normal stir speed of the magnetic bed stirrer until the next spinner command or the end of the method.

- Stop_Magnetic_Spinner – Stops or reduces the speed of the bed stirrer. If the command is followed by the maximum stir speed then the speed of the bed stirrer will be limited to the specified value, otherwise stirring of the bed stirrer will be stopped.

e.g.

```
Stop_Magnetic_Spinner 150
```

- Set_Magnetic_Spinner NNN – Sets the stirring speed of the bed stirrer.
- Resume_Magnetic_Spinner – Resumes normal stirring of the bed stirrer.

30.6 Example

```
LHMethod Dispense Standard Sample /Transfer_liquid_from_a_vessel_to_a_plate.
```

```
Move to Top ZOFFSET 0mm
```

```
Bottom ZOFFSET 1mm Speed 15mm/sec
```

```
Spit VOLUME Rate 25ul/sec
```

```
TOP ZOFFSET 0mm
```

```
Move to Clear Speed 50mm/sec
```

```
EndLHMethod
```

```
LHMethod Aspirate Standard Sample /Transfer_liquid_from_a_vessel_to_a_plate.
```

```
Move to Top ZOFFSET 0mm
```

```
Move to Bottom ZOFFSET 1mm
```

```
Suck VOLUME Rate 25ul/sec
```

```
Move to Clear Speed 50mm/sec
```

```
EndLHMethod
```

31 APPENDIX – EDITING LABWARE GEOMETRIES

Labware geometries can be viewed and new geometries created from the **Geometry** tab in the **Liquid handler\Maintenance** page.

The geometry wizard can help to enter the data required to define the geometry of a plate or bottle. Consideration also needs to be given to how the plate or bottle will be supported in a consistent position and to whether there are any other obstacles to reliable operation with a given type of labware. If in doubt consult an application specialist for help.

Errors in the geometry will cause liquid handling to fail



If the geometry for labware is incorrect the liquid handler may fail to perform the desired operation and may collide with other elements of the system – potentially damaging them or itself.

The screenshot shows the software interface for managing labware geometries. The main window title is "Run X, Created 14/10/2018 - under 200 Runtime - Emulated". The menu bar includes Experiment, Edit, Notes, Reports, Configuration, Help. The toolbar has icons for Run, Stop, Pause, and various system controls. The left sidebar has sections for Labware setup (e.g., Load tube racks, Set for deferred priming), Bioreactors (e.g., Prime bioreactor inlets, Connect bioreactors), and a section for Labware settings. The central panel is titled "Liquid handler\Maintenance" and "Liquid Handler maintenance". It has tabs for Log, App/Dsp, File, Calc, Links, Locations, Navigate, System Tools, and Geometry. The Geometry tab is active, showing a table of existing geometries. The table columns are Type, Name, Wells, Volume (ml), Dead volume (ml), Max. height, and Last updated. The data in the table is as follows:

Type	Name	Wells	Volume (ml)	Dead volume (ml)	Max. height	Last updated
8 well plate	CELLCOAT 8 WELL	8	2	0		13/05/2018 01:54
8 well plate	SIRK TUBE RACK	8	40	0		25/06/2018 10:45
96 well plate	TAP 96 WELL PLATE	96	0.5	0		25/06/2018 10:45
24 well plate	CMV CENTRIFUGE TUBE RACK	24	12	0		25/06/2018 09:51
24 well plate	CELL COUNTER 24 CUP HOLDER	24	2	0		25/04/2018 09:51
Large bottle	1L BOTTLE WITH FLEA	1	1000	215		25/04/2018 05:51
Small bottle	15ML BOTTLE WITH FLEA	1	1.75	27		25/04/2018 05:51
Small bottle	15ML BOTTLE	1	1.75	11		25/04/2018 05:51
24 well plate	ZMV CENTRIFUGE TUBE RACK	24	2	0		25/06/2018 09:51
Large bottle	1L BOTTLE	1	1000	40		25/04/2018 05:51
24 well plate	TAP 24 WELL PLATE	24	8	0		25/04/2018 05:51

Below the table are buttons for "Create geometry" (with arrows pointing to it) and "Edit geometry" (with arrows pointing to it). To the right of the table is a vertical toolbar with "Delete geometry" (with an arrow pointing to it).

Geometries are shown in last updated order by default. The table can be sorted by selecting the column header or filtered by selecting the button.

The edit button on the summary table allows geometries to be modified. When editing predefined Sartorius geometries only the dead volume can be modified.

The delete button on the summary table allows user defined geometries to be deleted. Custom geometries can only be deleted if they are not defined in the process.

New custom geometries can be created by selecting the appropriate button in the Create geometry panel.

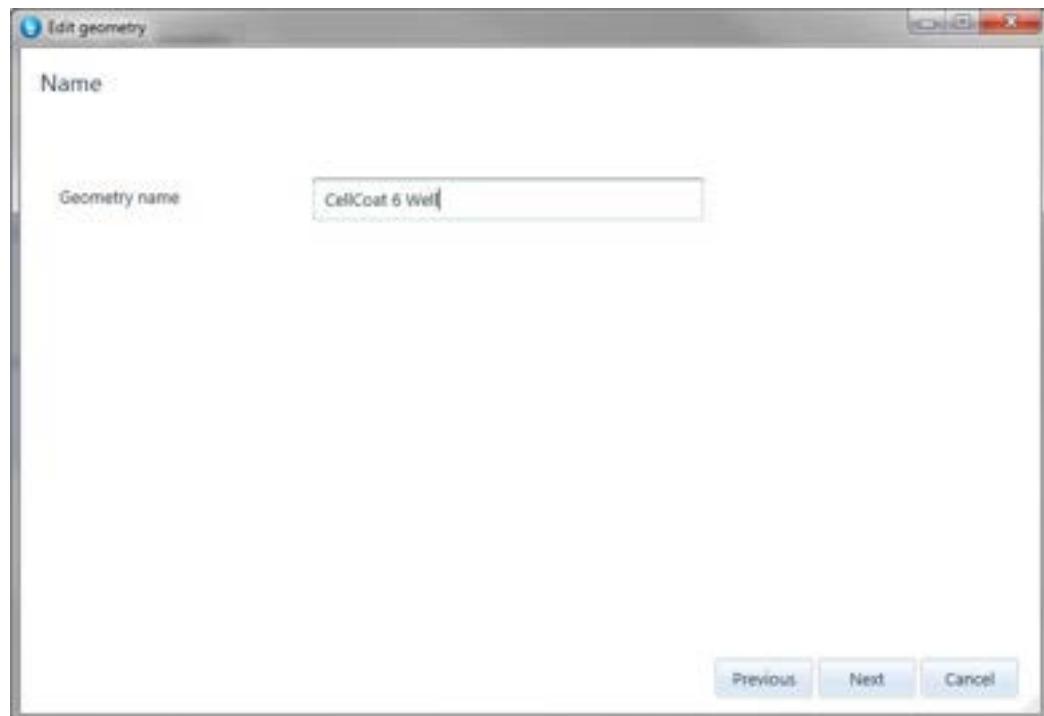
31.1 Plate geometry definition

To create a new geometry definition select **1 well plate**, **4 well plate**, **6 well plate**, **12 well plate** or **24 well plate** from the **Create geometry** panel. The plate wizard will guide you through the process of creating a new plate. Pressing **Cancel** at any point in the wizard will abort the creation of the new plate geometry. Use the **Next** and **Previous** buttons to navigate forwards and backwards through the wizard dialogs

