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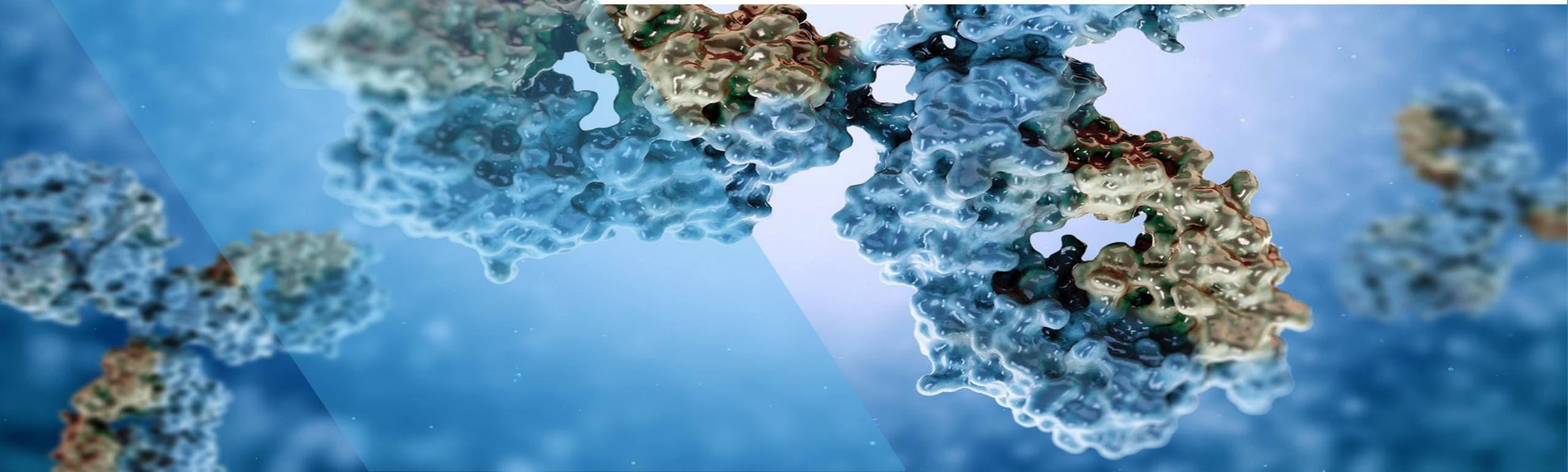
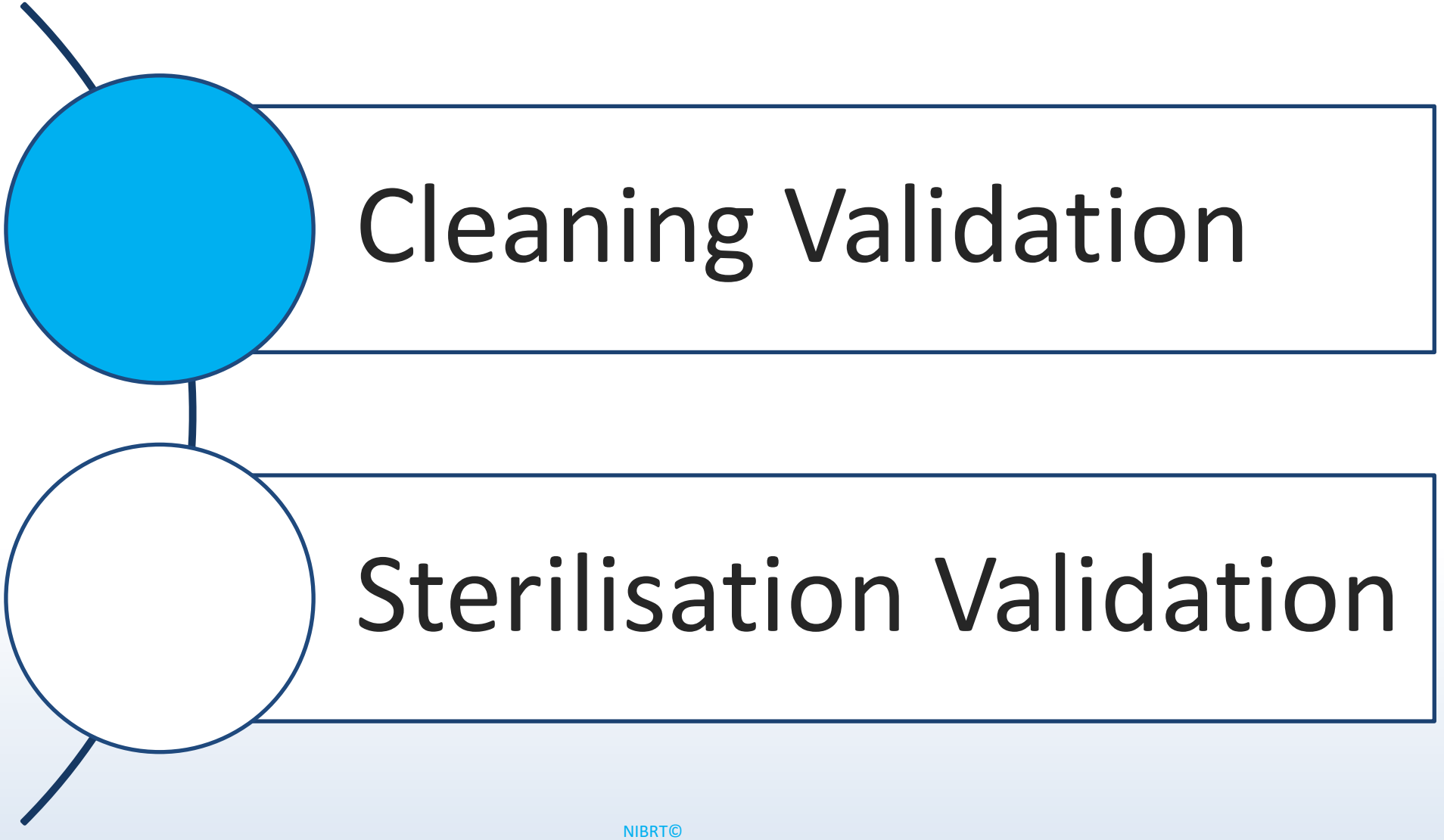
Lecture 6: Cleaning & Sterilisation Validation



MCQ 2

- The second MCQ for your Biopharmaceutical Validation module will take place on **12th March 2025**.
- There will be **20 multiple choice questions** (one correct answer for each) and once you begin your attempt you will have **50 minutes to complete.**
- The questions will cover **lectures 4-6.** The MCQ is worth **10% of your overall grade.**
- You will have **one attempt** only and the 50 minutes will begin when you click "start attempt".
- The MCQ will be on Moodle. **It will be open from 7.30am on 12th March to 7.30am on 13th March 2025.**
- IMPORTANT: attempts outside of this time frame will not be accepted except in certain extenuating circumstances. This is to ensure fairness to all students.

Topics



Cleaning in the Biopharma Industry

Why is it necessary?

