WHO GOOD MANUFACTURING PRACTICES FOR
STERILE PHARMACEUTICAL PRODUCTS
PROPOSAL FOR REVISION

Please address comments on this proposal, by 10 October 2009, to
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SCHEDULE FOR THE PROPOSED ADOPTION PROCESS OF DOCUMENT QAS/09.295:
WHO GOOD MANUFACTURING PRACTICES FOR STERILE PHARMACEUTICAL PRODUCTS.
PROPOSAL FOR REVISION

<table>
<thead>
<tr>
<th>Event</th>
<th>Date/Year</th>
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<tbody>
<tr>
<td>Discussion on the need to update WHO GMP for sterile pharmaceutical products took place during the meeting of inspectors collaborating in the WHO Prequalification Programme.</td>
<td>2-3 April 2009</td>
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<tr>
<td>A first draft proposal for revision was prepared by Dr G. Farquharson, UK</td>
<td>6 April 2009</td>
</tr>
<tr>
<td>An update of the proposed draft revision, based on all input and feedback received, was circulated among inspectors serving the Prequalification Programme, prepared by Dr I. Streipa</td>
<td>6 April-15 May 2009</td>
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<tr>
<td>Mailing (wide distribution) of document for comments</td>
<td>May 2009</td>
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<tr>
<td>Discussion during informal consultation on WHO guidelines for medicines quality assurance, quality control laboratories and transfer of technology of feedback received</td>
<td>27-31 July 2009</td>
</tr>
<tr>
<td>Review of comments received and mailing of new working document</td>
<td>September 2009</td>
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<tr>
<td>Discussion during WHO Expert Committee on Specifications for Pharmaceutical Preparations</td>
<td>12-16 October 2009</td>
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<td>Further consultation if necessary</td>
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INTRODUCTION


Following implementation of this WHO good manufacturing practices (GMP) within the context of the WHO Prequalification Programme, a proposal for revision is being submitted to take into consideration new developments. The proposal for revision of the above-mentioned guidance is being made to bring the WHO GMP into line with International Standardization Organization standard ISO 14644-1 and recent United States (US), Japanese, European Union (EU) and Pharmaceutical Inspection Co-operation Scheme (PIC/S) practices.

New chapters on "Isolator Technology" and "Blow/fill/seal technology" have been added to the document.

The chapter on "Finishing of sterile products" has been amended and provisions have been given for capping of vials.

The chapter entitled: "Manufacture of sterile preparations" has been amended and provisions have been given for clean room and clean air device monitoring.

Implementation of these new practices may need be undertaken for certain parts in a step-wise approach, especially the part relating to the provision for capping in a clean or sterile environment, as this is currently not implemented in most industries.

On the basis of the above, the following text is proposed to replace the previously published guidance.
WHO GOOD MANUFACTURING PRACTICES FOR STERILE PHARMACEUTICAL PRODUCTS.

CONTENTS

1. General considerations
2. Quality control
3. Sanitation
4. Manufacture of sterile preparations
5. Sterilization
6. Terminal sterilization
7. Aseptic processing and sterilization by filtration
8. Isolator technology
9. Blow/fill/seal technology
10. Personnel
11. Premises
12. Equipment
13. Finishing of sterile products

References

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 a manufacturing suite. 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:

(a) 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;

(b) 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.

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 such failure should be investigated and necessary action should be taken.

2.5 The use of rapid microbiological methods in order to replace the traditional microbiological methods, and in order to obtain earlier results about the microbiological quality of, e.g. water, environment, bioburden, could be considered if adequately validated.

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. Monitoring should be regularly undertaken in order to detect the contamination or the presence of an organism against which the cleaning procedure is ineffective. In view of its limited effectiveness, ultraviolet light should not be used as a substitute for chemical disinfection. Where disinfectants are used more than one type should be employed.

3.2 Disinfectants and detergents should be monitored for microbiological 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. Cleaning and disinfectant procedures should be validated.

3.4 In order to control the microbiological cleanliness of the cleanliness grades A-D in operation, the clean areas should be monitored. Where aseptic operations are performed, monitoring should be frequent and methods such as settle plates and volumetric air and surface sampling (e.g. swabs and contact plates) should be used. The zones should not be contaminated through the sampling methods used in the operations. The results of monitoring should be considered when batch documentation for release of the finished product is reviewed. Both surfaces and personnel should be monitored after critical operations.

3.5 Levels (limits) of detection of microbiological contamination should be established for the purpose of setting alert and action levels, 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.

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 environmental cleanliness level in the operational state in order to minimize the risks of particulate or microbial contamination of the product or materials being handled.
4.2 Detailed information on methods for determining the microbiological and particulate cleanliness of air, surfaces, etc. is not given here.

ISO 14644-1 should be used for classification of cleanliness by airborne particle (determination of number of sample locations, calculation of sample size and evaluation of classification from the data obtained). Table 2 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 are distinguished here, 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 (guidance value) at a defined test position 15-30 cm below the terminal filter or air distributor system. The uniformity and effectiveness of the unidirectional airflow shall be demonstrated by undertaking airflow visualization tests.

