SLOVENSKI STANDARD
SIST EN 12353:2013

01-julij-2013

Nadomešča:
SIST EN 12353:2006

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

iTeh STANDARD PREVIEW
(standards.iteh.ai)

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

SIST EN 12353:2013
https://standards.iteh.ai/catalog/standards/sist/81798c92-2941-48cd-bab4-31e22e9227c9/sist-en-12353-2013

Antiseptiques et désinfectants chimiques - Conservation des organismes test utilisés pour la détermination de l'activité bactéricide (Legionella inclus), mycobactéricide, sporicide, fongicide et virucide (bacteriophages inclus)

Ta slovenski standard je istoveten z: **EN 12353:2013**

ICS:

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

SIST EN 12353:2013 **en,fr,de**

2003-01.Slovenski inštitut za standardizacijo. Razmnoževanje celote ali delov tega standarda ni dovoljeno.

SIST EN 12353:2013

iTeh STANDARD PREVIEW
(standards.iteh.ai)

SIST EN 12353:2013
https://standards.iteh.ai/catalog/standards/sist/81798c92-2941-48cd-bab4-31e22e9227c9/sist-en-12353-2013

SIST EN 12353:2013

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 12353

February 2013

ICS 11.080.20; 71.100.35

Supersedes EN 12353:2006

English Version

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

Antiseptiques et désinfectants chimiques - Conservation des organismes test utilisés pour la détermination de l'activité bactéricide (Legionella inclus), mycobactéricide, sporicide, fongicide et virucide (bacteriophages inclus)

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

This European Standard was approved by CEN on 14 December 2012.

iTeh STANDARD PREVIEW

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 CEN-CENELEC Management Centre or to any CEN member.

(standards.iteh.ai)

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 CEN-CENELEC Management Centre has the same status as the official versions.

SIST EN 12353:2013
https://standards.iteh.ai/catalog/standards/sist/81798c92-2941-48cd-bab4-

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.

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

Management Centre: Avenue Marnix 17, B-1000 Brussels

© 2013 CEN All rights of exploitation in any form and by any means reserved worldwide for CEN national Members.

Ref. No. EN 12353:2013: E

SIST EN 12353:2013

Contents

Page

iTeh STANDARD PREVIEW
(standards.iteh.ai)
SIST EN 12353:2013
https://standards.iteh.ai/catalog/standards/sist/81798c92-2941-48cd-bab4-31e22e9227c9/sist-en-12353-2013

SIST EN 12353:2013

Foreword

This document (EN 12353:2013) 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 August 2013, and conflicting national standards shall be withdrawn at the latest by August 2013.

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

This document supersedes EN 12353:2006.

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 Legionella, mycobacteria, bacteriophages and viruses are new and were added. Data obtained by using the former version of EN 12353 are still valid.

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

iTeh STANDARD PREVIEW
(standards.iteh.ai)
SIST EN 12353:2013
https://standards.iteh.ai/catalog/standards/sist/81798c92-2941-48cd-bab4-31e22e9227c9/sist-en-12353-2013

SIST EN 12353:2013

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

iTeh STANDARD PREVIEW
(standards.iteh.ai)
SIST EN 12353:2013
https://standards.iteh.ai/catalog/standards/sist/81798c92-2941-48cd-bab4-31e22e9227c9/sist-en-12353-2013

SIST EN 12353:2013

1 Scope

This European Standard 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 European 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.

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

2 Normative references

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

iTeh STANDARD PREVIEW

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

(standards.iteh.ai)

SIST EN 12353:2013
https://standards.iteh.ai/catalog/standards/sist/81798c92-2941-48cd-bab4-31e22e9227c9/sist-en-12353-2013

3 Terms and definitions

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

4 Requirements

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

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.

SIST EN 12353:2013

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 origin (culture collection), taxonomic name and reference number, date of receipt and batch number of the freeze dried test organisms shall be recorded (5.11.2).