- Biopharmaceuticals are administered intravenously
- Risk of contamination must be reduced
- Sterility & cleanability become the highest priority

Regular Cleaning of Equipment

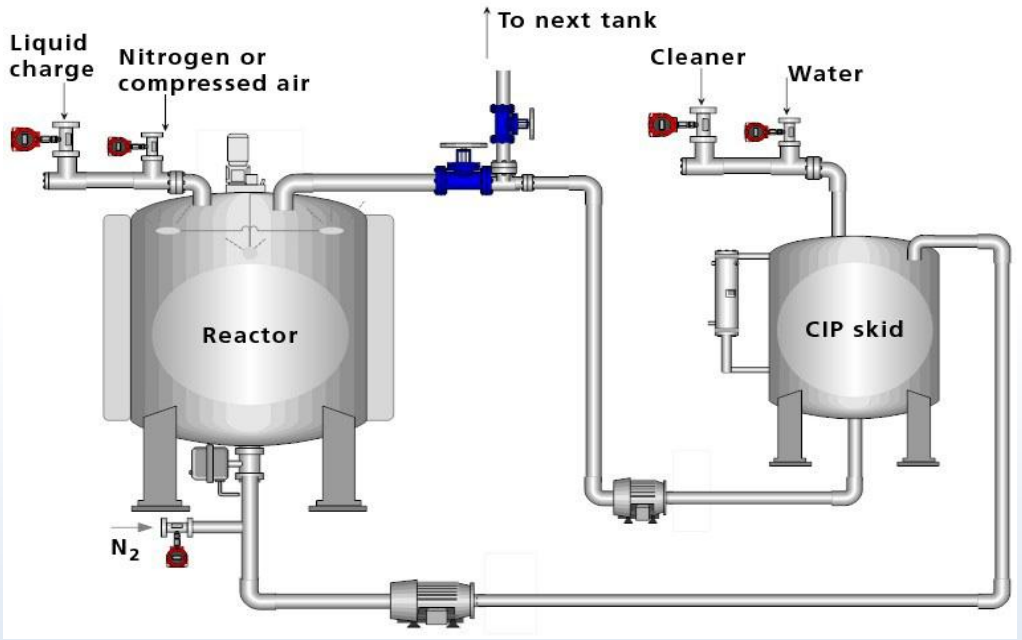
- Reduces and controls bioburden
- Prevents cross contamination
- Sets baseline for subsequent steaming and sterilising

Types of Cleaning

https://amcrasto.wordpress.com/tag/cleaning-validation/

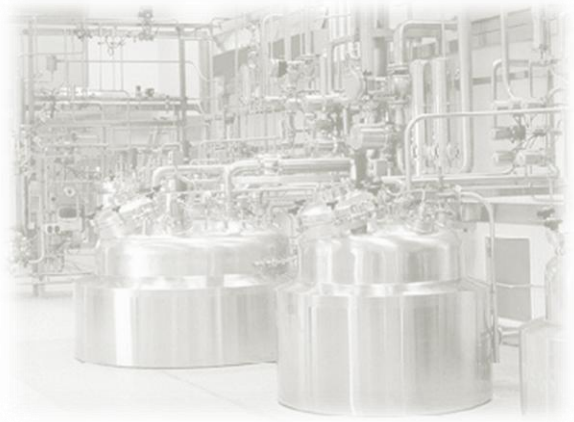
1. Manual Cleaning:
   - variation between operators

2. Automated Cleaning
   - can be validated
     i.e. less variation



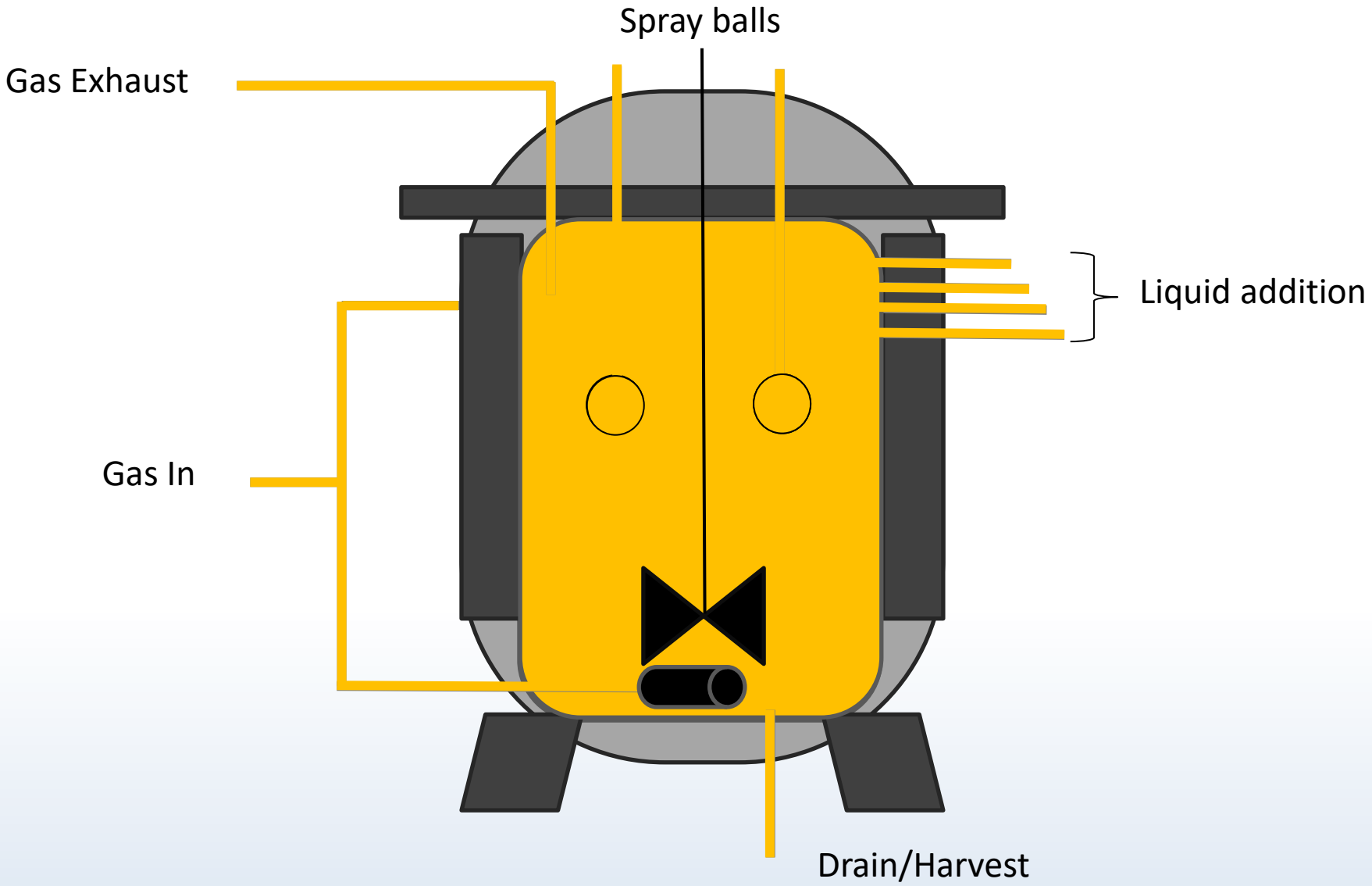
Cleaning Strategies

- **Clean in Place (CIP)**
  - Equipment stays in place (at point of process use) and cleaning solutions are routed through hoses and piping from Utilities to the equipment.
  - E.g. bioreactors and large immobile vessels