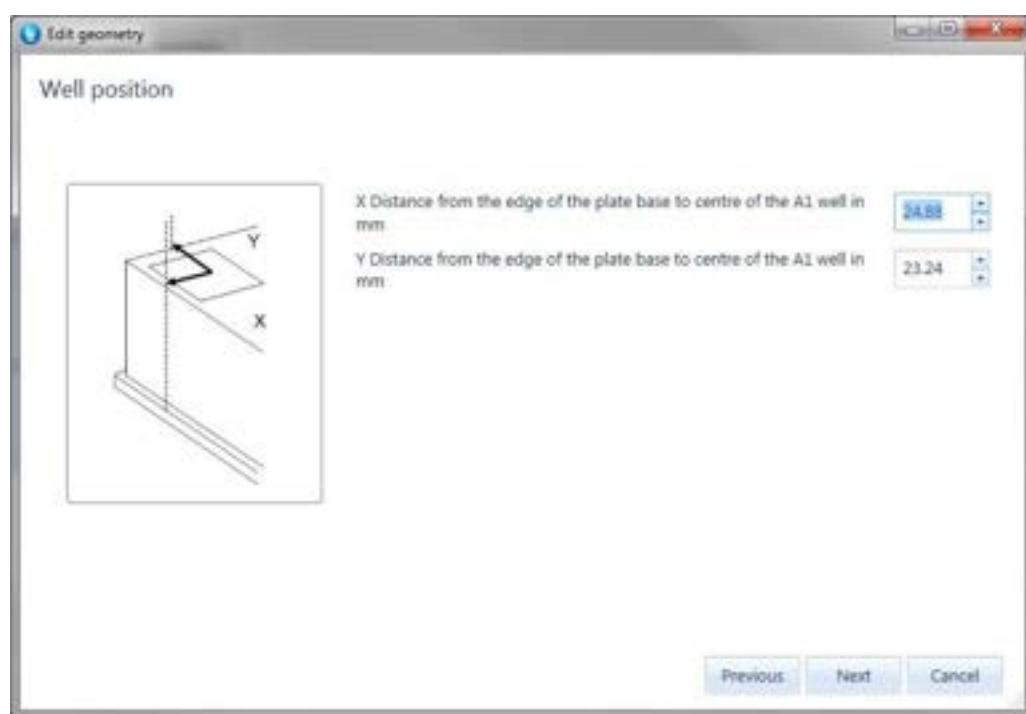
- 1) Confirmation of the plate geometry that is being created.



