
**Sterilization of health care products —
Biological indicators —**

Part 5:

**Biological indicators for low-temperature
steam and formaldehyde sterilization
processes**

Stérilisation des produits de santé — Indicateurs biologiques —

*Partie 5: Indicateurs biologiques pour la stérilisation à la vapeur d'eau et
au formaldéhyde à basse température*



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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 11138-5 was prepared by Technical Committee ISO/TC 198, *Sterilization of health care products*.

ISO 11138 consists of the following parts, under the general title *Sterilization of health care products — Biological indicators*:

- *Part 1: General requirements*
- *Part 2: Biological indicators for ethylene oxide sterilization processes*
- *Part 3: Biological indicators for moist heat sterilization processes*
- *Part 4: Biological indicators for dry heat sterilization processes*
- *Part 5: Biological indicators for low-temperature steam and formaldehyde sterilization processes*

Introduction

ISO 11138-1 specifies production, labelling, test methods and performance requirements for the manufacture of biological indicators including inoculated carriers and suspensions intended for use in validation and monitoring sterilization processes. This part of 11138 gives specific requirements for those biological indicators intended for use in low-temperature steam and formaldehyde sterilization processes.

The intent of providing requirements in the ISO 11138 series of International Standards is to provide general requirements and requirements for test methods. This series of International Standards represents the current “state-of-the-art” according to the experts representing manufacturers, users and regulatory authorities involved in developing the standard. The intent is not to promote the use of biological indicators where such use is not advised, but to provide common requirements for the production of those biological indicators that are known to be in use today.

Standards exist providing general requirements for the validation and control of low-temperature steam and formaldehyde sterilization (see ISO 14937)¹⁾.

NOTE Some countries or regions may have published standards covering requirements for sterilization or biological indicators.

Advice on selection, use and interpretation of results when using biological indicators can be found in ISO 14161.

1) Although ISO/TC 198 has agreed to develop a standard applicable to dry heat processes, it was not available for reference at the time this document was prepared.

Sterilization of health care products — Biological indicators —

Part 5:

Biological indicators for low-temperature steam and formaldehyde sterilization processes

1 Scope

This part of ISO 11138 provides specific requirements for test organisms, suspensions, inoculated carriers, biological indicators and test methods intended for use in assessing the performance of sterilization processes employing low-temperature steam and formaldehyde as the sterilizing agent.

NOTE 1 Requirements for validation and control of low-temperature steam and formaldehyde sterilization processes are provided by ISO 14937.

NOTE 2 Requirements for work place safety may be provided by national or regional regulations.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 11138-1:2006, *Sterilization of health care products — Biological indicators — Part 1: General requirements*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 11138-1 and the following apply.

3.1

low-temperature steam and formaldehyde sterilization

process incorporating forced air removal, which allows exposure of wrapped goods to steam at sub-atmospheric pressure, and thus at temperatures $< 100\text{ }^{\circ}\text{C}$, with the admission of formaldehyde gas, keeping the sterilizing agent in a steady state throughout the hold time

4 General requirements

The requirements of ISO 11138-1 apply.

5 Test organism

5.1 The test organisms shall be spores of *Geobacillus stearothermophilus* or other strains of microorganisms of demonstrated equivalent performance as required by this part of ISO 11138.

NOTE 1 *Bacillus stearothermophilus* has been reclassified as *Geobacillus stearothermophilus*.

NOTE 2 *Geobacillus stearothermophilus* NCIB 8224, DSM 6790, ATCC 7953, ATCC 10149 and ATCC 12980 have been found to be suitable.

5.2 If a test organism other than *Geobacillus stearothermophilus* is used, the suitability of the resistance of that test organism shall be determined.

6 Suspension

The requirements of ISO 11138-1 apply.

7 Carrier and primary packaging

7.1 The suitability of the carrier and primary packaging materials for biological indicators for use in low-temperature steam and formaldehyde sterilization processes shall be demonstrated in accordance with the requirements of ISO 11138-1:2006, 5.2 and Annex B.

NOTE Carriers based on filter paper might not be suitable because of the chemisorption of formaldehyde on cellulose surfaces.

7.2 The exposure conditions for establishing compliance shall be:

- a) minimum exposure temperature: $\geq 5\text{ }^{\circ}\text{C}$ above the manufacturer's stated maximum temperature;
- b) maximum exposure temperature: as stated by the manufacturer; if not stated by the manufacturer, the maximum exposure temperature shall be $\geq 100\text{ }^{\circ}\text{C}$;
- c) exposure time: $\geq 160\text{ min}$.

