

# Manufacture of sterile active pharmaceutical ingredients

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## **1. Acknowledgements**

This guide on manufacture of sterile Active Pharmaceutical Ingredients has been written by a task force from the Active Pharmaceuticals Ingredients Committee of CEFIC.

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## **2. Introduction**

Active Pharmaceutical Ingredients (API's), used as ingredients in sterile medicinal products, must be sterile unless the final dosage form is terminally sterilised, or produced by a process including a sterilising filtration step. API's intended for use in parenteral products must also comply with relevant specifications on pyrogens or bacterial endotoxins.

The manufacture of sterile API's must be strictly controlled in order to minimise the risk of contamination with micro-organisms, endotoxins and particles. If the final dosage form is not to be sterilised by filtration, the API's should be practically free of particles.

### **3. Glossary**

#### **Active Pharmaceutical Ingredient (API)**

Any substance or mixture of substances intended to be used in the manufacture of a drug (medicinal) product and that when used in the production of a drug becomes an active ingredient of the drug product. Such substances are intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment or prevention of disease or to effect the structure and function of the body

Note: Active pharmaceutical ingredients are usually first obtained in the crude state. Subsequent production operations convert the crude material to the final API that meets the pharmacopoeial and/or similar requirements.

A sterile API is an API that has been subjected to additional processing steps to remove micro-organisms, particles and/or endotoxins.

#### **Aseptic processing**

Handling sterile materials in a controlled environment, in which the air supplies, materials, equipment and personnel are regulated to control microbial and particulate contamination to acceptable levels.

#### **Closed system**

A closed system is sterilised-in-place or sterilised while closed prior to use, and is pressure and/or vacuum tight to some predefined leak rate. Such a system can be utilised for its intended purpose without breach to the integrity of the system, can be adapted for fluid transfers in and/or out while maintaining asepsis, and is connectable to other closed systems without loss of integrity.

#### **Open system**

A system that fails to meet one or more of the criteria which define a closed system.

#### **Process simulation**

*Without microbiological growth media*

Method of evaluating an aseptic process using an appropriate placebo material employing methods which closely approximate those used for sterile materials.

Method of evaluating an aseptic process using a microbial growth medium employing methods that closely approximate those used for sterile materials.

### **Sterile campaign**

Series of consecutive batches of the same API manufactured following sterilisation of the complete production plant. The equipment is held sterile for the duration of the manufacture of these batches.

### **Validation**

Action of proving and documenting that any procedure, process, equipment, activity or system will, with a high degree of assurance, lead to the expected results.

## **4. Scope**

Most GMP guides for API's do not provide specific guidance on the manufacture of sterile API's. This CEFIC document provides this additional guidance which is unique to the manufacture and handling of sterile APIs. The manufacture of API intermediates used in sterile processes falls outside the scope of this document and is covered by general GMP guidelines for API's such as the CEFIC document "GMP guidelines on manufacture of Bulk Pharmaceutical Chemicals" dated August 1996.

As with all CEFIC documents, this guidance document has been written to assist industry in certain aspects of manufacturing under the GMP regime.

## **5. General principles for the manufacture of sterile API's**

It is considered a general GMP requirement that the manufacture of all intermediates and API's should be carried out under conditions that minimise the risk of contamination by the manufacturing environment. Manufacture of sterile API's should take place in equipment, designed to be easily operated, cleaned and sterilised by personnel trained in performing aseptic processes.

Clean room classifications are defined in the Addendum to this document.

Whenever the process is open and the sterile API is exposed, this should be within a grade A area or in a grade A local workplace with a grade B background.

The standards for equipment design and environmental control required for the manufacture of sterile API's are been described in chapter 6 of this document.

Sterile API's can be manufactured by terminal sterilisation or by aseptic processing; terminal sterilisation is the method of choice. The two techniques are described in more detail in chapter 8 of this document.

## **6. Premises, equipment and environment**

### **6.1 Premises**

#### **6.1.1 General**

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

To reduce accumulation of dust and to facilitate cleaning, there should be no uncleanable recesses and a minimum of projecting ledges, shelves, cupboards and equipment. Doors should be designed to avoid uncleanable recesses; sliding doors may be undesirable for this reason.

