Annex 1

Manufacture of Sterile Medicinal Products

Document map

Section Number	General overview
1. Scope	Additional areas (other than sterile medicinal products) where the general principles of the annex can be applied.
2. Principle	General principles as applied to the manufacture of medicinal products.
3. Pharmaceutical Quality System (PQS)	Highlights the specific requirements of the PQS when applied to sterile medicinal products.
4. Personnel	Guidance on the requirements for specific training, knowledge and skills. Also gives guidance to the qualification of personnel.
5. Premises	General guidance regarding the specific needs for premises design and also guidance on the qualification of premises including the use of barrier technology.
6. Equipment	General guidance on the design and operation of equipment.
7. Utilities	Guidance with regards to the special requirements of utilities such as water, air and vacuum.
8. Production and specific technologies	Discusses the approaches to be taken with regards to aseptic and terminal sterilisation processes. Also discusses different technologies such as lyophilization and Blow Fill Seal (BFS) where specific requirements may be required. Discusses approaches to sterilization of products, equipment and packaging components.
9. Viable and non-viable environmental and process monitoring	This section differs from guidance given in section 5 in that the guidance here applies to ongoing routine monitoring with regards to the setting of alert limits and reviewing trend data. The section also gives guidance on the requirements of Aseptic Process Simulation.
10. Quality control (QC)	Gives guidance on some of the specific Quality Control requirements relating to sterile medicinal products.
11. Glossary	Explanation of specific terminology.

1 Scope

The manufacture of sterile medicinal products covers a wide range of product types, (sterile active substance through to finished dosage form), batch sizes (single unit to multiple units), processes (from highly automated systems to manual processes), primary packaging materials and technologies (e.g. biotechnology, classical small molecule manufacturing and closed systems). This Annex provides general guidance that should be used for all sterile medicinal products and sterile active substances, via adaption, using the principles of Quality Risk Management (QRM), to ensure that microbial, particulate and pyrogen contamination associated with microbes is prevented in the final product.

The intent of the Annex is to provide guidance for sterile medicinal products. However some of the principles and guidance, such as contamination control strategy, room qualification, classification, monitoring and gowning, may be used to support the manufacture of other products that are not intended to be sterile (such as certain liquids, creams, ointments and low bioburden biological intermediates) but where the control of microbial, particulate and pyrogen contamination, to reduce it as far as possible, is considered important.

2 Principle

The manufacture of sterile products is subject to special requirements in order to minimize risks of microbiological, particulate and pyrogen contamination. The following key areas should be considered:

a) Facility, equipment and process design must be optimized qualified and validated according to Annex 11 and Annex15 of EU GMP. The use of appropriate current technologies should be implemented to ensure protection and control of the product from potential extraneous sources of particulate and microbial contamination such as personnel, materials and the surrounding environment.

b) Personnel must have appropriate skills, training and attitudes with a specific focus on the principles involved in the protection of sterile product during the manufacturing, packaging and distribution processes.

c) Processes and monitoring systems for sterile product manufacture must be designed, commissioned, qualified and monitored by personnel with appropriate process, engineering and microbiological knowledge.

Processes, equipment, facilities and manufacturing activities should be managed in accordance with QRM principles that provide a proactive means of identifying, scientifically evaluating and controlling potential risks to quality. Risk assessments should be used to justify alternative approaches to those specified in this Annex only if these alternative approaches meet or surpass the intent of this Annex.

Quality Assurance is particularly important, and manufacture of sterile products must strictly follow carefully established and validated methods of manufacture and control. A contamination control strategy should be implemented across the facility in order to assess the effectiveness of all the control and monitoring measures employed. This assessment should lead to corrective and preventative actions being taken as necessary.

The strategy should consider all aspects of contamination control and its life cycle with ongoing and periodic review and update of the strategy as appropriate.

Contamination control and steps taken to minimise the risk of contamination from microbial and particulate sources are a series of successively linked events or measures. These are typically assessed, controlled and monitored individually but these many sources should be considered holistically.

The development of such strategies requires thorough technical and process knowledge. Potential sources of contamination are attributable to microbiological and cellular debris (e.g. pyrogens/endotoxins) as well as particulate matter (glass and other visible and sub-visible particles).

Elements to be considered within such a documented contamination control strategy should include (but not be limited to):

a) Design of both the plant and process.

b) Equipment and facilities.

c) Personnel.

d) Utilities.

e) Raw Materials Control – including in-process controls.

f) Product containers and closures.

g) Vendor approval – such as key component suppliers, sterilization of components and single use systems, and services.

h) For outsourced services, such as sterilization, sufficient evidence should be provided to the contract giver to ensure the process is operating correctly.

i) Process risk assessment.

j) Process validation.

k) Preventative maintenance – maintaining equipment and premises (planned and unplanned maintenance) to a standard that will not add significant risk of contamination.

l) Cleaning and disinfection.

m) Monitoring systems - including an assessment of the feasibility of the introduction of scientifically sound, modern methods that optimize the detection of environmental contamination.

n) Prevention – Trending, investigations, corrective and preventive actions (CAPA), root cause determination and the need for more robust investigational tools.

o) Continuous improvement based on information from the above systems.

The manufacturer should take all steps and precautions necessary to assure the sterility of the products manufactured within its facilities. Sole reliance for sterility or other quality aspects must not be placed on any terminal process or finished product test.

Note 1:
This guidance does not lay down detailed methods for determining the microbiological and particulate cleanliness of air, surfaces etc. Reference should be made to other documents such as the EN/ISO Standards and Pharmacopoeial monographs for more detailed guidance.

Note 2:
Where national legislation permits, additional guidance regarding the preparation of unlicensed sterile medicinal products normally performed by healthcare establishments for direct supply to patients, reference may be made to the Annex 1: “Guidelines on the standards required for the sterile preparation of medicinal products” of the PIC/S guide to good practices for the preparation of medicinal products in healthcare establishments, PE 010.

3 Pharmaceutical Quality System (PQS)

3.1 The manufacture of sterile medicinal products is a complex activity that requires additional controls and measures to ensure the quality of products manufactured. Accordingly, the manufacturer's Pharmaceutical Quality System (PQS) should encompass and address the specific requirements of sterile product manufacture and ensure that all activities are effectively controlled so that all final products are free from microbial and other contamination. In addition to the PQS requirements detailed in chapter 1 of the EU GMPs, the PQS for sterile product manufacturers should also ensure that:

a) There is an effective risk management system integrated into the product life cycle to minimise microbial contamination to ensure the safety, quality and efficacy of sterile manufactured product, including assurance of sterility.

b) The manufacturer has sufficient knowledge and expertise in relation to the products manufactured and the manufacturing methods employed.

c) Root cause analysis of procedural, process or equipment failure is key to ensure that the risk to product is correctly understood and suitable corrective and preventative actions are implemented.

d) Risk assessment is performed to identify, assess, eliminate (where applicable) and control contamination risks to prevent contamination, to monitor and detect contamination, and to establish process requirements and acceptance criteria for all elements of a sterile manufacturing process. The risk assessment should be documented and should include the rationale for decisions taken in relation to mitigating risks, discounting of potential risks and residual risk. The risk assessment should be reviewed regularly as part of on-going quality management, during change control and during the periodic product quality review.

e) Processes associated with the finishing and transport of sterile products should not compromise the finished sterile product in terms of container integrity or pose a risk of contamination and ensure that medicinal products are stored and maintained in accordance with registered storage conditions.

f) Persons responsible the quality release of sterile medicines should have appropriate access to manufacturing and quality information and possess adequate knowledge and experience in the manufacture of sterile dosage forms and their critical quality attributes in order to be able to ascertain that the medicines have been manufactured in accordance with the registered specification and are of the required safety, quality and efficacy.

3.2 Investigations should be performed into non-conformities, such as sterility test failures or environmental monitoring excursions or deviations from established procedures, with a specific focus regarding the potential impact to sterility, to not only the specific batch concerned but also any other potentially impacted batch. The reasons for including or excluding product from the scope of the investigation should be clearly recorded and justified within the investigation.

4 Personnel

4.1 The manufacturer should ensure that there are sufficient appropriate personnel, suitably qualified and experienced in the manufacture and testing of sterile medicines and any of the specific manufacturing technologies used in the site's manufacturing operations, to ensure compliance with Good Manufacturing Practice applicable to the manufacture of sterile medicinal products.

4.2 Only the minimum number of personnel required should be present in cleanrooms. The maximum number of operators in critical areas should be determined based on QRM principles, documented in the contamination control strategy, and validated during activities such as initial qualification and aseptic process simulations, so as not to compromise sterility assurance. This is particularly important during aseptic processing. Inspections and controls should be conducted outside the clean areas as far as possible.

4.3 All personnel (including those performing cleaning and maintenance) employed in such areas should receive regular training, qualification (including sampling of the operators bioburden, using methods such as contact plates, at key locations e.g. hands arms and chest) and assessment in disciplines relevant to the correct manufacture of sterile products. This training should include reference to hygiene, cleanroom practices, contamination control, aseptic techniques, and potential safety implications to the patient of a loss of product sterility and in the basic elements of microbiology.

4.4 The personnel working in a grade A/B cleanroom should be trained for aseptic gowning and aseptic practices. Compliance with aseptic gowning procedures should be assessed and confirmed and this should be periodically reassessed at least annually and should involve both visual and microbiological assessment (using additional locations such as arms and chest). Only trained personnel who have passed the gowning assessment and have participated in a successful aseptic process simulation (APS) test, during which they performed their normal duties, should be authorized to enter any grade A/B area, in which aseptic operations will be conducted, or are being conducted, whilst unsupervised. The microbial monitoring of personnel in the grade A/B area should be performed to assess their aseptic behaviour. This monitoring should take place immediately after completion of a critical intervention and upon each exit from the cleanroom. It should be noted that there should also be an ongoing continuous monitoring program for personnel including some consideration of periodic monitoring under the supervision of the quality unit.

4.5 There should be systems in place for disqualification of personnel from entry into cleanrooms, based on aspects including ongoing assessment and/or the identification of an adverse trend from the personnel monitoring program. Once disqualified, retraining and requalification is required before permitting the operator to have any further involvement in aseptic practices. This should include consideration of participation in a successful Aseptic Process Simulation (APS).

4.6 Manufacturers should establish written procedures outlining the process by which outside staff who have not received such training (e.g. building or maintenance contractors) need to be brought into grade A/B areas. Access by these persons should only be given in exceptional circumstances, evaluated and recorded in accordance with the PQS.

4.7 High standards of personal hygiene and cleanliness are essential. Personnel involved in the manufacture of sterile preparations should be instructed to report any specific health conditions or ailments which may cause the shedding of abnormal numbers or types of contaminants and therefore preclude clean room access; periodic health checks for such conditions should be performed. Actions to be taken with regard to personnel who could be introducing an undue microbiological hazard should be described in procedures decided by a designated competent person.

4.8 Staff who have been engaged in the processing of human or animal tissue materials or of cultures of micro-organisms, other than those used in the current manufacturing process, or any activities that may have a negative impact to quality, e.g. microbial contamination, should not enter sterile product areas unless rigorous, clearly defined and effective entry procedures have been followed.

4.9 Wristwatches, make-up and jewellery and other personal items such as mobile phones should not be allowed in clean areas.

4.10 Changing and hand washing should follow a written procedure designed to minimize contamination of clean area clothing or carry-through of contaminants to the clean areas. Garments should be visually checked for cleanliness and integrity prior to entry to the clean room. For sterilized garments, particular attention should be taken to ensure that garments and eye coverings have been sterilized and that their packaging is integral before use. Re-usable garments should be replaced based at a set frequency determined by qualification or if damage is identified.

4.11 The clothing and its quality should be appropriate for the process and the grade of the working area. It should be worn in such a way as to protect the product from contamination.

4.12 The description of clothing required for each grade is given below:

a) Grade D: Hair, beards and moustaches should be covered. A general protective suit and appropriately disinfected shoes or overshoes should be worn. Appropriate measures should be taken to avoid any contamination coming from outside the clean area.

b) Grade C: Hair, beards and moustaches should be covered. A single or two-piece trouser suit gathered at the wrists and with high neck and appropriately disinfected or sterilized shoes or overshoes should be worn. They should shed virtually no fibres or particulate matter.

c) Grade A/B: Sterile headgear should totally enclose hair and facial hair; it should be tucked into the neck of the sterile suit; a sterile face mask and sterile eye coverings should be worn to cover all facial skin and prevent the shedding of droplets and particles. Appropriate sterilized, non-powdered rubber or plastic gloves and sterilized footwear should be worn. Trouser-legs 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. Garments should be packed and folded in such a way as to allow operators to change into the garments with contact to the outer surfaces of the garment reduced to a minimum.

