



Design of Biopharmaceutical Production Facilities

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Abstract

In this report, the design of a biopharmaceutical production facility created for the BP3 module is described in detail. Based on the given user requirements specifications (URS), the plant has been designed to assure the production of four products (products A, B, C and D) based on hamster ovary (CHO) cell-derived monoclonal antibodies (mAb) in compliance with Swiss and US cGMP requirements. To fulfil the given URS, several calculations were made which showed that 72 batches with a production volume of 2000L were needed to reach the titer specified for the mAb production facility. Further calculations were made to determine the necessary seed, as well as production lines. To maximize the utilization of the plant, an occupancy list was created to and a staggered production process was selected. The results indicated that two inoculum seed lines, one 50L stainless steel bioreactor and one 200L single-use bioreactor, as well as three 2000L single-use bioreactors should be used to meet the goals set by the URS. Based on these assumptions, a four-story production facility was designed with a warehouse and an entrance attached to the main part of the building. Additionally, a height concept, a zoning concept, an and an area schedule were set and a 2- and 3-dimensional model of the building was created to visualize the facility including equipment. Lastly to operate the production facility, 85 full time equivalents will be necessary.

List of Abbreviations

Abbreviation	Meaning
API	Active pharmaceutical ingredient
BDS	Bulk drug substance
BP	Buffer preparation
BSL	Bio safety level
cGMP	Current good manufacturing practice
CHO	Chinese hamster ovary
CNC	Controlled not classified
DCS	Distribution control
DS	Drug substance
DSP	Downstream process
E&L	Extractables and leachables
HVAC	Heating, Ventilation, and Air conditioning
mAb	Monoclonal antibody
MP	Media preparation
PCS	Process control safety
PW	Purified water
QC	Quality control
SAP	Systems, application & products in data processing
SCADA	Supervisory control and data acquisition
SUS	Single-use system
SUT	Single-use technology
URS	User requirement specification
USP	Upstream process
WFI	Water for injection

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1 Introduction

This report describes the conceptual design of a biopharmaceutical production facility based on a Chinese hamster ovary (CHO) cell-derived monoclonal antibody (mAb) manufacture. The report follows the user requirement specifications (URS) which is explained in detail in the appendix. The case study centres on a biopharmaceutical facility that will allow the production of four different mAbs and describes the planning of the entire production process, including upstream processing (USP) and down-stream processing (DSP). The facility tends to employ single-use systems (SUS) and technology (SUT) which allows for a better process by decreased contamination risk in a cheaper and more versatile process.

1.1 Single-use technology (SUT)

As the name suggests, single use technology (SUT) deals with the use of materials that are disposed of after single use. SUT offers several advantages for a process and is being implemented in various areas of biologics and therapeutic vaccine production.

The usage of presterilized, gamma-irradiated equipment eliminates the need for clean-in-place (CIP) and steam-in-place (SIP) operations on the production containers. This prevents downtime through fast replacement of the equipment, as well as energy saving and a reduction of the solvent consumption. The cost of producing pure steam for SIP processes can be avoided, which leads to a significant cost reduction in a facility. (Parrish M. Galliher, 2018) Additionally, it minimizes the risk of cross-contamination between different products making it a good fit for facilities designed to produce multiple products. Furthermore, a cleaning validation is not required, also reducing work effort for the quality control (QC) department of a production site. Also, the turnover time can be reduced significantly the single use containers are preassembled. (Eibl & Eibl, 2019, Lopes, 2015)

Besides the many advantages SUT has to offer, there are also some major disadvantages hindering the use of SU-systems in some production processes. One of the major disadvantages are extractables and leachables (E&L). For instance, parts of the plastic materials of a SU-system could be released into the product, affecting its overall quality and safety. E&L events can occur under certain conditions, which is why the materials for a particular process should be carefully chosen, with the process conditions in mind (David J. Pollard, Alain Pralong, 2018). E&L substances must be reduced to a minimum and kept under a certain threshold, as E&L leaching into a product line is always inevitable. E&L substances could have toxic effects depending on their toxicity and dose within a batch. Another disadvantage would be the material limitations, as there is no SU-bioreactor available with a volume over 6000L. This section still favours stainless steel bioreactors. Furthermore, the usage of the SU-equipment is restricted to one usage, after which the equipment will be disposed.

Performing a production process with multiple SU-bioreactors throughout the year accumulates a large amount of plastic waste compared to the stainless-steel variant. (Eibl & Eibl, 2019)

Finally, it is also worth mentioning that by choosing a SUS for a biopharmaceutical production facility the dependence on the supplier of such systems increases dramatically. As a matter of fact, the equipment of different companies is in general incompatible. Therefore, it is almost impossible to switch the supplier. (Eibl & Eibl, 2019)

Table 1: Advantages and disadvantages of single-use technology (SUT) in production processes of biologics and other pharmaceutical products. (Eibl & Eibl, 2019)

Advantages	Disadvantages
<ul style="list-style-type: none"> • No CIP- and SIP process needed • Lower labour and utility expanses • Preassembled • Lowering the risk of cross contamination • Quality control on the equipment goes to the provider of the SU-technology • Reduced facility footprint 	<ul style="list-style-type: none"> • Release of E&L into the product • Up-scaling is limited through material limitations • Higher waste production • Staff needed with SUT handling skills • Harder to automate the process • Restricted availability of disposable sensors • Dependence on supplier

1.2 Application of Single-use technology

As already mentioned, SUT finds its purpose in areas of efficient and sterile manufacturing processes, such as the production of antibodies against certain diseases or for cancer treatment medication. The easily replaceable bioreactors reduce time and manpower for the preparation or rehabilitation of a production line, as well as the control of cleanliness. The application of SUT also minimizes the duration of qualification procedures regarding new production facilities. This becomes particularly beneficial when time is a crucial factor to not only save money but also lives, as it was the case in the development of a COVID-19 vaccination for instance (Gareth Macdonald, 2020).

1.3 Group 3 organisation

The group number 3 was supervised by M.Sc. Fruhar Mozaffari. Subgroups were built to tackle the different tasks given, during the project. The assignment of the members to the different groups were conducted by the project management team. Besides the project management team, the additional subgroups were USP, DSP, media preparation (MP), as well as clean utilities and logistics. The whole organigram is illustrated in Figure 1. Each participant's name is written to their respective subgroups as well as their affiliated school.

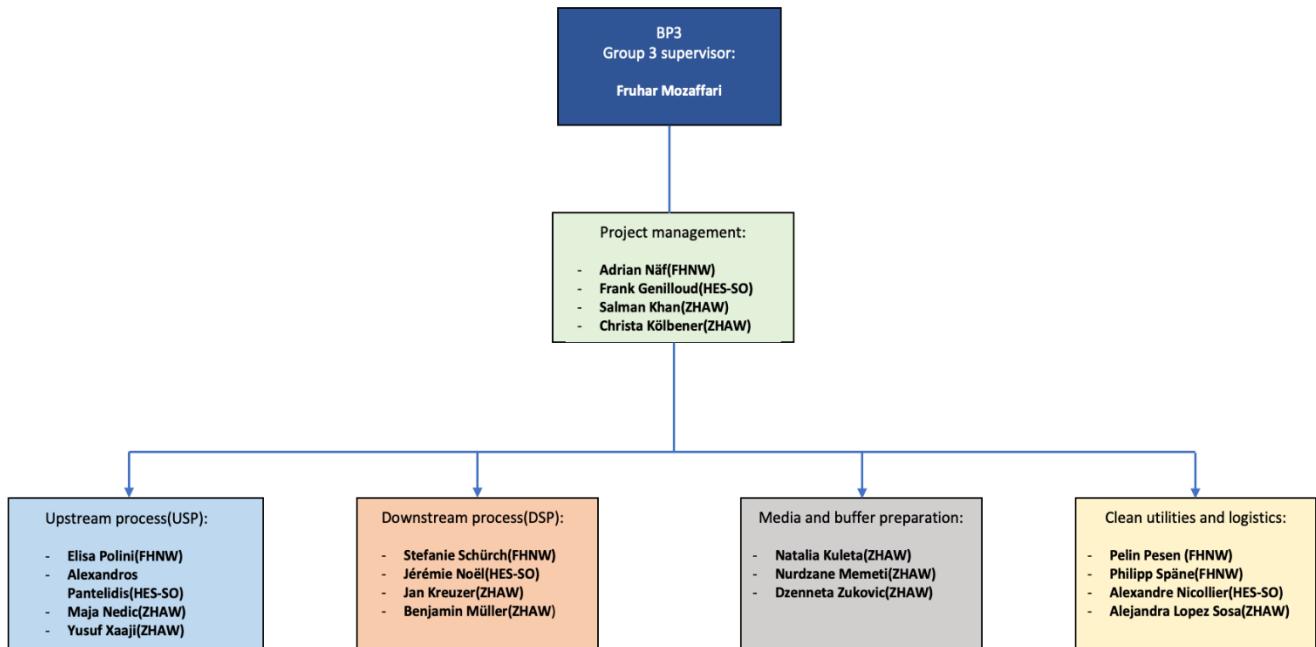


Figure 1: Organigram of group 3. The member of each subgroup are inside of each subgroup fields and their affiliated schools, from the MSLS-alliance, are written next to the respective names. The group supervisor is Fruhar Mozaffari.

2 Project Management

This chapter focuses on aspects of production facility planning that falls within the scope of project management tasks.

2.1 Occupancy list

To create an occupancy list for the production facility, calculation had to be made for the products and the number of batches to reach a certain amount of product within a year's time. In this case, the goal was the production of four different mAbs (labeled as A, B, C and D). All the products shall be produced by the same CHO platform; thus, the same medium will be used. The amount of product (kg/year), titer (g/L) and yield (g/g) was specified by the URS and is listed in the Table 2. The calculation of the required batch amounts was summarized and has shown that 72 batches are required to achieve the amount of product specified by the URS. Parts of this calculation was used to create an occupancy list. More details and the used formula may be found in chapter 3.2.

Table 2: Requirements from the URS and calculations for the required batches regarding the four mAb products (A to D).

	Amount [kg/year]	Titer [g/L]	Yield [g/g]	Batch volume [L]	Batches/year	Batches/ year (rounded)
Product A	100	4	0.684	2000	18.274	19
Product B	70	3	0.706	2000	16.525	17
Product C	70	2	0.64	2000	27.343	28
Product D	40	4	0.64	2000	7.812	8
Total batches						72

Production should take place 320 days per year with 45 days off-time according to the URS. Additionally, the 72 batches were accounted to calculate the required seed and production lines as well as the number of bioreactors to meet the requirements and prevent unnecessary purchase of equipment. The results of the calculations are seen in the Table 3. The upstream process as a whole will take 24 days, which then will go over to the downstream process for additional four days. This displays the production time for one batch. To produce the 72 required batches, two inoculum production lines are needed, as well as three production bioreactors. The calculations will be explained in more detail in chapter 3.2 of the upstream process.

Table 3: Required production lines and estimated operational time of the production facility within a year.

Process step	Time [d]	Production lines / USP step	Production lines / USP step (rounded)
Inoculum production[2L]	7	2	2
Seed bioreactor 1 [20L]	3	1	1
Seed bioreactor 2 [200L]	3	1	1
Production bioreactor[2000L]	11	2.88	3
Down stream	4		1
Total production days	307		

The occupancy list of the USP was meant to be used for the production of all 4 products (A to D) and is seen in Figure 2. It shows the planning for the first 34 days of production. A staggered production process was chosen to maximize facility utilisation. The production process consists of two inoculum seeds, as previously mentioned. The whole process starts with the inoculation of the first inoculum seed reactor for 7 days. After 4 days the second inoculum line will be started to perform a staggered process. After the 7 days of inoculum production, the inoculum will be transferred into the first seed reactor, where the cell culture will be cultivated in a 50 L bioreactor for three days. Afterwards, the cell culture will be moved to the second seed reactor for further cultivation. For the second seed a 200 L bioreactor will be used for another three days of cultivation. The last station during UP will be in one of the three 2000L production bioreactor, where the main antibody production will take place. The production lasts for 7 days, making it a total of 24 days to produce one batch. Full utilization of the facility will be achieved on day 22 of upstream process. After every end of a production step a changeover will be performed for material and cell culture transfer, cleaning of the equipment and/or replacement of the SUT equipment. The changeover is marked in orange. A rough overview of the downstream process is shown in the last part of the figure. This should give an idea of how much time is needed until one whole process is finished.

After the USP, the DSP will take place. The whole DSP process lasts for four days (rounded) after every end of an upstream batch. Figure 3 shows every step performed during the downstream process with the needed time to perform a step-in hour (rounded) and in what kind of room it will be performed (V+ or V-). Ten purification steps will be performed for the downstream process to be finished and are as follows: step 1 protein capture for 6 hours, step 2 virus inactivation for 7 hours, step 3 depth end filtration for 4 hours, step 4 cation exchange chromatography (CIEX) for 11 hours, step 5 pH-adjustment for 25 hours, step 6 anion exchange chromatography (AIEX) for 9 hours and step 7 nanofiltration for 17 hours. Step 7 will be the last step of the process which will be performed in a room with V+ classification. After step 7 the product will be transported in a room with V- classification where step 8 diafiltration/ultrafiltration will be performed for 13 hours. For step 9 and 10 a

0.2 µm filtration will be done for 4 hours following the bulk filling and freezing of the product, which will take 2 hours.

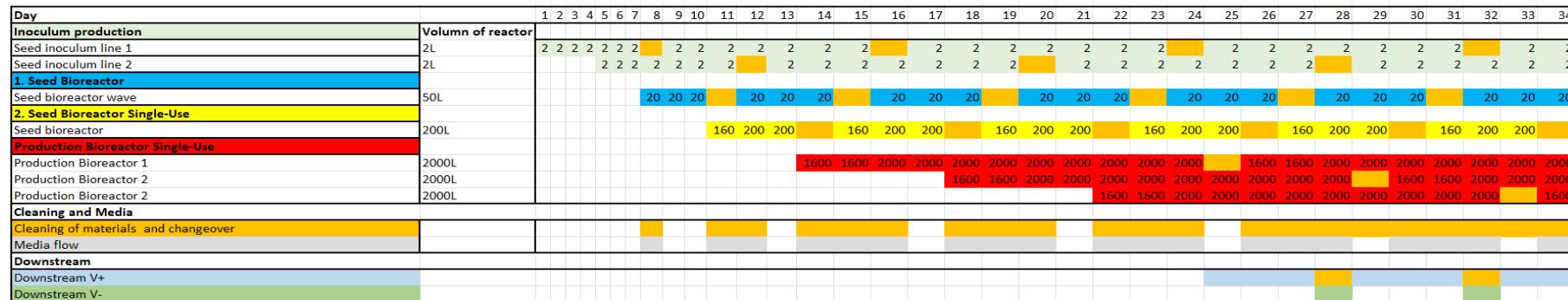


Figure 2: Occupancy list of USP, DSP with media and buffer flow.

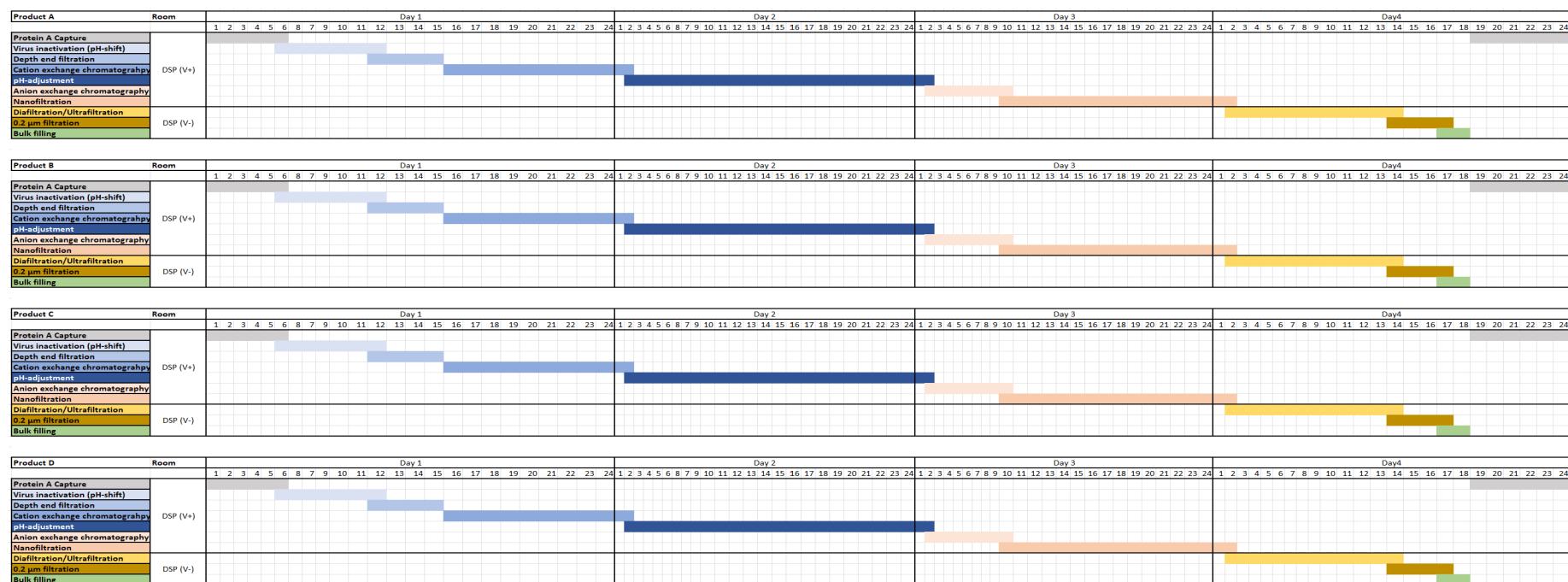


Figure 3: Occupancy list specific for DSP. Every step, which will be conducted is listed, with the estimated time in hours to perform the downstream steps.

2.2 Block flow diagram

The whole product process may be displayed in a block flow diagram. This allows to show the volumes, concentrations and flows, in a step-by-step illustration. For each product a block flow diagram was made, showing the USP and the DSP of the respective product as well as the used equipment in the respective steps and in or outflow of media and buffer. The block flow diagram covers the same steps that were already roughly described in the chapter 2.1 through the occupancy list. The difference in each block flow diagram is the titer of each product.

The block flow diagram in Figure 4 is for the product A. Product A has a titer of 4 g/L. For product B the titer is set at 3 g/L and the block flow diagram is seen in Figure 5. Figure 6 shows the block flow diagram of product C with a titer of 2 g/L and Figure 7 is for the product D with a titer of 4 g/L.

The first step is the inoculum production with a starting volume of 2 L for the first passage. The inoculum production will be performed in 2L shake flasks. After two days the second passage will be performed with additional two days of cultivation. Afterwards a third passage will be performed with 3 days cultivation, marking it the last step of the inoculum production. The second step will be performed in a 50 L seed bioreactor with 2L of the cell culture from the previous step and start volume of 16 L. In addition, 4L of medium will be added during the three days cultivation. The 20L of cell culture will then be transferred into a 200L STR seed bioreactor for the third step. Cultivation will be performed in this reactor for 3 days, with a starting volume of 160 L. On day two of the cultivation, 40 L of feed medium will be added to the process. Step 4 will take place in a 2000 L STR seed bioreactor, for the production of the mAbs. The working volume in this step will start with 1600 L, which also includes the 200L of inoculum from the previous step. Another 400L of feed medium will be added to the process on day 3. The process continues for another 8 days, summing it up to 11 days of cell cultivation for producing the desired product. Afterwards the harvested product will be transported to the DSP for purification, marking the harvest as the last step of the USP part.