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

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

Sinks and drains should be prohibited in grade A/B areas. Drains in grade C areas should be sanitizable. Floor drains in lower grade clean rooms should be fitted with traps or water seals to prevent back-flow. Air breaks should be fitted between any equipment or sinks and the drains.

#### **6.1.2 Changing rooms**

Changing rooms leading into class A and B environment should be designed as airlocks. There should be physical separation of the different stages of changing to minimise microbial and particulate contamination of clean room clothing. Changing rooms for all classes 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.

#### **6.1.3 Air systems**

Where airlocks are used, both airlock doors should not be opened simultaneously. An interlocking system or a visual and/or audible warning system should be operated to prevent the opening of more than one door at a time.

A filtered air supply should maintain a positive pressure and air flow relative to surrounding areas of a lower grade under all operational conditions and should flush the area effectively. Adjacent rooms of different grades should have a pressure differential of 10 - 15 Pascals (guidance values). Particular attention should be paid to the protection of the zone of greatest risk, that is, the immediate environment in which an API and sterilised product contact components are exposed.

The various recommendations regarding air supplies and pressure differentials may need to be modified in the case of highly toxic or radioactive products, whilst maintaining the required environmental control. Decontamination of facilities and treatment of air leaving a clean area may be necessary for some operations.

It should be demonstrated that air-flow patterns do not present a contamination risk, e.g. care should be taken to ensure that air flows do not distribute particles from a particle-generating person, operation or equipment to a zone of higher product risk.

A warning system should be provided to indicate failure in the air supply. Indicators of pressure differences should be fitted between areas where these differences are important. These pressure differences should be recorded regularly or otherwise documented. Continuous monitoring of pressure difference of grade A and grade B areas is recommended.

## **6.2 Equipment**

### **6.2.1 General**

Aseptic manufacturing of sterile API's should preferably take place in equipment operated under positive pressure relative to the surrounding area. If not, leak tests should be performed for critical steps at an established frequency and after maintenance and repair.

All gasses entering or leaving the sterilised equipment should be sterilised.

Testing of the integrity of all vent and gas filters should be carried out on an established frequency and after replacement.

Any aseptic connections made to the sterile plant must be carried out in a grade A environment. The suitability of such procedures should be validated.

Water treatment plants and distribution systems should be designed, constructed and maintained to ensure a reliable source of water of an appropriate quality. They should not be operated beyond their designed capacity. Water for injections (WFI) should be produced, stored and distributed in a manner that prevents microbial contamination and growth, for example by constant circulation at a temperature above 70°C.

All equipment such as sterilisers, air handling and filtration systems, air, vent and gas filters, water treatment, storage and distribution systems should be subject to validation and planned maintenance.

All support systems should be validated or qualified individually to ensure that each system is performing within the required specifications. Potential sources of contamination with micro-organisms, endotoxins and particulate matter should be identified and - whenever possible - eliminated.

Appropriate preventive maintenance and calibration procedures for equipment and support systems should be in place to ensure that the systems remain in control.

### **6.2.2 Cleaning**

Equipment to be used in the aseptic processes should be cleaned as appropriate. The final rinse of such equipment must be done using water that does not contribute in endotoxins, microbial contamination or particles. The use of Water For Injections is recommended.

Cleaning of the plant and of the equipment must be validated. Such validation should include setting specifications and testing of bio-burden, endotoxins and particulate matter.

Equipment to be used in aseptic processes should be cleaned using validated methods. Such validation should include the setting of specifications and the testing for residual product, bio-burden, endotoxins and particulate matter. The final rinse of such equipment must be done using water that does not contribute endotoxins, microbial contamination or particles. The use of WFI is recommended.

### **6.2.3 Sterilisation of equipment**

All process equipment, including pipework, that comes into contact with sterile process materials, should be cleaned and sterilised before use according to validated procedures. This should be done after complete reassembling whenever possible.

Sterilisation of equipment by heat is the method of choice. To obtain the highest possible assurance of sterility, sterilisation with steam of assembled equipment (“steam in place”) is preferred.

A combination of physical measurements and biological indicators should be used to validate the sterilisation process.

