ANNEX

to Decision of the Eurasian Economic
Commission's Council
No. _____ dated _____, 20___

A M E N D M E N T S
to the Good Manufacturing Practice of the Eurasian Economic Union

Annex No. 1 to the above Rules shall read as follows:

"Annex No. 1
to the Good Manufacturing Practice of the
Eurasian Economic Union

MANUFACTURE OF STERILE PRODUCTS

1. Scope

Sterile product manufacturing covers a wide range of sterile product types (active substance, excipient, primary packaging material and finished dosage form), packed sizes (single unit to multiple units), processes (from highly automated systems to manual processes) and technologies (for example, biotechnology, classical small molecule manufacturing systems and closed systems). This Annex provides general guidance that should be used in the design and control of facilities, equipment, systems and procedures used for the manufacture of all sterile products applying the principles of Quality Risk Management (QRM), to ensure that microbial, particulate and endotoxin/pyrogen contamination is prevented in the final product.

Quality Risk Management applies this document in its entirety and will not, normally, be referred to in specific paragraphs. Where specific limits or

frequencies or ranges are specified, these should be considered as a minimum requirement. They are stated due to historical regulatory experience with respect to issues that have been identified and have impacted the safety of patients.

The intent of the Annex is to provide guidance for the manufacture of sterile products. However, some of the principles and guidance, such as contamination control strategy, design of premises, cleanroom classification, qualification, validation, monitoring and personnel 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 and reduction of microbial, particulate and endotoxin/pyrogen contamination is considered important. If a manufacturer elects to apply guidance herein to non-sterile products, the manufacturer should clearly document which principles have been applied and acknowledge that compliance with those principles should be demonstrated.

2. Principle

2.1. The manufacture of sterile products is subject to special requirements in order to minimize risks of microbial, particulate and endotoxin/pyrogen contamination. The following key areas should be taken into account:

i. Facility, equipment and process should be appropriately designed, qualified and/or validated and where applicable, subjected to ongoing verification according to the relevant sections of the Eurasian Economic Union's Good Manufacturing Practice Rules approved by Decision No. 77 of the Eurasian Economic Commission's Council dated November 3, 2016 (hereinafter, the Rules).

ii. The use of appropriate technologies (for example, Restricted Access Barriers Systems (RABS), isolators, robotic systems, rapid/alternative methods and continuous monitoring systems) should be considered to increase the protection of the product from potential extraneous sources of endotoxin/pyrogen, particulate and microbial contamination such as personnel, materials and the surrounding environment, and assist in the rapid detection of potential contaminants in the environment and the product.

iii. Personnel shall have adequate qualifications and experience, training and behavior with a specific focus on the principles involved in the protection of sterile product during the manufacturing, packaging and distribution processes.

iv. Processes and monitoring systems for sterile product manufacture should be designed, commissioned, qualified, monitored and regularly reviewed by personnel with appropriate process, engineering and microbiological knowledge.

v. Raw materials and packaging materials should be adequately controlled and tested to ensure that level of bioburden and endotoxin/pyrogen are suitable for use.

2.2. Processes, equipment, facilities and manufacturing activities should be managed in accordance with Quality Risk Management principles to provide a preventive (proactive) means of identifying, scientifically evaluating and controlling potential risks to quality. Where alternative approaches are used, these should be supported by appropriate rationale, risk assessment and mitigation, and should meet the intent of this Annex.

Quality Risk Management priorities should first and foremost include appropriate design of the facility, equipment and processes, followed by the implementation of well-designed procedures, and finally application of monitoring systems as the element that demonstrates that the design and

procedures have been correctly implemented and continue to perform in line with expectations. Monitoring or testing alone does not give assurance of sterility.

2.3. A Contamination Control Strategy (CCS) should be implemented across the facility in order to define all critical control points and assess the effectiveness of all the controls (design, procedural, technical and organizational) and monitoring measures employed to manage risks to medicinal product quality and safety. The combined strategy of the contamination control strategy should establish robust assurance of contamination prevention. The contamination control strategy should be actively reviewed and, where appropriate, updated and should drive continual improvement of the manufacturing and control methods. Its efficiency should form part of the periodic management review. Where existing control systems are in place and are appropriately managed, these may not require replacement but should be referenced in the contamination control strategy and the associated interactions between systems should be understood.

2.4. Contamination control and steps taken to minimize the risk of contamination from microbial, endotoxin/pyrogen and particle sources includes a series of interrelated events and measures. These are typically assessed, controlled and monitored individually but their collective effectiveness should be considered together.

2.5. The development of the contamination control strategy requires detailed technical and process knowledge. Potential sources of contamination are attributable to microbial and cellular debris (e.g. pyrogen, endotoxin) as well as particulate (e.g. glass and other visible and sub-visible particles).

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

i. Design of both the plant and processes including the associated documentation;

ii. Premises and equipment;

iii. Personnel;

iv. Utilities;

v. Raw material controls — including in-process controls;

vi. Product containers and closures;

vii. Vendor approval (e.g. key component suppliers, sterilization of components and single use systems), and critical service providers;

viii. Management of outsourced activities and availability/transfer of critical information between parties, e.g. contract sterilization services.

ix. Process risk management;

x. Process validation;

xi. Validation of sterilization processes;

xii. Preventative maintenance — maintaining equipment, utilities and premises (planned and unplanned maintenance) to a standard that will ensure there is no additional risk of contamination;

xiii. Cleaning and disinfection;

xiv. Monitoring systems — including an assessment of the feasibility of the introduction of scientifically sound, alternative methods that optimize the detection of environmental contamination;

xv. Prevention mechanisms — trend analysis, detailed investigation, root cause determination, corrective and preventive actions (CAPA) and the need for comprehensive investigational tools;

xvi. Continuous improvement based on information derived from the above.

2.6. The contamination control strategy should consider all aspects of contamination control with ongoing and periodic review resulting in updates

within the pharmaceutical quality system as appropriate. Changes to the systems in place should be assessed for any impact on the contamination control strategy before and after implementation.

2.7. 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 should not be placed on any terminal process or finished product test.

3. Pharmaceutical Quality System (PQS)

3.1. The manufacture of sterile products is a complex activity that requires specific controls and measures to ensure the quality of products manufactured. Accordingly, the manufacturer's PQS should encompass and address the specific requirements of sterile product manufacture and ensure that all activities are effectively controlled so that the risk of microbial, particulate and endotoxin/pyrogen contamination is minimized in sterile products. In addition to the PQS requirements detailed in Chapter 1 of the Rules, the pharmaceutical quality system for sterile product manufacture should also ensure that:

i. An effective risk management system is integrated into all areas of the product life cycle with the aim to minimize microbial contamination and to ensure the quality of sterile products manufactured.

ii. The manufacturer has sufficient knowledge and expertise in relation to the products manufactured and the equipment, engineering and manufacturing methods employed that have an impact on product quality.

iii. Root cause analysis of procedural, process or equipment failure is performed in such a way that the risk to product is correctly identified and understood so that suitable corrective and preventive actions (CAPA) are implemented.

iv. Risk management is applied in the development and maintenance of the contamination control strategy, to identify, assess, reduce/eliminate (where applicable) and control contamination risks. Risk management should be documented and should include the rationale for decisions taken in relation to risk reduction and acceptance of residual risk.

v. Senior management should effectively oversee the state of control throughout the facility and product life cycle. The results of risk management should be reviewed regularly as part of the ongoing quality management, during change, in the event of a significant emerging problem, and during the periodic product quality review.

vi. Processes associated with the finishing, storage and transport of sterile products should not compromise the sterile product. Aspects that should be considered include: container/closure integrity, risks of contamination and avoidance of degradation by ensuring that products are stored and maintained in accordance with the registered storage conditions.

vii. Persons responsible for the certification/release of sterile products have appropriate access to manufacturing and quality information and possess adequate knowledge and experience in the manufacture of sterile products and the associated critical quality attributes. This is in order to allow such persons to determine if the sterile products have been manufactured in accordance with the registered specifications and approved process and are of the required quality.

3.2. All non-conformities, such as sterility test failures, environmental monitoring excursions or deviations from established procedures should be adequately investigated before certification/release of the batch. The investigation should determine the potential impact upon process and product quality and whether any other processes or batches are potentially impacted.

The reason for including or excluding a product or batch from the scope of the investigation should be clearly justified and recorded.

4. Premises

4.1. The manufacture of sterile products should be carried out in appropriate cleanrooms, entry to which should be through change rooms that act as airlocks for personnel and airlocks for equipment and materials. Cleanrooms and change rooms should be maintained to an appropriate cleanliness standard and supplied with air that has passed through filters of an appropriate efficiency. Controls and monitoring should be scientifically justified and should effectively evaluate the state of environmental conditions of cleanrooms, airlocks and pass-through hatches.

4.2. The various operations of component preparation, product preparation and filling should be carried out with appropriate technical and operational separation measures within the cleanroom or facility to prevent mix up and contamination.

4.3. Restricted Access Barrier Systems (RABS) or isolators are beneficial in assuring required conditions and minimizing microbial contamination associated with direct human interventions in the critical zone. Their use should be considered in the Contamination Control Strategy (CCS). Any alternative approaches to the use of Restricted Access Barrier Systems or isolators should be justified.

4.4. For the manufacture of sterile products, there are four grades of cleanroom/zones:

Grade A: The critical zone for high-risk operations (for example, aseptic processing line, filling zone, stopper bowl, open primary packaging or for making aseptic connections under the protection of first air). Typically, such conditions are provided by a localized airflow protection (e.g. using

unidirectional airflow workstations within Restricted Access Barrier Systems or isolators). The maintenance of unidirectional airflow should be demonstrated and qualified across the whole of the grade A zone. Direct intervention (e g. without the protection of barrier and glove port technology) into the grade A zone by operators should be minimized by premises, equipment, process and procedural design.

Grade B: For aseptic preparation and filling, this is the background cleanroom for grade A (where it is not an isolator). Air pressure differences should be continuously monitored. Cleanrooms of lower grade than grade B can be considered where isolator technology is used (in accordance with paragraph 4.20).

Grade C and D: These are cleanrooms used for carrying out less critical stages in the manufacture of aseptically filled sterile products or as a background for isolators. They can also be used for the preparation/filling of terminally sterilized products (see Section 8 for the specific details on terminal sterilization activities).

4.5. In cleanrooms and critical zones, all exposed surfaces should be smooth, impervious and unbroken in order to minimize the shedding or accumulation of particles or micro-organisms.

4.6. To reduce accumulation of dust and to facilitate cleaning there should be no recesses that are difficult to clean effectively, therefore projecting ledges, shelves, cupboards and equipment should be kept to a minimum. Doors should be designed to avoid recesses that cannot be cleaned. Sliding doors may be undesirable for this reason.

4.7. Materials used in cleanrooms, both in the construction of the room and for items used within the room, should be selected to minimize generation of particles and to permit the repeated application of cleaning, disinfectant and sporicidal agents (in the case of their use).

4.8. Ceilings should be designed and sealed to prevent contamination from the space above them.

4.9. Sinks and drains should be prohibited in the grade A and grade B areas. In other cleanrooms, air breaks should be fitted between the machine or sink and the drains. Floor drains in lower grade cleanrooms should be fitted with traps or water seals designed to prevent back flow. They and should be regularly cleaned, disinfected and maintained.

4.10. The transfer of equipment and materials into and out of the cleanrooms and critical zones is one of the greatest potential sources of contamination. Any activities with the potential to compromise the cleanliness of cleanrooms or the critical zone should be assessed and if they cannot be eliminated, appropriate controls should be implemented.

4.11. The transfer of materials, equipment, and components into the grade A or B areas should be carried out via a unidirectional process. Whenever possible, items should be sterilized and passed into these areas through double-ended sterilizers (e.g. through a double-door autoclave or depyrogenation oven/tunnel) sealed into the wall. Where sterilization upon transfer of the items is not possible, a procedure which achieves the same objective of not introducing contamination should be validated and implemented, (for example, using an effective transfer disinfection process, rapid transfer systems for isolators or, for gaseous or liquid materials, a bacteria-retentive filter). The removal of items from the grade A and B areas (for example, materials, waste, environmental samples) should be carried out via a separate unidirectional process. If this is not possible, time-based separation of movement (incoming/exiting material) by special procedure should be considered and controls applied to avoid potential contamination of incoming items.

4.12. Airlocks should be designed and used to provide physical separation and to minimize microbial and particle contamination of the

different areas. Airlocks should be present and used for material and personnel moving between different cleanliness grades. Wherever possible, airlocks used for personnel movement should be separated from those used for material movement. Where this is not practical, time-based separation of movement (personnel/material) by procedure should be considered. Airlocks should be flushed effectively with filtered air to ensure that the grade of the cleanroom is maintained. The final stage of the airlock should, in the “at rest” state, be of the same cleanliness grade (viable and total particle) as the cleanroom into which it leads. The use of separate change rooms for entering and leaving the grade B area is desirable. Where this is not practical, time-based separation of activities (ingress/egress) by procedure should be considered. Where the contamination control strategy indicates that the risk of contamination is high, separate change rooms for entering and leaving production areas should be used. Airlocks should be designed as follows:

i. Personnel airlocks: Areas of increasing cleanliness used for entry of personnel (e.g. from the grade D area to the grade C area to the grade B area). In general hand washing facilities should be provided only in the first stage of the changing room and not be present in changing rooms directly accessing the grade B area.

ii. Material airlocks: used for materials and equipment transfer.

- Only materials and equipment that have been included on an approved list and assessed during validation of the transfer process should be transferred into the grade A or grade B areas via an airlock or pass-through hatches. Equipment and materials (intended for use in the grade A area) should be protected when transiting through the grade B area. Any unapproved items that require transfer should be pre-approved in exceptional cases. Appropriate risk assessment and mitigation measures should be applied and recorded as per the

manufacturer's CCS and should include a specific disinfection and monitoring programme approved by quality assurance.

- Pass-through hatches should be designed to protect the higher-grade environment, for example by effective flushing with an active filtered air supply.

- The movement of material or equipment from lower grade or unclassified area to higher-grade clean areas should be subject to cleaning and disinfection commensurate with the risk and in line with the contamination control strategy.

4.13. For pass-through hatches and airlocks (for material and personnel), the entry and exit doors should not be opened simultaneously. For airlocks leading to the grade A and grade B areas, an interlocking system should be used. For airlocks leading to grade C and D areas, a visual and/or audible warning system should be operated as a minimum. Where required to maintain area segregation, a time delay between the closing and opening of interlocked doors should be established.

4.14. Cleanrooms should be supplied with a filtered air supply that maintains a positive pressure and/or an airflow relative to the background environment of a lower grade under all operational conditions. The specified air supply should flush the area effectively. Adjacent rooms of different cleanliness grades should have an air pressure difference of a minimum of 10 Pascals (standard value). Particular attention should be paid to the protection of the critical zone. The recommendations regarding air supplies and pressures may need to be modified where it is necessary to contain certain materials (e.g. pathogenic, highly toxic or radioactive products or live viral or bacterial materials). Such modifications may include positively or negatively pressurized airlocks that prevent the hazardous material from contaminating surrounding areas. Decontamination of facilities and systems (e.g. the

cleanrooms and the heating, ventilation, and air-conditioning (HVAC) systems) and the treatment of air leaving a clean area, may be necessary for some operations. Where containment requires air to flow into a critical zone, the source of the air should be from an area of the same or higher grade.

4.15. Airflow patterns within cleanrooms and zones should be visualized to demonstrate that there is no ingress from lower grade to higher grade areas and that air does not travel from less clean areas (such as the floor) or over operators or equipment that may transfer contamination to the higher grade areas. Where unidirectional airflow is required, visualization studies should be performed to determine compliance, (see paragraphs 4.4 & 4.19). When filled, closed products are transferred to an adjacent cleanroom of a lower grade via a small egress point, airflow visualization studies should demonstrate that air does not ingress from the lower grade cleanrooms to the grade B area. Where air movement is shown to be a contamination risk to the clean area or critical zone, corrective actions, such as design improvement, should be implemented. Airflow pattern studies should be performed both at rest and in operation (e.g. simulating operator interventions). Video recordings of the airflow patterns should be retained. The outcome of the air visualization studies should be documented and considered when establishing the facility's environmental monitoring programme.