Only one DSP line is needed for the purification of all four products, since the sequential USP approach provides a new batch each 3.7 days and the total DSP time is 3.8 days. By temporally separating the processes, any contamination between products is avoided. For the first chromatography step (protein A capture) the material is introduced into a ÄKTA™ ready XL system, which is flushed and washed by different buffers provided directly from the buffer storage suites over an aseptic connection to a buffer management system. The eluted and purified product is collected in two 2000 L tanks. The material is then aseptically connected to an Allegro™ MVP system for virus inactivation, which is also tied to the buffer management system. The same Allegro™ MVP system is used to pump the virus inactivated product through three connected depth filters. The filtrate is collected in a 2000L and 2500L tank, which are subsequently transported with a drive unit to a second ÄKTA™ ready XL system for cation exchange chromatography (CIEX). The purified product is eluted into a 2000 L tank and transported to a second Allegro™ MVP system for pH adjustment. This step is

performed directly in the stirrable 2000 L tank and with buffers provided from the buffer suites in totes. This tank is then brought to a third ÄKTA™ ready XL system for anion exchange chromatography (AIEX). The eluted and purified product is collected in a 2000L tank. Both, CIEX and AIEX ÄKTA systems are managed by one buffer management system. The nanofiltration step is performed on the same Allegro™ MVP system as the pH adjustment step, to which two nanofiltration capsules must be installed. The virus filtered product is collected in a 2000L tank, which is ready to be transported to the 'DSP V-' room.

Once the product arrives in the viral free zone, it is further processed in a fully automated centrasette TFF system, which is also supplied with buffer by a buffer management system linked to the adjacent buffer storage suites. The ultra- and diafiltrated material is directly connected to one depth filter and then collected in a 500 L tote. The purified bulk material is filled and transported by trolley to the DSP freezing room and finally to the storage room.

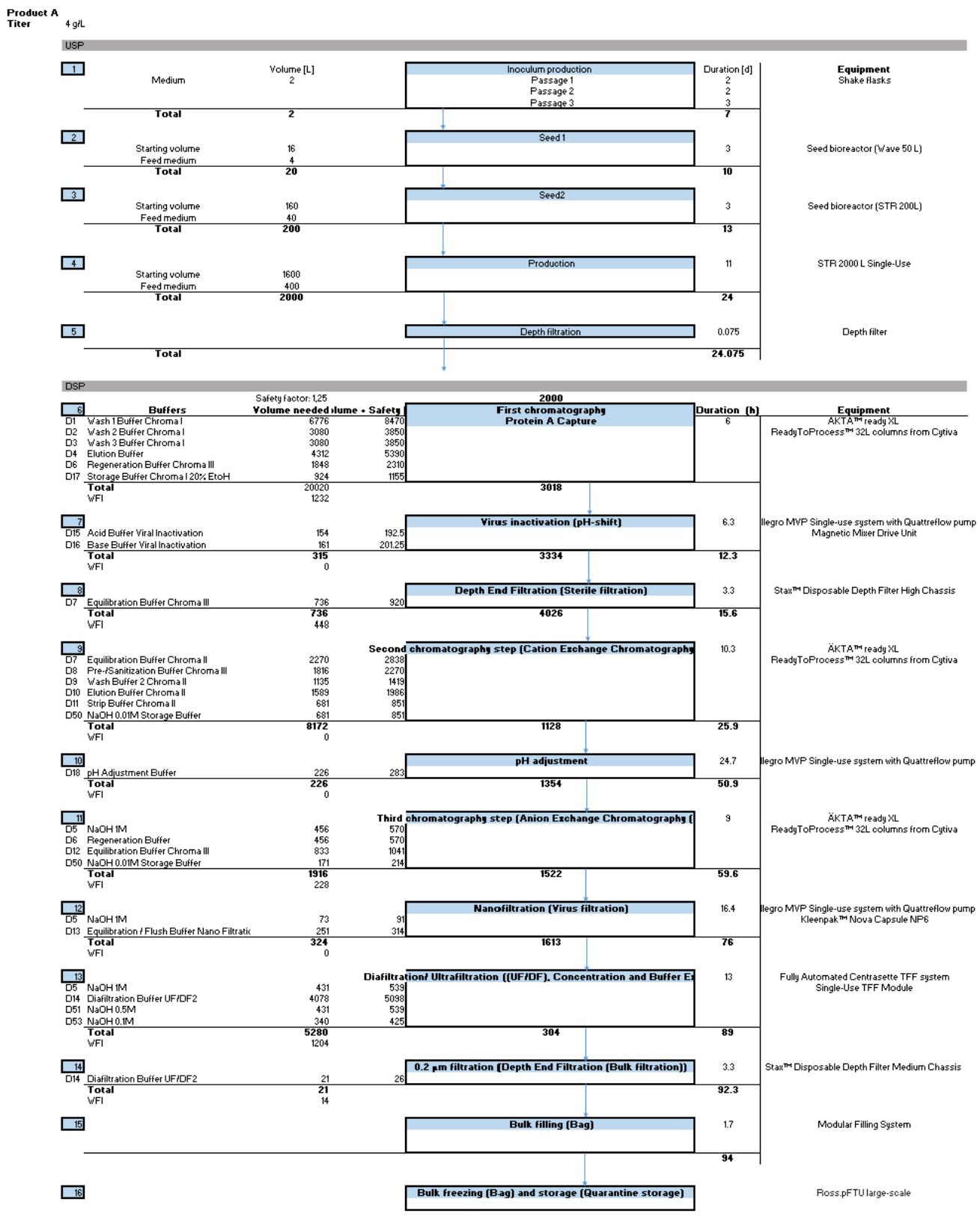


Figure 4: Block flow diagram of product A.

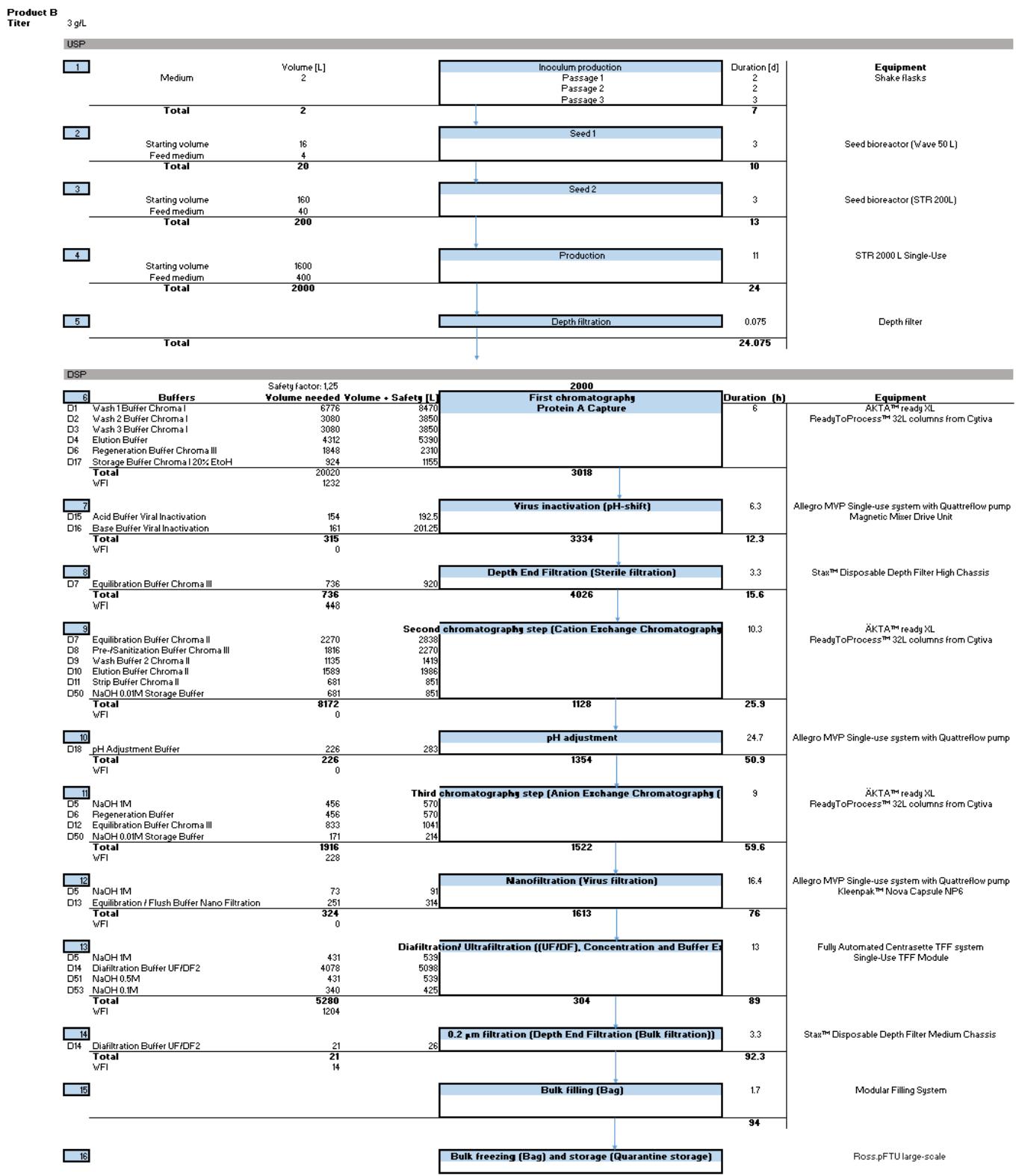


Figure 5: Block flow diagram of product B.

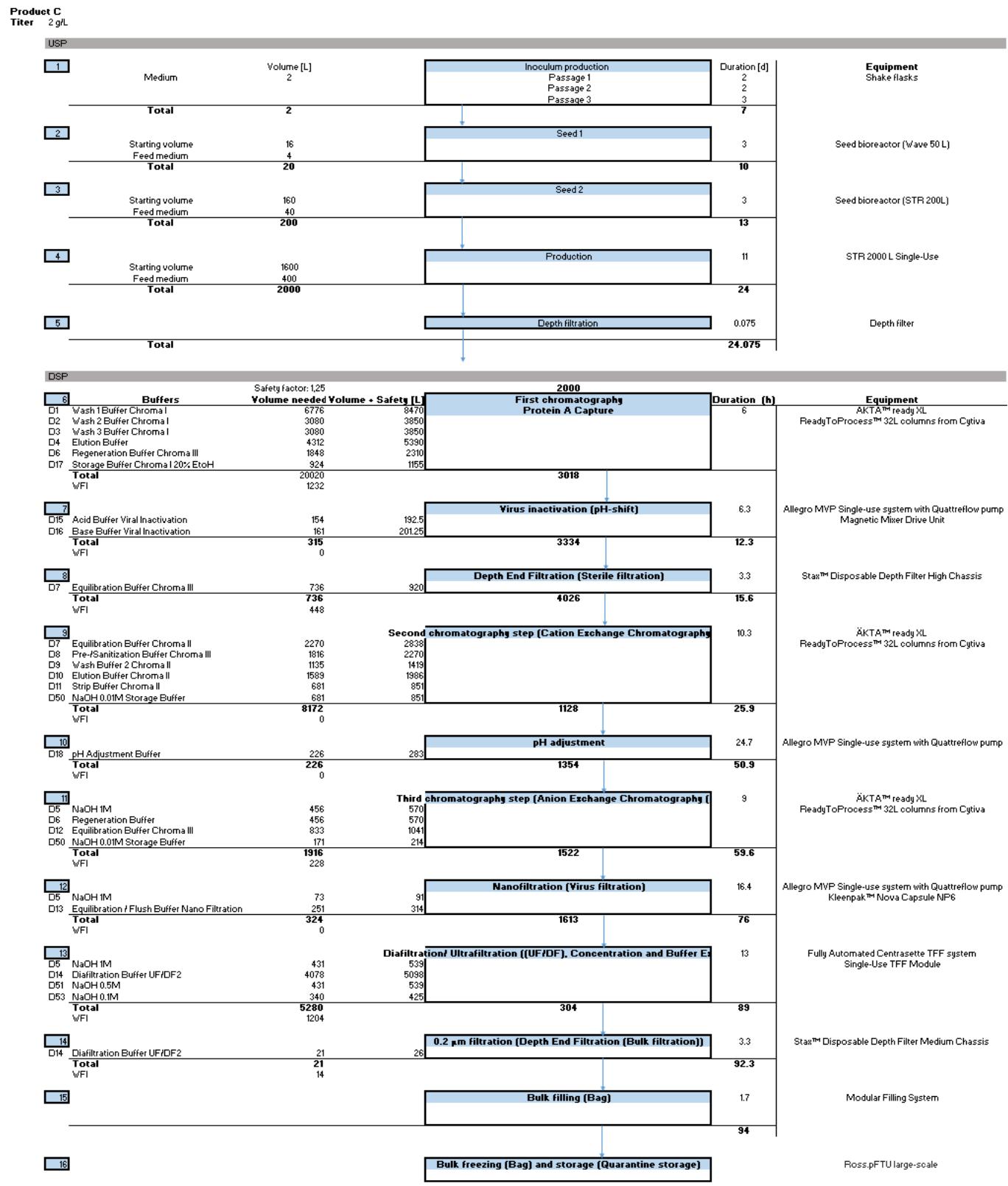


Figure 6: Block flow diagram of product C.

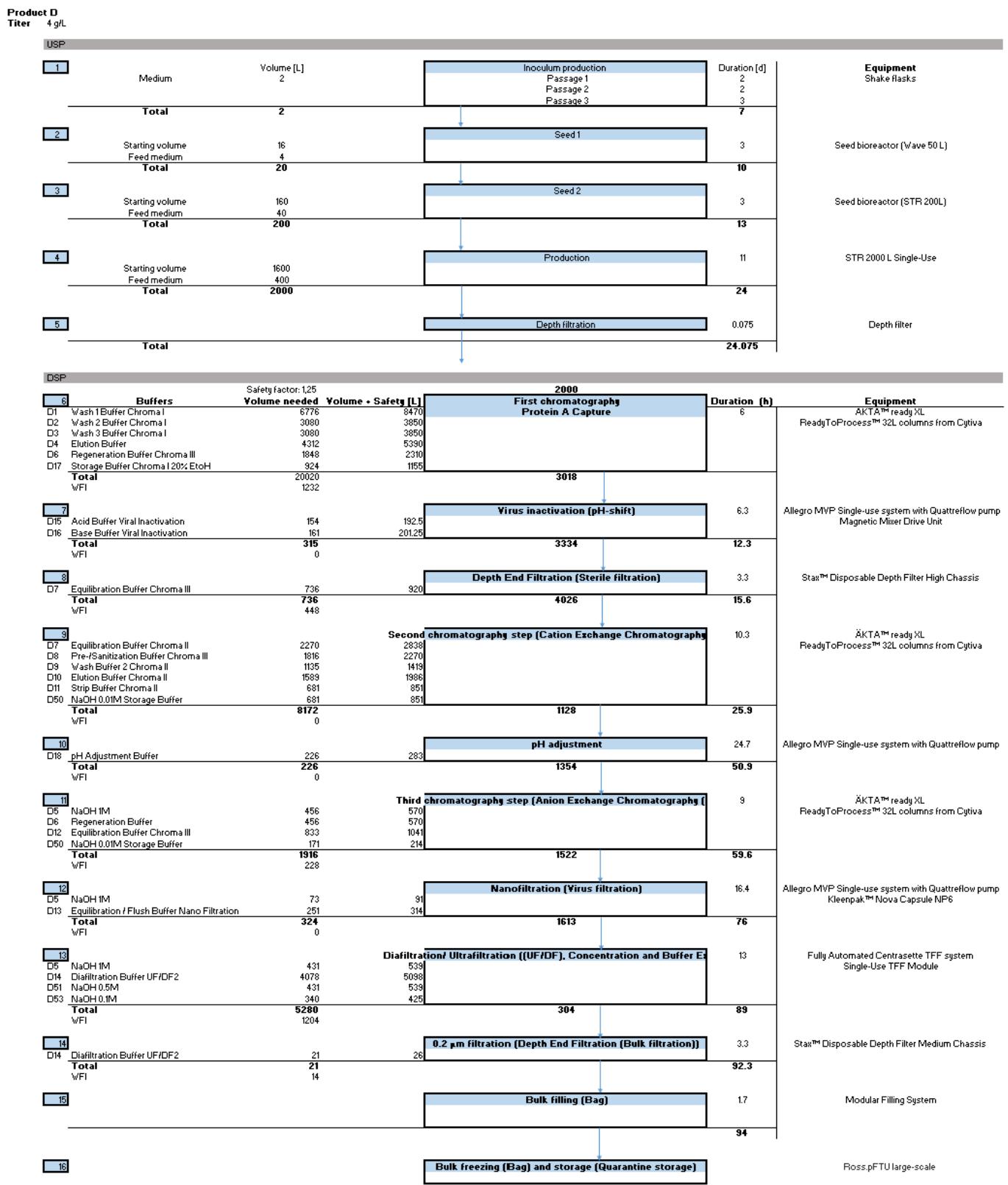


Figure 7: Block flow diagram of product D.

2.3 Area schedule and room list

A HakoBio drawing of the production facility was used to estimate the area of every room. Table 4 shows a detailed list of every room, sorted by area.