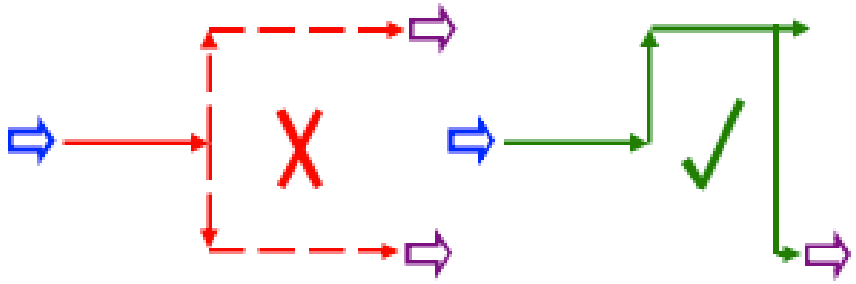


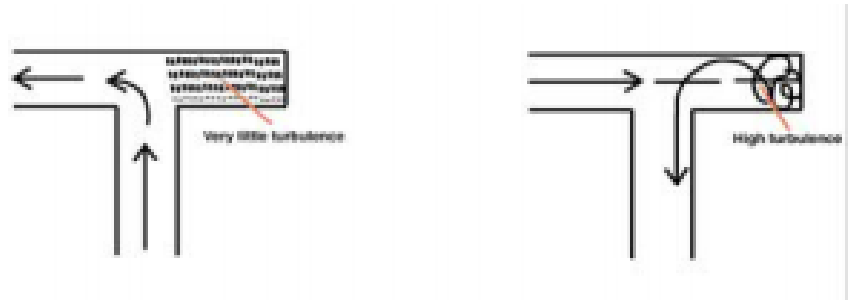
- **Clean out of Place (COP)**
  - Partswasher: Similar to a dishwasher used in homes. Parts and glassware are loaded into a chamber and solutions are sprayed onto the parts.
  - COP stations for mobile vessels
  - Sonicating baths e.g. some sparger heads

CIP of Vessel



Mains/Pipework CIP





Design considerations:

- Split routes – total route coverage
- Avoid Dead ends
- Avoid tees
- Refer to ASME BPE guide

Standard is 1.5-2.1 m/s velocity

- Important to ensure turbulent flow for flat velocity profile and thin boundary layer
- Turbulent flow minimises ineffective movement and cleaning in boundary layer.
- Turbulent flow also cleans out dead legs, sample points and instrumentation better than laminar profile – These elements are still preferably avoided

Cleaning Validation

- There are two main factors which affect the cleaning of a vessel:
  1. The physical design of both the vessel and the associated cleaning system/components.
  2. The cleaning cycle and its parameters

- Both of these factors need to be considered and validated appropriately .

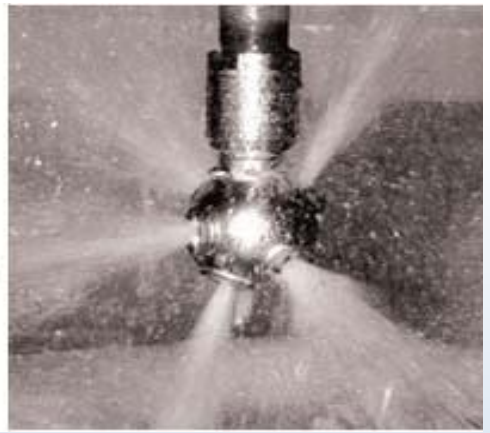
Physical Design: Spray Ball Devices

Spray device selection:

- deliver solutions to all locations
- vessel size
- vessel geometry
- agitator, spargers, dip tubes, sample ports
- shadowing

Spray ball can be:

- fixed or rotating



Physical Design: Coverage Testing

- Carried out during **FAT/OQ**
- Tests spray ball functionality
  - i.e. ability to deliver solution to **all** parts of vessel
  - Coverage test is not a demonstration of how clean the vessel is, but how **cleanable** it is with the current spray ball design
- Fluorescent solution (riboflavin) is sprayed on all internal surfaces.
- A water cycle is run through the spray ball
- Once the cycle is complete, the vessel is opened, and all internal surfaces are inspected using a UV light
- If the spray ball design and location is effective, all riboflavin should have been removed during the water cycle, and not fluorescence should be observed.

Internal surfaces of a vessel covered with Riboflavin

What conditions?

- short (5 mins), ambient water rinse

Coverage Test Failure – e.g. FAT, OQ

- Incorrect design of the spray ball
- Incorrect design of lines or ports of the vessel
- Grease or other residues in the vessel
- Spray ball not installed correctly
- Supply water pressure not sufficient
- Blockage of spray ball

Identify & fix root cause – repeat test

Cleaning Cycle Development

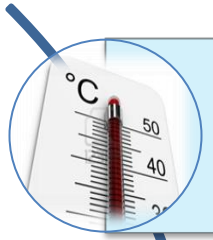
- Different recipes tested
- Effective cleaning cycle is developed which
  - Removes all residues
  - Leaves equipment dry

- Least amount of water
- Coolest temperature
- Shortest amount of time

Saves money

Cleaning Cycle Parameters

Ideally, cleaning cycles should optimise the parameters below, to generate the most efficient cleaning cycle possible in terms of cost, energy and efficacy.



TEMPERATURE

Some chemicals and flushes work better at hot temps, but can a cycle be completed as effectively at 60°C rather than 80°C?

CHEMICAL CONCENTRATION

Need to use the optimal concentration of chemical to effectively clean the surface, but is it preferable and safer to use a chemical at the lowest effective concentration?

FLOW RATE & PRESSURE

High flow rates and pressurised liquid streams can physically remove soils, but ideally, we want to use as little water as possible – time and energy!

CONTACT TIME

We need to allow chemicals time to “work”, but ideally, this should be as short a time as possible. Longer contact time is necessary at lower temps and lower concentration.

Cleaning Validation

- Must be performed *"to confirm the effectiveness of any cleaning procedure for all product contact equipment"*
  EUGMP Vol 4. Annex 15
- Once the cleaning cycle has been developed, it needs to be formally validated.
- Can be performed before, or in tandem with Process Validation (PV).
- Consumes a considerable amount of time and resources.
- Validation testing to demonstrate cycle efficacy include:

Visual Inspection

- Riboflavin Testing – coverage test.
- Visual "dryness" check after cycle completion.

Indirect Sampling

- also known as rinse sampling, performed on the WFI rinse prior to blow-down. Samples taken for TOC, conductivity, bioburden and endotoxin.

Online testing

- Examples of PAT – online conductivity and TOC meters

Direct sampling

- Swab sampling for TOC and bioburden

Validation - Acceptance Criteria for Dryness

- Dry, no water droplets visible
- Isolated water droplets visible
- Numerous water droplets visible, no pooling
- Pooling evident at bottom of vessel

https://ispe.org/pharmaceutical-engineering/september-october-2017/proof-closure-life-cycle-closed-systems

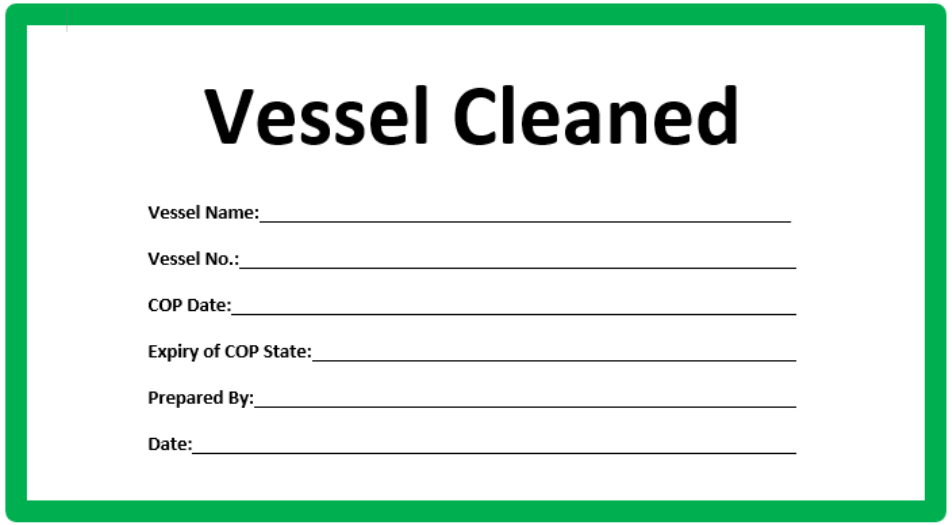
What to Sample For?

