# EUROPEAN PHARMACOPOEIA

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- 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.

**01/2005:50102**

## 5.1.2. BIOLOGICAL INDICATORS OF STERILISATION

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 $5 \times 10^5$ 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 $1 \times 10^5$ 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 $1 \times 10^7$ 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 $5 \times 10^5$ 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.

**01/2005:50103**

## 5.1.3. EFFICACY OF ANTIMICROBIAL PRESERVATION

If a pharmaceutical preparation does not itself have adequate antimicrobial activity, antimicrobial preservatives may be added, particularly to aqueous preparations, to prevent proliferation or to limit microbial contamination which, during normal conditions of storage and use, particularly for multidose containers, could occur in a product and present

a hazard to the patient from infection and spoilage of the preparation. Antimicrobial preservatives must not be used as a substitute for good manufacturing practice.

The efficacy of an antimicrobial preservative may be enhanced or diminished by the active constituent of the preparation or by the formulation in which it is incorporated or by the container and closure used. The antimicrobial activity of the preparation in its final container is investigated over the period of validity to ensure that such activity has not been impaired by storage. Such investigations may be carried out on samples removed from the final container immediately prior to testing.

During development of a pharmaceutical preparation, it shall be demonstrated that the antimicrobial activity of the preparation as such or, if necessary, with the addition of a suitable preservative or preservatives provides adequate protection from adverse effects that may arise from microbial contamination or proliferation during storage and use of the preparation.

The efficacy of the antimicrobial activity may be demonstrated by the test described below. The test is not intended to be used for routine control purposes.



## TEST FOR EFFICACY OF ANTIMICROBIAL PRESERVATION

The test consists of challenging the preparation, wherever possible in its final container, with a prescribed inoculum of suitable micro-organisms, storing the inoculated preparation at a prescribed temperature, withdrawing samples from the container at specified intervals of time and counting the organisms in the samples so removed.

The preservative properties of the preparation are adequate if, in the conditions of the test, there is a significant fall or no increase, as appropriate, in the number of micro-organisms in the inoculated preparation after the times and at the temperatures prescribed. The criteria of acceptance, in terms of decrease in the number of micro-organisms with time, vary for different types of preparations according to the degree of protection intended (see Tables 5.1.3.-1/2/3).

*Test micro-organisms*

| | |
|---|---|
| *Pseudomonas aeruginosa* | ATCC 9027; NCIMB 8626; CIP 82.118. |
| *Staphylococcus aureus* | ATCC 6538; NCTC 10788; NCIMB 9518; CIP 4.83. |
| *Candida albicans* | ATCC 10231; NCPF 3179; IP 48.72. |
| *Aspergillus niger* | ATCC 16404; IMI 149007; IP 1431.83. |

Single-strain challenges are used and the designated micro-organisms are supplemented, where appropriate, by other strains or species that may represent likely contaminants to the preparation. It is recommended, for example, that *Escherichia coli* (ATCC 8739; NCIMB 8545; CIP 53.126) is used for all oral preparations and *Zygosaccharomyces rouxii* (NCYC 381; IP 2021.92) for oral preparations containing a high concentration of sugar.

### Preparation of inoculum

Preparatory to the test, inoculate the surface of agar medium B (*2.6.12*) for bacteria or agar medium C without the addition of antibiotics (*2.6.12*) for fungi, with the recently grown stock culture of each of the specified micro-organisms. Incubate the bacterial cultures at 30-35 °C for 18-24 h, the culture of *C. albicans* at 20-25 °C for 48 h, and the culture of *A. niger* at 20-25 °C for 1 week or until good sporulation is obtained. Subcultures may be needed after revival before the micro-organism is in its optimal state, but it is recommended that their number be kept to a minimum.

To harvest the bacterial and *C. albicans* cultures, use a sterile suspending fluid, containing 9 g/l of *sodium chloride R*, for dispersal and transfer of the surface growth into a suitable vessel. Add sufficient suspending fluid to reduce the microbial count to about $10^8$ micro-organisms per millilitre. To harvest the *A. niger* culture, use a sterile suspending fluid containing 9 g/l of *sodium chloride R* and 0.5 g/l of *polysorbate 80 R* and adjust the spore count to about $10^8$ per millilitre by adding the same solution.

Remove immediately a suitable sample from each suspension and determine the number of colony-forming units per millilitre in each suspension by plate count or membrane filtration (*2.6.12*). This value serves to determine the inoculum and the baseline to use in the test. The suspensions shall be used immediately.

### METHOD

To count the viable micro-organisms in the inoculated products, use the agar medium used for the initial cultivation of the respective micro-organisms.

Inoculate a series of containers of the product to be examined, each with a suspension of one of the test organisms to give an inoculum of $10^5$ to $10^6$ micro-organisms per millilitre or per gram of the preparation. The volume of the suspension of inoculum does not exceed 1 per cent of the volume of the product. Mix thoroughly to ensure homogeneous distribution.

Maintain the inoculated product at 20-25 °C, protected from light. Remove a suitable sample from each container, typically 1 ml or 1 g, at zero hour and at appropriate intervals according to the type of the product and determine the number of viable micro-organisms by plate count or membrane filtration (*2.6.12*). Ensure that any residual antimicrobial activity of the product is eliminated by dilution, by filtration or by the use of a specific inactivator. When dilution procedures are used, due allowance is made for the reduced sensitivity in the recovery of small numbers of viable micro-organisms. When a specific inactivator is used, the ability of the system to support the growth of the test organisms is confirmed by the use of appropriate controls.

