British Pharmacopoeia Volume V
Appendices

Appendix XVIII Methods of Sterilisation

(Ph. Eur. general texts 5.1.1, 5.1.2 and 5.1.5)

Methods of preparation of sterile products

(Ph. Eur. method 5.1.1)

Sterility is the absence of viable micro-organisms. The sterility of a product cannot be guaranteed by testing; it has to be assured by the application of a suitably validated production process. It is essential that the effect of the chosen sterilisation procedure on the product (including its final container or package) is investigated to ensure effectiveness and the integrity of the product and that the procedure is validated before being applied in practice. It is recommended that the choice of the container is such as to allow the optimum sterilisation to be applied. Failure to follow meticulously a validated process involves the risk of a non-sterile product or of a deteriorated product. Revalidation is carried out whenever major changes in the sterilisation procedure, including changes in the load, take place. It is expected that the principles of good manufacturing practice (as described in, for example, the European Community Guide to GMP) will have been observed in the design of the process including, in particular, the use of:

— qualified personnel with appropriate training,

— adequate premises,

— suitable production equipment, designed for easy cleaning and sterilisation,

— adequate precautions to minimise the bioburden prior to sterilisation,

— validated procedures for all critical production steps,

— environmental monitoring and in-process testing procedures.

The precautions necessary to minimise the pre-sterilisation bioburden include the use of components with an acceptable low degree of microbial contamination. Microbiological monitoring and setting of suitable action limits may be advisable for ingredients which are liable to be contaminated because of their origin, nature or method of preparation.

The methods described here apply mainly to the inactivation or removal of bacteria, yeasts and moulds. For biological products of animal or human origin or in cases where such material has been used in the production process, it is necessary during validation to demonstrate that the process is capable of the removal or inactivation of relevant viral contamination. Guidance on this aspect is provided in, for example, the appropriate European Community Notes for Guidance.

Wherever possible, a process in which the product is sterilised in its final container (terminal sterilisation) is chosen. When a fully validated terminal sterilisation method by steam, dry heat or ionising radiation is used, parametric release, that is the release of a batch of sterilised items based on process data rather than on the basis of submitting a sample of the items to sterility testing, may be carried out, subject to the approval of the competent authority.

If terminal sterilisation is not possible, filtration through a bacteria-retentative filter or aseptic processing is used; wherever possible, appropriate additional treatment of the product (for example, heating of the product) in its final container is applied. In all cases, the container and closure are required to maintain the sterility of the product throughout its shelf-life.

Sterility Assurance Level (SAL)

Where appropriate reference is made within the methods described below, to a "sterility assurance level" or "SAL". The achievement of sterility within any one item in a population of items submitted to a sterilisation process cannot be guaranteed nor can it be demonstrated. The inactivation of micro-organisms by physical or chemical means follows an exponential law; thus there is always a finite statistical probability that a micro-organism may survive the sterilising process. For a given process, the probability of survival is determined by the number, types and resistance of the micro-organisms present and by the environment in which the organisms exist during treatment. The SAL of a sterilising process is the degree of assurance with which the process in question renders a population of items sterile. The SAL for a given process is expressed as the probability of a non-sterile item in that population. An SAL of 10^{-6}, for example, denotes a probability of not more than one viable micro-organism in 1×10^{6} sterilised items of the final product. The SAL of a process for a given product is established by appropriate validation studies.

Methods and conditions of sterilisation

Sterilisation may be carried out by one of the methods described below. Modifications to, or combinations of, these methods may be used provided that the chosen procedure is validated both with respect to its effectiveness and the integrity of the product including its container or package.

For all methods of sterilisation the critical conditions of the operation are monitored in order to confirm that the previously determined required conditions are achieved throughout the batch during the whole sterilisation process. This applies in all cases including those where the reference conditions are used.

Terminal sterilisation

For terminal sterilisation it is essential to take into account the non-uniformity of the physical and, where relevant, chemical conditions within the sterilising chamber. The location within the sterilising chamber that is least accessible to the sterilising agent is determined for each loading configuration of each type and size of container or package (for example, the coolest location in an autoclave). The minimum lethality delivered by the sterilising cycle and the reproducibility of the cycle are also determined in order to ensure that all loads will consistently receive the specified treatment.

Having established a terminal sterilisation process, knowledge of its performance in routine use is gained wherever possible, by monitoring and suitably recording the physical and, where relevant, chemical conditions achieved within the load in the chamber throughout each sterilising cycle.