Equipment sterilisation records, showing that the validation criteria have been met, should be available for each sterilisation run. Results should be recorded (preferably in an equipment log) and the equipment should be status labelled.

The validity of the sterilisation procedures should be demonstrated at an established frequency and after significant modification of procedures or equipment.

#### **6.2.3.1 Sterilisation of equipment by moist heat**

Each heat sterilisation cycle should be recorded on a time/temperature chart with a suitable scale, or using other appropriate equipment with suitable accuracy and precision. The position of the temperature probes used for controlling and/or recording should have been determined during validation and, where applicable should be checked against a second independent temperature probe located at the same position.

All steam sterilisation processes must be validated using a combination of temperature mapping and biological indicator studies.

Precautions should be taken against contamination of the sterilised equipment during cooling and prior to use.

Care should be taken to ensure that steam used for sterilisation is of suitable quality and does not contain additives at a level which could cause contamination of the equipment. The quality of the steam condensate should meet the chemical, biological and particulate standards defined for WFI.

Any air admitted to the equipment for cooling etc., must be first passed through a microbiologically retentive filter.

#### 6.2.3.1.1 Autoclave sterilisation

Both temperature and pressure should be used to monitor the sterilisation process. The autoclave should at least be monitored at the coolest point determined during the validation, normally the condensate drain.

#### 6.2.3.1.2 Sterilisation using Steam In Place.

It is recommended that temperature and if possible pressure are monitored during steam in place sterilisation. Temperature probes should be fitted at representative points in the equipment, normally the coolest points (determined during validation). For equipment fitted with a condensate drain, it may also be necessary to record the temperature at this position, throughout the sterilisation cycle.

#### 6.2.3.2 Sterilisation of equipment by dry heat

Each heat sterilisation cycle should be recorded on a time/temperature chart with a suitable large scale or by other appropriate equipment with suitable accuracy and precision. The position of the temperature probes used for controlling and recording should have been determined during validation, and where applicable, should be checked against a second independent temperature probe located at the same position.

The equipment to be sterilised should allow the air in the equipment to circulate and the maintenance of a positive pressure to prevent the entry of non-sterile air. Any air admitted should be passed through a microbiologically retentive filter.

Precautions should be taken against contamination of the sterilised equipment during cooling and waiting before use.

Dry heat sterilisation processes are normally validated using a combination of temperature mapping and biological indicator studies. Where the process is intended to sterilise and remove endotoxins, biological indicator studies can be replaced by endotoxin challenge tests.

#### 6.2.3.3 Fumigation Sterilisation of equipment

Fumigation Sterilisation e.g. using hydrogen peroxide, peracetic acid, ethylene oxide or formaldehyde, should only be used when no other method is feasible.

Since these methods generally provide only surface sterilisation; effective penetration of the sterilising agent into porous parts of the equipment such as gaskets, membranes etc. should be guaranteed by validation.

During validation, the sterilisation cycle should be monitored with suitable biological indicators. The number of biological indicators used should be sufficient to provide assurance of adequate gas distribution and of sterilant penetration into critical, difficult to sterilise positions within the equipment.

For each sterilisation cycle, records should be made of all critical parameters, for example time, temperature, humidity and volume of sterilant used.

The conditions and time required for removal of gas to safe levels at the end of a sterilisation cycle should be established during validation.

### **6.3 Environment [clean rooms]**

#### **6.3.1 General**

The manufacture of sterile APIs is generally conducted within clean rooms, the grade required (A, B or C) being dependant on the type of processing carried out.

Clean rooms should be properly designed and equipped to maintain the relevant air quality both in terms of particulates and microbial levels. Air supplies should be filtered through HEPA (High Efficiency Particulate Air) filters with an appropriate efficiency. The number of air changes should be related to the classification of the room, to the equipment and to the number of persons present in the worst situation. An air pressure differential 10-15 Pascals [higher air change rates may be necessary e.g. when handling API's which generate many particles] is recommended.

HEPA filters should be regularly tested for integrity, using a suitable aerosol challenge test. An initial test should be done when the HEPA units are installed, thereafter integrity testing should be repeated with a suitable frequency. Consideration should be given to the frequency of change of the HEPA filters.