**Grade B:** In aseptic preparation and filling, 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.

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 HEPA filters should be subjected to installed filter leakage test in accordance with ISO 14644-3 at least twice a year. 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.

4.6 Clean room and clean air device classification

(a) Clean rooms and clean air devices should be classified in accordance with EN ISO 14644.

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>
<thead>
<tr>
<th>Grade</th>
<th>At rest</th>
<th>In operation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5 µm</td>
<td>5.0µm</td>
</tr>
<tr>
<td>A</td>
<td>3 520</td>
<td>20</td>
</tr>
<tr>
<td>B</td>
<td>3 520</td>
<td>29</td>
</tr>
<tr>
<td>C</td>
<td>352 000</td>
<td>2 900</td>
</tr>
<tr>
<td>D</td>
<td>3 520 000</td>
<td>29 000</td>
</tr>
</tbody>
</table>
Notes:

(1) 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.

(2) 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 design of the areas and their associated environmental control systems shall be designed to achieve both the “at rest” and “in operation” states.

(b) For classification purposes EN/ISO 14644-1 methodology defines both the minimum number of sample locations and the sample size based on the class limit of the largest considered particle size, and the method for evaluation of the data collected. For classification of Grade A zones (at rest and operational) and Grade B zones (at rest), the minimum sample volume is based on the ISO 5 limit for the number of particles $\geq 0.5 \mu m$ (3520). Similarly, for classification of Grade B (operational), Grade C (at rest and operational), and Grade D (at rest), the minimum sample volume is based on the class limits for particles $\geq 0.5 \mu m$ shown in Table 2.

(c) Portable particle counters with a short length of sample tubing should be used for classification purposes to avoid the loss of particles $\geq 5.0 \mu m$. Isokinetic sample heads shall be used in unidirectional airflow systems.

(d) “In operation” classification may be demonstrated during normal operations, simulated operations or during media fills as worst-case simulation is required for this. EN ISO 14644-2 provides information on testing to demonstrate continued compliance with the assigned cleanliness classification.

4.7 Clean room and clean air device monitoring

(a) Clean rooms and clean air devices should be routinely monitored 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.

(b) 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, e.g. live organisms and radiological hazards. In such cases monitoring during routine equipment set up operations should be undertaken prior to exposure to the risk. Monitoring during simulated operations should also be performed. The Grade A zone should be monitored at such a frequency and with suitable sample size 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 $\geq 5.0 \mu m$ particles at the point of fill when filling is in progress, due to the generation of particles or droplets from the product itself.

(c) 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 such a frequency and with suitable sample size that changes in levels of contamination and any system deterioration would be captured and alarms triggered if alert limits are exceeded.

(d) 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 to 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.

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

(f) The airborne particle conditions given in Table 2 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 2 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.

(g) In order to demonstrate control of the cleanliness of the various clean areas during
operation, they should be monitored for airborne particles and microbiological
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 assessment of the process and contamination risk.

(h) 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.

(i) 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.

(j) Examples of operations to be carried out in the various grades are given in Table 3 (see
also paragraphs 4.14–4.22).

<table>
<thead>
<tr>
<th>Grade</th>
<th>Examples of operations for terminally sterilized products</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(see paragraphs 4.14–4.17)</td>
</tr>
<tr>
<td>A</td>
<td>Filling of products, when unusually at risk</td>
</tr>
<tr>
<td>C</td>
<td>Preparation of solutions, when unusually at risk. Filling of products</td>
</tr>
<tr>
<td>D</td>
<td>Preparation of solutions and components for subsequent filling</td>
</tr>
</tbody>
</table>
4.10 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.

4.11 Recommended limits for microbiological monitoring of clean areas during operation is given in Table 3.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Grade Examples of operations for aseptic preparations (see paragraphs 4.18–4.22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Aseptic preparation and filling</td>
</tr>
<tr>
<td>C</td>
<td>Preparation of solutions to be filtered</td>
</tr>
<tr>
<td>D</td>
<td>Handling of components after washing</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Grade</th>
<th>Air sample (CFU/m³)</th>
<th>Settle plates (diameter 90 mm) (CFU/4 hours)</th>
<th>Contact plates (diameter 55 mm) (CFU/plate)</th>
<th>Glove print (5 fingers) (CFU/glove)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>C</td>
<td>100</td>
<td>50</td>
<td>25</td>
<td>-</td>
</tr>
<tr>
<td>D</td>
<td>200</td>
<td>100</td>
<td>50</td>
<td>-</td>
</tr>
</tbody>
</table>

Notes
(a) these are average values,
(b) individual settle plates may be exposed for less than 4 hours.

4.12 Appropriate alert and action limits should be set for the results of particulate and microbiological monitoring. If these limits are exceeded, investigation should be initiated, and the appropriate corrective actions should be taken, as prescribed in the operating procedures.

4.13 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 microbiological contamination (bioburden) found.

**Terminally sterilized products**

4.14 Components and most products should be prepared in at least a grade D environment in order 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, should 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.
4.15 The filling of products for terminal sterilization should generally be done in at least a grade C environment.

