Health Canada Santé Canada

Annex 1 to the Good manufacturing practices guide - Manufacture of sterile drugs (GUI-0119)

Version 2

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Canada

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To obtain additional information, please contact:

Health Canada
Tel.: 613-957-2991
Toll free: 1-866-225-0709
Fax: 613-941-5366
TTY: 1-800-465-7735
Email: hc.publications-publications.sc@canada.ca

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Date implemented: The implementation period for most of the requirements outlined in this new guidance will end on April 1, 2024. For the requirements outlined in the lyophilization section, the implementation period will end on August 25, 2024.

Disclaimer: This document does not constitute legislation. If there is any inconsistency or conflict between the legislation and this document, the legislation takes precedence. This is an administrative document intended to facilitate compliance by the regulated party with the legislation and the applicable administrative policies.

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Purpose

This document provides guidance for fabricating and packaging/labelling sterile drugs.

It is an annex to the current edition of the Guidelines on Good Manufacturing Practices for Drugs (GUI-0001). It will help you understand and comply with good manufacturing practices (GMP) for sterile drugs. Refer to the glossary for terms used in this guide.

The interpretations in this document have been adopted from those published by the Pharmaceutical Inspection Cooperation Scheme (PIC/S) in the PIC/S GMP Guide Annexes.

The international norms referenced in this document (for example, International Organization for Standardization (ISO) standards) were applicable at the time it was drafted. Relevant updates of these norms will be reflected in a future version of this document.

These guidelines apply to these types of sterile drugs:

- biological
- veterinary
- pharmaceutical
- radiopharmaceutical

The scope of this document does not include establishment licensing.

To comply with GMP requirements in order to get an establishment licence, consult:

- Guidance on drug establishment licences (GUI-0002)

For guidelines for active pharmaceutical ingredients (APIs), consult:

- Good manufacturing practices for active pharmaceutical ingredients (GUI-0104).

Introduction

These guidelines interpret the requirements for manufacturing sterile drugs in Part C, Division 2, section C.02.029 of the *Food and Drug Regulations* (regulations).

Health Canada is an active participating member of the Pharmaceutical Inspection Cooperation Scheme (PIC/S). In 2018, we adopted the PIC/S guidance Annex 1, Manufacture of sterile medicinal products, which describes how to manufacture sterile drugs in compliance with C.02.029 of the regulations. This 2018 adoption enabled transition to the 2023 version of the guidance.

Health Canada prepared this guidance in cooperation with the European Medicines Agency (EMA), World Health Organization (WHO) and PIC/S in order to align with global standards, all while assuring the highest quality document possible. The document is subject to parallel adoption by the European Commission, WHO and PIC/S.

Guidance documents like this one help industry and health care professionals understand how to comply with regulations. They also provide guidance to Health Canada staff so that the regulations are enforced fairly, consistently and effectively across Canada.

Health Canada inspects establishments to assess their compliance with the *Food and Drugs Act* (act) and associated regulations. Our inspectors will use this document as a guide to assess your compliance with GMP requirements for sterile drugs.

These guidelines are not the only way to interpret GMP regulations and do not cover every possible case. Other ways of complying with GMP regulations will be considered with proper scientific justification. Also, as new technologies emerge, different approaches may be called for.

Guidance documents are administrative and do not have the force of law. Because of this, they allow for a flexible approach. Use this guide to help you develop specific approaches to meet your unique needs.

Principle

1. Scope

The manufacture of sterile drugs covers a wide range of:

- sterile product types, such as:
  - active substance
  - excipient
  - primary packaging material
  - finished dosage form
- packed sizes, from single unit to multiple units
- processes from highly automated systems to manual processes
- technologies, such as:
  - biotechnology
  - classical small molecule manufacturing systems
  - closed systems

This annex gives general guidance on applying the principles of quality risk management (QRM) in the design and control of facilities, equipment, systems and procedures used to manufacture all sterile drugs. Applying QRM ensures that microbial, particulate and endotoxin/pyrogen contamination is prevented in the final product.

Note: QRM applies to this entire document. Specific limits or frequencies or ranges should be considered as a minimum requirement. They are being provided due to historical regulatory experience of issues that have been identified and have impacted the safety of patients.

This annex provides guidance for the manufacture of sterile drugs. However, some of the principles and guidance may be followed when manufacturing other products that are not intended to be sterile, but where it's considered important to control and reduce microbial, particulate and endotoxin/pyrogen contamination. The principles and guidance include contamination control strategy, design of premises, cleanroom classification, qualification, validation, monitoring and gowning of personnel. These other products include, for example, certain liquids, creams, ointments and low bioburden biological intermediates.

If you as a manufacturer choose to apply this guidance to non-sterile drugs, you should clearly document which principles you have followed and how you have demonstrated the compliance with those principles.

2. Principle

2.1 The manufacture of sterile drugs is subject to special requirements in order to minimize the risk of microbial, particulate and endotoxin/pyrogen contamination.

The following key areas should be considered.

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 Good manufacturing practices guide for drug products (GUI-0001).
    - Consider using appropriate technologies (for example, restricted access barriers systems (RABS), isolators, robotic systems, rapid/alternative methods and continuous monitoring systems) to help:
        - protect the product from potential extraneous sources of endotoxin/pyrogen, particulate and microbial contamination such as personnel, materials and surrounding environment
        - detect potential contaminants in the environment and the product quickly

ii. **Personnel** should have adequate qualifications, experience, behaviour and training, with specific knowledge of the principles related to contamination control during the manufacturing, packaging and distribution processes.

iii. **Processes and monitoring systems** should be designed, commissioned, qualified, monitored and regularly reviewed by personnel with appropriate process, engineering and microbiological knowledge.

iv. **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 QRM principles to provide a proactive means of identifying, scientifically evaluating and controlling potential risks to quality. Alternative approaches should be supported by appropriate rationale, risk assessment and mitigation, and meet the intent of this annex.

QRM priorities should include appropriate design of the facility, equipment and processes, followed by the implementation of well-designed procedures, and finally application of monitoring systems. These monitoring systems demonstrate 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. A CCS defines critical control points and assesses the effectiveness of all controls (design, procedural, technical and organizational) and monitoring measures used to manage risks to drug quality and safety. The CCS should establish robust assurance of contamination prevention.

The CCS should be actively reviewed and updated, where appropriate. The CCS should drive the continual improvement of manufacturing and control methods. Its effectiveness should form part of the periodic management review. Existing control systems that are in place and appropriately managed may not need to be replaced but should be referenced in the CCS. As well, 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 include a series of interrelated events and measures. These are typically assessed, controlled and monitored individually, but their collective effectiveness should be considered.

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

Elements to be considered within a CCS should include:

i. design of the plant and processes, including 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 for key component suppliers, sterilization of components and single use systems (SUS), and critical service providers
viii. management of outsourced activities and availability/transfer of critical information between parties (for example, contract sterilization services)
ix. process risk management
x. process validation
xi. validation of sterilization processes
xii. preventative maintenance to bring 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 scientifically sound, alternative methods that optimize the detection of environmental contamination
xv. prevention mechanisms, such as trend analysis, detailed investigation, root cause determination, corrective and preventive actions (CAPA), and comprehensive investigational tools
xvi. continuous improvement based on all of this information

2.6 The CCS should consider all aspects of contamination control. Ongoing and periodic review should take place, with updates to the pharmaceutical quality system as appropriate. Changes to systems in place should be assessed for any impact on the CCS before and after implementation.

2.7 The manufacturer should take the necessary steps and precautions 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 drugs is a complex activity that requires specific controls and measures to ensure the quality of products manufactured.

The manufacturer's pharmaceutical quality system (PQS) should outline the specific requirements of sterile drug manufacturing. The PQS should also ensure that all activities are effectively controlled, to minimize the risk of microbial, particulate and endotoxin/pyrogen contamination in sterile drugs.

For details on the PQS requirements, consult section 4 of:

- Good manufacturing practices guide for drug products (GUI-0001)

The PQS for sterile drug manufacturing should also ensure that:

i. an effective risk management system is integrated into all areas of the product lifecycle with the aim to minimize microbial contamination and ensure the quality of sterile drugs manufactured
ii. the manufacturer has sufficient knowledge and expertise of the products as well as the equipment, engineering and manufacturing methods 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, and that suitable CAPA are implemented
iv. risk management is applied when developing and maintaining the CCS, to identify, assess, reduce/eliminate (where applicable) and control contamination risks
    - risk management should be documented and include the rationale for decisions taken related to risk reduction and acceptance of residual risk
v. senior management oversee the state of control throughout the facility and product lifecycle
    - includes a regular review of risk management outcome as part of the ongoing quality management, during change, for a significant emerging problem and during periodic product quality review
vi. processes for the finishing, storage and transport of sterile drugs do not compromise the products
    - consider container integrity, risks of contamination and avoidance of degradation
    - ensure that products are stored and maintained according to authorized storage conditions
vii. persons responsible for certifying/releasing sterile drugs have appropriate access to manufacturing and quality information, and have adequate knowledge and experience in the manufacture of sterile drugs and the associated critical quality attributes
    - able to determine if sterile drugs have been manufactured according to authorized 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 a batch is certified/released. This investigation should determine the potential impact on process and product quality and if other processes or batches are potentially impacted.

The manufacturer should justify and record the reason for including or excluding a product or batch from the scope of the investigation.

4. Premises

4.1 The manufacture of sterile drugs should take place in appropriate cleanrooms. Entry to these should be through change rooms that act as airlocks for personnel, 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 effectively evaluate the state of environmental conditions of cleanrooms, airlocks and pass-through hatches.

4.2 Component preparation, product preparation and filling operations 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 CCS. The manufacturer should justify the use of alternative approaches to RABS or isolators.

4.4 There are 4 grades of cleanroom/zone:

Grade A: This cleanroom is 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). Normally, such conditions are provided by a localized airflow protection, such as unidirectional airflow workstations within RABS or isolators. The maintenance of unidirectional airflow should be demonstrated and qualified across the entire grade A area. Premises, equipment, process and procedures should be designed to minimize direct intervention by operators (for example, without the protection of barrier and glove port technology) into the grade A area.

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 (refer to the information on background environment for isolators under 4.20 in the Barrier technologies section).