Note: This is minimum guidance and higher standards of clothing may be required dependent on the processes performed in the specific area.
4.13 Outdoor clothing should not be brought into changing rooms leading to grade B and C rooms. It is recommended that facility suits, including dedicated socks be worn before entry to change rooms for grade C and B. Where clothing is reused this should be considered as part of the qualification.

4.14 For every worker in a grade A/B area, clean sterilized protective garments (including eye coverings and masks) of an appropriate size should be provided at each work session. Gloves should be regularly disinfected during operations. Garments and gloves should be changed at least for every working session.

4.15 Clean area clothing should be cleaned, handled and worn in such a way that it does not gather additional contaminants which can later be shed. These operations should follow written procedures. Separate laundry facilities for such clothing are desirable. Inappropriate treatment of clothing will damage fibres and may increase the risk of shedding of particles. After washing and before sterilization, garments should be checked for integrity.

4.16 Activities in clean areas, especially when aseptic operations are in progress, should be kept to a minimum and movement of personnel should be controlled and methodical to avoid excessive shedding of particles and organisms due to over-vigorous activity. Operators performing aseptic operations should adhere to strict aseptic technique at all times. To prevent changes in air currents that introduce lower quality air, movement adjacent to the critical area should be restricted and the obstruction of the path of the unidirectional airflow must be avoided. The ambient temperature and humidity should be set to prevent shedding due to operators becoming too cold (leading to excessive movement) or too hot.

5 Premises

5.1 The manufacture of sterile products should be carried out in clean areas, entry to which should be through airlocks for personnel and/or for equipment and materials. Clean areas should be maintained to an appropriate cleanliness standard and supplied with air which has passed through filters of an appropriate efficiency.

5.2 The various operations of component preparation, product preparation and filling should be carried out with appropriate technical and operational separation measures within the clean area.

5.3 For the manufacture of sterile medicinal products 4 grades of clean room can be distinguished.

> Grade A: The local zone for high risk operations, e.g. filling zone, stopper bowls, open ampoules and vials, making aseptic connections. Normally, such conditions are provided by a localised air flow protection, such as laminar air flow work stations or isolators. Unidirectional air flow systems should provide a homogeneous air speed in a range of 0.36 – 0.54 m/s (guidance value), the point at which the air speed

measurement is taken should be clearly justified in the protocol. During initial qualification and requalification air speeds may be measured either close to the terminal air filter face or at the working height, Where ever the measurement is taken it is important to note that the key objective is to ensure that air visualization studies should correlate with the airspeed measurement to demonstrate air movement that supports protection of the product and open components with unidirectional air at the working height, where high risk operations and product and components are exposed. The maintenance of unidirectional airflow should be demonstrated and validated across the whole of the grade A area. Entry into the grade A area by operators should be minimized by facility, process and procedural design.

<u>Grade B</u>: For aseptic preparation and filling, this is the background environment for the grade A zone. In general, only grade C cleanrooms should interface with the grade B aseptic processing area.
Lower grades can be considered where isolator technology is used (refer to clause 5.19-5.20).

<u>Grade C and D</u>: Clean areas for carrying out less critical stages in the manufacture of sterile products.

5.4 In clean areas, all exposed surfaces should be smooth, impervious and unbroken in order to minimize the shedding or accumulation of particles or micro-organisms and to permit the repeated application of cleaning agents, and disinfectants, where used.

5.5 To reduce accumulation of dust and to facilitate cleaning there should be no uncleanable recesses and a minimum of projecting ledges, shelves, cupboards and equipment. Doors should be designed to avoid uncleanable recesses.

5.6 Materials liable to generate fibres should not be permitted in clean areas

5.7 False ceilings should be designed and sealed to prevent contamination from the space above them.

5.8 Sinks and drains should be prohibited in grade A/B areas. In other areas air breaks should be fitted between the machine or sink and the drains. Floor drains in lower grade rooms should be fitted with traps or water seals to prevent back flow and should be regularly cleaned and disinfected.

5.9 Airlocks should be designed and used to provide physical separation and to minimize microbial and particulate contamination of the different areas, and should be present for material and personnel moving from different grades, typically airlocks used for personnel movement are separate to those used for material movement. They should be flushed effectively with filtered air. The final stage of the airlock should, in the at-rest state, be the same grade as the area into which it leads. The use of separate changing rooms for entering and leaving clean areas is generally desirable.

a) Personnel airlocks. A cascade concept should be followed for personnel (e.g. from grade D to grade C to grade B). In general hand washing facilities should be provided only in the first stage of the changing rooms.

b) Material airlocks (used for materials and equipment).

i. Pass through hatches without active filtered air supply should be avoided. If necessary, provisions and procedures should be in place to avoid any risk of contamination (e.g. by the incoming material or by entering air).

ii. For airlocks leading to grade A and B areas, only materials and equipment that have been included as part of the qualification list should be allowed to be transferred into the grade A/B area via the air lock or pass through; the continuity of grade A should be maintained in the aseptic core when the materials have to be transferred from grade B to grade A areas, consideration should be given to listing these items on an authorized list. Any unapproved items that require transfer should be an exception. Appropriate risk evaluation and mitigation strategies should be applied and recorded as per the manufacturer's contamination control strategy and should include a specific sanitisation and monitoring regime approved by quality assurance.

iii. The movement of material from clean not classified (CNC) to grade C should be based on QRM principles, with cleaning and disinfection commensurate with the risk.

5.10 Both airlock doors should not be opened simultaneously. The opening of more than one door at a time should be prevented, for airlocks leading to grade A and B an interlocking system should usually be used; for airlocks leading to grade C and D at least a visual and/or audible warning system should be operated. Where required to maintain zone segregation, a time delay between the closing and opening of interlocked doors should be established.

5.11 A HEPA or ULPA filtered air supply should maintain a positive pressure and an air flow relative to surrounding areas of a lower grade under all operational conditions and should flush the area effectively. Adjacent rooms of different grades should have a pressure differential of 10 - 15 Pascals (guidance values). Particular attention should be paid to the protection of the zone of greatest risk, that is, the immediate environment to which a product and cleaned components which contact the product are exposed. The recommendations regarding air supplies and pressure differentials may need to be modified where it becomes necessary to contain some materials, e.g. pathogenic, highly toxic, radioactive or live viral or bacterial materials or products. Decontamination of facilities, e.g. the clean rooms and HVAC, and the treatment of air leaving a clean area may be necessary for some operations.

5.12 It should be demonstrated that air-flow patterns do not present a contamination risk, e.g. care should be taken to ensure that air flows do not distribute particles from a particle-generating person, operation or machine to a zone of higher product risk.
Air flow patterns should be visualised in grade A/B areas to evaluate if airflow is unidirectional. Where unidirectional air flow is not demonstrated, corrective actions, such as design improvements, should be implemented. In the other areas, the need to demonstrate the air flow patterns should be based on a risk assessment. Air flow pattern studies should be performed under dynamic conditions. Video recordings of the airflow patterns are recommended. The outcome of the air visualisation studies should be considered when establishing the facility's environmental monitoring program.

5.13 A warning system should be provided to indicate failure in the air supply and reduction of pressure differentials below set limits. Indicators of pressure differences should be fitted between areas, based on QRM principles. These pressure differences should be recorded regularly or otherwise documented.

5.14 Consideration should be given to designing facilities that permit observation of activities from outside the clean areas, e.g. through the provision of windows or remote camera access with a complete view of the area and processes to allow observation and supervision without entry.

Barrier Technologies

5.15 Isolator or Restricted Access Barrier System (RABS) technologies, and the associated processes, should be designed so as to provide maximum protection of the grade A environment. The transfer of materials into and out of the RABS or isolator is one of the greatest potential sources of contamination and therefore the entry of additional materials following sterilisation should be minimized. Any activities that potentially compromise the sterility assurance of the critical zone should be assessed and controls applied if they cannot be eliminated.

5.16 The design of the RABS or isolator shall take into account all critical factors associated with these technologies, including the quality of the air inside and the surrounding area, the materials and component transfer, the decontamination, disinfection or sterilization processes and the risk factors associated with the manufacturing operations and materials, and the operations conducted within the critical zone.

5.17 The critical zone of the RABS or isolator used for aseptic processes should meet grade A with unidirectional air flow. Under certain circumstances turbulent airflow may be justified in a closed isolator when proven to have no negative impact on the product. The design of the RABS and open isolators should ensure a positive airflow from the critical zones to the surrounding areas; negative pressure isolators should only be used when containment of the product is considered essential.

5.18 For RABS, the background environment should meet grade B. For open RABS, or where doors may be very rarely opened during processing, and studies should be performed to demonstrate the absence of air ingress.

5.19 For open, positive pressure isolators or closed isolators with decontamination by a sporicidal agent, the surrounding area should correspond to a minimum of grade D. The disinfection regime should be included as a key consideration when performing the risk assessment to design the contamination control strategy for an isolator.

5.20 For isolators, the required background environment can vary depending on the design of the isolator, its application and the methods used to achieve bio-decontamination.
The decision as to the supporting background environment should be documented in a risk assessment where additional risks are identified, such as for negative pressure isolators. Where items are introduced to the isolator after disinfection then a higher grade of background should be considered.

5.21 Glove systems, as well as other parts of an isolator, are constructed of various materials that can be prone to puncture and leakage. The materials used shall be demonstrated to have good mechanical and chemical resistance. Integrity testing of the barrier systems and leak testing of the isolator and the glove system should be performed using visual, mechanical and physical methods. They should be performed at defined periods, at a minimum of the beginning and end of each batch, and following any intervention that may affect the integrity of the unit.

5.22 Decontamination processes of an isolator or RABS should be validated and controlled in accordance with defined parameters. Evidence should also be available to demonstrate that the agent does not affect any process performed in the isolator or RABS, such as having an adverse impact on product or sterility testing.

Clean room and clean air device qualification

5.23 Clean rooms and clean air devices (clean areas) for the manufacture of products should be qualified according to the required characteristics of the environment. Each manufacturing operation requires an appropriate environmental cleanliness level in the operational state in order to minimize the risks of particulate or microbial contamination of the product or materials being handled.

Note: Classification is a method of assessing the level of air cleanliness against a specification for a cleanroom or clean area device by measuring the airborne particle concentration. The classification is part of the qualification of a clean area.

5.24 Clean rooms and clean air devices should be qualified in accordance with Annex 15 of EU GMP. Reference for the classification of the clean rooms and clean air devices can be found in the ISO 14644 series of standards.

5.25 For classification, the airborne particles equal to or greater than 0.5 µm should be measured. This measurement should be performed both at rest and in operation. The maximum permitted airborne particle concentration for each grade is given in table 1.

Table 1: **Maximum permitted airborne particle concentration during classification**

	Maximum permitted number of particles equal to or greater than 0.5 µm		
Grade	At rest equal to or greater than 0.5 µm per m^3	In operation equal to or greater than 0.5 µm per m^3	ISO classification in operation/at rest
A	3 520	3 520	5/5
B	3 520	352 000	5/7
C	352 000	3 520 000	7/8
D	3 520 000	Not defined[(a)]	8

[a] For grade D, no “in operation” limits are defined; the company should establish in operation limits based on a risk assessment and on historical data, where applicable.

5.26 For initial classification the minimum number of sampling locations can be found in ISO 14644 Part 1. However, a higher number of samples and sample volume is typically required for the aseptic processing room and the immediately adjacent environment (grade A/B) to include consideration of all critical processing locations such as point of fill stopper bowls. With the exception of the aseptic processing room, the sampling locations should be distributed evenly throughout the area of the clean room. For later stages of qualification and classification, such as performance qualification, locations should be based on a documented risk assessment and knowledge of the process and operations to be performed in the area

a) The “in operation” and “at rest” states should be defined for each clean room or suite of clean rooms.

b) The definition of “at rest” is the room complete with all HVAC systems, utilities functioning and with manufacturing equipment installed as specified but without personnel in the facility and the manufacturing equipment is static.

c) The “in operation” state is the condition where the installation is functioning in the defined operating mode with the specified number of personnel working.

d) “In operation” classification, qualification and requalification may be performed during normal operations, simulated operations or during aseptic process simulations (where worst case simulation is required).

e) The particle limits given in Table 1 above for the “at rest” state should be achieved after a “clean up” period on completion of operations. The "clean up" period should be determined during the initial classification of the rooms.

f) In order to meet “in operation” conditions these areas should be designed to reach certain specified air-cleanliness levels in the “at rest” occupancy state.

