WHO Technical Report Series, No. 961, 2011

Annex 6

WHO good manufacturing practices for sterile pharmaceutical products

Introduction

Following implementation of these WHO good manufacturing practices (GMP) guidelines (*1*) within the context of the WHO Prequalification of Medicines Programme, clarifying, editorial modifications have been proposed. These changes were adopted for maintenance purposes. In order to ease reading the full guideline has been reproduced again as an Annex to the current report of the WHO Expert Committee on Specifications for Pharmaceutical Preparations.

WHO good manufacturing practices for sterile pharmaceutical products

1. General considerations

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

1.2 The various operations of component preparation (such as those involving containers and closures), product preparation, filling and sterilization should be carried out in separate areas within the clean area. These areas are classified into four grades (see section 4).

1.3 Manufacturing operations are divided here into two categories:

— first, those where the product is terminally sterilized; and
— second, those which are conducted aseptically at some or all stages.

2. Quality control

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

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

- for products that have been filled aseptically, samples should include containers filled at the beginning and end of the batch and after any significant interruption of work;
- for products that have been heat sterilized in their final containers, consideration should be given to taking samples from that part of the load that is potentially the coolest.

2.3 The sterility of the finished product is assured by validation of the sterilization cycle in the case of terminally sterilized products, and by "media simulation" or "media fill" runs for aseptically processed products. Batch-processing records and, in the case of aseptic processing, environmental quality records, should be examined in conjunction with the results of the sterility tests. The sterility test procedure should be validated for a given product. Pharmacopoeial methods should be used for the validation and performance of the sterility test. In those cases where parametric release has been authorized in place of sterility testing special attention should be paid to the validation and the monitoring of the entire manufacturing process.

2.4 For injectable products the water for injection and the intermediate, if appropriate, and finished products should be monitored for endotoxins, using an established pharmacopoeial method that has been validated for each type of product. For large-volume infusion solutions, such monitoring of water or intermediates should always be done, in addition to any tests required by an approved monograph for the finished product. When a sample fails a test, the cause of the failure should be investigated and necessary action should be taken. Alternative methods to those in the pharmacopoeias may be used if they are validated, justified and authorized.

2.5 The use of rapid microbiological methods to replace the traditional microbiological methods, and to obtain earlier results on the microbiological quality of, for example, water, the environment or bioburden, could be considered if appropriately validated and if a comparative assessment of the proposed rapid method is performed against the pharmacopoeial method.

3. Sanitation

3.1 The sanitation of clean areas is particularly important. They should be cleaned frequently and thoroughly in accordance with an approved written programme. Where disinfectants are used, more than one type should be employed. Monitoring should be regularly undertaken to detect contamination or the presence of an organism against which the cleaning procedure is ineffective. Interactions between different cleaning materials should be validated. Appropriate cleaning validation should be carried out to ensure disinfectant residuals can be detected and are removed by the cleaning process.

3.2 Disinfectants and detergents should be monitored for microbial contamination; dilutions should be kept in previously cleaned containers and should only be stored for defined periods unless sterilized. Disinfectants and detergents used in Grade A and B areas should be sterile before use.

3.3 A disinfectant programme should also include a sporicidal agent since many common disinfectants are ineffective against spores. The effectiveness of cleaning and disinfectant procedures should be demonstrated.

3.4 Fumigation of clean areas may be useful for reducing microbial contamination in inaccessible places.

4. Manufacture of sterile preparations

4.1 Clean areas for the manufacture of sterile products are classified according to the required characteristics of the environment. Each manufacturing operation requires an appropriate level of environmental

cleanliness in the operational state to minimize the risks of particulate or microbial contamination of the product or materials being handled.

4.2 Detailed information on methods for determining the microbiological and particulate cleanliness of air, surfaces, etc., is not given in these guidelines.

ISO 14644-1 (*2*) should be used for classification of cleanliness according to concentration of airborne particles (determination of number of sample locations, calculation of sample size and evaluation of classification from the data obtained). Table 1 should also be used to define the levels to be used as the basis for monitoring clean areas for airborne particles.

4.3 For the manufacture of sterile pharmaceutical preparations, four grades of clean areas are distinguished as follows:

- *Grade A*: The local zone for high-risk operations, e.g. filling and making aseptic connections. Normally such conditions are achieved by using a unidirectional airflow workstation. Unidirectional airflow systems should provide a homogeneous air speed of 0.36–0.54 m/s (guidance value) at a defined test position 15–30 cm below the terminal filter or air distributor system. The velocity at working level should not be less than 0.36 m/s. The uniformity and effectiveness of the unidirectional airflow should be demonstrated by undertaking airflow visualization tests.
- *Grade B*: In aseptic preparation and filling, this is the background environment for the Grade A zone.
- *Grades C and D*: Clean areas for carrying out less critical stages in the manufacture of sterile products or carrying out activities during which the product is not directly exposed (i.e. aseptic connection with aseptic connectors and operations in a closed system).

A unidirectional airflow and lower velocities may be used in closed isolators and glove boxes.

4.4 In order to reach the B, C and D air grades the number of air changes should be appropriate for the size of the room and the equipment and personnel present in it.