Grades C and D: These are cleanrooms used for carrying out less critical stages in the manufacture of aseptically filled sterile drugs or a background for isolators. They can also be used for preparing/filling terminally sterilized products. (For details on terminal sterilization activities, refer to the Production and specific technologies section).

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

4.6 To reduce dust accumulation and facilitate cleaning, there should be no recesses that make it difficult to clean effectively. 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 to construct the cleanroom and items used in the room should generate minimal particles. They should permit repeated application of cleaning, disinfectant and sporicidal agents.

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 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 should be regularly cleaned, disinfected and maintained.

4.10 The transfer of equipment and materials in and out of cleanrooms and critical zones is one of the greatest potential sources of contamination. Any activities that could potentially compromise the cleanliness of cleanrooms or the critical zone should be assessed. If activities cannot be eliminated, appropriate controls should be implemented.

4.11 A unidirectional process should be followed to transfer materials, equipment and components into grade A or B areas. Where possible, items should be sterilized and passed into these areas through double-ended sterilizers (for example, 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 that does not introduce contamination should be validated and implemented. Examples of procedures include using an effective transfer disinfection process, rapid transfer systems for isolators or, for gaseous or liquid materials, a bacteria-retentive filter.

A separate, unidirectional process should be followed to remove materials, waste and environmental samples from grade A and B areas. If this is not possible, consider moving incoming and exiting material at different times and applying controls to avoid potential contamination of incoming items.

4.12 Airlocks should be designed to provide physical separation, minimize microbial and particle contamination of the different areas, and used when material and personnel move between different grades. Wherever possible, airlocks used for personnel movement should be separated from those used for material movement. Where this is not practical, consider moving the personnel and materials at different times.

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, consider time based separation of ingress and

egress activities. Where the CCS 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 (for example, from grade D to grade C to grade B areas). In general, hand washing facilities should be provided only in the first stage of the changing room, not 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 B areas through an airlock or pass-through hatches. Equipment and materials intended for use in the grade A area should be protected when moving through the grade B area. Unapproved items that require transfer should be pre-approved as an exception. Appropriate risk assessment and mitigation measures should be applied and recorded as per the manufacturer's CCS. They should include a specific disinfection and monitoring program approved by quality assurance.
- Pass-through hatches should be designed to protect the higher-grade environment, for example, by flushing with an active filtered air supply.
- The movement of material or equipment from lower-grade or unclassified areas to higher-grade clean areas should be cleaned and disinfected in keeping with the risk and in line with the CCS.

4.13 Entry and exit doors for all pass-through hatches and airlocks (for material and personnel) should not be opened at the same time. For airlocks leading to grade A and 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, there should be a time delay between when the interlocked doors close and open.

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 filtered air supply should flush the area effectively. Adjacent rooms of different grades should have an air pressure difference of at least 10 Pascals (guidance value). Particular attention should be paid to protecting the critical zone.

The recommendations for air supplies and pressures may need to be modified where certain materials, such as pathogenic, highly toxic or radioactive products or live viral or bacterial materials, must be contained. Modifications may include positively or negatively pressurized airlocks that prevent the hazardous material from contaminating surrounding areas. For some operations, it may be necessary to decontaminate facilities (for example, cleanrooms and heating, ventilation, air conditioning (HVAC) systems) and treat the air leaving a clean area. 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's no ingress from lower- to higher-grade areas
- air doesn't travel from less clean areas (such as the floor) or over operators or equipment that may transfer contamination to higher-grade areas

Where unidirectional airflow is required, visualization studies should be done to determine compliance. For information on grades of cleanroom/zone, refer to 4.4 in the Premises section. For information on design of technology and processes, refer to 4.19 in the Barrier technologies section.

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 conducted both "at rest" and "in operation" (for example, simulating operator interventions) and the video recordings of airflow patterns retained. The outcome of these studies should be documented and considered when work is undertaken to establish the environmental monitoring program for the facility.

4.16 Indicators of air pressure differences should be fitted between cleanrooms and/or between isolators and their background. The CCS should consider set-points and air pressure differences that have been identified as critical.

Critical air pressure differences 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 developed outlining 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 allow observation and supervision of production activities from outside the grade A and B areas (through windows or remote cameras with a full view of the area and processes). This requirement should be considered when designing new facilities or refurbishing existing facilities.

Barrier technologies

4.18 Isolators or RABS, which are different technologies, and the associated processes, should be designed to provide protection, by separating the grade A environment from the environment of the surrounding room. The 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 technology and processes that are in place in the critical zone should ensure that appropriate conditions are maintained 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 it's essential to contain the product (for example, radiopharmaceutical products) and 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 transferring 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 a risk assessment and justified in the CCS.
b Key considerations when performing the risk assessment for the CCS of an isolator should include, for example, the:
- bio-decontamination program
- extent of automation
- impact of glove manipulations that may potentially compromise 'first air' protection of critical process points
- 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 before the final bio-decontamination of the isolator

c. Where additional process risks are identified, a higher grade of background should be considered unless appropriately justified in the CCS.
d. Airflow pattern studies should be performed at the interfaces of open isolators to demonstrate the absence of air ingress.

ii. **RABS:**
The background environment 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 shown to have appropriate mechanical and chemical resistance. The CCS should indicate how frequent gloves should be replaced.

i. **Isolators:**
a. Leak testing of the glove system should be performed using a methodology demonstrated to be suitable for the task and criticality. Testing should be done at defined intervals. In general, glove integrity testing should be done, at a minimum, at the beginning and end of each batch or campaign. Additional glove integrity testing may be necessary depending on the validated campaign length.
b. Glove integrity monitoring should include a visual inspection associated with each use and following any manipulation that may affect the integrity of the system.
c. 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.
d. Integrity/leak testing of isolator systems should be performed at defined intervals.

ii. **RABS:**
Gloves used in the grade A area should be sterilized before installation and sterilized or effectively bio-decontaminated by a validated method before 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 tested for integrity 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 before the bio-decontamination step is essential. Any residues that remain may inhibit the effectiveness of the decontamination process.

Evidence should be available to demonstrate that the cleaning and bio-decontamination agents used do not have an 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, and include a sporicidal agent in a suitable form (for example, 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 validated method. The method should include all areas of the interior surfaces and ensure the environment is suitable 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 should be qualified according to the required characteristics of the environment. Each manufacturing operation requires an appropriate environmental cleanliness level in the operational state to minimize the risk of contaminating 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 either of the following:

- Guide to validation – Drugs and supporting activities (GUI-0029) (Health Canada)
- PIC/S Annex 15 – Qualification and validation (PIC/S)

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 Health Canada's Guide to validation – drugs and supporting activities (GUI-0029) (or alternatively PIC/S Annex 15 – Qualification and validation), 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 for 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 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. It's 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 conducted to avoid any impact on process or product quality. For example, initial classification should take place during simulated operations and reclassification during simulated operations or 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 permitted number of particles/m^3 equal to or greater than the tabulated size			
	≥ 0.5 µm		≥5.0 µm	
	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 these 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 Part 1. For the aseptic processing area and background environment (grade A and 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 through a documented risk assessment process and knowledge of the process and operations being performed in the area.

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

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

ii The "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 performing or simulating routine operational work.

iii The total particle limits given in Table 1 for the "at rest" state should be achieved after a "clean-up" period once operations and line clearance/cleaning activities have been completed. 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, including the location for air speed measurement, should be clearly justified in the qualification protocol. Air speed should be designed, measured and maintained to ensure that appropriate unidirectional air movement protects the product and open components at the working position (for example, where high-risk operations occur and product and/or components are exposed). Unidirectional airflow systems should provide a homogeneous air speed in a range of 0.36 to 0.54 m/s (guidance value) at the working position, unless otherwise scientifically justified in the CCS. 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 for 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: Recommended limits for microbial contamination

Grade	Air sample cfu/m³	Settle plates (diam. 90 mm), cfu/4 hours[a]	Contact plates (diam. 55 mm), cfu/plate
A	< 1	< 1	< 1
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.

Notes: 1. All methods indicated for a specific grade in the table should be used for qualifying the area of that specific grade. If a method tabulated is not used or alternative methods are used, the approach taken should be appropriately justified. 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, correlated to cfu. 3. For the qualification of personnel gowning, the limits given for contact plates and glove prints in Table 6 should apply. 4. Sampling methods should not pose a contamination risk to manufacturing operations.

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

i cleanroom classification (total particle concentration)
ii integrity test of final filters
iii airflow volume measurement
iv verification of air pressure difference between rooms
v air velocity test

Note: For grades B, C and D areas, the air velocity test should be performed according to a risk assessment documented as part of the CCS. However, it is required for filling zones that have unidirectional airflow (for example, 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.

Maximum time intervals for requalification are:

- 6 months for grade A and B areas
- 12 months for grade C and D areas

Appropriate requalification consisting of at least the above tests should also be carried out after:

- completion of remedial action to rectify an out-of-compliance equipment or facility condition
- changes to equipment, facility or processes

The change management process should be used to determine the significance of a change. Examples of changes to be considered include the following:

i interruption of air movement that affects installation
ii change in design of the cleanroom or the operational setting parameters of the HVAC system
iii special maintenance that affects installation (for example, changing final filters)

Disinfection

4.33 Disinfecting the cleanrooms is particularly important. Cleanrooms should be cleaned and disinfected thoroughly in accordance with a written program.

For disinfection to be effective, prior cleaning to remove surface contamination should be performed. Cleaning programs should effectively remove disinfectant residues. More than 1 type of disinfecting agent should be used to ensure that where they have different modes of action, their combined usage is effective against bacteria and fungi. Disinfection should also include using a sporicidal agent periodically.

There should be regular monitoring to assess the effectiveness of the disinfection program and detect changes in types of microbial flora (for example, organisms resistant to the disinfection regime currently in use).

4.34 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. Such studies should also support the in-use expiry periods of prepared solutions.

4.35 Disinfectants and detergents used in grade A and B areas should be sterile before they are used. Disinfectants used in Grade C and D should be sterile where determined in the CCS. Disinfectants and detergents that are diluted/prepared by the sterile drug manufacturer should be done in a manner that prevents contamination and 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 appropriate vendor qualification.