5.27 The microbial load of the clean rooms should be determined as part of the clean room qualification. The recommended maximum limits for microbial contamination during qualification for each grade are given in table 2.

Table 2: **Recommended limits for microbial contamination in operation**

Grade	air sample cfu/m^3	settle plates (diameter 90 mm) cfu/4 hours [a]	contact plates (diameter 55 mm) cfu/plate
A[b]	1	1	1
B	10	5	5
C	100	50	25
D	200	100	50

[a] Individual settle plates may be exposed for less than 4 hours. Where settle plates are exposed for less than 4 hours the limits in the table should still be used, no

recalculation is necessary. Settle plates should be exposed for the duration of critical operations and changed as required after 4 hours.

(b) It should be noted that for grade A the expected result should be 0 cfu recovered; any recovery of 1 cfu or greater should result in an investigation.

Note: For qualification of personnel, the limits given for contact plates and glove prints in table 6 should be applied.

5.28 Clean room qualification (including classification) should be clearly differentiated from operational process environmental monitoring.

5.29 Clean rooms should be requalified periodically and after changes to equipment, facility or processes based on the principles of QRM. For grade A and B zones, the maximum time interval for requalification is 6 months. For grades C and D, the maximum time interval for requalification is 12 months.

5.30 Other characteristics, such as temperature and relative humidity, depend on the product and nature of the operations carried out. These parameters should not interfere with the defined cleanliness standard.

Disinfection

5.31 The disinfection of clean areas is particularly important. They should be cleaned and disinfected thoroughly in accordance with a written programme (for disinfection to be effective, cleaning to remove surface contamination must be performed first)., More than one type of disinfecting agent should be employed, and should include the periodic use of a sporicidal agent. Disinfectants should be shown to be effective for the duration of their in use shelf-life taking into consideration appropriate contact time and the manner in and surfaces on which they are utilized. Monitoring should be undertaken regularly in order to show the effectiveness of the disinfection program and to detect the development of resistant and/or spore forming strains. Cleaning programs should be effective in the removal of disinfectant residues.

5.32 Disinfectants and detergents should be monitored for microbial contamination; dilutions should be kept in previously cleaned containers and should only be stored for defined periods. Disinfectants and detergents used in grade A and B areas should be sterile prior to use.

5.33 Disinfectants should be shown to be effective when used on the specific facilities, equipment and processes that they are used in.

5.34 Fumigation or vapour disinfection of clean areas such as Vapour Hydrogen Peroxide (VHP) may be useful for reducing microbiological contamination in inaccessible places.

6 Equipment

6.1 A written, detailed description of the equipment design should be produced (including diagrams as appropriate) and kept up to date. It should describe the product and other critical gas and fluid pathways and controls in place.

6.2 Equipment monitoring requirements should be determined during qualification. Process alarm events should be reviewed and approved and evaluated for trends.

6.3 As far as practicable equipment, fittings and services should be designed and installed so that operations, maintenance, and repairs can be carried out outside the clean area, if maintenance has to be performed in the clean area then precautions such as additional disinfection and additional environmental monitoring should be considered. If sterilization is required, it should be carried out, wherever possible, after complete reassembly.

6.4 When equipment maintenance has been carried out within the clean area, the area should be cleaned, disinfected and/or sterilized where appropriate, before processing recommences if the required standards of cleanliness and/or asepsis have not been maintained during the work.

6.5 The cleaning process should be validated so that it can be demonstrated that it:

a) Can remove any residues that would otherwise create a barrier between the sterilizing agent and the equipment surfaces.

b) Prevents chemical and particulate contamination of the product during the process and prior to disinfection.

6.6 All critical surfaces that come into direct contact with sterile materials should be sterile.

6.7 All equipment such as sterilizers, air handling and filtration systems, water treatment, generation, storage and distribution systems should be subject to qualificion, monitoring and planned maintenance; their return to use should be approved.

6.8 A conveyor belt should not pass through a partition between a grade A or B area and a processing area of lower air cleanliness, unless the belt itself is continually sterilized (e.g. in a sterilizing tunnel).

6.9 Particle counters should be qualified (including sampling tubing). Portable particle counters with a short length of sample tubing should be used for qualification purposes. Isokinetic sample heads shall be used in unidirectional airflow systems.

6.10 Where unplanned maintenance of equipment critical to the sterility of the product is to be carried out, an assessment of the potential impact to the sterility of the product should be performed and recorded.

7 Utilities

7.1 The nature and amount of controls associated with utilities should be commensurate with the risk associated with the utility determined via risk assessment.

7.2 In general higher risk utilities are those that:

a) Directly contact product e.g. compressed gases.

b) Contact materials that ultimately will become part of the product.

c) Control contamination of surfaces that contact the product.

d) Or otherwise directly impact the product.

7.3 Utilities should be installed, operated and maintained in a manner to ensure the utility functions as expected.

7.4 Results for critical parameters of the high risk utility should be subject to regular trend analysis to ensure that system capabilities remain appropriate.

7.5 Current drawings should be available that identify critical system attributes such as: pipeline flow, pipeline slopes, pipeline diameter and length, tanks, valves, filters, drains and sampling points.

7.6 Pipes and ducts and other utilities should be installed so that they do not create recesses, unsealed openings and surfaces which are difficult to clean.

Water systems

7.7 Water treatment plants and distribution systems should be designed, constructed and maintained to minimize the risk of microbial contamination and proliferation so as to ensure a reliable source of water of an appropriate quality. Water produced should comply with the current monograph of the relevant Pharmacopeia.

7.8 Water for injections (WFI) should be produced from purified water, stored and distributed in a manner which prevents microbial growth, for example by constant circulation at a temperature above 70°C. Where the WFI is produced by methods other than distillation further techniques post Reverse osmosis (RO) membrane should be considered such as nanofiltration, and ultra-filtration.

7.9 Water systems should be validated to maintain the appropriate levels of physical, chemical and microbial control, taking seasonal variation into account.

7.10 Water flow should remain turbulent through the pipes to prevent microbial adhesion.

7.11 The water system should be configured to prevent the proliferation of microorganisms, e.g. sloping of piping to provide complete drainage and the avoidance of dead legs. Where filters are included in the system, special attention should be taken with regards to the monitoring and maintenance of these filters.

7.12 Where WFI storage tanks are equipped with hydrophobic bacteria retentive vent filters the filters should be sterilized, and the integrity of the filter tested before and after use.

7.13 To prevent the formation of biofilms, sterilization or disinfection or regeneration of water systems should be carried out according to a predetermined schedule and also when microbial counts exceed action and alert limits. Disinfection of a water system with chemicals should be followed by a validated rinsing procedure. Water should be analyzed after disinfection/regeneration; results should be approved before the start of use of the water system.

7.14 A suitable sampling schedule should be in place to ensure that representative water samples are obtained for analysis on a regular basis.

7.15 Regular ongoing chemical and microbial monitoring of water systems should be performed with alert limits based on the qualification that will identify an adverse trend in the performance of the systems. Sampling should include all outlets and user points at a specified interval. A sample from the worst case sample point, e.g. the end of the distribution loop return, should be included each time the water is used for manufacturing and manufacturing processes. A breach of an alert limit should trigger review and follow-up, which might include investigation and corrective action. Any breach of an action limit should lead to a root cause investigation and risk assessment.

7.16 WFI systems should include continuous monitoring systems such as Total Organic Carbon (TOC) and conductivity.

Steam used for sterilization

7.17 Purified water, with a low level of endotoxin, should be used as the minimum quality feed water for the pure steam generator.

7.18 Steam used for sterilization processes should be of suitable quality and should not contain additives at a level which could cause contamination of product or equipment. The quality of steam used for sterilization of porous loads and for Steam-In-Place (SIP) should be assessed periodically against validated parameters. These parameters should include consideration of the following examples: non-condensable gases, dryness value (dryness fraction), superheat and steam condensate quality.

Compressed gases and vacuum systems

7.19 Compressed gases that come in direct contact with the product/container primary surfaces should be of appropriate chemical, particulate and microbiological purity, free from oil with the correct dew point specification and, where applicable, comply with appropriate pharmacopoeial monographs. Compressed gases must be filtered through a sterilizing filter (with a nominal pore size of a maximum of 0.22µm) at the point of use. Where used for aseptic manufacturing, confirmation of the integrity of the final sterilization gas filter should be considered as part of the batch release process.

7.20 There should be prevention of backflow when any vacuum or pressure system is shut off.

Cooling systems

7.21 Major items of equipment associated with hydraulic and cooling systems should, where possible, be located outside the filling room. Where they are located inside the filling room there should be appropriate controls to contain any spillage and/or cross contamination associated with the hydraulics of cooling system fluids.

7.22 Any leaks from the cooling system must be detectable (i.e. an indication system for leakage). In addition, there must be adequate cooling flow within the system.

7.23 The cooling circuit should be subject to leak testing both periodically and following any maintenance.

7.24 There should be periodic cleaning/disinfection of both the vacuum system and cooling systems.

8 Production and Specific Technologies

Terminally sterilized products

8.1 Preparation of components and most products should be done in at least a grade D environment in order to give a low risk of microbial, pyrogen and particulate contamination, so that the product is suitable for filtration and sterilization. Where the product is at a high or unusual risk of microbial contamination, (for example, because the product actively supports microbial growth and/or must be held for a long periods before sterilisation and/or is not processed mainly in closed vessels), then preparation should be carried out in a grade C environment.

8.2 Filling of products for terminal sterilization should be carried out in at least a grade C environment.

8.3 Where the product is at an unusual risk of contamination from the environment because, for example, the filling operation is slow, the containers are wide necked or are necessarily exposed for more than a few seconds beforeclosing, or the product is held for extended periods prior to terminal sterilization, then the product should be filled in a grade A zone with at least a grade C background. Preparation and filling of ointments, creams, suspensions and emulsions should generally be carried out in a grade C environment before terminal sterilization.

8.4 Processing of the bulk solution should include a filtration step to reduce bioburden levels and particulates prior to filling into the final product containers.

8.5 Examples of operations to be carried out in the various grades are given in table 3.

Table 3: **Examples of operations and grades they should be performed in for terminally sterilized products**

A	Filling of products, when unusually at risk.
C	Preparation of solutions, when unusually at risk. Filling of products.
D	Preparation of solutions and components for subsequent filling.

Aseptic preparation

8.6 Aseptic processing is the handling of sterile product, containers and/or devices in a controlled environment, in which the air supply, materials and personnel are regulated to prevent microbial contamination. Additional requirements apply to Restricted Access Barrier Systems (RABS) and isolators (refer clauses 5.15-5.22).

8.7 The aseptic process should be clearly defined. The risks associated with the aseptic process, and any associated requirements, should be identified, assessed and appropriately controlled. The site's contamination control strategy should clearly define the acceptance criteria for these controls, requirements for monitoring and the review of their effectiveness. Methods and procedures to control these risks should be described and implemented. Residual risks should be justified.

8.8 Precautions to minimise microbiological, pyrogen and particulate contamination should be taken, as per the site’s contamination control strategy, during the preparation of the aseptic environment, during all processing stages, including the stages before and after filter sterilization, and until the product is sealed in its final container. Materials liable to generate fibres should not be permitted in clean areas.

8.9 Where possible, the use of equipment such as RABS, isolators or closed systems, should be considered in order to reduce the need for interventions into the grade A environment and minimize the risk of contamination. Automation of processes should also be considered to remove the risk of contamination by interventions (e.g. dry heat tunnel, automated lyophilizer loading, SIP).