4.5 High-efficiency particulate air (HEPA) filters should be subjected to an installed filter leakage test in accordance with ISO 14644-3 (*3*) at a recommended interval of every 6 months, but not exceeding 12 months. The purpose of performing regular leak tests is to ensure the filter media, filter frame and filter seal are free from leaks. The aerosol selected for HEPA leak testing should not support microbial growth and should be composed of a sufficient number or mass of particles. HEPA filter patching is allowed at the filter manufacturer and in situ operation provided that the patch sizes and procedures follow the recommendations of ISO 1822-4 (*4*).

Clean room and clean-air device classification

4.6 Clean rooms and clean-air devices should be classified in accordance with ISO 14644 (*2–3, 5–7*).

4.6.1 Classification should be clearly differentiated from operational process environmental monitoring. The maximum permitted airborne particle concentration for each grade is given in Table 1.

Table 1
Maximum permitted airborne particle concentrat

	Maximum permitted number of particles per m^3 greater than or equal to the tabulated size			
	At rest[a]		In operation[b]	
Grade	**0.5 µm**	**5.0 µm**	**0.5 µm**	**5.0 µm**
A	3 520	20	3 520	20
B	3 520	29	352 000	2 900
C	352 000	2 900	3 520 000	29 000
D	3 520 000	29 000	Not defined	Not defined

[a] The "at rest" state is the condition where the installation is complete with equipment installed and operating in a manner agreed upon by the customer and supplier, but with no personnel present.

[b] The "in operation" state is the condition where the installation is functioning in the defined operating mode and the specified number of personnel is present. The areas and their associated environmental control systems should be designed to achieve both the "at rest" and "in operation" states.

4.6.2 For classification purposes in Grade A zones, a minimum sample volume of 1 m^3 should be taken per sample location. Referring to Table 1, for Grade A the airborne particle classification is ISO 4.8 dictated by the limit for particles ≥ 5.0 µm. For Grade B (at rest) the airborne particle classification is ISO 5 for both particle sizes considered. For Grade C (at rest and in operation) the airborne particle classification is ISO 7 and ISO 8, respectively. For Grade D (at rest) the airborne particle classification is ISO 8. For classification purposes ISO 14644-1 (*2*) methodology defines both the minimum number of sample locations and the sample size based on the class limit of the largest particle size considered and the method of evaluation of the data collected. The sample volume should be determined according to ISO 14644-1 (*2*) clause B.4.2. However, for lower grades (Grade C in operation and Grade D at rest) the sample volume per location should be at least 2 litres and the sample time per location should be not less than 1 minute.

4.6.3 Portable particle counters with a short length of sample tubing should be used for classification purposes to avoid the loss of particles ≥ 5.0 µm. Isokinetic sample heads should be used in unidirectional airflow systems.

4.6.4 "In operation" classification may be demonstrated during normal operations, simulated operations or during media fills as worst-case simulation

is required for this. ISO 14644-2 (*6*) provides information on testing to demonstrate continued compliance with the assigned cleanliness classification.

Clean room and clean-air device monitoring

4.7 Clean rooms and clean-air devices should be routinely monitored while in operation and the monitoring locations based on a formal risk analysis study and the results obtained during the classification of rooms and/or clean-air devices.

4.7.1 For Grade A zones, particle monitoring should be undertaken for the full duration of critical processing, including equipment assembly, except where justified by contaminants in the process that would damage the particle counter or present a hazard, for example, live organisms and radiological hazards. In such cases monitoring during routine equipment set-up operations should be undertaken before exposure to the risk. Monitoring during simulated operations should also be performed. The Grade A zone should be monitored at a frequency and sample size such that all interventions, transient events and any system deterioration would be captured and alarms triggered if alert limits are exceeded. It is accepted that it may not always be possible to demonstrate low levels of ≥ 5.0 μm particles at the point of fill when filling is in progress, due to the generation of particles or droplets from the product itself.

4.7.2 It is recommended that a similar system be used for Grade B zones, although the sample frequency may be decreased. The importance of the particle monitoring system should be determined by the effectiveness of the segregation between the adjacent Grade A and B zones. The Grade B zone should be monitored at a frequency and with a sample size such that changes in levels of contamination and any deterioration of the system would be captured and alarms triggered if alert limits are exceeded.

4.7.3 Airborne particle monitoring systems may consist of independent particle counters; a network of sequentially accessed sampling points connected by manifold to a single particle counter; or multiple small particle counters located near monitoring points and networked to a data acquisition system. Combinations of systems can also be used. The system selected should be appropriate for the particle size considered.

Where remote sampling systems are used, the length of tubing and the radii of any bends in the tubing should be considered in the context of particle losses in the tubing. The selection of the monitoring system should take account of any risk presented by the materials used in the manufacturing operation, for example, those involving live organisms or radiopharmaceuticals.

4.7.4 The sizes of samples taken for monitoring purposes using automated systems will usually be a function of the sampling rate of the system used.

It is not necessary for the sample volume to be the same as that used for formal classification of clean rooms and clean-air devices.