4.16. Indicators of air pressure differences should be fitted between cleanrooms and/or between isolators and their background. Set points and the criticality of air pressure differences should be considered within the CCS. Air pressure differences identified as critical should be continuously monitored and recorded. A warning system should be in place to instantly indicate and warn operators of any failure in the air supply or reduction of air pressure differences (below set limits for those identified as critical). The warning signal should not be overridden without assessment and a procedure should be

available to outline the steps to be taken when a warning signal is given. Where alarm delays are set, these should be assessed and justified within the CCS. Other air pressure differences should be monitored and recorded at regular intervals.

4.17. Facilities should be designed to permit observation of production activities from outside the grade A and B areas (e.g. through the provision of windows or remote cameras with a full view of the area and processes to allow observation and supervision without entry). This requirement should be considered when designing new facilities or during refurbishment of existing facilities.

Barrier Technologies

4.18. Isolators or RABS (representing different technologies) and the associated processes, should be designed to provide protection through separation of the grade A environment from the environment of the surrounding room. All hazards introduced from entry or removal of items during processing should be minimized and supported by high capability transfer technologies or validated systems that robustly prevent contamination and are appropriate for the respective technology.

4.19. The design of the technology and processes used should ensure appropriate conditions are maintained in the critical zone to protect the exposed product during operations.

i. Isolators:

a. The design of open isolators should ensure grade A conditions with first air protection in the critical zone and unidirectional airflow that sweeps over and away from exposed products during processing;

b. The design of closed isolators should ensure grade A conditions with adequate protection for exposed products during processing; Airflow may not be fully unidirectional in closed isolators where simple operations are

conducted. However, any turbulent airflow should not increase risk of contamination of the exposed product. Where processing lines are included in closed isolators, grade A conditions should be ensured with first air protection in the critical zone and unidirectional airflow that sweeps over and away from exposed products during processing;

c. Negative pressure isolators should only be used when containment of the product is considered essential (e.g. radiopharmaceutical products). Specialized risk control measures should be applied to ensure the critical zone is not compromised.

ii. RABS:

The design of RABS should ensure grade A conditions with unidirectional airflow and first air protection in the critical zone. A positive airflow from the critical zone to the supporting background environment should be maintained.

4.20. The background environment for isolators or RABS should ensure the risk of transfer of contamination is minimized.

i. Isolators:

a. The background environment for open isolators should generally correspond to a minimum of grade C. The background for closed isolators should correspond to a minimum of grade D. The decision on the background classification should be based on risk assessment and justified in the CCS.

b. Key considerations when performing the risk assessment for the contamination control strategy of an isolator should include (but are not limited to); the bio-decontamination programme, the extent of automation, the impact of glove manipulations that may potentially compromise ‘first air’ protection of critical process points, the impact of potential loss of barrier/glove integrity, transfer mechanisms used and activities such as set-up or maintenance that may require the doors to be opened prior to the final bio-decontamination of the

isolator. Where additional process risks are identified, a higher grade of background should be considered unless appropriately justified in the contamination control strategy.

c. Airflow pattern studies should be performed at the interfaces of open isolators to demonstrate the absence of air ingress.

ii. RABS:

The background environment for RABS used for aseptic processing should correspond to a minimum of grade B and airflow pattern studies should be performed to demonstrate the absence of air ingress during interventions (including door openings if applicable).

4.21. The materials used for glove systems (for both isolators and RABS), should be demonstrated to have appropriate mechanical and chemical resistance. The frequency of glove replacement should be defined within the contamination control strategy.

i. Isolators:

a. For isolators, leak testing of the glove system should be performed using a methodology demonstrated to be suitable for the task and criticality. The testing should be performed at defined intervals. Generally glove integrity testing should be performed at a minimum frequency of the beginning and end of each batch or campaign. Additional glove integrity testing may be necessary depending on the validated campaign length.

Glove integrity monitoring should include a visual inspection associated with each use and following any manipulation that may affect the integrity of the system.

For manual aseptic processing activities where single unit or small batch sizes are produced, the frequency of integrity verification may be based on other criteria, such as the beginning and end of each manufacturing session.

b. Integrity/leak testing of isolator systems should be performed at defined intervals.

ii. RABS:

For RABS, gloves used in the grade A area should be sterilized before installation and sterilized or effectively bio-decontaminated by a validated method prior to each manufacturing campaign. If exposed to the background environment during operation, disinfection using an approved methodology following each exposure should be completed. Gloves should be visually examined with each use, and integrity testing should be performed at periodic intervals.

4.22. Decontamination methods (cleaning and bio-decontamination, and where applicable inactivation for biological materials) should be appropriately defined and controlled. The cleaning process prior to the bio-decontamination step is essential; any residues that remain may inhibit the effectiveness of the decontamination process. Evidence should also be available to demonstrate that the cleaning and bio- decontamination agents used do not have adverse impact on the product produced within the RABS or isolator.

i. For isolators:

The bio-decontamination process of the interior should be automated, validated and controlled within defined cycle parameters. It should include a sporicidal agent in a suitable form (e.g. gaseous or vaporized form). Gloves should be appropriately extended with fingers separated to ensure contact with the agent. Methods used (cleaning and sporicidal bio-decontamination) should render the interior surfaces and critical zone of the isolator free from viable microorganisms.

ii. For RABS:

The sporicidal disinfection should include the routine application of a sporicidal agent using a method that has been validated and demonstrated to

robustly include all areas of the interior surfaces and ensure a suitable environment for aseptic processing.

Cleanroom and clean air equipment qualification

4.23. Cleanrooms and clean air equipment such as unidirectional airflow units (UDAFs), RABS and isolators, used for the manufacture of sterile 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 risk of contamination of the product or materials being handled. Appropriate cleanliness levels in the “at rest” and “operational” states should be maintained.

4.24. Cleanrooms and clean air equipment should be qualified using methodology in accordance with the requirements of Annex No. 15 to the Rules. Cleanroom qualification (including classification) should be clearly differentiated from operational environmental monitoring.

4.25. Cleanroom and clean air equipment qualification is the overall process of assessing the level of compliance of a classified cleanroom or clean air equipment with its intended use. As part of the qualification requirements of Annex No. 15 to the Rules, the qualification of cleanrooms and clean air equipment should include (where relevant to the design/operation of the installation):

i. Installed filter system leakage and integrity testing;

ii. Airflow tests — volume and velocity;

iii. Air pressure difference test;

iv. Airflow direction test and visualization;

v. Microbial airborne and surface contamination;

vi. Temperature measurement test;

vii. Relative humidity test;

viii. Recovery test;

ix. Containment leak test.

Reference for the qualification of the cleanrooms and clean air equipment can be found in the ISO 14644 series of standards.

4.26. Cleanroom classification is part of the cleanroom qualification and is a method of assessing the level of air cleanliness against a specification for a cleanroom or clean air equipment by measuring the total particle concentration. Classification activities should be scheduled and performed in order to avoid any impact on process or product quality. For example, initial classification should be performed during simulated operations and reclassification performed during simulated operations or during aseptic process simulation (APS).

4.27. For cleanroom classification, the total of particles equal to or greater than 0.5 and 5 μm should be measured. This measurement should be performed both at rest and in simulated operations in accordance with the limits specified in Table 1.

Table 1

Maximum permitted total particle concentration for classification

Grade	Maximum limits for total particle ≥ 0.5 μm/m^3		Maximum limits for total particle ≥ 5 μm/m^3	
	at rest	in operation	at rest	in operation
A	3 520	3 520	Not specified[(a)]	Not specified[(a)]
B	3 520	352 000	Not specified[(a)]	2 930
C	352 000	3 520 000	2 930	29 300
D	3 520 000	Not predetermined[(b)]	29 300	Not predetermined[(b)]

[(a)] Classification including 5 μm particles may be considered where indicated by the CCS or historical trends.

[(b)] For grade D, in operation limits are not predetermined. The manufacturer should establish in operation limits based on a risk assessment and routine data where applicable.

4.28. For classification of the cleanroom, the minimum number of sampling locations and their positioning can be found in ISO 14644-1. For the aseptic processing area and the background environment (the grade A and grade B areas, respectively), additional sample locations should be considered and all critical processing areas such as the point of fill and container closure feeder bowls should be evaluated. Critical processing locations should be determined by documented risk assessment and knowledge of the process and operations to be performed in the area.

4.29. Cleanroom classification should be carried out in the “at rest” and “in operation” states.

i. The definition of “at rest” state is the condition whereby the installation of all the utilities is complete including any functioning HVAC system, with the main manufacturing equipment installed as specified but not operating and without personnel present in the room.

ii. The definition of “in operation” state is the condition where the installation of the cleanroom is complete, the HVAC system fully operational, equipment installed and functioning in the manufacturer’s defined operating mode with the maximum number of personnel present performing or simulating routine operational work.

iii. The total particle limits given in Table 1 above for the “at rest” state should be achieved after a “clean up” period on completion of operations and line clearance/cleaning activities. The "clean up" period (guidance value of less than 20 minutes) should be determined during the qualification of the rooms, documented and adhered to in procedures to reinstate a qualified state of cleanliness if disrupted during operation.

4.30. The speed of air supplied by unidirectional airflow systems should be clearly justified in the qualification protocol including the location for air

speed measurement. Air speed should be designed, measured and maintained to ensure that appropriate unidirectional air movement provides protection of the product and open components at the working position (e.g. where high-risk operations occur and where product and/or components are exposed). Unidirectional airflow systems should provide a homogeneous air speed in a range of 0.36 — 0.54 m/s (guidance value) at the working position, unless otherwise scientifically justified in the contamination control strategy. Airflow visualization studies should correlate with the air speed measurement.

4.31. The microbial contamination level of the cleanrooms should be determined as part of the cleanroom qualification. The number of sampling locations should be based on a documented risk assessment and the results obtained from room classification, air visualization studies and knowledge of the process and operations to be performed in the area. The maximum limits for microbial contamination during qualification for each grade are given in Table 2. Qualification should include both “at rest” and “in operation” states.

Table 2

Maximum permitted microbial contamination level during qualification

Grade	Air sample, CFU/m^3	Settle plates (diameter 90 mm) CFU/4 hours[(a)]	Contact plates (diameter 55 mm) CFU/plate
A	No growth		
B	10	5	5
C	100	50	25
D	200	100	50

(a) Settle plates should be exposed for the duration of operations and changed as required after a maximum of 4 hours. Exposure time should be based on recovery studies and should not allow desiccation of the media used.

Note 1: All methods indicated for a specific grade in the table should be used for qualifying the area of that specific grade. If one of the methods tabulated is not used, or alternative methods are used, the approach taken should be appropriately justified.

Note 2: Limits are applied using CFU throughout the document. If different or new technologies are used that present results in a manner different from CFU, the manufacturer should scientifically justify the limits applied and where possible correlate them to CFU.

Note 3: For the qualification of personnel gowning, the limits given for contact plates and glove prints in Table 6 should apply.
Note 4: Sampling methods should not pose a risk of contamination to the manufacturing operations.

4.32. The requalification of cleanrooms and clean air equipment should be carried out periodically following defined procedures. The requalification should include at a minimum the following:

Cleanroom classification (total particle concentration);

Integrity test of final filters;

Airflow volume measurement;

Verification of air pressure difference between rooms;

Air velocity test (Note: For grade B, C and D the air velocity test should be performed according to a risk assessment documented as part of the contamination control strategy. However, it is required for filling zones supplied with unidirectional airflow (e.g. when filling terminally sterilized products or background to grade A and RABS). For grades with non-unidirectional airflow, a measurement of recovery testing should replace velocity testing).

The maximum time interval for requalification of grade A and B areas, is 6 months.

The maximum time interval for requalification of grade C and D areas, is 12 months.

Appropriate requalification consisting of at least the above tests should also be carried out following completion of remedial action implemented to rectify an out of compliance equipment or facility condition or after changes to equipment, facility or processes as appropriate. The significance of a change should be determined through the change management process. Examples of changes to be considered include but are not limited to the following:

i. Interruption of air movement which affects the operation of the installation.

ii. Change in the design of the cleanroom or of the operational setting parameters of the Heat, Ventilation and Air Conditioning (HVAC) system.

iii. Special maintenance which affects the operation of the installation (e.g. change of final filters).

Disinfection

4.33. The disinfection of cleanrooms is particularly important. Cleanrooms should be cleaned and disinfected thoroughly in accordance with a written programme. For disinfection to be effective, prior cleaning to remove surface contamination should be performed. Cleaning programmes should effectively remove disinfectant residues. More than one type of disinfecting agent should be employed to ensure that where they have different modes of action, their combined usage is effective against bacteria and fungi. Disinfection should include the periodic use of a sporicidal agent. Monitoring should be undertaken regularly in order to assess the effectiveness of the disinfection programme and to detect changes in types of microbial flora (e.g. organisms resistant to the disinfection regime currently in use).

4.34. The disinfection process should be validated. Validation studies should demonstrate the suitability and effectiveness of disinfectants in the specific manner in which they are used and on the type of surface material, or representative material if justified, and should support the in-use expiry periods of prepared solutions.

4.35. Disinfectants and detergents used in grade A and grade B areas should be sterile prior to use. Disinfectants used in grade C and D may also be required to be sterile where determined in the contamination control strategy. Where the disinfectants and detergents are diluted / prepared by the sterile product manufacturer, this should be done in a manner to prevent

contamination and they should be monitored for microbial contamination. Dilutions should be kept in previously cleaned containers (and sterilized where applicable) and should only be stored for the defined period. If the disinfectants and detergents are supplied “ready-made” then results from certificates of analysis or conformance can be accepted subject to successful completion of the appropriate vendor qualification.

4.36. Where fumigation or vapor disinfection (e.g. Vapor-phase Hydrogen Peroxide) of cleanrooms and associated surfaces are used, the effectiveness of any fumigation agent and dispersion system should be understood and validated.

5. Equipment

5.1. A written, detailed description of the equipment design should be available (including process and instrumentation diagrams as appropriate). This should form part of the initial qualification package and be kept up to date.

5.2. Equipment monitoring requirements should be defined in “user requirements specifications” during early stages of development, and confirmed during qualification. Process and equipment alarm events should be acknowledged and evaluated for trends. The frequency at which alarms are assessed should be based on their criticality (with critical alarms reviewed immediately).

5.3. As far as practicable, equipment, fittings and services should be designed and installed so that operations, maintenance, and repairs can be performed outside the cleanroom. If maintenance has to be performed in the cleanroom, and the required standards of cleanliness and/or asepsis cannot be maintained, then precautions such as restricting access to the work area to specified personnel, generation of clearly defined work protocols and

maintenance procedures should be considered. Additional cleaning, disinfection and environmental monitoring should also be considered. If sterilization of equipment is required, it should be carried out, wherever possible, after complete reassembly.

5.4. The cleaning process should be validated to be able to:

i. Remove any residue or debris that would detrimentally impact the effectiveness of the disinfecting agent used.

ii. Minimize chemical, microbial and particulate contamination of the product during the process and prior to disinfection.

5.5. For aseptic processes, direct and indirect product contact parts should be sterilized. Direct product contact parts are those that the product passes through, such as filling needles or pumps. Indirect product contact parts are equipment parts that do not contact the product, but may come into contact with other sterilized surfaces, the sterility of which is critical to the overall product sterility (e.g. sterilized items such as stopper bowls and guides, and sterilized components).

5.6. All equipment such as sterilizers, air handling systems (including air filtration) and water systems should be subject to qualification, monitoring and planned maintenance. Upon completion of maintenance, their return to use should be approved.

5.7. 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.

5.8. 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).

5.9. Particle counters, including sampling tubing, should be qualified. The manufacturer’s recommended specifications should be considered for tube

diameter and bend radii. Tube length should typically be no longer than 1 meter unless justified and the number of bends should be minimized. Portable particle counters with a short length of sample tubing should be used for classification purposes. Isokinetic sampling heads should be used in unidirectional airflow systems. They should be oriented appropriately and positioned as close as possible to the critical location to ensure that samples are representative.

6. Utilities

6.1. The nature and extent of controls applied to utility systems should be commensurate with the risk to product quality associated with the utility. The impact should be determined via a risk assessment and documented as part of the contamination control strategy.

6.2. In general, higher risk utilities are those that:

i. Directly contact product, for example, water for washing and rinsing, gases and steam for sterilization.

ii. Contact materials that will ultimately become part of the product.

iii. Contact surfaces that come into contact with the product.

iv. Otherwise directly impact the product.

6.3. Utilities should be designed, installed, qualified, operated, maintained and monitored in a manner to ensure that the utility system functions as expected.