1. Conductivity
2. Microbes - Endotoxin
3. Protein - Drug substance
4. TOC – Total Organic Carbon

Clean/Dirty Hold Times

NIBRT:

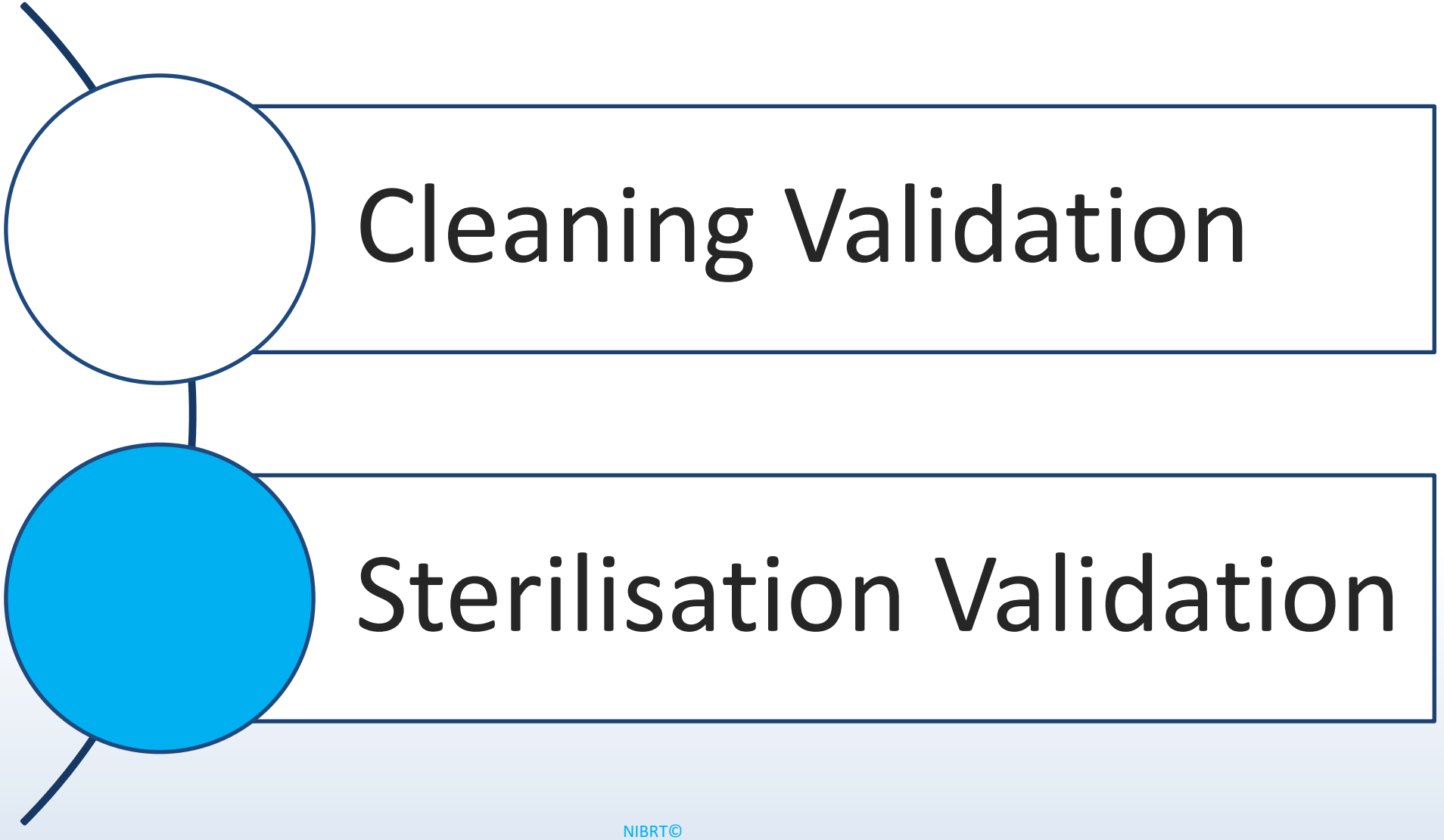
- Clean hold time: ≤ 14 days
- Dirty hold time: ≤ 3 days
- Steam hold time: ≤ 24hrs



- How are these validated?

- What happens if hold times are exceeded?

Topics



Sterilization vs. Depyrogenation

- Sterilization: Validated process used to render a product free of living microorganisms including bacterial endospores.

- Depyrogenation: Removal or inactivation of bacterial endotoxin. Endotoxins are toxins present within certain bacterial cell walls, which are released when the cell is broken down.

- An overkill approach is applied when sterilizing - assurance of 10^6 probability of non-sterility.

Sterilisation Methods

- **Steam** - ~121°C – shorter exposure times, higher kill rate, more complicated system design
- **Dry Heat** - ~400°C – longer exposure times, lower kill rate, simpler system design

- Vaporised Hydrogen Peroxide (VHP) – ok for sterilizing background work surfaces (e.g. internal surfaces of filling line) but not suitable for direct and indirect product contact parts.

- Radiation - typically used for single use plastics

1. Dry Heat Sterilization & Depyrogenation

- Dry heat is the method of choice for sterilizing items which will tolerate high temperatures
- Generally less complicated than steam processes
- Higher temperature and/or longer exposure times are required
- Microbial lethality associated with dry heat is much lower than that for saturated steam at the same temperature

Depyrogenation

- Refers to the removal of pyrogens – defined as “any substance that can cause a fever. Bacterial pyrogens include endotoxins and exotoxins”
- There is no uniform regulation in relation to the time and temperature required for depyrogenation
- 3 log step reduction of endotoxins is required by the USP and FDA
- No European regulation for depyrogenation of final containers for parenterals

2. Steam Sterilization - Steam In Place (SIP)

- Widely adopted method of in-line sterilisation for process equipment.
- Very effective if done right – needs to be considered at early concept stage and considerations incorporated into facility design.

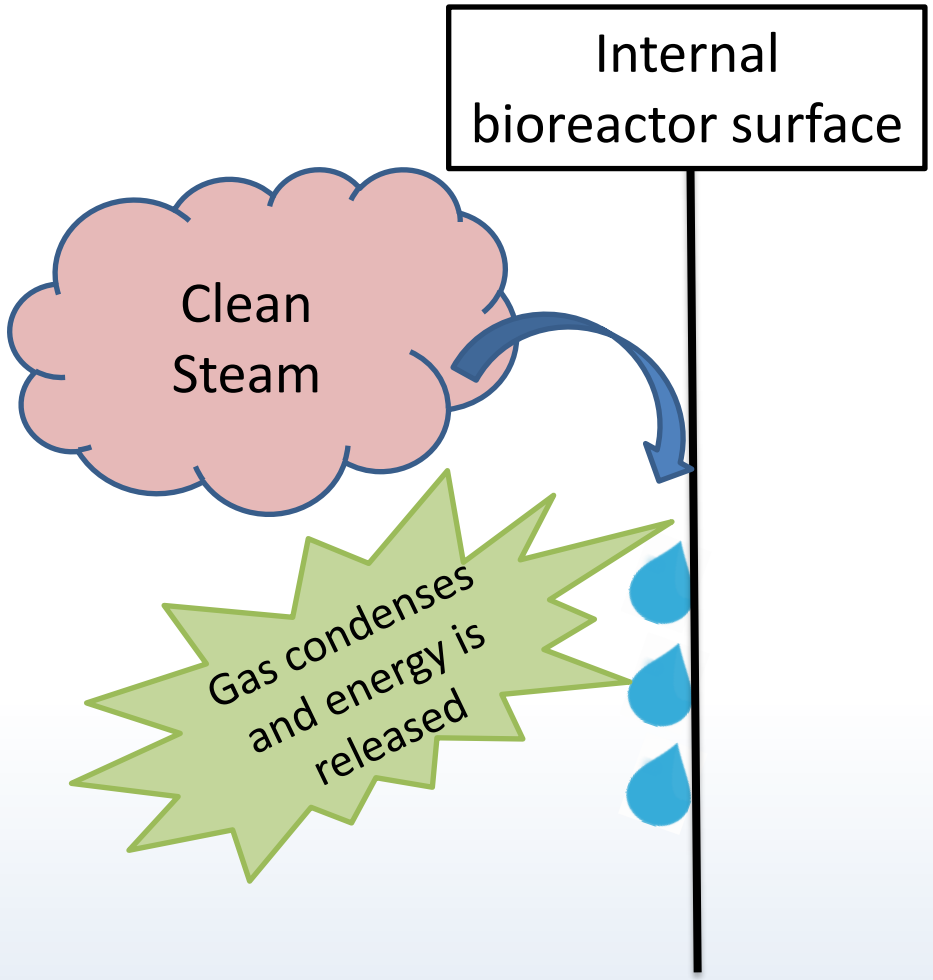
- Critical design parameters:
  - Proper steam distribution and contact with **all** surfaces
  - Non-condensable gas removal – Steam traps.
  - Continuous condensate removal.