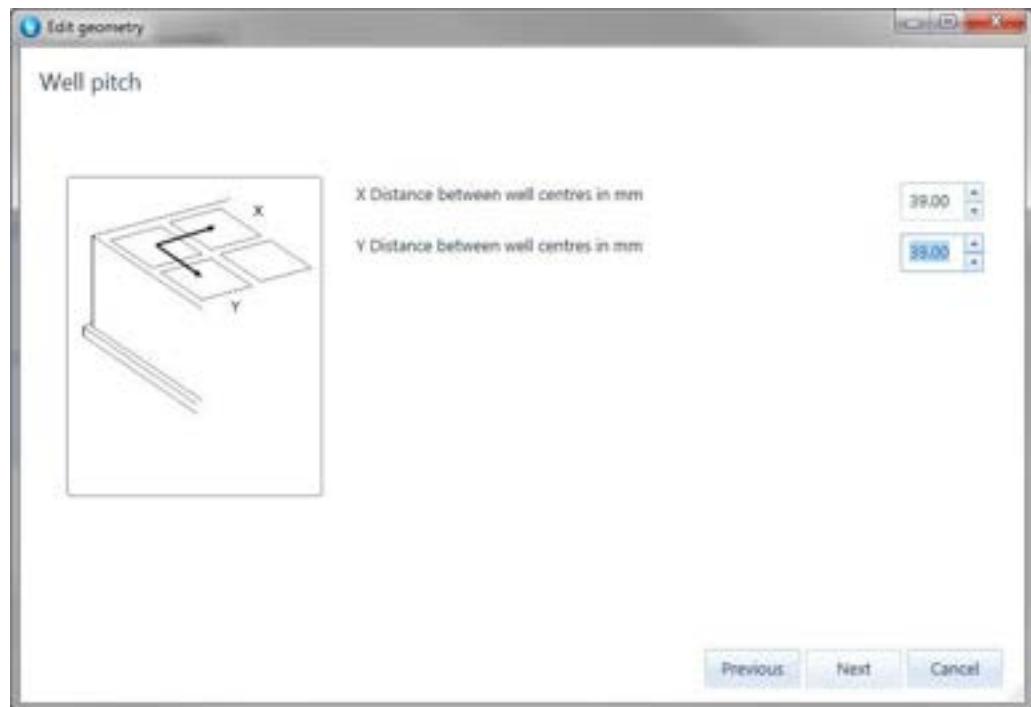
- 2) Enter the name of the geometry



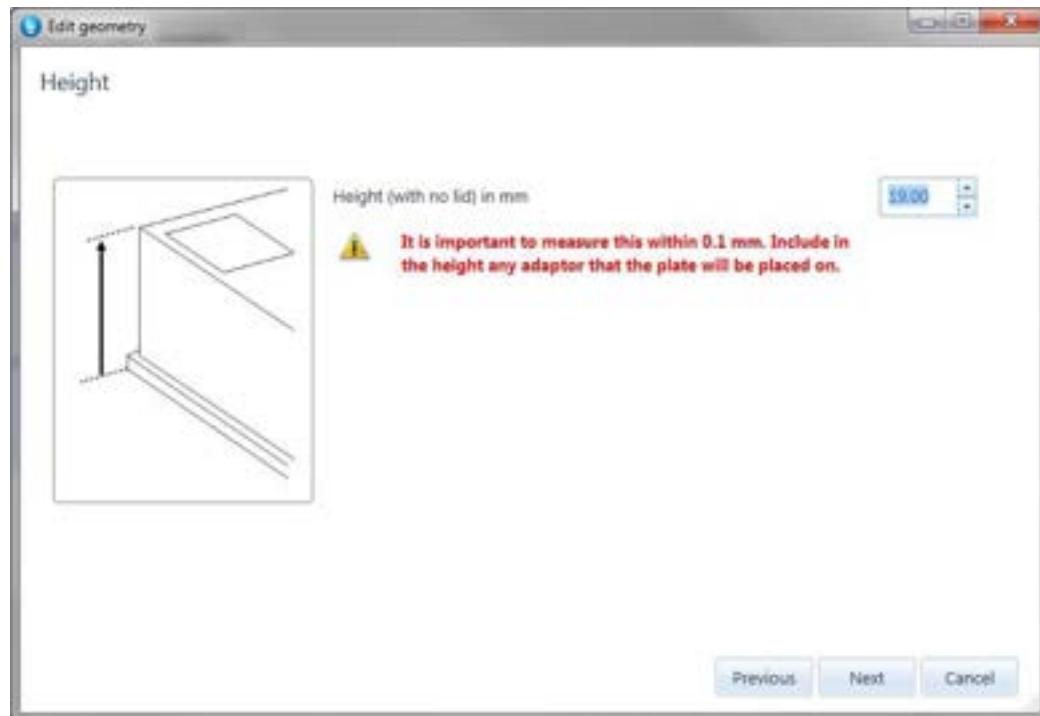
- 3) Enter the A1 well offset



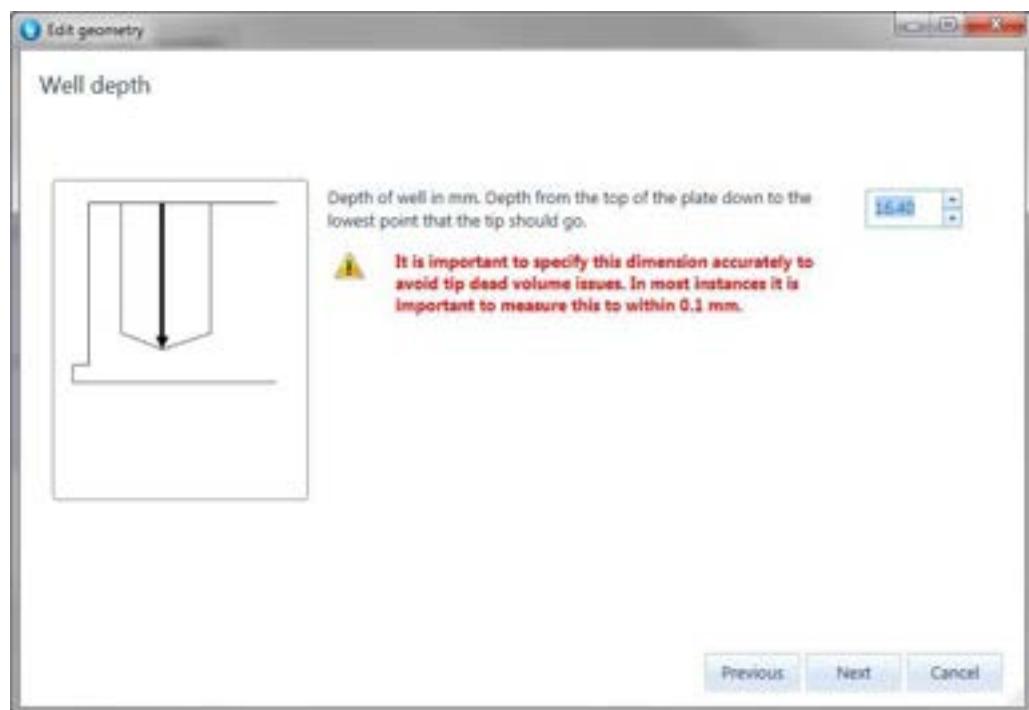
4) Enter the well pitch



- 5) Enter the height of the plate. The height of the plate adaptor supplied with the system is 5mm.



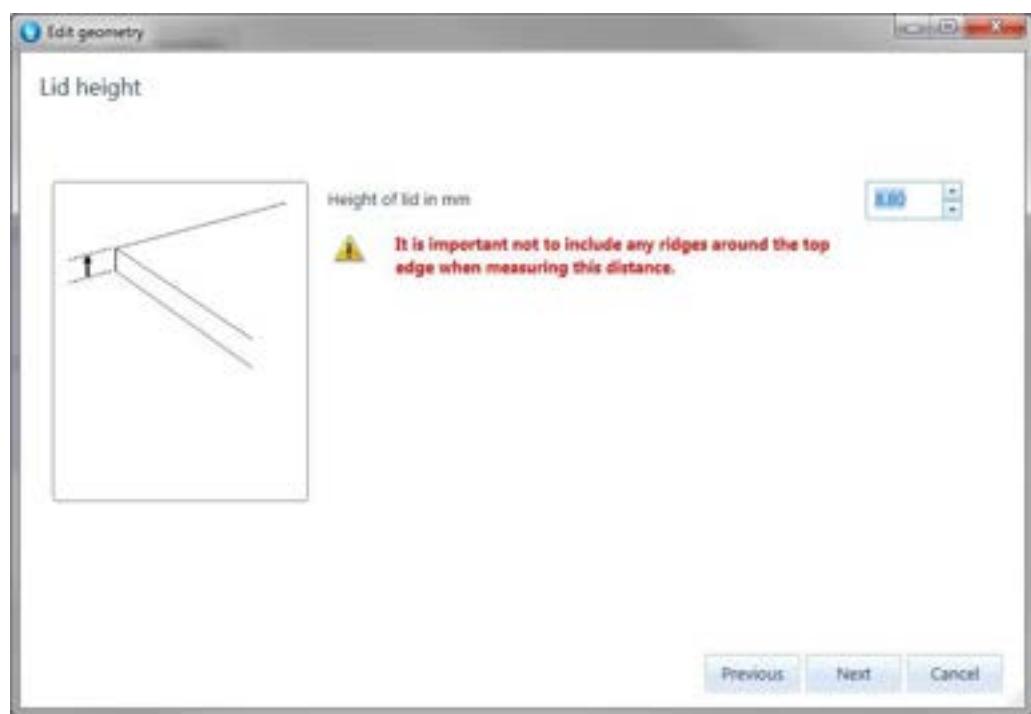
- 6) Enter the well depth. The tip should not touch the plate at its lowest point.



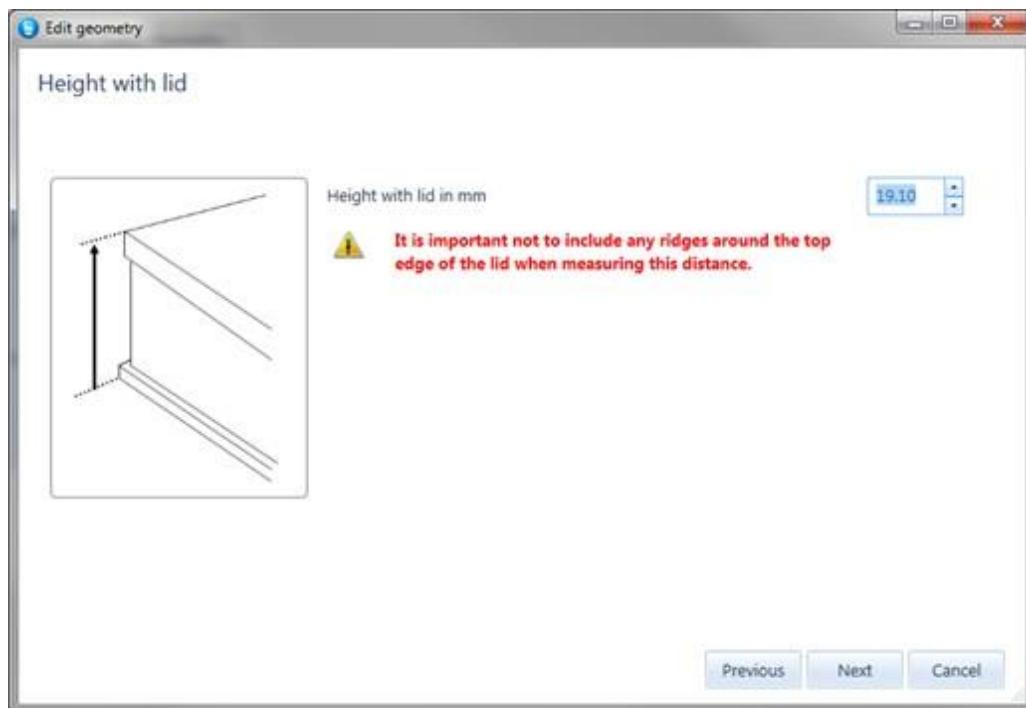
- 7) Select the appropriate radio button. If the plate does not have a lid the **Lid height** and **Height with lid** dialogs are skipped.



- 8) Enter the height of the plate lid.



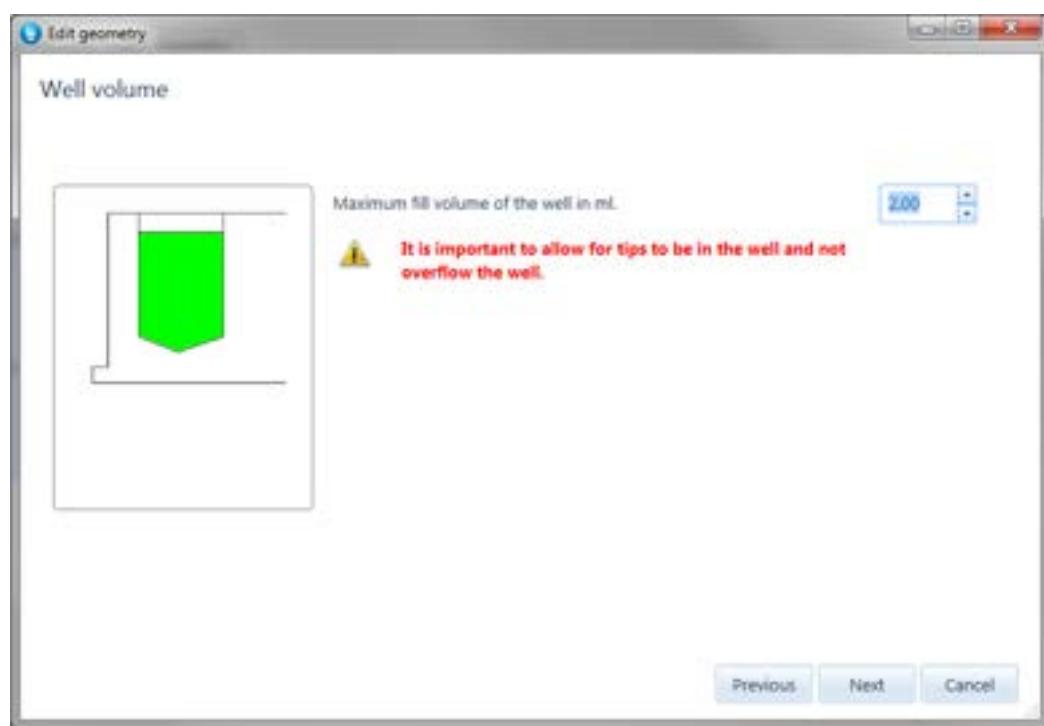
- 9) Enter the height of the plate with the lid on. The height should include the adaptor plate if it is being used with the plate.