6.4. Results for critical parameters and critical quality attributes of high risk utilities should be subject to regular trend analysis to ensure that system capabilities remain appropriate.

6.5. Records of utility system installation should be maintained throughout the system’s life-cycle. Such records should include current

drawings and schematic diagrams, construction material lists and system specifications. Typically, important information includes attributes such as:

i. Pipeline flow direction, slopes, diameter and length.

ii. Tank and vessel details.

iii. Valves, filters, drains, sampling and user points.

6.6. Pipes, ducts and other utilities should not be present in cleanrooms. If unavoidable, then they should be installed so that they do not create recesses, unsealed openings and surfaces which are difficult to clean. Installation should allow cleaning and disinfection of outer surface of the pipes.

Water systems

6.7. Water treatment plant and distribution systems should be designed, constructed, installed, commissioned, qualified, monitored and maintained to prevent microbiological contamination and to ensure a reliable source of water of an appropriate quality. Measures should be taken to minimize the risk of presence of particulates, microbial contamination/proliferation and endotoxin/pyrogen (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 given to their monitoring and maintenance. Water produced should comply with the current monograph of the relevant Pharmacopoeia.

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

6.9. Water flow should remain turbulent through the pipes in water distribution systems to minimize the risk of microbial adhesion, and subsequent biofilm formation. The flow rate should be established during qualification and be routinely monitored.

6.10. Water for injections (WFI) should be produced from water meeting specifications that have been defined during the qualification process. WFI

should be stored and distributed in a manner which minimizes the risk of microbial growth (for example, by constant circulation at a temperature above 70 °C). WFI should be produced by distillation or by a purification process that is equivalent to distillation. This may include reverse osmosis coupled with other appropriate techniques such as electrodeionization (EDI), ultrafiltration or nanofiltration.

6.11. Where WFI storage tanks are equipped with hydrophobic bacteria retentive vent filters, the filters should not be a source of contamination and the integrity of the filter tested before installation and after use. Controls should be in place to prevent condensation formation on the filter (e.g. by heating).

6.12. To minimize the risk of biofilm formation, sterilization, disinfection or regeneration of water systems should be carried out according to a predetermined schedule and as a remedial action following out-of-limit or specification results. Disinfection of a water system with chemicals should be followed by a validated rinsing/flushing procedure. Water should be tested after disinfection/regeneration. Chemical testing results should be approved before the water system is returned to use and microbiological/endotoxin results verified to be within specification and approved before batches manufactured using water from the system are considered for certification/release.

6.13. Regular ongoing chemical and microbial monitoring of water systems should be performed to ensure that the water continues to meet compendial expectations. Alert levels should be based on the initial qualification data and thereafter periodically reassessed on data obtained during subsequent re-qualifications, routine monitoring, and investigations. Review of ongoing monitoring data should be carried out to identify any adverse trend in system performance. Sampling programmes should reflect the requirements of the contamination control strategy and should include all

outlets and points of use, at a specified interval, to ensure that representative water samples are obtained for analysis on a regular basis. Sample plans should be based on the qualification data, should consider the potential worst case sampling locations and should ensure that at least one representative sample is included every day of the water that is used for manufacturing processes.

6.14. Alert level excursions should be documented and reviewed, and include an investigation to determine whether the excursion is a single (isolated) event or if results are indicative of an adverse trend or system deterioration. Each action limit excursion should be investigated to determine the probable root causes and any potential impact on the quality of products and manufacturing processes as a result of the use of the water.

6.15. WFI systems should include continuous monitoring systems such as Total Organic Carbon (TOC) and conductivity, as these may give a better indication of overall system performance than discrete sampling. Sensor locations should be based on risk.

Steam used as a direct sterilizing agent

6.16. Feed water to a pure steam (clean steam) generator should be appropriately purified. Pure steam generators should be designed, qualified and operated in a manner to ensure that the quality of steam produced meets defined chemical and endotoxin levels.

6.17. Steam used as a direct sterilizing agent should be of suitable quality and should not contain additives at a level that could cause contamination of product or equipment. For a generator supplying pure steam used for the direct sterilization of materials or product-contact surfaces (e.g. porous hard-good autoclave loads), steam condensate should meet the current monograph for WFI of the relevant Pharmacopoeia (microbial testing is not mandatory for steam condensate). A suitable sampling schedule should be in place to ensure that representative pure steam is obtained for analysis on a regular basis. Other

aspects of the quality of pure steam used for sterilization should be assessed periodically against validated parameters. These parameters should include the following (unless otherwise justified): non-condensable gases, dryness value (dryness fraction) and superheat.

Gas and vacuum systems

6.18. Gases that come in direct contact with the product/primary container surfaces should be of appropriate chemical, particulate and microbial quality. All relevant parameters, including oil and water content, should be specified, taking into account the use and type of the gas, the design of the gas generation system and, where applicable, comply with the current monograph of the relevant Pharmacopoeia or the product quality requirement.

6.19. Gases used in aseptic processes should be filtered through a sterilizing grade filter (with a nominal pore size of a maximum of 0.22 μm) at the point of use. Where the filter is used on a batch basis (e g. for filtration of gas used for overlay of aseptically filled products) or as product vessel vent filter, then the filter should be integrity tested and the results reviewed as part of the batch certification/release process. Any transfer pipework or tubing that is located after the final sterilizing grade filter should be sterilized. When gases are used in the process, microbial monitoring of the gas should be performed periodically at the point of use.

6.20. Where backflow from vacuum or pressure systems poses a potential risk to the product, there should be mechanism(s) to prevent backflow when the vacuum or pressure system is shut off.

Heating and cooling and hydraulic systems

6.21. Major items of equipment associated with hydraulic, heating and cooling systems should, where possible, be located outside the filling room. There should be appropriate controls to contain any spillage and/or cross contamination associated with the system fluids.

6.22. Any leaks from these systems that would present a risk to the product should be detectable (e.g. an indication system for leakage).

7. Personnel

7.1. The manufacturer should ensure that there are sufficient appropriate personnel, suitably qualified, trained and experienced in the manufacture and testing of sterile products, and any of the specific manufacturing technologies used in the site's manufacturing operations, to ensure compliance with good manufacturing practices applicable to the manufacture and handling of sterile products.

7.2. Only the minimum number of personnel required should be present in cleanrooms. The maximum number of operators in cleanrooms should be determined, documented and considered during activities such as initial qualification and aseptic process simulation, so as not to compromise sterility assurance.

7.3. All personnel (including those performing cleaning, maintenance, monitoring and those that access cleanrooms) should receive regular training, gowning qualification and assessment in disciplines relevant to the correct manufacture of sterile products. This training should include the basic elements of microbiology and hygiene, with a specific focus on cleanroom practices, contamination control, aseptic techniques and the protection of sterile products (for those operators entering the grade B cleanrooms and/or intervening into grade A) and the potential safety implications to the patient if the product is not sterile. The level of training should be based on the criticality of the function and area in which the personnel are working.

7.4. The personnel accessing grade A and B areas should be trained for aseptic gowning and aseptic behaviors. Compliance with aseptic gowning procedures should be confirmed by assessment and periodic reassessment at

least annually, and should involve both visual and microbial assessment (using monitoring locations such as gloved fingers, forearms, chest and hood (facemask / forehead). See paragraph 9.30 for the expected limits). The unsupervised access to the grade A and grade B areas where aseptic operations are or will be conducted should be restricted to appropriately qualified personnel, who have passed the gowning assessment and have participated in a successful aseptic process simulation (APS).

7.5. Unqualified personnel should not enter grade B cleanrooms or grade A in operation. If needed in exceptional cases, manufacturers should establish written procedures outlining the process by which unqualified personnel are brought into the grade B and A areas. An authorized person from the manufacturer should supervise the unqualified personnel during their activities and should assess the impact of these activities on the cleanliness of the area. Access by these persons should be assessed and recorded in accordance with the PQS.

7.6. There should be systems in place for the disqualification of personnel from working in or given unsupervised entry into cleanrooms that is based on aspects including ongoing assessment and/or identification of an adverse trend from the personnel monitoring programme and/or after being implicated in a failed aseptic process simulation. Once disqualified, retraining and requalification should be completed before permitting the operator to have any further involvement in aseptic practices. For operators entering grade B cleanrooms or performing intervention into grade A, this requalification should include consideration of participation in a successful aseptic process simulation.

7.7. High standards of personal hygiene and cleanliness are essential to prevent excessive shedding or increased risk of introduction of microbial contamination. Personnel involved in the manufacture of sterile products

should be instructed to report any specific health conditions or ailments that may cause the shedding of abnormal numbers or types of contaminants and therefore preclude cleanroom access. Health conditions and actions to be taken with regard to personnel who could be introducing an undue microbial hazard should be provided by the designated competent person and described in procedures.

7.8. Personnel 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 clean areas unless clearly defined and effective decontamination and entry procedures have been followed and documented.

7.9. Wristwatches, make-up, jewelry, other personal items such as mobile phones and any other non-essential items should not be allowed in clean areas. Electronic devices used in cleanrooms, for example, mobile phones and tablets, that are supplied by the manufacturer solely for use in the cleanrooms, may be acceptable if suitably designed to permit cleaning and disinfection commensurate with the grade in which they are used. The use and disinfection of such equipment should be included in the contamination control strategy.

7.10. Cleanroom gowning and hand washing should follow a written procedure designed to minimize contamination of cleanroom clothing and/or the transfer of contaminants to the clean areas.

7.11. 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. When the type of clothing chosen needs to provide the operator protection from the product, it should not compromise the protection of the product from contamination. Garments should be visually

checked for cleanliness and integrity immediately prior to and after gowning. Gown integrity should also be checked upon exit. For sterilized garments and eye coverings, particular attention should be taken to ensure they have been subject to the sterilization process, are within their specified hold time and that the packaging is visually inspected to ensure it is integral before use. Reusable garments (including eye coverings) should be replaced if damage is identified, or at a set frequency that is determined during qualification studies. The qualification of garments should consider any necessary garment testing requirements (including damage to garments that may not be identified by visual inspection alone).

7.12. Clothing should be chosen to limit shedding due to operators' movement.

7.13. A description of typical clothing required for each cleanliness grade is given below:

i. Grade B (including access / interventions into grade A): appropriate garments that are dedicated for use under a sterilized suit should be worn before gowning (see paragraph 7.14). Appropriately sterilized, non-powdered, rubber or plastic gloves should be worn while donning the sterilized garments. Sterile headgear should enclose all hair (including facial hair) and where separate from the rest of the gown, it should be tucked into the neck of the sterile suit. A sterile facemask and sterile eye coverings (e.g. goggles) should be worn to cover and enclose all facial skin and prevent the shedding of droplets and particles. Appropriate sterilized footwear (e.g. over-boots) should be worn. Trouser legs should be tucked inside the footwear. Garment sleeves should be tucked into a second pair of sterile gloves worn over the pair worn while donning the gown. The protective clothing should minimize shedding of fibers or particles and retain particles shed by the body. The particle shedding and the particle retention efficiencies of the garments should be assessed

during the garment qualification. Garments should be packed and folded in such a way as to allow operators to don the gown without contacting the outer surface of the garment and to prevent the garment from touching the floor.

ii. 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 shoes or overshoes should be worn. They should minimize the shedding of fibers and particles.

iii. 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 ingress of contaminants from outside the clean area.

iv. Additional gowning including gloves and facemask may be required in grade C and D areas when performing activities considered to be a contamination risk as defined by the contamination control strategy (CCS).

7.14. Cleanroom gowning should be performed in change rooms of an appropriate cleanliness grade to ensure gown cleanliness is maintained. Outdoor clothing including socks (other than personal underwear) should not be brought into changing rooms leading directly to grade B and C areas. Single or two-piece facility trouser suits, covering the full length of the arms and the legs, and facility socks covering the feet, should be worn before entry to change rooms for grades B and C. Facility suits and socks should not present a risk of contamination to the gowning area or processes.

7.15. Every operator entering grade B or A areas should gown into clean, sterilized protective garments (including eye coverings and masks) of an appropriate size at each entry. The maximum period for which the sterilized gown may be worn before replacement during a shift should be defined as part of the garment qualification.

7.16. Gloves should be regularly disinfected during operations. Garments and gloves should be changed immediately if they become damaged and present any risk of product contamination.

7.17. Reusable clean area clothing should be cleaned in a laundry facility adequately segregated from production operations, using a qualified process ensuring that the clothing is not damaged and/or contaminated by fibers or particles during the repeated laundry process. Laundry facilities used should not introduce risk of contamination or cross-contamination. Inappropriate handling and use of clothing may damage fibers and increase the risk of shedding of particles. After washing and before packing, garments should be visually inspected for damage and visual cleanliness. The garment management processes should be evaluated and determined as part of the garment qualification programme and should include a maximum number of laundry and sterilization cycles.

7.18. Activities in clean areas that are not critical to the production processes should be kept to a minimum, especially when aseptic operations are in progress. Movement of personnel should be slow, controlled and methodical to avoid excessive shedding of particles and organisms due to over-vigorous activity. Operators performing aseptic operations should adhere to aseptic technique at all times to prevent changes in air currents that may introduce air of lower quality into the critical zone. Movement adjacent to the critical zone should be restricted and the obstruction of the path of the unidirectional (first air) airflow should be avoided. A review of airflow visualization studies should be considered as part of the training programme.

8. Production and Specific Technologies

Terminally sterilized products

8.1. Preparation of components and materials should be performed in at least a grade D cleanroom in order to limit the risk of microbial, endotoxin/ pyrogen and particle contamination, so that the product is suitable for sterilization. Where the product is at a high or unusual risk of microbial contamination (e.g. the product actively supports microbial growth, the product must be held for long periods before filling or the product is not processed mostly in closed vessels), then preparation should be carried out in at least a grade C environment. Preparation of ointments, creams, suspensions and emulsions should be carried out in at least a grade C environment before terminal sterilization. Specific guidance regarding terminally sterilized veterinary medicinal products can be found within Annex No. 4 of the GMP to the Rules.

8.2. Primary packaging containers and components should be cleaned using validated processes to ensure that particle, endotoxin/pyrogen and bioburden contamination is appropriately controlled.

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

8.4. Where the contamination control strategy identifies that 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 before closing, then the product should be filled in grade A with at least a grade C background.

8.5. Processing of the bulk solution should include a filtration step with a microorganism retaining filter, where possible, to reduce bioburden levels and particles prior to filling into the final product containers and there should be a maximum permissible time between preparation and filling.

8.6. Examples of operations to be carried out in the various grades are given in Table 3.

Table 3

Examples of operations and grades for terminally sterilized preparation and processing operations

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

Aseptic preparation and processing

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 CCS 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. Accepted residual risks should be formally documented.

8.8. Precautions to minimize microbial, endotoxin/pyrogenic and particle 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 bulk product sterilization), and until the product is sealed in its final container. The presence of materials liable to generate particles and fibers should be minimized in cleanrooms.

8.9. Where possible, the use of equipment such as RABS, isolators or other systems, should be considered in order to reduce the need for critical interventions into grade A and to minimize the risk of contamination. Robotics and automation of processes can also be considered to eliminate direct human critical interventions (for example, dry heat tunnel, automated lyophilizer loading, sterilization in place).

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

Table 4

Examples of operations and grades for aseptic preparation and processing operations

Grade A	- Aseptic assembly of filling equipment - Connections made under aseptic conditions (where sterilized product contact surfaces are exposed) that are post the final sterilizing grade filter. These connections should be sterilized by steam-in-place (SIP) whenever possible - Aseptic compounding and mixing - Replenishment of sterile bulk product, containers and closures - Removal and cooling of unprotected (e.g. with no packaging) items from sterilizers - Staging and conveying of sterile primary packaging components in the aseptic filling line while not wrapped - Aseptic filling, sealing of containers such as ampoules, vial closure, transfer of open or partially stoppered vials - Loading of a lyophilizer
Grade B	- Background support for grade A (when not in an isolator) - Conveying or staging, while protected from the surrounding environment, of equipment, components and ancillary items for introduction into grade A
Grade C	- Preparation of solutions to be filtered including sampling and dispensing
Grade D	- Cleaning of equipment - Handling of components, equipment and accessories after washing (cleaning) - Assembly under HEPA filtered airflow of cleaned components, equipment and accessories prior to sterilization - Assembly of closed and sterilized SUS using intrinsic sterile connection devices

8.11. For sterile products where the final formulation cannot be filtered, the following should be considered:

i. All product and component contact equipment should be sterilized prior to use.

ii. All raw materials or intermediates should be sterilized and aseptically added.

iii. Bulk solutions or intermediates should be sterilized.