4.7.5 The airborne particle conditions given in Table 1 for the "at rest" state should be achieved in the absence of the operating personnel after a short "clean-up" or "recovery" period of about 15–20 minutes (guidance value), after completion of the operations. The particulate conditions given in Table 1 for Grade A "in operation" should be maintained in the zone immediately surrounding the product whenever the product or open container is exposed to the environment. The "clean-up" or "recovery" test should demonstrate a change in particle concentration by a factor of 100 within the prescribed time (ISO 14644-3 clause B.12) (*3*).

4.7.6 In order to demonstrate control of the cleanliness of the various clean areas during operation, they should be monitored for airborne particles and microbial contamination. In addition to "at rest" and "in operation" classification, airborne particles should be monitored periodically "in operation" at critical locations. The sampling plan need not be the same as that used for classification. Locations and sample sizes should be determined based on an assessment of the process and contamination risk.

4.7.7 The monitoring of Grade C and D areas in operation should be performed in accordance with the principles of quality risk management. The requirements and alert/action limits will depend on the nature of the operations carried out, but the recommended "clean-up period" should be attained.

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

4.7.9 Examples of operations to be carried out in the various grades are given in Table 2 (see also sections 4.12–4.20).

Table 2

Examples of operations to be carried out in the various grades

Grade	Examples of operations for terminally sterilized products (see sections 4.12–4.15)
A	Filling of products when unusually at risk
C	Preparation of solutions when unusually at risk. Filling of products
D	Preparation of solutions and components for subsequent filling

Grade	Examples of operations for aseptic preparations (see sections 4.16–4.20)
A	Aseptic preparation and filling
C	Preparation of solutions to be filtered
D	Handling of components after washing

4.8 To control the microbiological cleanliness of Grades A–D in operation the clean areas should be monitored. Where aseptic operations are performed, monitoring should be frequent using methods such as settle plates, volumetric air and surface sampling (e.g. swabs and contact plates). Sampling methods used in operation should not interfere with zone protection. Results from monitoring should be considered when reviewing batch documentation for finished product release. Surfaces and personnel should be monitored after critical operations. Additional microbiological monitoring is also required outside production operations, e.g. after validation of systems, cleaning and sanitization.

4.9 Levels of detection of microbial contamination should be established for the purpose of setting alert and action limits and for monitoring the trends in environmental cleanliness in the facility. Limits expressed in colony-forming units (CFU) for the microbiological monitoring of clean areas in operation are given in Table 3. The sampling methods and numerical values included in the table are not intended to represent specifications, but are for information only.

Table 3
Recommended limits for microbial contamination[a]

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

CFU, colony-forming units.
[a] These are average values.
[b] Individual settle plates may be exposed for less than 4 hours.

4.10 Appropriate alert and action limits should be set for the results of particulate and microbiological monitoring. If the action limits are exceeded or a trend is identified in the alert limits, investigation should be initiated and the appropriate corrective actions should be taken, as prescribed in the operating procedures.

4.11 The area grades as specified in sections 4.12 to 4.20 should be selected by the manufacturer on the basis of the nature of the process operations being performed and validation runs (e.g. aseptic media fills or others types of process simulations) are used to establish processing hold times and a maximum fill duration. The determination of an appropriate process area environment and a time limit should be based on the microbial contamination (bioburden) found.

Terminally sterilized products

4.12 Components and most products should be prepared in at least a Grade D environment to ensure low microbial bioburden and particulate counts prior to filtration and sterilization. Where the product is at unusual risk of microbial contamination (e.g. because it actively supports microbial growth, must be held for a long period before sterilization, or is necessarily processed mainly in open vessels), the preparation should generally be done in a Grade C environment.

4.13 The filling of products for terminal sterilization should generally be done in at least a Grade C environment.

4.14 Where the product is at unusual risk of contamination from the environment (e.g. because the filling operation is slow, the containers are wide-necked or are necessarily exposed for more than a few seconds before sealing), the filling should be done in a Grade A zone with at least a Grade C background.

4.15 The preparation and filling of ointments, creams, suspensions and emulsions should generally be done in a Grade C environment before terminal sterilization.

Aseptic preparation

4.16 Components after washing should be handled in at least a Grade D environment. The handling of sterile starting materials and components, unless subjected to sterilization or filtration through a microorganism-retaining filter later in the process, should be undertaken in a Grade A environment with a Grade B background.

4.17 The preparation of solutions which are to be sterile-filtered during the process should be undertaken in a Grade C environment (unless a closed system is used, in which case a Class D environment may be justifiable). If not sterile-filtered (therefore an aseptic manipulation) the preparation of materials and products should be undertaken in a Grade A environment with a Grade B background.

4.18 The handling and filling of aseptically prepared products, as well as the handling of exposed sterile equipment, should be undertaken in a Grade A environment with a Grade B background.

4.19 The transfer of partially closed containers, as used in freeze-drying, before stoppering is completed, should be undertaken either in a Grade A environment with a Grade B background or in sealed transfer trays in a Grade B environment.

4.20 The preparation and filling of sterile ointments, creams, suspensions and emulsions should be undertaken in a Grade A environment with a Grade B background when the product is exposed and is not subsequently filtered.

Processing

4.21 Precautions to minimize contamination should be taken during all processing stages, including the stages before sterilization.