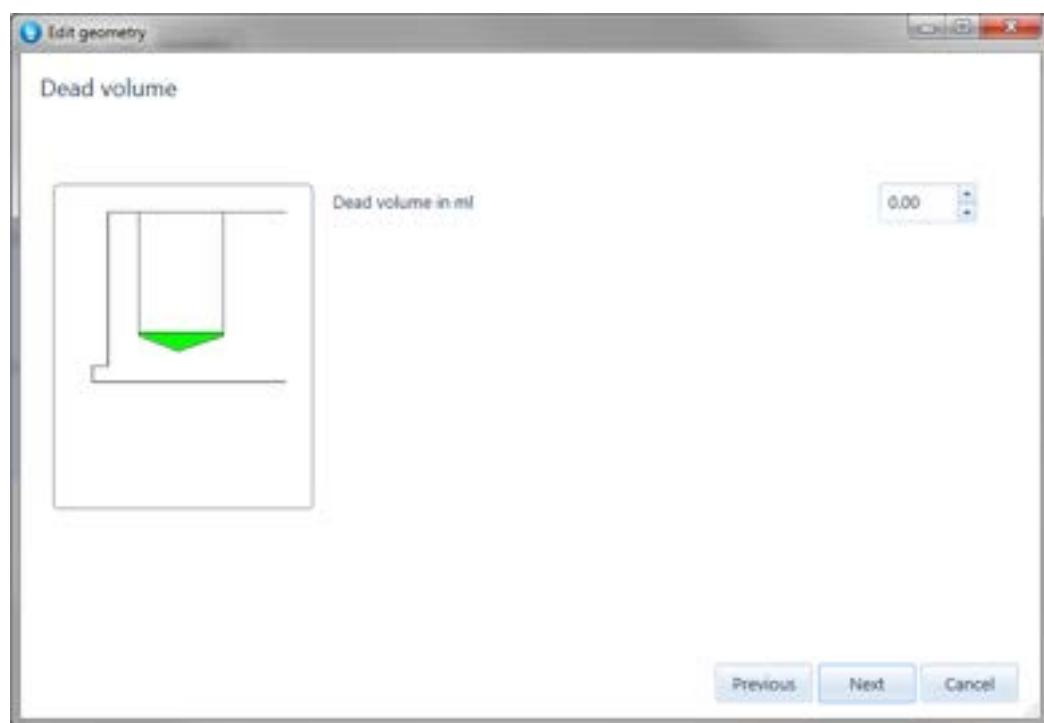
- 10) Specify the tip types(s) that are to be used with the plate.



- 11) Enter the maximum fill volume for a well. If the plate could be used as liquid source the volume should account for the volume of liquid that will be displaced by an empty tip and the volume reduced accordingly.



- 12) Enter the dead volume for the plate – that is the volume of liquid that cannot be removed using the liquid handler. If the plate is only to be used for sampling this volume can be zero.



- 13) Summary dialog displaying the properties of the plate that is about to be created. Press the **Finish** button to create the new plate geometry.



- 14) Labware summary table showing the newly created plate geometry. Editing the geometry will invoke the creation wizard but with the existing values filled in.

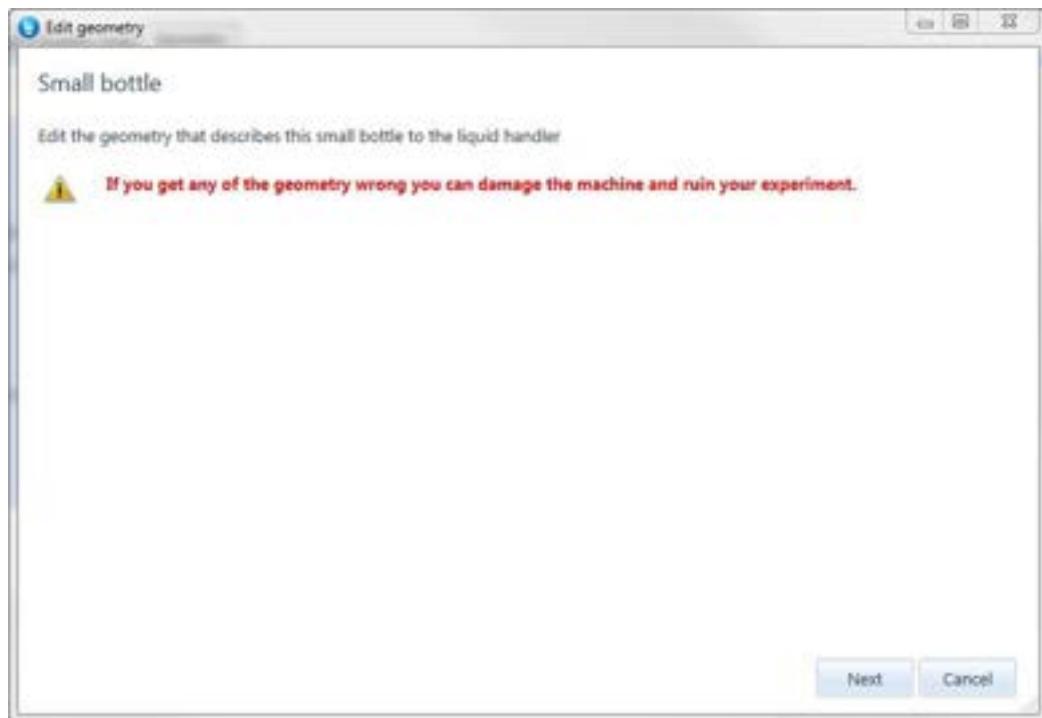
Type	Name	Wells	Volume (ml)	Dead Volume (ml)	Has lid	Last updated	
6 well plate	CELLCOAT 6 WELL	6	2	0	✓	13/07/2016 01:14	
6 well plate	50ML TUBE RACK	6	45	0	✓	21/06/2016 10:45	
96 well plate	TAP 96 WELL PLATE	96	0.5	0	✓	21/06/2016 10:45	
24 well plate	15ML CENTRIFUGE TUBE RACK	24	11	0	✓	20/04/2016 05:53	
24 well plate	CELL COUNTER 24 CUP HOLDER	24	2	0	✓	20/04/2016 05:53	