8.12. The unwrapping, assembly and preparation of sterilized equipment, components and ancillary items with direct or indirect product contact should be treated as an aseptic process and performed in grade A with a grade B background. The filling line set-up and filling of the sterile product should be treated as an aseptic process and performed in grade A with a grade B background. Where an isolator is used, the background should be in accordance with paragraph 4.20.

8.13. Preparation and filling of sterile products such as ointments, creams, suspensions and emulsions should be performed in grade A with a grade B background when the product and components are exposed to the environment and the product is not subsequently filtered (via a sterilizing grade filter) or terminally sterilized. Where an isolator or RABS is used, the background should be in accordance with paragraph 4.20.

8.14. Aseptic connections should be performed in grade A with a grade B background unless subsequently sterilized in place or conducted with intrinsic sterile connection devices that minimize any potential contamination from the immediate environment. Intrinsic sterile connection devices should be designed to mitigate risk of contamination.

Where an isolator is used, the background should be in accordance with paragraph 4.20. Aseptic connections should be appropriately assessed and their effectiveness verified. For requirements regarding intrinsic sterile connection devices see paragraphs 8.129 and 8.130.

8.15. Aseptic manipulations (including non-intrinsic sterile connection devices) should be minimized through the use of engineering design solutions

such as preassembled and sterilized equipment. Whenever feasible, product contact piping and equipment should be pre-assembled, and sterilized in place.

8.16. There should be an authorized list of allowed and qualified interventions, both inherent and corrective, that may occur during production (see paragraph 9.34). Interventions should be carefully designed to ensure that the risk of contamination of the environment, process and product is effectively minimized. The process of designing interventions should include the consideration of any impact on air-flows and critical surfaces and products. Engineering solutions should be used whenever possible to minimize incursion by operators during the intervention. Aseptic technique should be observed at all times, including the appropriate use of sterile tools for manipulations. The procedures listing the types of inherent and corrective interventions, and how to perform them, should be first evaluated via risk management and aseptic process simulation and be kept up to date. Non-qualified interventions should only be used in exceptional circumstances, with due consideration of the risks associated with the intervention and with the authorization of the quality unit. The details of the intervention conducted should be subject to risk assessment, recorded and fully investigated under the manufacturer's PQS. Any non-qualified interventions should be thoroughly assessed by the quality department and considered during batch disposition.

8.17. Interventions and stoppages should be recorded in the batch record. Each line stoppage or intervention should be sufficiently documented in batch records with the associated time, duration of the event, and operators involved (refer to paragraph 9.34).

8.18. The duration of each aspect of aseptic preparation and processing should be minimized and limited to a defined and validated maximum time, including:

i. The holding time between equipment, component, and container cleaning, drying and sterilization.

ii. The holding time for sterilized equipment, components, and containers before use and during filling/assembly.

iii. The holding time for a decontaminated environment, such as the RABS or isolator before use.

iv. The time between the start of the preparation of a product and its sterilization or filtration through a microorganism-retaining filter (if applicable), through to the end of the aseptic filling process. There should be a maximum permissible time for each product that takes into account its composition and the prescribed method of storage

v. The holding time for sterilized product prior to filling

vi. The aseptic processing time

vii. The filling time

8.19. Aseptic operations (including aseptic process simulation (APS)) should be observed on a regular basis by personnel with specific expertise in aseptic processing to verify the correct performance of operations including operator behavior in the cleanroom and address inappropriate practices if detected.

Finishing of sterile products

8.20. Open primary packaging containers should be maintained under grade A conditions with the appropriate background for the technology as described in paragraph 4.20. For partially stoppered vials or prefilled syringes (see paragraph 8.126).

8.21. Final containers should be closed by appropriately validated methods.

8.22. Where final containers are closed by fusion, e.g. Blow-Fill-Seal (BFS), Form-Fill-Seal (FFS), Small and Large Volume Parenteral (SVP and

LVP) bags, glass or plastic ampoules, the critical parameters and variables that affect seal integrity should be evaluated, determined, effectively controlled and monitored during operations. Glass ampoules, Blow-Fill-Seal units and small volume containers (≤ 100 ml) closed by fusion should be subject to 100% integrity testing using validated methods. For large volume containers (> 100 ml) closed by fusion, reduced sampling may be acceptable where scientifically justified and based on data demonstrating the consistency of the existing process, and a high level of process control. It should be noted that visual inspection is not considered as an acceptable integrity test method.

8.23. Samples of products using systems other than fusion should be taken and checked for integrity using validated methods. The frequency of testing should be based on the knowledge and experience of the container and closure systems being used. A scientifically justified sampling plan should be used. The sample size should be based on information such as supplier management, packaging component specifications and process knowledge.

8.24. Containers sealed under vacuum should be tested for maintenance of vacuum after an appropriate pre-determined period prior to certification/release and during shelf life.

8.25. The container closure integrity validation should take into consideration any transportation or shipping requirements that may negatively impact the integrity of the container (for example, by decompression or extreme temperatures).

8.26. Where the equipment used to crimp vial caps can generate large quantities of non-viable particle, measures to prevent particle contamination such as locating the equipment at a physically separate station equipped with adequate air extraction should be taken.

8.27. Vial capping of aseptically filled products can be undertaken as an aseptic process using sterilized caps or as a clean process outside the aseptic

processing area. Where the 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. The supporting background environment of grade A air supply should meet at least grade D requirements. Where capping is a manual process, it should be performed under grade A conditions either in an appropriately designed isolator or in grade A with a grade B background.

8.28. Where capping of aseptically filled sterile product 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 qualified, automated methods for stopper height detection should be in place.

8.29. Where human intervention is required at the capping station, appropriate technological and organizational measures should be used to prevent direct contact with the vials and to minimize microbial contamination. RABS and isolators may be beneficial in assuring the required conditions.

8.30. All filled containers of parenteral products should be inspected individually for extraneous contamination or other defects. Defect classification and criticality should be determined during qualification and based on risk and historical knowledge. Factors to consider include, but are not limited to, the potential impact of the defect to the patient and the route of administration. Different defect types should be categorized and batch performance analyzed. Batches with unusual levels of defects, when compared with routine defect numbers for the process (based on routine and trend data), should be investigated. A defect library should be generated and maintained which captures all known classes of defects. The defect library should be used for the training of production and quality assurance personnel. Critical defects shall not be detected during subsequent selection of samples and supervision of acceptable containers. Any critical defect identified subsequently should

trigger an investigation as it indicates a possible failure of the original inspection process.

8.31. When inspection is performed manually, it should be conducted under suitable and controlled conditions of illumination and background. Inspection rates should be appropriately controlled and qualified. Operators performing the inspection should undergo visual inspection qualification (whilst wearing corrective lenses, if these are normally worn) at least annually. The qualification should be undertaken using appropriate samples from the manufacturer's defect library 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, container size or fatigue). The qualification should also include consideration of eyesight checks. Operator distractions should be minimized and frequent breaks, of an appropriate duration, should be taken from inspection.

8.32. Where automated methods of inspection are used, the process should be validated to detect known defects (which may impact product quality or safety) and be equal to, or better than, manual inspection methods. The performance of the equipment should be challenged using representative defects prior to start up and at regular intervals throughout the batch.

8.33. Results of the inspection should be recorded and defect types and numbers trended. Reject levels for the various defect types should also be trended based on statistical principles. Impact to product on the market should be assessed as part of the investigation when adverse trends are observed.

Sterilization

8.34. 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 sterile filtration process and/or aseptic processing. Where it is not possible for a product to undergo

terminal sterilization, consideration should be given to using post-aseptic processing terminal heat treatment, combined with aseptic process to give improved sterility assurance.

8.35. The selection, design and location of the equipment and cycle/programme used for sterilization should be based on scientific principles and data which demonstrate repeatability and reliability of the sterilization process. All parameters should be defined, and where critical, these should be controlled, monitored and recorded.

8.36. All sterilization processes should be validated. Validation studies should take into account the product composition, storage conditions and maximum time between the start of the preparation of a product or material to be sterilized and its sterilization. Before any sterilization process is adopted, its suitability for the product and equipment, and its efficacy in consistently achieving the desired sterilizing conditions in all parts of each type of load to be processed should be validated notably by physical measurements and where appropriate by Biological Indicators (BI). For effective sterilization, the whole of the product, and surfaces of equipment and components should be subject to the required treatment and the process should be designed to ensure that this is achieved.

8.37. Particular attention should be given when the adopted product 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.

8.38. Validated loading patterns should be established for all sterilization processes and load patterns should be subject to periodic revalidation. Maximum and minimum loads should also be considered as part of the overall load validation strategy.

8.39. The validity of the sterilizing process should be reviewed and verified at scheduled intervals based on risk. Heat sterilization cycles should be revalidated with a minimum frequency of at least annually for load patterns that are considered worst case. Other load patterns should be validated at a frequency justified in the contamination control strategy (CCS).

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

8.41. There should be mechanisms in place to detect a sterilization cycle that does not conform to the validated parameters. Any failed sterilization or sterilization that deviated from the validated process (for example, have longer or shorter phases such as heating cycles) should be investigated.

8.42. Suitable BIs placed at appropriate locations should be considered as an additional method to support the validation of the sterilization process. Biological indicators should be stored and used according to the manufacturer's instructions. Where BIs are used to support validation and/or to monitor a sterilization process (e.g. with ethylene oxide), positive controls should be tested for each sterilization cycle. If biological indicators are used, strict precautions should be taken to avoid transferring microbial contamination to the manufacturing or other testing processes. BI results in isolation should not be used to override other critical parameters and process design elements.

8.43. The reliability (validity) of biological indicators is important. Suppliers should be qualified and transportation and storage conditions should be controlled in order that BI quality is not compromised. Prior to use of a new batch/lot of BIs, the population, purity and identity of the indicator organism of the batch/lot should be verified. For other critical parameters, e.g. D-value, Z-value, the batch certificate provided by the qualified supplier can normally be used.

8.44. There should be a clear means of differentiating products, equipment and components, which have not been subjected to the sterilization process from those which have. Equipment such as baskets or trays used to carry products, other items of equipment and/or components should be clearly labelled (or electronically tracked) with the product name and 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 material, component, equipment) has passed through a sterilization process. However, these indicators show only that the sterilization process has occurred; they do not indicate product sterility or achievement of the required sterility assurance level.

8.45. Sterilization records should be available for each sterilization run. Each cycle should have a unique identifier. Their conformity should be reviewed and approved as part of the batch certification/release procedure.

8.46. Where required, 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 sterilized items are not used immediately after sterilization, these should be stored using appropriately sealed packaging and a maximum hold time should be established. Where justified, components that have been packaged with multiple sterile packaging layers need not be stored in a cleanroom if the integrity and configuration of the sterile pack allows the items to be readily disinfected during transfer by operators into grade A (e.g. by the use of multiple sterile coverings that can be removed at each transfer from lower to higher grade). Where protection is achieved by containment in sealed packaging, this packaging process should be undertaken prior to sterilization.

8.47. Where materials, equipment, components and ancillary items are sterilized in sealed packaging and then transferred into grade A, 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. The use of rapid transfer port technology should also be considered. These methods should be demonstrated to effectively control the potential risk of contamination of the grade A and grade B areas 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 B and grade A areas.

8.48. Where materials, equipment, components and ancillary items are sterilized in sealed packaging or containers, the packaging should be qualified for minimizing the risk of particulate, microbial, endotoxin/pyrogen or chemical contamination, and for compatibility with the selected sterilization method. The packaging sealing process should be validated. The validation should consider the integrity of the sterile protective barrier system, the maximum hold time before sterilization and the maximum shelf life assigned to the sterilized items. The integrity of the sterile protective barrier system for each of the sterilized items should be checked prior to use.

8.49. For materials, equipment, components and ancillary items that are not a direct or indirect product contact part and 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 programme.

Sterilization by heat

8.50. Each heat sterilization cycle should be recorded either electronically or by hardcopy, using equipment with suitable accuracy and precision. The system should have safeguards and/or redundancy in its control

and monitoring instrumentation to detect a cycle not conforming to the validated cycle parameter requirements and abort or fail this cycle (for example, by the use of duplex/double probes connected to independent control and monitoring systems).

8.51. The position of the temperature probes used for controlling and/or recording should be determined during the validation and selected based on system design and in order to correctly record and represent routine cycle conditions. Validation studies should be designed to demonstrate the suitability of system control and recording probe locations, and should include the verification of the function and location of these probes by the use of an independent monitoring probe located at the same position during validation.

8.52. The whole of the load should reach the required temperature before measurement of the sterilizing time-period starts. For sterilization cycles controlled by using a reference probe within the load, specific consideration should be given to ensuring the load probe temperature is controlled within defined temperature range prior to cycle commencement.

8.53. After completion of the high temperature phase of a heat sterilization cycle, precautions should be taken against contamination of a sterilized load during cooling. Any cooling liquid or gas that comes into contact with the product or sterilized material should be sterilized.

8.54. 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. Further guidance regarding parametric release is provided in Annex No. 17 of the Rules.

Moist heat sterilization

8.55. Moist heat sterilization can be achieved using steam, (direct or indirect contact), but also includes other systems such as superheated water

systems (cascade or immersion cycles) that could be used for containers that may be damaged by other cycle designs (for example, Blow-Fill-Seal containers, plastic bags).

8.56. The items to be sterilized, other than products in sealed containers, should be dry, packaged in a protective barrier system which allows removal of air and penetration of steam and prevents recontamination after sterilization. All loaded items should be dry upon removal from the sterilizer. Load dryness should be confirmed by visual inspection as a part of the sterilization process acceptance.

8.57. For porous cycles (hard goods), time, temperature and pressure should be used to monitor the process and be recorded. Each sterilized item should be inspected for damage, packaging material integrity and moisture on removal from the autoclave. Any item found not to be fit for purpose should be removed from the manufacturing area and an investigation performed.

8.58. For autoclaves capable of performing pre-vacuum sterilization cycles, the temperature should be recorded at the chamber drain throughout the sterilization period. Load probes may also be used where appropriate but the controlling system should remain related to the load validation. For steam in place systems, the temperature should be recorded at appropriate condensate drain locations throughout the sterilization period.

8.59. Validation of porous cycles should include a calculation of equilibration time, exposure time, correlation of pressure and temperature and the minimum/maximum temperature range during exposure. Validation of cycling with liquids shall include temperature, time, and/or F_0. Critical processing parameters should be subject to defined limits (including appropriate tolerances) and be confirmed as part of the sterilization validation and routine cycle acceptance criteria.

8.60. Leak tests on the sterilizer should be carried out periodically (normally weekly) when a vacuum phase is part of the cycle or the system is returned, post-sterilization, to a pressure lower than the environment surrounding the sterilizer.

8.61. There should be adequate assurance of air removal prior to and during sterilization when the sterilization process includes air purging (e.g. porous autoclave loads, lyophilizer chambers). For autoclaves, this should include an air removal test cycle (normally performed on a daily basis) or the use of an air detector system. Loads to be sterilized should be designed to support effective air removal and be free draining to prevent the build-up of condensate.

8.62. Distortion and damage of non-rigid containers that are terminally sterilized, such as containers produced by Blow-Fill-Seal (BFS) or Form-Fill-Seal (FFS) technologies, should be prevented by appropriate cycle design and control (for instance setting correct pressure, heating and cooling rates and loading patterns).

8.63. Where steam in place systems are used for sterilization (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 locations during routine use to ensure all areas are effectively and reproducibly sterilized. These locations should be demonstrated as being representative of, and correlated with, the slowest to heat locations during initial and routine validation. Once a system has been sterilized by steam in place, it should remain integral and where operations require, maintained under positive pressure or otherwise equipped with a sterilizing vent filter prior to use.

8.64. In fluids load cycles where superheated water is used as the heat transfer medium, the heated water should consistently reach all of the required contact points. Initial qualification studies should include temperature mapping of the entire load. There should be routine checks on the equipment to ensure that nozzles (where the water is introduced) are not blocked and drains remain free from debris.

8.65. Validation of the sterilization of fluids loads in a superheated water autoclave should include temperature mapping of the entire load and heat penetration and reproducibility studies. All parts of the load should heat up uniformly and achieve the desired temperature for the specified time. Routine temperature monitoring probes should be correlated to the worst case positions identified during the qualification process.