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 European Standard 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.

iTeh STANDARD PREVIEW
(standards.iteh.ai)

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.

SIST EN 12353:2013
https://standards.iteh.ai/catalog/standards/sist/81798c92-2941-48cd-bab4-31e22e9227c9/sist-en-12353-2013

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.

SIST EN 12353:2013

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.

iTeh STANDARD PREVIEW

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

(standards.iteh.ai)

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

SIST EN 12353:2013
https://standards.iteh.ai/catalog/standards/sist/81798c92-2941-48cd-bab4-31d22e922a30/sist-en-12353-2013

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.

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)).

---

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.

SIST EN 12353:2013

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 sterilise 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 sterilise 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 i.u./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.	

iTeh STANDARD PREVIEW
(standards.iteh.ai)

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:

SIST EN 12353:2013
https://standards.iteh.ai/catalog/standards/sist/81798c92-2941-48cd-bab4-31e22e9227c9/sist-en-12353-2013

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

5.2.2.12 Phosphate-buffered saline solution (PBS)

Sodium chloride (NaCl)	8,00 g
Potassium chloride (KCl)	0,20 g
Disodium hydrogen phosphate, 12-hydrate (Na_2HPO_4 x $12H_2O$)	2,89 g
Potassium phosphate, monobasic (KH_2PO_4)	0,20 g
Water (5.2.2.2)	to 1 000,0 ml

5.2.2.13 Foetal calf serum (FCS)

FCS has to be certified free of viruses and mycoplasma.

Extraneous viruses and mycoplasma may interfere with cell and virus growth resulting in false results.

5.2.2.14 Earle's BSS

Sodium chloride (NaCl)	68,0 g
Potassium chloride (KCl)	4,0 g

---

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.

SIST EN 12353:2013

Calcium chloride ($CaCl_2$)	2,0 g
Magnesium sulphate, 7-hydrate ($MgSO_4$ x $7H_2O$)	2,0 g
Sodium hydrogenphosphate, 2-hydrate (NaH_2PO_2 x $2H_2O$)	1,4 g
Glucose	10,0 g
Phenol red, 1 % (5.2.2.15)	20,0 ml
Water (5.2.2.2)	to 1 000,0 ml

$CaCl_2$ should be dissolved separately in 100 ml of water (5.2.2.2) and added to the other dissolved reagents just before the solution is brought to its final volume. The solution is 10-fold concentrated. It is sterilized by membrane filtration through a 0,22 µm Millipore or Seitz-type filter[3] and can be stored at (4 ± 1) °C for 4 weeks.

For use the solution is diluted 10-fold with water (5.2.2.2) and buffered by the addition of 2,5 % of an 8,8 % Sodium hydrogen carbonate ($NaHCO_3$) solution.

5.2.2.15 Phenol red, 1 % solution

a) A 1,0 N Sodium hydroxide (NaOH) solution is prepared.

b) 10 g of alcohol soluble Phenol red, European Pharmacopeia [2] are placed in a 100 ml flask (5.3.2.12); 20 ml of the NaOH solution are added, mixed and allowed to stand for a few minutes.

c) The dissolved dye is transferred in a 1 000 ml volumetric flask (5.3.2.12).

d) Additional 10 ml amounts of the NaOH solution are added to the flask and the dissolved material is added to the volumetric flask. No more than a total of 70 ml of the NaOH solution should be used.

e) The solution is brought to a final volume of 1 000 ml with water (5.2.2.2) and stored at room temperature.

iTeh STANDARD PREVIEW
(standards.iteh.ai)
SIST EN 12353:2013
https://standards.iteh.ai/catalog/standards/sist/81798c92-2941-48cd-bab4-31e22e9227c9/sist-en-12353-2013

5.2.2.16 Sodium bicarbonate (8,8 % w/v solution)

Dissolve 8,8 g sodium bicarbonate in water (5.2.2.2) to 100 ml and sterilize by autoclaving (5.3.2.1a)). Store at (4 ±1) °C.