31.2 Bottle geometry definition

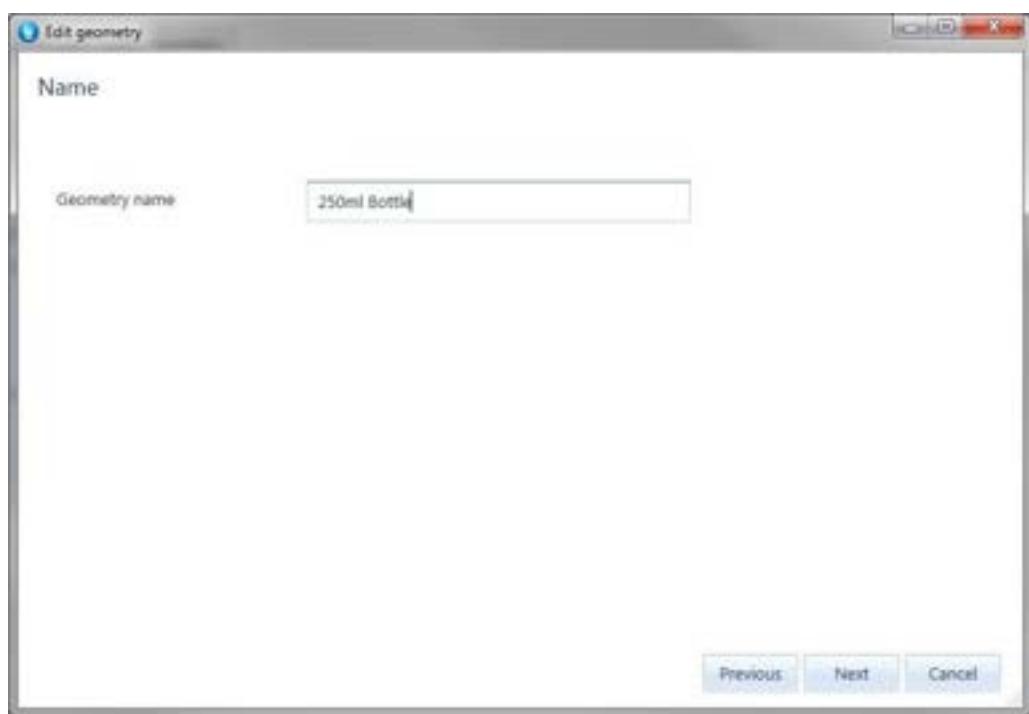
To create a new bottle geometry wizard select **Small bottle** or **Large bottle** from the **Create geometry** panel. The bottle wizard will guide you through the process of creating a new bottle.

Press the **Cancel** button at any point in the wizard to abort the creation of the new plate geometry. Use the **Next** and **Previous** buttons to navigate forwards and backwards through the wizard dialogs

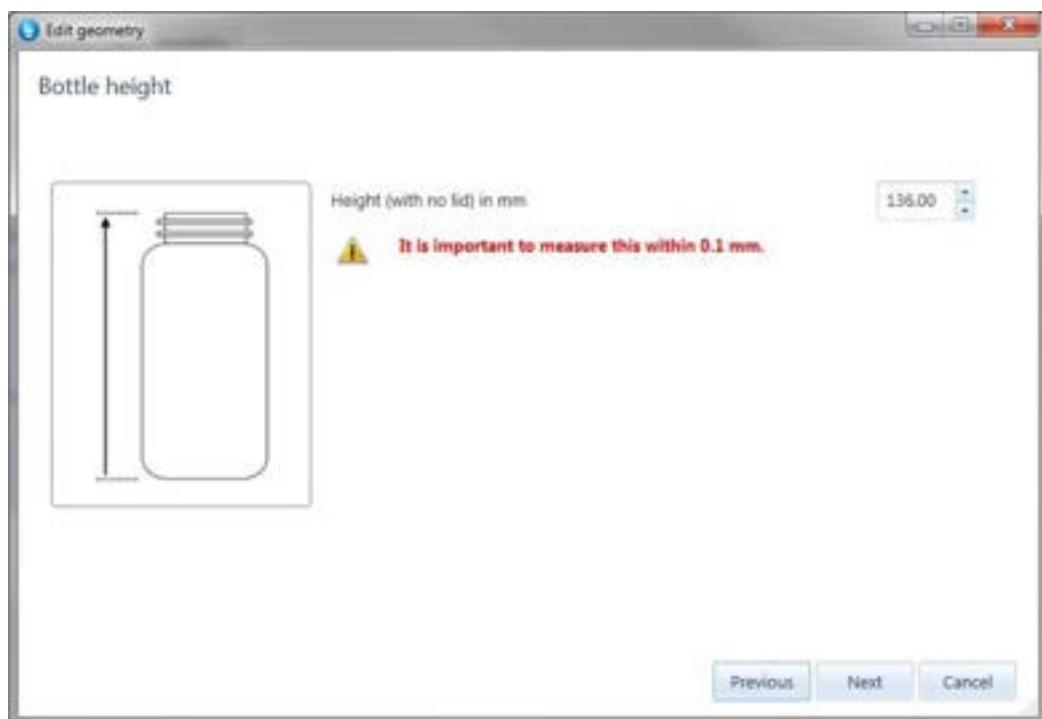
- 1) Confirmation of the bottle geometry that is being created.



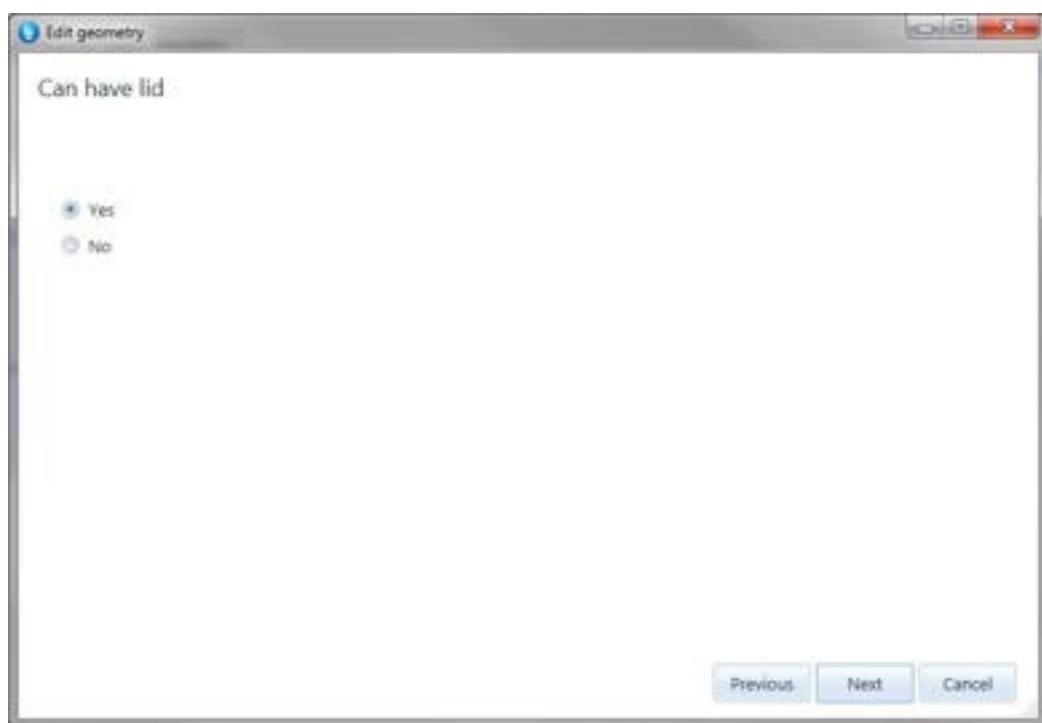
- 2) Enter the name of the geometry.