8.10 Examples of operations to be carried out in the various environmental grades are given in the table 4.

Table 4: **Examples of operations and which grades they should be performed in**

A	Critical processing zone. Aseptic assembly of filling equipment. Aseptic connections (should be sterilized by steam-in-place whenever feasible). Aseptic compounding and mixing. Replenishment of sterile product, containers and closures. Removal and cooling of items from heat sterilizers. Staging and conveying of sterile primary packaging components. Aseptic filling, sealing, transfer of open or partially stoppered vials, including interventions. Loading and unloading of a lyophilizer
B	Direct support zone for the critical processing (grade A) zone. Transport and preparation of packaged equipment, components and ancillary items for introduction into the grade A zone. Removal of sealed product from the grade A zone.
C	Preparation of solutions to be filtered.
D	Cleaning of equipment. Handling of components, equipment and accessories after washing. Assembly of cleaned equipment to be sterilized.

Note: If Isolators are used then a risk assessment should determine the necessary background environment grade; at least a minimum of grade D should be used. Refer clauses 5.19-5.20.

8.11 Where the product is not subsequently sterile filtered, the preparation of equipment, components and ancillary items and products should be done in a grade A environment with a grade B background.

8.12 Preparation and filling of sterile products such as ointments, creams, suspensions and emulsions should be performed in a grade A environment, with a grade B background, when the product and components are exposed and the product is not subsequently filtered or sterilized.

8.13 Unless subsequently sterilized by steam-in-place or conducted with validated intrinsic sterile connection devices, aseptic connections should be performed in a grade A environment with a grade B background (or in an isolator with a suitable background), in a way that minimizes the potential contamination from the immediate environment, e.g. from operators or boundaries with lower grades. Aseptic connections, including those performed to replace equipment, should be appropriately assessed and their effectiveness verified as acceptable by process simulation tests. (For requirements regarding intrinsic sterile connection devices (refer clause 8.115).

8.14 The transfer of partially closed containers to a lyophilizer, should be done under grade A conditions (e.g. HEPA filtered positive pressure) at all times and, where possible, without operator intervention. Portable transfer systems (e.g. transfer carts, portable Laminar Flow Work Stations, etc.) should ensure that the integrity of transfer system is maintained and the process of transfer should minimize the risk of contamination.

8.15 Aseptic manipulations (including non-intrinsic aseptic connections) should be minimized using engineering solutions such as the use of preassembled and sterilized equipment. Whenever feasible, product contact piping and equipment should be pre-assembled, then cleaned and sterilized in place. The final sterile filtration should be carried out as close as possible to the filling point and downstream of aseptic connections wherever possible

8.16 The duration for each aspect of the aseptic manufacturing process should be limited to a defined and validated maximum, including:

a) Time between equipment, component, and container cleaning, drying and sterilization.

b) Holding time for sterilized equipment, components, and containers prior to and during filling/assembly.

c) The time between the start of the preparation of a solution and its sterilization or filtration through a micro-organism-retaining filter. There should be a set maximum permissible time for each product that takes into account its composition and the prescribed method of storage.

d) Aseptic assembly.

e) Holding sterile product prior to filling.

f) Filling.

g) Maximum exposure time of sterilized containers and closures in the critical processing zone (including filling) prior to closure.

Finishing of sterile products

8.17 Partially stoppered vials or prefilled syringes should be maintained under grade A conditions (e.g. use of isolator technology, grade A with B background, with physical segregation from operators) or grade A LAF carts (with suitable grade B background environment and physical segregation from operators) at all times until the stopper is fully inserted.

8.18 Containers should be closed by appropriately validated methods. Containers closed by fusion, e.g. Form-Fill-Seal Small Volume Parenteral (SVP) & Large Volume Parenteral (LVP) bags, glass or plastic ampoules, should be subject to 100% integrity testing. Samples of other containers should be checked for integrity utilising validated methods and in accordance with QRM, the frequency of testing should be based on the knowledge and experience of the container and closure systems being used. A statistically valid sampling plan should be utilized. It should be noted that visual inspection alone is not considered as an acceptable integrity test method.

8.19 Containers sealed under vacuum should be tested for maintenance of vacuum after an appropriate, pre-determined period and during shelf life.

8.20 The container closure integrity validation should take into consideration any transportation or shipping requirements.

8.21 As the equipment used to crimp vial caps can generate large quantities of non-viable particulates, the equipment should be located at a physically separate station equipped with adequate air extraction.

8.22 Vial capping can be undertaken as an aseptic process using sterilized caps or as a clean process outside the aseptic core. Where this latter approach is adopted, vials should be protected by grade A conditions up to the point of leaving the aseptic processing area, and thereafter stoppered vials should be protected with a grade A air supply until the cap has been crimped. Where capping is a manual process it must be performed in grade A conditions with a grade B background.

8.23 In the case where capping is conducted as a clean process with grade A air supply protection, vials with missing or displaced stoppers should be rejected prior to capping. Appropriately validated, automated methods for stopper height detection should be in place. Microbial ingress studies (or alternative methods) should be utilized to determine the acceptable stopper height displacement.

8.24 Where human intervention is required at the capping station, appropriate technology should be used to prevent direct contact with the vials and to minimize microbial contamination.

8.25 RABS and isolators may be beneficial in assuring the required conditions and minimising direct human interventions into the capping operation.

8.26 All filled containers of parenteral products should be inspected individually for extraneous contamination or other defects. QRM principles should be used for determination of defect classification and criticality. Factors to consider include, but are not limited, to the potential impact to the patient of the defect and the route of administration. Different defect types should be categorized and batch performance analyzed. Batches with unusual levels of defects, when compared to routine defect levels for the process, should lead to investigation and consideration of partial or the whole rejection of the batch concerned. A defect library should be generated and maintained which captures all known defects. The defect library can be used as a training tool for production and quality assurance personnel. Critical defects should not be identified during any subsequent sampling of acceptable containers as it indicates a failure of the original inspection process.

8.27 When inspection is done manually, it should be done under suitable and controlled conditions of illumination and background. Inspection rates should be appropriately validated. Operators performing the inspection should undergo robust visual inspection qualification (whilst wearing corrective lenses, if these are normally worn) at least annually. The qualification should be undertaken using appropriate sample sets and taking into consideration worst case scenarios (e.g. inspection time, line speed (where the product is transferred to the operator by a conveyor system), component size or fatigue at the end of shift) and should include consideration of eyesight checks. Operator distractions should be removed and frequent breaks of appropriate duration from inspection should be taken.

8.28 Where automated methods of inspection are used, the process should be validated to detect known defects with sensitivity equal to or better than manual inspection methods and the performance of the equipment checked prior to start up and at regular intervals.

8.29 Results of the inspection should be recorded and defect types and levels trended. Reject rates for the various defect types should also be trended. Investigations should be performed as appropriate to address adverse trends or discovery of new defect types. Impact to product on the market should be assessed as part of this investigation.

Sterilization

8.30 Where possible, finished product should be terminally sterilized using a validated and controlled sterilization process as this provides a greater assurance of sterility than a validated and controlled sterilizing filtration process and/or aseptic processing. Where it is not possible for a product to undergo a sterilisation, consideration should be given to using terminal bioburden reduction steps, such as heat treatments (pasteurization), combined with aseptic processing to give improved sterility assurance.

8.31 The selection, design and location of the equipment and cycle/programme used for sterilization should be decided using QRM principles. Critical parameters should be defined, controlled, monitored and recorded.

8.32 There should be mechanisms in place to detect a cycle that does not conform to the validated parameters. Any failed or atypical sterilization cycles must be formally investigated.

8.33 All sterilization processes should be validated. Particular attention should be given when the adopted sterilization method is not described in the current edition of the Pharmacopoeia, or when it is used for a product which is not a simple aqueous solution. Where possible, heat sterilization is the method of choice. Regardless, the sterilization process must be in accordance with the registered marketing and manufacturing specifications.

8.34 Before any sterilization process is adopted, its suitability for the product and equipment and its efficacy in achieving the desired sterilizing conditions in all parts of each type of load to be processed should be demonstrated by physical measurements and by biological indicators where appropriate.

8.35 The validity of the process should be verified at scheduled intervals, with a minimum of at least annually. Revalidation of the sterilization process should be conducted whenever significant modifications have been made to the product, product packaging, sterilization load configuration, sterilizing equipment or sterilization process parameters.

8.36 For effective sterilization, the whole of the material and equipment must be subjected to the required treatment and the process should be designed to ensure that this is achieved.

8.37 Routine operating parameters should be established and adhered to for all sterilization processes, e.g. physical parameters and loading patterns, etc.

8.38 Suitable biological indicators (BIs) placed at appropriate locations may be considered as an additional method for monitoring the sterilization. BIs should be stored and used according to the manufacturer's instructions. Prior to use of a new batch/lot of BIs, the quality of the batch/lot should be verified by confirming the viable spore count and identity. Where BIs are used to validate and/or monitor a sterilization process (e.g. for Ethylene Oxide), positive controls should be tested for each sterilization cycle, with strict precautions in place to avoid transferring microbial contamination from BIs, including preventing positive control BIs from contaminating BIs exposed to the sterilization cycle. If biological indicators are used, strict precautions should be taken to avoid transferring microbial contamination to the manufacturing or other testing processes.

8.39 There should be a clear means of differentiating products, equipment and components, which have not been sterilized from those which have. Each basket, tray or other carrier of products, items of equipment or components should be clearly labelled with the material name, its batch number and an indication of whether or not it has been sterilized. Indicators such as autoclave tape, or irradiation indicators may be used, where appropriate, to indicate whether or not a batch (or sub-batch) has passed through a sterilization process. However, these indicators show only that the sterilization process has occurred; they do not necessarily indicate product sterility or achievement of the required sterility assurance level.

8.40 Sterilization records should be available for each sterilization run. They should be reviewed and approved as part of the batch release procedure.

8.41 Where possible, materials, equipment and components should be sterilized by validated methods appropriate to the specific material. Suitable protection after sterilization should be provided to prevent recontamination. If items sterilized "in house" are not used immediately after sterilization, these should be stored, using appropriately sealed packaging, in at least a grade B environment, a maximum hold period should also be established. Components that have been packaged with multiple sterile packaging layers need not be stored in grade B (where justified) if the integrity and configuration (e.g. multiple sterile coverings that can be removed at each transfer from lower to higher grade) of the sterile pack allows the items to be readily disinfected during transfer into the grade A zone. Where protection is achieved by containment in sealed packaging this process should be undertaken prior to sterilisation.

8.42 Transfer of materials, equipment, and components into an aseptic processing area should be via a unidirectional process (e.g. through a double-door autoclave, a depyrogenation oven, effective transfer disinfection, or, for gaseous or liquid materials, a bacteria-retentive filter).

8.43 Where materials, equipment, components and ancillary items are sterilized in sealed packaging and then transferred into the grade A/B area, this should be done using appropriate, validated methods (for example, airlocks or pass through hatches) with accompanying disinfection of the exterior of the sealed packaging. These methods should be demonstrated to be effective in not posing an unacceptable risk of contamination of the grade A/B area and, likewise, the disinfection procedure should be demonstrated to be effective in reducing any contamination on the packaging to acceptable levels for entry of the item into the grade A/B area. Packaging may be multi-layered to allow removal of a single layer at each interface to a higher grade.

8.44 Where materials, equipment, components and ancillary items are sterilized in sealed packaging or containers, the integrity of the sterile protective barrier should be qualified for the maximum hold time, and the process should include inspection of each sterile item prior to its use to ensure that the sterile protective measures have remained integral.

8.45 For materials, equipment, components and ancillary items that are necessary for aseptic processing but cannot be sterilized, an effective and validated disinfection and transfer process should be in place. These items once disinfected should be protected to prevent recontamination. These items, and others representing potential routes of contamination, should be included in the environmental monitoring program.

8.46 When a depyrogenation process is used for any components or product contact equipment, validation studies should be performed to demonstrate that the process will result in a minimum 3 log reduction in endotoxin. There is no additional requirement to demonstrate sterilization in these cases.

Sterilization by heat

8.47 Moist heat sterilization utilises clean steam, typically at lower temperatures and shorter duration than dry heat processes, in order to sterilize a product or article. Moist heat sterilization is primarily effected by latent heat of condensation and the quality of steam is therefore important to provide consistent results. The reduced level of moisture in dry heat sterilization process reduces heat penetration which is primarily effected by conduction. Dry heat processes may be utilized to sterilize or control bioburden of thermally stable materials and articles. Dry heat sterilization is of particular use in the removal of thermally robust contaminants such as pyrogens and is often utilized in the preparation of aseptic filling components. Moist heat sterilization processes may be utilized to sterilize or control bioburden (for non-sterile applications) of thermally stable materials, articles or products and is the preferred method of sterilization, where possible.