The environment within a classified area should be monitored at an established frequency, during and after production. Additional monitoring may be necessary during or after critical operations, maintenance, cleaning and sanitation/sterilisation. Monitoring techniques used may include settle plates, volumetric air sampling, contact plates and swabbing. Particle counting must be carried out at a frequency appropriate to the grade of clean room. Particulate monitoring should be carried out before and after production and during production where feasible.

Special attention should be given to the movement of personnel and materials into and out of the classified areas. Such movements should be described in detail in written procedures.

#### **6.3.2 Sanitization of clean rooms**

## Manufacture of sterile active pharmaceutical ingredients

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The sanitization of clean areas is particularly important. They should be cleaned thoroughly in accordance with a written programme. Where disinfectants are used, more than one type should be employed. Microbiological monitoring should be undertaken regularly to check the efficacy of the sanitization and to detect the development of resistant strains.

Disinfectants and detergents should be monitored for microbial contamination; dilutions should be kept in previously cleaned containers and should only be stored for defined periods.

Disinfectants and detergents used in grades A and B areas should be sterilised prior to use.

Fumigation of clean areas may be useful for reducing microbiological contamination.

## **7. Personnel**

### **7.1 General**

Only the minimum number of personnel required should be present in clean areas; this is particularly important during aseptic processing.

All personnel (including those concerned with cleaning and maintenance) employed in such areas, should receive regular training in disciplines relevant to the manufacture of sterile API's. Training should include reference to hygiene and to the basic elements of microbiology. Staff entering the area who have not received such training (e.g. building or maintenance contractors) must be closely supervised.

High standards of personal hygiene and cleanliness are essential. Personnel involved in the manufacture of sterile ingredients should be instructed to report any condition that may cause the shedding of abnormal numbers or types of micro-organisms; periodic health checks for such conditions are necessary. Actions to be taken about personnel who could be introducing undue microbiological hazard should be decided by a designated, competent person.

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

Wristwatches, make-up and jewellery should not be worn in 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 API from contamination, and, if necessary, to protect the operator from exposure to the API.

### **7.2 Clothing required for the individual grades**

Grade A/B: Headgear should totally enclose hair and, where relevant, beard and moustache; it should be tucked into the neck of the suit; a face mask should be worn to prevent the shedding of droplets. Appropriate sterilised, non-powdered rubber or plastic gloves and sterilised 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 retain particles shed by the body.

Outdoor clothing should not be brought into changing rooms leading to grade A, grade B or grade C rooms. For every worker in a grade A or grade B area, clean sterile protective garments should be provided at each work session, or at least once a day if monitoring results justify this. Gloves should be regularly disinfected during operations. Masks and gloves should be changed at least at every working session.

Clean area clothing should be cleaned and handled in such a way that it does not gather additional contaminants that can later be shed. Laundry facilities should not contaminate the garments with particles. These operations should follow written procedures. Separate laundry facilities for such clothing are desirable. Inappropriate treatment of clothing may damage fibres and increase the risk of shedding of particles.

Grade C: Hair and where relevant beard and moustache should be covered. A single or two-piece trouser suit, gathered at the wrists and with high neck and appropriate shoes or overshoes should be worn. They should shed virtually no fibres or particulate matter.

## **8. Processing**

### **8.1 General**

Precautions to minimise contamination should be taken during all processing stages before, during and after sterilisation. Once the sterile API has been produced, special attention should be given during subsequent handling e.g. during charging and discharging lyophilisers, blenders, milling equipment etc.

Process equipment should be designed to minimise the generation of particulate matter. Aseptic processing plants may be classified as either closed or open. A closed plant is one where no aseptic connections are made post sterilisation. A closed plant is operated at positive pressure or at ambient or negative pressure if leak tested to a defined appropriate standard. If the criteria for a closed plant are not met, the system is described as open.