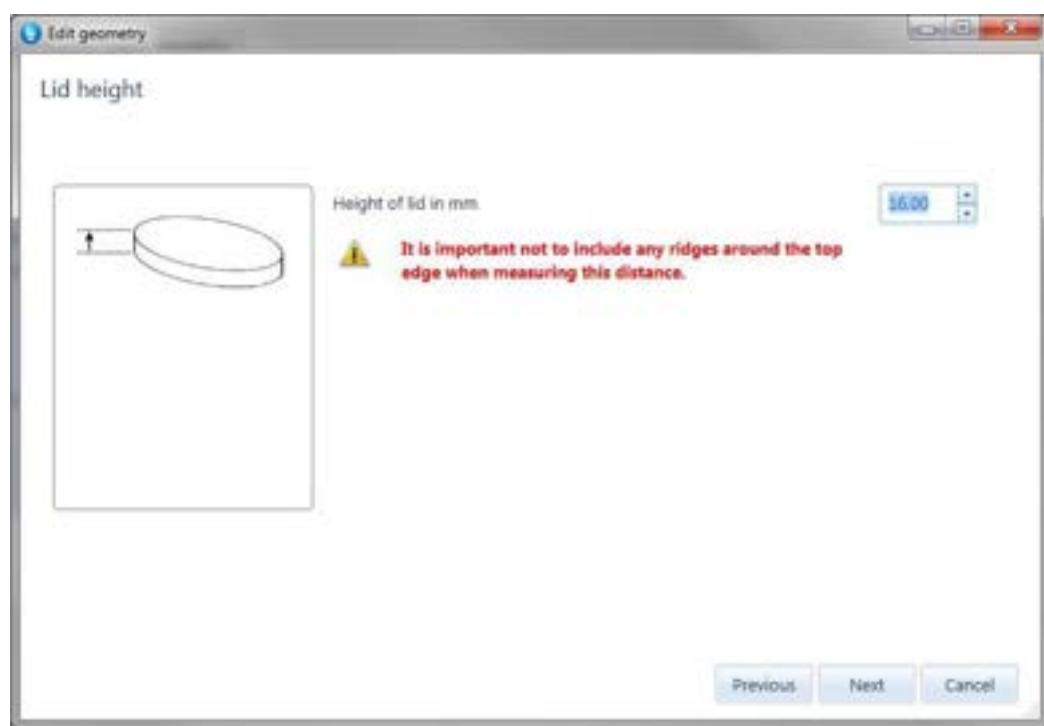
- 3) Enter the height of the bottle.



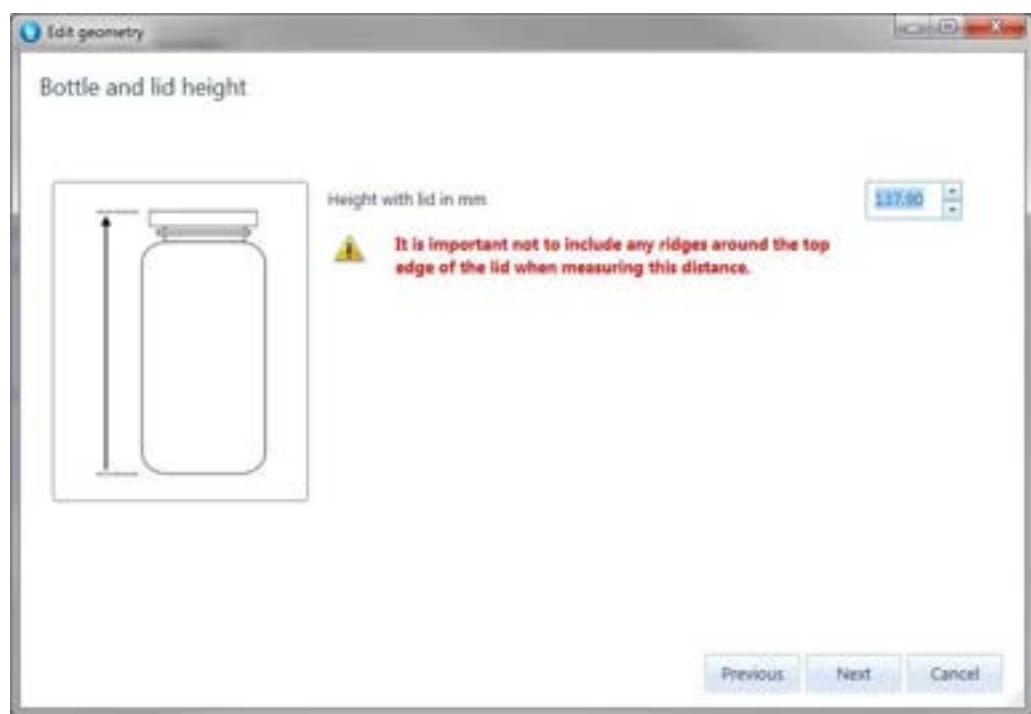
- 4) Select the appropriate radio button. If the bottle does not have a lid the **Lid height** and **Bottle and lid height** dialogs are skipped.



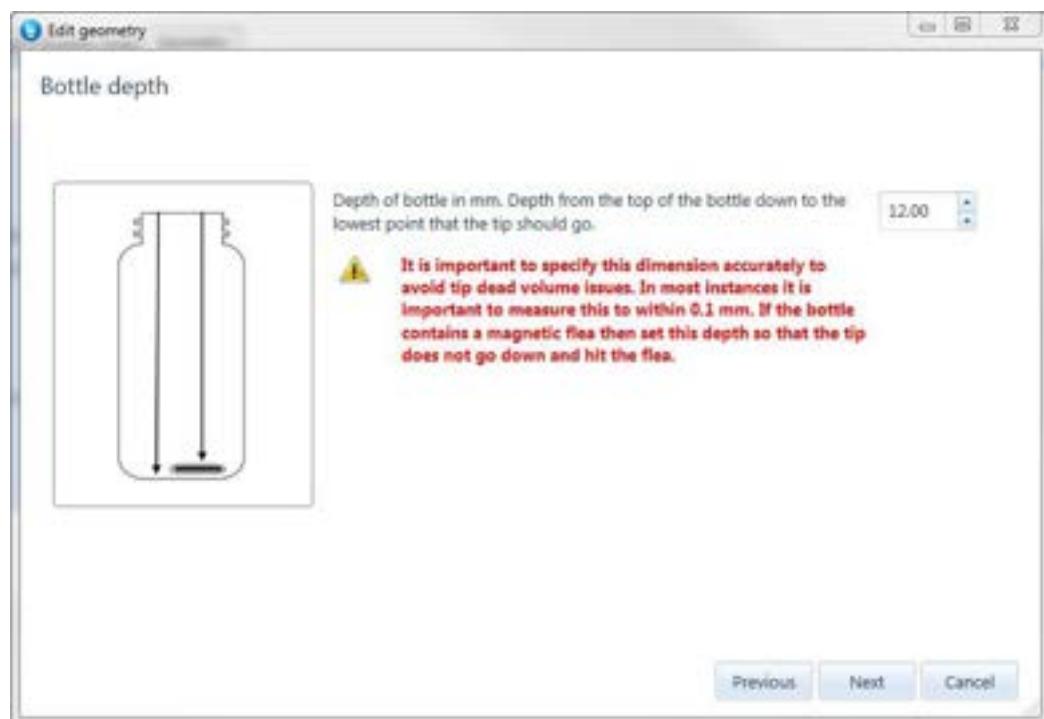
- 5) Enter the height of the lid. The standard bottle lids supplied with the system are 16mm in height.



- 6) Enter the height of the bottle with the lid. The standard bottle lids supplied with the system add 1.9mm to the height of a bottle.