Table 4: Area schedule and room list

Area	Room	BSL	Hygi- enic zone	Level	Area [m ²]	Room height [m]	Room volume [m ³]	Air ex- change rate [h ⁻¹]	Airflow [m ³ /hour]
Media	Medium preparation	none	D	Ground floor	28	3	83	20	1660
	Medium & feed preparation	none	D	Ground floor	41	3	122	20	2440
	Buffer preparation & storage	none	D	Ground floor	187	4	560	20	11200
	Medium cold storage	none	D	Ground floor	49	3	146	20	2920
USP	Personnel Airlock Inoculation Lab	1	C	Ground floor	6	3	17	30	510
	Inoculum Production lab	1	C	Ground floor	40	3	119	30	3570
	Cell expansion, Production	1	D	Ground floor	145	3	434	20	8680
	Harvest	1	D	Ground floor	30	3	91	20	1820
	Corridor	none	D	Ground floor	36	3	107	20	2140
	Corridor	none	D	Ground floor	36	3	109	20	2180
	Corridor	none	CNC	Ground floor	34	3	101	10	1010
DSP	Personnel Airlock USP -DSP V+	none	C	Ground floor	28	3	83	30	2490
	Personnel Airlock DSP V- - freezing	none	C	Ground floor	18	3	53	30	1590
	Personnel Airlock DSP V- - freezing	none	D	Ground floor	8	3	25	20	500
	Personnel Airlock DSP V+ - Corridor	none	C	Ground floor	6	3	17	30	510
	Personnel Airlock DSP V+ - Corridor	none	D	Ground floor	6	3	17	20	340
	Material Airlock USP QC - DSP V+	none	C	Ground floor	14	3	43	30	1290
	Material Airlock Buffer storage - DSP V+	none	C	Ground floor	6	3	17	30	510
	Material Airlock DSP V- - Freezing	none	C	Ground floor	6	3	17	30	510
	Material Airlock DSP V- - Freezing	none	D	Ground floor	8	3	25	20	500
	DSP room V+	none	C	Ground floor	209	3	627	30	18810
	DSP room V-	none	C	Ground floor	68	3	203	30	6090
	DSP cleaning	none	D	Ground floor	25	3	76	20	1520
	DSP freezing room	none	CNC	Ground floor	27	3	82	10	820
	DSP storage room	none	CNC	Ground floor	39	3	116	10	1160
QK	QC Lab	none	CNC	Ground floor	70	3	210	10	2100
Utilities	HVAC	none	NC	2 nd floor	*	5	*	5	*
	Ambient Heating & Cooling	none	NC	2 nd floor	*	5	*	5	*
	Waste treatment	none	NC	Basement	*	3	*	5	*
	Portable Water and Purified Water	none	NC	Basement	*	3	*	5	*
	WFI/Steam Production	none	NC	Basement	*	3	*	5	*
	Clean compressed Air	none	NC	Outside	10	4	40	Outside	Outside
	Process Gases	none	NC	Outside	30	4	120	Outside	Outside
	Emergency Generators	none	NC	Basement	*	3	*	5	*

Area	Room	BSL	Hygi- enic zone	Level	Area [m ²]	Room height [m]	Room volume [m ³]	Air ex- change rate [h ⁻¹]	Airflow [m ³ /hour]
Ware- house	Warehouse	none	NC	Ground floor	500	7	3500	5	17'500
Facility Management	Reception	none	NC	Ground floor	*	5	*	5	*
	Offices	none	NC	1 st floor	*	5	*	5	*
	Cafeteria	none	NC	1 st floor	*	5	*	5	*
	Toilets Men	none	NC	1 st floor	*	5	*	5	*
	Toilets Women	none	NC	1 st floor	*	5	*	5	*
	Stairs	none	NC	1 st floor	*	5	*	5	*
	Elevator	none	NC	1 st floor	*	5	*	5	*
	Data Storage	none	NC	Basement	*	5	*	5	*

* values are not precisely defined

2.3.1 Biosafety level (BSL)

Recombinant organisms produced by genetic manipulation are considered biological hazards if they can survive in the environment or in living organisms and pose a health risk (Miller & Bergmann, 1993). The safe handling of infectious organisms or biological materials that may harbor them, including the safe handling of recombinant and synthetic DNA (rDNA) materials, is regulated by various national institutional committees („Appendix B – Large Scale Biosafety Guidelines“, 2010). In the United States, for example, this is administered by the Center for Disease Control (CDC), which has defined safety levels from the lowest level 1 (BSL-1) to the highest level 4 (BSL-4). The same biosafety levels are established in the European Union (Council Directive 90/679/EEC, 1990). In the URS, the production site is defined as BSL-2. Level 2 is applied for work with agents with moderate hazard potential. The following procedures / practices are prescribed (Joseph, 2018):

- Standard cGMP / microbiological practices (including gloves, protective clothing, eye protection and other personal protective equipment (PPE)) as for BSL-1.
- Personnel are trained in handling pathogenic agents and are guided by competent scientists.
- Restricted access to active work areas while working
- Extreme precautions are taken when handling contaminated sharps
- Procedures that may generate infectious aerosols or splashes are performed in biological safety cabinets or other physical containment facilities

2.3.2 Area classification

Bioproduction facilities are usually designed according to a shell-type control concept. Thereby, the most critical process activities are performed in clean rooms with a higher cleanliness standard, which are surrounded by areas of lower classifications. Control of the environment within a facility is basically divided into three degrees:

- Not classified (NC)
- Controlled-Non-Classified (CNC) rooms, where temperature, pressure and humidity are monitored. CNC rooms are generally acceptable for closed process systems. In this case, the product is protected by the system operation and is therefore independent of the room environment.
- Classified rooms, where the air is validated to a certain level of purity, so-called "clean rooms". Cleanliness is defined by the number of particles in a volume of air and rooms are divided into classes of A, B, C and D, with A being the cleanest. Clean rooms are relevant for open processes, where the room environment is part of the product protection strategy (Joseph, 2018).

The classification of the given rooms can be found in the area schedule Table 4 and in Figure 8, Figure 9 and Figure 10.

2.3.3 Zone Concept

Figure 8, Figure 9 and Figure 10 present a schematic view of the zone concept for the production site. In these schemes, the dimensions are not representative to the real production facility. The zone concept was designed in a way to avoid cross-contamination when moving the product to and from the different production steps. The different colours of the rooms correspond to their hygiene zones, with D-class in yellow, C-class in green, Controlled-not-Classified (CNC) in grey and non-classified (NC) in white. The grey-yellow boxes represent the airlocks, which are necessary for material and personnel to change rooms with different hygiene classes safely. The personnel movements are depicted with blue arrows, the material with red arrows, the waste in green and finally the product flow is represented by purple arrows.

The following Figure 8Figure 8 depicts the zone concept of the actual biopharmaceutical production facility, as well as its extensions consisting of the warehouse and the restaurant/cafeteria located on the ground floor. It is shown that only DSP V-/+ and the inoculum lab are specified as class C. The administration, quality control, DSP freezing and storage are CNC. On the other hand, the cleaning room, Media/Buffer preparation and storage, as well as the USP production and harvest operate under class D requirements.

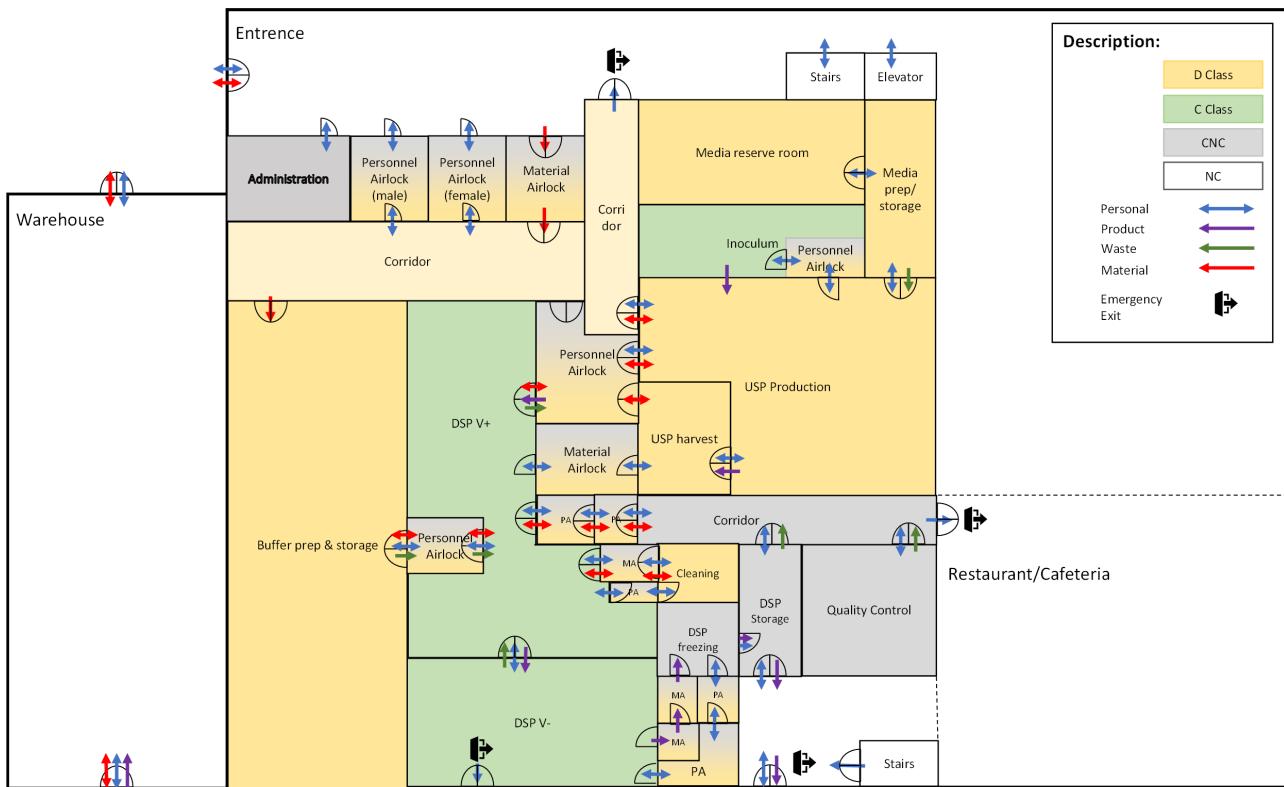


Figure 8: Zone concept of the biopharmaceutical production facility regarding the ground floor.

Employees who enter the building may reach the specified area through a personnel airlock. Material that is required for production can either be brought into the zone D by passing the material airlock or directly from the warehouse. To reach the more strictly classified zone C both material and personnel must cross another airlock.

In the Figure 9 the zone concept of the 1st floor of the main building is visible. As there are no critical processes performed here, it makes no sense to apply a classified zone on this floor. Furthermore, the operation under classified areas causes higher costs and the additional clothing maybe uncomfortable for the employees. Therefore, the entire floor is a non-classified area.

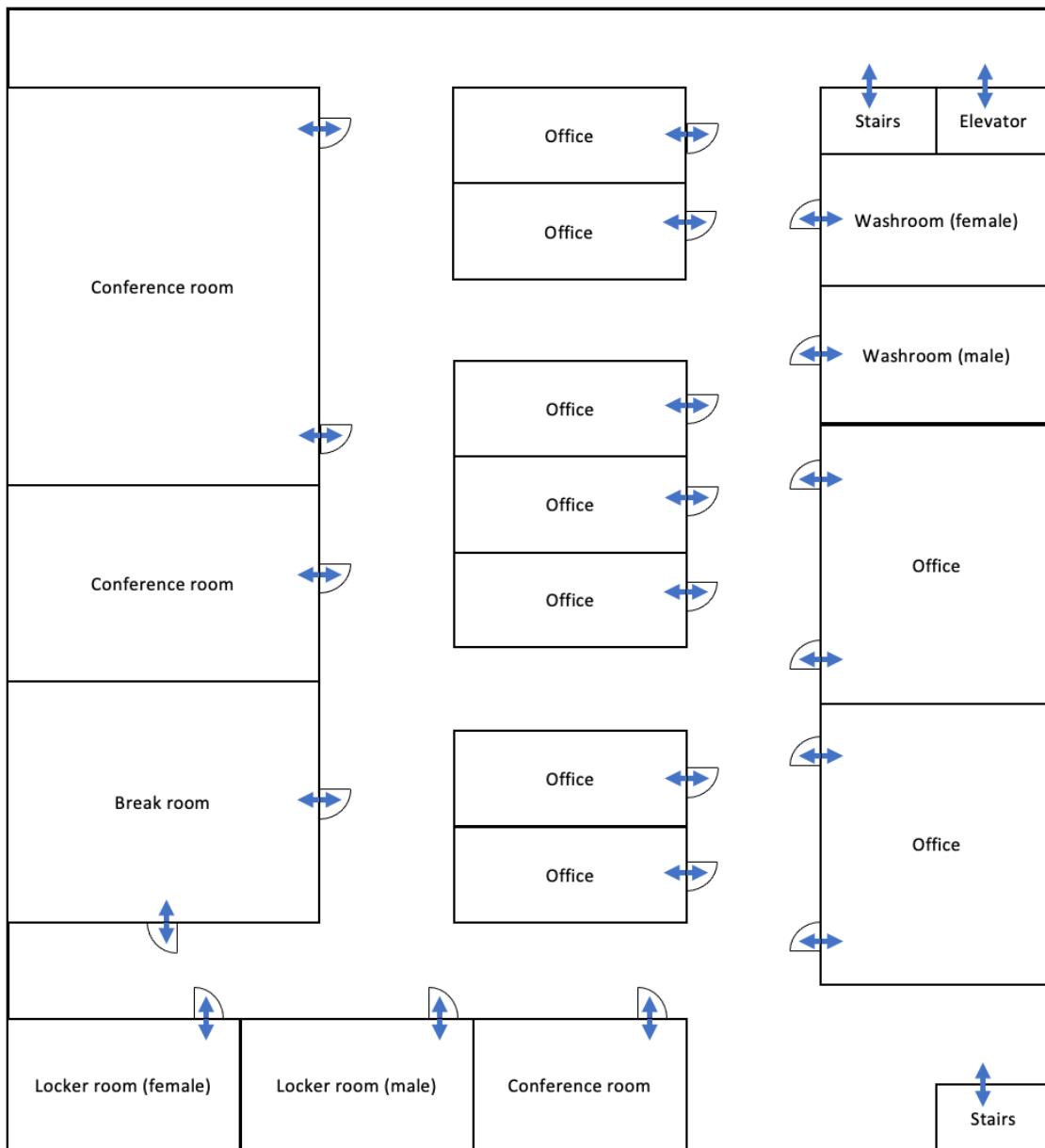


Figure 9: Zone concept of the 1st floor. Mostly offices and conference rooms are located on this floor.

The zone concept of the 2nd floor is shown in the Figure 10. On this level the HVAC system is installed. Since no product critical process steps are executed here either, the entire floor is a NC area.

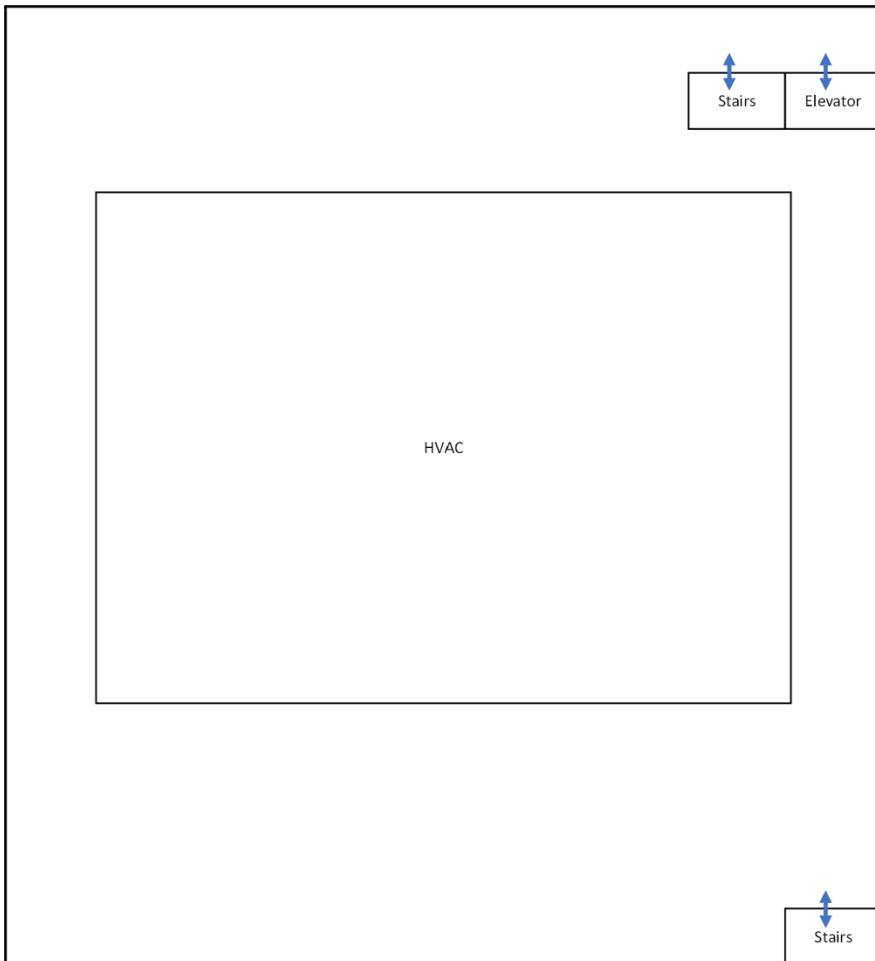


Figure 10: Zone concept of the 2nd floor. This level contains the HVAC system.

2.4 Height Concept

The height concept defines how many floors the production facility needs and the height of every specific floor. As it is shown in the Figure 11 production facility consists of 4 floors, a warehouse attached to the building and an entrance at the production floor (ground floor). The height of the floors was defined according to the necessary equipment that will be installed on them. The different heights are listed in the Table 5.

Production of the different mAb products and their respective quality control will take place on the ground floor. The overall height of the production floor will be 3 meters with exception to the media- and buffer-preparation/storage room for the reason, that these sections have the highest containers in the production area. Additional crawl space for maintenance of the air ventilation system is implemented. The basement houses different utilities that a production facility needs to be fully functional and technical areas for data storage or power generators. Several areas were allocated for WFI-production, which will be used throughout the mAb production processes, as well as for containment and waste neutralisation. Power and emergency generators are also required to provide electricity to the whole facility in case of power outages. The first floor consists of offices for the employees

and conference rooms. A height of 5 meters was determined for this floor to leave enough space for interior design ideas to build a pleasing atmosphere for the future employees. The second floor builds an additional area for technical utilities but is more specifically planned for air handling units (AHUs) and other HVAC utilities. Some of these devices are connected to production rooms through ventilation systems, to provide clean air. As already mentioned, the warehouse is attached on the sides of the building and will be used to store materials. Storage of the materials in shelves makes the work deployment of certain equipment more efficient and eases the overview of the inventory. A height of 7 meters will provide enough volume for the storage of all the single-use and reusable goods, as well as raw materials. Also attached to the main body of the building is the entrance. The entrance should also include a cafeteria and cantina for lunch breaks and after noon breaks, as well as giving the employees the possibility to have a breakfast. Keeping it near the production area will decrease non-productive time for the employees, due to the little distance to the break room.

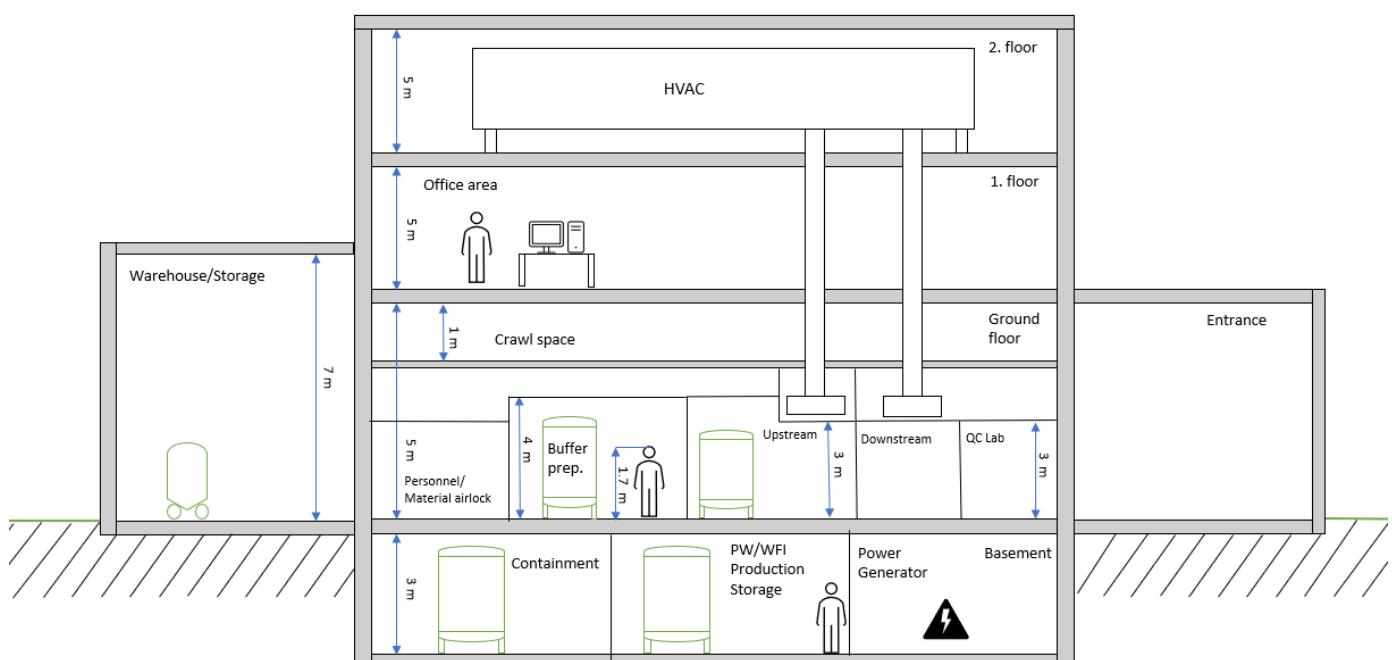


Figure 11: Height concept of the designed production facility for mAb production. The different floors, with their respective heights can be seen, as well as the heights of the attached buildings e.g., warehouse.