Dry heat sterilization

8.66. Dry heat sterilization utilizes high temperatures of air or gas to sterilize a product or article. Dry heat sterilization is of particular use in the thermal removal of difficult-to-eliminate thermally robust contaminants such as endotoxin/pyrogen and is often used in the preparation of components for aseptic filling. The combination of time and temperature to which product, components or equipment are exposed should produce an adequate and reproducible level of lethality and/or endotoxin/pyrogen inactivation/removal when operated routinely within the established limits. The process may be operated in an oven or in a continuous tunnel process, e.g. for sterilization and depyrogenation of glass containers.

8.67. Dry heat sterilization/depyrogenation tunnels should be configured to ensure that airflow protects the integrity and performance of the grade A sterilizing zone by maintaining appropriate pressure differentials and airflow through the tunnel. Air pressure difference profiles should be assessed. The impact of any airflow change should be assessed to ensure the heating profile

is maintained. All air supplied to the tunnel should pass through at least a HEPA filter and periodic tests (at least biannually) should be performed to demonstrate air 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 are not limited to:

i. Belt speed or dwell time within the sterilizing zone

ii. Temperature — minimum and maximum temperatures

iii. Heat penetration into the material/article

iv. Heat distribution (uniformity)

v. Airflows determined by air pressure difference profiles correlated with the heat distribution and penetration studies

8.68. When a thermal process is used as part of the depyrogenation process for any component or product contact equipment/material, validation studies should be performed to demonstrate that the process provides a suitable F_h value and results in a minimum 3 $\log_{10}$ reduction in endotoxin concentration. When this is attained, there is no additional requirement to demonstrate sterilization in these cases.

8.69. Containers spiked with endotoxin should be used during validation and should be carefully managed with a full reconciliation performed. Containers should be representative of the materials normally processed (in respect to composition of the packaging materials, porosity, dimensions, nominal volume). Endotoxin quantification and recovery efficiency should also be demonstrated.

8.70. Dry heat ovens are typically employed to sterilize or depyrogenate primary packaging components, starting materials or active substances but may be used for other processes. They should be maintained at a positive pressure relative to lower grade clean areas throughout the sterilization and

post sterilization hold process unless the integrity of the packaging is maintained. All air entering the oven should pass through a HEPA filter. Critical process parameters that should be considered in qualification and/or routine processing should include, but are not limited to:

i. Temperature

ii. Exposure period (time)

iii. Chamber pressure (for maintenance of over pressure)

iv. Air speed

v. Air quality within the oven

vi. Heat penetration of material/article (slow to heat spots)

vii. Heat distribution (uniformity)

viii. Load pattern and configuration of articles to be sterilized/depyrogenated including minimum and maximum loads.

Sterilization by radiation

8.71. Sterilization by radiation is used mainly for the sterilization of heat sensitive materials and products. Ultraviolet irradiation is not an acceptable method of sterilization. Guidance regarding ionizing radiation sterilization can be found within Annex No. 12 to the Rules.

8.72. Validation procedures should ensure that the effects of variation in density of the product and packages are considered.

Sterilization with ethylene oxide

8.73. 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 result in the reduction of any residual ethylene oxide gas and reaction products to defined acceptable limits for the given product or material.

8.74. 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, porosity and quantity of packaging materials can significantly affect the process.

8.75. Before exposure to the gas, materials should be brought into equilibrium with the humidity and temperature required by the process. Where steam is used to condition the load for sterilization, it should be of an appropriate quality. The time required for this should be balanced against the opposing need to minimize the time before sterilization.

8.76. Each sterilization cycle should be monitored with suitable Bis, using the appropriate number of test units distributed throughout the load at defined locations that have been shown to be worst case locations during validation.

8.77. Critical process parameters that could be considered as part of the sterilization process validation and routine monitoring include, but are not limited to:

i. Concentration of ethylene oxide
ii. Pressure
iii. Quantity of ethylene oxide gas used
iv. Relative humidity
v. Temperature
vi. Exposure time

8.78. After sterilization, the load should be aerated to allow ethylene oxide 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 ethylene oxide sterilization process validation.

Filter sterilization of products which cannot be sterilized in their final container

8.79. If the product cannot be sterilized in its final container, solutions or liquids should be sterilized by filtration through a sterile sterilizing grade filter (with a nominal pore size of a maximum of 0.22 μm that has been appropriately validated to obtain a sterile filtrate) and subsequently aseptically filled into a previously sterilized container. The selection of the filter used should ensure that it is compatible with the product and as described in the marketing authorization (see paragraph 8.135).

8.80. Suitable bioburden reduction prefilters 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 final sterilizing filter. Due to the potential additional risks of a sterile filtration process, as compared with other sterilization processes, an additional filtration through a sterile sterilizing grade filter, as close to the point of fill as possible, should be considered as part of an overall contamination control strategy.

8.81. The selection of components for the filtration system and their interconnection and arrangement within the filtration system, including pre-filters, should be based on the critical quality attributes of the product, justified and documented. The filtration system should minimize the generation of fibers and particles, not cause or contribute to unacceptable levels of impurities, or possess characteristics that otherwise alter the quality and efficacy of the product. Similarly, the filter characteristics should be compatible with the fluid and not be adversely affected by the product to be filtered. Adsorption of product components and extraction/leaching of filter components should be evaluated (see paragraph 8.135).

8.82. The filtration system should be designed to:

i. Allow operation within validated process parameters;

ii. Maintain the sterility of the filtrate;

iii. Minimize the number of aseptic connections required between the final sterilizing grade filter and the final filling of the product;

iv. Allow cleaning procedures to be conducted as necessary;

v. Allow sterilization procedures, including sterilization in place, to be conducted as necessary;

vi. Permit in-place integrity testing, of the 0.22 pm final sterilizing grade filter, preferably as a closed system, both prior to, and following filtration as necessary. In-place integrity testing methods should be selected to avoid any adverse impact on the quality of the product.

8.83. Sterile filtration of liquids should be validated in accordance with relevant Pharmacopoeia requirements. Validation can be grouped by different strengths or variations of a product but should be done under worst-case conditions. The rationale for grouping should be justified and documented.

8.84. During filter validation, wherever possible, the product to be filtered should be used for bacterial retention testing of the sterilizing grade filter. 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.

8.85. Filtration parameters that should be considered and established during validation should include, but are not limited to:

i. The wetting fluid used for filter integrity testing:

- It should be based on the filter manufacturer's recommendation or the fluid to be filtered. The appropriate integrity test value specification should be established;
- If the system is flushed or integrity tested in-situ with a fluid other than the product, appropriate actions are taken to avoid any deleterious effect on product quality.

ii. Filtration process conditions including:

- Fluid pre-filtration holding time and effect on bioburden;
- Filter conditioning, with fluid if necessary;
- Maximum filtration time/total time filter is in contact with the fluid;
- Maximum operating pressure;
- Flow rate;
- Maximum filtration volume;
- Temperature;
- The time taken to filter a known volume of bulk solution and the pressure difference to be used across the filter.

8.86. Routine process controls should be implemented to ensure adherence to validated filtration parameters. Results of critical process parameters should be included in the batch record, including but not limited to the minimum time taken to filter a known volume of bulk solution and pressure difference across the filter. Any significant difference from critical parameters during manufacturing should be documented and investigated.

8.87. The integrity of the sterilized filter assembly should be verified by integrity testing before use (pre-use post sterilization integrity test or PUPSIT). It should be done to check for damage and loss of integrity caused by the filter preparation prior to use. A sterilizing grade filter that is used to sterilize a fluid should be subject to a non-destructive integrity test post-use prior to removal of the filter from its housing. The integrity test process should be validated and test results should correlate to the microbial retention capability of the filter established during validation. Examples of tests that are used include bubble point, diffusive flow, water intrusion or pressure hold test. It is recognized that PUPSIT may not always be possible after sterilization due to process constraints (e.g. the filtration of very small volumes of solution). In these cases,

an alternative approach may be taken providing that a thorough risk assessment has been performed and compliance is achieved by the implementation of appropriate controls to mitigate any risk of a non-integral filtration system. Points to consider in such a risk assessment should include but are not limited to:

i. In depth knowledge and control of the filter sterilization process to ensure that the potential for damage to the filter is minimized;

ii. In depth knowledge and control of the supply chain to include:

- Contract sterilization facilities;
- Defined transport mechanisms;
- Packaging of the sterilized filter, to prevent damage to the filter during transportation and storage.

iii. In depth process knowledge such as:

- The specific product type, including particle burden and whether there exists any risk of impact on filter integrity values, such as the potential to alter integrity-testing values and therefore prevent the detection of a non-integral filter during a post-use filter integrity test;
- Pre-filtration and processing steps, prior to the final sterilizing grade filter, which would remove particle burden and clarify the product prior to the sterile filtration.

8.88. The integrity of critical sterile gas and air vent filters (that are directly linked to the sterility of the product) should be verified by testing after use, with the filter remaining in the filter assembly or housing.

8.89. The integrity of non-critical air or gas vent filters should be confirmed and recorded at appropriate intervals. Where gas filters are in place for extended periods, integrity testing should be carried out at installation and prior to replacement. The maximum duration of use should be specified and

monitored based on risk (e.g. considering the maximum number of uses and heat treatment/ sterilization cycles permitted as applicable).

8.90. For gas filtration, unintended moistening or wetting of the filter or filter equipment should be avoided.

8.91. If the sterilizing filtration process has been validated as a system consisting of multiple filters to achieve the sterility for a given fluid, the filtration system is considered to be a single sterilizing unit and all filters within the system should satisfactorily pass integrity testing after use.

8.92. In a redundant filtration system (where a second redundant sterilizing grade filter is present as a backup but the sterilizing process is validated as only requiring one filter), post-use integrity test of the primary sterilizing grade filter should be performed and if demonstrated to be integral, then a post-use integrity test of the redundant (backup) filter is not necessary. However, in the event of a failure of the post-use integrity test on the primary filter, post-use integrity test on the secondary (redundant) filter should be performed, in conjunction with an investigation and risk assessment to determine the reason for the primary filter test failure.

8.93. Bioburden samples should be taken from the bulk product and immediately prior to the final sterile filtration. In case where a redundant filtration set-up is used, it should be taken prior to the first filter. Systems for taking samples should be designed so as not to introduce contamination.

8.94. Liquid sterilizing grade filters should be discarded after the processing of a single batch and the same filter should not be used continuously for more than one working day unless such use has been validated.

8.95. Where campaign manufacture of a product has been appropriately justified in the contamination control strategy and validated, the filter user should:

i. Assess and document the risks associated with the duration of filter use for the sterile filtration process for a given fluid;

ii. Conduct and document effective validation and qualification studies to demonstrate that the duration of filter use for a given sterile filtration process and for a given fluid does not compromise performance of the final sterilizing grade filter or filtrate quality;

iii. Document the maximum validated duration of use for the filter and implement controls to ensure that filters are not used beyond the validated maximum duration. Records of these controls should be maintained.

iv. Implement controls to ensure that filters contaminated with fluid or cleaning agent residues, or considered defective in any other way, are removed from use.

Form-Fill-Seal (FFS)

8.96. The conditions for Form-Fill-Seal (FFS) machines used for terminally sterilized products should comply with the environmental requirements of paragraphs 8.3 and 8.4 of this Annex. The conditions for FFS machines used in aseptic manufacture should comply with the environmental requirements of paragraph 8.10 of this Annex.

8.97. Contamination of the packaging films used in the FFS process should be minimized by appropriate controls during component fabrication, supply and handling. Due to the criticality of packaging films, procedures should be implemented to ensure that the films supplied meet defined specifications and are of the appropriate quality, including material thickness and strength, microbial and particulate contamination, integrity and artwork, as relevant. The sampling frequency, the bioburden and, where applicable, endotoxin/pyrogen levels of packaging films and associated components should be defined and controlled within the PQS and considered in the contamination control strategy.

8.98. Particular attention should be given to understanding and assessing the operation of the equipment, including set-up, filling, sealing and cutting processes, so that critical process parameters are understood, validated, controlled and monitored appropriately.

8.99. Any product contact gases, e.g. those used to inflate the container or used as a product overlay, should be appropriately filtered, as close to the point of use as possible. The quality of gases used and the effectiveness of gas filtration systems should be verified periodically in accordance with paragraphs 6.18 and 6.19.

8.100. The controls identified during qualification of FFS should be in alignment with the contamination control strategy. Aspects to be considered include but are not limited to:

i. Determination of critical zone boundaries;

ii. Environmental control and monitoring, both of the machine and the background in which it is placed;

iii. Personnel gowning requirements;

iv. Integrity testing of the product filling lines and filtration systems (as relevant);

v. Duration of the batch or filling campaign;

vi. Control of packaging films, including any requirements for film decontamination or sterilization;

vii. Cleaning-in-place and sterilization-in-place of equipment as necessary;

viii. Machine operation, settings and alarm management (as relevant).

8.101. Critical process parameters for FFS should be determined during equipment qualification and should include, but are not limited to:

i. Settings for uniform package dimensions and cutting in accordance with validated parameters;

ii. Setting, maintenance and monitoring of validated forming temperatures (including preheating and cooling), forming times and pressures as relevant;

iii. Setting, maintenance and monitoring of validated sealing temperatures, sealing temperature uniformity across the seal, sealing times and pressures as relevant;

iv. Environmental and product temperature;

v. Batch-specific testing of package seal strength and uniformity;

vi. Settings for correct filling volumes, speeds and uniformity;

vii. Settings for any additional printing (batch coding), embossing or debossing to ensure that unit integrity and quality is not compromised;

viii. Methods and parameters for integrity testing of filled containers (see paragraph 8.22).

8.102. Appropriate procedures for the verification, monitoring and recording of FFS critical process parameters and equipment operation should be applied during production.

8.103. Operational procedures should describe how forming and sealing issues are detected and rectified. Rejected units or sealing issues should be recorded and investigated.

8.104. Appropriate maintenance procedures should be established based on risk, and include maintenance and inspection plans for tooling critical to the effectiveness of unit sealing. Any issues identified that indicate a potential product quality concern should be documented and investigated.

Blow-Fill-Seal (BFS)

8.105. Blow-Fill-Seal (BFS) equipment used for the manufacture of products which are terminally sterilized should be installed in at least a grade D environment. The conditions at the point of fill should comply with the environmental requirements of paragraphs 8.3 and 8.4.

8.106. BFS technology used for aseptic processing:

i. For shuttle type equipment used for aseptic filling, the parison is open to the environment and therefore the areas where parison extrusion, blow-molding and sealing take place should meet grade A conditions at the critical zones. The filling environment should be designed and maintained to meet grade A conditions for viable and total particle limits both at rest and when in operation;

ii. For rotary-type equipment used for aseptic filling, the parison is generally closed to the environment once formed, the filling environment within the parison should be designed and maintained to meet grade A conditions for viable and total particle limits both at rest and when in operation;

iii. The equipment should be installed in at least a grade C environment, provided that grade A/B clothing is used. The microbiological monitoring of operators wearing grade A/B clothing in a grade C area, should be performed in accordance with risk management principles, and the limits and monitoring frequencies applied with consideration of the activities performed by these operators.

8.107. Due to the generation of particles from polymer extrusion and cutting during operation, and the restrictive size of critical filling zones of BFS equipment, in operation monitoring of total particle for BFS equipment is not expected. However, data should be available to demonstrate that the design of the equipment ensures that critical zones of the filling process environment would meet grade A conditions in operation.

8.108. Viable environmental monitoring of BFS processes should be risk-based, and designed in accordance with Section 9 of this Annex. In operation viable monitoring should be undertaken for the full duration of critical processing, including equipment assembly. For rotary-type BFS

equipment, it is acknowledged that monitoring of the critical filling zone may not be possible.

8.109. The environmental control and monitoring programme should take into consideration the moving parts and complex airflow paths generated by the BFS process and the effect of the high heat outputs of the process, (e.g. through the use of airflow visualization studies and/or other equivalent studies). Environmental monitoring programmes should also consider factors such as air-filter configuration, air-filter integrity, cooling systems integrity (see paragraph 6.21), equipment design and qualification.

8.110. Air or other gases that make contact with critical surfaces of the container during extrusion, formation or sealing of the molded container should undergo appropriate filtration. The quality of gas used and the effectiveness of gas filtration systems should be verified periodically in accordance with paragraphs 6.18 and 6.19.