8.48 In those cases where parametric release has been authorized, a robust system should be applied to the product lifecycle validation and the routine monitoring of the manufacturing process. This system should be periodically reviewed.

8.49 Each heat sterilization cycle should be recorded on a time/temperature chart with a sufficiently large scale or by other appropriate equipment with suitable accuracy and precision. Monitoring and recording systems should be independent of the controlling system.

8.50 The position of the temperature probes used for controlling and/or recording should have been determined during the validation (which should include heat distribution and penetration studies), and, where applicable, also checked against a second independent temperature probe located at the same position.

8.51 Chemical or biological indicators may also be used, but should not take the place of physical measurements.

8.52 Sufficient time must be allowed for the whole of the load to reach the required temperature before measurement of the sterilizing time-period is commenced. This time must be determined for each type of load to be processed.

8.53 After the high temperature phase of a heat sterilization cycle, precautions should be taken against contamination of a sterilized load during cooling. Any cooling fluid or gas in contact with the product should be sterilized unless it can be shown that any leaking container would not be approved for use.

Moist heat sterilization

8.54 Time, temperature and pressure should be used to monitor the process. Each item sterilized should be inspected for damage, seal and packaging material integrity and moisture on removal from the autoclave. Seal and packaging integrity should also be inspected immediately prior to use. Any items found not to be fit for purpose should be removed from the manufacturing area and an investigation performed.

8.55 System and cycle faults should be registered and recorded by the control and monitoring system and appropriate actions taken prior to release of the process.

8.56 For sterilizers fitted with a drain at the bottom of the chamber, it may also be necessary to record the temperature at this position throughout the sterilization period. For Steam-In-Place (SIP) systems, it may also be necessary to record the temperature at condensate drain locations throughout the sterilization period.

8.57 Validation should include a consideration of equilibration time, exposure time, correlation of pressure and temperature and maximum temperature range during exposure for porous cycles and temperature, time and F_o for fluid cycles. These critical parameters should be subject to defined limits (including appropriate tolerances) and be confirmed as part of sterilization validation and routine cycle acceptance criteria. Revalidation should be performed annually.

8.58 There should be frequent leak tests on the system to be sterilized when a vacuum phase is part of the cycle or the system is returned, post-sterilization, to a pressure equivalent to or lower than the environment surrounding the sterilized system. The frequency of testing should be based on the principles of QRM.

8.59 When the sterilization process includes air purging (e.g. porous autoclave loads, lyophilizer chambers) there should be adequate assurance of air removal prior to and during sterilization. Loads to be sterilized should be designed to support effective air removal and be free draining to prevent the build-up of condensate.

8.60 The items to be sterilized, other than products in sealed containers, should be dry, wrapped in a material which allows removal of air and penetration of steam but which prevents recontamination after sterilization. All load items should be dry upon removal from the sterilizer. Load dryness should be confirmed as a part of sterilization process acceptance.

8.61 Distortion and damage of flexible containers, such as containers produced by Blow-Fill-Seal and Form-Fill-Seal technology that are terminally sterilized, should be prevented by setting correct counter pressure and loading patterns.

8.62 Care should be taken to ensure that materials or equipment are not contaminated after the sterilization exposure phase of the cycle due to the introduction of non-sterile air into the chamber during subsequent phases; typically only sterile filtered air would be introduced into the chamber during these phases.

8.63 Where Sterilization in place (SIP) systems are used, (for example, for fixed pipework, vessels and lyophilizer chambers), the system should be appropriately designed and validated to assure all parts of the system are subjected to the required treatment. The system should be monitored for temperature, pressure and time at appropriate critical locations during routine use, this is to ensure all areas are effectively and reproducibly sterilized; these critical locations should be demonstrated as being representative, and correlated with, the slowest to heat locations during initial and routine validation. Once a system has been sterilized by SIP it should remain integral prior to use, the maximum duration of the hold time should be qualified.

Dry heat sterilization

8.64 The combination of time and temperature to which product, components and equipment are exposed should produce an adequate and reproducible level of lethality and/or pyrogen (endotoxin) inactivation/removal when operated routinely within the established tolerances.

8.65 Dry heat sterilization or depyrogenation tunnels are typically employed to prepare components for aseptic filling operations but may be used for other processes. Tunnels should be configured to ensure that airflow patterns protect the integrity and performance of the sterilizing zone, by maintaining a stable pressure differential and airflow pattern through the tunnel from the higher grade area to the lower grade area. All air supplied to the tunnel should pass through a HEPA filter; periodic tests should be performed to demonstrate filter integrity. Any tunnel parts that come into contact with sterilized components should be appropriately sterilized or disinfected. Critical process parameters that should be considered during validation and/or routine processing should include, but may not be limited to:

a) Belt speed or dwell time within sterilising zone.

b) Temperature – Minimum and maximum temperatures.

c) Heat penetration of material/article.

d) Heat distribution/uniformity.

e) Airflows – correlated with the heat distribution and penetration studies.

8.66 When using endotoxin spiked containers these need to be carefully managed with a full reconciliation performed. Endotoxin quantification and recovery efficiency should also be demonstrated.

8.67 Dry heat ovens are typically employed to sterilize or depyrogenate primary packaging components, finished materials or APIs but may be used for other processes. They should be maintained at a positive pressure to lower grade areas. All air entering the oven should pass through a HEPA filter. Critical process parameters that should be considered in validation qualification and/or routine processing should include, but may not be limited to:

a) Temperature.

b) Exposure period/time.

c) Chamber pressure.

d) Heat penetration of material/article (slow to heat spots and different loads).

e) Heat distribution/uniformity.

8.68 For dry heat sterilization of starting materials and intermediates the same principles should be applied. Consideration should be given to factors affecting heat penetration such as the container type, size and packing matrix.

Sterilization by radiation

8.69 Guidance regarding ionising radiation sterilization can be found within Annex 12 of the EU GMP.

8.70 Radiation sterilization is used mainly for the sterilization of heat sensitive materials and products. Many medicinal products and some packaging materials are radiation-sensitive, so this method is permissible only when the absence of deleterious effects on the product has been confirmed. Ultraviolet irradiation is not normally an acceptable method of sterilization.

8.71 Validation procedures should ensure that the effects of variations in density of the packages are considered.

Sterilization with ethylene oxide

8.72 This method should only be used when no other method is practicable. During process validation it should be shown that there is no damaging effect on the product and that the conditions and time allowed for degassing to reduce any residual ethylene oxide (EO) gas and reaction products to defined acceptable limits for the type of product or material.

8.73 Direct contact between gas and microbial cells is essential; precautions should be taken to avoid the presence of organisms likely to be enclosed in material such as crystals or dried protein. The nature and quantity of packaging materials can significantly affect the process.

8.74 Before exposure to the gas, materials should be brought into equilibrium with the humidity and temperature required by the process. The time required for this should be balanced against the opposing need to minimize the time before sterilization.

8.75 Each sterilization cycle should be monitored with suitable biological indicators, using the appropriate number of test pieces distributed throughout the load unless parametric release has been authorized by the National Competent Authority.

8.76 Critical process variables that should be considered as part of sterilization process validation and routine monitoring include, but are not limited to: EO gas concentration, relative humidity, temperature and EO gas pressure and exposure time.

8.77 After sterilization, the load should be aerated to allow EO gas and/or its reaction products to desorb from the packaged product to predetermined levels. Aeration can occur within a sterilizer chamber and/or in a separate aeration chamber or aeration room. The aeration phase should be validated as part of the overall EO sterilization process validation.

Filtration of medicinal products which cannot be sterilized in their final container

8.78 If a liquid product cannot be terminally sterilized by a microbiocidal process, it should be sterilized by filtration through a sterile, sterilizing grade filter (with nominal pore size of 0.22 micron (or less) or with at least equivalent micro-organism retaining properties), and subsequently aseptically filled into a previously sterilized container, the selection of the filter used should ensure that it is compatible with the product, see 8.119.. Suitable bioburden reduction and/or sterilizing grade filters may be used at multiple points during the manufacturing process to ensure a low and controlled bioburden of the liquid prior to the primary sterilizing grade filter. Due to the potential additional risks of a sterilizing filtration process as compared to other sterilization processes, a second filtration through a sterile, sterilising grade filter (positioned as per clause 8.15), immediately prior to filling, is advisable

8.79 The selection of components for the filtration system (including air, gas and vent filters) and their interconnection and arrangement within the filtration system, including pre-filters, should be based on the critical quality attributes of the products, documented and justified. The filtration system should not generate fibres, unacceptable levels of impurities or otherwise alter the quality and efficacy of the product. Similarly, the filter characteristics should not be adversely affected by the product to be filtered. Adsorption of product components and extraction/leaching of filter components should be evaluated (see Single-Use-Systems, Clauses 8.117-8.119).

8.80 The filtration system should be designed to:

a) Allow operation within validated process parameters.

b) Maintain the sterility of the filtrate.

c) Minimise the number of aseptic connections required between the sterilizing filter and the final filling of the product.

d) Allow cleaning procedures to be conducted as necessary.

e) Allow sterilization procedures, including SIP, to be conducted as necessary. The sterilization procedures should be validated to ensure achievement of a target sterilization assurance level (SAL) of 10^{-6} or better (e.g. 10^{-7}).

f) Permit in-place integrity testing, preferably as a closed system, prior to filtration as necessary. In-place integrity testing methods should be selected to avoid any adverse impact on the quality of the product.

8.81 Liquid-sterilizing filtration should be validated during initial processvalidation. Validation can be grouped by different strengths or variations of a product, but should be done under worst-case conditions. The rational for grouping fluids should be justified and documented.

8.82 Wherever possible, the product to be filtered should be used for bacterial retention testing. Where the product to be filtered is not suitable for use in bacterial retention testing, a suitable surrogate product should be justified for use in the test. The challenge organism used in the bacterial retention test should be justified.

8.83 Filtration parameters that should be considered in validation and routine processing should include but are not limited to:

a) If the system is flushed or integrity tested in-situ with a fluid other than the product, then flushing with the product should be part of the process.

b) The wetting fluid used for filter integrity testing based on filter manufacturer's recommendation or the fluid to be filtered. For the latter, the appropriate integrity test value specification should be established.

c) Filtration process conditions including:

   i. Fluid prefiltration holding time and effect on bioburden.

   ii. Filter conditioning, with fluid if necessary.

   iii. Maximum filtration time/total time filter is in contact with fluid.

   iv. Flow rate.

   v. Filtration volume.

   vi. Temperature.

   vii. The time taken to filter a known volume of bulk solution and the pressure difference to be used across the filter. Any significant differences from those validated to those observed during routine manufacturing should be noted and investigated. Results of these checks should be included in the batch record.

8.84 The integrity of the sterilized filter assembly should be verified by testing before use, in case of damage and loss of integrity caused by processing, and should be verified by on line testing immediately after use by an appropriate method such as a bubble point, diffusive flow, water intrusion or pressure hold test. It is recognised that for small batch sizes, this may not be possible; in these cases an alternative approach may be taken as long as a formal risk assessment has been performed and compliance is achieved. There should be written integrity test methods, including acceptance criteria, and failure investigation procedures and justified conditions under which the filter integrity test can be repeated. Results of the integrity tests (including failed and repeated tests) should be included in the batch record.

8.85 The integrity of critical sterile gas and air vent filters in the filter assembly should be verified by testing after use. The integrity of non-critical air or gas vent filters should be confirmed and recorded at appropriate intervals.

8.86 For gas filtration, the avoidance of unintended moistening or wetting of the filter or filter equipment is important. This can be achieved by the use of hydrophobic filters.

8.87 Where serial filtration (one filtration is followed by a subsequent filtration) is a process requirement the filter train is considered to be a sterilizing unit and all sterilizing-grade filters within it should satisfactorily pass integrity testing both before use, in case of damage during processing, and after use.

8.88 Where a redundant sterilizing filter is used, the additional filter does not require post-integrity testing unless the primary sterilizing filter fails, in which case the redundant filter must then satisfactorily pass post-use integrity testing. Bioburden samples should be taken prior to the first filter and the sterilizing filter, systems for taking samples should be designed so as not to introduce contamination.