Table 5: Heights of the different rooms based on the height concept, in meters.

Room	Height [m]
Basement	3
Personnel/Material airlock	3
Buffer Preparation	4
Upstream	3
Downstream	3
Warehouse Storage	7
Crawl space	1
Entrance	5
Office	5
HVAC room	5

2.4.1 3D and 2D layout of the production area

With the program HakoBio a 3D layout of the production area, as well as the media and buffer preparation area were made. This is seen in the Figure 12. Dependent on the room classification an air lock needs to be crossed to get to another room. Therefore, a personal and a material airlock was inserted. For example, to enter the USP inoculum production (class C) from the USP production area (class D) an air lock must be crossed. This also applies for the transition from an USP area (mostly classification D) to DSP V+/- area (classification C).

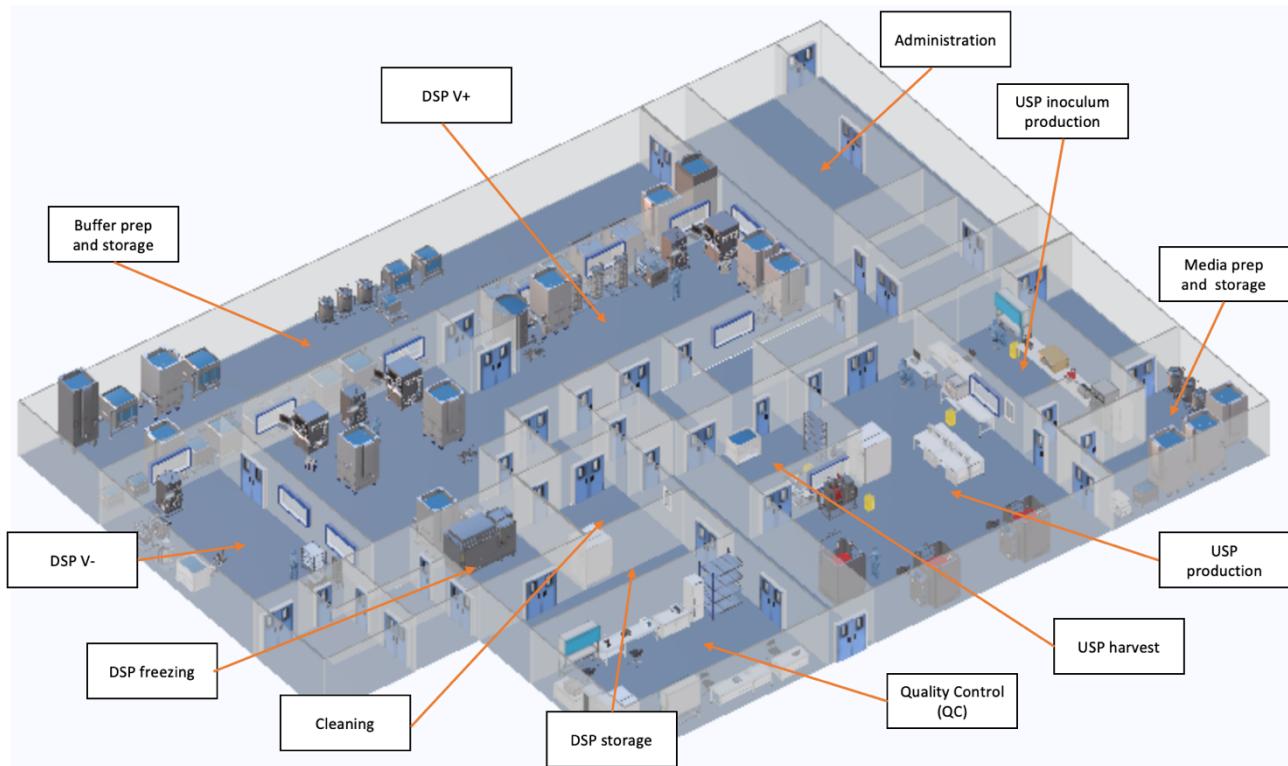


Figure 12: 3D layout of the production area made using the program HakoBio. The different room names are written in the respective blocks, pointing with an orange arrow to the location.

In Figure 13 a 2D layout of the production area is seen. The orange arrows indicate the production flow. Additionally, numbers were added indicating the different steps, which were taken until the final product is achieved. Step 1 starts with the inoculum production and the production ends with step 21, where the mAb product is stored. The whole process and the equipment will be explained in the USP (chapter 3) and DSP (chapter 4) chapters.

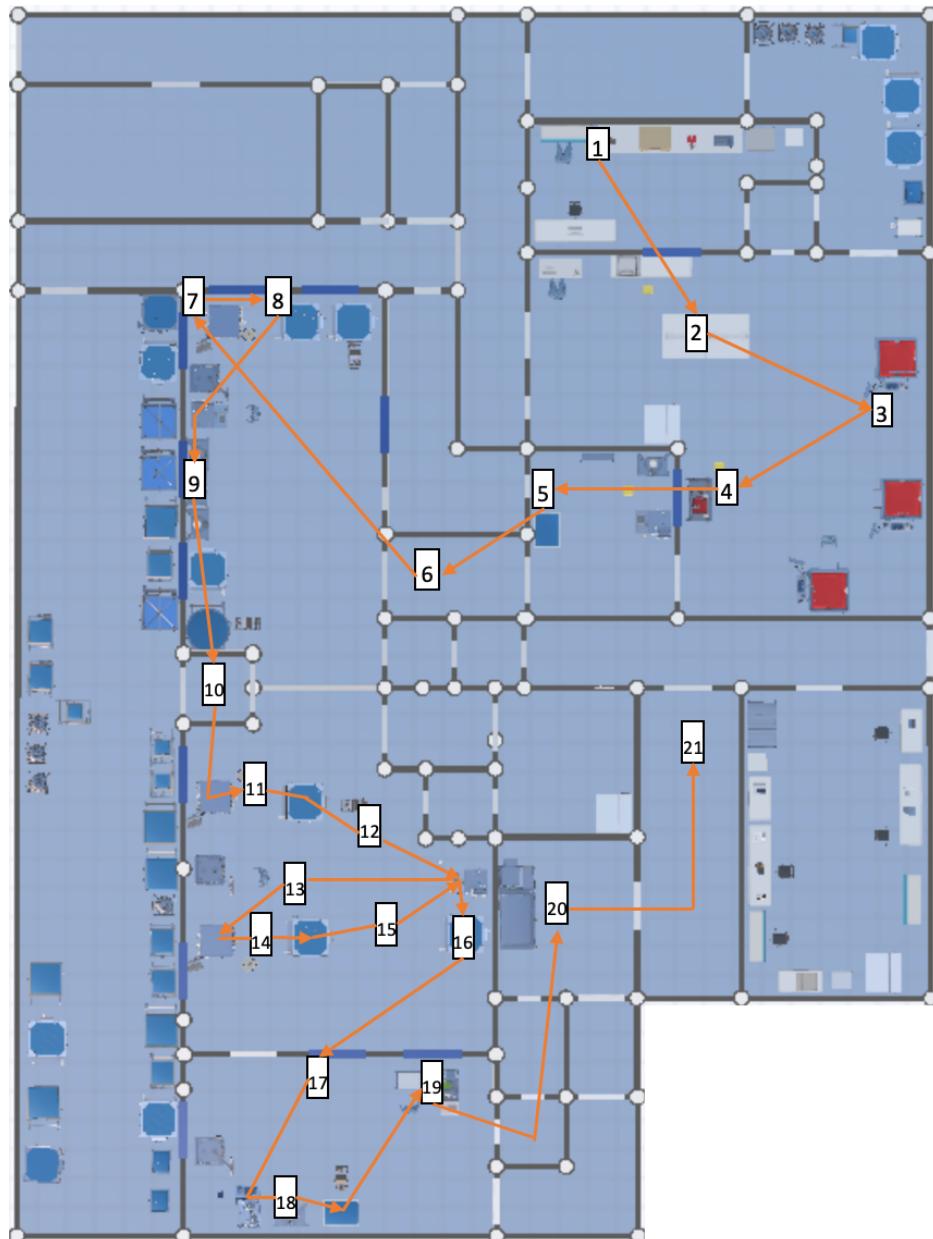


Figure 13: 2D layout of the production area made with the program HakoBio. The orange arrows indicate the product flow and the number in the blocks indicates the steps taken during the production.

2.5 Staff requirement

The total number of Full Time Equivalents (FTE) in shift mode for each department of the production facility was calculated based on the required working days per week, the number of shifts and the number of FTE per shift following the Equation 1 below. The results are depicted in the Table 6.

Equation 1: Formula to calculate the required staff, dependent on the workdays, number of shifts and employees in an area per shift.

$$FTE = \frac{\left(\left(5 \cdot \frac{days}{week} \right) \cdot number\ of\ shifts \cdot \frac{FTE}{shift} \right)}{21}$$

In addition, the amount of FTE per area was determined by summing the FTE in shift mode, the FTE day team and the FTE manager. Thus, the number of employees that must be hired corresponds to a total of 84.6 FTE. Furthermore, the maximum of Full Time Equivalents, which are simultaneously at the facility is 51.

Table 6: FTE estimation in different areas of occupations, in the designed facility.

Area	Working days	Shifts	FTE per Shift	Total FTE (Shift)	FTE Day Team	FTE Manager	Total FTE
USP	7	2	3	10.0	1.0	0.5	11.5
DSP	7	3	3	15.0	1.0	0.5	16.5
Buffer preparation	5	1	2	2.4	0.5	0.5	3.4
Media preparation	5	1	2	2.4	0.5	0.5	3.4
Clean utilities	7	1	2.5	4.2	0.0	0.25	4.4
Black utilities	7	1	1.5	2.5	0.0	0.25	2.8
Warehouse/logistics	5	2	2	4.8	0.0	0.25	5.0
Building Management	5	1	2	2.4	1.0	0.25	3.6
QA	5	1	0	0.0	4.0	1.0	5.0
QC	7	3	2	10.0	0.0	1.0	11.0
Process Development	5	1	0	0.0	6.0	1.0	7.0
Administration	5	1	0	0.0	10.0	1.0	11.0
Total FTE							84.6
Max. FTE simultaneously in Facility							51

3 Upstream Process

3.1 Introduction

Engineered mammalian cells are used for the production of monoclonal antibody (mAb). The preliminary steps in their generation are comprised in Upstream Processing (USP). USP includes cell culture seed development, inoculum, bioreactor culturing and harvest (Joseph, 2018). The crucial aim of USP is to scale up the volume of culture systems up to several thousand litres, in order to produce kilograms of the desired mAb in a facility designed to reduce the risk of contamination.

The workflow for this case study is represented in Figure 14 and it can be summarized as follows.

The seed train is characterized by three passages performed in 7 days to obtain a final volume of 2 L in shake flask. Then the content of the 2 L shake flask is transferred into the 50 L wave bag bioreactor together with 14 L of basal medium and 4 L of feed medium to reach a final volume of 20 L. After 3 days cells are transferred into the seed STR 200 bioreactor with 140 L of basal medium, on the second day of cultivation in this bioreactor 40 L of feed medium are added. Cells are re-collected 3 days after start. At this point cells are introduced in STR 2000 bioreactor, and 1400 L of basal medium are added. Feeding is started 3 days after the beginning of production with 400 L of medium to achieve a final volume of 2000 L. According to our calculations (refer to chapter 3.2) three STR 2000 bioreactors are necessary to produce 72 batches per year. Once completed the production phase, cells are harvested by a filtration step with depth filter before sending the product to the downstream processing (Schirmer et al., 2021).

3.2 Calculations

To determine the number of batches that will be needed to achieve the required production, the following equations were used (Equation 2):

Equation 2: Total amount of batches per year needed, of all the four mAb products (A to D), to fulfill the URS of the production facility.

$$\begin{aligned} \text{Total required batches per year } \left[\frac{\text{batch}}{\text{year}} \right] &= \sum \frac{\text{Required amount per year } \left[\frac{\text{kg}}{\text{year}} \right]}{\text{Amount of product per batch } \left[\frac{\text{kg}}{\text{batch}} \right]} = \\ \frac{100 \left[\frac{\text{kg}}{\text{year}} \right]}{5.472 \left[\frac{\text{kg}}{\text{batch}} \right]} + \frac{70 \left[\frac{\text{kg}}{\text{year}} \right]}{4.236 \left[\frac{\text{kg}}{\text{batch}} \right]} + \frac{70 \left[\frac{\text{kg}}{\text{year}} \right]}{2.56 \left[\frac{\text{kg}}{\text{batch}} \right]} + \frac{40 \left[\frac{\text{kg}}{\text{year}} \right]}{4.12 \left[\frac{\text{kg}}{\text{batch}} \right]} &= 19 + 17 + 28 + 8 = 72 \left[\frac{\text{batch}}{\text{year}} \right] \end{aligned}$$

Knowing the number of batches needed, the number of batches that can be done (in one year) using one bioreactor has been calculated as seen in Equation 3:

Equation 3: Calculation for number of batches needed to produce the defined amount of mAb product A according to the USR, for a year.

$$\text{Number of batches } [-] = \frac{\text{operational readiness } [d]}{\text{length of production in bioreactor } [d]} = \frac{320 d - 13 d}{(11 + 1) d} = 25.58 \approx 25$$

Where the operational readiness and the production time in the bioreactor is given by the Operational requirements in the URS document. One day has been added to the production time for cleaning purposes. It is then possible to calculate the number of bioreactor lines that will be necessary to reach the yearly production rate with Equation 4:

Equation 4: Calculation for the number of required production bioreactors.

$$\text{Number of bioreactor lines } [-] = \frac{\text{Total required batches per year } \left[\frac{\text{batch}}{\text{year}} \right]}{\text{batches per year per reactor } [\text{batch}]} = \frac{72}{25} = 2.88 \approx 3$$

With the number of bioreactor lines, the minimum number of seed trains can be calculated as follows (Equation 5):

Equation 5: Calculation for the number of required seed lines.

$$\text{Number of seed lines } [-] = \frac{\text{number of bioreactor lines } [-] * \text{length of a seeding process } [d]}{\text{length of production in bioreactor } [d]} = \frac{3 * (3 + 1) d}{(11 + 1) d} = 1$$

In this calculation, an additional day has been added to each step to account for the cleaning and preparations of the utilities. Only one seed line will be necessary to meet the production requirements.

Finally, the number of inoculum lines can be determined with the following formula (Equation 6):

Equation 6: Calculation for the number of required inoculum lines.

$$\text{Number of inoculum lines } [-] = \frac{\text{number of bioreactor lines } [-] * \text{length of an inoculum process } [d]}{\text{length of production in bioreactor } [d]} = \frac{3 * (7 + 1) d}{(11 + 1) d} = 2$$

An additional day has also been used in each step to account for the cleaning and preparation of the utilities. In this case, two inoculum lines will be required. These results are the basis on which the rest of the production plan will be designed.

3.3 Plant on a page

For the upstream process, a plant on a page was designed (refer to Figure 14). The main goal is to get an overview over the whole process. Also, the necessary equipment can be listed that way. The plant on a page consists of four steps: the inoculum step, seed 1, seed 2, the production step and a depth filtration step. The detailed description for each step can be found in chapter 3.1. As mentioned, three production lines are planned to be used therefore three STR 2000 bioreactors were added. Only the lines of one production reactor were drawn to avoid clutter. The media and feed container are shown, however those are to be transferred from the media preparation room.

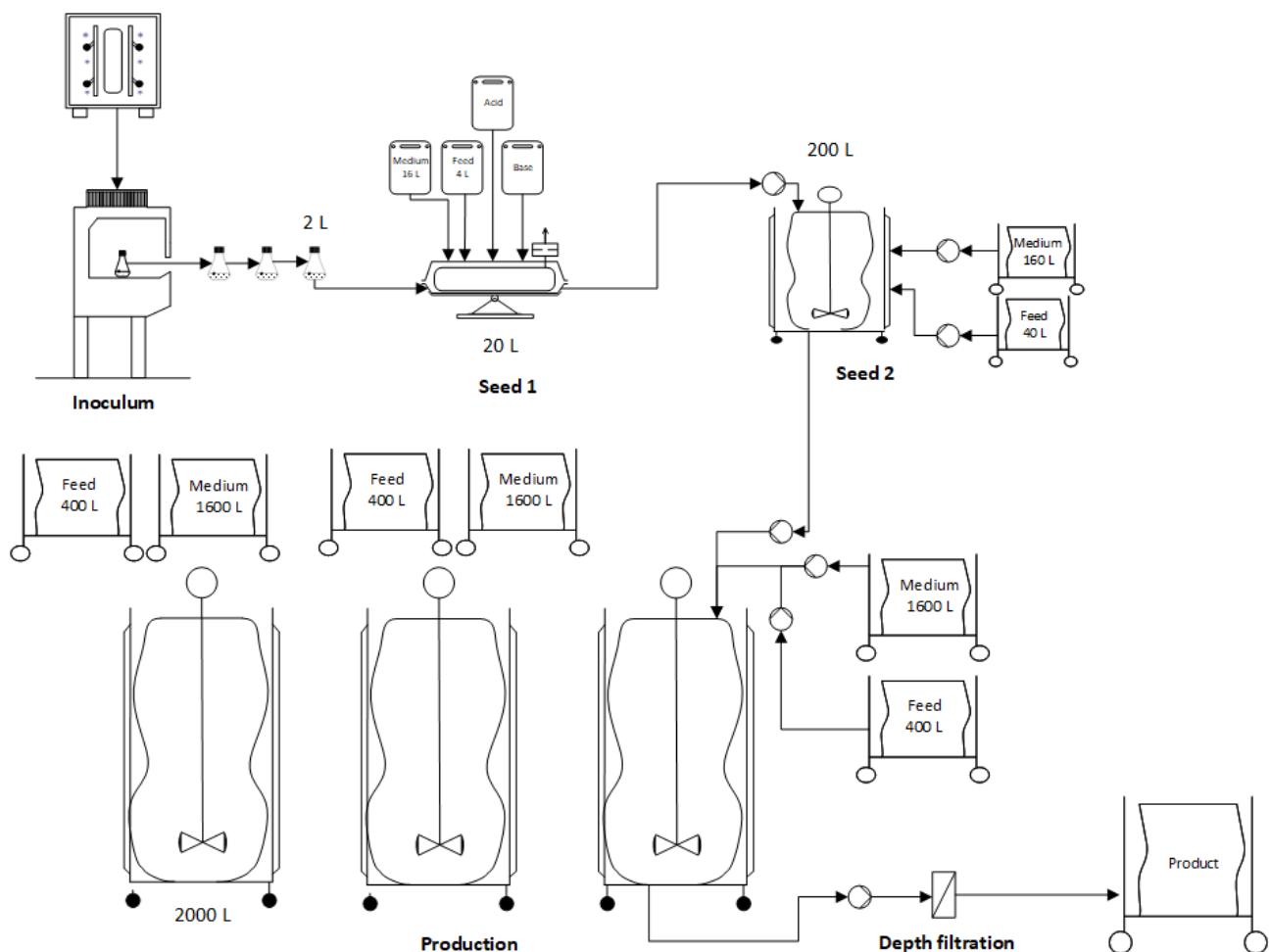


Figure 14: USP Plant on a page

3.4 HakoBio room concept

For the simulation and visualization of the room concept, the HakoBio software from PALL Corporation was used. The images are shown as 3D visualization and represent the inoculum room (1), seeding (2) and production (3) room, followed by the transfer (4) to the harvest room and depth filtration process (5). Finally, the production is transferred further to the DSP (6) Lastly transferring the production further to the DSP (6) (Figure 15). The devices and production equipment used for this design are described and shown in chapter 3.5. The production process starts in the inoculation room, in cleanliness class C. Followed by a material and personnel airlock, the seeding and USP process are in the same room. This room is categorized as cleanliness class D. Hereby the process is divided into the seeding train and production process. Last of all, the harvesting is performed in a separate room, also classified as class D.