Measures should be taken to minimise the particulate contamination of the final API. The interval between the washing and drying and the sterilisation of components, product containers and equipment as well as between their sterilisation and use should be minimised and subject to a time-limit appropriate to the storage conditions. The time between the start of the preparation of a solution and its sterilisation or filtration through a micro-organism-retaining filter should be minimised. There should be a validated maximum permissible time for storage of each API before sterilisation, taking into account its composition and the prescribed method of storage. The bioburden of material to be sterilised should be monitored before sterilisation. There should be working limits on contamination immediately before sterilisation that are related to the efficiency of the method to be used. Where appropriate the levels of pyrogens or endotoxins should be monitored and working limits set.

Components, containers, equipment and any other articles required in a clean area where aseptic work takes place should be sterilised and passed into the area by a procedure which achieves the objective of not introducing contamination. Water sources, water treatment equipment and treated water should be monitored regularly for chemical and biological contamination and, as appropriate, for endotoxins. Records should be maintained of the results of the monitoring and of any action taken.

Activities in clean areas and especially when aseptic operations are in progress should be kept to a minimum and movement of personnel should be controlled and methodical, to avoid excessive shedding of particles and organisms. The ambient temperature and humidity should not be uncomfortably high because of the nature of the garments worn.

Aseptically manufactured, aqueous solutions or moist API's ("wet cakes") without preservation properties, should be processed within an established period.

Bulk sterile API aseptic processing is often done on campaign basis, the maximum length of which should be validated both in open and in closed systems.

Batch release procedures must take account campaign working e.g. filter integrity testing at the end of the campaign.

### 8.2 Aseptic manufacturing of API's

API's, required to be sterile and not resistant to heat or radiation, can only be manufactured under strict aseptic conditions.

Usually, sterility is achieved by dissolving the non-sterile active pharmaceutical ingredient in a solvent, followed by filtration through a sterilising filter [nominal pore size  $\leq 0,22 \mu\text{m}$ ]. The preparation of such a solution should be done in grade C environment. The sterile filtrate is further processed in sterilised equipment.

E.g. after precipitation, the substance is isolated and washed in a sterile centrifuge or on a sterile filter, dried and - if necessary - milled and blended using sterile equipment before it is packed in its final, sterilised container.

Solutions may also be sterile filtered and subsequently spray dried or lyophilised under aseptic conditions.

Throughout aseptic processing, operations should be carried out wherever possible in closed equipment in classified environments.

Solutions may be sterilised by passage through filters with a nominal pore size of less than or equal to  $0,22\mu\text{m}$ . The sterilisation of the API by filtration is a critical step and must be validated accordingly. Filters must be shown to microbiologically retentive under worst case process conditions using actual product solutions where ever possible. It must also be demonstrated that filters used neither significantly absorb any component from, nor release any significant contaminant into the solution being filtered.

The integrity of assembled sterilising filters must be confirmed before and after each use using an integrity test that is correlated with microbial retention. The time required to filter a known volume of solution and the maximum pressure differential across the filter should be established during validation and deviations from these parameters during routine use must be recorded and investigated.

The length of use of the same filter and maximum waiting times, should be established during process development and confirmed by validation.

### **8.2.1 Validation of aseptic processing of API's**

Aseptic processes must be validated, and this normally involves the use of process simulation. The setting of acceptance criteria for the validation of an API process is complicated by the small number of product containers filled. Whilst the usual acceptance criteria of <0.1% non sterile units cannot be directly applied, the

acceptance criteria set should be based upon sound scientific rationale, and should give an equivalent level of sterility assurance.

#### **8.2.1.1 Validation of aseptic processing in closed systems**

The sterilisation process of closed systems should be validated using biological indicators and thermocouples. Process simulation may not be necessary for closed systems.

#### **8.2.1.2 Validation of aseptic processing in open systems**

Validation of aseptic processing in open systems should be validated by means of process simulation tests. Process simulation should imitate, as closely as possible, the routine aseptic manufacturing process and include all critical manufacturing steps. Depending on the design of the equipment, the process can be simulated using the whole equipment in one run or the simulation can be split up into unit operations.

The use of an inert, non inhibiting substance, suitable to be conveyed through the entire installation, is preferred.

Process simulation should be repeated at defined intervals and after significant modifications of the equipment and/or the process. Validity criteria for the simulation process should be established on sound statistical grounds, taking into account accepted sterility assurance levels (SAL).