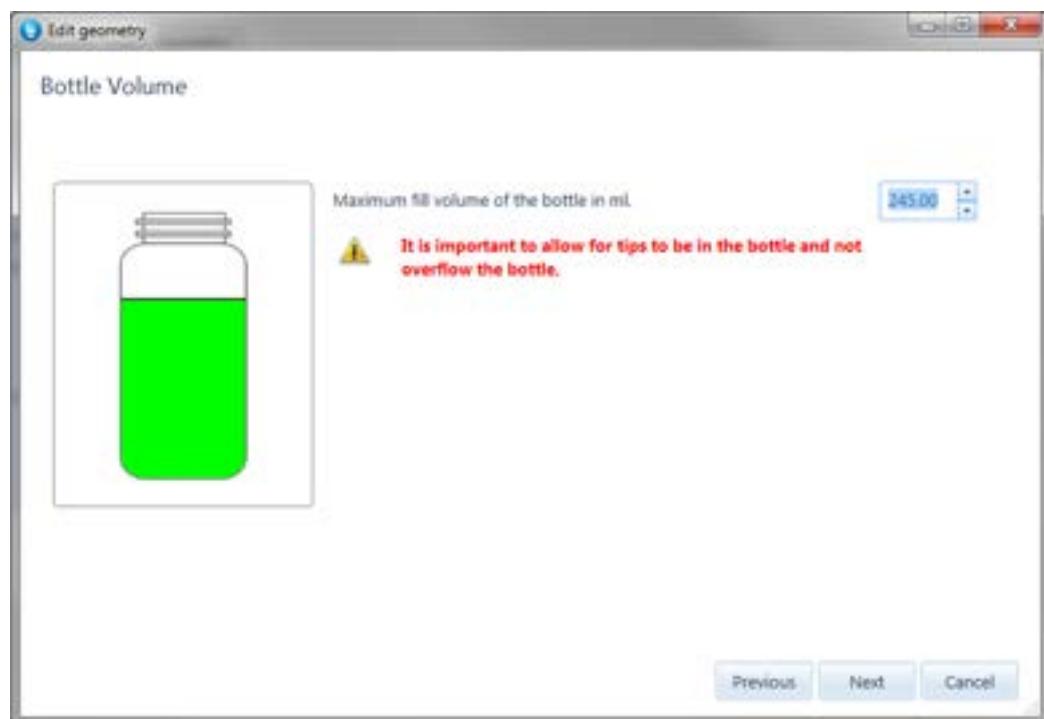
- 7) Enter the depth of the bottle



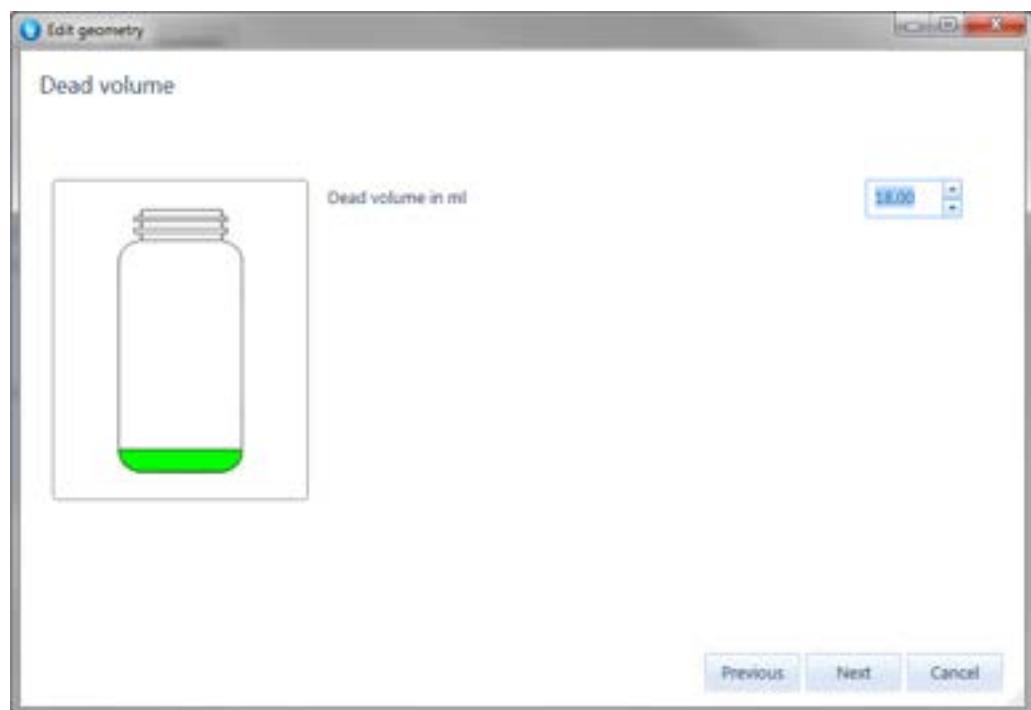
- 8) Specify the tip type(s) that are to be used with the bottle.



- 9) Enter the maximum fill volume for the bottle.



- 10) Enter the dead volume for the bottle – that is the volume of liquid that cannot be removed by the liquid handler.



- 11) Summary dialog displaying the properties of the bottle that is about to be created.
Pressing the **Finish** button creates the new bottle geometry.

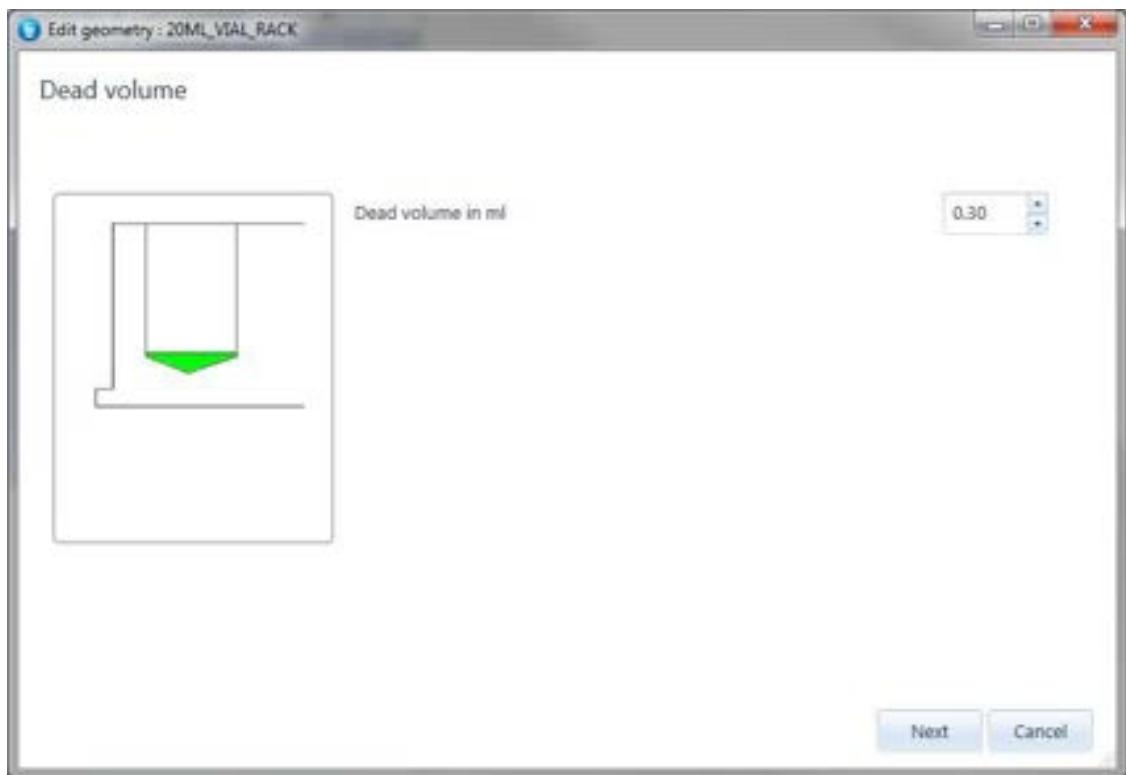


- 12) Labware summary table showing the newly created bottle geometry. Editing the geometry will invoke the creation wizard but with the existing values filled in.

Type	Name	Wells	Volume (ml)	Dead Volume (ml)	Has lid	Last updated	
Small bottle	250ML BOTTLE	1	245	18	✓	13/07/2016 04:47	
6 well plate	CELLCOAT 6 WELL	6	2	0	✓	13/07/2016 01:14	
6 well plate	50ML TUBE RACK	6	45	0	✓	21/06/2016 10:45	
96 well plate	TAP 96 WELL PLATE	96	0.5	0	✓	21/06/2016 10:45	
24 well plate	15ML CENTRIFUGE TUBE RACK	24	11	0	✓	20/04/2016 05:53	
24 well plate	CELL COUNTER 24 CUP HOLDER	24	2	0	✓	20/04/2016 05:53	
Large bottle	1L BOTTLE WITH FLEA	1	1000	115	✓	20/04/2016 05:53	

31.3 Dead volume definition

The dead volumes for Sartorius standard labware can be modified by selecting the edit button on the labware summary table. The wizard is entered at the Dead volume dialog and the **Previous** button is not shown. Pressing **Next** the wizard progresses as above.



