Clean Room (Mikrobiological)

Actions to avoid microbiological contamination in clean room areas

Personnel

Personnel should not only have knowledge and current practical and theoretical experience in the preparation of sterile products, but also in microbiology.

This includes:

- Regular reassessment of knowledge and revision/retraining where necessary, e.g. the aseptic technique should be periodically tested by performing media fill simulations.
- Regular observation of aseptic technique: Swap tests should be performed.

Environment

The contamination risk of the manufactured drug defines the Cleanroom Grade in which the manufacturing takes place.

Aseptic products are the most at risk for contamination, as no terminal sterilization takes place. Therefore, the environment should be monitored, and only authorized people should have access. Unless there is a proper justification, the background environment for LFCs and BSCs should meet grade B requirements and grade D required for pharmaceutical isolators.

Clothing:

Clothing should protect the product from contamination. So, there are different requirements for each clean room grade based on the contamination risk of the product.

For Example:

- Grade D: Hair, arms and, where relevant, beard and moustache should be covered. A general protective suit and appropriate shoes or overshoes should be worn. Appropriate measures should be taken to avoid any contamination coming from outside the clean area.
- Grade C: Hair, arms and, where relevant, beard and moustache should be covered. A single or two-piece trouser suit, gathered at the wrists and with high neck and appropriate shoes or overshoes should be worn. They should shed virtually no fibres or particulate matter.
- Grade A/B: Headgear should totally enclose hair and, where relevant, beard and moustache; it should be tucked into the neck of the suit; a facemask should be worn to prevent the shedding of droplets. Appropriate sterilised, non-powdered rubber or plastic gloves and sterilised or disinfected footwear should be worn. Trouser bottoms should be tucked inside the footwear and garment sleeves into the gloves. The protective clothing should shed virtually no fibres or particulate matter and retain particles shed by the body.
- Gloves should be regularly disinfected during the manufacturing processes. Gloves and masks should be changed at least at every working session. It is important to visually check that garments are in good condition and that the seams are sealed.

Cleaning:

- Clean areas should be regularly cleaned according to a documented and approved procedure. Any staff performing cleaning duties should have received documented training beforehand.
- Dedicated equipment should be used to minimize microbiological contamination. Cleaning and disinfecting agents should not contain viable microorganisms and those used in Grade A and B areas should be spore free and sterile. Therefore, periodic use of

sporicidal cleaning agents should be considered to reduce contamination from spore forming microorganisms.
- The effectiveness of cleaning should be routinely evaluated, by microbiological surface sampling e.g. contact plates or swabs.

<u>Aseptic Processing:</u>

Actions to avoid microbiological contamination:

Materials:

- Define Disinfection processes for starting materials. Only sterile materials should be taken into grade A or B areas e.g. settle plates, swabs, and cleaning materials.
- All starting materials, components and packaging materials should be visually checked before use
- Physical, chemical and microbiological quality control tests should be performed based on a risk assessment
- Microbiological analysis does not have to be performed on each batch. Alternatively, a regular microbiological analysis of the units produced over a certain period of time or a regular program of media fills may be acceptable. Any growth should be investigated and documented.

Process:

- Perform Standard aseptic processing techniques, e.g. not-touching critical surfaces, correct positioning of materials within the laminar air flow, and use of specific pieces of equipment and regular sanitization of gloves
- Limited number of people present in the room at the same time
- Process should be validated by using broth or a similar nutrient media to simulate the aseptic procedure (media fills) and should be performed initially as well as subsequently on a regular basis, according to the risk, and whenever significant modifications have been made to the equipment or to the process.

<u>Monitoring:</u>

How to monitor microbiological contamination:

- Media fill simulations for personnel before start and on a regular basis

- Periodic monitoring for particles and bioburden (contact plates) should be considered.
- The justification for the frequency of microbiological monitoring should be documented.
- Reduced monitoring frequency may be justified by:
    - Use of closed systems during preparation
    - Immediate use of prepared products
    - Terminal sterilisation of products
    - Decrease of workload (less operational activities to be observed)

<u>Recommended frequencies of microbiological monitoring (Pic Annex 1 Guidelines On The Standards Required For The Sterile Preparation Of Medicinal Products):</u>

	Direct working environment (Grade A zone)	Background environment
Settle plates	Every working session	Weekly
Glove finger dabs	At the end of each working session	At the end of each working session
Surface samples (swabs or contact plates)	Weekly	Monthly
Active air samples	Quarterly	Quarterly

<u>Recommended limits for microbiological monitoring of clean areas in operation (Pic Annex 1 Guidelines On The Standards Required For The Sterile Preparation Of Medicinal Products):</u>[a]

Grade	Air sample (cfu/m3)	Settle plates, diam. 90mm (cfu/4hours)[b]	Contact plates, diam. 55mm (cfu/plate)	Glove print, 5 fingers (cfu/glove)
A	<1	<1	<1	<1
B	10	5	5	5
C	100	50	25	-
D	200	100	50	-

(a) These are average values (b) Individual settle plates may be exposed for less than 4 hours in which case the limits should be appropriately reduced.

Sources:

- PIC/S Guide To Good Practices For The Preparation Of Medicinal Products In Healthcare Establishments, Annex 1 Guidelines On The Standards Required For The Sterile Preparation Of Medicinal Products, PE 010-4, March 2014
- EU GMP Annex 1, GUIDELINES The Rules Governing Medicinal Products in the European Union Volume 4 EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use, European Commission Brussels, 22.8.2022 C(2022) 5938 final