4.36 Where fumigation or vapour disinfection (for example, vapour-phase hydrogen peroxide) is used for cleanrooms and associated surfaces, the effectiveness of the 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" and during early stages of development and then 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 how crucial they are, with those that are critical reviewed immediately.

5.3 As much as is 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 should be considered. These could include restricting access to the work area to specified personnel and generating clearly defined work protocols and maintenance procedures. Additional cleaning, disinfection and environmental monitoring should also be considered. Equipment that must be sterilized 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 before disinfection

5.5 For aseptic processes, direct and indirect product contact parts should be sterilized. Direct 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 where the sterility is critical to the overall product sterility. Examples of indirect product contact parts are 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 The potential impact of unplanned maintenance of equipment critical to the sterility of the product should be assessed 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 is continually sterilized (for example, in a sterilizing tunnel).

5.9 Particle counters, including sampling tubing, should be qualified. The manufacturer's recommended specifications for tube diameter and bend radii

should be considered. Tube length should typically be no longer than 1 m 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, 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 using a risk assessment and documented as part of the CCS.

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

i. contact the product directly (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. directly impact the product in other ways

6.3 Utilities should be designed, installed, qualified, operated, maintained and monitored 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 lifecycle. 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 this is unavoidable, they should be installed so that they do not create recesses, unsealed openings and surfaces that are difficult to clean. Installation should allow the outer surface of the pipes to be cleaned and disinfected.

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 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 (for example, sloping of piping to provide complete drainage and avoid dead legs). Filters that are included in the system should be monitored and maintained. Water produced should comply with the current monograph of the relevant Pharmacopeia.

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

6.9 In water distribution systems, water flow should remain turbulent through the pipes 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. The WFI should be stored and distributed in such a manner as to minimize the risk of microbial growth (for example, by constant circulation at a temperature above 70°C). WFI should be produced by distillation or 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 should be tested before installation and after use. Controls should be in place to prevent condensation from forming on the filter (for example, 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 in use again. Microbiological/endotoxin results should be within specification and approved before batches manufactured using water from the system are certified/released.

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. These should be reassessed periodically on data obtained during subsequent re-qualifications, routine monitoring and investigations. Ongoing monitoring data should be reviewed to identify an adverse trend in system performance.

Sampling programs should reflect the CCS requirements. They should also include all outlets and points of use, at a specified interval, to ensure that representative water samples are obtained for regular analysis. Sample plans should:

- be based on the qualification data
- consider the potential worst case sampling locations
- ensure at least 1 representative sample is included for each day the water is used for manufacturing processes

6.14 Alert level excursions should be documented and reviewed. An investigation to determine whether the excursion is a single (isolated) event or if results point to an adverse trend or deterioration of the system should be conducted. 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 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. It should not contain additives at a level that could contaminate the product or equipment. For a generator supplying pure steam used for the direct sterilization of materials or product-contact surfaces (for example, porous hard-good autoclave loads), steam condensate should meet the current monograph for WFI of the relevant Pharmacopeia (microbial testing is not mandatory for steam condensate). A suitable sampling schedule should be in place to ensure that representative pure steam is analyzed regularly. 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.

Gases 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, considering the use and type of the gas and the design of the gas generation system. Where applicable, gases should 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. When the filter is used on a batch basis (for example, to filter gas used for overlay of aseptically filled products) or as a product vessel vent filter, the filter should be tested for integrity and the results reviewed as part of the batch certification/release process. 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 (for instance, an indication system for leakage).

7. Personnel

7.1 There should be sufficient appropriate personnel, suitably qualified, trained and experienced in the manufacture and testing of sterile drugs, and any of the specific manufacturing technologies used in the site's manufacturing operations. This requirement ensures compliance with GMP applicable to the manufacture and handling of sterile drugs.

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 APS, so as not to compromise sterility assurance.

7.3 All personnel, including cleaning, maintenance and monitoring staff and those who access cleanrooms, should receive regular training, gowning qualification and assessment in disciplines relevant to the correct manufacture of sterile drugs. Training should include the basic elements of microbiology and hygiene. There should be a specific focus on:

- cleanroom practices
- contamination control
- aseptic techniques
- protection of sterile drugs (for those operators entering the grade B cleanrooms and/or intervening into grade A)
- potential safety implications to the patient if the product is not sterile

The level of training should be based on how critical the function is and on the 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 behaviours. Compliance with aseptic gowning procedures should be confirmed by assessment and periodic reassessment at least annually. Assessment should involve both visual and microbial assessment (using monitoring locations such as gloved fingers, forearms, chest and hood (facemask/forehead). Refer to 9.30 in the Environmental and personnel monitoring - viable particle section for the expected limits (Table 6). The unsupervised access to the grade A and 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 APS.

7.5 Unqualified personnel should not enter grade B cleanrooms or grade A areas in operation. 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 assess the impact of those activities on the cleanliness of the area. Access by these persons should be assessed and recorded in accordance with the pharmaceutical quality system (PQS).

7.6 There should be systems in place to prevent disqualified personnel from working in or accessing cleanrooms without supervision. The systems that are put in place should be based on ongoing assessment and/or identification of an adverse trend from the personnel monitoring program and/or after a failed APS. Once disqualified, retraining and requalification should be completed before permitting the operator to have any further involvement in aseptic practices. Requalification for operators entering grade B cleanrooms or performing intervention in grade A areas should include participation in a successful APS.

7.7 High standards of personal hygiene and cleanliness are essential to prevent excessive shedding or increased risk of introducing microbial contamination. Personnel should be instructed to report any specific health conditions or ailments that may cause the shedding of abnormal numbers or types of contaminants and be prevented from accessing cleanrooms. Health conditions and actions to be taken should be provided by the designated competent person and described in procedures.

7.8 Personnel who have been engaged in the following activities should not enter clean areas unless clearly defined and effective decontamination and entry procedures have been followed and documented:

- processing human or animal tissue materials
- cultures of micro-organisms, other than those used in the current manufacturing process
- any activities that may have a negative impact on quality (such as microbial contamination)

7.9 Wristwatches, make-up, jewellery, mobile phones and other non-essential items should not be allowed in clean areas. Electronic devices, such as mobile phones and tablets, that are supplied by the manufacturer solely for use in the cleanrooms may be acceptable if they can be cleaned and disinfected according to the grade in which they are used. The use and disinfection of such equipment should be included in the CCS.

7.10 Cleanroom gowning and hand washing should follow a written procedure and be 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. Clothing that protects the operator from the product should not compromise the protection of the product from contamination. Garments should be visually checked for cleanliness and integrity immediately before and after gowning. Gown integrity should also be checked upon exit. Particular attention should be paid to ensuring that sterilized garments and eye coverings have been subject to the sterilization process and are within their specified hold time. Their packaging should be visually inspected to ensure it has not been compromised. Reusable garments (including eye coverings) should be replaced if damaged or at a frequency that has been established 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 operator's movement.

7.13 Clothing that is required for each cleanliness grade is described as follows.

i) **For grade B** (including access/interventions into grade A):
- garments that are dedicated for use under a sterilized suit should be worn before gowning
  - refer to the paragraph 7.14 on cleanroom gowning
- sterilized, non-powdered, rubber or plastic gloves should be worn while donning the sterilized garments
- sterile headgear that encloses all hair (including facial) should be tucked into the neck of the sterile suit if separate from the gown
- sterile face mask and sterile eye coverings (such as goggles) should be worn to cover and enclose all facial skin and prevent the shedding of droplets and particles
- appropriate sterilized footwear (such as over-boots) should be worn
- trouser legs should be tucked inside footwear
- garment sleeves should be tucked into a second pair of sterile gloves worn over the pair worn while donning the gown
- protective clothing should minimize shedding of fibres or particles and retains particles shed by the body
  - Assess particle shedding and particle retention efficiencies of the garments during the garment qualification.
  - Pack and fold garments 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) **For grade C:**
- hair, beards and moustaches should be covered
- single or 2-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 fibres and particles.

iii) **For 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) Note: Additional gowning, including gloves and face mask, may be required in grade C and D areas when performing activities considered to be a contamination risk, as defined by the CCS.

7.14 Cleanroom gowning should be performed in change rooms of an appropriate cleanliness grade to maintain gown cleanliness. Outdoor clothing including socks (other than personal underwear) should not be brought into changing rooms that lead directly to grade B and C areas. Single or 2-piece facility trouser suits covering the full length of the arms and the legs and facility socks covering the feet should

be worn before entering 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 put on 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 or present any risk of product contamination.

7.17 Reusable clothing for clean areas should be cleaned in a laundry facility that is adequately segregated from production operations. Cleaning should be conducted using a qualified process to ensure the clothing does not become damaged and/or contaminated by fibres or particles during repeated laundering. Laundry facilities should not introduce risk of contamination or cross-contamination. Inappropriate handling and use of clothing may damage fibres 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 program and should include a maximum number of laundry and sterilization cycles.

7.18 Activities in clean areas that are not critical to 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 that could result from over-vigorous activity. Operators performing aseptic operations should adhere to aseptic technique at all times to prevent changes in air currents that may introduce lower-quality air into the critical zone. Movement next to the critical zone should be restricted. The path of the unidirectional (first air) airflow should not be obstructed.

A review of airflow visualization studies should be part of the training program.

8. Production and Specific Technologies

Terminally sterilized products

8.1 Components and materials should be prepared in at least a grade D cleanroom to limit the risk of microbial, endotoxin/pyrogen and particle contamination, and render the product suitable for sterilization. A product that is at a high or unusual risk of microbial contamination (for example, actively supports microbial growth, must be held for long periods before filling, is not processed mostly in closed vessels,) should be prepared in at least a grade C environment. Ointments, creams, suspensions and emulsions should be prepared in at least a grade C environment before terminal sterilization.

For guidance on terminally sterilized drugs, please consult:

- Process validation: Gaseous sterilization for pharmaceuticals (GUI-0007)
- Process validation: Irradiation sterilization for pharmaceuticals (GUI-0009)
- Process validation: Moist heat sterilization for pharmaceuticals (GUI-0010)

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 Products for terminal sterilization should be filled in at least a grade C environment.