- Also need to consider vent filters for gas inlets/outlets – must be installed before steaming – therefore must be suitable for steaming – must remain dry for batch
- Use hydrophobic materials – prevent blockage by humidity

Steam In Place

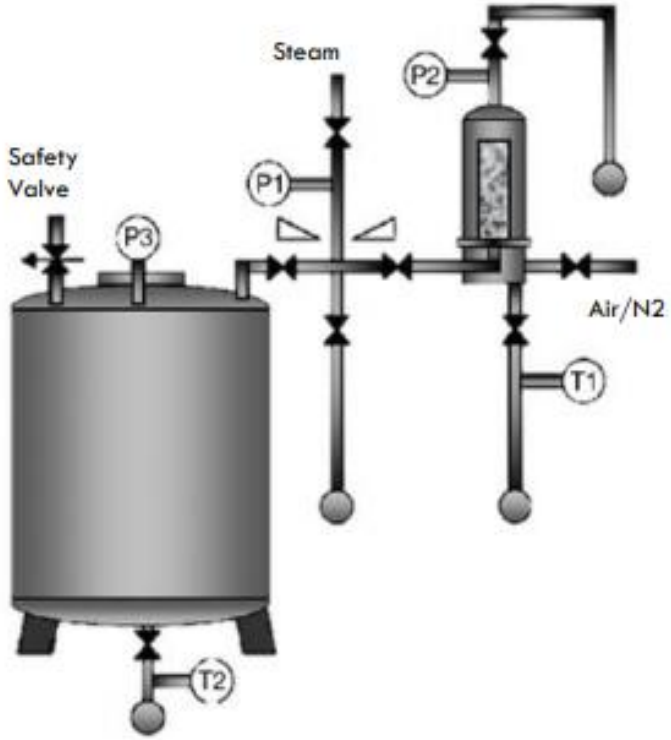
Moist heat sterilisation

- Clean steam
- Uses latent heat of steam
- Contacts a surface and gas condenses releasing heat to the surface
- Microbes destroyed on the surface



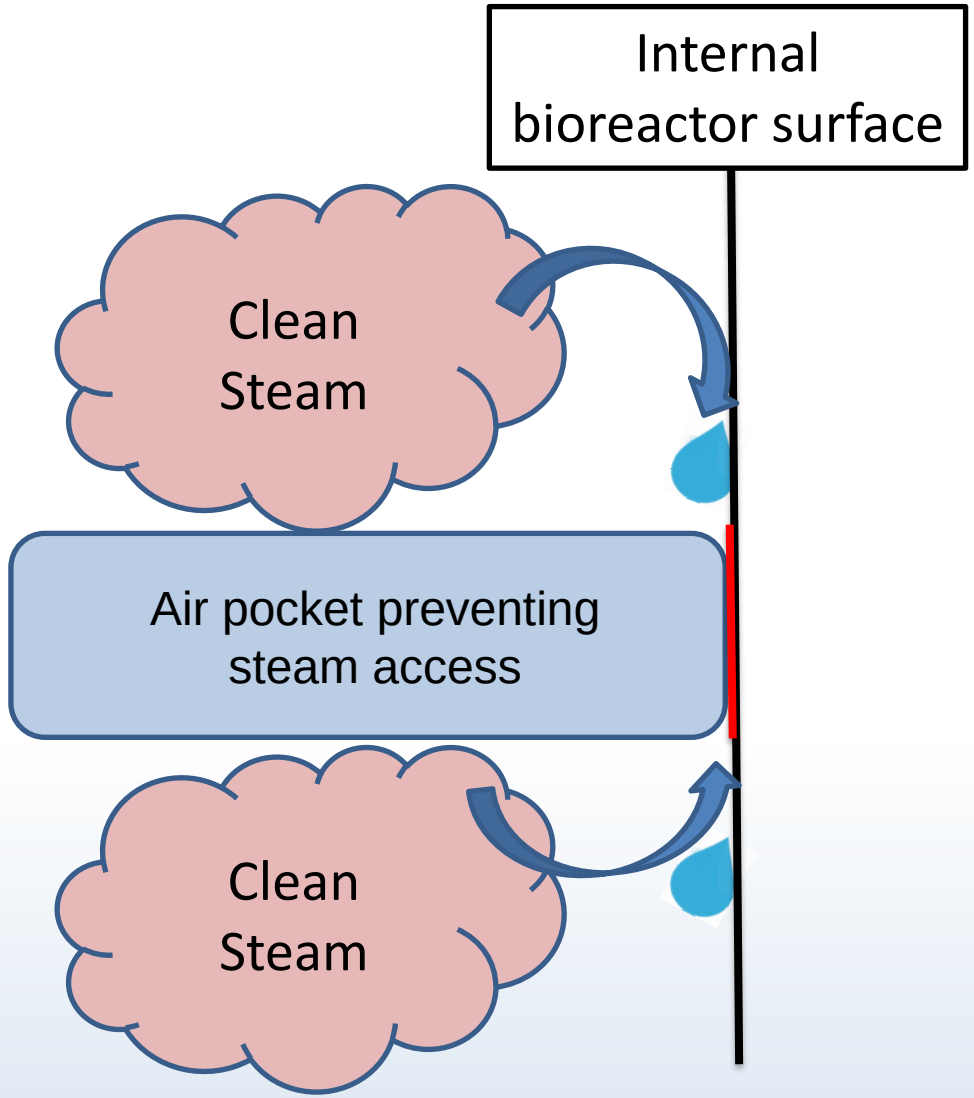
SIP Consideration

- Large amounts of Steam are needed per Cycle
  - Steam vessel first – Vent filter limits flowrate
- Appropriate placement and usage of steam traps
  - Remove non-condensable gases
- Correctly sloped/placed drains
  - Ensure adequate condensate removal
- Efficient SIP procedure and cycle
  - Test pressure, validation study and post SIP assurance