4.22 In general, preparations containing live microorganisms should not be made, nor should containers be filled in areas used for the processing of other pharmaceutical products. However, if the manufacturer can demonstrate and validate effective containment and decontamination of the live microorganisms, the use of multiproduct facilities may be justifiable. Vaccines consisting of dead organisms or of bacterial extracts may be dispensed into containers in the same premises as other sterile pharmaceutical products, provided that the inactivation procedure has been properly validated.

When multiproduct facilities are used to manufacture sterile preparations containing live microorganisms and other sterile pharmaceutical products, the manufacturer should demonstrate and validate the effective decontamination of the live microorganisms, in addition to precautions taken to minimize contamination.

4.23 Validation of aseptic processing should include a process simulation test using a nutrient medium (media fill). Selection of the nutrient medium should be made based on dosage form of the product and selectivity, clarity, concentration and suitability for sterilization of the nutrient medium.

4.24 The process simulation test should imitate as closely as possible the routine aseptic manufacturing steps except where the activity may lead to any potential microbial contamination.

4.25 Process simulation tests should be performed as part of validation by running three consecutive satisfactory simulation tests. These tests should be repeated at defined intervals and after any significant modification to the heating, ventilation and air-conditioning (HVAC) system, equipment or process. Process simulation tests should incorporate activities and interventions known to occur during normal production as well as the worst-case situation. The process simulation tests should be representative of each shift and shift changeover to address any time-related and operational features.

4.26 The number of containers used for media fills should be sufficient to enable a valid evaluation. For small batches the number of containers for media fills should at least equal the size of the product batch. The target should be zero growth and the following should apply:

- when filling fewer than 5000 units, no contaminated units should be detected.
- when filling 5000–10 000 units:
  — one contaminated unit should result in an investigation, including consideration of a repeat media fill;

  - two contaminated units are considered cause for revalidation following investigation;
- when filling more than 10 000 units:
  - one contaminated unit should result in an investigation;
  - two contaminated units are considered cause for revalidation following investigation.

4.27 For any run size, intermittent incidents of microbial contamination may be indicative of low-level contamination that should be investigated. Investigation of gross failures should include the potential impact on the sterility assurance of batches manufactured since the last successful media fill.

4.28 Care should be taken to ensure that any validation does not compromise the processes.

4.29 Water sources, water-treatment equipment and treated water should be monitored regularly for chemicals, biological contamination and contamination with endotoxins to ensure that the water complies with the specifications appropriate to its use. Records should be maintained of the results of the monitoring and of any action taken (*8*).

4.30 Activities in clean areas, especially when aseptic operations are in progress, should be kept to a minimum and the movement of personnel should be controlled and methodical, so as to avoid excessive shedding of particles and organisms due to over-vigorous activity. As far as possible, personnel should be excluded from Grade A zones. The ambient temperature and humidity should not be uncomfortably high because of the nature of the garments worn and to reduce the risk of contamination liberated from the personnel.

4.31 The presence of containers and materials liable to generate fibres should be minimized in clean areas and avoided completely when aseptic work is in progress.

4.32 Components, bulk-product containers and equipment should be handled after the final cleaning process in such a way as to ensure that they are not recontaminated. The stage of processing of components as well as the bulk-product containers and equipment should be properly identified.

4.33 The interval between the washing and drying and the sterilization of components, bulk-product containers and equipment, as well as between sterilization and use, should be as short as possible and subject to a time-limit appropriate to the validated storage conditions.

4.34 The time between the start of the preparation of a solution and its sterilization or filtration through a bacteria-retaining filter should be as short as possible. A maximum permissible time should be set for each product that takes into account its composition and the prescribed method of storage.

4.35 Any gas that is used to purge a solution or blanket a product should be passed through a sterilizing filter.

4.36 The bioburden should be monitored before sterilization. There should be working limits on contamination immediately before sterilization, which are related to the efficiency of the method to be used. Bioburden assay should be performed on each batch for both aseptically filled products and terminally sterilized products. Where overkill sterilization parameters are set for terminally sterilized products, bioburden might be monitored only at suitable scheduled intervals. For parametric release systems, bioburden assay should be performed on each batch and considered as an in-process test. Where appropriate, the level of endotoxins should be monitored. All solutions, in particular large-volume infusion fluids, should be passed through a microorganism-retaining filter, if possible sited immediately before filling.

4.37 Components, bulk-product containers, equipment, and any other articles required in a clean area where aseptic work is in progress, should be sterilized and wherever possible passed into the area through double-ended sterilizers sealed into the wall. Other procedures that prevent the introduction of contamination may be acceptable in some circumstances.

4.38 The efficacy of any new processing procedure should be validated and the validation should be repeated at regular intervals thereafter or when any significant change is made in the process or equipment.

5. Sterilization

5.1 Whenever possible products intended to be sterile should be terminally sterilized by heat in their final container. Where it is not possible to carry out terminal sterilization by heating due to the instability of a formulation or incompatibility of a pack type (necessary to the administration of the product, e.g. plastic eye-dropper bottles), a decision should be taken to use an alternative method of terminal sterilization following filtration and/or aseptic processing.