8.89 Liquid sterilizing filters should be discarded after the processing of a single lot. The same filter should not be used for more than one working day unless such use has been validated.

Form-Fill-Seal

8.90 Form-Fill-Seal (FFS) units include blow moulding from thermoplastic granulate and thermoforming from thermoplastic film typically known as Blow-Fill-Seal (BFS) and Vertical-Form-Fill-Seal (VFFS) respectively. VFFS process is an automated filling process, typically for terminally sterilized processes, that may utilize a single or dual web system which constructs the primary container out of a flat roll of thermoplastic film while simultaneously filling the formed bags with product and sealing the filled bags in a continuous process. All such containers are considered to be sealed by fusion and, as such, fall under the requirement to perform 100% integrity testing.

8.91 Process parameters relating to seal integrity should be validated and appropriately controlled. Critical parameters include, but are not limited to: seal strength, seal uniformity, sealing temperatures, pressures, sealing times and dwell time for filling. Seal strength and uniformity should be monitored routinely.

8.92 Samples of filled containers should be tested for general performance e.g. ease-of-opening, and seal uniformity. Sample size and frequency should be based on the principles of QRM.

Blow-Fill-Seal technology

8.93 Blow-Fill-Seal (BFS) units are purpose built machines in which, in one continuous operation, containers are formed from a thermoplastic granulate, filled and then sealed, all by the one automatic machine, see glossary for full definition.

8.94 Risk management principles should be used to justify the machine's design and operational controls. These controls should be in alignment with the site's contamination control strategy. Aspects to be considered should include (but are not limited to):

a) Determination of the "critical zone" that should be protected from contamination, and its control.

b) Environmental control and monitoring, both of the BFS machine and the background in which it is placed.

c) Integrity testing of the BFS product pathways.

d) Duration of the batch or filling campaign.

e) Control of polymer starting material.

f) Cleaning-in-place and sterilization-in-place of equipment, and air and product pathways.

8.95 Shuttle and Rotary-type equipment used for aseptic production which is fitted with an effective grade A air shower should be installed in at least a grade C environment, provided that grade A/B clothing is used.

8.96 For Shuttle–type equipment, the environment should comply with the viable and non-viable limits at rest and the viable limit only when in operation. The shuttle zone should meet grade A viable limits.

8.97 For Rotary-type equipment the environment should comply with the viable and non-viable limits "at rest". It is not normally possible to perform environmental monitoring within the parison during operation" Monitoring of the background environment should be performed in accordance with risk management principles

8.98 The environmental control and monitoring program should take into consideration the complex gas flow paths generated by the BFS process and the effect of the high heat outputs of the process.

8.99 In addition, for Shuttle-type designs, the area between parison cutting and mould sealing should be covered by a flow of HEPA filtered or sterile air of appropriate quality to provide grade A at the critical zone.

8.100 Blow-Fill-Seal equipment used for the production of products which are terminally sterilized should be installed in at least a grade D environment.

8.101 External particle and microbial contamination of the polymer should be prevented by appropriate design, control, and maintenance of the polymer storage and distribution systems.

8.102 Interventions requiring cessation of filling and/or blowing and sealing and, where required, re-sterilization of the filling machine should be clearly defined and well described in the aseptic filling procedure, and included in the aseptic process simulation (refer clause 9.36).

8.103 Process validation should take into consideration critical operating parameters and variables of the equipment that impact on the quality of the product, e.g. filling speed, extrusion temperature, filling times.

8.104 Samples of filled containers should be tested for general performance e.g. ease-of-opening and wall thickness; sample size and frequency should be based on the principles of QRM.

Lyophilization

8.105 Lyophilization is a critical process step and all activities that can affect the sterility of the product or material need to be regarded as extensions of the aseptic processing of that sterilized product or material. The lyophilization equipment and its processes should be designed so as to ensure product or material sterility is maintained during lyophilization by preventing microbiological and particulate contamination between the filling operation and completion of lyophilization process. All control measures in place should be determined by the site's contamination control strategy.

8.106 The lyophilizer should be sterilized before each load. The lyophilizer should be protected from contamination after sterilization.

8.107 Where there is a closing system for partially closed containers, the surfaces of any equipment protruding into the chamber to effect sealing should also be sterilized.

8.108 Lyophilization trays should be checked to ensure that they are not misshapen and damaged.

8.109 The maximum permitted leakage of air into the lyophilizer should be specified.

8.110 The integrity of the system should be monitored periodically along with consideration of the leak rate test.

8.111 With regard to loading and unloading the lyophilizer:

a) The loading pattern within the lyophilizer should be specified and documented.

b) Transport to the lyophilizer and loading of filled product, or other equipment into the lyophilizer should take place under a grade A environment.

c) Airflow patterns should not be adversely affected by transport devices and venting of the loading zone. Unsealed containers should be maintained under grade A environment.

d) Where seating of the stoppers is not completed prior to opening the lyophilizer chamber, product removed from the lyophilizer should remain under a grade A environment during subsequent handling.

e) Utensils used during transfer to, loading and unloading of, the lyophilizer (such as trays, bags, placing devices, tweezers, etc.) should be subjected to a validated sterilization process.

Closed systems

8.112 Closed systems can be both single use systems (SUS) (i.e. disposable) and fixed systems (such as vessels with fixed pipework). Guidance in this section is equally applicable to both systems.

8.113 The use of closed systems can reduce the risk of both microbial and chemical contamination due to interventions.

8.114 It is critical to ensure the sterility of product contact surfaces of closed systems used for aseptic processing. The design and selection of any closed system used for aseptic processing must ensure maintenance of sterility. Tubing/pipework that is not assembled prior to sterilization should be designed to be connected aseptically, e.g. by intrinsic aseptic connectors or fusion systems.

8.115 Appropriate systems should be in place to assure the integrity of those components used. The manner in which this is conducted should be determined based on QRM principles. Appropriate system integrity tests should be considered when there is a risk of compromising product sterility.

8.116 The background in which closed systems are located will vary. If there is a high risk that the system will not remain integral during processing it should be located in a grade A environment. If the system can be shown to remain integral at every usage then lower grades, including grade D, can be considered.

Single use systems

8.117 Single use systems (SUS) are those technologies used in manufacture of sterile medicinal products which are designed to replace reusable equipment. SUS are typically defined systems made up of components such as bags, filters, tubing, connectors, storage bottles and sensors.

8.118 There are some specific risks associated with SUS which include, but are not limited to:

a) Interaction between the product and product contact surface (adsorption, leachable and extractables).

b) More fragile than fixed reusable systems.

c) Increase in number and complexity of manual operations and connections made.

d) Design of the assembly.

e) Performance of the pre-use integrity testing for sterilizing grade filters. (Refer to clause 8.84.)

f) Integrity testing.

g) Pin-hole and leakage.

h) The potential for compromising the system at the point of opening the outer packaging.

i) Assessment of suppliers of disposable systems (including sterilization of these disposable systems.

j) Risk of particulate contamination.

8.119 The compatibility of materials used for product contact surfaces with the products should be ensured under the process conditions by evaluating e.g. adsorption and reactivity to the product.

8.120 Extractable profile data obtained from the supplier of the components of SUS may be useful to ensure that extractables and leachables from the SUS do not alter the quality of the product. A risk assessment should be conducted for each component to evaluate the applicability of the extractable profile data. For components considered to be at high risk to leachables, including those taking up leachables extensively or those stored for longer periods, an assessment of leachable profile studies, including safety concerns, and should be taken into consideration, as necessary. If applying simulated processing conditions these should accurately reflect the actual processing conditions and be based on a scientific rationale.

8.121 SUS should be designed so as to maintain integrity during the intended operational conditions and duration, especially the structural integrity of the single use components under extreme process and transport conditions such as during freeze and thaw processes. This should include verification that intrinsic aseptic connections (both heat and mechanical) remain integral under these conditions.

8.122 Acceptance procedures should be established and implemented for SUS corresponding to the risks or criticality of the products and its processes. On receipt, a visual inspection of outer packaging (e.g. appearance of exterior carton, product pouches), label printing, and attached documents (e.g. Certificate of Analysis, radiation certificate) should be carried out. Prior to use, each piece of SUS should be checked to ensure that they have been manufactured and delivered in accordance with the approved specification.

8.123 Critical manual handling operation of SUS, such as assembling and connecting, should be subject to appropriate controls and verified during the aseptic process simulation test.

9 Viable and non-viable environment & process monitoring

General

9.1 The site's environmental and process monitoring program forms part of the overall contamination control strategy designed to minimise the risk of microbial and particulate contamination.

9.2 This program is typically comprised of the following elements:

a) Environmental monitoring – non viable.
b) Environmental monitoring – viable.

c) Aseptic process simulation (aseptically manufactured product only).

9.3 These key elements provide information with regards to the process and facility capabilities with respect to the maintenance of sterility assurance. The information from these systems should be used for routine batch release and for periodic assessment during process review or investigations.

Environmental monitoring

9.4 In order to establish a robust environmental monitoring program, i.e. locations, frequency of monitoring and incubation conditions (e.g. time, temperature(s) and aerobic and or anaerobic), appropriate risk assessments should be conducted based on detailed knowledge of the process inputs, the facility, equipment, specific processes, operations involved and knowledge of the typical microbial flora found, consideration of other aspects such as air visualization studies should also be included. These risk assessments should be re-evaluated at defined intervals in order to confirm the effectiveness of the site's environmental monitoring program, and they should be considered in the overall context of the trend analysis and the contamination control strategy for the site.

9.5 Routine monitoring for clean rooms, clean air devices and personnel should be performed "in operation" throughout all critical stages, including equipment set up. The locations, frequency, volume and duration of monitoring should be determined based on the risk assessment and the results obtained during the qualification.
9.6 Monitoring should also be performed outside of operations within the area, e.g. pre disinfection, post disinfection, prior to start of manufacturing and after a shutdown period etc., in order to detect potential incidents of contamination which may affect the controls within the areas. The number of samples and frequency of monitoring should be considered in the context of the risk assessments and contamination control strategy.

9.7 For grade A monitoring, it is important that sampling should be performed at locations posing the highest risk of contamination to the sterile equipment surfaces, container-closures and product in order to evaluate maintenance of aseptic conditions during critical operations.

9.8 Appropriate alert and action limits should be set for the results of particulate and microbiological monitoring. Alert levels should be established based on results of Performance Qualification (PQ) tests or trend data and should be subject to periodic review.

9.9 The alert limits for grade B, c and D should be set based on the area performance, with the aim to have limits lower than those specified as action limits, in order to minimise risks associated and identify potential changes that may be detrimental to the process.

9.10 If action limits are exceeded operating procedures should prescribe a root-cause investigation followed by corrective and preventive action. If alert limits are exceeded, operating procedures should prescribe scrutiny and follow-up, which might include investigation and corrective action.
9.11 Surfaces and personnel should be monitored after critical operations. Results from monitoring should be considered when reviewing batch documentation for finished product release.

Non-viable monitoring

9.12 Non-viable particle monitoring systems should be established to obtain data for assessing potential contamination risks and to maintain the environment for sterile operations in the qualified state.

9.13 The recommended limits for airborne particle concentration in monitoring for each grade are given in Table 5.

Table 5: **Recommended limits for airborne particle concentration for the monitoring of non-viable contamination**

Grade	**Recommended maximum limits for particles ≧ 0.5 μm/m^3**		**Recommended maximum limits for particles ≧ 5 μm/m^3**	
	in operation	at rest	in operation	at rest
A	3 520	3 520	20	20
B	352 000	3 520	2 900	29
C	3 520 000	352 000	29 000	2 900
D	Set a limit based on the risk assessment	3 520 000	Set a limit based on the risk assessment	29 000

Note 1: The particle limits given in the table for the “at rest” state should be achieved after a short “clean up” period defined during qualification in an unmanned state after the completion of operations (see 5.26e).

Note 2: With regards to the monitoring of 5.0 μm, the limit of 20 is selected due to the limitations of monitoring equipment. It should be noted that alert limits should also be set based on historical and qualification data, such that frequent sustained recoveries below the action limit should also trigger an investigation.

9.14 For grade A zones, particle monitoring should be undertaken for the full duration of critical processing, including equipment assembly.

9.15 The grade A zone should be monitored continuously and with a suitable sample size (at least 28 litres (a cubic foot) per minute) so that all interventions, transient events and any system deterioration would be captured and alarms triggered if alert limits are exceeded.