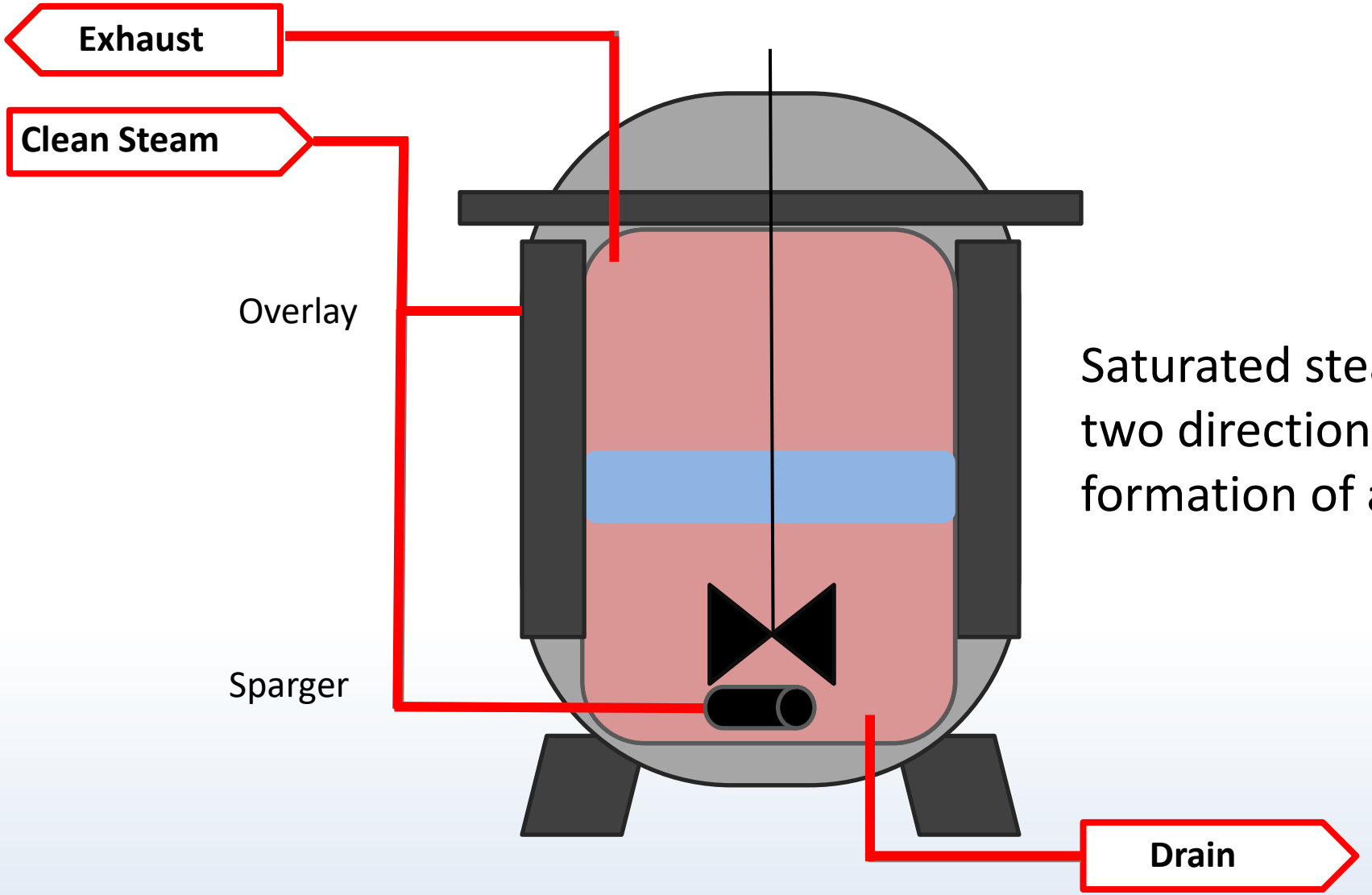


Air Removal

- Air is a mixture of non-condensable gas
- Air is an excellent insulator
- A small layer can prevent steam accessing a surface
- ∴ As much air must be removed as possible

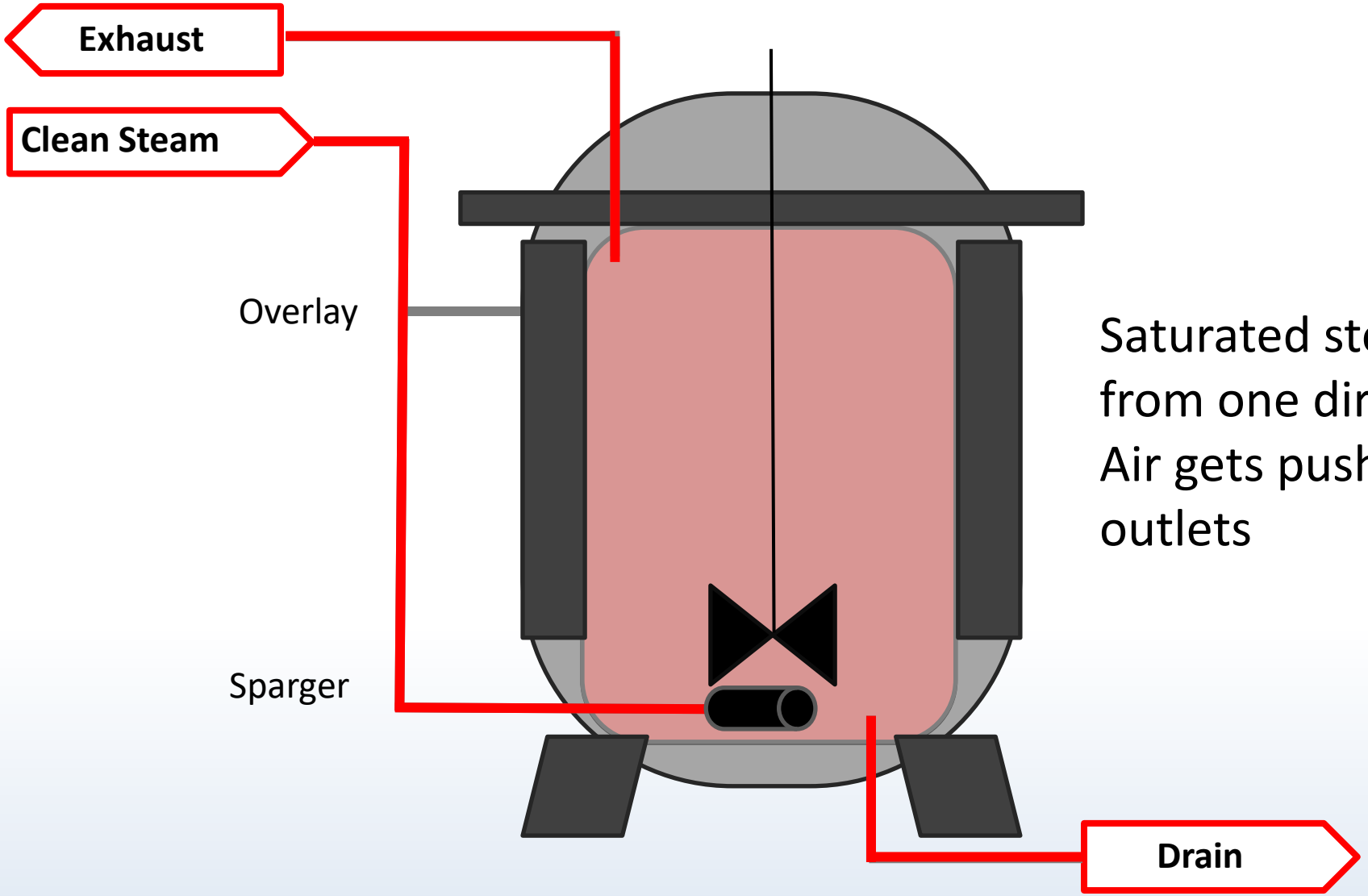


What you don't want: Bi-Directional Steam



Saturated steam from two directions risks the formation of air pockets

What you do want: Uni-Directional Steam



Saturated steam introduced from one direction.
Air gets pushed to multiple outlets