Steam sterilisation (Heating in an autoclave) Sterilisation by saturated steam under pressure is preferred, wherever applicable, especially for aqueous preparations. For this method of terminal sterilisation the reference conditions for aqueous preparations are heating at a minimum of 121 °C for 15 min. Other combinations of time and temperature may be used provided that it has been satisfactorily demonstrated that the process chosen delivers an adequate and reproducible level of lethality when operating routinely within the established tolerances. The procedures and precautions employed are such, as to give an SAL of 10^{-6} or better. Guidance concerning validation by means of the F_0 concept is provided below *(5.1.5)*.

Knowledge of the physical conditions (temperature and pressure) within the autoclave chamber during the sterilisation procedure is obtained. The temperature is usually measured by means of temperature-sensing elements inserted into representative containers together with additional elements at the previously established coolest part of the loaded chamber. The conditions throughout each cycle are suitably recorded, for example, as a temperature-time chart, or by any other suitable means.

Where a biological assessment is carried out, this is obtained using a suitable biological indicator *(5.1.2)*.

Dry heat sterilisation For this method of terminal sterilisation the reference conditions are a minimum of 160 °C for at least 2 h. Other combinations of time and temperature may be used provided that it has been satisfactorily demonstrated that the process chosen delivers an adequate and reproducible level of lethality when operated routinely within the established tolerances. The procedures and precautions employed are such as to give an SAL of 10^{-6} or better.

Dry heat sterilisation is carried out in an oven equipped with forced air circulation or other equipment specially designed for the purpose. The steriliser is loaded in such a way that a uniform temperature is achieved throughout the load. Knowledge of the temperature within the steriliser during the sterilisation procedure is usually obtained by means of temperature-sensing elements inserted into representative containers together with additional elements at the previously established coolest part of the loaded steriliser. The temperature throughout each cycle is suitably recorded.

Where a biological assessment is carried out, this is obtained using a suitable biological indicator *(5.1.2)*.

Dry heat at temperatures greater than 220 °C is frequently used for sterilisation and depyrogenation of glassware. In this case demonstration of a 3-log reduction in heat resistant endotoxin can be used as a replacement for biological indicators *(5.1.2)*.

Ionising radiation sterilisation Sterilisation by this method is achieved by exposure of the product to ionising radiation in the form of gamma radiation from a suitable radioisotopic source (such as cobalt 60) or of a beam of electrons energised by a suitable electron accelerator.

In some countries there are regulations that lay down rules for the use of ionising radiation for sterilisation purposes, for example, in the appropriate European Community Notes for Guidance.

For this method of terminal sterilisation the reference absorbed dose is 25 kGy. Other doses may be used provided that it has satisfactorily been demonstrated that the dose chosen delivers an adequate and reproducible level of lethality when the process is operated routinely within the established tolerances. The procedures and precautions employed are such as to give an SAL of 10^{-6} or better.

During the sterilisation procedure the radiation absorbed by the product is monitored regularly by means of established dosimetry procedures that are independent of dose rate. Dosimeters are calibrated against a standard source at a reference radiation plant on receipt from the supplier and at suitable intervals of not longer than one year thereafter.

Where a biological assessment is carried out, this is obtained using a suitable biological indicator *(5.1.2)*.

Gas sterilisation This method of sterilisation is only to be used where there is no suitable alternative. It is essential that penetration by gas and moisture into the material to be sterilised is ensured and that it is followed by a process of elimination of the gas under conditions that have been previously established to ensure that any residue of gas or its transformation products in the sterilised product is below the concentration that could give rise to toxic effects during use of the product. Guidance on this aspect with respect to the use of ethylene oxide is provided, for example, in the appropriate European Community Notes for Guidance.

Wherever possible, the gas concentration, relative humidity, temperature and duration of the process are measured and recorded. Measurements are made where sterilisation conditions are least likely to be achieved, as determined at validation.

The effectiveness of the process applied to each sterilisation load is checked using a suitable biological indicator *(5.1.2)*.

A suitable sample of each batch is tested for sterility *(2.6.1)* before the batch is released.

Filtration

Certain active ingredients and products that cannot be terminally sterilised may be subjected to a filtration procedure using a filter of a type that has been demonstrated to be satisfactory by means of a microbial challenge test using a suitable test micro-organism. A suspension of *Pseudomonas diminuta* (ATCC 19146, NCIMB 11091 or CIP 103020) may be suitable. It is recommended that a challenge of at least 10^7 CFU per cm^2 of active filter surface is used and that the suspension is prepared in tryptone soya broth which, after passage through the filter, is collected aseptically and incubated aerobically at 32 °C. Such products need special precautions. The production process and environment are designed to minimise microbial contamination and are regularly subjected to appropriate monitoring procedures. The equipment, containers and closures and, wherever possible, the ingredients are subjected to an appropriate sterilisation process. It is recommended that the filtration process is carried out as close as possible to the filling point. The operations following filtration are carried out under aseptic conditions.