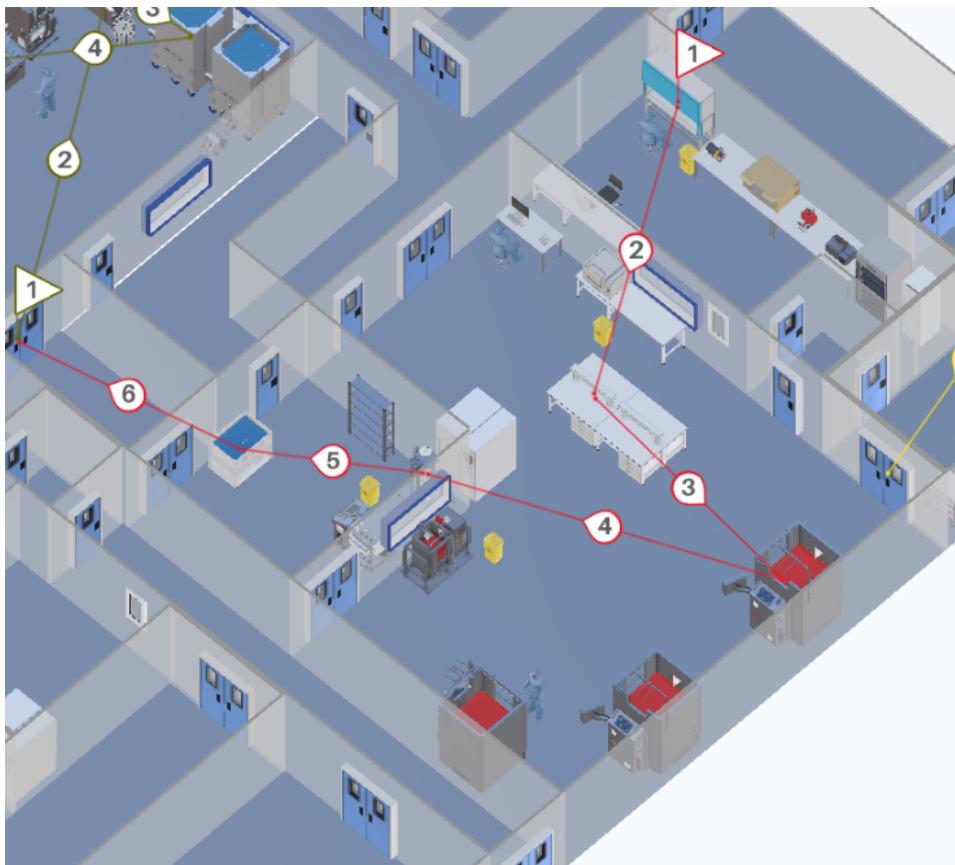
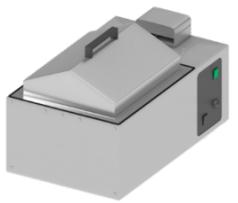


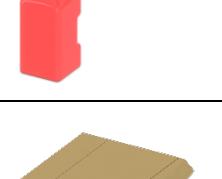
Figure 15: 3D layout of the USP process (created with HakoBio). The process flow is shown with the red process line, starting in the inoculum room (1), seeding (2) and production (3) room, followed by the transfer (4) to the harvest room (5), where the depth filtration is performed. Lastly transferring the production further to the DSP (6).

3.5 Size and functions of USP system

In this paragraph, Table 7 shows the equipment required for USP in mAb production. The table highlights the types of material, their quantity, power consumption and size. The analysis of the equipment needed, and their size is crucial for the biopharmaceutical facility design.

Table 7: Equipment for USP in mAb production

INOCULUM LAB				
Equipment	Size (WxDxH) [m]	Power consump-tion [W]	Quantitiy	Photo
Laminar Flow Hood	1.78 x 0.59 x 2.20	69	1	
Nucleo-Counter® NC-200™	0.46 x 0.26 x 0.26	20	1	
Combined refrigerator-freezer	0.6 x 0.7 x 2.01	2900	1	
Large-Capacity Reach-In CO2 Incubator	0.96 x 0.92 x 2.03		1	
SWBR17 SHEL LAB Shaking Water bath	0.65 x 0.41 x 0.4	900	1	

Large Benchtop	2.83 x 0.80 x 0.80	-	1	
Large Laboratory Bench	2.49 x 0.98 x 0.80	-	2	
Chair	0.66 x 0.66 x 0.86	-	1	
Desktop Computer	0.57 x 0.32 x 0.50	200	1	
Biohazard trash	0.4 x 0.67 x 0.33	-	1	
Inverted microscope	0.34 x 0.51 x 0.61	3	1	
Stool	0.4 x 0.35 x 0.59	-	1	
Cell culture media	0.12 x 0.12 x 0.28	-	1	
INFORS Multitron II Stackable incubation shaker	1.1 x 0.88 x 0.55	330	1	

USP – SEEDING				
Equipment	Size (WxDxH) [m]	Power consump- tion [W]	Quantity	Photo
Allegro™ STR 200 L Single- Use Jacketed Stirred Tank Bioreactor	1.62 x 1.17 x 2.12	-	1	
Allegro™ XRS 25 Biore- actor System	0.90 x 0.60 x 0.62	-	1	
Double Benchtop	1.52 x 1.55 x 1.18	-	2	
Biohazard trash	0.4 x 0.67 x 0.33	-	1	

USP - PRODUCTION				
Equipment	Size (WxDxH) [m]	Power consumption [W]	Quantity	Photo
Allegro™ STR 2000 L Single-Use Stirred Tank Bioreactor	1.78 x 1.74 x 2.93	-	3	
Desktop Computer	0.57 x 0.32 x 0.50	200	1	
Corded telephone	0.24 x 0.3 x 0.04	2	1	
Biohazard trash	0.4 x 0.67 x 0.33	-	2	
Small laboratory bench	1.51 x 0.69 x 0.81	-	1	
Chair	0.66 x 0.66 x 0.86	-	1	

DEPTH FILTRATION				
Equipment	Size (WxDxH) [m]	Power con-sumption [W]	Quantitiy	Photo
Stax™ Disposable Depth Filter System	0.80 x 1.15 x 1.94	-	1	
Allegro MVP Single-use system with Watson Marlow pump	0.96 x 1.28 x 1.13	-	1	
Allegro™ 3D 2000 L Modular Tote	1.26 x 1.07 x 3.01	-	1	
Biohazard trash	0.4 x 0.67 x 0.33	-	1	
Stock Shelves	1.40 x 0.30 x 2.00	-	1	

4 Downstream Process

4.1 Introduction

The downstream process (DSP) is the second important step of the core manufacturing process and it aims to render the product to its final bulk formation. For this purpose, the clarified product is isolated, concentrated and polished in several steps and then packed in closed and transportable containers for fill-finish activities (Joseph, 2018).

For this case study work it was decided to physically segregate the main downstream activities in two separate suites, one for all activities prior to the virus filtration (nanofiltration) and one for all following activities, such as diafiltration, ultrafiltration and bulk filling. To highlight the separation, the rooms were labelled as 'DSP V+' and 'DSP V-', both corresponding to cleanroom class C. The rationale for this design is to avoid any cross-contamination of the post-viral process material with potentially contaminated pre-viral material. The filled product is then fed through material locks into a CNC room for bulk freezing before being stored in an adjacent storage room. Further process support activities, such as washing clean and staging, are to take place in a separate class D cleaning room adjacent to the DSP V+ room. The DSP is constantly monitored by three full-time staff members, two of whom work primarily in the pre-viral and one in the post-viral filtration zone. The following subchapters provide more in-depth information on the process, the premises and the equipment used in DSP.

4.2 Plant on a page

The Figure 16 shows the plant on a page for the DSP. The layout was created based on the occupancy list and the provided process description. After harvesting the product will be transported in a mobile tote to the DSP V+ room, where it makes its journey through the subsequent purification steps. Upon completion of the nanofiltration, the material is then transferred into the DSP V- room for ultra-/diafiltration (UF/DF) and filling. Most activities in the DSP require buffers, which are provided as concentrates from the buffer suites and pumped through the wall into the DSP rooms via aseptic connections. The concentrates are diluted *in-situ* to the respective working concentrations using a buffer management system. Such a set-up allows buffers to be ready on demand and mitigates the risk of introducing contaminants through needless buffer handling and transport. On the plant on a page, the buffer management systems are visualized as mixing valves, which are connected to multiple unit operations. However, there are separate ÄKTA systems for each chromatography step, to increase reproducibility and flexibility. A detailed process description is provided in the next subchapter.

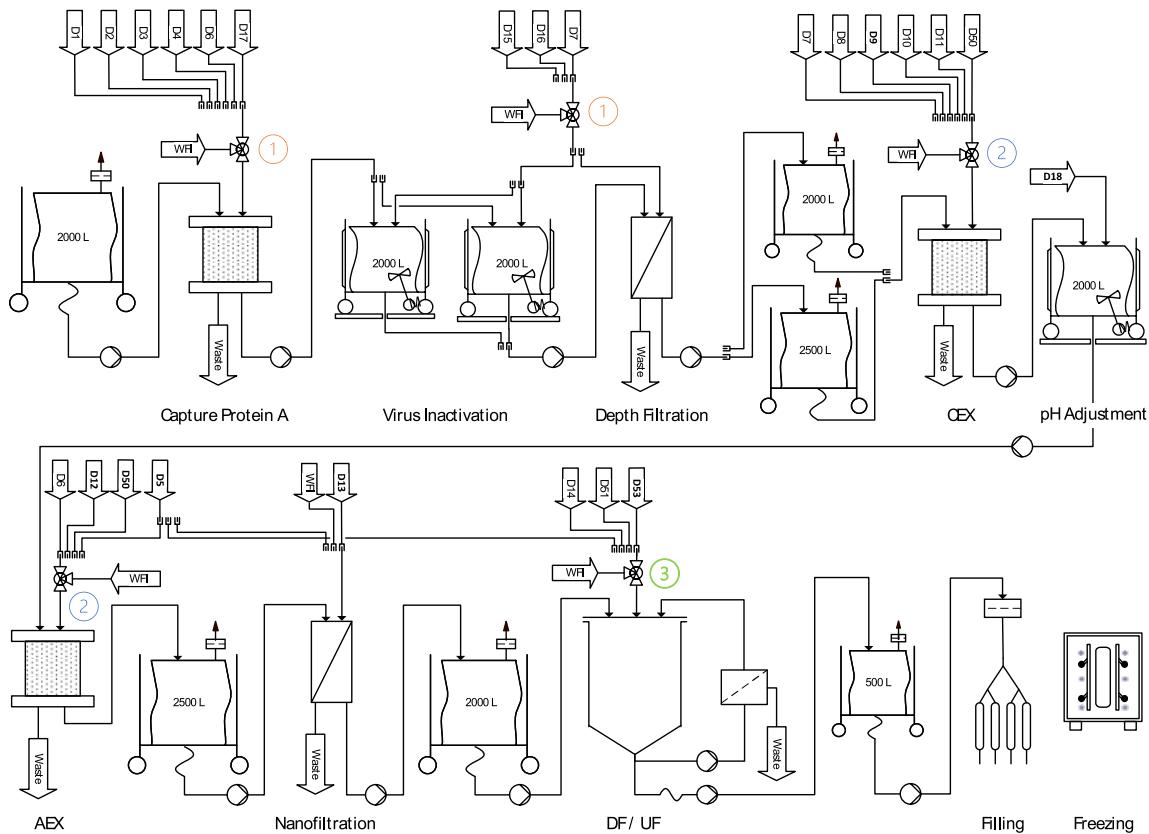


Figure 16: DSP Plant on a page.

There are three buffer management systems. The first buffer management system (1, orange) supplies buffers for protein A capture and virus inactivation. The second buffer management system (2, blue) supplies buffers for CEX and AEX. The third buffer management system (3, green) supplies buffers for DF/UF. The pH adjustment and nanofiltration buffers are provided in transportable totes to the DSP suites.

4.3 Hako Bio room concept

One DSP line was planned for the purification of all four products, since the sequential USP approach provides a new batch each 3.7 days and the total DSP time is 3.8 days. Therefore, DSP can start processing a new batch as soon as the previous batch is in the fill and finish phase. By temporally separating the processes, any contamination between products is avoided. The HakoBio layout of the DSP rooms can be found in Figure 17. The material flow is marked as green and the corresponding process steps are described in the following paragraph.

The harvested product from the upstream processing is transported to the DSP V+ suite via a material airlock (1). For the first chromatography step (protein A capture) the material is introduced into a ÄKTA™ ready XL system, which is flushed and washed by different buffers provided by a buffer management system (2). The eluted and purified product is collected in two 2000 L tanks (3). The material is then aseptically connected to an Allegro™ MVP system for virus inactivation, which is also tied to the buffer management system (4). The same Allegro™ MVP system is used to pump

the virus inactivated product through three interconnected depth filters containing 21 Stax filters with 1m^2 area each (5). The filtrate is collected in a 2000L and 2500L tank, which are subsequently transported with a drive unit to a second ÄKTA™ ready XL system for cation exchange chromatography (CIEX) (6). The purified product is eluted into a 2000 L tank (7) and transported to a second Allegro™ MVP system for pH adjustment (8). This step is performed directly in the stirrable 2000 L tank and with buffers provided from the buffer suites in totes. This tank is then brought to a third ÄKTA™ ready XL system for anion exchange chromatography (AIEX) (9). The eluted and purified product is collected in a 2000L tank (10). Both, CIEX and AIEX ÄKTA systems are managed by one buffer management system. The nanofiltration step is then performed on the same Allegro™ MVP system as the pH adjustment step, to which two nanofiltration capsules must be installed (11). The virus filtered product is collected in a 2000L tank (12), which is ready to be transported to the 'DSP V-' room (13).

Once the product arrives in the viral free zone, it is further processed in a fully automated centrasette TFF system, which is also supplied with buffers by a buffer management system (14). The ultra- and diafiltrated material is directly connected to one depth filter containing 4 Stax filters with 0.5 m^2 area each (15) and then collected in a 500 L tote (16). The purified bulk material is filled (17) and transported with a trolley through a material airlock into the DSP freezing room (18) and subsequent storage room.

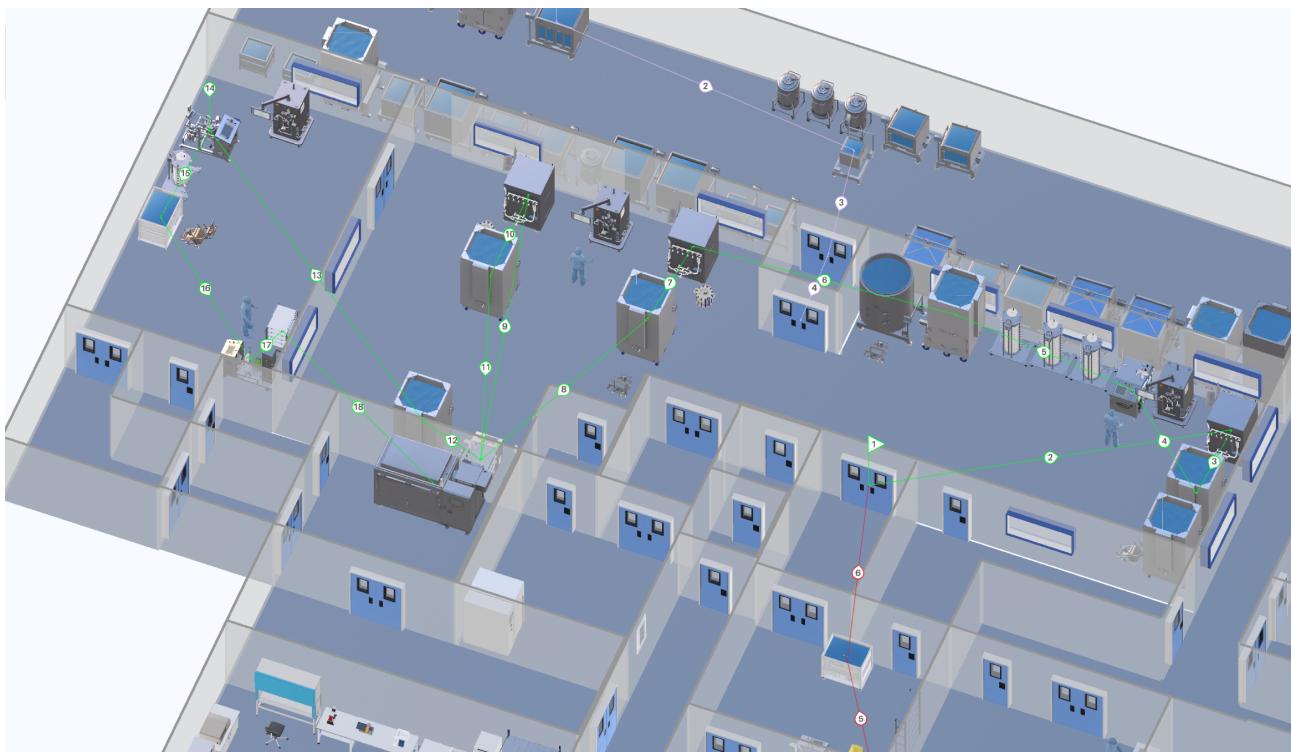


Figure 17: 3D layout of the DSP process (created with HakoBio). The process flow is shown with the green process line and subdivided in 18 process steps.

4.4 Size and function of DSP systems

The required equipment for the DSP rooms, their size and quantity are provided in Table 8, Table 9, Table 10 and Table 11. The DSP relies heavily on single-use peripheral elements, such as connectors, aseptic transfer systems, tank liners and valves. Among the single-use equipment worth mentioning is the Allegro™ Connect Buffer Management System. Concentrated buffer solutions from the buffer cold storage room are coupled via an aseptic connection across the wall to the buffer management system in the DSP suites, where they are mixed with WFI and directly available to supply the equipment's. Furthermore, three ÄKTA™ ready XL systems are located in the DSP V+ room, each dedicated to one chromatography step. This setup allows for more flexibility, especially in cases where upstream or downstream process times could be shortened and several ÄKTA™ ready XL systems could be operated in parallel. Finally, it is worth mentioning the Allegro™ MVP disposable system. This device is an excellent all-rounder for several DSP steps such as virus inactivation, pH adjustment, depth filtration and nanofiltration. Overall, the single-use approach generates higher operating costs and stronger dependence on suppliers. However, the greater flexibility and smaller footprint of the plant (no CIP/SIP required) are a significant advantage, even for DSP. Furthermore, single-use systems are safer due to the decreased risk of microbial contamination, which is of absolute priority in a multiproduct facility.

Table 8: Required equipment for the DSP V+ area.