SIP Cycle

Pre-Cycle

- Pressure hold test,
- Flush out non-condensable gases and heat system

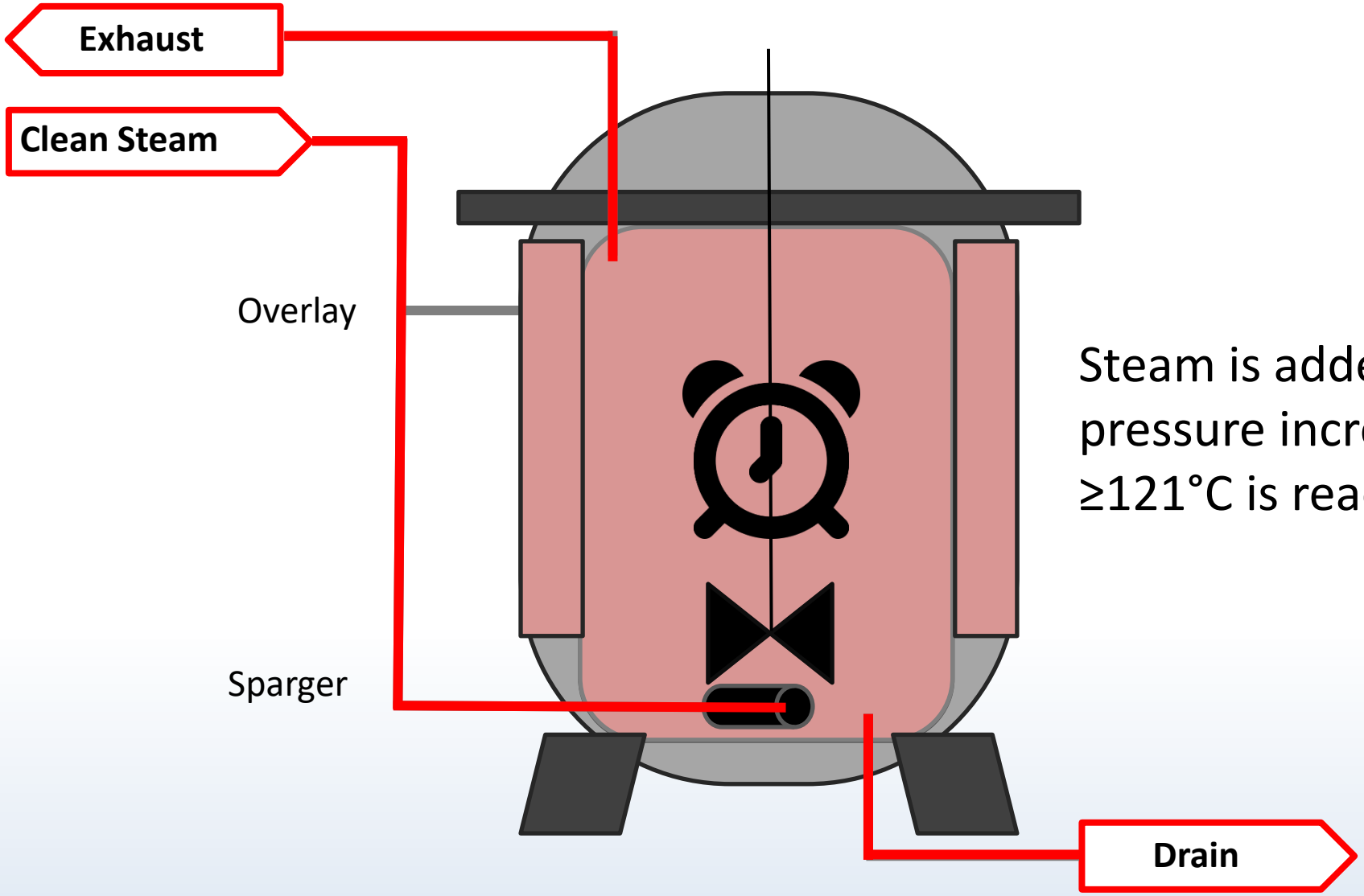
Steam Cycle

- Continues for time based on validation study
- Temperature and pressure are monitored to maintain saturated steam conditions
- Condensate continuously drains due to system design and is replaced with fresh steam
- Air is removed continuously through bleed valves and steam traps

Post SIP

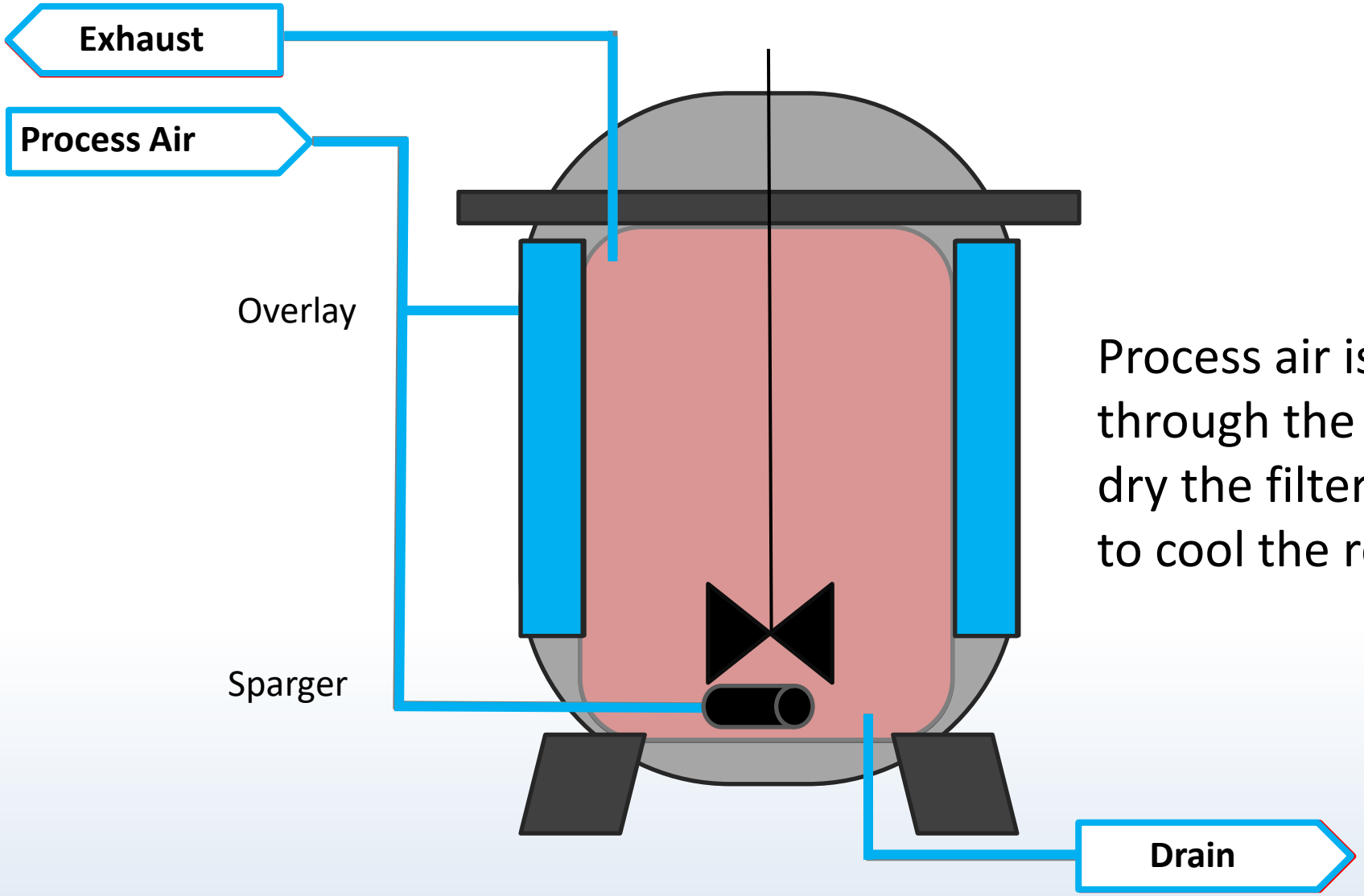
- Venting with sterile gas (typically process air) to remove condensate
- Retain positive pressure during cooling to avoid vacuum conditions
- May choose to integrity test vent filter for assurance

