SLOVENSKI STANDARD
oSIST prEN 12353:2019

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Kemična razkužila in antiseptiki - Shranjevanje preskusnih organizmov za določanje baktericidnega (vključno Legionella), mikobaktericidnega, sporocidnega, fungicidnega in virucidnega (vključno bakteriofagi) delovanja

Chemical disinfectants and antiseptics - Preservation of test organisms used for the determination of bactericidal (including Legionella), mycobactericidal, sporicidal, fungicidal and virucidal (including bacteriophages) activity

Chemische Desinfektionsmittel und Antiseptika - Aufbewahrung von Testorganismen für die Prüfung der bakteriziden (einschließlich Legionella), mykobakteriziden, sporiziden, fungiziden und viruziden (einschließlich Bakteriophagen) Wirkung

Ta slovenski standard je istoveten z: **prEN 12353**

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07.100.99	Drugi standardi v zvezi z mikrobiologijo	Other standards related to microbiology
71.100.35	Kemikalije za dezinfekcijo v industriji in doma	Chemicals for industrial and domestic disinfection purposes

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EUROPEAN STANDARD

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English Version

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This draft European Standard is submitted to CEN members for enquiry. It has been drawn up by the Technical Committee CEN/TC 216.

If this draft becomes a European Standard, 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.

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Recipients of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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European foreword

This document (prEN 12353:2019) has been prepared by Technical Committee CEN/TC 216 “Chemical disinfectants and antiseptics”, the secretariat of which is held by AFNOR.

This document is currently submitted to the CEN Enquiry.

This document will supersede EN 12353:2013.

The document was revised to adapt it to the latest state of science, to correct errors and ambiguities. The following are the significant technical changes since the last edition:

— the methods of preservation of viruses are described more detailed (5.8.3);

— the description of BCYE Agar for *Legionella* was added (5.2.2.24);

— the information on source of strains was added (5.10.2);

— the information of the storage of documentation was added (5.11.6);

— the IP Number for fungi was deleted (see A.4);

— the used virus strains are re-drafted (see A.5).

The changes mentioned above have no impact on the test results obtained with reference to the previous version. Those results are still valid.

Introduction

Standardized tests for the determination of bactericidal (incl. *Legionella pneumophila*), mycobactericidal, sporicidal, fungicidal and virucidal (incl. bacteriophages) 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 document aims to describe methods for preservation of test organisms used for such purposes.

1 Scope

This document specifies methods for keeping test organisms used and defined in European Standards for the determination of bactericidal (incl. *Legionella pneumophila*), mycobactericidal, sporicidal, fungicidal and virucidal (incl. bacteriophages) 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 document is established.

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

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

NOTE 3 A specific part on the preservation of bacterial spores could be added once the results of the ongoing ring trials are available.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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.

EN 14885, *Chemical disinfectants and antiseptics — Application of European Standards for chemical disinfectants and antiseptics*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN 14885 apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- IEC Electropedia: available at http://www.electropedia.org/
- ISO Online browsing platform: available at http://www.iso.org/obp

4 Requirements

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

The purity and identity of the preserved test organism shall be verified during the preparation and regularly during the storage, except for viruses where only the identity is checked before the stock virus suspension is stored.

The preserved test organism – except viruses - 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, EN 14348 for mycobacteria, EN 13623 for *Legionella pneumophila*, EN 14476 for viruses or EN 13610 for dairy bacteriophages.

5 Methods

5.1 Principle

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

From this sample 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 documentation on the test organisms should follow 5.10.2 and 5.11.3.

5.2.2 Culture media and reagents

5.2.2.1 General

The formulas of all media and reagents are given in case commercial ready-to-use material is not used. It is to be checked that each commercial supplier has established an appropriate quality control system.

All weights of chemical substances given in this document refer to the anhydrous salts unless otherwise stated. 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.

To improve reproducibility, it is recommended that whenever possible, 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.

All specified pH values are measured at (20 ± 1) °C.

For each culture medium, cell culture 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. If distilled water of adequate quality is not available, water for injections (see bibliographic reference [1]) can be used.

Sterilize in the autoclave (5.3.2.1a). Sterilization is not necessary if the water is used for e.g. preparation of culture media and subsequently sterilized.

5.2.2.3 Tryptone Soya Broth (TSB) for bacteria, except *Legionella*

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 (5.3.2.1a). After sterilization the pH of the medium shall be equivalent to 7,2 ± 0,2.

5.2.2.4 Malt Extract Broth (MEB) for fungi

Malt extract broth, consisting of:

Malt extract (food grade, e.g. Christomalt powder from Difal or equivalent that is not highly purified and not only based on maltose, e.g.

malt extract from OXOID)[1]	20,0 g
Water (5.2.2.2)	to 1 000,0 ml

Sterilize in the autoclave (5.3.2.1a). After sterilization the pH of the medium shall be equivalent to 5,6 ± 0,2.

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 (5.3.2.1a). After sterilization the pH of the solution shall be equivalent to 6,9 ± 0,2.

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

If justified, any other equivalent cryoprotectant solution may be used, e.g. for *Legionella* (5.5.2).

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)	800,0 ml

Treat in the autoclave (5.3.2.1a) for a holding time of only 10 min and cool to 45 °C. Add under aseptic conditions 100 ml Middlebrook ADC enrichment to obtain approximately 1 000,0 ml. The pH of the medium shall be equivalent to 6,6 ± 0,2.

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[1] This information is given for the information of users of this standard and does not constitute an endorsement of the products named. Corresponding products supplied by other manufacturers may be used if they can be shown to lead to the same results.

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 (5.3.2.1a).

5.2.2.8 DMSO as cryoprotectant for cell culture freezing

Dimethyl sulphoxide (DMSO) is used to help protect the cells from rupture by the formation of ice crystals.

Since DMSO is toxic it should be handled with care. It can be absorbed through the skin and may cause irritation and/or burns. It is teratogenic and an allergen. Latex gloves should be worn when handling it.

5.2.2.9 Glutamine solution, 3 %

Dissolve 12 g Glutamine in 400 ml of water (5.2.2.2) and sterilize by membrane filtration. The solution is stored at (−20 ± 1) °C.

5.2.2.10 TV (Trypsin-Versene)

Dissolve 0,05 g Trypsin in 100 ml of 0,53 mM EDTA (Ethylene diamine tetra acetic acid) and sterilize by membrane filtration. Store at (4 ± 1) °C.

5.2.2.11 Antibiotic suspension

Chemicals

50 million units Penicillin-G	(eg Sigma PEN-K[2])
50 g Streptomycin sulphate (approx. equal to 750 IU/mg)	(eg Sigma Cat: 56501[2])
25 × 500,000 units Mycostatin	(eg Nystatin: E R Squibb 59150[2])

Water (5.2.2.2) to 2,5 l.

Preparation

Dissolve vial contents of antibiotics in water (5.2.2.2) and fill up to 2,5 l.

Dispense aseptically into 50 ml and 5 ml aliquots.

Store at – 20 °C. Shake the bottle after thawing.

Use 5 ml per litre of medium to give a final concentration of:

Penicillin	100 units/ml
Streptomycin	100 µg/ml
Mycostatin	25 units/ml

[2] This information is given for the information of users of this standard and does not constitute an endorsement of the products named. Corresponding products supplied by other manufacturers may be used if they can be shown to lead to the same results.