SLOVENSKI STANDARD
SIST EN 12353:2006

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Kemična razkužila in antiseptiki – Shranjevanje preskusnih organizmov za določanje baktericidnega, sporocidnega in fungicidnega delovanja

Chemical disinfectants and antiseptics - Preservation of test organisms used for the determination of bactericidal, mycobactericidal, sporicidal and fungicidal activity

Chemische Desinfektionsmittel und Antiseptika - Aufbewahrung von Testorganismen für die Prüfung der bakteriziden, mykobakteriziden, sporiziden und fungiziden Wirkung

Antiseptiques et désinfectants chimiques - Conservation des microorganismes d'essai utilisés pour la détermination de l'activité bactéricide, mycobactéricide, sporicide et fongicide

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Chemical disinfectants and antiseptics - Preservation of test organisms used for the determination of bactericidal, mycobactericidal, sporicidal and fungicidal activity

Antiseptiques et désinfectants chimiques - Conservation des microorganismes d'essai utilisés pour la détermination de l'activité bactéricide, mycobactéricide, sporicide et fongicide

Chemische Desinfektionsmittel und Antiseptika - Aufbewahrung von Testorganismen für die Prüfung der bakteriziden, mykobakteriziden, sporiziden und fungiziden Wirkung

This European Standard was approved by CEN on 23 February 2006.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

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Foreword

This European Standard (EN 12353:2006) has been prepared by Technical Committee CEN/TC 216 “Chemical disinfectants and antiseptics”, the secretariat of which is held by AFNOR.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by December 2006, and conflicting national standards shall be withdrawn at the latest by December 2006.

This European Standard supersedes EN 12353:1999.

It was revised to include mycobacteria and spore-forming bacteria and to harmonize the structure and wording with other CEN/TC 216 standards.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

Introduction

Standardized tests for the determination of bactericidal, mycobactericidal, sporicidal and fungicidal activity of chemical disinfectants and antiseptics necessitate the use of test organisms whose purity and identity have been verified and whose biological behaviour remains stable. Therefore it is essential to specify the storage requirements.

This European Standard aims at describing methods for preservation of test organisms used for such purposes.

1 Scope

This European Standard specifies methods for keeping test organisms used and defined in European Standards for the determination of bactericidal, mycobactericidal, sporicidal and fungicidal activity of chemical disinfectants and antiseptics drawn up by CEN/TC 216. These methods for keeping test organisms can only be carried out in connection with at least one of those standards where a reference to this standard is established.

NOTE 1 Annex A (informative) contains a non-exhaustive list of test organisms for which this standard can be applied.

NOTE 2 European Standards (EN and prEN) where this European Standard is referenced are listed in the Bibliography.

2 Normative references

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

ISO 4793, *Laboratory sintered (fritted) filters – Porosity grading, classification and designation.*

3 Terms and definitions

For the purposes of this European Standard, the following terms and definitions apply.

3.1
bactericidal activity
capability of a product to produce a reduction in the number of viable bacterial cells of relevant test organisms under defined conditions

3.2
conidium
asexual fungal spore, produced exogenously from a hyphal tip

NOTE Conidiospore is a synonym for conidium.

3.3
fungicidal activity
capability of a product to produce a reduction in the number of viable vegetative yeast cells and mould spores of relevant test organisms under defined conditions

3.4
mycobactericidal activity
capability of a product to produce a reduction in the number of viable mycobacterial cells of relevant test organisms under defined conditions

3.5
product
chemical agent or formulation used as a chemical disinfectant or antiseptic

3.6
sporicidal activity
capability of a product to produce a reduction in the number of viable bacterial spores of relevant test organisms under defined conditions

3.7
test organism
strain of a micro-organism selected for testing products within a standardized test

NOTE For the purpose of this European Standard the term micro-organism includes vegetative bacteria, bacterial spores, fungi, fungal spores and viruses.

3.8
yeasticidal activity
capability of a product to produce a reduction in the number of viable yeast cells of relevant test organisms under defined conditions

4 Requirements

Each test organism specified in a CEN/TC 216 European Standard and referred to in this standard shall be handled as described in this standard.