8.111. Particulate and microbial contamination of the polymer granulate should be prevented by appropriate design, control, and maintenance of the polymer granulate storage, sampling and distribution systems.

8.112. The capability of the extrusion system to provide appropriate sterility assurance for the molded container should be understood and validated. The sampling frequency, the bioburden and, where applicable, endotoxin/pyrogen levels of the raw polymer should be defined and controlled within the PQS and considered in the contamination control strategy.

8.113. Interventions requiring cessation of filling and/or extrusion, molding and sealing and, where required, re-sterilization of the filling machine should be clearly defined and described in the filling procedure, and included in the aseptic process simulation as relevant (see paragraphs 9.34, 9.35 and 9.36).

8.114. The controls identified during qualification of BFS should be in alignment with the site's contamination control strategy. Aspects to be considered include but are not limited to:

i. Determination of critical zone boundaries;

ii. Environmental control and monitoring, both of the machine and the background in which it is placed;

iii. Personnel gowning requirements;

iv. Integrity testing of the product filling lines and filtration systems (as relevant);

v. Duration of the batch or filling campaign;

vi. Control of polymer granulate, including distribution systems and critical extrusion temperatures;

vii. Cleaning-in-place and sterilization-in-place of equipment as necessary;

viii. Machine operation, settings and alarm management (as relevant).

8.115. Critical process parameters for BFS should be determined during equipment qualification and should include, but are not limited to:

i. Clean-in-place and sterilization-in-place of product pipelines and filling needles (mandrels);

ii. Setting, maintenance and monitoring of extrusion parameters, including temperature, speed and extruder throat settings for parison thickness;

iii. Setting, maintenance and monitoring of mold temperatures, including rate of cooling where necessary for product stability;

iv. Preparation and sterilization of ancillary components added to the molded unit, e.g. bottle caps;

v. Environmental control, cleaning, sterilization and monitoring of the critical extrusion, transfer and filling areas as relevant;

vi. Batch-specific testing of package wall-thickness at critical points of the container;

vii. Settings for correct filling volumes, speeds and uniformity;

viii. Settings for any additional printing (batch coding), embossing or debossing to ensure that unit integrity and quality is not compromised;

ix. Methods and parameters for integrity testing of 100% of all filled containers (see paragraph 8.22);

x. Settings for cutters or punches used to remove waste plastic surrounding filled units (flash removal).

8.116. Appropriate procedures for the verification, monitoring and recording of BFS critical process parameters and equipment operation should be applied during production.

8.117. Operational procedures should describe how blowing, forming and sealing issues are detected and rectified. Rejected units or sealing issues should be recorded and investigated.

8.118. Where the BFS process includes the addition of components to molded containers (e.g. addition of caps to Large Volume Parenteral bottles), these components should be appropriately decontaminated and added to the process using a clean, controlled process.

i. For aseptic processes, the addition of components should be performed under grade A conditions, to ensure the sterility of critical surfaces, using pre-sterilized components.

ii. For terminally sterilized products, the validation of terminal sterilization processes should ensure the sterility of all critical product pathways between the component and molded container, including areas that are not wetted during sterilization.

iii. Testing procedures should be established and validated to ensure the effective sealing of components and molded containers.

8.119. Appropriate maintenance procedures should be established based on risk, and include maintenance and inspection plans for items critical to unit sealing, integrity and sterility.

8.120. The molds used to form containers are considered critical equipment and any changes or modification to molds should result in an assessment of finished product container integrity, and where the assessment indicates, should be supported by validation. Any issues identified that indicate a potential product quality concern should be documented and investigated.

Lyophilization

8.121. 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 the sterilized product. The lyophilization equipment and its processes should be designed to ensure that product or material sterility is maintained during lyophilization by preventing microbial and particle contamination between the filling of products for lyophilization, and completion of lyophilization process. All control measures in place should be determined by the site's contamination control strategy.

8.122. The sterilization of the lyophilizer and associated equipment (e.g. trays, vial support rings) should be validated and the holding time between the sterilization cycle and use appropriately challenged during aseptic process simulation (see paragraph 9.33). The lyophilizer should be sterilized regularly, based on system design. Re-sterilization should be performed following maintenance or cleaning. Sterilized lyophilizers and associated equipment should be protected from contamination after sterilization.

8.123. Lyophilizers and associated product transfer and loading/unloading areas should be designed to minimize operator intervention as far as possible. The frequency of lyophilizer sterilization should be determined based on the design and risks related to system contamination

during use. Lyophilizers that are manually loaded or unloaded with no barrier technology separation should be sterilized before each load. For lyophilizers loaded and unloaded by automated systems or protected by closed barrier systems, the frequency of sterilization should be justified and documented as part of the contamination control strategy.

8.124. The integrity of the lyophilizer should be maintained following sterilization and during lyophilization. The filter used to maintain lyophilizer integrity should be sterilized before each use of the system and its integrity testing results should be part of the batch certification/release. The frequency of vacuum/leak integrity testing of the chamber should be documented and the maximum permitted leakage of air into the lyophilizer should be specified and checked at the start of every cycle.

8.125. Lyophilization trays should be checked regularly to ensure that they are not misshapen or damaged.

8.126. Points to consider for the design of loading (and unloading, where the lyophilized material is still unsealed and exposed), include but are not limited to:

i. The loading pattern within the lyophilizer should be specified and documented;

ii. The transfer of partially closed containers to a lyophilizer should be undertaken under grade A conditions at all times and handled in a manner designed to minimize direct operator intervention. Technologies such as conveyor systems or portable transfer systems (e.g. clean air transfer carts, portable unidirectional airflow workstations) should be used to ensure that the cleanliness of the system used to transfer the partially closed containers is maintained. Alternatively, where supported by validation, trays closed in grade A and not reopened whilst in the grade B area may be used to protect partially stoppered vials (e.g. appropriately closed boxes);

iii. Airflow patterns should not be adversely affected by transport devices and venting of the loading zone;

iv. Unsealed containers (such as partially stoppered vials) should be maintained under grade A conditions and should normally be separated from operators by physical barrier technology or any other appropriate measures;

v. Where seating of the stoppers is not completed prior to opening the lyophilizer chamber, product removed from the lyophilizer should remain under grade A conditions during subsequent handling;

vi. Utensils used during loading and unloading of the lyophilizer (e g. trays, bags, placing devices, tweezers) should be sterile.

Closed systems

8.127. The use of closed systems can reduce the risk of microbial, particle and chemical contamination from the adjacent environment. Closed systems should always be designed to reduce the need for manual manipulations and the associated risks.

8.128. It is critical to ensure the sterility of all product contact surfaces of closed systems used for aseptic processing. The design and selection of any closed system used for aseptic processing should ensure maintenance of sterility. Connection of sterile equipment (e.g. tubing/pipework) to the sterilized product pathway after the final sterilizing grade filter should be designed to be connected aseptically (e.g. by intrinsic sterile connection devices).

8.129. Appropriate measures should be in place to ensure the integrity of components used in aseptic connections. The means by which this is achieved should be determined and captured in the contamination control strategy. Appropriate system integrity tests should be considered when there is a risk of compromising product sterility. Supplier assessment should include

the collation of data in relation to potential failure modes that may lead to a loss of system sterility.

8.130. The background environment in which closed systems are located should be based on their design and the processes undertaken. For aseptic processing and where there are any risks that system integrity may be compromised, the system should be located in grade A. If the system can be shown to remain integral at every usage (e.g. via pressure testing and/or monitoring) then a lower classified area may be used. Any transfer between classified areas should be thoroughly assessed (see paragraph 4.10). If the closed system is opened (e.g. for maintenance of a bulk manufacturing line) then this should be performed in a classified area appropriate to the materials (e.g. grade C for terminal sterilization processes, or grade A for aseptic processing) or be subject to further cleaning and disinfection (and sterilization in case of aseptic processes).

Single-Use Systems (SUS)

8.131. SUS are those technologies used in manufacture of sterile products which are used as an alternative to reusable equipment. SUS can be individual components or made up of multiple components such as bags, filters, tubing, connectors, valves, storage bottles and sensors. Single use systems should be designed to reduce the need for manipulations and complexity of manual interventions.

8.132. There are some specific risks associated with SUS which should be assessed as part of the contamination control strategy. These risks include but are not limited to:

i. The interaction between the product and product contact surface (such as adsorption, or leachables and extractables);

ii. The fragile nature of the system compared with fixed reusable systems;

iii. The increase in the number and complexity of manual operations (including inspection and handling of the system) and connections made;

iv. The complexity of the assembly;

v. The performance of the pre- and post-use integrity testing for sterilizing grade filters (see paragraph 8.87);

vi. The risk of holes and leakage;

vii. The potential for compromising the system at the point of opening the outer packaging;

viii. The risk of particle contamination.

8.133. Sterilization processes for SUS should be validated and shown to have no adverse impact on system performance.

8.134. Assessment of suppliers of disposable systems including sterilization is critical to the selection and use of these systems. For sterile SUS, verification of sterility assurance should be performed as part of the supplier qualification and evidence of sterilization of each unit should be checked on receipt.

8.135. The adsorption and reactivity of the product with product contact surfaces should be evaluated under process conditions.

8.136. The extractable and leachable profiles of the SUS and any impact on the quality of the product especially where the system is made from polymer-based materials should be evaluated. An assessment should be carried out for each component to evaluate the applicability of the extractable profile data. For components considered to be at high risk from leachables, including those that may absorb processed materials or those with extended material contact times, an assessment of leachable profile studies, including safety concerns, should be taken into consideration. If applying simulated processing conditions, these should accurately reflect the actual processing conditions and be based on a scientific rationale.

8.137. SUS should be designed to maintain integrity throughout processing under the intended operational conditions. Attention to the structural integrity of the single use components is necessary where these may be exposed to more extreme conditions (e.g. freezing and thawing processes) either during routine processing or transportation. This should include verification that intrinsic sterile connection devices (both heat sealed and mechanically sealed) remain integral under these conditions.

8.138. Acceptance criteria should be established and implemented for SUS corresponding to the risks or criticality of the products and its processes. On receipt, each piece of SUS should be checked to ensure that they have been manufactured, supplied and delivered in accordance with the approved specification. A visual inspection of the outer packaging (e.g. appearance of exterior carton, product pouches), label printing, and review of attached documents (e.g. certificate of conformance and proof of sterilization) should be carried out and documented prior to use.

8.139. Critical manual handling operations of SUS such as assembly and connections should be subject to appropriate controls and verified during aseptic process simulation.

9. Environmental and Process Monitoring

General information

9.1. The site’s environmental and process monitoring programme forms part of the overall contamination control strategy and is used to monitor the controls designed to minimize the risk of microbial and particle contamination. It should be noted that the reliability of each of the elements of the monitoring system (viable, non- viable and aseptic process simulation) when taken in isolation is limited and should not be considered individually to be an indicator

of asepsis. When considered together, the results help confirm the reliability of the design, validation and operation of the system that they are monitoring.

9.2. This programme is typically comprised of the following elements:

i. Environmental monitoring — total particle;

ii. Environmental and personnel monitoring — viable particle;

iii. Temperature, relative humidity and other specific characteristics;

iv. APS (aseptically manufactured product only).

9.3. The information from these systems should be used for routine batch certification/release and for periodic assessment during process review or investigation. This applies for both terminal sterilization and aseptic processes, however, the criticality of the impact may differ depending upon the product and process type.

Environmental and Process Monitoring

9.4. An environmental monitoring programme should be established and documented. The purpose of the environmental monitoring programme, is to:

i. Provide assurance that cleanrooms and clean air equipment continue to provide an environment of appropriate air cleanliness, in accordance with design and regulatory requirements;

ii. Effectively detect excursions from environmental limits triggering investigation and assessment of risk to product quality.

Risk assessments should be performed in order to establish this comprehensive environmental monitoring programme, i.e. sampling locations, frequency of monitoring, monitoring methods and incubation conditions (e.g. time, temperature(s), aerobic and/or anaerobic conditions).

These risk assessments should be conducted based on detailed knowledge of; the process inputs and final product, the facility, equipment, the criticality of specific processes and steps, the operations involved, routine

monitoring data, monitoring data obtained during qualification and knowledge of typical microbial flora isolated from the environment.

The risk assessment should include the determination of critical monitoring locations, those locations where the presence of microorganisms during processing may have an impact upon product quality, (e.g. grade A, aseptic processing areas and the grade B areas that directly interface with the grade A area). Consideration of other information such as air visualization studies should also be included. These risk assessments should be reviewed regularly in order to confirm the effectiveness of the site's environmental monitoring programme. The monitoring programme should be considered in the overall context of the trend analysis and the contamination control strategy for the site.

9.5. Routine monitoring of cleanrooms, clean air equipment and personnel should be performed in operation throughout all critical stages of processing, including equipment set-up.

9.6. Other characteristics, such as temperature and relative humidity, should be controlled within ranges that align with product/processing/personnel requirements and support maintenance of defined cleanliness standards (e.g. grade A or B).

9.7. The monitoring of grade A should demonstrate the maintenance of aseptic processing conditions during critical operations. Monitoring should be performed at locations posing the highest risk of contamination to the sterile equipment surfaces, containers, closures and product. The selection of monitoring locations and the orientation and positioning of sampling devices should be justified and appropriate to obtain reliable data from the critical zones.

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

9.9. Appropriate alert levels and action limits should be set for the results of viable and total particle monitoring. The maximum total particle action limits are described in Table 5 and the maximum viable particle action limits are described in Table 6. However, more stringent action limits may be applied based on data trending, the nature of the process or as determined within the contamination control strategy. Both viable and total particle alert levels should be established based on results of cleanroom qualification tests and periodically reviewed based on ongoing trend data.

9.10. Alert levels for grade A (total particle only) grade B, grade C and grade D should be set such that adverse trends (e g. a numbers of events or individual events that indicate a deterioration of environmental control) are detected and addressed.

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

i. Increasing numbers of excursions from action limits or alert levels;

ii. Consecutive excursions from alert levels;

iii. Regular but isolated excursion from action limits that may have a common cause, (e.g. single excursions that always follow planned preventative maintenance);

iv. Changes in microbial flora type and numbers and predominance of specific organisms. Particular attention should be given to organisms recovered that may indicate a loss of control, deterioration in cleanliness or organisms that may be difficult to control such as spore-forming microorganisms and mold fungi.

9.12. The monitoring of grade C and D cleanrooms in operation should be performed based on data collected during qualification and routine data to allow effective trend analysis. The requirements of alert levels and action

limits will depend on the nature of the operations carried out. Action limits may be more stringent than those listed in Table 5 and Table 6.

9.13. If action limits are exceeded, operating procedures should prescribe a root cause investigation, an assessment of the potential impact to product (including batches produced between the monitoring and reporting) and requirements for corrective and preventive actions. If alert levels are exceeded, operating procedures should prescribe assessment and follow-up, which should include consideration of an investigation and/or corrective actions to avoid any further deterioration of the environment.

Environmental monitoring. Total particle content

9.14. A total particle monitoring program should be established to obtain data for assessing potential contamination risks and to ensure the maintenance of the environment for sterile operations in a qualified state.

9.15. The limits for environmental monitoring of airborne particle concentration for each graded area are given in Table 5.

Table 5:

Maximum permitted total particle concentration for monitoring.

Grade	Maximum limits for total particle ≥ 0.5 μm/m^3		Maximum limits for total particle ≥ 5 μm/m^3	
	at rest	in operation	at rest	in operation
A	3 520	3 520	29	29
B	3 520	352 000	29	2 930
C	352 000	3 520 000	2 930	29 300
D	3 520 000	Not predetermined[(a)]	29 300	Not predetermined[(a)]

[(a)] For grade D, in operation limits are not predetermined. The manufacturer should establish in operation limits based on a risk assessment and on routine data, where applicable.

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 (guidance value of less than 20 minutes) in an unmanned state, after the completion of operations (see paragraph 4.29).

Note 2: The occasional indication of macro particle counts, especially ≥ 5 μm, within grade A may be considered to be false counts due to electronic noise, stray light, coincidence loss, 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 system,

equipment failure, or may also be diagnostic of poor practices during machine set-up and routine operation.

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

9.17. The grade A area should be monitored continuously (for particles ≥0.5 and ≥5 μm) and with a suitable sample flow rate (at least 28 liters (1 ft^3) per minute) so that all interventions, transient events and any system deterioration is captured. The system should frequently correlate each individual sample result with alert levels and action limits at such a frequency that any potential excursion can be identified and responded to in a timely manner. Alarms should be triggered if alert levels are exceeded. Procedures should define the actions to be taken in response to alarms including the consideration of additional microbial monitoring.