Sterilisation Plateau (121°C)



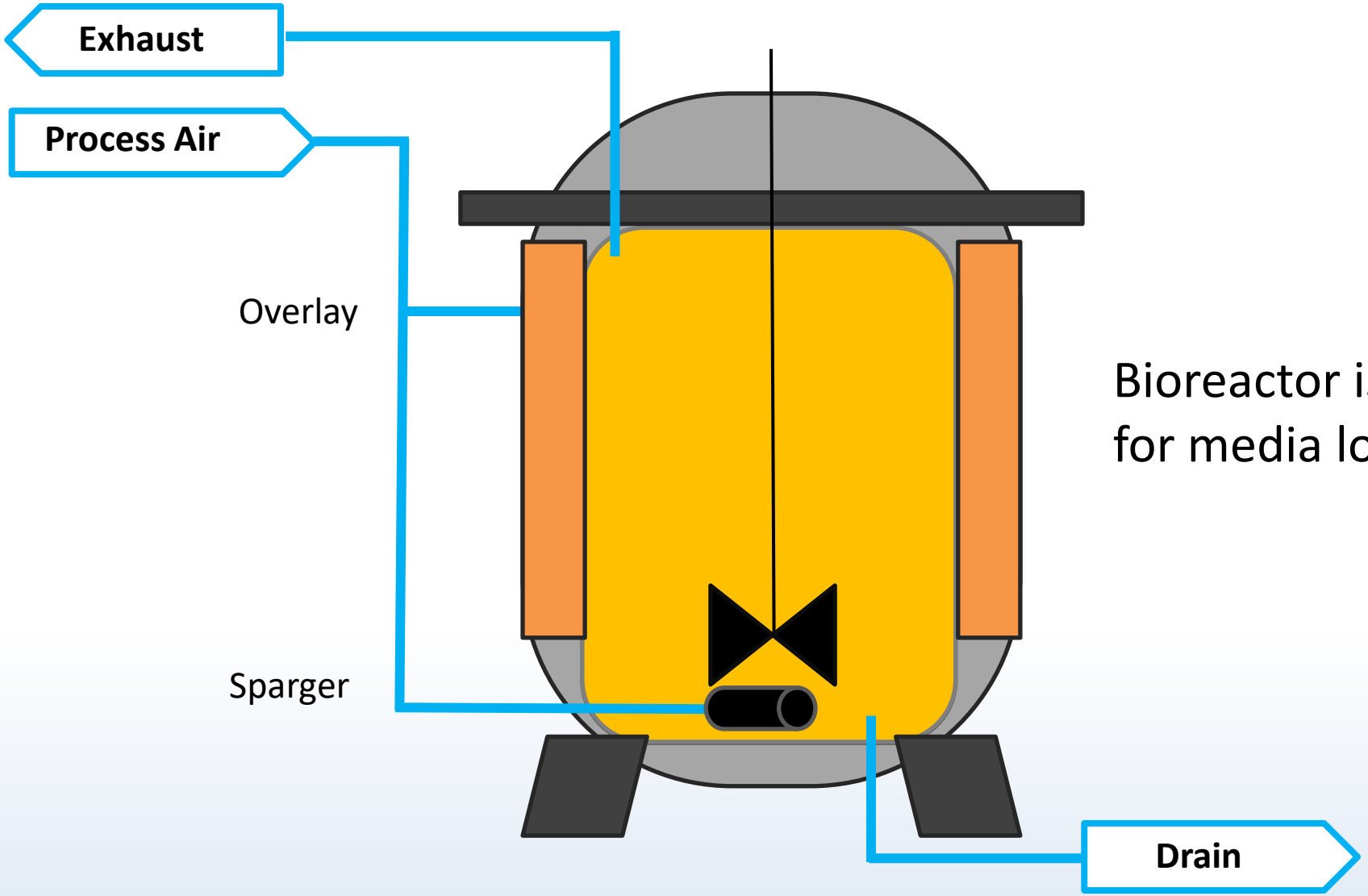
Steam is added and the pressure increases until ≥121°C is reached

Filter Drying and Cooldown



Process air is fed through the lines to dry the filters and then to cool the reactor

Awaiting Loading



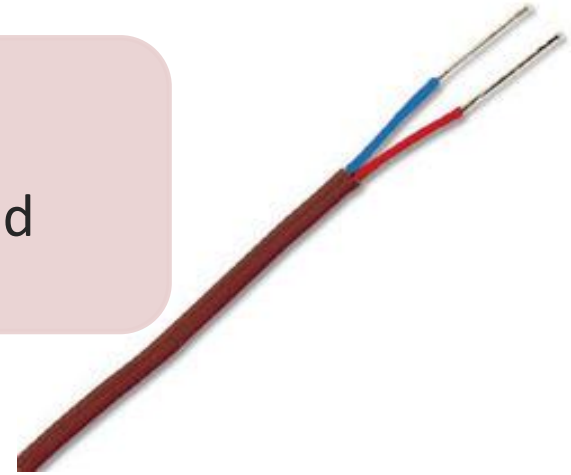
Bioreactor is ready for media loading

Steam Sterilization Parameters & Principles

Steam Sterilization Validation

1. Thermocouples

- Verify correct temperature, pressure, and time are reached



2. Biological Indicators

- Verify all organisms are killed

Thermocouples

- Two dissimilar metals – insulated wires
- Hot junction / cold junction
- Generates EMF within the wires
- This current is dependent on temperature
- Current EU and US regulatory requirements - EN285,

  HTM2010, HTM 01-05, EN17665, PDA Technical Report 01.

Biological Indicators

- **Contain bacterial endospores**
  - *Geobacillus stearothermophilus*
  - *Bacillus atrophaeus*
- **Spore strips**
  - inoculated with 10^6 spores

- **Incubated after steaming cycle**
- **Some BIs contain growth medium & colour indicator**

Which Locations?

- Worst case scenarios
- Coldest point? – steam rises, condensate drains
- Areas of difficult steam penetration
- Places that may trap air pockets

3. Autoclave Steam Sterilization – Steam Out of Place (SOP)

- Smaller equipment – hard/wrapped items or liquid load
- Shorter times and lower temperatures than dry sterilization
- Also uses latent heat of steam – direct steam contact is required
- Cost-effective
- A dedicated pure steam supply is recommended

Autoclave Cycle

- **Pre-Conditioning:** Air is removed, and load is humidified and heated
- **Exposure:** Temperature is raised, and Exposure time is conducted
- **Post-Conditioning:** load is cooled and dried, chamber is brought to atmospheric pressure
- Cycles will vary with individual applications – vital to ensure a suitable cycle is being used

Autoclave Validation Tests

- Similar to the SIP of vessels and larger systems, autoclaves will be tested during validation studies (and periodic re-validation studies) using thermocouples and biological indicators.

The following tests are also typically carried out on a regular basis post-validation as part of routine monitoring:

- Leak rate test (Before use) – check that autoclave is sealed and has no leaks
- Bowie-Dick (Daily) – check penetration power of steam
- Air detector function (Weekly) – ensure air detector functionality
- Monitoring of chamber and printouts

- Routine monitoring tests must be validated as fit-for-use/suitable during system development/validation.

Thank You