Solutions are passed through a bacteria-retentive membrane with a nominal pore size of 0.22 µm or less or any other type of filter known to have equivalent properties of bacteria retention. Appropriate measures are taken to avoid loss of solute by adsorption on to the filter and to avoid the release of contaminants from the filter. Attention is given to the bioburden prior to filtration, filter capacity, batch size and duration of filtration. The filter is not used for a longer period than has been approved by validation of the combination of the filter and the product in question.

The integrity of an assembled sterilising filter is verified before use and confirmed after use by carrying out tests appropriate to the type of filter used and the stage of testing, for example bubble-point, pressure hold or diffusion rate tests.

Due to the potential additional risks of the filtration method as compared with other sterilisation processes, a prefiltration through a bacteria-retentative filter may be advisable in cases where a low bioburden cannot be ensured by other means.

Aseptic preparation

The objective of aseptic processing is to maintain the sterility of a product that is assembled from components, each of which has been sterilised by one of the above methods. This is achieved by using conditions and facilities designed to prevent microbial contamination. Aseptic processing may include aseptic filling of products into container/closure systems, aseptic blending of formulations followed by aseptic filling and aseptic packaging.

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

— environment,

— personnel,

— critical surfaces,

— container/closure sterilisation and transfer procedures,

— maximum holding period of the product before filling into the final container.

Process validation includes appropriate checks on all the above and checks on the process are regularly carried out by means of process simulation tests using microbial growth media which are then incubated and examined for microbial contamination (media fill tests). In addition, a suitable sample of each batch of any product that is sterilised by filtration and/or aseptically processed is tested for sterility *(2.6.1)* before the batch is released.

Biological indicators of sterilisation

(Ph. Eur. method 5.1.2)

Biological indicators are standardised preparations of selected micro-organisms used to assess the effectiveness of a sterilisation procedure. They usually consist of a population of bacterial spores placed on an inert carrier, for example a strip of filter paper, a glass slide or a plastic tube. The inoculated carrier is covered in such a way that it is protected from any deterioration or contamination, while allowing the sterilising agent to enter into contact with the micro-organisms. Spore suspensions may be presented in sealed ampoules. Biological indicators are prepared in such a way that they can be stored under defined conditions; an expiry date is set.

Micro-organisms of the same bacterial species as the bacteria used to manufacture the biological indicators may be inoculated directly into a liquid product to be sterilised or into a liquid product similar to that to be sterilised. In this case, it must be demonstrated that the liquid product has no inhibiting effect on the spores used, especially as regards their germination.

A biological indicator is characterised by the name of the species of bacterium used as the reference micro-organism, the number of the strain in the original collection, the number of viable spores per carrier and the *D*-value. The *D*-value is the value of a parameter of sterilisation (duration or absorbed dose) required to reduce the number of viable organisms to 10 per cent of the original number. It is of significance only under precisely defined experimental conditions. Only the stated micro-organisms are present. Biological indicators consisting of more than one species of bacteria on the same carrier may be used. Information on the culture medium and the incubation conditions is supplied.

It is recommended that the indicator organisms are placed at the locations presumed, or wherever possible, found by previous physical measurement to be least accessible to the sterilising agent. After exposure to the sterilising agent, aseptic technique is used to transfer carriers of spores to the culture media, so that no contamination is present at the time of examination. Biological indicators that include an ampoule of culture medium placed directly in the packaging protecting the inoculated carrier may be used.

A choice of indicator organisms is made such that:

a) the resistance of the test strain to the particular sterilisation method is great compared to the resistance of all pathogenic micro-organisms and to that of micro-organisms potentially contaminating the product,

b) the test strain is non-pathogenic,

c) the test strain is easy to culture.

After incubation, growth of the reference micro-organisms subjected to a sterilisation procedure demonstrates that the procedure has been unsatisfactory.

Steam sterilisation The use of biological indicators intended for steam sterilisation is recommended for the validation of sterilisation cycles. Spores of *Bacillus stearothermophilus* (for example, ATCC 7953, NCTC 10007, NCIMB 8157 or CIP 52.81) are recommended. The number of viable spores exceeds 5×10^5 per carrier. The *D*-value at 121 °C exceeds 1.5 min. It is verified that exposing the biological indicators to steam at 121 ± 1 °C for 6 min leaves revivable spores, and that there is no growth of the reference micro-organisms after the biological indicators have been exposed to steam at 121 ± 1 °C for 15 min.