9.18. It is recommended that a similar system be used for the grade B area although the sample frequency may be decreased. The grade B area should be monitored at such a frequency and with suitable sample size that the programme captures any increase in levels of contamination and system deterioration. If alert levels are exceeded, alarms should be triggered.

9.19. The selection of the monitoring system should take into account any risk presented by the materials used in the manufacturing operation (e.g. those involving live organisms, powdery products or radiopharmaceuticals) that may give rise to biological, chemical or radiation hazards.

9.20. In the case where contaminants are present due to the processes involved and would potentially damage the particle counter or present a hazard (e.g. live organisms, powdery products and radiation hazards), the frequency and strategy employed should be such as to assure the environmental classification both prior to and post exposure to the risk. An increase in viable particle monitoring should be considered to ensure comprehensive monitoring

of the process. Additionally, monitoring should be performed during simulated operations. Such operations should be performed at appropriate intervals. The approach should be defined in the contamination control strategy.

9.21. The size of monitoring samples taken 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 classification of cleanrooms and clean air equipment. Monitoring sample volumes should be justified.

Environmental and personnel monitoring. Viable particles

9.22. Where aseptic operations are performed, microbial monitoring should be frequent using a combination of methods such as settle plates, volumetric air sampling, glove, gown and surface sampling (e.g. swabs and contact plates). The method of sampling used should be justified within the contamination control strategy and should be demonstrated not to have a detrimental impact on grade A and B airflow patterns. Cleanroom and equipment surfaces should be monitored at the end of an operation.

9.23. Viable particle monitoring should also be performed within the cleanrooms when normal manufacturing operations are not occurring (e.g. post disinfection, prior to start of manufacturing, on completion of the batch and after a shutdown period), and in associated rooms that have not been used, in order to detect potential incidents of contamination which may affect the controls within the cleanrooms. In case of an incident, additional sample locations may be used as a verification of the effectiveness of a corrective action (e.g. cleaning and disinfection).

9.24. Continuous viable air monitoring in grade A (e.g. air sampling or settle plates) should be undertaken for the full duration of critical processing, including equipment (aseptic set-up) assembly and critical processing. A similar approach should be considered for grade B cleanrooms based on the

risk of impact on the aseptic processing. 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.

9.25. A risk assessment should evaluate the locations, type and frequency of personnel monitoring based on the activities performed and the proximity to critical zones. Monitoring should include sampling of personnel at periodic intervals during the process. Sampling of personnel should be performed in such a way that it will not compromise the process. Particular consideration should be given to monitoring personnel following involvement in critical interventions (at a minimum gloves, but may require monitoring of areas of gown as applicable to the process) and on each exit from the grade B cleanroom (gloves and gown). Where monitoring of gloves is performed after critical interventions, the outer gloves should be replaced prior to continuation of activity. Where monitoring of gowns is required after critical interventions, the gown should be replaced before further activity in the cleanroom.

9.26. Microbial monitoring of personnel in the grade A and grade B areas should be performed. Where operations are manual in nature (e.g. aseptic compounding or filling), the increased risk should lead to enhanced emphasis placed on microbial monitoring of gowns and justified within the contamination control strategy.

9.27. Where monitoring is routinely performed by manufacturing personnel, this should be subject to regular oversight by the quality unit (refer also to paragraph 8.19).

9.28. The adoption of suitable alternative monitoring systems such as rapid methods should be considered by manufacturers in order to expedite the detection of microbiological contamination issues and to reduce the risk to product. These rapid and automated microbial monitoring methods may be

adopted after validation has demonstrated their equivalency or superiority to the established methods.

9.29. Sampling methods and equipment used should be fully understood and procedures should be in place for the correct operation and interpretation of results obtained. Supporting data for the recovery efficiency of the sampling methods chosen should be available.

9.30. Action limits for viable particle contamination are shown in Table 6.

Table 6:

Maximum action limits for viable particle contamination

Grade	Air sample, CFU/m^3	Settle plates (diam. 90 mm) CFU /4 hours[(a)]	Contact plates (diameter - 55 mm), CFU per plate[(b)]	Glove print, Incl. 5 fingers on both hands, CFU / glove
A	No growth[(c)]			
B	10	5	5	5
C	100	50	25	-
D	200	100	50	-

[(a)] - Settle plates should be exposed to the media for the duration of operations (including equipment set-up) and changed as required after a maximum of 4 hours (exposure time should be based on validation including recovery studies and it should not have any negative effect on the suitability of the media used).
- For grade C and D areas, exposure time (with a maximum of 4 hours) and frequency should be based on QRM.
- Individual settle plates may be exposed for less than 4 hours.
[(b)] Contact plate limits apply to equipment, room and gown surfaces within the grade A and grade B areas. Routine gown monitoring is not normally required for grade C and D areas, depending on their function.
[(c)] It should be noted that for Class A, the presence of any growth should lead to conducting an investigation.
Note 1: It should be noted that the types of monitoring methods listed in the table above are examples and other methods can be used provided they meet the intent of providing information across the whole of the critical process where product may be contaminated (e.g. aseptic line set-up, aseptic processing, filling and lyophilizer loading).
Note 2: Limits are applied using CFU throughout the document. If different or new technologies are used that present results in a manner different from CFU, the manufacturer should scientifically justify the limits applied and where possible correlate them to CFU.

9.31. Microorganisms detected in the grade A and grade B areas should be identified to species level and the potential impact of such microorganisms on product quality (for each batch implicated) and overall state of control should be evaluated. Consideration should also be given to the identification of microorganisms detected in grade C and D areas (for example where action limits or alert levels are exceeded) or following the isolation of organisms that may indicate a loss of control, deterioration in cleanliness or that may be difficult to control such as spore-forming microorganisms and molds. Such work needs to be carried out at a sufficient frequency to maintain a current understanding of the typical flora of these areas.

Aseptic Process Simulation (APS) (also known as media fill)

9.32. Periodic verification of the effectiveness of the controls in place for aseptic processing should include an APS using a sterile nutrient media and/or surrogate in place of the product. The aseptic process simulation should not be considered as the primary means to validate the aseptic process or aspects of the aseptic process. The effectiveness of the aseptic process should be determined through process design, adherence to the pharmaceutical quality system and process controls, training, and evaluation of monitoring data. The selection of an appropriate nutrient medium and/or surrogate should be based on the ability of the medium and/or surrogate to mimic the physical characteristics of the product, assessed for the possibility of risks to product sterility during the aseptic process. Where processing stages may indirectly impact the viability of any introduced microbial contamination, (e.g. aseptically produced semi-solids, powders, solid materials, microspheres, liposomes and other formulations where product is cooled or heated or lyophilized), alternative procedures that represent the operations as closely as possible should be developed. Where surrogate materials, such as buffers, are

used in parts of the APS, the surrogate material should not inhibit the growth of any potential contamination.

9.33. The APS should imitate as closely as possible the routine aseptic manufacturing process and include all the critical manufacturing steps, specifically:

i. The APS should assess all aseptic operations performed subsequent to the sterilization and decontamination cycles of materials utilized in the process to the point where the container is sealed;

ii. For non-filterable formulations, any additional aseptic steps should be assessed;

iii. Where aseptic manufacturing is performed under an inert atmosphere, the inert gas should be substituted with air in the process simulation unless anaerobic simulation is intended;

iv. Processes requiring the addition of sterile powders should use an acceptable surrogate material in the same containers as those used in the process under evaluation;

v. Separate simulations of individual unit operations (e.g. processes involving drying, blending, milling and subdivision of a sterile powder) should be avoided. Any use of individual simulations should be supported by a documented justification and ensure that the sum total of the individual simulations continues to fully cover the whole process;

vi. The process simulation procedure for lyophilized products should represent the entire aseptic processing chain including filling, transport, loading, a representative duration of the chamber dwell, unloading and sealing under specified, documented and justified conditions representing worst case operating parameters;

vii. The lyophilization process simulation should mimic all aspects of the process, except those that may affect the viability or recovery of

contaminants. For instance, boiling-over or actual freezing of the solution should be avoided. Factors to consider in determining APS design include, where applicable:

- The use of air to break vacuum instead of nitrogen or other process gases;
- Replicating the maximum interval between sterilization of the lyophilizer and its use;
- Replicating the maximum period of time between filtration and lyophilization;
- Quantitative aspects of worst-case situations, e g. loading the largest number of trays, replicating the longest duration of loading where the chamber is open to the environment.

9.34. The APS should take into account various aseptic manipulations and interventions known to occur during normal production as well as worst-case situations. It should also take into account the following:

i. Inherent and corrective interventions representative of the routine process should be performed in a manner and frequency similar to that during the routine aseptic process;

ii. The inclusion and frequency of interventions in the APS should be based on assessed risks posed to product sterility.

9.35. APS should not be used to justify practices that pose unnecessary contamination risks.

9.36. In developing the APS plan, consideration should be given to the following:

i. Identification of worst case conditions covering the relevant variables, such as container size and line speed, and their impact on the process. The outcome of the assessment should justify the variables selected.

ii. Determining the representative sizes of container/closure combinations to be used for validation. Bracketing or matrix approach may be considered for validation of the same container/closure configuration for different products where process equivalence is scientifically justified;

iii. Maximum permitted holding times for sterile product and equipment exposed during the aseptic process;

iv. 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. The volume used should provide sufficient headspace to support potential microbial growth and ensure that turbidity can be detected during inspection;

v. The requirement for substitution of any inert gas used in the routine aseptic manufacturing process by air unless anaerobic simulation is intended. In these situations, inclusion of occasional anaerobic simulations as part of the overall validation strategy should be considered (see paragraph 9.33, indent iii).

vi. The selected nutrient media should be capable of growing a designated group of reference microorganisms as described by the relevant pharmacopoeia and suitably representative local isolates;

vii. The method of detection of microbial contamination should be scientifically justified to ensure that contamination is reliably detected;

viii. The process simulation should be of sufficient duration to challenge the process, the operators that perform interventions, shift changes and the capability of the processing environment to provide appropriate conditions for the manufacture of a sterile product;

ix. Where the manufacturer operates different or extended shifts, the aseptic process simulation should be designed to capture factors specific to

those shifts that are assessed to pose a risk to product sterility (for example, the maximum duration for which an operator may be present in the cleanroom);

x. Simulating normal aseptic manufacturing interruptions where the process is idle (e.g. shift changeovers, recharging dispensing vessels, introduction of additional equipment);

xi. Ensuring that environmental monitoring is conducted as required for routine production, and throughout the entire duration of the process simulation;

xii. 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;

xiii. The performance of "end of production or campaign APS" may be used as additional assurance or investigative purposes. However, their use should be justified in the contamination control strategy and should not replace routine aseptic imitation process. If used, it should be demonstrated that any residual product does not negatively impact the recovery of any potential microbial contamination.

9.37. For sterile active substances, batch size should be large enough to represent routine operation, simulate intervention operation at the worst case, and cover all surfaces that may come into contact with the sterile product. In addition, all the simulated materials (surrogates or growth medium) should be subjected to microbial evaluation. The simulation materials should be sufficient to satisfy the evaluation of the process being simulated and should not compromise the recovery of microorganisms.

9.38. APS should be performed as part of the initial validation, with at least three consecutive satisfactory simulation tests that cover all working shifts that the aseptic process may occur therein. However, after any significant modification to operational practices, facilities, services or equipment which are assessed to have an impact on the sterility assurance of the product (e.g. modification to the HVAC system, equipment, changes to process, number of shifts and numbers of personnel, major facility shut down). Normally, APS (periodic revalidation) should be repeated twice a year (approximately every six months) for each aseptic process, each filling line and each shift. Each operator should participate in at least one successful APS annually. Consideration should be given to performing an aseptic process simulation after the last batch prior to shut down, before long periods of inactivity or before decommissioning or relocation of a line.

9.39. Where manual operation (e.g. aseptic compounding or filling) occurs, each type of container, container closure and equipment train should be initially validated with each operator participating in at least 3 consecutive successful APS and revalidated with one APS approximately every 6 months for each operator. The APS batch size should mimic that used in the routine aseptic manufacturing process.

9.40. The number of units processed (filled) for APS 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 contamination control strategy. Typically, a minimum of 5000 to 10000 units are filled. For small batches (e.g. those under 5000 units), the number of containers for APS should at least equal the size of the production batch.

9.41. Filled APS units should be agitated, swirled or inverted before incubation to ensure contact of the media with all interior surfaces in the

container. All integral units from the aseptic process simulation should be incubated and evaluated, including units with cosmetic defects or those which have gone through nondestructive in-process control checks. If units are discarded during the process simulation and not incubated, these should be comparable with units discarded during a routine fill, and only if production SOPs clearly specify that units must be removed under the same circumstances (i.e. type of intervention; line location; specific number of units removed). In no case should more units be removed during a media fill intervention than would be cleared during a production run. Examples may include those that must be discarded during routine production after the set-up process or following a specific type of intervention. To fully understand the process and assess contamination risks during aseptic setup or mandatory line clearances, these units would typically be incubated separately, and would not necessarily be included in the acceptance criteria for the APS.

9.42. Where processes include materials that contact the product contact surfaces but are then discarded (e.g. product flushes), the discarded material should be simulated with nutrient media and be incubated as part of the APS, unless it can be clearly demonstrated that this waste process would not impact the sterility of the product.

9.43. Filled APS units should be incubated in a clear container to ensure visual detection of microbial growth. Where the product container is not clear (e g. amber glass, opaque plastic), clear containers of identical configuration may be substituted to aid in the detection of contamination. When a clear container of identical configuration cannot be substituted, a suitable method for the detection of microbial growth should be developed and validated. Microorganisms isolated from contaminated units should be identified to the species level when practical, to assist in the determination of the likely source of the contaminant.

9.44. Filled APS units should be incubated without unnecessary delay to achieve the best possible recovery of potential contamination. The selection of the incubation conditions and duration should be scientifically justified and validated to provide an appropriate level of sensitivity of detection of microbial contamination.

9.45. On completion of incubation:

i. Filled APS units should be inspected by personnel who have been appropriately trained and qualified for the detection of microbiological contamination. Inspection should be conducted under conditions that facilitate the identification of any microbial contamination.

ii. Samples of the filled units should undergo positive control by inoculation with a suitable range of reference organisms and suitably representative local isolates.

9.46. The target should be zero growth. Any contaminated unit should result in a failed aseptic process simulation and the following actions should be taken:

i. An investigation to determine the most probable root cause(s);

ii. Determination and implementation of appropriate corrective measures;

iii. A sufficient number of successful, consecutive repeat aseptic process simulation (normally a minimum of 3) should be conducted in order to demonstrate that the process has been returned to a state of control;

iv. A prompt review of all appropriate records relating to aseptic production since the last successful aseptic process simulation:

a) The outcome of the review should include a risk assessment of potential sterile breaches in batches manufactured since the last successful aseptic process simulation;

b) All other batches not released to the market should be included in the scope of the investigation. Any decision regarding their release status should consider the investigation outcome.

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

vi. Where the root cause investigation indicates that the failure was related to operator activity, actions to limit the operator's activities, until retrained and requalified, should be taken;

vii. Production should resume only after completion of successful revalidation.

9.47. All aseptic process simulation runs should be fully documented and include a reconciliation of units processed (for example, units filled, incubated and not incubated). Justification for filled and non-incubated units should be included in the documentation. All interventions performed during the aseptic process simulation should be recorded, including the start and end time of each intervention and the involved person. All microbial monitoring data as well as other testing data should be recorded in the aseptic process simulation batch record.

9.48. An aseptic process simulation run should be aborted only under circumstances in which written procedures require commercial lots to be equally handled. An investigation should be documented in such cases.

9.49. An aseptic process should be subject to a repeat of the initial validation when:

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

ii. There is a change to the process, equipment, procedures or environment that has the potential to affect the aseptic process or an addition of new product containers or container- closure combinations.

10. Quality Control

10.1. There should be personnel available with appropriate training and experience in microbiology, sterility assurance and knowledge of the processes to support the design of the manufacturing activities, environmental monitoring regime and any investigation assessing the impact of microbiologically linked events to the safety of the sterile product.

10.2. Specifications for raw materials, components and products should include requirements for microbial, particulate and endotoxin/pyrogen limits when the need for this has been indicated by monitoring and/or by the contamination control strategy.