The purity and identity of the preserved test organism shall be verified during the preparation and regularly during the storage.

The preserved test organism should be checked at regular intervals (at least in the case of longer storage than 14 months) to ensure that its susceptibility to products has not changed. As long as CEN/TC 216 has not developed specific tests for this purpose any suitable method can be used e.g. EN 1040 for bacteria, EN 1275 for fungi or EN 14348 for mycobacteria.

5 Methods

5.1 Principle

A freeze dried sample of the test organism is obtained from a culture collection. This sample is cultured, prepared for storage, filled into storage vessels and placed in the deep freeze.

From the deep freeze samples a stock culture is prepared and subsequently used to prepare working cultures for the test procedure. In some cases the working cultures are directly prepared from the deep freeze samples.

5.2 Materials and reagents

5.2.1 Test organisms

See Annex A for examples of test organisms.

The origin (culture collection), taxonomic name and reference number, date of receipt and batch number of the freeze dried test organisms shall be recorded (**5.9.2**).

5.2.2 Culture media and reagents

5.2.2.1 General

All weights of chemical substances given in this European Standard refer to the anhydrous salts. Hydrated forms may be used as an alternative, but the weights required shall be adjusted to allow for consequent molecular weight differences.

The reagents shall be of analytical grade and/or appropriate for microbiological purposes. They shall be free from substances that are toxic or inhibitory to the test organisms.

NOTE 1 To improve reproducibility, it is recommended that commercially available dehydrated material is used for the preparation of culture media. The manufacturer's instructions relating to the preparation of these products should be rigorously followed.

NOTE 2 For each culture medium and reagent a limitation for use should be fixed.

5.2.2.2 Water

The water shall be freshly glass distilled water and not demineralized water.

Sterilize in the autoclave [**5.3.2.1**a)].

NOTE 1 Sterilization is not necessary if the water is used for e.g. preparation of culture media and subsequently sterilized.

NOTE 2 If distilled water of adequate quality is not available, water for injections (see bibliographic reference [1]) can be used.

5.2.2.3 Tryptone Soya Broth (TSB) for bacteria

Tryptone soya broth, consisting of:

Tryptone, pancreatic digest of casein	17,0 g
Soya peptone, papaic digest of Soybean meal	3,0 g
Sodium chloride (NaCl)	5,0 g
Water (**5.2.2.2**)	800,0 ml
Dipotassium phosphate (K_2HPO_4)	2,5 g
Glucose	2,5 g
Water (**5.2.2.2**)	to 1 000,0 ml

Sterilize in the autoclave [**5.3.2.1**a)]. After sterilization the pH of the medium shall be equivalent to 7,2 ± 0,2 when measured at 20 °C ± 1 °C.

5.2.2.4 Malt Extract Broth (MEB) for fungi

Malt extract broth, consisting of:

Malt extract	20,0 g
Water (**5.2.2.2**)	to 1 000,0 ml

Sterilize in the autoclave [**5.3.2.1**a)]. After sterilization the pH of the medium shall be equivalent to 6,9 ± 0,2 when measured at 20 °C ± 1 °C

5.2.2.5 Cryoprotectant solution for bacteria, spore-forming bacteria, fungi

Cryoprotectant solution, consisting of:

Beef extract	3,0 g
Tryptone, pancreatic digest of casein	5,0 g

Glycerol ($C_3H_8O_3$) [2]	150,0 g
Water (**5.2.2.2**)	to 1 000,0 ml

Dissolve the constituents in boiling water. Sterilize in the autoclave [**5.3.2.1**a)]. After sterilization the pH of the solution shall be equivalent to 6,9 ± 0,2 when measured at 20 °C ± 1 °C.

NOTE 1 Any commercially available cryoprotectant containing glycerol for preservation of test organisms equivalent to the solution described above may be used.

NOTE 2 If justified, any other equivalent cryoprotectant solution may be used.