8.4 The CCS may identify that the product is at an unusual risk of contamination from the environment. For example, if the filling operation is slow, or the containers have wide necks or are necessarily exposed for more than a few seconds before closing, the product should be filled in grade A with at least a grade C background.

8.5 Processing the bulk solution should include a filtration step with a microorganism-retaining filter, where possible, to reduce bioburden levels and particles before filling into the final product containers. 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	Examples of operations for terminally sterilized products
A	filling products, when unusually at risk
C	preparing solutions, when unusually at risk filling products
D	preparing 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 contamination control strategy (CCS) should clearly define the:

- acceptance criteria for these controls
- requirements for monitoring
- 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 CCS:

- during the preparation of the aseptic environment
- during all processing stages (including the stages before and after bulk product sterilization)
- until the product is sealed in its final container

The presence of materials liable to generate particles and fibres should be minimized in cleanrooms.

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

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	Examples
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 whenever possible) - aseptic compounding and mixing - replenishing sterile bulk product, containers and closures - removing and cooling unprotected (for example, with no packaging) items from sterilizers - staging and conveying sterile primary packaging components in the aseptic filling line while not wrapped

	- aseptic filling, sealing containers such as ampoules, vial closure, transferring open or partially stoppered vials loading of a lyophilizer
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
C	- preparing solutions to be filtered, including sampling and dispensing
D	- cleaning equipment - handling components, equipment and accessories after cleaning - assembling under HEPA-filtered airflow of cleaned components, equipment and accessories before sterilization - assembling closed and sterilized single-use systems (SUS) using intrinsic sterile connection devices

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

i. all product and component contact equipment should be sterilized before use
ii. all raw materials or intermediates should be sterilized and aseptically added
iii. bulk solutions or intermediates should be sterilized

8.12 Unwrapping, assembling and preparing sterilized equipment, components and ancillary items with direct or indirect product contact should be:

- treated as an aseptic process
- performed in grade A with a grade B background

This also applies to the filling line set-up and filling of the sterile drug. Where an isolator is used, the background should be in accordance with the information on background environment in 4.20 of the Barrier technologies section.

8.13 Preparing and filling sterile drugs 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
- 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 the information on background environment in 4.20 of the Barrier technologies section.

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 the risk of contamination.

Where an isolator is used, the background should be in accordance with the paragraph on background environment in 4.20 of the Barrier technologies section. Aseptic connections should be appropriately assessed and their effectiveness verified. For requirements regarding intrinsic sterile connection devices, refer to 8.129 and 8.130 in the Closed systems section.

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 then 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 (refer to the general information in 9.34 of the Aseptic process simulation (APS) (also known as media fill section). Interventions should be carefully designed to effectively minimize the risk of contamination to the environment, process and product. When designing interventions, consider 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.

Procedures listing the types of inherent and corrective interventions, and how to perform them, should be first evaluated via risk management and APS and be kept up to date. Non-qualified interventions should only be used in exceptional circumstances, with consideration given to the risks associated with the intervention and with the quality unit's authorization. The details of the intervention conducted should be subject to risk assessment, recorded and fully investigated under the manufacturer's pharmaceutical quality system (PQS). 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. The associated time, duration of the event and operators involved should be noted in batch records. Refer to the general information in 9.34 of the Aseptic process simulation (APS) (also known as media fill section).

8.18 The duration of each aspect of aseptic preparation and processing should be minimized, with a maximum time defined and validated. Include the following:

i holding time between equipment, component and container cleaning, drying and sterilization
ii holding time for sterilized equipment, components and containers before use and during filling/assembly
iii holding time for a decontaminated environment, such as the RABS or isolator before use

iv 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 considers its composition and the prescribed method of storage.
v holding time for sterilized product prior to filling
vi aseptic processing time
vii filling time

8.19 Personnel with specific expertise in aseptic processing should observe aseptic operations (including APS) on a regular basis. They should verify the correct performance of operations, including operator behaviour in the cleanroom, and address any inappropriate practices they may detect.

Finishing of sterile drugs

8.20 Open primary packaging containers should be maintained under grade A conditions with the appropriate background for the technology (refer to the information on background environment in 4.20 of the Barrier technologies section). For partially stoppered vials or prefilled syringes, refer to 8.126 of the Lyophilization section).

8.21 Final containers should be closed following appropriately validated methods.

8.22 Where final containers are closed by fusion (for example, 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, BFS 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 if scientifically justified and should be based on data that demonstrates the consistency of the existing process and a high level of process control. Visual inspection is not 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. 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 to ensure the vacuum is maintained after an appropriate pre-determined period prior to certification/release and during shelf life.

8.25 When validating the integrity of the container closure, any transportation or shipping requirements that may negatively impact that integrity (for example, decompression or extreme temperatures) should be considered.

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

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. If choosing the latter approach, vials should be protected by grade A conditions up to the point of leaving the aseptic processing area, and then 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. Manual capping 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 an aseptically filled sterile drug 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 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 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 that captures all known classes of defects should be generated and maintained. The defect library should be used for training production and quality assurance personnel.

There should be no critical defects during subsequent sampling and inspection of acceptable containers. Any critical defect identified subsequently should trigger an investigation, as this would indicate a possible failure of the original inspection process.

8.31 Manual inspections 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 (and wear corrective lenses if these are normally worn) at least once a year. The qualification should be undertaken using appropriate samples from the manufacturer's defect library sets. Qualification should also take into consideration worst-case scenarios (for example, inspection time, line speed where

the product is transferred to the operator by a conveyor system, container size or fatigue) as well as eyesight checks. Operator distractions should be minimized and there should be frequent breaks, of an appropriate duration.

8.32 Automated inspection process should be validated for its ability to detect known defects (which may impact product quality or safety) and be as good as or better than manual inspection methods. The performance of the equipment should be challenged using representative defects before start-up and at regular intervals throughout the batch.

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

Sterilization

8.34 Where possible, the finished product should be terminally sterilized, using a validated and controlled sterilization process. This provides a greater assurance of sterility than a validated and controlled sterile filtration process and/or aseptic processing. Where it's 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/program used for sterilization should be based on scientific principles and data that demonstrate repeatability and reliability of the sterilization process. All parameters should be defined and, where critical, should be controlled, monitored and recorded.

8.36 All sterilization processes should be validated. Validation studies should consider the product composition, storage conditions and maximum time between when a product or material is being prepared to be sterilized and the start of sterilization.

The sterilization process should be validated for 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. Validation should be done using physical measurements and biological indicators (BI) where appropriate. For effective sterilization, the entire product and the 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 is used for a product that 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 re-validated periodically. 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 re-validated at least once a year for load patterns that are considered worst-case. Other load patterns should be validated at a frequency justified in the CCS.

8.40 Routine operating parameters should be established and adhered to for all sterilization processes (for example, physical parameters and loading patterns).

8.41 Mechanisms should be in place to detect a sterilization cycle that does not conform to the validated parameters. Any failed sterilization or one that deviated from the validated process (longer or shorter phases such as heating cycles, for example) 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. BIs 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 (for example, with ethylene oxide), positive controls should be tested for each sterilization cycle. If BIs 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 of BIs is important. Suppliers should be qualified and transportation and storage conditions should be controlled in order that BI quality is not compromised. Before using 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, such as D-value or 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 that have not been subjected to the sterilization process from those that have. Equipment such as baskets or trays used to carry products and other items of equipment and/or components should be clearly labelled (or electronically tracked) with the product name, 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 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 where the required sterility assurance level was achieved.

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 using validated methods appropriate for the specific material. Suitable protection after sterilization should be provided to prevent recontamination. Sterilized items

that are not used immediately after sterilization should be stored using appropriately sealed packaging and a maximum hold time should be established.

Where justified, components that are packaged with multiple sterile packaging layers do not need to be stored in a cleanroom if the integrity and configuration of the sterile pack allows the items to be readily disinfected when being transferred by operators into grade A areas (for example, 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 The transfer of materials, equipment, components and ancillary items that are sterilized in sealed packaging into grade A areas should be done using appropriate validated methods (for example, airlocks or pass-through hatches). The exterior of the sealed packaging should be disinfected as well. The use of rapid transfer port technology should also be considered.

The effectiveness of these methods to control the potential risk of contamination of the grade A and B areas should be demonstrated. Likewise, the effectiveness of the disinfection procedure used to reduce any contamination on the packaging to acceptable levels for entry of the item into the grade A and B areas should be demonstrated.

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 An effective and validated disinfection and transfer process should be in place 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. Once disinfected, these items should be protected to prevent recontamination. These items and others representing potential routes of contamination should be included in the environmental monitoring program.

Sterilization by heat

8.50 Each heat sterilization cycle should be recorded using equipment with suitable accuracy and precision (electronical or manual methods). The system should have safeguards and/or redundancy in its control and monitoring instrumentation to detect a cycle that does not meet the validated cycle parameter requirements and thus abort or fail the cycle. An example of a safeguard would be to use duplex/double probes connected to independent control and monitoring systems.

8. 51 The position of the temperature probes used to control and/or record should be determined during validation. The position should be selected based on system design and 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 to verify the function and location of these probes, by using an independent monitoring probe located at the same position used during validation.

8.52 The entire 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 a defined temperature range before the cycle starts.

8.53 After the high temperature phase of a heat sterilization cycle is complete, 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 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.

For further guidance on parametric release, please consult:

- Annex 17, Parametric release (PIC/S) - Guide to good manufacturing practice for medicinal products annexes

Moist heat sterilization

8.55 Moist heat sterilization can be achieved using steam (direct or indirect contact). Other systems such as superheated water systems (cascade or immersion cycles) could be used for containers that may be damaged by other cycle designs (for example, Blow-Fill-Seal containers, plastic bags).

8.56 Other than products in sealed containers, items to be sterilized should be dry and packaged in a protective barrier system that allows air to be removed, steam to penetrate and prevents recontamination after sterilization. All loaded items should be dry when they are removed from the sterilizer. Load dryness should be confirmed by visual inspection as a part of the sterilization process acceptance.