NOTE These conditions have been selected to represent a realistic challenge to the carrier while remaining within the practical limits of a low-temperature steam and formaldehyde sterilization process.

8 Inoculated carriers and biological indicators

The requirements of ISO 11138-1 apply.

9 Population and resistance

9.1 The manufacturer shall state the resistance characteristics according to ISO 11138-1:2006, 6.4.

9.2 The viable count shall be stated with increments $\leq 0,1 \times 10^n$ per unit (e.g. per ml of suspension, per inoculated carrier or per biological indicator).

9.3 For inoculated carriers and biological indicators, the viable count shall be $\geq 1,0 \times 10^5$.

9.4 The resistance shall be expressed as the *D* value in minutes at 60 °C. The *D* value of each batch/lot of biological indicators or inoculated carriers shall be stated in minutes to one decimal place at 60 °C.

9.5 Suspensions, inoculated carriers or biological indicators containing *Geobacillus stearothermophilus* spores shall have a D_{60} value of ≥ 6 min when tested according to the conditions in Annex A. Other microorganisms shall have D values supporting the application.

9.6 The resistance of a biological indicator may also be indicated by the term F_{BIO} value (see ISO 11138-1:2006, 3.7).

The resistance characteristics specified in this part of ISO 11138 and any other part of ISO 11138 apply to the specific test conditions stated in the standards.

9.7 D values are determined according to methods given in Annexes C and D of ISO 11138-1:2006.

9.8 Determination of D value and survival-kill response characteristics are based on the process parameters in Annex A.

9.9 The survival-kill window can be calculated using the formulae in ISO 11138-1:2006, Annex E.

NOTE This information may be of value to the user when comparing different batches from the same manufacturer.

EXAMPLE

Using the formulae in ISO 11138-1:2006, Annex E with the minimum population and minimum D value requirements specified in this part of ISO 11138, the survival-kill response characteristics are:

— at 60 °C: survival time ≥ 18 min and kill time ≤ 54 min.

Annex A (normative)

Method for determination of resistance to low-temperature steam and formaldehyde

A.1 General

This method is based on a qualitative test on inoculated carriers immersed in an aqueous solution of formaldehyde. This method has been shown to provide more reproducible results than using a vapour phase, chamber method.

Specific requirements related to the test method are provided in A.3.

A.2 Inoculated carrier exposure conditions

A.2.1 The test system consists of test tubes filled with 10 ml of aqueous solution of formaldehyde and held in an automatically controlled temperature water bath. The test system shall be capable of maintaining the conditions specified for exposure periods between 1 min and 150 min to an accuracy of ± 10 s.

A.2.2 The formaldehyde concentration of the aqueous solution shall be established by use of analytical chemical methods.

A.2.3 The method shall be validated.

A.3 Method

A.3.1 Completely immerse the inoculated carriers in the test tubes filled with the formaldehyde solution at a concentration of $1 \text{ mol/l} \pm 0,01 \text{ mol/l}$ that has been pre-heated to $60 \text{ }^{\circ}\text{C} \pm 0,5 \text{ }^{\circ}\text{C}$.

A.3.2 Ensure that the inoculated carriers are completely immersed in the formaldehyde solution and do not float to the surface.

A.3.3 Use an aseptic technique when performing this test in order to prevent adventitious contamination.

A.3.4 At the end of the specified exposure time, remove the inoculated carriers from the formaldehyde solution.

A.3.5 Eliminate the excess liquid and immerse the carriers in the test tubes filled with a filtered solution of 2 % Na_2SO_3 for at least 10 min at ambient conditions in order to inactivate formaldehyde residues on the carriers. Close the test tubes.

Care should be taken to minimize agitation in the formaldehyde as well as in the neutralizer solution to prevent "wash off" of test organisms.

NOTE Histidine and cystein have been shown to be effective neutralization agents.

A.3.6 The growth medium shall be specified and qualified to ensure recovery of the test organisms.

NOTE Soybean Casein Digest Medium has been found suitable for this test.

A.3.7 Transfer the carriers into test tubes filled with 10 ml of the growth medium according to A.3.6. Close the test tubes.

A.3.8 Treat the test tubes for 60 min at 90 °C for heat activation of the spores.

A.3.9 At the end of the process, incubate the carriers (see ISO 11138-1:2006, Clause 7).

A.4 Determination of resistance

Resistance characteristics shall be determined according to methods given in Annexes C, D and E of ISO 11138-1:2006.

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