5.2 Sterilization can be achieved by the use of moist or dry heat, by irradiation with ionizing radiation (noting that ultraviolet irradiation is not normally an acceptable method of sterilization), by ethylene oxide (or other suitable gaseous sterilizing agents), or by filtration with subsequent aseptic filling of sterile final containers. Each method has its advantages and disadvantages. Where possible and practicable, heat sterilization is the method of choice. In any case the sterilization process must be in accordance with the marketing and manufacturing authorizations.

5.3 The microbial contamination of starting materials should be minimal and their bioburden should be monitored before sterilization. Specifications

should include requirements for microbiological quality when the need for this has been indicated by monitoring.

5.4 All sterilization processes should be validated. Particular attention should be paid when the adopted sterilization method is not in accordance with pharmacopoeial standards or other national standards, or when it is used for a preparation that is not a simple aqueous or oily solution, for example, colloidal suspensions.

5.5 Before any sterilization process is adopted, its suitability for the product and its efficacy in achieving the desired sterilizing conditions in all parts of each type of load to be processed should be demonstrated by physical measurements and by biological indicators, where appropriate. The validity of the process should be verified at scheduled intervals, at least annually, and whenever significant modifications have been made to the equipment. Records should be kept of the results.

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

5.7 Biological indicators should be considered only as an additional method of monitoring the sterilization process. They should be stored and used according to the manufacturer's instructions, and their quality checked by positive controls. If they are used, strict precautions should be taken to avoid any transfer of microbial contamination from them.

5.8 There should be a clear means of differentiating products that have not been sterilized from those which have. Each basket, tray, or other carrier of products or components should be clearly labelled with the name of the material, its batch number and an indication of whether or not it has been sterilized. Indicators such as autoclave tape may be used where appropriate to indicate whether or not a batch (or sub-batch) has passed through a sterilization process, but they do not give a reliable indication that the batch is in fact sterile.

5.9 Validated loading patterns should be established for all sterilization processes.

5.10 Sterilization records should be available for each sterilization run. They should be approved as part of the batch-release procedure.

6. Terminal sterilization

Sterilization by heat

6.1 Each heat-sterilization cycle should be recorded by means of appropriate equipment of suitable accuracy and precision, e.g. on a time/temperature chart with a suitably large scale. The temperature should

be recorded by a probe situated at the coolest part of the load or loaded chamber, this point having been determined during the validation; the temperature should preferably be checked against a second independent temperature probe located at the same position. Sterilization records should be available for each sterilization run and should be approved as part of the batch release procedure. Chemical or biological indicators may also be used but should not take the place of physical controls.

6.2 Sufficient time should be allowed for the whole of the load to reach the required temperature before measurement of the sterilizing time is started. This time should be determined for each type of load to be processed.

6.3 After the high-temperature phase of a heat sterilization cycle, precautions should be taken against contamination of a sterilized load during cooling. Any cooling fluid or gas in contact with the product should be sterilized.

Sterilization by moist heat

6.4 Both temperature and pressure should be used to monitor the process. Control instrumentation should normally be independent of monitoring instrumentation and recording charts. Where automated control and monitoring systems are used for these applications they should be validated to ensure that critical process requirements are met. System and cycle faults should be registered by the system and observed by the operator. The reading of the independent temperature indicator should be routinely checked against the reading on the chart recorder during the sterilization period. For sterilizers fitted with a drain at the bottom of the chamber, it may also be necessary to record the temperature at this position throughout the sterilization period. There should be regular leak tests on the chamber when a vacuum phase is part of the cycle.

6.5 The items to be sterilized, other than products in sealed containers, should be wrapped in a material that allows the removal of air and the penetration of steam but prevents recontamination after sterilization. Specially designed autoclavable stainless steel containers, that allow steam to enter and air to leave, can also be used. All parts of the load should be in contact with water or saturated steam at the required temperature for the required time.

6.6 Care should be taken to ensure that the steam used for sterilization is of suitable quality (chemical, microbiological and endotoxin analysis of condensate and physical examination of steam (such as dryness, superheat, and non-condensable gases) and does not contain additives at a level that could cause contamination of the product or equipment. Steam used for sterilization should be tested regularly.

Sterilization by dry heat

6.7 Sterilization by dry heat may be suitable for non-aqueous liquids or dry-powder products.

The process used should include air circulation within the chamber and the maintenance of a positive pressure to prevent the entry of non-sterile air. If air is supplied it should be passed through a microorganism-retaining filter (e.g. a HEPA filter). Where sterilization by dry heat is also intended to remove pyrogens, challenge tests using endotoxins are required as part of the validation.

Sterilization by radiation

6.8 Sterilization by radiation is used mainly for heat-sensitive materials and products. Many pharmaceutical products and some packaging materials are radiation-sensitive, so this method is permissible only when the absence of deleterious effects on the product has been confirmed experimentally. Ultraviolet irradiation is not an acceptable method for terminal sterilization.

6.9 If sterilization by radiation is done by an outside contractor, the manufacturer is responsible for ensuring that the requirements of section 6.8 are met and that the sterilization process is validated.

6.10 During the sterilization procedure the radiation dose should be measured. The dosimeters used for this purpose should be independent of the dose rate and should provide a quantitative measurement of the dose received by the product itself. Dosimeters should be inserted in the load in sufficient number and close enough together to ensure that there is always a dosimeter in the chamber. Where plastic dosimeters are used they should be used within the time-limit of their calibration. Dosimeter absorbance should be read shortly after exposure to radiation. Radiation-sensitive colour discs may be used to differentiate between packages that have been subjected to irradiation and those that have not; they are not indicators of successful sterilization. The information obtained should constitute part of the batch record.