DSP V+				
Equipment	Information	Quantity	Size (WxDxH) [m]	Photo
Allegro™ Connect Buffer Management System	In-line buffer dilution system Energy consumption: 230V	2	1.12x1.12x1.99	
2000 L Jacketed Cubical Tank with Load Cell	Stainless Steel Tank for Use with Magnetic Mixer Drive Unit	6	1.78x1.39x2.65	

2500 L Jacketed Cubical Tank with Load Cell	Stainless Steel Tank for Use with Magnetic Mixer Drive Unit	1	1.75x1.66x2.29	
ÄKTA™ ready XL	Single-use chromatography system Flow rates from 45 to 3500 L/h	3	1.28x1.15x1.95	
ReadyToProcess™ 32L columns from Cytiva	Chromatography columns for protein A capture	1	0.7x0.7x0.63	
ReadyToProcess™ 32L columns from Cytiva	Chromatography columns for CIEX	1	0.7x0.7x0.63	
ReadyToProcess™ 32L columns from Cytiva	Chromatography columns for AIEX	1	0.7x0.7x0.63	
Allegro™ MVP Single-use system with Quattreflow pump	Multipurpose applications Energy consumption: 230V	2	0.96x1.28x1.13	

Magnetic Mixer Drive Unit	Robust single-use mixing system	3	0.4x0.82x1.03	
Stax™ Disposable Depth Filter High Chassis-	Chassis for up to 10 Large Stax Disposable Depth Filters - 21 Depth filters used per batch	3	0.8x1.15x1.93	
Kleenpak™ Nova Capsule NP6	For 100L to 1000L Virus removal filters used with MVP Single-use system	2	0.24x0.24x0.35	

Table 9: Required equipment for the DSP V- area.

DSP V-				
Equipment	Information	Quantity	Size (WxDxH) [m]	Photo
Allegro™ Connect Buffer Management System	In-line buffer dilution system Energy consumption: 230V	1	1.12 x1.12x1.99	
Fully Automated Centrasette TFF system	Tangential Flow Filtration System	1	0.8x1.5x1.5	

Single-Use TFF Module	Single-Use Module for Concentration/ Diafiltration	1	0.23x0.25x0.09	
Stax™ Disposable Depth Filter Medium Chassis	Medium chassis for up to 5 Stax Disposable Depth Filters - 4 Depth filters used per batch	1	0.8x1.15x1.31	
Bulk Filling System		1	0.69x1.61x1.58	
Allegro™ 500L Plastic Tote with Trolley	Collapsible	1	1.22x0.87x1.23	
Bulk Filling Trolley		1	1.02x0.66x1.15	
Magnetic Mixer Drive Unit	Robust single-use mixing system	1	0.4x0.82x1.03	

Table 10: Required equipment for the DSP Freezing area.

DSP Freezing				
Equipment	Information	Quantity	Size (WxDxH) [m]	Photo
Ross.pFTU large-scale	Plate-based freeze-thaw unit with control unit	1	3.17x1.39x2.25	

Table 11: Required equipment for the DSP Cleaning area.

DSP Cleaning				
Equipment	Information	Quantity	Size (WxDxH) [m]	Photo
Sterilization Autoclave GSS-L 6710 EC1	Steam Sterilizer	1	0.66x1.00x0.70	

5 Media and Buffer preparation

Activities involving media preparation are carried out in separate rooms by operators who are outfitted with appropriate protective equipment. Because of the high particle concentration of this combination of dry media and buffer component, rooms are classified as hygienic zone D and are found on the ground floor. In this study case four products A, B, C, and D with the amount of 19, 17, 28 and 9, respectively were considered. The medium preparation consists of 1000 L tank and 2000 L tank and is 37 m². For the buffer preparation room there are it several tanks, in particular 50 L, 100 L, 500 L, 1'000 L, 1'500 L, 2'000 L, 3'000 L in the area of 192 m². The buffer cold storage room consists of 124 m² (Table 26).

5.1 Buffer estimation per batch

During this process, 72 batches per year were carried out for the four different products (A, B, C, D) which makes about 2 batches per week. Due to safety reasons and uncomplicated usage, the inline dilution system was used, except for the buffers D5, D13 and D18.

Inline dilution is a process of transferring buffer concentrate, through an aseptic transport system, and diluting it with water directly at the site of use. As a result of using this technique, the efficiency and flexibility are exceeded. It enables to lower facility's footprint, as well as reduced utilities and equipment costs in the long run. One of the biggest disadvantages is the insurance of the quality of mixed buffer, due to physical difficulties with controlling it. (ispe.org, 2019)

Table 12: Calculations of the required volumes of the different buffers per batch.

Number	Buffer	Volume per batch [L]	Volume per two batch (one week) [L]	Volume concentrate per week [L]	Storage
D1	Wash 1 Buffer Chroma I	6776	13552	2711	3000
D2	Wash 2 Buffer Chroma I	3080	6160	1232	1500
D3	Wash 3 Buffer Chroma I	3080	6160	1232	1500
D4	Elution Buffer Chroma I	4312	8624	1725	2000
D5	NaOH 1M	960	1920	-	2000
D6	Regeneration Buffer Chroma III	2304	4608	922	1000
D7	Equilibration Buffer Chroma III	3006	6012	1202	1500
D8	Pre-/ Sanitization Buffer Chroma III	1816	3632	726	1000
D9	Wash Buffer 2 Chroma II	1135	2270	454	500
D10	Elution Buffer Chroma II	1589	3178	636	1000
D11	Strip Buffer Chroma II	681	1362	272	400
D12	Equilibration Buffer Chroma III	833	1666	333	400
D13	Equilibration / Flush Buffer Nano Filtration	251	502	-	400
D14	Diafiltration Buffer UF/DF2	4099	8198	1640	2000
D15	Acid Buffer Viral Inactivation	154	308	62	100
D16	Base Buffer Viral Inactivation	161	322	64	100
D17	Chroma I Storage Buffer 20% EtOH	924	1848	370	400
D18	pH Adjustment Buffer	226	452	-	500
D50	NaOH 0.01 M Storage Buffer	852	1704	341	400
D51	NaOH 0.5 M	431	862	172	200
D53	NaOH 0.1 M	340	680	136	200

5.2 Plant on a page

Media and feed preparation has an important role in this process and especially for the upstream process that follows. The following volumes of media and feed (see Table 13) are required for the different bioreactors during the process:

Table 13: Volume data for medium and feed for the entire process

Bioreactor	Media Volume/batch [L]	Feed Volume/batch [L]
Seed Bioreactor 1	16	4
Seed Bioreactor 2	160	40
Production Bioreactor	3x 1'600	3x 400

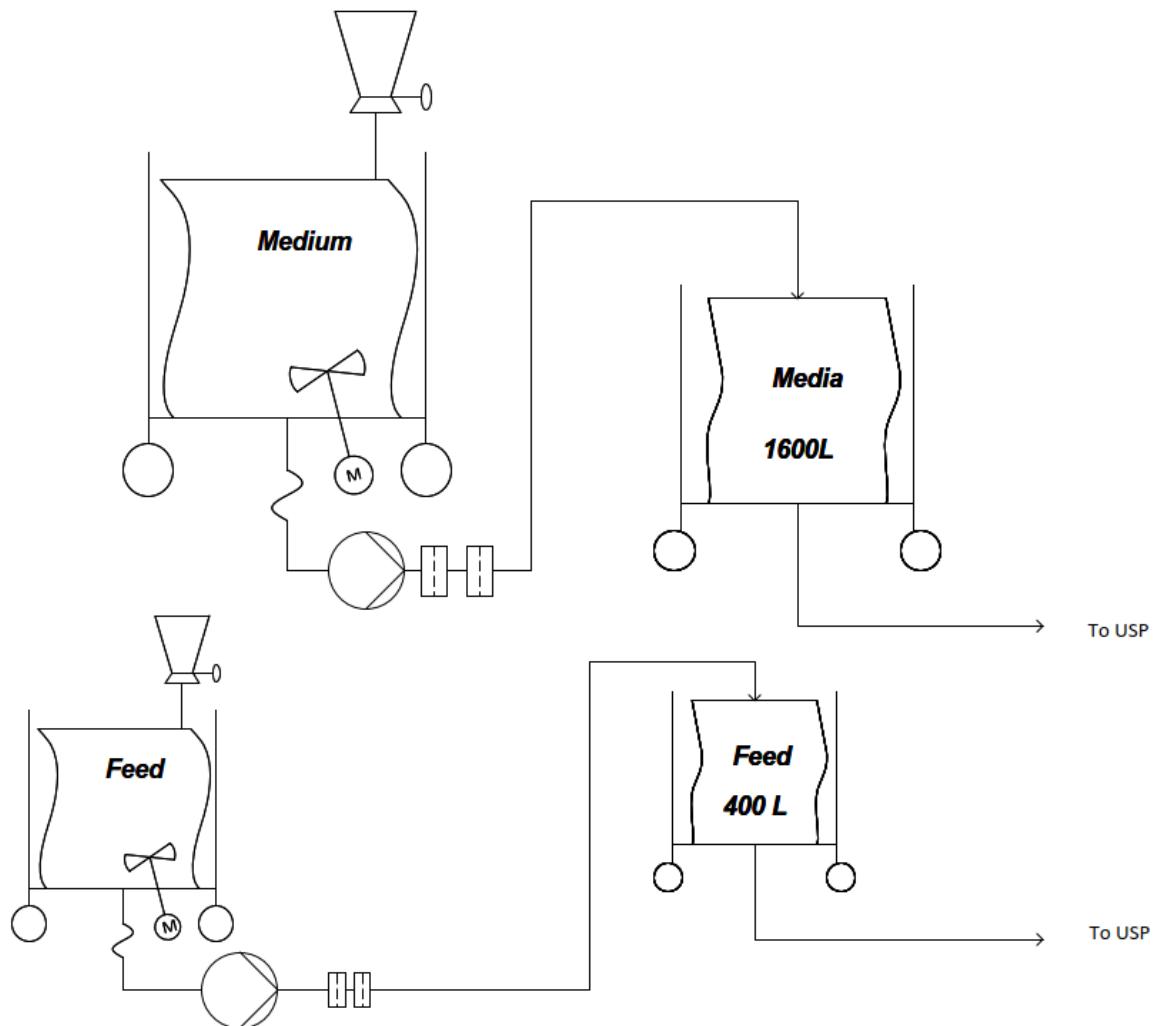


Figure 18: Plant on a page of media and feed preparation for the USP. Image created with Visio®.

The medium and feed are prepared in stirred tanks and then distributed into transportable bags, where each bioreactor in the upstream process receives the corresponding volume size. The plant on a page is showed on Figure 18.

Based on the calculations for the buffer preparation, a plant on a page (seen in Figure 19) was designed. Each buffer concentrate is produced in a large production reactor and then stored in a tank. The transport of the buffers to the DSP takes place through the inline dilution system. Buffer D5, D13 and D18 are transported through transportable bags and all other buffers through hard pipes.

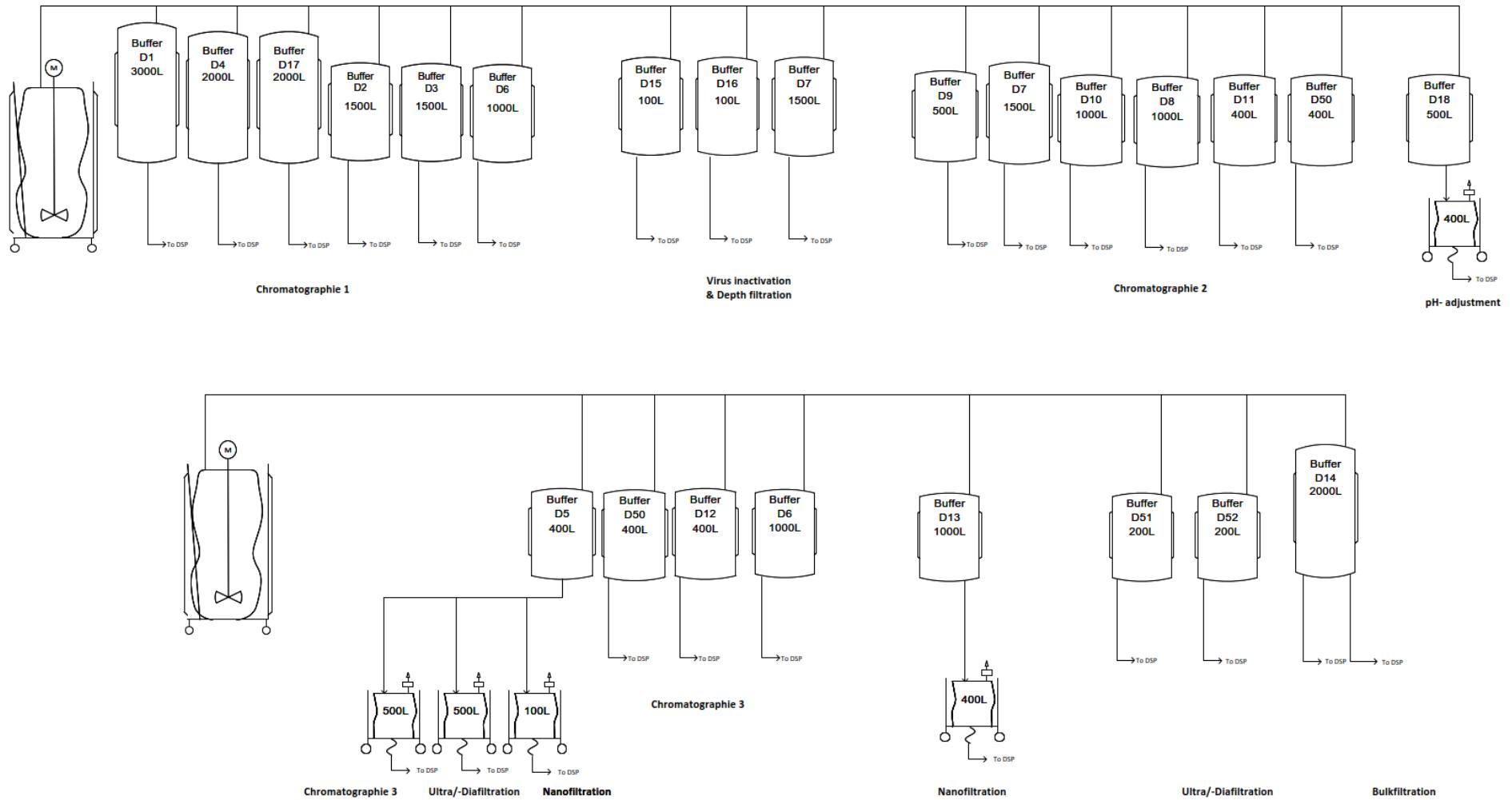


Figure 19: Plant on a page of buffer preparation for the DSP. Image was created with Visio®.

5.3 HakoBio room concept of media and buffer preparation

5.3.1 Room concept of Media & feed preparation & storage room

The room has 37 m² and is placed close to the USP production, to enable easier and shorter transport of already prepared media and feed to the upstream production. All the used tanks are going to be mobile, and ready for transit of needed materials. The room is depicted in the Figure 20.

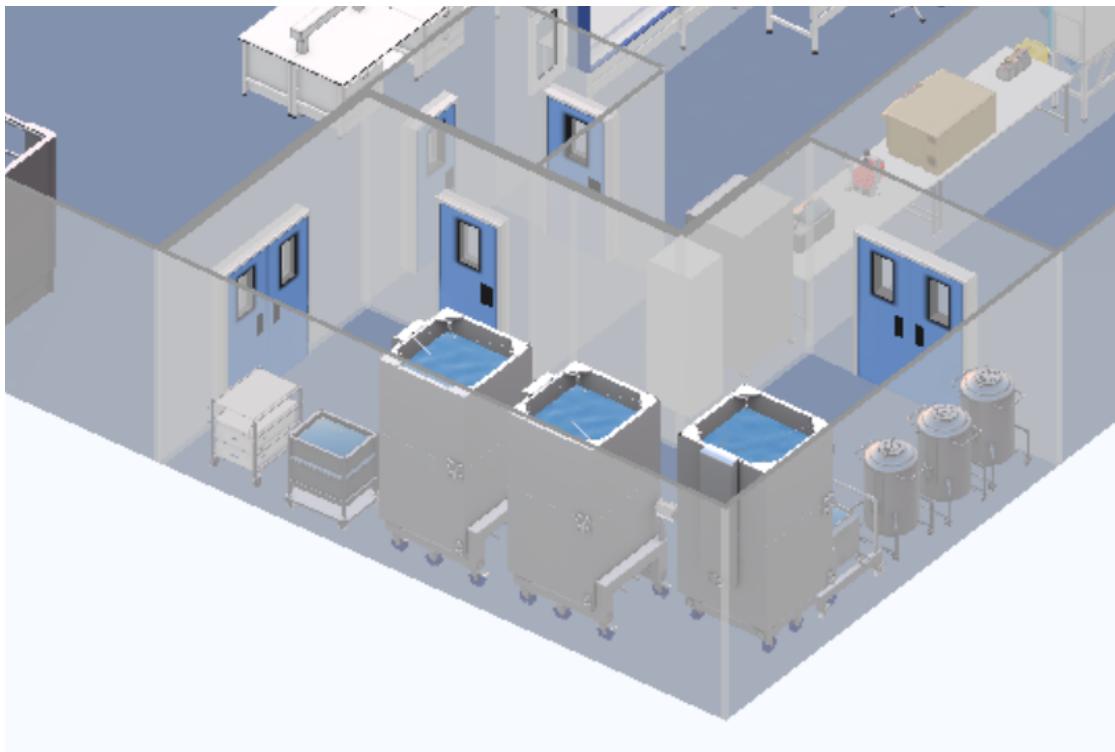


Figure 20: Media & feed preparation & storage room. Image created with HakoBio.

5.3.2 Room concept of buffer preparation & storage

The buffer preparation has 192 m² and storage room has 124 m² and is right next to the DPS production.

The inline dilution system will be used for 18 out of 21 buffers, which made it necessary to place those two rooms close to each other so that the process of transferring buffer into downstream production does not disturb other production steps or the work organization of the whole facility. For the remaining three buffers system with preparation in separate tanks and delivery with the use of mobile tanks was chosen, due to difficulties with the placement of the equipment in the DSP production.

Despite the current trend of working with single-use technologies, it was decided to use regular tanks for preparation and storage, due to the very small variation of the used buffers in between the products. The buffer prep and storage room are depicted in the Figure 21.

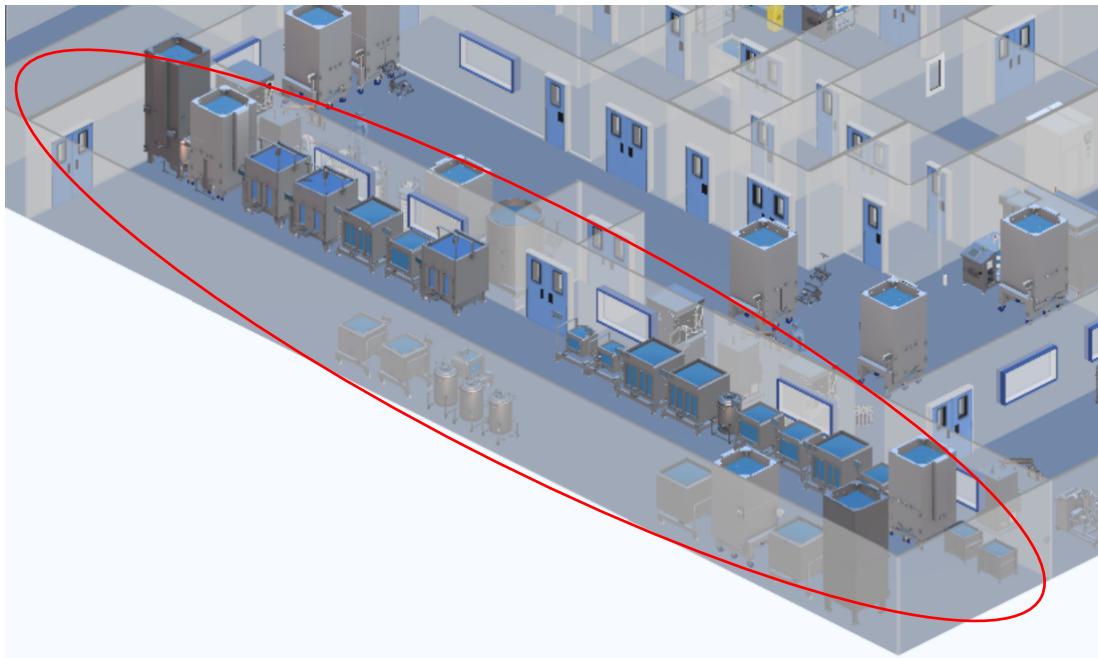


Figure 21: Buffer preparation & storage room. Image created with HakoBio.