8.57 For cycles, time, temperature and pressure should be used to monitor the process and be recorded. Each sterilized item that is removed from the autoclave should be inspected for damage, packaging material integrity and moisture. 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 cycles should include a calculation of equilibration time, exposure time, correlation of pressure and temperature, and minimum/maximum temperature range during exposure. Validation of fluid cycles should 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 before and during sterilization when the sterilization process includes air purging (for example, autoclave loads, lyophilizer chambers). For autoclaves, this involves an air removal test cycle (normally performed daily) or use of an air detector system. Loads to be sterilized should be designed to support effective air removal and be free draining to prevent condensation from building up.

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

8.63 Steam-in-place systems that are used for sterilization (for example, for fixed pipework, vessels and lyophilizer chambers) 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 correlate 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 For fluids load cycles where superheated water is used to transfer the heat, the heated water should consistently reach all of the required contact points. Initial qualification studies should include temperature mapping of the entire load. The equipment should be checked routinely to ensure that nozzles (where the water is introduced) are not blocked and drains are free of 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 correlate to the worst-case positions identified during the qualification process.

Dry heat sterilization

8.66 Dry heat sterilization uses 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. It 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 established limits. The process may be operated in an oven or in a continuous tunnel process (for example, 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. Tests (at least twice a year) should be performed periodically 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:

i belt speed or dwell time within the sterilizing zone
ii minimum and maximum temperatures
iii heat penetration of the material or article
iv heat distribution and uniformity
v airflows determined by air pressure difference profiles (correlated with heat distribution and penetration studies)

8.68 For a thermal process used as part of the depyrogenation process for any component or product contact equipment/material, validation studies should 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 be carefully managed with a full reconciliation performed. Containers should represent the materials normally processed (in terms of the 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 used to sterilize or depyrogenate primary packaging components, starting materials or active substances. They may also 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:

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 or article (slow-to-heat spots)
vii heat distribution and 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 to sterilize heat-sensitive materials and products. Ultraviolet irradiation is not an acceptable method of sterilization.

For guidance on ionizing radiation sterilization, please consult:

- Process validation: Irradiation sterilization for pharmaceuticals (GUI-0009)
- PIC/S Annex 12 - Use of ionising radiation in the manufacture of medicinal products

8.72 Validation procedures should consider the effects of variation in the density of the product and packages.

Sterilization with ethylene oxide

8.73 This method should only be used when no other method is practicable. Process validation should show that:

- the product has not been damaged
- the conditions and time allowed for degassing have reduced any residual ethylene oxide (EO) 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, such as crystals or dried protein, in material. 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. Steam used to condition the load for sterilization should be of an appropriate quality. The time required for this should be balanced against the 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 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:

i EO gas concentration
ii pressure
iii amount of EO gas used
iv relative humidity
v temperature
vi exposure time

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

Filter sterilization of products that 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 using 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). The product should then be aseptically filled into a previously sterilized container. The filter that is selected/used should be 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 CCS.

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 fibres and particles, not cause or contribute to unacceptable levels of impurities or otherwise not alter the product's quality and efficacy. Similarly, the filter characteristics should be compatible with the fluid and not be adversely affected by the product being 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 sterilizing 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 µm final sterilizing grade filter, preferably as a closed system, both before 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 Pharmacopeia 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, the product to be filtered should be used for bacterial retention testing of the sterilizing grade filter, wherever possible. 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:

i the wetting fluid used for filter integrity testing:
    - 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, such as:
    - 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 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 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), 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 undergo a non-destructive integrity test post-use before the filter is removed 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 include bubble point, diffusive flow, water intrusion and pressure hold.

PUPSIT may not always be possible after sterilization due to process constraints (for example, the filtration of very small volumes of solution). In these cases, an alternative approach may be taken providing that there has been a thorough risk assessment performed and compliance has been achieved by implementing appropriate controls to mitigate any risk of a non-integral filtration system.

Points to consider in such a risk assessment should include:

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 is a 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 before 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 before replacement. The maximum duration of use should be specified and monitored based on risk (for example, 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. 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 1 filter), a post-use integrity test of the primary sterilizing grade filter should be performed. If the filter is demonstrated to be integral, then a post-use integrity test of the redundant (backup) filter is not necessary. However, in the primary filter has failed, a post-use integrity test on the secondary (redundant) filter should be performed. This should be performed, along 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 before the final sterile filtration. If a redundant filtration set-up is used, the samples should be taken before 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. The same filter should not be used continuously for more than 1 working day unless such use has been validated.

8.95 Where campaign manufacture of a product has been appropriately justified in the CCS 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, including APS, to demonstrate that the duration of filter use for a given sterile filtration process and 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 FFS machines used for terminally sterilized products and in aseptic manufacture should comply with the environmental requirements of this annex as follows:

- grade for filling (see paragraph 8.3)

- product at unusual risk of contamination (see paragraph 8.4)
- examples of operations and grades for aseptic preparation and processing operations (Table 4- see paragraph 8.10)

8.97 Contamination of the packaging films used in the FFS process should be minimized by appropriate controls during component fabrication, supply and handling. As packaging films are critical, procedures should be implemented to ensure the films that are 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 CCS.

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

8.99 Any product contact gases (for example, those used to inflate the container or used as a product overlay) must be appropriately filtered. This should be done close to when they will be used. The quality of the gases used and the effectiveness of gas filtration systems should be verified periodically in accordance with:

- gases that come into direct contact with product/primary container (see paragraph 6.18)
- gases used in aseptic processes (see paragraph 6.19)

8.100 The controls identified during qualification of FFS should align with the CCS. Aspects to be considered include:

i. determining the boundaries of the critical zone
ii. environmental control and monitoring of the machine and the background in which the machine 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 include:

i. settings for uniform package dimensions and cutting in accordance with validated parameters
ii. setting, maintaining and monitoring validated forming temperatures (including pre-heating and cooling), forming times and pressures as relevant

iii setting, maintaining and monitoring validated sealing temperatures, sealing temperature uniformity across the seal, sealing times and pressures as relevant
iv environmental and product temperatures
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 is not compromised
viii methods and parameters for integrity testing of filled containers (see paragraph 8.22)

8.102 There should be appropriate procedures for verifying, monitoring and recording FFS critical process parameters and equipment operation 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

8.105 Blow-Fill-Seal equipment used for the manufacture of products that are terminally sterilized should be installed in at least a grade D environment. The conditions at the point of fill should comply with the following environmental requirements:

- grade for filling of products for terminal sterilization (see paragraph 8.3)
- product at unusual risk of contamination (see paragraph 8.4)

8.106 BFS used for aseptic processing:

i For shuttle-type equipment used for aseptic filling, the parison is open to the environment. Therefore, the areas where parison extrusion, blow-moulding 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 as long as grade A or B clothing is used. The microbiological monitoring of operators wearing grade A or B clothing in a grade C area, should be performed in accordance with risk management principles. The limits and monitoring

frequencies that are applied should take into consideration the activities performed by these operators.

8.107 Due to the generation of particles from polymer extrusion, 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 the requirements set out in the Environmental and process monitoring section. In-operation viable monitoring should be undertaken for the full duration of critical processing, including equipment assembly. For rotary-type BFS equipment, monitoring of the critical filling zone may not be possible.

8.109 The environmental control and monitoring program should consider the moving parts and complex airflow paths generated by the BFS process and the effect of the high heat outputs of the process (for example, through airflow visualization studies and/or other equivalent studies). Environmental monitoring programs should also consider factors such as the configuration and integrity of air filters, the integrity of cooling systems and equipment design and qualification. Refer to 6.21 in the heating and cooling and hydraulic systems section.

8.110 Air or other gases that come into contact with critical surfaces of the container during extrusion, formation or sealing of the moulded container should undergo appropriate filtration. The quality of gas used and the effectiveness of gas filtration systems should be verified periodically in accordance with:

- gases that come into direct contact with product/primary container (see paragraph 6.18)
- gases used in aseptic processes (see paragraph 6.19)

8.111 Appropriate design, control and maintenance of the polymer granulate storage, sampling and distribution systems should prevent particulate and microbial contamination of the polymer granulate.

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

8.113 Interventions requiring cessation of filling and/or extrusion, moulding and sealing and, where required, re-sterilization of the filling machine should be clearly defined. These interventions should also be described in the filling procedure and included in the APS as relevant. Refer to the information in the Aseptic process simulation (APS) (also known as media fill) section related to the following:

- aseptic manipulations and interventions (see paragraph 9.34)
- unnecessary contamination risks (see paragraph 9.35)
- developing the APS plan (see paragraph 9.36)

8.114 The controls identified during qualification of BFS should align with the site's CCS. Aspects to consider include:

i. determining the boundaries of the critical zone
ii. environmental control and monitoring of the machine and the background in which the machine 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 include:

i. clean-in-place and sterilization-in-place of product pipelines and filling needles (mandrels)
ii. setting, maintaining and monitoring extrusion parameters, including temperature, speed and extruder throat settings for parison thickness
iii. setting, maintaining and monitoring mould temperatures, including rate of cooling where necessary for product stability
iv. preparing and sterilizing ancillary components added to the moulded unit (for example, bottle caps)
v. environmental control, cleaning, sterilizing and monitoring the critical extrusion, transfer and filling areas as relevant
vi. batch-specific testing of the wall thickness of the package at critical points of the container
vii. settings for correct filling volumes, speeds and uniformity
viii. settings for 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
    - refer to 8.22 in the finishing of sterile products section
x. settings for cutters or punches used to remove waste plastic that surrounds the filled units (flash removal)

8.116 Appropriate procedures for verifying, monitoring and recording 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 adding components to moulded containers (for example, caps added to LVP bottles), the components should be appropriately decontaminated and added to the process using a clean, controlled process.

i For aseptic processes, components should be added 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 moulded container, including areas that are not wetted during sterilization.
iii Testing procedures should be established and validated to ensure that components and moulded containers are sealed effectively.

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

Lyophilization

8.121 Lyophilization is a critical process step. 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 maintain product or material sterility during lyophilization. This is done by preventing microbial and particle contamination between when the products are filled for lyophilization and the lyophilization process is complete. All control measures in place should be determined by the site's CCS.

8.122 The sterilization of the lyophilizer and associated equipment (for example, trays, vial support rings) should be validated and the holding time between the sterilization cycle and use should be appropriately challenged during APS. Refer to the information in 9.33 of the Aseptic process simulation section. 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 much 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 CCS.