6.11 Validation procedures should ensure that consideration is given to the effects of variations in the density of the packages.

6.12 Material-handling procedures should prevent any mix-up of irradiated and non-irradiated materials. Each package should carry a radiation-sensitive indicator to show whether or not it has been subjected to radiation treatment.

6.13 The total radiation dose should be administered within a predetermined period.

Sterilization by gases and fumigants

6.14 Sterilization by gases and fumigants should only be used for finished products where there is no suitable alternative.

6.15 Various gases and fumigants may be used for sterilization (e.g. ethylene oxide and hydrogen peroxide vapour). Ethylene oxide should be used only when no other method is practicable. During process validation it should be shown that the gas has no damaging effect on the product and that the conditions and time allowed for degassing are such as to reduce any residual gas and reaction products to defined acceptable limits for the type of product or material concerned. These limits should be incorporated in the specifications.

6.16 Direct contact between gas and microorganisms is essential; precautions should, therefore, be taken to avoid the presence of organisms likely to be enclosed in materials such as crystals or dried protein. The nature and quantity of packaging materials can significantly affect the proces.

6.17 Before exposure to the gas, materials should be brought into equilibrium with the humidity and temperature required by the process. This requirement should be balanced against the need to minimize the waiting time before sterilization.

6.18 Each sterilization cycle should be monitored with suitable biological indicators, using the appropriate number of test pieces distributed throughout the load. The information thus obtained should form part of the batch record.

6.19 Biological indicators should be stored and used according to the manufacturer's instructions and their performance checked by positive controls.

6.20 For each sterilization cycle, records should be made of the time taken to complete the cycle, of the pressure, temperature and humidity within the chamber during the process and of the gas concentration. The pressure and temperature should be recorded on a chart throughout the cycle. The records should form part of the batch record.

6.21 After sterilization, the load should be stored in a controlled manner in ventilated conditions to allow concentrations of residual gas and reaction products to fall to their prescribed levels. This process should be validated.

7. Aseptic processing and sterilization by filtration

7.1 The objective of aseptic processing is to maintain the sterility of a product that is assembled from components, each of which has been sterilized by one of the above methods (see sections 5 and 6).

7.2 The operating conditions should be such as to prevent microbial contamination.

7.3 In order to maintain the sterility of the components and the product during aseptic processing, careful attention needs to be given to:

— the environment;
— personnel;
— critical surfaces;
— container/closure sterilization and transfer procedures;
— the maximum holding period of the product before filling into the final container; and
— the sterilizing filter.

7.4 Certain solutions and liquids that cannot be sterilized in the final container can be filtered through a sterile filter of nominal pore size 0.22 micron (or less), or with at least equivalent microorganism-retaining properties, into a previously sterilized container. Such filters can remove bacteria and moulds, but not all viruses or mycoplasmas. Consideration should be given to complementing the filtration process with some degree of heat treatment. Filtration alone is not considered sufficient when sterilization in the final container is possible. Of the methods currently available, steam sterilization is preferred.

7.5 Owing to the potential additional risks of the filtration method as compared with other sterilization processes, a double-filter layer or second filtration through a further sterilized microorganism-retaining filter immediately prior to filling may be advisable. The final sterile filtration should be carried out as close as possible to the filling point.

7.6 The fibre-shedding characteristics of filters should be minimal (virtually zero). Asbestos-containing filters should not be used under any circumstances.

7.7 The integrity of the sterilized filter should be verified before use and should be confirmed immediately after use by an appropriate method such as a bubble point, diffusive flow or pressure hold test. The time taken to filter a known volume of bulk solution and the pressure difference to be used across the filter should be determined during validation and any significant differences from these during routine manufacturing should be noted and investigated. Results of these checks should be included in the batch record. The integrity of critical gas and air vent filters should be confirmed after use. The integrity of other filters should be confirmed at appropriate intervals. Consideration should be given to increased monitoring of filter integrity in processes that involve harsh conditions, e.g. the circulation of high-temperature air.

7.8 The same filter should not be used for more than one working day unless such use has been validated.

7.9 The filter should not affect the product either by removing ingredients from it or by releasing substances into it.

8. Isolator technology

8.1 The use of isolator technology to minimize human interventions in processing areas may result in a significant decrease in the risk of microbial contamination of aseptically manufactured products from the environment. There are many possible designs of isolators and transfer devices. The isolator and the background environment should be designed so that the required air quality for each zone can be realized. Isolators are constructed of various materials more or less prone to puncture and leakage. Transfer devices may vary from single-door to double-door designs to fully-sealed systems incorporating sterilization mechanisms.

8.2 The transfer of materials into and out of the unit is one of the greatest potential sources of contamination. In general the area inside the isolator is the local zone for high-risk manipulations, although it is recognized that unidirectional airflow may not exist in the working zone of all isolators and transfer devices.

8.3 The air classification required for the background environment depends on the design of the isolator and its application. It should be controlled, and for aseptic processing it should be at least Grade D.