9.16 It is recommended that a similar system be used for grade B zones although the sample frequency may be decreased. The design of the monitoring system should be based on risk assessment and be commensurate with the risk of the process to the product sterility assurance. The grade B zone should be monitored at such a frequency and with suitable sample sizes that the programme captures any change in levels of contamination and system deterioration. If alert limits are exceeded, alarms should be triggered.

9.17 The monitoring of grade C and D areas in operation should be performed in accordance with the principles of QRM to provide sufficient data to allow effective trend analysis. The requirements and alert/action limits will depend on the nature of the operations carried out.

9.18 The selection of the monitoring system should take account of any risk presented by the materials used in the manufacturing operation, for example those involving live organisms or radiopharmaceuticals that may give rise to biological or chemical hazards.

9.19 In the case where contaminants present due to the processes involved would damage the particle counter or present a hazard, e.g. live organisms and radiological hazards, the frequency and strategy employed should be such as to assure the environment classification both prior to and post exposure to the risk. Additionally, monitoring should be performed during simulated operations. Such operations should be performed at appropriately defined intervals. The approach should be defined in the contamination control strategy.

9.20 Where powdery products are manufactured, monitoring of particles may have to take into consideration an alternative monitoring scheme and frequency, e.g. monitoring for particle levels prior to and after the manufacturing process step.

9.21 The sample sizes taken for monitoring purposes using automated systems will usually be a function of the sampling rate of the system used. It is not necessary for the sample volume to be the same as that used for formal qualification of clean rooms and clean air devices.

9.22 Although monitoring of ≧ 5.0 µm particles are not required for room qualification and classification purposes, it is required for routine monitoring purposes as they are an important diagnostic tool for early detection of machine, equipment and HVAC failure.

9.23 The occasional indication of macro particle counts, especially ≧ 5.0 µm, may be considered false counts due to electronic noise, stray light, coincidence, etc. However, consecutive or regular counting of low levels may be indicative of a possible contamination event and should be investigated. Such events may indicate early failure of the room air supply filtration (HVAC) system, filling equipment failure, or may also be diagnostic of poor practices during machine set-up and routine operation.

9.24 Monitoring conditions such as frequency, sampling volume or duration, alert and action limits and corrective action including investigation should be established in each manufacturing area based on risk assessment.

Viable monitoring

9.25 Where aseptic operations are performed, microbiological monitoring should be frequent using a combination of methods such as settle plates, volumetric air, glove print and surface sampling (e.g. swabs and contact plates).

9.26 Monitoring should include sampling of personnel at periodic intervals during the process. Particular consideration should be given to monitoring personnel following involvement in critical interventions and on exit from the grade A/B processing area.

9.27 Continuous monitoring in grade A and B areas should be undertaken for the full duration of critical processing, including equipment (aseptic set up) assembly and filling operations (i.e., an understanding of function and interactions of each clean area). The monitoring should be performed in such a way that all interventions, transient events and any system deterioration would be captured and any risk caused by interventions of the monitoring operations is avoided.

9.28 Rapid microbial monitoring methods may be adopted after validation as long as they are demonstrated to be at least equivalent to the established methodology.

9.29 Sampling methods should not pose a risk of contamination to the manufacturing operations.

9.30 Additional microbiological monitoring should also be performed outside production operations, e.g. after validation of systems, cleaning and disinfection.

9.31 Recommended action limits for microbial contamination are shown in Table 6

Table 6: **Recommended maximum limits for microbial contamination**

Grade	**Air sample cfu/m^3**	**Settle plates (diam. 90 mm) cfu/4 hours [(a)]**	**Contact plates (diam. 55mm), cfu/ plate**	**Glove print 5 fingers on both hands cfu/ glove**
A[(b)]	1	1	1	1
B	10	5	5	5
C	100	50	25	-
D	200	100	50	-

[(a)] Individual settle plates may be exposed for less than 4 hours. Where settle plates are exposed for less than 4 hours the limits in the table should still be used. Settle plates should be exposed for the duration of critical operations and changed as required after 4 hours.

[(b)] It should be noted that for grade A the expected result should be 0 cfu recovered; any recovery of 1 cfu or greater should result in an investigation.

9.32 Monitoring procedures should define the approach to trending. Trends can include but are not limited to:

a) Increasing numbers of action or alert limit breaches.

b) Consecutive breaches or alert limits.

c) Regular but isolated breaches of limits that may have a common cause, for example single excursions that always follow planned preventative maintenance.

d) Changes in flora type and numbers.

9.33 If microorganisms are detected in a grade A or B zone, they should be identified to species level and the impact of such microorganisms on product quality (for each batch implicated) and state of control should be evaluated. Consideration may also be given to the identification of grade C and D contaminants and the requirements should be defined in the contamination control strategy.

Aseptic process simulation (APS)[1]

9.34 Periodic verification of the effectiveness of the controls in place for aseptic processing should include a process simulation test using a sterile nutrient media and/or placebo. Selection of an appropriate nutrient media should be made based on the ability of the media to imitate product characteristics at all processing stages. Where processing stages may indirectly impact the viability of any introduced microbial contamination, (e.g. sterile aseptically produced semi-solids, powders, solid materials, microspheres, liposomes and other formulations where product is cooled or heated or lyophilized, etc.), alternative surrogate procedures that represent the operations as closely as possible can be developed and justified. Where surrogate materials, such as buffers, are used in parts of the process simulation, the surrogate material should not inhibit the growth of any potential contamination.

9.35 The process simulation test should imitate as closely as possible the routine aseptic manufacturing process and include all the critical manufacturing steps. Specifically:

a) Process simulation tests should assess all aseptic operations performed subsequent to the sterilisation of materials utilised in the process.

b) For non-filterable formulations any additional aseptic steps should be assessed.

c) Aseptic manufacturing performed in a strict anaerobic environment should be evaluated with an anaerobic media in addition to aerobic evaluation.

d) Processes requiring the addition of sterile powders should employ an acceptable surrogate material in containers identical to those utilised in the process being evaluated.

e) Processes involving blending, milling and subdivision of a sterile powder require similar attention.

---

[1] For further details on the validation of aseptic processing, please refer to the PIC/S Recommendation on the Validation of Aseptic Processing (PI 007) For PICS version only

f) The process simulation test for lyophilized products should include the entire aseptic processing chain, including filling, transport, loading, chamber dwell, unloading and sealing. The process simulation should duplicate the lyophilization process, with the exception of freezing and sublimation, including partial vacuum and cycle duration and parameters as appropriate for the media. Boiling over or actual freezing of the solution should be avoided.

9.36 The process simulation testing should take into account various aseptic manipulations and interventions known to occur during normal production as well as worst-case situations, including:

a) Inherent interventions at the maximum accepted frequency per number of filled units.
b) Corrective interventions in representative number and with the highest degree of intrusion acceptable.

9.37 There should be an approved list of allowed interventions, both inherent and corrective, which may occur during production and in the APS. The procedures listing the types of inherent and corrective interventions, and how to perform them, should be updated, as necessary, to ensure consistency with the actual manufacturing activities.

9.38 In developing the process simulation test plan, risk management principles should be used and consideration should be given to the following:

a) Identification of worst case conditions covering the relevant variables and their microbiological impact on the process. The outcome of the assessment should justify the variables selected.

b) Determining the representative sizes of container/closure combinations to be used for validation. Bracketing or a matrix approach can be considered for initial validation of the same container/closure configuration.

c) The volume filled per container, which should be sufficient to ensure that the media contacts all equipment and component surfaces that may directly contaminate the sterile product.

d) Maximum permitted holding times for sterile product and associated sterile components exposed during the aseptic process.

e) Ensuring that any contamination is detectable.

f) The requirement for substitution of any inert gas used in the routine aseptic manufacturing process by air, unless anaerobic simulation is intended.

g) The duration of the process simulation filling run to ensure it is conducted over the maximum permitted filling time. If this is not possible, then the run should be of sufficient duration to challenge the process, the operators that perform interventions, and the capability of the processing environment to provide appropriate conditions for the manufacture of a sterile product.

h) Simulating normal aseptic manufacturing interruptions where the process is idle. In these cases, environmental monitoring should be conducted to ensure that grade A conditions have been maintained.

i) The special requirements and considerations for manually intensive operations.

j) Where campaign manufacturing occurs, such as in the use of barrier technologies or manufacture of sterile active substances, consideration should be given to designing and performing the process simulation so that it simulates the risks associated with both the beginning and the end of the campaign and demonstrating that the campaign duration does not pose any risk. If end of production campaign APS are used, then it should be demonstrated that any residual product does not negatively impact the recovery of any potential microbiological contamination.

k) Where barrier technologies (RABS, isolators, BFS, etc.) are used in the routine aseptic manufacturing process, the relative risk and unique aspects of these technologies should be taken into consideration when assessing the design of aseptic process simulation tests.

9.39 For sterile active substances, batch sizes should be large enough to represent routine operation, simulate intervention operation at the worst case, and cover potential contact surfaces. In addition, all the simulated materials (surrogates of growth medium) should be subjected to microbiological evaluation. The recovery rate from simulation materials should be sufficient to satisfy the evaluation of the process being simulated and should not compromise the recovery of micro-organisms.

9.40 Process simulation tests should be performed as initial validation, generally with three consecutive satisfactory simulation tests per shift, and after any significant modification to the HVAC system, equipment, major facility shut down, process and number of shifts, etc. Normally process simulation tests (periodic revalidation) should be repeated twice a year (approximately every six months) for each aseptic process and filling line, and at least annually for each operator. Consideration should be given to performing an APS after the last batch prior to shut down, before long periods of inactivity or before decommissioning or relocation of a line.

9.41 Where manual filling occurs, each product, container closure, equipment train and operator should be revalidated approximately every 6 months. The APS batch size should mimic that used in the routine aseptic manufacturing process. An aseptic process or filling should be subject to a repeat of the initial validation when:

a) Revalidation of the unique process has failed and corrective actions have been taken.

b) The specific aseptic process has not been in operation for an extended period of time..

c) A change to the process, equipment, personnel, procedures or environment that has potential to affect the aseptic process or the addition of new product containers or container-closure combinations.

9.42 The number of units processed (filled) for process simulation tests should be sufficient to effectively simulate all activities that are representative of the aseptic manufacturing process; justification for the number of units to be filled should be clearly captured in the PQS. For small batches, e.g. those under 5,000 units filled, the number of containers for media fills should at least equal the size of the production batch.

9.43 The target should be zero growth and any contaminated unit should result in an investigation (refer to clause 9.47) to determine the root cause (if possible) and to identify appropriate CAPA. Following implementation of CAPA, a repeat APS will be required to validate the effectiveness of the CAPA. The number of APS to be repeated should be determined using QRM principles taking into consideration the number and type of CAPA and the level of contamination found in the failed APS. Typically 3 successful consecutive repeat APS would be expected; any differences to this expectation should be clearly justified prior to repeat performance.

9.44 Filled APS units should be agitated, swirled or inverted before incubation to ensure contact of the media with all interior surfaces in the container. Cosmetic defects, non-destructive weight checks and all other units should be identified and incubated with the other units. Units discarded during the process simulation and not incubated should be comparable to units discarded during a routine fill.

9.45 Filled APS units should be incubated in a clear container to ensure visual detection of microbial growth. Microorganisms isolated from contaminated units should be identified to at least the genus, and to the species level when practical, to assist in the determination of the likely source of the contaminant. The selection of the incubation duration and temperature should be justified and appropriate for the process being simulated and the selected growth medium.

9.46 All products that have been manufactured on a line subsequent to the process simulation should be quarantined until a successful resolution of the process simulation has occurred.

9.47 In the case of a failed process simulation there should be a prompt review of all appropriate records relating to aseptic production since the last successful process simulation. The outcome of the review should include a risk assessment of the non-sterility for batches manufactured since the last successful process simulation, and the justification for the disposition of batches of product affected. Subsequent to a failed APS, in addition to a full investigation, production should resume only upon further successful APS unless adequately justified. The number of repeat successful APS prior to resuming production should also be justified.

9.48 Where results indicate that an operator may have failed qualification, actions to restrict entry of the operator to the aseptic processing areas should be taken.