4.16 Where the product is at unusual risk of contamination from the environment (e.g. because the filling operation is slow or 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.

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

Aseptic preparation

4.18 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.

4.19 The preparation of solutions which are to be sterile filtered during the process should be undertaken in a grade C environment; 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.

4.20 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.

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

4.22 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

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

4.24 Preparations containing live microorganisms should not be made or containers filled in areas used for the processing of other pharmaceutical products; however, 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 validated inactivation and cleaning procedures have been undertaken.

[Note from the Secretariat: During the consultation, it was questioned if it is acceptable to fill live attenuated viral vaccines such as Measles, Mumps, Rubella, Polio, etc. or inactivated vaccines in a same area by using same filling machine after inactivation and cleaning of the area and the machine on each filling batch? Feedback would be most welcome.]

4.25 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.

4.26 The process simulation test should imitate as closely as possible the routine aseptic manufacturing process and include all the critical subsequent manufacturing steps. It should also take
into account various interventions known to occur during normal production as well as worst-case situations.

4.27 Process simulation tests should be performed as initial validation with three consecutive satisfactory simulation tests per shift and repeated at defined intervals and after any significant modification to the HVAC system, equipment, process and number of shifts. Normally process simulation tests should be repeated twice a year per shift and process.

4.28 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 to 10 000 units:
  (a) one (1) contaminated unit should result in an investigation, including consideration of a repeat media fill,
  (b) two (2) contaminated units are considered cause for revalidation, following investigation.
- When filling more than 10 000 units:
  (a) one (1) contaminated unit should result in an investigation,
  (b) two (2) contaminated units are considered cause for revalidation, following investigation.

4.29 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.

4.30 Care should be taken that any validation does not compromise the processes.

4.31 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 (Water for pharmaceutical use\(^1\)).

4.32 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 contamination liberated from the personnel.

4.33 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.

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

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4.35 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.

4.36 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.

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

4.38 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.

4.39 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.

4.40 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 preferably 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, 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 particular advantages and disadvantages. Where possible and practicable, heat sterilization is the method of choice.

5.3 The microbiological 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 given when the adopted sterilization method is not in accordance with pharmacopoeial, 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 microbiological contamination from them.

5.8 There should be a clear means of differentiating products that have not been sterilized from
those that 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 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 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 Sterilization by moist heat (heating in an autoclave) is suitable only for water-wettable
materials and aqueous formulations. Both temperature and pressure should be used to monitor the
process. The temperature recorder should normally be independent of the controller, and there
should be an independent temperature indicator, the reading from which should be routinely
checked against 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.

**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. an 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 the sterilization of 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 carried out 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. The responsibilities of the radiation plant operator (e.g. for using the correct dose) should also be specified.

6.10 During the sterilization procedure, the radiation dose should be measured. For this purpose, the dosimeters used 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 employed, 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 of time.

**Sterilization by gases and fumigants**

6.14 This method of sterilization 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, 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 process.

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 porous load sterilization cycle should be monitored with suitable biological indicators, using the appropriate number of test pieces distributed throughout the load. The information so 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 under 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: (a) the environment; (b) the personnel; (c) the critical surfaces; (d) the container/closure sterilization and transfer procedures; (e) the maximum holding period of the product before filling into the final container; and (f) 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. With regard to methods currently available, steam sterilization is to be 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 via 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 filter should be verified before and 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 values should be noted and investigated. The results of these checks should be recorded 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 utilization of isolator technology to minimize human interventions in processing areas may result in a significant decrease in the risk of microbiological 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 the respective zones can be realized. Isolators are constructed of various materials more or less prone to puncture and leakage. Transfer devices may vary from a 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 air flow may not exist in the working zone of all such 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 carried out routinely and should include frequent leak testing of the isolator and 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/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
- 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. Inspections and controls should be conducted from outside such areas as far as possible.

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 microbiological hazards should be decided by a designated competent person.

10.5 Outdoor clothing should not be brought into clean areas, and personnel entering changing rooms should already be clad in standard factory protective garments. 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. 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 for every working session.

10.6 Wrist-watches, make-up/cosmetics and jewellery should not be worn in clean areas.

10.7 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/B. Personnel entry 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 face mask should be worn to prevent the shedding of droplets. Appropriate, 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.

10.8 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/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 in order 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 un-cleanable recesses and a minimum of projecting ledges, shelves, cupboards and equipment. Doors should be carefully designed to avoid un-cleanable recesses; sliding doors are undesirable for this reason.

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/B areas where aseptic operations are carried out. Where installed, they should be designed, located and maintained so as to minimize the risks of microbiological 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 microbiological 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 grade A/B areas is sometimes desirable. In general hand washing facilities should be provided only in the first stage of the changing rooms.
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 various 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 (refer to the WHO Water for pharmaceutical use guidance).

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, pre-determined period.

13.8 Filled containers of parenteral products should be inspected individually for extraneous contamination or other defects. When inspection is done visually, it should be done under suitable and controlled conditions of illumination and background. Operators doing the inspection should pass regular eye-sight 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.

**References**


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