8.4 Isolators should be introduced only after appropriate validation. Validation should take into account all critical factors of isolator technology, for example, the quality of the air inside and outside (background) the isolator, sanitization of the isolator, the transfer process and isolator integrity.

8.5 Monitoring should be done routinely and should include frequent leak testing of the isolator and the glove/sleeve system.

9. Blow/fill/seal technology

9.1 Blow/fill/seal units are purpose-built machines in which, in one continuous operation, containers are formed from a thermoplastic granulate, filled and then sealed, all by the one automatic machine. Blow/fill/seal equipment used for aseptic production which is fitted with an effective Grade A air shower may be installed in at least a Grade C environment, provided that Grade A or B clothing is used. The environment should comply with the viable and non-viable limits at rest and the viable limit only when in operation. Blow/fill/seal equipment used for the production of products which are terminally sterilized should be installed in at least a Grade D environment.

9.2 Because of this special technology, particular attention should be paid to at least the following:

— equipment design and qualification;

— validation and reproducibility of cleaning-in-place and sterilization-in-place;
— background clean room environment in which the equipment is located;
— operator training and clothing; and
— interventions in the critical zone of the equipment including any aseptic assembly prior to the commencement of filling.

10. Personnel

10.1 Only the minimum number of personnel required should be present in clean areas; this is particularly important during aseptic processes. As far as possible, inspections and controls should be conducted from outside such areas.

10.2 All personnel (including those concerned with cleaning and maintenance) employed in such areas should receive initial and regular training in disciplines relevant to the correct manufacture of sterile products, including hygiene and the basic elements of microbiology. When outside staff who have not received such training (e.g. building or maintenance contractors) need to be brought in, particular care should be taken over their instruction and supervision.

10.3 Staff who have been engaged in the processing of animal-tissue materials or of cultures of microorganisms other than those used in the current manufacturing process should not enter sterile-product areas unless rigorous and clearly defined decontamination procedures have been followed.

10.4 High standards of personal hygiene and cleanliness are essential and personnel involved in the manufacture of sterile preparations should be instructed to report any conditions that may cause the shedding of abnormal numbers or types of contaminants; periodic health checks for such conditions are desirable. The action to be taken in respect of personnel who might be introducing undue microbial hazards should be decided by a designated competent person.

10.5 Changing and washing should follow a written procedure designed to minimize the contamination of clean-area clothing or the carry-through of contaminants to clean areas. 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.

10.6 Outdoor clothing should not be brought into changing rooms leading to Grade B and C rooms. For every worker in a Grade A/B area, clean sterile (sterilized or adequately sanitized) protective garments should be provided at each work session. Gloves should be regularly disinfected during

operations. Masks and gloves should be changed at least every working session. Operators working in Grade A and B areas should wear sanitized goggles.

10.7 Wrist-watches, cosmetics and jewellery should not be worn in clean areas.

10.8 The clothing required for each grade is as follows:

- *Grade D*. The hair and, where relevant, beard and moustache should be covered. Protective clothing and appropriate shoes or overshoes should be worn. Appropriate measures should be taken to avoid any contamination from outside the clean area.
- *Grade C*. The hair and, where relevant, beard and moustache should be covered. A one-piece jumpsuit, gathered at the wrists and with a high neck, and appropriate shoes or overshoes should be worn. The clothing should shed virtually no fibres or particulate matter.
- *Grades A and B*. Entry of personnel into Grade A areas should be minimized. Headgear should totally enclose the hair and, where relevant, beard and moustache. A one-piece jumpsuit, gathered at the wrists and with a high neck, should be worn. The headgear should be tucked into the neck of the suit. A facemask should be worn to prevent the shedding of droplets. Sterilized, non-powdered gloves of appropriate material and sterilized or disinfected footwear should be worn. Trouser-bottoms should be tucked inside the footwear and garment sleeves into the gloves. The protective clothing should shed virtually no fibres or particulate matter and should retain particles shed by the body.

10.9 Clothing used in clean areas should be laundered or cleaned in such a way that it does not gather additional particulate contaminants that can later be shed. Separate laundry facilities for such clothing are desirable. If fibres are damaged by inappropriate cleaning or sterilization, there may be an increased risk of shedding particles. Washing and sterilization operations should follow standard operating procedures.

11. Premises

11.1 All premises should as far as possible be designed to avoid the unnecessary entry of supervisory or control personnel. Grade A and B areas should be designed so that all operations can be observed from outside.

11.2 In clean areas all exposed surfaces should be smooth, impervious and unbroken to minimize the shedding or accumulation of particles or microorganisms and to permit the repeated application of cleaning agents and disinfectants, where used.

11.3 To reduce the accumulation of dust and to facilitate cleaning, there should be no uncleanable recesses and a minimum of projecting ledges, shelves, cupboards and equipment. Doors should be carefully designed to avoid uncleanable recesses; sliding doors may be undesirable for this reason. Swing doors should open to the high-pressure side and be provided with self-closers. Exceptions are permitted based on egress and site environmental, health and safety containment requirements.

11.4 False ceilings should be sealed to prevent contamination from the void space above them.

11.5 Pipes and ducts and other utilities should be installed so that they do not create recesses, unsealed openings and surfaces that are difficult to clean. Sanitary pipes and fittings should be used and threaded pipe connections should be avoided.