9.49 All process simulation runs should be fully documented and include a reconciliation of units processed and changes in the custody of the APS batch. All interventions performed during the process simulations should be recorded, including the start and end of each intervention.

10 Quality Control (QC)

10.1 Microbiological contamination of starting materials should be minimal. Specifications should include requirements for microbiological quality when the need for this has been indicated by monitoring and/orby the contamination control strategy.

10.2 The bioburden assay should be performed on each batch for both aseptically filled product and terminally sterilized products and the results considered as part of the final batch review. There should be working limits on contamination immediately before sterilization, which are related to the efficiency of the method to be used.

10.3 Where overkill sterilization parameters are set for terminally sterilized products, bioburden should be monitored at suitable scheduled intervals.

10.4 For parametric release systems, the bioburden assay should be performed on each batch and considered as an in-process test. Where appropriate, the level of endotoxins should be monitored.

10.5 The sterility test applied to the finished product should only be regarded as the last in a series of control measures by which sterility is assured. The test should be validated for the product(s) concerned.

10.6 The sterility test should be performed under aseptic conditions, which are at least consistent with the standard of clean room required for the aseptic manufacture of pharmaceutical products.

10.7 Samples taken for sterility testing should be representative of the whole of the batch, but should in particular include samples taken from parts of the batch considered to be most at risk of contamination, for example:

a) Products which have been filled aseptically, samples should include containers filled at the beginning and end of the batch and after any significant intervention.

b) Products which have been heat sterilized in their final containers, consideration should be given to taking samples from the potentially coolest part of the load.

c) Each sterilized load should be considered as different batches and require a separate sterility test.

d) Products that have been lyophilized in different lyophilization loads..

Note: Where sterilization or lyophilization leads to separate sterility tests, consideration of performing separate testing for other finished product tests should also be given.

10.8 Any process (e.g. VHP) used to decontaminate sterility samples prior to testing should not negatively impact the sensitivity of the test method.

10.9 Media used for environmental monitoring and APS should be tested for its growth promotion capability, in accordance with a formal written program.

10.10 Environmental monitoring data generated in grade A and B areas should be reviewed as part of product batch release. A written plan should be available that describes the actions to be taken when data from environmental monitoring are found out of trend or out of specification.

10.11 The use of rapid microbial methods can also be considered. These methods should be validated for the product(s) or processes concerned and be approved in the registered product testing specification.

11 Glossary

Air lock - A small room with interlocked doors, constructed to maintain air pressure control between adjoining rooms (generally with different air cleanliness standards). The intent of an aseptic processing airlock is to preclude ingress of particulate matter and microorganism contamination from a lesser controlled area.

Alert Level - An established microbial or airborne particle level giving early warning of potential drift from normal operating conditions and triggers appropriate scrutiny and follow-up to address the potential problem. Alert levels are always lower than action levels and are established based on historical and qualification trend data and periodically reviewed.

Action Level - An established microbial or airborne particle level that, when exceeded, should trigger appropriate investigation and corrective action based on the investigation.

Aseptic Manufacturing Area - The classified part of a facility that includes the aseptic processing room and ancillary cleanrooms. For purposes of this document, this term is synonymous with “aseptic processing facility”.

Aseptic Processing Facility - A building, or segregated segment of it, containing cleanrooms in which air supply, materials, and equipment are regulated to control microbial and particle contamination.

Aseptic Processing Room - A room in which one or more aseptic activities or processes are performed.

Asepsis - A state of control attained by using an aseptic work area and performing activities in a manner that precludes microbiological contamination of the exposed sterile product.

Bacterial retention testing – This test is performed to validate that a filter can remove bacteria from a gas or solution. The test is usually performed using a standard organism, such as *Brevundimonas diminuta* at a minimum concentration of 10^7 Colony Forming Units/ml.

Bioburden - The total number of microorganisms associated with a specific item prior to sterilization.

Barrier - A physical partition that affords aseptic processing area (grade A) protection by partially separating it from the surrounding area such as RABS or isolators.

Biological Indicator (BI) - A population of microorganisms inoculated onto a suitable medium (e.g. solution, container or closure) and placed within appropriate sterilizer load locations to determine the sterilization cycle efficacy of a physical or chemical process. The challenge microorganism is selected based upon its resistance to the given process. Incoming lot D-value and microbiological count define the quality of the BI.

Blow-Fill-Seal - Blow-Fill-Seal (BFS) technology is a pharmaceutical filling process in which containers are formed from a thermoplastic granulate, filled with product, and then sealed in a continuous, integrated, automatic operation. The two most common types of BFS machines are the Shuttling machine (with Parison cut) and the Rotary machine (Closed

Parison) types. The equipment design, operation, and therefore controls for these differ. For Shuttling systems the processes of container extrusion and filling occur at two separate locations within the machine. The extrusion of the container parison occurs adjacent to the filling zone, the extruded plastic is collected from underneath the extruder head, is cut and formed and automatically transferred (usually by horizontal shuttling) to the filling and sealing zone. For Rotary design machines the filling needles are enclosed within the extruded parison and therefore there is limited exposure of the inner surfaces of the container to the external environment.

Clean Area - An area with defined particle and microbiological cleanliness standards.

Cleanroom - A room designed, maintained, and controlled to prevent particle and microbiological contamination of drug products. Such a room is assigned and reproducibly meets an appropriate air cleanliness classification.

Clean Non Classified (CNC) area - An area that does not meet any of the formal pre-determined grades of cleanliness included in the Annex, i.e. grades A to D, but where a manufacturer defined level of microbial control is still required. The area should be subject to a formal cleaning/disinfection regime and formal environmental monitoring program to achieve the defined level of control. The level, type and frequency of both the cleaning program and the environmental monitoring program (including contamination limits) should be based on a formal risk assessment (captured within the wider contamination control strategy) and should be commensurate with the specific risks to the processes and product performed manufactured within each CNC area.

It is possible that different CNC areas within the same facility may have different approaches to control and monitoring, based on differing risks to processes and products.

Clean Zone - See Clean Area.

Closed system – A system in which the sterile product is not exposed to the surrounding environment.

Colony Forming Unit (cfu) - A microbiological term that describes the formation of a single macroscopic colony after the introduction of one or more microorganisms to microbiological growth media. One colony forming unit is expressed as 1 cfu.

Commissioning – Activities to verify that equipment and systems are installed according to specification

Component - Any ingredient intended for use in the manufacture of a drug product, including those that may not appear in the final drug product.

Critical Area - An area designed to maintain sterility of sterile materials. Sterilized product, containers, closures, and equipment may be exposed in critical areas such as the grade A area or a closed system.

Critical surfaces - Surfaces that may come into contact with, or directly affect, a sterilized product or its containers or closures. Critical surfaces are rendered sterile prior to the start of the manufacturing operation, and sterility is maintained throughout processing.

Critical zone – See critical area

D value - The time (in minutes) of exposure at a given temperature that causes a one-log or 90 per cent reduction in the population of a specific microorganism.

Deadleg – length of pipe that is not part of the circuit that is greater than 3 internal pipe diameters

Decontamination - A process that eliminates viable bioburden via use of chemical agents.

Depyrogenation - A process used to destroy or remove pyrogens (e.g. endotoxin).

Disinfection – The process by which surface bioburden is reduced to a safe level or eliminated. Some disinfection agents are effective only against vegetative microbes, while others possess additional capability to effectively kill bacterial and fungal spores.

Dynamic - Conditions relating to clean area classification under normal production operations.

Endotoxin - A pyrogenic product (e.g. lipopolysaccharide) present in the bacterial cell wall. Endotoxin can lead to reactions in patients receiving injections ranging from fever to death.

Extractables - Chemical entities that migrate from the surface of the process equipment contacting with model solvents under appropriate testing conditions (e.g. kind of solvent, temperature) that exceed "worst case" process conditions.

Form Fill seal – Similar to Blow fill Seal, this involves the formation of a large tube formed from a flexible packaging material, in the filling machine, the tube is then filled to form large volume bags.

Gowning Qualification - A program that establishes, both initially and on a periodic basis, the capability of an individual to don the complete sterile gown in an aseptic manner.

Grade A air – Air which is passed through a filter qualified as capable of producing grade A non-viable quality air, but where there is no requirement to continuously perform non-viable monitoring or meet grade A viable monitoring limits.

HEPA filter - High efficiency particulate air filter with minimum 0.3 µm particle retaining efficiency of 99.97 percent.

HVAC - Heating, ventilation, and air conditioning.

Intervention - An aseptic manipulation or activity that occurs at the critical area.

Intrinsic sterile connection device - A device that removes the risk of contamination during the connection process; these can be mechanical or fusion devices.

Isokinetic sampling head – A sampling head designed to disturb the air as little as possible so that the same particles go into the nozzle as would have passed the area of the nozzle had it not been there.

Isolator - A decontaminated unit supplied with grade A (ISO 5) or higher air quality that provides uncompromised, continuous isolation of its interior from the external environment (e.g., surrounding cleanroom air and personnel). There are two major types of isolators:

Closed isolator systems exclude external contamination from the isolator's interior by accomplishing material transfer via aseptic connection to auxiliary equipment, rather than use of openings to the surrounding environment. Closed systems remain sealed throughout operations.

Open isolator systems are designed to allow for the continuous or semi-continuous ingress and/or egress of materials during operations through one or more openings. Openings are engineered (e.g., using continuous overpressure) to exclude the entry of external contamination into the isolator.

Laminar flow - An airflow moving in a single direction and in parallel layers at constant velocity from the beginning to the end of a straight line vector.

Leachables - Chemical entities that migrate into medicinal products from the product contact surface of the process equipment under actual product and process conditions.

Lyophilization A physical-chemical drying process designed to remove solvents from both aqueous and non-aqueous systems, primarily to achieve product or material stability. Lyophilization is synonymous to the term freeze-drying.

Manual Filling –Where the product is transferred into the final container by systems where operator intervention is required to complete the filling of each container e.g. pipetting liquids.

Operator - Any individual participating in the aseptic processing operation, including line set-up, filler, maintenance, or other personnel associated with aseptic line activities.

Overkill sterilization process - A process that is sufficient to provide at least a 12 log reduction of microorganisms having a minimum D value of 1 minute.

Pass through hatch – refer to airlock.

Pyrogen - A substance that induces a febrile reaction in a patient.

Qualification - Establishing documented evidence that provides a high degree of assurance that equipment or facilities will perform to the required specification detailed in the user requirement specification and the design qualification.

Restricted Access Barrier System (RABS) - A restricted access barrier system (RABS) provides an enclosed, but not closed, environment meeting defined cleanroom conditions using a rigid-wall enclosure and air overspill to separate its interior from the surrounding environment.

Active RABS: integral HEPA-filtered air supply

Passive RABS: air supply by ceiling mounted HEPA-filters.

Open RABS. Where there are vents in the barrier that allow air to move from the grade A to the grade B area.

Sterile Product - For purposes of this guidance, sterile product refers to one or more of the elements exposed to aseptic conditions and ultimately making up the sterile finished drug product. These elements include the containers, closures, and components of the finished drug product.

Sterilizing grade filter - A filter that, when appropriately validated, will remove a defined microbial challenge from a fluid stream, producing a sterile effluent.

Single Use Systems (SUS) - Systems in which some product contact components are used only once (i.e. single use components) to replace reusable equipment such as stainless steel transfer lines or bulk containers. SUS covered in this document are those that are used in manufacturing processes of sterile medicinal products (e.g. sterile API, sterile bio bulk, sterile finish dosage), and are typically made up of components such as bags, filters, tubing, connectors, storage bottles and sensors.

Terminal sterilization - The application of a lethal sterilizing agent to finished product within a sealed container to achieve a predetermined sterility assurance level (SAL) of 10^{-6} or better (i.e. the theoretical probability of there being a single viable microorganism present on or in a sterilized unit is equal to or less than 1×10^{-6} (one in a million)).

ULPA filter - Ultra-low penetration air filter with minimum 0.3 µm particle retaining efficiency of 99.999 per cent.

Unidirectional flow - An airflow moving in a single direction, in a robust and uniform manner, and at sufficient speed, to reproducibly sweep particles away from the critical processing or testing area.

Validation - Establishing documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes.

Worst case - A set of conditions encompassing upper and lower processing limits and circumstances, including those within standard operating procedures, that pose the greatest chance of process or product failure (when compared to ideal conditions). Such conditions do not necessarily induce product or process failure.