5.2.2.6 Middlebrook 7 H 9 broth with 10 % ADC enrichment and glycerol as reconstituent and cryoprotectant solution for mycobacteria (MADC)

Middlebrook 7 H 9 broth, consisting of:

Middlebrook 7 H 9 broth powder	4,7 g
Glycerol ($C_3H_8O_3$) [2]	100,0 ml
Water (**5.2.2.2**)	750,0 ml

Treat in the autoclave [**5.3.2.1**a)] for a holding time of only 10 min and cool to 45 °C. Add under aseptic conditions 100 ml Middlebrook ADC enrichment and then sterilized water (**5.2.2.2**) to 1 000,0 ml. The pH of the medium shall be equivalent to 6,6 ± 0,2 when measured at 20 °C ± 1 °C.

5.2.2.7 Polysorbate 80 solution

Polysorbate 80 solution, consisting of:

Polysorbate 80	0,5 g
Water (**5.2.2.2**)	to 1 000,0 ml

Sterilize in the autoclave [**5.3.2.1**a)].

5.3 Apparatus and glassware

5.3.1 General

Sterilize all glassware and parts of the apparatus that will come into contact with the culture media and reagents or the sample, except those which are supplied sterile, by one of the following methods:

a) by moist heat, in the autoclave [**5.3.2.1**a)];

b) by dry heat, in the hot air oven [**5.3.2.1**b)].

5.3.2 Usual microbiological laboratory equipment[1] and in particular, the following:

5.3.2.1 Apparatus for sterilization:

a) for moist heat sterilization, an autoclave capable of being maintained at $\left(121^{+3}_{0}\right)$ °C for a minimum holding time of 15 min;

b) for dry heat sterilization, a hot air oven capable of being maintained at $\left(180^{+5}_{0}\right)$ °C for a minimum holding time of 30 min, at $\left(170^{+5}_{0}\right)$ °C for a minimum holding time of 1 h or at $\left(160^{+5}_{0}\right)$ °C for a minimum holding time of 2 h.

5.3.2.2 Water baths, capable of being controlled at 20 °C ± 1 °C and at 45 °C ± 1 °C if pour plate technique is used.

5.3.2.3 Incubator, capable of being controlled at either 36 °C ± 1 °C or at 37 °C ± 1 °C (for bacteria and mycobacteria) or at 30 °C ± 1 °C (for fungi).

NOTE For mycobacteria a CO_2 – incubator and a temperature of 36 °C ± 1 °C is better suited. If a CO_2 – incubator is not used, the inoculated plates should be protected from drying by sealing with insulating tape or packing them into polyethylene bags.

5.3.2.4 pH-meter, having an inaccuracy of calibration of no more than ± 0,1 pH units at 20 °C ± 1 °C.

NOTE A puncture electrode or a flat membrane electrode should be used for measuring the pH of the agar media (**5.4.2**).

5.3.2.5 Fritted filter: porosity of 40 µm to 100 µm (ISO 4793).

5.3.2.6 Electromechanical agitator e.g. Vortex® mixer[2].

5.3.2.7 Forceps or wire.

5.3.2.8 Refrigerator, capable of being controlled at 2 °C to 8 °C.

5.3.2.9 Graduated pipettes, of nominal capacities 10 ml and 1 ml and 0,1 ml. Calibrated automatic pipettes may be used.

5.3.2.10 Petri dishes (plates), of size 90 mm to 100 mm.

5.3.2.11 Glass or ceramic beads, (3 mm to 4 mm in diameter).

5.3.2.12 Volumetric flasks.

5.3.2.13 Equipment for deep freezing test organisms, at a temperature of –70 °C or less – including cryovials of nominal capacity of 0,5 ml (min) to 2,0 ml (max).

5.3.2.14 Centrifuge (2 000 g_N).

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1) Disposable equipment is an acceptable alternative to reusable glassware.

2) Vortex® is an example of a suitable product available commercially. This information is given for the convenience of users of this standard and does not constitute an endorsement by CEN of this product.