Dry heat sterilisation Spores of *Bacillus subtilis* (for example, var. *niger* ATCC 9372, NCIMB 8058 or CIP 77.18) are recommended for the preparation of biological indicators. The number of viable spores exceeds 1×10^5 per carrier and the D-value at 160 °C is approximately 1 min to 3 min. Dry heat at temperatures greater than 220 °C is frequently used for sterilisation and depyrogenation of glassware. In this case, demonstration of a 3 log reduction in heat resistant bacterial endotoxin can be used as a replacement for biological indicators.

Ionising radiation sterilisation Biological indicators may be used to monitor routine operations, as an additional possibility to assess the effectiveness of the set dose of radiation energy, especially in the case of accelerated electron sterilisation. The spores of *Bacillus pumilus* (for example, ATCC 27.142, NCTC 10327, NCIMB 10692 or CIP 77.25) are recommended. The number of viable spores exceeds 1×10^7 per carrier. The *D*-value exceeds 1.9 kGy. It is verified that there is no growth of the reference micro-organisms after the biological indicators have been exposed to 25 kGy (*minimum absorbed dose*).

Gas sterilisation The use of biological indicators is necessary for all gas sterilisation procedures, both for the validation of the cycles and for routine operations. Gas sterilisation is widely used for medical devices, isolators, chambers, etc. Use for such purposes is outside the scope of the European Pharmacopoeia. The use of spores of *Bacillus subtilis* (for example, var. *niger* ATCC 9372, NCIMB 8058 or CIP 77.18) is recommended for ethylene oxide. The number of viable spores exceeds 5×10^5 per carrier. The parameters of resistance are the following: the *D*-value exceeds 2.5 min for a test cycle involving 600 mg/l of ethylene oxide, at 54 °C and at 60 per cent relative humidity. It is verified that there is no growth of the reference micro-organisms after the biological indicators have been exposed to the test cycle described above for 60 min and that exposing the indicators to a reduced temperature cycle (600 mg/l, 30 °C and 60 per cent relative humidity) for 15 min leaves revivable spores. Exposing the indicators to 600 mg/l of ethylene oxide at 54 °C for 60 min without humidification must leave revivable spores to ensure that the biological indicator is able to reveal insufficient humidification.

Application of the F_0 concept to steam sterilisation of aqueous preparations

(Ph. Eur. method 5.1.5)

The following chapter is published for information.

The F_0 value of a saturated steam sterilisation process is the lethality expressed in terms of the equivalent time in minutes at a temperature of 121 °C delivered by the process to the product in its final container with reference to micro-organisms possessing a theoretical *Z*-value of 10.

The total F_0 of a process takes account of the heating up and cooling down phases of the cycle and can be calculated by integration of lethal rates with respect to time at discrete temperature intervals.

When a steam sterilisation cycle is chosen on the basis of the F_0 concept, great care must be taken to ensure that an adequate assurance of sterility is consistently achieved. In addition to validating the process, it may also be necessary to perform continuous, rigorous microbiological monitoring during routine production to demonstrate that the microbiological parameters are within the established tolerances so as to give an SAL of 10^{-6} or better.

In connection with sterilisation by steam, the *Z*-value relates the heat resistance of a micro-organism to changes in temperature. The *Z*-value is the change in temperature required to alter the *D*-value by a factor of 10.

The *D*-value (or decimal reduction value) is the value of a parameter of sterilisation (duration or absorbed dose) required to reduce the number of viable organisms to 10 per cent of the original number. It is only of significance under precisely defined experimental conditions.

The following mathematical relationships apply:

$$F_0 = D_{121}\,(\log N_0 - \log N) = D_{121} \log IF$$

D_{121} = *D*-value of the reference spores (*5.1.2*) at 121 °C,

N_0 = initial number of viable micro-organisms,

N = final number of viable micro-organisms,

IF = inactivation factor.

$$Z = \frac{T_2 - T_1}{\log D_1 - \log D_2}$$

D_1 = *D*-value of the micro-organism at temperature T_1,

D_2 = *D*-value of the micro-organism at temperature T_2.

$$IF = \frac{N_0}{N} = 10^{t/D}$$

t = exposure time,

D = *D*-value of micro-organism in the exposure conditions.