32 APPENDIX – UPDATING BIOREACTOR FIRMWARE

To install the firmware:

- 1) Start the Mass Flash program.



Figure 542 Starting the Mass Flash program from the Windows Start menu

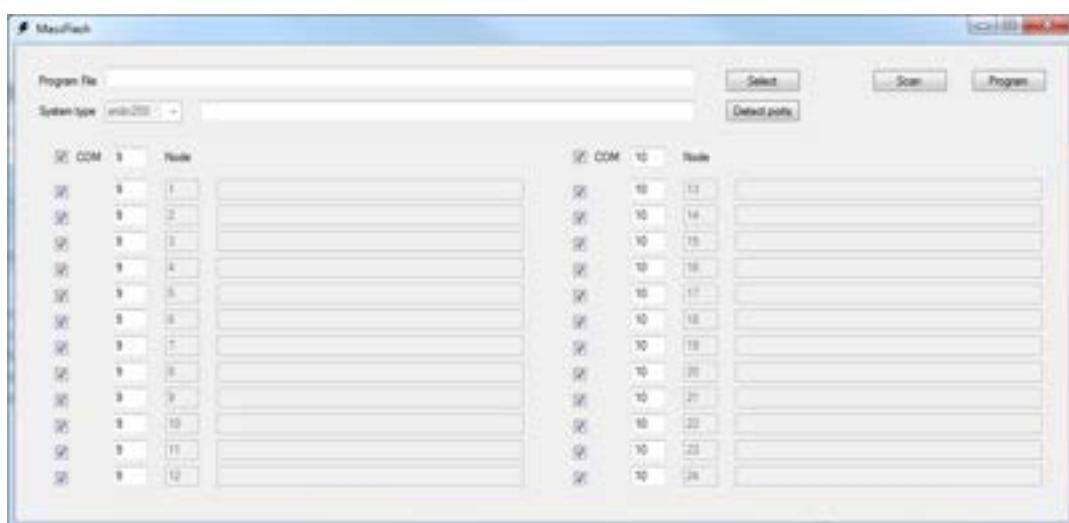


Figure 543 Initial screen of MassFlash program

- 2) Enter into the MassFlash.exe program the COM ports on the system (9 and 10). On the demo systems that is ports 4 and 6.

- 3) Select the check box for the top two boards
- 4) Press **Scan** – the screen should show the existing version details from the two bioreactors on the system

Press **Select** and browse for the firmware to put on the system. The firmware is in the file “B-0092_PegasusBioreactorControlBoard_v2.8.7-R8.2.bin or similar included with the installation. The application should now look like the figure below.



Figure 544 MassFlash ready to program

- 5) Press **Program** and the application should program the boards. While the boards are being programmed a progress bar should be displayed.



Figure 545 MassFlash while programming showing dark 'console' window

- 6) Once the boards have been programmed the screen should look as below.

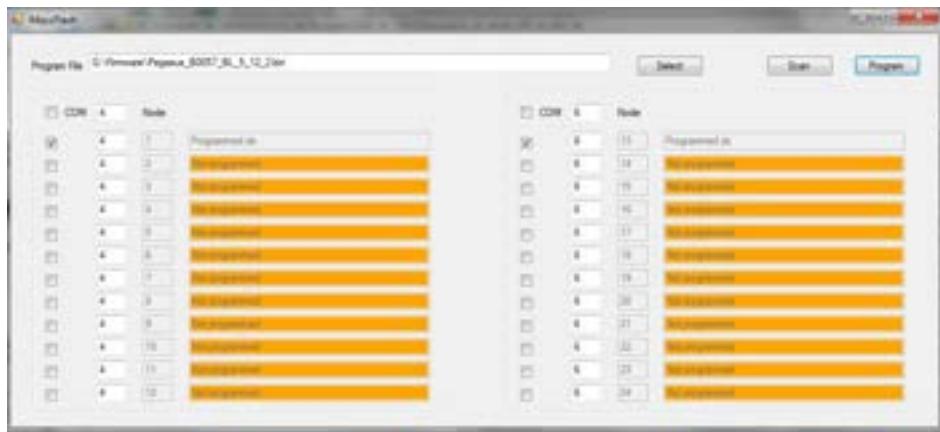


Figure 546 MassFlash after programming

- 7) If programming something other than a bioreactor control board then the port associated with the device can be found by using the Detect ports button.



Figure 547 Detect ports scanning

Once the scan is completed a list of the ports nodes and devices found is displayed.

6 control boards found on 4 COM ports		
Port	Node	Board type
COM5	1	B0090_Reader
COM9	1	B0092_PEG
COM9	2	B0092_PEG
COM10	1	B0092_PEG
COM10	2	B0092_PEG
COM15	1	B0090

Figure 548 Detected ports

33 APPENDIX – AUTOMATIC EXPORT OF LABWARE AUDIT REPORTS

The system can be configured to automatically export the audit records for transfers to and from labware to files. This allows external software to be created that uses the records to understand automatically the content of plates being taken for sampling.

To configure the option set **Labware_Audit_Export_Directory** in SystemOptions.txt to the directory where the files are to be created.

Each file contains a line of headers followed by a single audit record. The file is a csv file with the following columns:

- Component – the component that raised the audit record, in this context typically “Liquid handler”
- Time – the time associated with the transfer
- Source – the sort of actor that caused the action, in this context typically “Step”
- Action – label for what was being done e.g. “Sample”
- Value – values of parameters associated with the action
- Source – name of the source e.g. “Bioreactor 12”
- SourceBarcode – any barcode associated with source as a whole
- SourceLocation – location of the source
- SourceWell – name of the well within a multi-welled item
- SourceWellBarcode – any barcode associated with the well
- SourceWellLiquid – any liquid associated with the well
- Target – name of the target e.g. “Sample”
- TargetBarcode – any barcode associated with source as a whole
- TargetLocation – location of the target
- TargetWell – name of the well within a multi-welled item
- TargetWellBarcode – any barcode associated with the well
- TargetWellLiquid – any liquid associated with the well
- Volume – the volume of the transfer
- ExtraInfo – any extra information about the action

34 APPENDIX – LABWARE

Labware name	Labware Part No.	Adaptor
1L Bottle	001-2G51	
175ml Bottle	001-2G50	Supplied with system
1L Bottle With Flea	001-2G51 Fleas 001-2G89	
175ml Bottle with Flea	001-2G50 Fleas 001-2G88	Supplied with system
Stericap 1L Bottle	Millipore 1L Stericup receiver flask SC00B10RE	
Stericap 250ml Bottle	Millipore 250ml Stericup receiver flask SC00B02RE	Supplied with system
Packard tube rack	Packard tubes	
20ml Vial rack	Sample/Scintillation vials	
15ml centrifuge tube rack	001-2G97	
2ml centrifuge tube rack	001-2G96	
Cell Counter 24 Cup Holder	Beckman Coulter Vi-CELL 4mL Sample Cup, Roche Cedex sample cups	
50ml tube	Conical centrifuge tube (Corning, Cole-Parmer, Falcon)	001-8G22
50ml tube rack	Conical centrifuge tube (Corning, Cole-Parmer, Falcon)	
TAP 1 well plate	A-0068	001-2G57
TAP 4 well plate	A-0067	001-2G57
TAP 24 well plate	A-0038	001-2G57
TAP 48 well plate	Axygen™ P-5ML-48-C-S	001-5G67
TAP 48 well plate no adapter	Axygen™ P-5ML-48-C-S	
TAP 96 well plate	Axygen™ P-2ML-SQ-C-S	001-5G67
TAP 96 well plate no adaptor	Axygen™ P-2ML-SQ-C-S	