5.2.2.17 Eagle's minimum essential medium (MEM) for cell cultures

MEM is used for growth and maintenance of cell cultures. First prepare a stock solution. For use, the stock solution is diluted 10-fold with water (5.2.2.2). 1 % of the 3 % Glutamine solution (= 0,03 %) (5.2.2.9), Antibiotic suspension (5.2.2.11), and 2,5 % of a 8,8 % Sodium bicarbonate solution (5.2.2.16) are added. An appropriate concentration of foetal calf serum (FCS, (5.2.2.13); 10 % for growth, 2 % for maintenance) is added before use.

The following solutions are prepared:

Solution A

	per litre stock solution
L-Arginine HCl	1,05 g
L-Histidine HCl	0,31 g
Lysine HCl	0,58 g
Tryptophane	0,10 g
L-Phenylalanine	0,32 g
L-Threonine	0,48 g
L-Leucine	0,52 g

[3] Millipore® and Seitz® are examples of suitable products 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.

SIST EN 12353:2013

L-Valine	0,46 g
L-Isoleucine	0,52 g
L-Methionine	0,15 g

These amino acids are dissolved with gentle heating (80 °C) in 200 ml of 0,075 N HCl. 0,075 N HCl is prepared by adding 1,5 ml of commercial C.P. HCl (11.9 N) to 236,6 ml water. Take 200 ml from the prepared 238,1 ml.

Solution B

	per litre stock solution
L-Tyrosine	0,36 g
L-Cysteine	0,24 g

These two amino acids are dissolved in 200 ml of 0,075 N hydrochloric acid (HCl) by heating up to 80 °C for 2 h and subsequently cooling to 20 °C.

Solution C

	per litre stock solution
Nìcotinamide	0,20 g
Pyridoxal	0,20 g
Thiamine	0,20 g
Pantothenic acid	0,20 g
Choline	0,20 g
Inositol	0,40 g
Riboflavin	0,02 g

iTeh STANDARD PREVIEW
(standards.iteh.ai)

These reagents are dissolved in approximately 175 ml of water (5.2.2.2) then brought to a final volume of 200 ml with water (5.2.2.2). The solution is dispensed in 10 ml volumes.

SIST EN 12353:2013
https://standards.iteh.ai/catalog/standards/sist/81798c02-2941-48cd-bbb4-31e22e9227c9/sist-en-12353-2013

NOTE The solutions A, B and C are 10-fold concentrated preparations and can be stored in the refrigerator (5.3.2.8).

Solution D

Dissolve 200 mg of Biotin in 150 ml of water (5.2.2.2). To increase stability upon storage, 1 ml of 1 N hydrochloric acid (HCl) is added.

The total volume is brought to 200 ml with water (5.2.2.2) and the solution is dispensed in 10 ml aliquots and stored at (-20 ± 1) °C.

Solution E

Dissolve 200 mg Folic acid (crystalline) in 200 ml of 10 fold diluted Earle's BSS (5.2.2.14), pH = 7,4. The solution is dispensed in 10 ml amounts and stored at (-20 ± 1) °C.

Preparation of the final mixture of Eagle's MEM

a) The following reagents are dissolved in 200 ml Solution B :

Sodium chloride (NaCl)	68,0 g
Potassium chloride (KCl)	4,0 g
Magnesium sulphate heptahydrate ($MgSO_4 x 7H_2O$)	2,0 g

b) 1,4 g of Sodium dihydrogen orthophosphate monohydrate (NaH_2PO_4 x H_20) are dissolved in 55 ml of water (5.2.2.2) and added to the solution a).

c) 10 g of Glucose dissolved in 50 ml of water (5.2.2.2) and 20 ml of a 1 % Phenol red solution (5.2.2.15) are added to the solution b).