The procedure is validated to verify its ability to demonstrate the required reduction in count of viable micro-organisms.

### CRITERIA OF ACCEPTANCE

The criteria for evaluation of antimicrobial activity are given in Tables 5.1.3.-1/2/3 in terms of the log reduction in the number of viable micro-organisms against the value obtained for the inoculum.

Table 5.1.3.-1. - *Parenteral and ophthalmic preparations*

| | | Log reduction | | | | |
|---|---|---|---|---|---|---|
| | | 6 h | 24 h | 7 d | 14 d | 28 d |
| Bacteria | A | 2 | 3 | - | - | NR* |
| | B | - | 1 | 3 | - | NI** |
| Fungi | A | - | - | 2 | - | NI |
| | B | - | - | - | 1 | NI |

*NR: no recover
**NI: no increase

The A criteria express the recommended efficacy to be achieved. In justified cases where the A criteria cannot be attained, for example for reasons of an increased risk of adverse reactions, the B criteria must be satisfied.

Table 5.1.3.-2. - *Topical preparations*

| | | Log reduction | | | |
|---|---|---|---|---|---|
| | | 2 d | 7 d | 14 d | 28 d |
| Bacteria | A | 2 | 3 | - | NI |
| | B | - | - | 3 | NI |
| Fungi | A | - | - | 2 | NI |
| | B | - | - | 1 | NI |

The A criteria express the recommended efficacy to be achieved. In justified cases where the A criteria cannot be attained, for example for reasons of an increased risk of adverse reactions, the B criteria must be satisfied.

Table 5.1.3.-3. - *Oral preparations*

| | Log reduction | |
|---|---|---|
| | 14 d | 28 d |
| Bacteria | 3 | NI |
| Fungi | 1 | NI |

The above criteria express the recommended efficacy to be achieved.

**01/2005:50104**

## 5.1.4. MICROBIOLOGICAL QUALITY OF PHARMACEUTICAL PREPARATIONS

*The following chapter is published for information.*

In the manufacture, packaging, storage and distribution of pharmaceutical preparations, suitable means must be taken to ensure their microbiological quality. The pharmaceutical preparations should comply with the criteria given below.

**Category 1**

*Preparations required to be sterile by the relevant monograph on the dosage form and other preparations labelled sterile.*

– Test for sterility (*2.6.1*).

**Category 2**

*Preparations for topical use and for use in the respiratory tract except where required to be sterile and transdermal patches.*

– Total viable aerobic count (*2.6.12*). Not more than $10^2$ micro-organisms (aerobic bacteria plus fungi) per gram, per millilitre or per patch (including the adhesive and backing layer).
– Transdermal patches: absence of enterobacteria and certain other gram-negative bacteria, determined on 1 patch (including the adhesive and backing layer). Other preparations: not more than $10^1$ enterobacteria and certain other gram-negative bacteria per gram or per millilitre (*2.6.13*).
– Absence of *Pseudomonas aeruginosa*, determined on 1 g, 1 ml or one patch (including the adhesive and backing layer) (*2.6.13*).
– Absence of *Staphylococcus aureus*, determined on 1 g, 1 ml or one patch (including the adhesive and backing layer) (*2.6.13*).

**Category 3**

A. *Preparations for oral and rectal administration.*
– Total viable aerobic count (*2.6.12*). Not more than $10^3$ bacteria and not more than $10^2$ fungi per gram or per millilitre.
– Absence of *Escherichia coli* (1 g or 1 ml) (*2.6.13*).

B. *Preparations for oral administration containing raw materials of natural (animal, vegetable or mineral) origin for which antimicrobial pretreatment is not feasible and for which the competent authority accepts microbial contamination of the raw material exceeding $10^3$ viable micro-organisms per gram or per millilitre. Herbal medicinal products described in category 4 are excluded.*
– Total viable aerobic count (*2.6.12*). Not more than $10^4$ bacteria and not more than $10^2$ fungi per gram or per millilitre.
– Not more than $10^2$ enterobacteria and certain other gram-negative bacteria per gram or per millilitre (*2.6.13*).
– Absence of *Salmonella* (10 g or 10 ml) (*2.6.13*).
– Absence of *Escherichia coli* (1 g or 1 ml) (*2.6.13*).
– Absence of *Staphylococcus aureus* (1 g or 1 ml) (*2.6.13*).

**Category 4**

*Herbal medicinal products consisting solely of one or more herbal drugs (whole, reduced or powdered).*

A. *Herbal medicinal products to which boiling water is added before use.*
– Total viable aerobic count (*2.6.12*). Not more than $10^7$ bacteria and not more than $10^5$ fungi per gram or per millilitre.
– Not more than $10^2$ *Escherichia coli* per gram or per millilitre (*2.6.13*, using suitable dilutions).

B. *Herbal medicinal products to which boiling water is not added before use.*
– Total viable aerobic count (*2.6.12*). Not more than $10^5$ bacteria and not more than $10^4$ fungi per gram or per millilitre.
– Not more than $10^3$ enterobacteria and certain other gram-negative bacteria per gram or per millilitre (*2.6.13*).
– Absence of *Escherichia coli* (1 g or 1 ml) (*2.6.13*).
– Absence of *Salmonella* (10 g or 10 ml) (*2.6.13*).

**01/2005:50105**

## 5.1.5. APPLICATION OF THE $F_0$ CONCEPT TO STEAM STERILISATION OF AQUEOUS PREPARATIONS

*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 *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