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 time the system is used and the results of integrity testing should be part of the batch certification/release. The frequency of vacuum/leak integrity testing of the chamber should be documented. 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 the following:

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 (for example, clean air transfer carts, portable unidirectional airflow workstations) should be used to maintain the cleanliness of the system used to transfer the partially closed containers. Alternatively, where supported by validation, trays closed in grade A and not reopened while in the grade B area may be used to protect partially stoppered vials (for example, 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 be separated from operators by physical barrier technology or 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 (for example, trays, bags, placing devices, tweezers) should be sterile.

Closed systems

8.127 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 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 sterility is maintained. Connection of sterile equipment (for example, tubing/pipework) to the sterilized product pathway after the final sterilizing grade filter should be designed to be connected aseptically (for example, 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 CCS. 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 a grade A area. If it can be shown that the integrity of the system is maintained at every usage (for example, via pressure testing and/or monitoring), a lower classified area may be used. Any transfer between classified areas should be thoroughly assessed (refer to 4.10 of the Premises section). If the closed system is opened (for example, for maintenance of a bulk manufacturing line), then this should be either:

- performed in a classified area appropriate to the materials
    - for example, 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 Single-use systems (SUS) are used in the manufacture of sterile drugs 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 Specific risks associated with SUS should be assessed as part of the CCS. These risks include:

i. interaction between the product and product contact surface (such as adsorption, leachables and extractables)
ii. fragile nature of the system compared with fixed reusable systems
iii. increase in the number and complexity of manual operations (including inspection and handling of the system) and connections
iv. complexity of the assembly
v. performance of pre- and post-use integrity testing for sterilizing grade filters
    - refer to information on the integrity of the sterilized filter assembly, see paragraph 8.87
vi. potential for holes and leakage
vii. potential for compromising the system at the point of opening the outer packaging
viii. potential for 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, sterility assurance should be verified 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. Each component should be assessed 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 considered. Simulated processing conditions 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 (such as freezing and thawing processes) either during routine processing or transportation. The integrity of intrinsic sterile connection devices (both heat-sealed and mechanically sealed) should be verified under these conditions.

8.138 Acceptance criteria should be established and implemented for SUS and correspond to the risks or criticality of a product and its processes. On receipt, each piece of SUS should be checked to ensure it has been manufactured, supplied and delivered in accordance with the approved specification. The outer packaging (exterior carton, product pouches) and label should be visually inspected and attached documents (for example, certificate of conformance and proof of sterilization) should be reviewed and documented before the product is used.

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

9. Environmental & process monitoring

General

9.1 The site's environmental and process monitoring program forms part of the overall contamination control strategy (CCS) and is used to monitor the controls designed to minimize the risk of microbial and particle contamination. Note: When taken in isolation, the reliability of each element of the monitoring system (viable, non-viable and APS) is limited and should not be considered an indicator of asepsis. When considered together, the results help confirm the reliability of the design, validation and operation of the system being monitored.

9.2 In general, this program consists 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 to 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 – overview

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

i give 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 that trigger an investigation and assessment of risk to product quality

Risk assessments should be used to establish the comprehensive environmental monitoring program. They should cover sampling locations, frequency of monitoring, monitoring methods and incubation conditions (such as time, temperature(s), aerobic and/or anaerobic conditions).

Risk assessments should be based on detailed knowledge of the following:

- the process inputs and final product
- the facility and equipment
- criticality of specific processes and steps
- the operations involved
- routine monitoring data
- monitoring data obtained during qualification
- typical microbial flora isolated from the environment

Risk assessments should:

- determine critical monitoring locations
- determine locations where the presence of microorganisms during processing may have an impact upon product quality (for example, grade A areas, aseptic processing areas and grade B areas that directly interface with a grade A area
- include other information such as air visualization studies
- confirm the effectiveness of the site's environmental monitoring program through regular reviews
    - The monitoring program should be considered in the overall context of the trend analysis and the CCS for the site.

9.5 Cleanrooms, clean air equipment and personnel should be routinely monitored throughout all critical stages of processing, including equipment set-up and when in operation.

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

9.7 The monitoring of grade A areas should demonstrate that aseptic processing conditions are maintained during critical operations. Locations that pose the highest risk of contamination to the sterile equipment surfaces, containers, closures and product should be monitored. Monitoring locations and the orientation and positioning of sampling devices should be justified and appropriate for obtaining 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 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 CCS. Both viable and total particle alert levels should be established based on cleanroom qualification test results 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 areas should be set in order to detect, and address, adverse trends (such as number of or individual events that indicate a deterioration of environmental control).

9.11 Monitoring procedures should define the trending approach. Trends should include:

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 (for example, 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 that may be difficult to control such as spore-forming microorganisms and molds

9.12 Grades C and D cleanrooms should be monitored in operation 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 being carried out. Action limits may be more stringent than those listed in tables 5 and 6.

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

Environmental monitoring - total particle

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

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

Table 5: Maximum permitted total particle concentration for monitoring

Grade	Maximum permitted number of particles/m^3 equal to or greater than the tabulated size			
	≥ 0.5 µm		≥5.0 µm	
	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.

Notes:
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 operations have been completed. Refer to the information on cleanroom classification (see paragraph 4.29).
2. The occasional indication of macro particle counts, especially ≥ 5 µm, within grade A may be considered to be false counts due to such things as electronic noise, stray light or coincidence loss. However, consecutive or regular counting of low levels may indicate a possible contamination event and should be investigated. Such events may indicate early failure of the room air supply filtration system or equipment. They may also indicate poor practices during machine set-up and routine operation.

9.16 For grade A, particles should be monitored 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 litres (1 ft^3) per minute) in order to capture all interventions, transient events and any system deterioration. The system should frequently correlate individual sample results with alert levels and action limits at the frequency that would make it possible to identify, and respond to any potential excursion in a timely manner. Alarms should be triggered if alert levels are exceeded, and procedures should outline the actions that should be taken in response to alarms, such as additional microbial monitoring.

9.18 A similar system can 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 so that any increase in contamination levels and system deterioration is captured by the program. If alert levels are exceeded, alarms should be triggered.

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

9.20 Where contaminants are present due to the processes involved that would potentially damage the particle counter or present a hazard (for example, live organisms, powdery products and radiation hazards), the frequency and strategy used should be such as to assure the environmental classification both before and after being exposed to the risk. Increased viable particle monitoring should be considered to ensure comprehensive monitoring of the process. Simulated operations should also be monitored and performed at appropriate intervals. The approach should be defined in the CCS.

9.21 The size of monitoring samples taken using automated systems is usually a function of the system's sampling rate. It's 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 particle

9.22 Where aseptic operations are performed, microbial monitoring should be done frequently using a combination of methods such as settle plates, volumetric air sampling, glove, gown and surface sampling (for example, swabs and contact

plates). The CCS should justify the method of sampling that is chosen and demonstrate that the method does not have a detrimental impact on grades A and B airflow patterns. Cleanroom and equipment surfaces should be monitored at the end of an operation.

9.23 Viable particles in the cleanrooms should also be monitored when normal manufacturing operations (such as post-disinfection, before manufacturing starts, after the batch is completed and after a shutdown period) are not taking place. Associated rooms that have not been used should also be monitored for viable particles.

Viable particles monitoring is done to detect potential incidents of contamination that may affect the controls in the cleanrooms. In case of an incident, additional sample locations may be used to verify the effectiveness of a corrective action (for example, cleaning and disinfection).

9.24 Continuous viable air monitoring in the grade A area (for example, air sampling or settle plates) should be undertaken for the full duration of critical processing, including equipment (aseptic set-up) assembly. A similar approach should be considered for grade B cleanrooms based on the risk of impact on the aseptic processing. The monitoring should capture all interventions, transient events and system deterioration and avoid any risk caused by monitoring operations.

9.25 A risk assessment should evaluate the locations, type and frequency of personnel monitoring based on the activities performed and the proximity of personnel to critical zones. Monitoring should involve personnel at periodic intervals during the process, but not to compromise the process. In particular, personnel should be monitored after they have been involved in critical interventions. At a minimum, gloves should be monitored, but areas of gown as applicable to the process may also be monitored. Where monitoring of gloves is performed after critical interventions, the outer gloves should be replaced before the activity resumes. Where monitoring of gowns is required after critical interventions, the gown should be replaced before further activity takes place in the cleanroom. In addition, the personnel should be monitored each time they exit the grade B cleanroom (gloves and gown).

9.26 Personnel in grade A and B areas should be monitored for microbial contamination. Where operations are manual in nature (for example, aseptic compounding or filling), there should be increased emphasis on microbial monitoring gowns due to increased risk. This should be justified within the CCS.

9.27 Routine monitoring by manufacturing personnel should be subject to regular oversight by the quality unit. Refer also to the information in the paragraph 8.19.

9.28 Manufacturers should consider adopting suitable alternative monitoring systems such as rapid methods in order to expedite the detection of microbiological contamination issues and reduce the risk to product. Rapid and automated microbial monitoring methods may be adopted after validation has demonstrated they are equal or superior to established methods.

9.29 Sampling methods and equipment used should be fully understood. There should be procedures for operating the equipment correctly and for interpreting the sampling results. There should also be data available to support the recovery efficiency of the sampling methods chosen.

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

Table 6: Action limits for microbial contamination

Grade	Air sample cfu/m³	Settle plates (diam. 90 mm), cfu/4 hours[a]	Contact plates (diam. 55 mm), cfu/plate[b]	Glove print, including 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 in grade A and B areas 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 not negatively affect 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 B areas. Routine gown monitoring is not normally required for grade C and D areas, depending on their function.
[c] For grade A areas, any growth should result in an investigation.

Notes: 1. The types of monitoring methods listed in Table 6 are examples. Other methods may be used if they provide information throughout the entire critical process where the product may be contaminated (for example, aseptic line set-up, aseptic processing, filling and lyophilizer loading). 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 grade A and B areas should be identified to species level. The potential impact of such microorganisms on product quality (for each batch implicated) and overall state of control should be evaluated. Microorganisms detected in grade C and D areas should also be identified, for example:

- where action limits or alert levels are exceeded
- following the isolation of organisms that may:
    - indicate a loss of control, deterioration in cleanliness
    - be difficult to control such as spore-forming microorganisms and molds
    - be 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 APS should not be 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 pharmaceutical quality system and process controls, training and evaluation of monitoring data. Selection of an appropriate nutrient media and/or surrogate should be made based on the ability of the media and/or surrogate to imitate physical product characteristics that pose a risk to product sterility during the aseptic process.