11.6 Sinks and drains should be avoided wherever possible and should be excluded from Grade A and B areas where aseptic operations are carried out. Where installed they should be designed, located and maintained so as to minimize the risks of microbial contamination; they should be fitted with effective, easily cleanable traps and with air breaks to prevent backflow. Any floor channels should be open and easily cleanable and be connected to drains outside the area in a manner that prevents the ingress of microbial contaminants.

11.7 Changing rooms should be designed as airlocks and used to provide physical separation of the different stages of changing and so minimize microbial and particulate contamination of protective clothing. They should be flushed effectively with filtered air. The final stage of the changing room should, in the at-rest state, be the same grade as the area into which it leads. The use of separate changing rooms for entering and leaving clean areas is sometimes desirable. In general hand-washing facilities should be provided only in the first stage of the changing rooms.

There should not be a change of more than one grade between airlocks or passages and changing rooms, i.e. a Grade D passage can lead to a Grade C airlock, which leads to a Grade B changing room, which leads to a Grade B clean room. Changing rooms should be of a sufficient size to allow for ease of changing. Changing rooms should be equipped with mirrors so that personnel can confirm the correct fit of garments before leaving the changing room.

11.8 Airlock doors should not be opened simultaneously. An interlocking system and a visual and/or audible warning system should be operated to prevent the opening of more than one door at a time.

11.9 A filtered air supply should be used to maintain a positive pressure and an airflow relative to surrounding areas of a lower grade under all operational conditions; it should flush the area effectively. Adjacent rooms

of different grades should have a pressure differential of approximately 10–15 Pascals (guidance value). Particular attention should be paid to the protection of the zone of greatest risk, i.e. the immediate environment to which the product and the cleaned components in contact with it are exposed. The recommendations regarding air supplies and pressure differentials may need to be modified where it becomes necessary to contain certain materials, e.g. pathogenic, highly toxic, radioactive or live viral or bacterial materials or products. The decontamination of the facilities and the treatment of air leaving a clean area may be necessary for some operations.

11.10 It should be demonstrated that airflow patterns do not present a contamination risk; for example, care should be taken to ensure that particles from a particle-generating person, operation or machine are not conveyed to a zone of higher product risk.

11.11 A warning system should be operated to indicate failure in the air supply. Indicators of pressure differentials should be fitted between areas where this difference is important, and the pressure differentials should be regularly recorded and failure alarmed.

11.12 Consideration should be given to restricting unnecessary access to critical filling areas, e.g. Grade A filling zones, by means of a physical barrier.

12. Equipment

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

12.2 Whenever possible, equipment used for processing sterile products should be chosen so that it can be effectively sterilized by steam or dry heat or other methods.

12.3 As far as possible, equipment fittings and services should be designed and installed so that operations, maintenance and repairs can be carried out outside the clean area. Equipment that has to be taken apart for maintenance should be re-sterilized after complete reassembly, wherever possible.

12.4 When equipment maintenance is carried out within a clean area, clean instruments and tools should be used and the area should be cleaned and disinfected again, where appropriate, before processing recommences, if the required standards of cleanliness and/or asepsis have not been maintained during the maintenance work.

12.5 All equipment such as sterilizers, air-handling and filtration systems, air vent and gas filters, water treatment, generation, storage and distribution

systems should be subject to validation and planned maintenance; their return to use should be approved.

12.6 Water-treatment plants and distribution systems should be designed, constructed and maintained so as to ensure a reliable source of water of an appropriate quality. They should not be operated beyond their designed capacity. Consideration should be given to including a testing programme in the maintenance of a water system. Water for injection should be produced, stored and distributed in a manner which prevents the growth of microorganisms, e.g. by constant circulation at a temperature above 70 °C or not more than 4 °C (*8*).

13. Finishing of sterile products

13.1 Containers should be closed by appropriately validated methods. Containers closed by fusion, e.g. glass or plastic ampoules, should be subject to 100% integrity testing. Samples of other containers should be checked for integrity according to appropriate procedures.

13.2 The container closure system for aseptically filled vials is not fully integral until the aluminum cap has been crimped into place on the stoppered vial. Crimping of the cap should, therefore, be performed as soon as possible after stopper insertion.

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

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

13.5 Vials with missing or displaced stoppers should be rejected prior to capping. Where human intervention is required at the capping station, appropriate technology should be used to prevent direct contact with the vials and to minimize microbial contamination.

13.6 Restricted access barriers and isolators may be beneficial in assuring the required conditions and minimizing direct human interventions into the capping operation.

13.7 Containers sealed under vacuum should be tested for maintenance of that vacuum after an appropriate, predetermined period.

13.8 Filled containers of parenteral products should be inspected individually for extraneous contamination or other defects. When inspection is carried

out visually this should be done under suitable and controlled conditions of illumination and background. Operators doing the inspection should pass regular eyesight checks, using personal corrective lenses (e.g. spectacles or contact lenses) as required, and be allowed frequent breaks from inspection. Where other methods of inspection are used, the process should be validated and the performance of the equipment checked at intervals. Results should be recorded.

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Further reading

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