The efficacy of any new procedure should be validated, and the validation verified at scheduled intervals, based on performance history, or when a significant change is made to the process or equipment.

### **8.3 Terminal sterilisation of API's**

Bioburden, endotoxin and particulate levels must be controlled in terminally sterilised APIs. The final steps of processing must be carried out in a grade C environment.

Terminal sterilisation can be accomplished by dry heat, by moist heat and by radiation. Sterilisation procedures and precautions employed should give a "sterility assurance level" (SAL) of 10<sup>-6</sup> or better.

### **8.3.1 Sterilisation of the API by dry heat**

If an API can withstand the lengthy time and high temperature necessary for dry heat sterilisation, this is the method of choice to achieve sterility.

The validation of such a sterilisation process should include heat penetration and distribution studies related to cycle times and temperatures. Suitable biological indicators should be used.

The effect, if any, of the sterilisation process on the stability and performance of the API must also be established.

### **8.3.2 Moist Heat [steam] sterilisation of the API**

Steam sterilisation is an acceptable method of sterilisation for those aqueous API's that can withstand high temperature and high moisture conditions. Clean steam should be used [clean steam has to be made from purified water with a system where the condensate also complies with compendial purified water specifications].

The validation of the sterilisation process should include measurement of heat penetration into the aqueous API solution and temperature distribution. Suitable biological indicators may be used to demonstrate the sterilising properties of the process.

The effect, if any, of the steam sterilisation process on the stability and performance of the API must also be established.

### **8.3.3 Sterilisation of the API by radiation**

Some heat sensitive ingredients may be resistant to gamma radiation from a suitable radio-isotopic source or a beam of electrons.

For this method the reference absorbed dose must be greater than 25 kGy. During sterilisation the radiation absorbed by the ingredient is monitored by means of established dosimetry procedures, independent of the dose rate. When, additionally, a biological assessment is carried out, suitable biological indicators should be used.

The radiation procedure must be validated. Validation procedures should include all variations in weights to be used and all types of packaging materials to be used.

The effect, if any, of the radiation process on the stability and performance of the API must be established.

### **8.3.4 Sterilisation of the API by gas**

Sterilisation by gas e.g. ethylene oxide, or formaldehyde, is not a recognised method of terminal sterilisation. The effect of gas is limited to a treatment of the surface of the active pharmaceutical ingredient. Moreover, there is the possibility of chemical reactions and the risk of residuals.

#### **8.4 Sterile API handling**

Exposure of sterile API's to the environment (e.g. filling, sampling and dispensing) must be done in a grade A area.

#### **8.5 Finishing of sterile active pharmaceutical ingredients**

Containers used for sterile APIs, should be sterile, airtight and tamperproof. If the container is intended to be opened on more than one occasion, it must be so designed that it remains airtight after re-closure.

Containers for API's should be made of inert, non-shedding, sterilizable, cleanable materials such as glass, plastic, aluminium or stainless steel. The compatibility of each combination of container-closure and ingredient should be demonstrated experimentally. The integrity of the container after filling and during storage should be validated. Such validation should include a microbiological penetration test.

The quality of containers and closures depends on the type of API it will contain and should comply with pharmacopoeial specifications, as appropriate. The cleaning process of the containers and closures should be validated to show a suitable reduction in endotoxins and particulate matter.

Aseptically manufactured API's should be filled into their final containers under grade A conditions. Containers should be closed immediately after filling and sampling to avoid contamination and uptake of moisture.

## **9. Quality Unit activities**

A full review of production and monitoring data from the whole process (or campaign where applicable) should be considered as part of the release procedure of the API. In case of a sterility failure in a campaign, all other batches of that campaign must be included in a failure investigation.

If alert or action limits are exceeded, a procedure prescribing appropriate preventive, investigative and corrective actions, should be followed.

The sterility test applied to a sample of a batch of the sterile API, should only be regarded as the last in a series of control measures by which sterility is assured.

Sterility test samples should be taken in accordance with pharmacopoeial requirements. Samples should be representative of the container filling process and, in the case of terminal sterilisation by heat, they should be taken from the coolest part of the sterilising equipment.