10.3. The bioburden assay should be performed on each batch for both aseptically filled product and terminally sterilized products. The results should be considered as part of the final batch review. There should be defined limits for bioburden immediately before the final sterilizing grade filter or the terminal sterilization process, which are related to the efficiency of the method to be used. Samples should be taken to be representative of the worst-case scenario (for example, at the end of hold time). Where overkill sterilization parameters are set for terminally sterilized products, bioburden should be monitored at suitable scheduled intervals.

10.4. For products authorized for parametric release, a supporting pre-sterilization bioburden monitoring programme for the filled product prior to initiating the sterilization cycle should be developed and the bioburden assay should be performed for each batch. The sampling locations of filled units before sterilization should be based on a worst case scenario and be

representative of the batch. Any organisms found during bioburden testing should be identified and their impact on the effectiveness of the sterilizing process determined. Where appropriate, the level of endotoxin/pyrogen should be monitored.

10.5. The sterility test applied to the finished product should only be regarded as the last in a series of critical control measures by which sterility is assured. It cannot be used to assure sterility of a product that does not meet its design, procedural or validation parameters. The test should be validated for the product concerned.

10.6. The sterility test should be performed under aseptic conditions. 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:

i. For products which have been filled aseptically, samples should include containers filled at the beginning and end of the batch. Additional samples, for example, taken after critical interventions should be considered based on risk;

ii. For products which have been heat sterilized in their final containers, samples taken should be representative of the worst case locations (e.g. the potentially coolest or slowest to heat part of each load);

iii. For products which have been lyophilized, samples taken from different lyophilization loads.

Note: Where the manufacturing process results in sub-batches (e.g. for terminally sterilized products) then sterility samples from each sub-batch should be taken and a sterility test for each sub-batch performed. Consideration should also be given to performing separate testing for other finished product tests.

10.7. For some products it may not be possible to obtain a sterility test result prior to release because the shelf life of the product is too short to allow completion of a sterility test. In these cases, the additional considerations of design of the process and additional monitoring and/or alternative test methods required to mitigate the identified risks. Such aspects should be assessed and documented.

10.8. Any process (e.g. Vaporized Hydrogen Peroxide, Ultra Violet) used to decontaminate the external surfaces of sterility samples prior to testing should not negatively impact the sensitivity of the test method or the reliability of the sample.

10.9. Media used for product testing should be quality control tested according to the related Pharmacopoeia before use. Media used for environmental monitoring and aseptic process simulation should be tested for growth promotion before use, using a scientifically justified and designated group of reference microorganisms and including suitably representative local isolates. Media quality control testing should normally be performed by the end user. Any reliance on outsourced testing or supplier testing of media should be justified. As to transportation and shipping conditions, these should be thoroughly considered in this case.

10.10. Environmental monitoring data and trend data generated for classified areas should be reviewed as part of product batch certification/release. A written procedure should be available that describes the actions to be taken when data from environmental monitoring are found out of trend or exceeding the established limits. For products with short shelf life, the environmental data for the time of manufacture may not be available. In these cases, the compliance should include a review of the most recent available data. Manufacturers of these products should consider the use of rapid/alternative methods.

10.11. Where rapid and automated microbial methods are used for general manufacturing purposes, these methods should be validated for the product(s) or processes concerned.

Definitions

"Action limit" — An established relevant measure (e.g. microbial, or airborne particle limits) that, when exceeded, should trigger appropriate investigation and corrective action based on the investigation;

"Airlock" — An enclosed space with interlocked doors, constructed to maintain air pressure control between adjoining rooms (generally with different air cleanliness standards). The intent of an airlock is to preclude ingress of particle matter and microorganism contamination from a lesser controlled area;

"Alert level" — An established relevant measure (e.g. microbial, or airborne particle levels) giving early warning of potential drift from normal operating conditions and validated state, which does not necessarily give grounds for corrective action but triggers appropriate scrutiny and follow-up to address the potential problem. Alert levels are established based on routine and qualification trend data and are periodically reviewed. The alert level can be based on a number of parameters including adverse trends, individual excursions above a set limit and repeat events;

"Asepsis" — A state of control attained by using an aseptic work area and performing activities in a manner that precludes microbial contamination of the exposed sterile product;

"Aseptic preparation (processing)" — The handling of sterile product, containers and/or devices in a process-control environment in which the air

supply, materials and personnel are regulated to prevent microbial, endotoxin/pyrogen and particle contamination;

"Aseptic Process Simulation (APS)" — A simulation of the entire aseptic manufacturing process in order to verify the capability of the process to assure product sterility. Includes all aseptic operations associated with routine manufacturing, e.g. equipment assembly, formulation, filling, freeze-drying (lyophilization) and sealing processes as necessary;

"Bacterial retention testing" — This test is performed to validate that a filter can remove bacteria from a gas or liquid. The test is usually performed using a standard organism, such as *Brevundimonas diminuta* at a minimum concentration of 10^7 Colony Forming Units/cm^2;

"Barrier" — A physical partition that affords aseptic processing area (usually grade A) protection by separating it from the background environment. Such systems frequently use in part or totally the Barrier Technologies known as Restricted Access Barrier System (RABS) or isolators;

"Bioburden" — The total number of microorganisms associated with a specific item such as personnel, manufacturing environments (air and surfaces), equipment, product packaging, raw materials (including water), in-process materials, or finished products;

"Bio-decontamination" — A process that eliminates viable bioburden via use of sporicidal chemical agents capable of inactivating microbial spores;

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

"Blow-Fill-Seal (BFS)" — A technology 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 Shuttle type (with Parison cut) and the Rotary type (Closed Parison);

"Campaign manufacture" — A manufacture of a series of batches of the same product in sequence in a given period of time with strict adherence to established and validated control measures;

"Classified area" — An area that contains a number of cleanrooms (see cleanroom definition);

"Clean area" — An area with defined particle and microbiological cleanliness standards usually containing a number of joined cleanrooms;

"Cleaning" — A process for removing contamination e.g. product residues or disinfectant residues;

"Cleanroom classification" — A method of assessing the level of air cleanliness against a specification for a cleanroom or clean air equipment by measuring the total particle concentration in the air;

"Cleanroom qualification" — A method of assessing the level of compliance of a classified cleanroom or clean air equipment with its intended use;

"Cleanroom" — A room designed, maintained, and controlled to prevent particle and microbial contamination of drug products. Grade A means a Grade A zone;

"Closed system" — A system in which the product is not exposed to the surrounding environment. For example, this can be achieved by the use of bulk product holders (such as tanks or bags) that are connected to each other by pipes or tubes as a system, and where used for sterile products, the full system is sterilized after the connections are made. Examples of these can be (but are

not limited to) large scale reusable systems, such as those seen in active substance manufacturing, or disposable bag and manifold systems, such as those seen in the manufacture of biological products. Closed systems are not opened until the conclusion of an operation. The use of the term “closed systems” in this Annex does not refer to systems such as RABS or isolators;

"Colony Forming Unit (CFU)" — A microbiological term that describes a single detectable colony that originates from one or more microorganisms. Colony forming units are typically expressed as CFU per ml for liquid samples, CFU per m^3 for air sample and CFU per sample for samples captured on solid medium such as settle or contact plates;

"Contamination Control Strategy (CSS)" — A planned set of controls for microorganisms, endotoxin/pyrogen and particles, derived from current product and process understanding that assures process performance and product quality. The controls can include parameters and attributes related to active substance, excipient and drug product materials and components, facility and equipment operating conditions, in-process controls, finished product specifications, and the associated methods and frequency of monitoring and control;

"Contamination" — The undesired introduction of impurities of a microbiological nature (quantity and type of microorganisms, pyrogen), or of foreign particle matter, into or onto a raw material, intermediate, active substance or drug product during production, sampling, packaging or repackaging, storage or transport with the potential to adversely impact product quality;

"Corrective intervention" — An intervention that is performed to correct or adjust an aseptic process during its execution. These may not occur at a set frequency in the routine aseptic process. Examples include such as clearing

component jams, stopping leaks, adjusting sensors, and replacing equipment components;

"Critical intervention" — An intervention (corrective or inherent) into the critical zone;

"Critical surfaces" — Surfaces that may come directly into contact with, or directly affect, a sterile 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" — A location within the aseptic processing area in which product and critical surfaces are exposed to the process environment;

"Dead leg" — Length of non-circulating pipe (where fluid may remain static) that is greater than 3 internal pipe diameters;

"Decommission" — When a process, equipment or cleanroom are closed and they will not be used again;

"Decontamination" — The overall process of removal or reduction of any contaminants (chemical, waste, residue or microorganisms) from an area, object, or person. The method of decontamination used (e.g. cleaning, disinfection, sterilization) should be chosen and validated to achieve a level of cleanliness appropriate to the intended use of the item decontaminated;

"Depyrogenation" — A process designed to remove or inactivate pyrogenic material (for example, endotoxin) to a specified minimum quantity;

"Disinfection" — The process by which the reduction of the number of microorganisms is achieved by the irreversible action of a product on their structure or metabolism, to a level deemed to be appropriate for a defined purpose;

"D-value" — The value of a parameter of sterilization (duration or absorbed dose) required to reduce the number of viable organisms to 10 per cent of the original number;

"Endotoxin" — A pyrogenic product (i.e. lipopolysaccharide) present in the Gram negative bacterial cell wall. Endotoxin can lead to reactions in patients receiving injections. These reactions can range from fever to death.

"Equilibration time" — Period which elapses between the attainment of the sterilization temperature at the reference measurement point and the attainment of the sterilization temperature at all points within the load;

"Extractables" — Chemical entities that migrate from the surface of the process equipment, exposed to an appropriate solvent at extreme conditions, into the product or material being processed;

"Filter Integrity test" — A test to confirm that a filter (product, gas or HVAC filter) retain their retentive properties and have not been damaged during handling, installation or processing;

"First Air" — Refers to filtered air that has not been interrupted (by various factors, e.g. on the part of operators) prior to contacting exposed product and product contact surfaces with the potential to add contamination to the air prior to reaching the critical zone;

"Form-Fill-Seal (FFS)" — An automated filling process, typically used for terminally sterilized products, which constructs the primary container out of a continuous flat roll of packaging film while simultaneously filling the formed container with product and sealing the filled containers in a continuous process. FFS processes may utilize a single web system (where a single flat roll of film is wrapped around itself to form a cavity), or a dual web system (where two flat rolls of film are brought together to form a cavity), often with the aid of vacuum molds or pressurized gases. The formed cavity is filled, sealed and cut into sections. Films typically consist of a polymeric material, polymeric coated foil or other suitable material;

"Gowning qualification" — A programme that establishes, both initially and on a periodic basis, the capability of an individual to don the complete gown;

"Grade A air supply" — Air which is passed through a filter qualified as capable of producing grade A total particle quality air, but where there is no requirement to perform continuous total particle monitoring or meet grade A viable monitoring limits. Specifically used for the protection of fully stoppered vials where the cap has not yet been crimped;

"HEPA filter" — High efficiency particulate air filter specified in accordance with a relevant international standard;

"Inherent interventions" — An intervention that is an integral part of the aseptic process and is required for either set-up, routine operation and/or monitoring (e.g. aseptic assembly, container replenishment, environmental sampling). Inherent interventions are required by procedure or work instruction for the execution of the aseptic process;

"Intrinsic sterile connection device" — A device that reduces the risk of contamination during the connection process. These devices can be mechanical or fusion sealing;

"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 if the nozzle had not been there (i.e. the sampling condition in which the mean velocity of the air entering the sample probe inlet is nearly the same (± 20 percent) as the mean velocity of the airflow at that location);

"Isolator" — An enclosure capable of being subject to reproducible interior bio-decontamination, with an internal work zone meeting grade A conditions 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:

i. Closed isolator systems exclude external contamination of 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;

ii. 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 contaminant into the isolator;

"Leachables" — Chemical entities that migrate into products from the product contact surface of the process equipment or containers under normal condition of use and/or storage;

"Local isolates" — Suitably representative microorganisms of the site that are frequently recovered through environmental monitoring within the classified zone/areas especially grade A and B areas, personnel monitoring or positive sterility test results;

"Lyophilization" — A physical-chemical drying process designed to remove solvents, by way of sublimation, from both aqueous and non-aqueous systems, primarily to achieve product or material stability. Lyophilization is synonymous to the term 'freeze-drying';

"Manual aseptic processing" — An aseptic process where the operator manually compounds, fills, places and/or seals an open container with sterile product;

"Operator" — Any individual participating in the processing operation, including line set-up, filling, maintenance, or other personnel associated with manufacturing activities;

"Overkill sterilization" — A process that is sufficient to provide at least a 12 $\log_{10}$ reduction of microorganisms having a minimum D-value of 1 minute.

"Parison" — The “tube” of polymer extruded by the BFS machine from which containers are formed;

"Pass-through hatch" — Synonymous with airlock (see airlock definition) but typically smaller in size;

"Patient" — Human or animal including participants in a clinical trial;

"Post-aseptic processing terminal heat treatment" — A terminal moist heat process employed after aseptic processing which has been demonstrated to provide a sterility assurance level (SAL) $\leq 10^{-6}$ but where the requirements of steam sterilization (for example, F0≥8 min) are not fulfilled. This may also be beneficial in the destruction of viruses that may not be removed through filtration;

"Pyrogen" — A substance that induces a febrile reaction in patients receiving injections;

"Rapid Transfer System/Port (RTP)" — A System used for the transfer of items into Restricted Access Barrier System (RABS) or isolators that minimizes the risk to the critical zone (for example, a rapid transfer container with an alpha/beta port);

"Raw material" — Any ingredient intended for use in the manufacture of a sterile product, including those that may not appear in the final drug product;

"Restricted Access Barrier System (RABS) — A system that provides an enclosed, but not fully sealed, environment meeting defined air quality conditions (for aseptic processing grade A), and using a rigid-wall enclosure and integrated gloves to separate its interior from the surrounding cleanroom environment. The inner surfaces of the RABS are disinfected and

decontaminated with a sporicidal agent. Operators use gloves, half suits, Rapid Transfer System/Ports (RTPS) and other integrated transfer ports to perform manipulations or convey materials to the interior of the Restricted Access Barrier System (RABS). Depending on the design, doors are rarely opened, and only under strictly pre-defined conditions;

"Single Use Systems (SUS)" — Systems in which product contact components are used only once to replace reusable equipment such as stainless steel transfer lines or bulk containers. SUS covered in this Annex No. 1 are those that are used in manufacturing processes of sterile products and are typically made up of disposable components such as bags, filters, tubing, connectors, storage bottles and sensors;

"Sporicidal agent" — An agent that destroys bacterial and fungal spores when used in sufficient concentration for specified contact time. It is expected to kill all vegetative microorganisms;

"Sterile Product" — For the purpose of this annex, sterile product refers to one or more of the sterilized elements exposed to aseptic conditions and ultimately making up the sterile active substance or finished sterile product. These elements include the containers, closures, and components of the finished drug product. Or, a product that is rendered sterile by a terminal sterilization process;

"Sterilizing grade filter" — A filter that, when appropriately validated, will remove a defined microbial challenge from a fluid or gas producing a sterile effluent. Usually such filters have a pore size equal or less than 0.22 μm;

"Terminal Sterilization" — The application of a lethal sterilizing agent or conditions to a product in its final container to achieve a predetermined sterility assurance level (SAL) of 10^{-6} or better (e.g. the theoretical probability

of there being a single viable microorganism present on or in a sterilized unit is equal to or less than 1 x 10^{-6} (i.e. one in a million));

"Turbulent airflow" — Air that is not unidirectional. Turbulent air in cleanrooms should flush the cleanroom via mixed flow dilution and ensure maintenance of acceptable air quality;

"Unidirectional Airflow (UDAF) unit" — A cabinet supplied with filtered unidirectional airflow (previously referred to as a Laminar Airflow Unit or LAF).

"Unidirectional airflow" — 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;

"Water system" — A system for producing, storing and distributing water, usually compliant to a specific pharmacopoeia grade (e.g. purified water and water for injection (WFI)).

"Worst case" — A set of conditions encompassing processing limits and circumstances, including those within standard operating procedures, that pose the greatest chance of process or product failure (when compared with ideal conditions). Such conditions have the highest potential to, but do not necessarily always result in product or process failure;

"Z-value" — The temperature difference that leads to a 10-fold change in the D-value of the biological indicators.

———————