Where processing stages may indirectly impact the viability of any introduced microbial contamination (for example, 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.

i. The APS should assess all aseptic operations performed subsequent to the sterilization and decontamination cycles of materials used 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 (for example, processes involving drying, blending, milling and subdividing a sterile powder) should be avoided. Any use of individual simulations should be supported by a documented justification, and ensure that the individual simulations combined continue 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. The lyophilization process simulation should be conducted 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:

- using 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
- considering quantitative aspects of worst-case situations (for example, 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 consider various aseptic manipulations and interventions known to occur during normal production as well as worst-case situations.

i Inherent and corrective interventions representing 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 The APS should not be used to justify practices that pose unnecessary contamination risks.

9.36 In developing the APS plan, the manufacturer should consider:

i identifying 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 validating the same container/closure configuration for different products where process equivalence is scientifically justified
iii determining maximum permitted holding times for sterile product and equipment exposed during the aseptic process
iv ensuring that the volume filled per container should be sufficient to ensure that the media contacts all equipment and component surfaces that may directly contaminate the sterile drug
    - the volume used should provide sufficient headspace to support potential microbial growth and ensure that turbidity can be detected during inspection
v requiring that air substitute any inert gas used in the routine aseptic manufacturing process unless anaerobic simulation is intended
    - consider including occasional anaerobic simulations as part of the overall validation strategy
        - see paragraph 9.33 point iii
vi ensuring that the selected nutrient media are capable of growing a designated group of reference microorganisms as described by the relevant pharmacopeia and suitably representative local isolates

vii providing scientific justification for the method used to detect microbial contamination to ensure that contamination is reliably detected

viii ensuring the process simulation is 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 drug

ix ensuring that where the manufacturer operates different or extended shifts, the APS is designed to capture factors specific to those shifts that are assessed to pose a risk to product sterility

- for example, the maximum length of time an operator may be present in the cleanroom

x simulating normal aseptic manufacturing interruptions where the process is idle

- for example, shift changeovers, recharging dispensing vessels, introducing additional equipment

xi ensuring that environmental monitoring is conducted as required for routine production and throughout the entire duration of the process simulation

xii ensuring that where campaign manufacturing occurs, such as in the use of barrier technologies or manufacture of sterile active substances, consideration is given to designing and performing the process simulation so that it simulates the risks associated with the beginning and the end of the campaign

- 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 CCS and should not replace routine APS.

- 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 drug. All the simulated materials (surrogates or growth medium) should also 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 micro-organisms.

9.38 APS should be performed as part of the initial validation. There should be at least 3 consecutive satisfactory simulation tests that cover all working shifts that the aseptic process may occur in. These tests should also be performed right after operational practices, facilities, services or equipment that are assessed to have an impact on the sterility assurance of the product have been significantly modified. Examples include:

- modifications to the HVAC system or to equipment
- changes to process, number of shifts and personnel
- a major facility shut-down

Normally, APS (periodic revalidation) should be repeated twice a year (about every 6 months) for each aseptic process, each filling line and each shift. Each operator should participate in at least one successful APS annually. An APS should be performed after the last batch before shut-down, before long periods of inactivity or before a line is decommissioned or relocated.

9.39 Where manual operation (for example, 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. They should be revalidated with 1 APS about 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 represent the aseptic manufacturing process. The number of units to be filled should be justified in the CCS. Typically, a minimum of 5,000 to 10,000 units are filled. For small batches (those under 5,000 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 APS should be incubated and evaluated, including units with cosmetic defects or those that have gone through non-destructive in-process control checks. Units that are discarded during the process simulation and not incubated should be comparable to units discarded during a routine fill, and only if production SOPs clearly specify that units must be removed under the same circumstances (for example, 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 usually be incubated separately. They 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 (for example, product flushes), the discarded material should be simulated with nutrient media and incubated as part of the APS, unless it can be shown that this waste process does 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. For product containers that are not clear (amber glass, opaque plastic), clear containers of identical configuration may be substituted to help detect contamination. If a clear container of identical configuration cannot be used as a substitute, a suitable method for detecting microbial growth should be developed and validated. Microorganisms isolated from contaminated units should

be identified to the species level when practical, to help determine 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 (and duration) of the incubation conditions should be scientifically justified and validated to provide an appropriate level of sensitivity for detecting microbial contamination.

9.45 When incubation is completed:

i. Filled APS units should be inspected by personnel who have been appropriately trained and qualified to detect 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 APS and the following actions 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 APS (a minimum of 3) to demonstrate that the process has been returned to a state of control
iv. a prompt review of all appropriate records on aseptic production since the last successful APS:
    a. The outcome of the review should include a risk assessment of potential sterile breaches in batches manufactured since the last successful APS.
    b. All other batches not released to the market should be included in the scope of the investigation.
        - Any decision on their release status should consider the investigation outcome.
v. the quarantine of all products that have been manufactured on a line subsequent to a process simulation failure until the process simulation failure has been successfully resolved
vi. actions taken to limit the operator's activities, until the person has been retrained and requalified, where the root cause investigation has determined that the failure was related to operator activity
vii. production to resume only after revalidation has been completed successfully

9.47 All APS 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 APS should be recorded, including the start and end time of each intervention and the person involved. All microbial monitoring data as well as other testing data should be recorded in the APS batch record.

9.48 An APS run should be aborted only when written procedures require commercial lots to be handled in the same way. In such cases, an investigation should be documented.

9.49 An aseptic process should have to undergo a repeat of the initial validation when:

i. the specific aseptic process has not been in operation for an extended period of time
ii. a change to the process, equipment, procedures or environment that has the potential to affect the aseptic process
    - new product containers or container-closure combinations have been added

10. Quality Control (QC)

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

10.2 Specifications for raw materials, components and products should include requirements for microbial, particulate and endotoxin/pyrogen limits when monitoring and/or the contamination control strategy (CCS) have indicated the need.

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 that represent the worst-case scenario (such as at the end of hold time) should be taken. 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 program for the filled product prior to initiating the sterilization cycle should be developed. 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 A sterility test conducted on the finished product should be viewed as the last in a series of critical control measures taken to assure sterility. A sterility test 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. In particular samples should be taken from parts of the batch considered to be most at risk of contamination, for example:

- For products that have been filled aseptically, samples should include containers filled at the beginning and end of the batch. Additional samples, such as those taken after critical interventions, should be considered based on risk.

ii For products that have been heat sterilized in their final containers, samples should represent the worst-case locations (for example, the potentially coolest or slowest to heat part of each load).
iii For products that have been lyophilized, samples should be taken from different lyophilization loads.

Note: Where the manufacturing process results in sub-batches (for example, for terminally sterilized products), sterility samples from each sub-batch should be taken and a sterility test conducted for each sub-batch. Other finished product tests should be done separately.

10.7 It may not be possible to obtain a sterility test result for some products before they are released because the shelf life is too short to allow a sterility test to be completed. In these cases, the additional process design considerations as well as additional monitoring and/or alternative test methods required to mitigate identified risks should be assessed and documented.

10.8 Any process (for example, vaporized hydrogen peroxide, ultraviolet) used to decontaminate the external surfaces of sterility samples before 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 Pharmacopeia before use. Media used for environmental monitoring and APS should be tested for growth promotion before use, using a scientifically justified and designated group of reference microorganisms and including suitably representative local isolates. The end user should perform media quality control testing. Outsourced testing or supplier testing of media should be justified and transportation and shipping conditions should be thoroughly considered.

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

10.11 Rapid and automated microbial methods used for general manufacturing purposes should be validated for the product(s) or processes concerned.

Glossary

Acronyms and abbreviations

API: active pharmaceutical ingredient
cfu: colony forming unit
EO: ethylene oxide
GMP: good manufacturing practices
PIC/S: Pharmaceutical Inspection Cooperation/Scheme
RABS: restricted access barrier systems
SOP: standard operating procedure
WFI: water for injection

Definitions

These definitions explain how terms are used in this document. Definitions quoted from other documents are identified in brackets at the end of the definition. If there is a conflict with a definition in the *Food and Drugs Act or Food and Drug Regulations*, the definition in the act or regulations prevails. More applicable definitions can be found in the Good manufacturing practices guide for drug products (GUI-0001).

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.

Action limit:
An established relevant measure (for example, microbial or airborne particle limits) that, when exceeded, should trigger appropriate investigation and corrective action based on the investigation.

Alert level:
An established relevant measure (for example, 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 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.

Aseptic preparation/processing:
The handling of sterile drug, containers and/or devices in a controlled 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 (for example, equipment assembly, formulation, filling, lyophilization and sealing processes as necessary).

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 drug.

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 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 by using sporicidal chemical agents.

Biological indicators (BI):
A population of microorganisms inoculated onto a suitable medium (for example, solution, container or closure) and placed within a sterilizer, 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 BI.

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 2 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. (Refer to the definition for "cleanroom".)

Cleaning:
A process for removing contamination (for example, product residues and disinfectant residues).

Clean area:
An area with defined particle and microbiological cleanliness standards that usually contains a number of joined cleanrooms.

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

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.

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

Closed system:
A system in which the product is not exposed to the surrounding environment. For example, this can be achieved by using 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 drugs, the full system is sterilized after the connections are made. Examples of these can be 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 end of an operation.

The use of the term "closed systems" in this Annex does not refer to systems such as RABS or isolator systems.

Colony forming unit (cfu):
A microbiological term to describe a single detectable colony that originates from 1 or more microorganisms. Colony forming units are typically expressed as:

- cfu per ml for liquid samples
- cfu per m^3 for air samples
- cfu per sample for samples captured on solid medium
    - such as settle or contact plates

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. Can occur during production, sampling, packaging or repackaging, storage or transport. Has the potential to adversely impact product quality.

Contamination control strategy (CCS):
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. 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
- associated methods and frequency of monitoring and control

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 clearing component jams, stopping leaks, adjusting sensors and replacing equipment components.