Sterility testing should be carried out according to the general pharmacopoeial methods. The sterility test should be validated for the API concerned. In case of a sterility failure the result can only be invalidated if laboratory error can be proven. Whenever possible, other critical tests such as endotoxins and particulate matter, should also be undertaken according to pharmacopoeial methods.

Samples taken for testing and retention should be representative of the whole batch and include samples taken at the beginning, middle and end of the filling operation.

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### **ADDENDUM**

For the manufacture of sterile API's three air grades can be distinguished.

#### **1. Grade A**

The local zone for high risk operations, e.g. filling and aseptic connections. Normally such conditions are provided by a laminar air flow work station. Laminar air flow systems should provide an homogeneous air speed of 0.45 m/s +/- 20% (guidance value) at the working position.

#### **2. Grade B**

The background environment for grade A zone operations such as aseptic preparation and filling.

#### **3. Grade C**

Clean areas for carrying out less critical stages in the manufacture of sterile API's or the surrounding environment for enclosed processes.

### Airborne particulate classification for grades A, B and C:

|       | at rest (b)                                                |       | in operation |        |
|-------|------------------------------------------------------------|-------|--------------|--------|
|       |                                                            |       |              |        |
| Grade | maximum permitted number of particles/m3 equal to or above |       |              |        |
|       | 0,5 µm                                                     | 5 µm  | 0,5 µm       | 5 µm   |
|       |                                                            |       |              |        |
| A     | 3 500                                                      | 0     | 3 500        | 0      |
| B(a)  | 3 500                                                      | 0     | 350 000      | 2 000  |
| C(a)  | 350 000                                                    | 2 000 | 3 500 000    | 20 000 |

#### Notes:

- (a) In order to reach the B and C air grades, the number of air changes should be related to the size of the room and the equipment and personnel present in the room. The air system should be provided with appropriate filters such as HEPA for grades A, B and C.
- (b) The guidance given for the maximum permitted number of particles in the “at rest” condition corresponds approximately to the US Federal Standard 209 E and the ISO classifications as follows: grades A and B correspond with class 100, M 3.5, ISO 5; grade C with class 10 000, M 5.5, ISO 7.

The particulate conditions given in the table for the “at rest” state should be achieved in the unmanned state after a short “clean up” period of 15-20 minutes (guidance value), after completion of operations. The particulate conditions for grade A (in operation) given in the table should be maintained in the zone immediately surrounding the API whenever the API or open container is exposed to the environment. It is accepted that it may not always be possible to demonstrate conformity with particulate standards at the point of fill when filling is in progress, due to the generation of particles or droplets from the API itself. In such case particle measurement should be done before and after the process and meet the “at rest” schedule.

Recommend limits for microbiological monitoring of clean areas in operation.

| Recommended limits for microbial contamination (a) |                                  |                                                   |                                               |                                       |
|----------------------------------------------------|----------------------------------|---------------------------------------------------|-----------------------------------------------|---------------------------------------|
| GRADE                                              | air sample<br>cfu/m <sup>3</sup> | settle plates<br>(diam. 90 mm),<br>cfu/4 hours(b) | contact plates<br>(diam. 55 mm),<br>cfu/plate | glove print<br>5 fingers<br>cfu/glove |
| A                                                  | < 1                              | < 1                                               | < 1                                           | < 1                                   |
| B                                                  | 10                               | 5                                                 | 5                                             | 5                                     |
| C                                                  | 100                              | 50                                                | 25                                            | -                                     |

#### Notes:

- (a) These are average values.
- (b) Individual settle plates may be exposed for less than 4 hours.

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

| Grade | Examples of operations for terminally sterilised API's                 |
|-------|------------------------------------------------------------------------|
| A     | Filling of API's, when unusually at risk.                              |
| C     | Preparation of solutions, when unusually at risk.<br>Filling of API's. |

| Grade | Examples of operations for aseptic API's                 |
|-------|----------------------------------------------------------|
| A     | Aseptic preparation and filling.<br>Aseptic connections. |
| C     | Preparation of solutions to be filtered.                 |

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\* Please contact the secretary of APIC at CEFIC