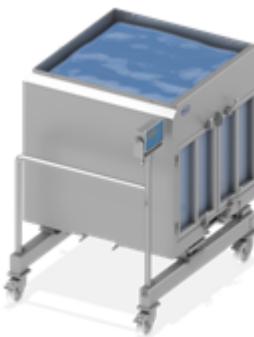
5.4 Size and function of MP and BP systems

The equipment needed for media and buffer preparation and storage in mAb manufacturing is listed in this paragraph. Table 14 and Table 15 show the different sorts of materials, as well as their number and size. For the design of a biopharmaceutical facility, a review of the equipment required, and its scale is critical.

Table 14: Size and Function of Media Systems

Media and feed storage and preparation room			
Equipment	Quantity	Size in m	Photo
2000 L Jacketed Cubical Tank with Load Cell	3	1.78 x 1.39 x 2.65	
200 L stainless steel Bioreactor with Trolley	1	0.82 x 0.65 x 1.2	
2 x 20 L Biocontainers with Trolley	1	0.71x1.05x0.98	
500 L formulation tank	3	0.72 x 0.83 x 1.39	
100 L Jacketed Cubical Tank with Load Cell, Stainless Steel	1	1 x 0.86 x 1.58	

Table 15: Size and Function of Buffer Systems

Buffer storage and preparation room			
Equipment	Quantity	Size in m	Photo
3000 L Jacketed Cubital Tank with Load Cell	1	1.64 x 1.40 x 3.56	
2000 L Jacketed Cubical Tank with Load Cell	2	1.78 x 1.39 x 2.65	
1500 L Jacketed Cubical Tank with Load Cell	3	1.62 x 1.39 x 2.17	
1000 L Jacketed Cubical Tank with Load Cell	3	1.53 x 1.29 x 1.60	

500 L Formulation tank	4	0.72 x 0.83 x 1.39	
400 L Jacketed Cu-bical Tank with Load Cell	5	1.27 x 1.01 x 1.58	
200 L stainless steel Bioreactor with Trolley	3	0.82 x 0.65 x 1.2	
100 L stainless steel Tank	3	1.00 x 0.86 x 1.58	

6 Quality Control

A rock-solid quality control (QC) is needed to produce four different mAb products. The QC will perform quality analysis of many different parameters, which are essential to the end quality of a product. Analysis will be performed on the raw materials, intermediate products which will be obtained during each step during the process and the end product itself. To fulfil these tasks a laboratory was designed with equipment's such as a Cell counter, Spectrophotometer and other analytical devices listed in the chapter below. Since Hakobio does not support analytical apparatus such as Liquid Chromatography, they have not been included. A total of 16 FTEs will be working in the Quality department, 5 for the QA and 11 for the QC.

6.1 HakoBio room concept of the QC Laboratory

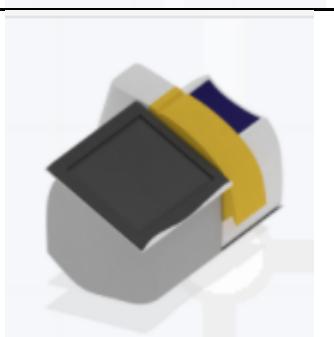
The laboratory has a footprint of 70 square meters, with an occupancy of 22%. It has been designed to allow the QC staff to work comfortably on their task, with room to move around and space on the tables next to the instruments for samples and flasks. The Figure 22 shows the 3D model of the room and its contents. A fridge, a freezer and shelving have been attributed to have all the necessary reagents and solvents at hand, as well as a autoclave to destroy biowaste inside the lab directly. All the contents of the QC laboratory are described in Table 16.



Figure 22: HakoBio 3D Drawing of the QC Lab

6.2 Size and functions of QC systems

Table 16: Size and Function of the QC Lab

Quality Control Systems			
Equipment	Quantity	Size in m	Photo
Laminar Flow Hood	2	1.78 x 0.59 x 2.2	 A blue and white laminar flow hood unit with a blue front panel and a white base.
Nucleocounter NC-200 Automated Cell Counters	1	0.46 x 0.26 x 0.26	 A grey and yellow automated cell counter device.
Leica DM750 Optical Microscope	1	0.2 x 0.37 x 0.44	 A white Leica DM750 optical microscope.
3250 Single Sample Osmometer	1	0.33 x 0.46 x 0.41	 A white 3250 single sample osmometer.

V-730 UV-Vis Spectrophotometer	1	0.47 x 0.44 x 0.23	
Midrics Complete Bench Scale	1	0.32 x 0.39 x 0.1	
SevenCompact pH meter S220	1	0.35 x 0.36 x 0.38	
Laptop	1	0.34 x 0.24 x 0.24	
Allegra X-15R Centrifuge	1	0.77 x 0.63 x 0.36	
Freezer MDF-U333	1	0.75 x 0.62 x 1.64	

Lab Combined refrigerator-freezer	1	0.6 x 0.7 x 2.01	
Sterilization Autoclave GSS-L 6710 EC1	1	0.66 x 1 x 0.7	
Chair	4	0.66 x 0.66 x 0.86	
Large Benchtop	2	2.83 x 0.8 x 0.8	
Small Benchtop	2	1.52 x 0.81 x 0.8	

7 Clean facility utilities

7.1 List of necessary clean and technical utilities

The biopharmaceutical manufacturing uses a multitude various, precise processes that require different resources and energies, for example water, electricity, and compressed air. These can be classified into “Clean Utilities and Technical Utilities (also called Black Utilities)” categories (see Table 17) (Joseph, 2018).

Table 17: List of clean and technical utilities.

Clean Utilities	Technical Utilities (Black Utilities)
Water for Injection (WFI)	Wastewater Collection/ Inactivation (Bio / non-Bio)
Purified Water (PW)	Water (Portable, Cooling, Heating and Fire Water)
Clean Steam	Compressed Air
Clean Compressed air	HVAC
Process Air	Electrical Power
Process Gases (N ₂ , O ₂ , CO ₂)	Technical Steam (Black Steam – Fresh / Used) Waste (Plastics / Paper / Other) Gasoline Nitrogen Liquid

7.2 HVAC

The HVAC system should provide for the worker a well-appointed working environment as well as the provider of rooms and the manufacturing process with the required grade of cleanliness and pressurization to ease cGMP and BSL requirements for manufacturing. The basic four HVAC system functions are depicted in Table 18 (Joseph, 2018).

Table 18: HVAC system and their respective functions.

HVAC system	Functions
Maintain room cleanliness	<ul style="list-style-type: none"> Control of airborne particles, dust, and micro-organisms <ul style="list-style-type: none"> Performed through air filtration using high-efficiency particulate air (HEPA) filters
Maintain room pressure	<ul style="list-style-type: none"> Air flow must come from the cleaner area toward the adjoining space <ul style="list-style-type: none"> To reduce the chance of airborne contamination Achieved by more air into the cleaner space than is mechanically removed from that same space
Maintain space moisture	<ul style="list-style-type: none"> Controlled by cooling air to dew point temperatures or by using desiccant humidifiers <ul style="list-style-type: none"> Can affect the efficacy and stability of drugs
Maintain space temperature	<ul style="list-style-type: none"> Can affect production <ul style="list-style-type: none"> Directly by impacting chemical or biochemical reactions Indirectly by fostering growth of microbial contaminants in the process or on workers

7.3 Clean utilities

Clean utilities have a direct impact on the quality of the product; therefore, they are defined as the necessary requirements by the production process. The cleanliness of the utilities must be as pure as possible to make sure that no novel contaminant is introduced into the production process. Clean utilities comprise (Joseph, 2018):

Table 19: Functions of clean utilities

Clean Utilities	Utilized for
WFI and PW	<ul style="list-style-type: none"> To produce buffers and cell culture media For CIP operations
Clean Steam	<ul style="list-style-type: none"> For sterilization of product contacting surface
Clean Compressed Air	<ul style="list-style-type: none"> For blow down of transfer pipes and drying of product contacting surface after cleaning and sterilizing Pneumatic valves within the transfer pipe network and unit operations
Clean Process Gases	<ul style="list-style-type: none"> Within the cell culture processes

7.3.1 List of equipment used for clean utilities

A list of the main equipment used for clean utilities is provided in the Table 20. The table is intended to show a simplified summary of all the necessary equipment lists for clean utilities. The major components in a clean utility are shown, followed by where they can be found (stages). Then all equipment necessary for production / generation is listed below (Joseph, 2018).

Table 20: List of equipment for clean utilities.

PW	WFI	Clean Steam	Clean Compressed air	Process Air	Process Gases	Liquid Nitrogen
Basement	Basement	Basement	Basement	2nd Floor	Outside	Outside
Heat exchanger	Heat exchanger	Distillation Column	Filters	Filters	Storage Tanks for all gases	Storage Tank
Storage Tank	Storage Tank	Compressor	Pneumatic Valve	Cooler	Pneumatic Valve	Pneumatic Valve
Reverse Osmosis	Distillation Column	-	Storage	Heater + Reheaters	-	-
Nano Filtration	-	-	Compressor	Humidifier	-	-
Water Treatment	-	-	-	Mixing Chambers	-	-

7.3.2 Water requirements

In Table 21 the total amount of water (in liters, L) required for USP and DSP is shown. These numbers have been estimated from the numbers given by the DSP, USP block flow diagrams and the URS.

Table 21: Liters of water required for USP and DSP.

	WFI*	PW*	Solid Waste*	Liquid Waste*	Liquid Biological**
DSP	3541	-	625	42000	-
USP	-	2500	-	-	2500

7.4 WFI, PW and clean steam

The biopharmaceutical manufacturing uses water, which needs to be suitable for the process steps. This generally means it must be as clean as necessary regarding bioburden, particles and soluble. The kind of clean water which is necessary for each step is determined by the process step. The purity is measured by conductivity, pH, total organic carbon, and endotoxin content. The designer must consider the different grades of water production depending to the United States Pharmacopeia (USP), WHO, or European Pharmacopoeia (EUPH) or other region-specific guidelines which are portable water, purified water (PW) and water for injection (WFI). Each kind of water is “cleaner” as the type before (Joseph, 2018).

Portable water is basically tap water while PW and WFI are generated in a cascade in a continuous manner (see Figure 23). The process is guided under GMP and starts with drinkable water of the local provider, also defined, or described as potable water. The potable water is filtered by Nanofiltration and followed by UV sterilization. Then it will be transferred to the reverse osmosis system.

Reverse osmosis is used to produce WFI. In order to have a continuous flow even if filters must be exchanged 2 reverse osmosis system are installed as well as 2 nanofiltration systems. The PW can be used for USP, DSP, MP and BP. Clean steam is also generated from PW via distillation and is predominantly used for cleaning. For the WFI generation, the PW goes through a multi-distillation column which saves energy. WFI is also used in USP, DSP, MP and BP (Joseph, 2018).

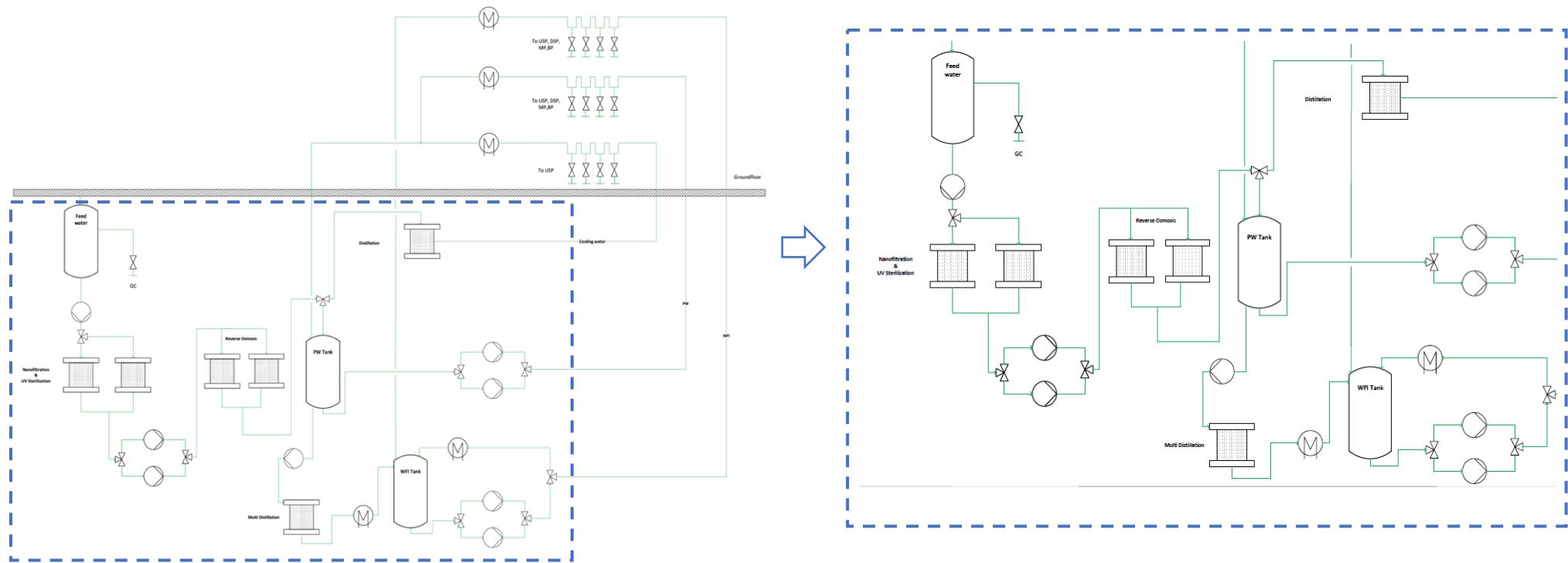


Figure 23: Plant on a page of PW, WFI and clean steam systems. Designed with Visio®. The right figure zooms in the production part of the WFI-plant.

7.4.1 Characteristics of the system

Dead legs need to be minimized to avoid stagnant water (bioburden), material = Stainless Steel 316L, constant turbulent flow needs to be achieved through the pumps to reduce bacterial growth, a positive pressure between the loop and Points of use (POUs) needs to be guaranteed to avoid contamination, adequate slope of piping toward the drains needs to be achieved to allow complete drainage, air breaks to wastewater need to be installed to eliminate backwash, two pumps are used for redundancy.

There are two types of steams which are required within the biopharmaceutical facility (Joseph, 2018). The difference is seen in Table 22.

Table 22: Types of steam and their function.

Kind of steam	Production	Used for
Technical/ Black steam	Produced from a boiler	Heating of non-product contacting surfaces and systems
Clean steam	Generated from treated water free of volatile additives	Thermal disinfection or sterilization processes For sterilization of products, and more typically equipment

A steam produced by a boiler is pressurized and used to produce the clean steam of the clean steam distillation column.

7.5 Technical utilities

Technical utilities, also known as black utilities, are used for the direct support of the process operation, while it does not have a direct contact with the product. Technical utilities may comprise the elements listed in Table 23 (Joseph, 2018).

Table 23: Technical utilities functions

Technical Utilities	Utilized for
Potable water	<ul style="list-style-type: none"> To higher grades of water To use within the domestic systems of a facility
Fire Water	<ul style="list-style-type: none"> For the safety
Cooling/chilled water/glycol	<ul style="list-style-type: none"> For non-product contact cooling applications
Hot water/technical steam	<ul style="list-style-type: none"> Required for non-product contact heating applications
Electrical power	-
Natural or liquefied gas	<ul style="list-style-type: none"> Needed for firing gas boilers
Wastewater collection/inactivation	-

7.6 Waste

During biopharmaceutical processes waste is always generated. The majority of the waste is from the production process steps and can be classified in biological waste and non-biological waste. For instance, the kind of waste in an upstream process can be generated during the cell growth media, bioreactor, media culture hold bags, filters, and tubing or during the steam in place process a steam waste can be generated. Waste can be differentiated to solids, liquids, and gases. The kind of waste is summarized in the following Table 24 with their impact (Joseph, 2018).

Table 24: Types of waste and their impact.

Waste type	Impact of waste
Non-Bioactive Waste	<ul style="list-style-type: none"> • Directly discarded or slightly treated to diminish the environmental impact
Liquids	<ul style="list-style-type: none"> • Non-biological liquids waste <ul style="list-style-type: none"> ◦ Collection within a waste tank followed by chemical treatments • Harmful waste <ul style="list-style-type: none"> ◦ Further purification processing may be required
Solids	<ul style="list-style-type: none"> • Biologically exposed solid waste <ul style="list-style-type: none"> ◦ Sanitized either via an autoclave or chemically • Non-active solid waste <ul style="list-style-type: none"> ◦ Can be double-bagged and taken out of the facility to landfill or incineration sites, depending on local practices • Like non-biological liquid waste: some deactivation may require by some means prior to exiting the facility.
Exhausted Air	<ul style="list-style-type: none"> • Non-hazardous exhausted air <ul style="list-style-type: none"> ◦ From vent filters not hazardous to health or environment • Odorous or solvent vapors <ul style="list-style-type: none"> ◦ Deodorization or organic solvent emission reduction
Hazardous Waste De-contamination	<ul style="list-style-type: none"> • Can be solid and liquid waste • Decontamination systems <ul style="list-style-type: none"> ◦ Must ensure inactivation of all microorganisms, including survival structures ◦ Processes must be validated by microbial challenge testing

7.7 Zone Concept Basement

The zone concept of the basement is shown below in Figure 24. In here, the rooms corresponding to clean and technical utilities can be found. Both the room design and selection of equipment were performed in HakoBio's platform. Due to lack of availability of the actual equipment described in the previous section, the equipment portrayed was selected as an alternative. The electricity room was left empty for the previously mentioned reason.