Critical surfaces:
Surfaces that may come directly into contact with, or directly affect, a sterile drug 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 environment.

Critical intervention:
An intervention (corrective or inherent) into the critical zone.

D-value:
The value of a parameter of sterilization (duration or absorbed dose) required to reduce the number of viable organisms to 10% of the original number.

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 will not be used again.

Decontamination:
The overall process of removing or reducing any contaminants (chemical, waste, residue or microorganisms) from an area, object or person. The method of decontamination used (for example, cleaning, disinfection, sterilization) should be chosen and validated to achieve a level of cleanliness appropriate to the intended use of the item decontaminated. (Refer to the definition for "bio-decontamination".)

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.

Endotoxin:
A pyrogenic product (for example, lipopolysaccharide) present in the gram negative bacterial cell wall. Endotoxin can lead to reactions in patients receiving injections ranging from fever to death.

Equilibration time:
Period that 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.

First air:
Filtered air that has not been interrupted prior to contacting exposed product and product contact surfaces with the potential to add contamination to the air before reaching the critical zone.

Filter integrity test:
A test to confirm that a filter (product, gas or HVAC filter) retains its retentive properties and has not been damaged during handling, installation or processing.

Form-Fill-Seal (FFS):
An automated filling process, typically used for terminally sterilized products. The process 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 use:

- single web system
    - where a single flat roll of film is wrapped around itself to form a cavity or

- dual web system
    - where 2 flat rolls of film are brought together to form a cavity, often with the aid of vacuum moulds 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 program that establishes, both initially and on a periodic basis, the capability of an individual to don the complete gown.

Grade A air supply:
Air that 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 to protect 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 (for example, 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 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. For example: the sampling condition in which the mean velocity of the air entering the sample probe inlet is nearly the same (± 20%) 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 (for example, surrounding cleanroom air and personnel). There are 2 major types of isolators:

- **Closed isolator systems** exclude external contamination of the isolator's interior by accomplishing material transfer by aseptic connection to auxiliary equipment, rather than use of openings to the

surrounding environment. Closed systems remain sealed throughout operations.

- **Open isolator systems** are designed to allow for the continuous or semi-continuous ingress and/or egress of materials during operations through one or more openings. Openings are engineered (using continuous overpressure, for example) 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 with the term freeze-drying.

Manual aseptic processing:
An aseptic process where the operator manually compounds, fills, places and /or seals an open container with the sterile drug.

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" but typically smaller in size. (Refer to the definition for "airlock".)

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, $F_0 \geq 8$ min) are not fulfilled. This may also be beneficial in destroying viruses that may not have been removed through filtration.

Pyrogen:
A substance that induces a febrile reaction in patients receiving injections.

Rapid transfer system/port (RTP):
A system used to transfer items into RABS or isolators that minimizes the risk to the critical zone. An example would be a rapid transfer container with an alpha/beta port.

Radiopharmaceutical:
"A drug that exhibits spontaneous disintegration of unstable nuclei with the emission of nuclear particles or photons." (C.03.201)

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

Restricted access barrier system (RABS):
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, RTPs and other integrated transfer ports to perform manipulations or convey materials to the interior of the RABS. Depending on the design, doors are rarely opened, and only under strictly predefined 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 document are those that are used in manufacturing processes of sterile products. 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 drug:
In this guidance, sterile drug refers to 1 or more of the sterilized elements exposed to aseptic conditions and ultimately making up the sterile active substance or finished sterile drug. 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 to 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. For example: 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 (1 in a million).

Turbulent airflow:
Air that is not unidirectional. Turbulent air in cleanrooms should flush the cleanroom via mixed flow dilution and ensure acceptable air quality is maintained.

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.

Unidirectional airflow (UDAF) unit:
A cabinet supplied with filtered unidirectional airflow (previously referred to as a laminar airflow unit, or LAF).

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.

Water system:
A system for producing, storing and distributing water, usually compliant to a specific pharmacopeia grade (for example, purified water and water for injection, or WFI).

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

References

- Food and Drugs Act
- Food and Drug Regulations
- Good manufacturing practices guide for drug products (GUI-0001)
- Process validation: Aseptic processes for pharmaceuticals (GUI-0006)
- Guidance on drug establishment licences (GUI-0002)
- Good manufacturing practices for active pharmaceutical ingredients (GUI-0104)
- Guide to validation – Drugs and supporting activities (GUI-0029)
- Annex 17, Parametric Release (PIC/S) - Guide to good manufacturing practice for medicinal products annexes
- Process validation: Gaseous sterilization for pharmaceuticals (GUI-0007)
- Process validation: Irradiation sterilization for pharmaceuticals (GUI-0009)
- Process validation: Moist heat sterilization for pharmaceuticals (GUI-0010)
- ISO standards
    - ISO 14644-1: Cleanrooms and associated controlled environments – Part 1: Classification of air cleanliness by particle concentration
    - ISO 14644-2: Cleanrooms and associated controlled environments – Part 2: Monitoring to provide evidence of cleanroom performance related to air cleanliness by particle concentration
    - ISO14644-3: Cleanrooms and associated controlled environments – Part 3: Test methods
    - ISO 14644-4: Cleanrooms and associated controlled environments – Part 4: Design, construction and start-up
    - ISO 14644-5: Cleanrooms and associated controlled environments – Part 5: Operations
- PIC/S Annex 12 - Use of ionising radiation in the manufacture of medicinal products
- PIC/S Annex 15 – Qualification and validation
- PIC/S GMP guide annexes

Note: The ISO standards referenced in this document were applicable at the time of drafting. Future revisions of these standards do not automatically apply to this document. Relevant updates will be reflected in a future version.

Questions and answers

Does the supervisor of a sterile drug manufacturing facility need to have a degree in microbiology?

Section C.02.029 (b) "Sterile drugs" of the Food and Drug Regulations requires that "a drug that is intended to be sterile shall be produced under the supervision of personnel trained in microbiology." The expression "trained in microbiology" does not mean that this person must have a university degree in microbiology. However, the person must have taken university courses in microbiology.

If water that has already been used in compounding is later found to contain endotoxins, what actions need to be taken?

Water can be used for production before obtaining microbiological test results, but the results of these tests must be available before final release of the product. Good manufacturing practices permit release only after raw material and finished product testing is completed and results show the product complies with its specifications.

The appropriate action would include an investigation into:

- the potential sources of endotoxins
- the sanitation and maintenance of the water system

Are sterile products in amber glass and plastic ampoules exempt from 100% inspection?

No. You must inspect each final container of injections. The 100% inspection test does not limit itself to particulate matter. It also includes, for example, sealing defects, charring, glass defects, underfills and overfills, and missing print. Please refer to interpretation in the section on Finishing of sterile drugs. For parenteral products, there are more requirements for packaging (for example, the immediate container must be of a material and construction that allows visual or electronic inspection of the drug). Please refer to section C.01.069 "Limits of Variability" in the *Food and Drug Regulations*.

What are the room classification requirements for preparing containers and other packaging materials to be used in fabricating sterile drugs?

Normally, you would prepare (for example, clean, wash) containers and packaging materials in a "clean" room (Grades C or D). Afterwards, for drugs sterilized by filtration (and not further subjected to terminal sterilization in their final containers), you must depyrogenate and sterilize (using double-ended sterilizers or any other validated method) the containers and materials used before introducing them into aseptic rooms. The depyrogenation step can be done using pyrogen-free water for injection (WFI) for the last rinse before sterilization or by performing the depyrogenation and sterilization in one operation using a dry heat oven. Filling of these products normally takes place in a Grade A area with a Grade B background.

For products that are terminally sterilized, you do not have to use containers and packaging materials that are sterile. However, those in direct contact with the product should be free of pyrogen. This is usually done by using pyrogen-free WFI for the last rinse of these materials, unless they are later depyrogenated by another method (for example, using a dry heat oven).

Also, the initial bioburden of these materials should meet pre-established limits, based on sound science. Keep the risk of contamination during their introduction in filling areas to a minimum.

For the validation of moist heat sterilization cycles, will the new standards include the use of prions as the organism of choice (instead of Geobacillus stearothermophilus)?

At the present time, the scientific and pharmaceutical community recognizes the spores of Geobacillus stearothermophilus as the organisms of choice for validating moist heat sterilization cycles. The use of prions (infectious proteins) could be inadequate because their detection and quantification (which is based on animal models) is very difficult. Also, these proteins are very hard to destroy and could present a danger should they accidentally be spread in a plant.

According to the monograph on parenteral products (0520) of the 10th edition of the *European Pharmacopoeia* (Ph. Eur.), injections for veterinary use with a volume dose of less than 15 mL are exempted from bacterial endotoxins/pyrogen testing by the European Union (EU). Is this interpretation correct? If so, would this EU exemption be applicable in Canada?

Yes, this interpretation is correct. But this exemption does not apply in Canada.

As per section C.01.067 (1) "Limits of Variability" in the regulations, each lot of a drug for parenteral use must be tested for the presence of pyrogens using an acceptable method. Each lot must be found to be non-pyrogenic. The bacterial endotoxins and pyrogen test methods described in the United States Pharmacopeia (USP) and Ph. Eur. are considered acceptable methods for that purpose.

For all parenteral drug products, the bacterial endotoxins test should be preferred over the pyrogen test, unless the latter is shown to be justified (more appropriate) or has been approved by a review directorate. The specification of all drug products for parenteral use intended for the Canadian market should include a test for bacterial endotoxins or pyrogens. The current EU "15 mL exemption" does not apply in Canada.

The only acceptable exemptions are those provided in section C.01.067 (2) "Limits of Variability." In other words, not testing a parenteral drug product for the presence of pyrogens would be considered acceptable only if documentation is available to show that the parenteral drug product is inherently pyrogenic or that it cannot be tested by any of the methods.

What is Health Canada's position on pooling samples within the same batch (for example, 7 samples in 1 pool) for testing for sterility? The European Pharmacopoeia (Ph. Eur.) does not mention explicitly a pooling of samples for testing for sterility.

It is acceptable to pool samples for sterility testing with the membrane filtration method. But it is not acceptable to pool samples if you use the direct inoculation method. Exceptions can be tolerated when the volume of the sample pool does not exceed 10% of the culture medium volume.