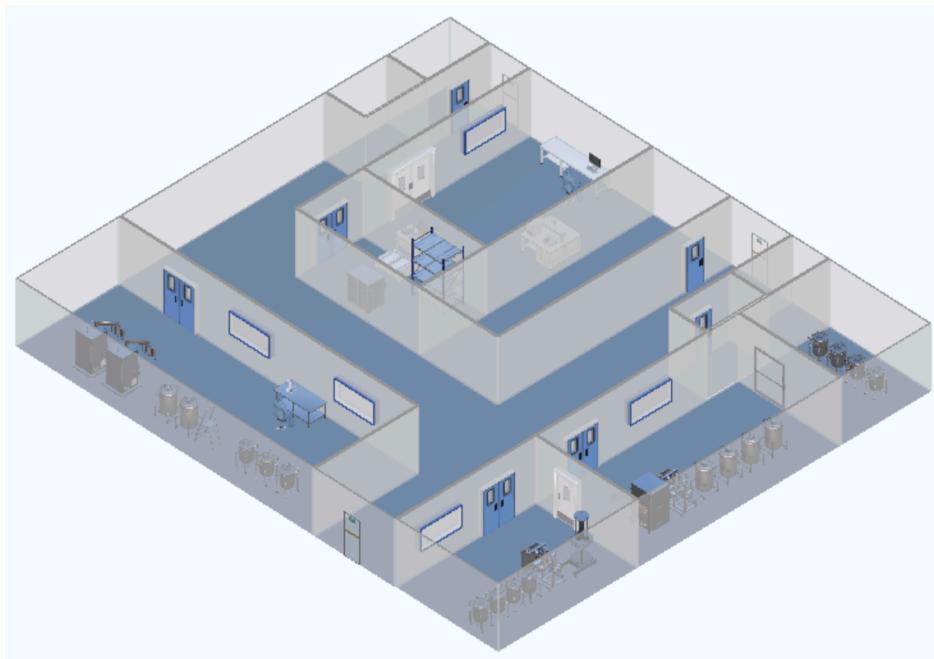
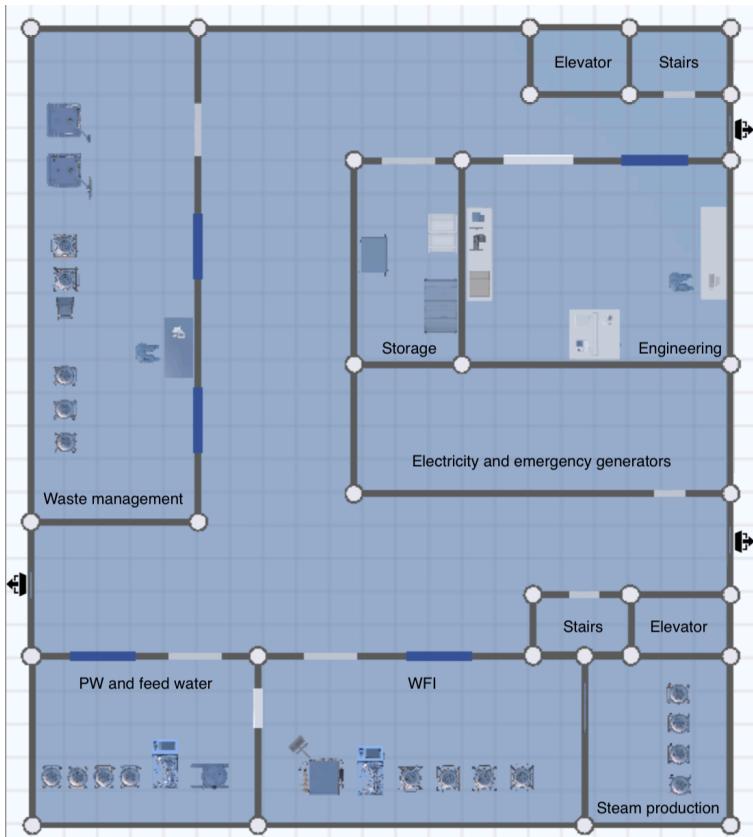


Figure 24: Room concept of the basement designed with HakoBio. The basement is depicted in 2D (upper image) and 3D (lower image).

7.7.1 Points of use

The points of use are depicted in Figure 25 and indicated per process in Table 25. The first step to design the points of use zone concept, was to determine which utilities were required for the different processes and their respective rooms. For the clean utilities, rooms such as media preparation, buffer preparation & storage, Inoculum, USP production, USP harvest and DSP V+ have points of use for both Water for Injection (WFI) and Potable Water (PW). For the quality room, only PW has been supplied. Points of use for Clean compressed air are present in the USP production and USP harvest for cell cultivation and fermentation processes. This clean utility is also present in buffer preparation & storage, and DSP V+, with the last one requiring two points of use. For the process air, a point of use in the USP production room was included. In addition to this, a supply for process gases (N_2 , O_2 , CO_2) are present in the USP production, USP harvest and inoculum rooms. A single point of use for clean steam is set in buffer preparation & storage. The technical facilities such as biological, liquid, and solid waste are distributed across the main rooms of buffer and media preparation, USP and DSP. Since biological active waste must be inactivated prior to disposal, a waste management room was design to carry out the inactivation.

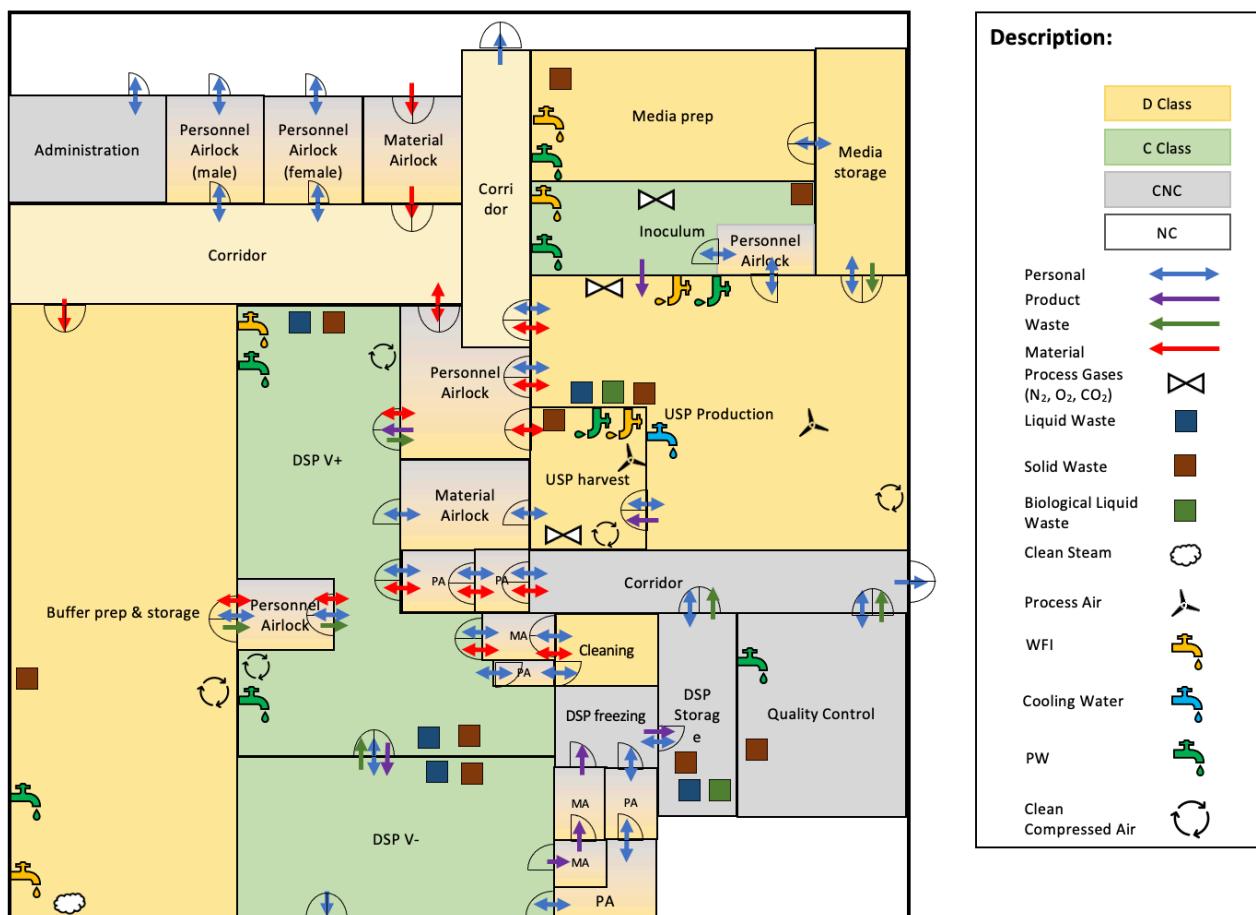


Figure 25: HVAC and points of use in the zone concept of the production area of the ground floor.

Table 25: Points of use of clean and technical utilities per process

Process	Step	Clean utilities							Technical utilities				
		WFI	PW	Clean steam	CO2	O2	N2	Liquid nitrogen	Clean compressed air	Heating/Cooling/Portable/ Fire water	Water waste	Solid waste	Electrical power
										Biological	Non-Biological	Biological	Non-biological
USP	Inoculation production	x	x		x					x		x	x
	Seed train	x	x		x	x	x	x	x	x		x	x
	Seed production	x	x		x	x	x	x	x	x		x	x
	Depth-end filtration	x								x		x	x
DSP	Protein A chromatography	x	x							x	x	x	x
	Virus inactivation (pH shift)	x	x							x		x	x
	Depth-end filtration (Sterile filtration)	x	x							x	x	x	x
	Second chromatography step (CIEX)	x	x							x	x	x	x
	pH adjustment	x	x									x	x
	Third chromatography step (AIEX)	x	x							x	x	x	x
	Nanofiltration (virus filtration)	x	x							x		x	x
	Diafiltration/Ultrafiltration (UF/DF), concentration & buffer exchange	x	x							x	x	x	x
	0.2 µm filtration (Depth End Filtration (Bulk filtration))	x	x							x	x	x	x
	Bulk filling (Bag)	x	x			x				x		x	x
	Bulk freezing (Bag) and storage (Quarantine storage)					x							x
Media & buffer preparation		x	x							x		x	x
Buffer preparation		x	x							x		x	x

8 References

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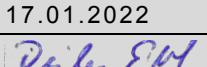
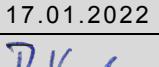
Appendix

Table 26 Calculations of the required volumes of the different buffers per batch.

Number	Buffer	Product A Vol- ume/batch [L]	Product B Volume/batch [L]	Product C Volume/batch [L]	Product D Volume/batch [L]
D1	Wash 1 Buffer Chroma I	6776	6776	6776	6776
D2	Wash 2 Buffer Chroma I	3080	3080	3080	3080
D3	Wash 3 Buffer Chroma I	3080	3080	3080	3080
D4	Elution Buffer Chroma I	4312	4312	4312	4312
D5	NaOH 1M	960	960	960	960
D6	Regeneration Buffer Chroma III	2304	2304	2304	2304
D7	Equilibration Buffer Chroma III	3006	3006	3006	3006
D8	Pre-/ Sanitization Buffer ChromallII	1816	1816	1816	1816
D9	Wash Buffer 2 Chroma II	1135	1135	1135	1135
D10	Elution Buffer Chroma II	1589	1589	1589	1589
D11	Strip Buffer Chroma II	681	681	681	681
D12	Equilibration Buffer Chroma III	833	833	833	833
D13	Equilibration / Flush Buffer Nano Filtration	251	251	251	251
D14	Diafiltration Buffer UF/DF2	4099	4099	4099	4099
D15	Acid Buffer Viral Inactivation	154	154	154	154
D16	Base Buffer Viral Inactivation	161	161	161	161
D17	Chroma I Storage Buffer 20% EtOH	924	924	924	924
D18	pH Adjustment Buffer	226	226	226	226
D50	NaOH 0.01 M Storage Buffer	852	852	852	852
D51	NaOH 0.5 M	431	431	431	431
D53	NaOH 0.1 M	340	340	340	340

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	Biochemical Engineering and Cell Cultivation Techniques	2021.01

Title	Design of Biopharmaceutical Production Facilities
Object/ Project	Master of Science in Life Sciences / Module BP3
Purpose	The URS describes the tasks and requirements of a Biopharmaceutical Production Facility producing in Single-Use-Technology.
Area of application	Planning and realization of a biopharmaceutical production site, Front-End Engineering - Conceptual Design
Status	Version 3
Relevant documents	Schedule_BP3_AS21.pdf Process description_BP3_AS21_Group_3.pdf Workshop Guideline_BP3_AS21.pdf

-	Created	Checked	Approved	Replaced	New version
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Date	17.01.2022	17.01.2022	17.01.2022		
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	Biochemical Engineering and Cell Cultivation Techniques	2021.01

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1 Introduction

1.1 Project background

In the cooperative Module BP3 “Design of Biopharmaceutical Production Facilities”, part of the consecutive master’s programme in Life Sciences, students acquire basic knowledge in the field of Front-End Engineering regarding the design of a modern, flexible biopharmaceutical production facility, which makes use of single-use-technology.

The design of the biopharmaceutical production facility is based on Chinese hamster ovary (CHO) cell-derived monoclonal antibody (mAb) processes. As part of a group work, the students will create a conceptual design of a production facility. Information on yields, productivity and technologies on the process is given. The work on the concept study is supported by specialist contributions from industry representatives and a given case study.

1.2 Purpose of document

The URS is a planning document and describes the requirements of a project, type of processes (cell culture generated Drug Substance (DS), purification of DS to deliver Bulk Drug Substance (BDS) or Active Pharmaceutical Ingredient (API)), type of equipment to be used (Single-use technology (SUT)), capacities (number of batches per year, batch size, weight (kg) of product / year). Furthermore, the URS describes the available boundaries of the project (availability of Quality control (QC) laboratories, warehouses, dispensing, offices etc.). In addition to the user requirements, the document also provides a list of requirements (functional and non-functional) which are essential for the successful realization of the project goal.

2 Process / Product requirement

Information on production cell lines media (chemically defined media) and feeding strategies are confidential.

Information on the process is given in the document "Process description_BP3_AS21".

The product is the conceptual design (concept study) of a flexible biopharmaceutical production facility according to the URS. The concept study must be submitted in the form of a project folder.

The aim is to use single-use systems (SUSs) and SUTs wherever possible to ensure the flexibility of the biopharmaceutical production facilities!

The media and buffer preparation (MP/BP) should take place on site.

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3 Operational requirement

- Facility
 - Operational readiness 320 days per year
 - Maintenance interruption of 45 days per year
 - Greenfield facility
 - Horizontal arrangement of all process suites
 - With support of unit operations (MP/BP) alongside in an adjacent room (devices in the working suites to be connected with airlock through the wall)
 - Describe all available auxiliary areas (QC lab, office, warehouse etc.)
- Process control
 - Incoming inspection
 - In process control
 - End product control
 - Electronical batch control
- Automation
 - Enterprise Resource Planning: Systems, Applications and Products in Data Processing (SAP)
 - Manufacturing Execution system: SAP
 - Process control: The Distributed Control System (DCS) is based on PCS7 and will be used for recipe control and batch management. The DCS will send all necessary data to control the different process units to the equipment and will receive all data which are needed for a central batch report.
 - Equipment Control level: Each Package-Unit is equipped with a local Programmable Logic Controller (PLC) and an Human Machine Interface (HMI). All unit operation related functionality will run on the local skid. The Package-Units will be integrated into the DCS level to enable full recipe control. All I/Os are connected to the PLC. The Automation Hardware is based on Siemens.
- Products:
 - Four products (product A, product B, product C, product D)
 - All products are based on the same CHO cell platform and use the same production medium.
 - The mAbs are produced in Switzerland for the Swiss, European and US markets.

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- Product A
 - Amount [kg/year]: 100
 - Titer [g/L]: 4
 - Yield: 0.684
- Product B
 - Amount [kg/year]: 70
 - Titer [g/L]: 3
 - Yield: 0.706
- Product C
 - Amount [kg/year]: 70
 - Titer [g/L]: 2
 - Yield: 0.64
- Product D
 - Amount [kg/year]: 40
 - Titer [g/L]: 4
 - Yield: 0.64
- Shift operation:
 - Upstream Processing (USP): 2-shift operation + on-call service
 - Downstream Processing (DSP): 3-shift operation
 - QC, in process control, final product control: 3-shift operation
 - Utilities: 1-shift operation + on-call service

4 GMP requirement and compliance

4.1 GMP requirement

Facility design and operation must meet the Current Good Manufacturing Practice (cGMP) requirements of Switzerland and the USA.

- Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use:
 - EU-GMP, Annex 2
- Clean room and air device classification:

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- EU-GMP, Annex 1
- EN ISO 14644-4:2001

4.2 GMP compliance

- Concept of segregation
 - For the zone concept, take into consideration that the segregation practices form the fundamental design strategy for the prevention of cross-contamination as well as protection of quality of the final bulk and intermediate products throughout the manufacturing process.
 - Note the critical flows within the production facility:
 - Raw material flow
 - Product flow
 - Personnel flow
 - Waste flow
 - Air flow

5 Biosafety

- Biosafety Level (BSL)
 - Up to BSL 2

6 Deliverables

The creation of a project folder with the following documents (chapters) is required:

1. URS (add)
2. Plant on a page
3. Occupancy list
4. List of necessary clean facility utilities
5. Area schedule, Biosafety Level and Room list
6. Size of functions and of systems
7. Zone concept
8. Height concept
9. Staff estimation
10. Presentation of the intermediate state of the case study
11. Definitions and abbreviations

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Technical drawings and sketches can be assigned to the individual chapters.

7 Definitions and abbreviations

7.1 Definitions

Area schedule	<p>Is a document which summarizes all the building spaces incorporated within the project.</p>
Biosafety Level	<p>Describes a set of biocontainment precautions required for the isolation of dangerous biological agents within an enclosed laboratory facility.</p>
Occupancy list	<p>Occupancy list is a diagram of the occupancy of a system over a defined, temporal period.</p>
Circulation area	<p>Area (or volume) required to operate a system and assure material flow and personnel flow (horizontal and vertical)</p>
Clean utilities	<p>Ubiquitous in biopharma manufacturing, especially aseptic manufacturing, clean utilities describe utilities which are required to meet specific quality requirements in order to ensure that they are used safely during manufacturing. Typical clean utilities found in biopharma facilities are:</p> <ul style="list-style-type: none"> • Purified Water (PW) • Water for Injection (WFI) • Clean Steam • Clean Compressed Air • Process Gases (e.g., nitrogen, oxygen, carbon dioxide) <p>Clean utilities are considered high-risk systems, as they usually come into direct or indirect contact with the product. They, therefore, must be carefully designed to guarantee that utility and product quality requirements are consistently met. Well controlled clean utilities are critical for regulatory compliance.</p>
Clean room class	<p>Cleanrooms are classified according to the number and size of particles permitted per volume of air.</p> <p>see: EU-GMP, Annex 1 and EN ISO 14644-4:2001:</p>

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Height concept	Height required to set up and operate a system. The required room height is derived from the required height for operating the system.
Plant on a page	Flow diagram representing the procedure, configuration and function of a process plant or plant section.
Room list	List of all necessary rooms
Size of functions	Area (or volume) required to perform a function (task).
Size of systems	Area (or volume) required to set up and operate a system (plant).
Staff estimation	Assessment of the necessary number of employees to operate the factory.
User requirements specification	<p>The document, URS, plays a pivotal role in the entire system development life cycle and is required for both business (investment protection) and regulatory reasons (defining intended purpose).</p> <p>Within the document, the qualification team sets the pharmaceutical and technical requirements for qualification representatives, equipment managers and, in the case of larger qualification projects, the qualification team. The specifications represent a basic document for the entire product life cycle of a plant.</p>
Zone concept	Zone concept is a segregation of the building spaces based on environmental control. While the environment within a facility is always controlled to a certain degree, it is usually split into two distinct environmental envelopes, namely; spaces deemed controlled-not-classified (CNC), where temperature, pressure, and humidity are generally controlled and monitored, and classified areas, within which air cleanliness is additionally controlled, monitored and validated to conform to a specific cleanliness level. The latter are often referred to as "clean rooms", where the cleanliness is defined as a minimum number of airborne particles allowed within a volume of air or room.

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7.2 Abbreviations

API	Active Pharmaceutical Ingredient
BDS	Bulk Drug Substance
BP	Buffer prep (buffer preparation)
BSL	Biosafety Level
cGMP	Current Good Manufacturing Practice
CHO	Chinese hamster ovary
CNC	Controlled-Not-Classified
DCS	Distributed control system
DS	Drug Substance
DSP	Downstream Processing
HVAC	Heating, Ventilation and Air Conditioning
(I/O)	Inputs and Outputs
mAb	Monoclonal antibody
MP	Media prep (media preparation)
PCS	Process Control System
PW	Purified Water
QC	Quality control
SAP	Systems, Applications & Products in Data Processing
SCADA	Supervisory control and data acquisition
SUS	Single-use system
SUT	Single-use technology
URS	User requirements specification
USP	Upstream Processing
WFI	Water for Injection