SLOVENSKI STANDARD
oSIST prEN 1650:2018

01-junij-2018

Kemična razkužila in antiseptiki - Kvantitativni suspenzijski preskus za vrednotenje fungicidnega delovanja ali delovanja kemičnih razkužil in antiseptikov na kvasovke v živilski in drugih industrijah, gospodinjstvu in javnih ustanovah - Preskusna metoda in zahteve (faza 2, stopnja 1)

Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas - Test method and requirements (phase 2, step 1)

Chemische Desinfektionsmittel und Antiseptika - Quantitativer Suspensionsversuch zur Bestimmung der fungiziden oder levuroziden Wirkung chemischer Desinfektionsmittel und Antiseptika in den Bereichen Lebensmittel, Industrie, Haushalt und öffentliche Einrichtungen - Prüfverfahren und Anforderungen (Phase 2, Stufe 1); Deutsche und Englische Fassung prEN 1650:2016

Antiseptiques et désinfectants chimiques - Essai quantitatif de suspension pour l□évaluation de l□activité fongicide ou levuricide des antiseptiques et des désinfectants chimiques utilisés dans le domaine de l□agro-alimentaire, dans l□industrie, dans les domaines domestiques et en collectivité - Méthode d□essai et prescriptions

Ta slovenski standard je istoveten z: **prEN 1650**

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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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Will supersede EN 1650:2008+A1:2013

English Version

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

CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

Contents

Page

European foreword

This document (prEN 1650:2018) 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 1650:2008+A1:2013.

The main changes in relation to EN 1650:2008+A1:2013 are:

— inclusion of hand hygiene;

— handrub and handwash test conditions and test requirements have been harmonized with EN 13624;

— interfering substance for breweries, soft drinks, cosmetics and cleaning in place have been deleted. A sentence to allow additional interfering substance for specific applications has been added;

— the obligatory conditions (temperature and contact time) have been deleted. The text has been harmonized with EN 13624 keeping specified time intervals and temperature steps.

Introduction

This European Standard specifies a suspension test for establishing whether a chemical disinfectant or antiseptic has or does not have a fungicidal or yeasticidal activity in the fields described in the scope.

This laboratory test takes into account practical conditions of application of the product including contact time, temperature, test organisms and interfering substance, i.e. conditions which may influence its action in practical situations.

The conditions are intended to cover general purposes and to allow reference between laboratories and product types. Each utilization concentration of the chemical disinfectant or antiseptic found by this test corresponds to defined experimental conditions. However, for some applications the recommendations of use of a product may differ and therefore additional test conditions should be used.

1 Scope

This document specifies a test method and the minimum requirements for fungicidal or yeasticidal activity of chemical disinfectant and antiseptic products that form a homogeneous, physically stable preparation when diluted with hard water or - in the case of ready-to-use-products - with water. Products can only be tested at a concentration of 80 % or less as some dilution is always produced by adding the test organisms and interfering substance.

This document applies to products that are used in food, industrial, domestic and institutional areas excluding areas and situations where disinfection is medically indicated and excluding products used on living tissues except those for hand hygiene in the above considered areas. The following areas are at least included:

a) processing, distribution and retailing of:

   1) food of animal origin:
      - milk and milk products;
      - meat and meat products;
      - fish, seafood, and related products;
      - eggs and egg products;
      - animal feeds;
      - etc.

   2) food of vegetable origin:
      - beverages;
      - fruits, vegetables and derivatives (including sugar, distillery ...);
      - flour, milling and baking;
      - animal feeds;
      - etc.

b) institutional and domestic areas:
   - catering establishments;
   - public areas;
   - public transports;
   - schools;
   - nurseries;
   - shops;
   - sports rooms;
   - waste containers (bins ...);
   - hotels;
   - dwellings;
   - clinically non-sensitive areas of hospitals;
   - offices;
   - etc.

c) other industrial areas:

— packaging material;

— biotechnology (yeast, proteins, enzymes, ...);

— pharmaceutical;

— cosmetics and toiletries;

— textiles;

— space industry, computer industry;

— etc.

EN 14885 specifies in detail the relationship of the various tests to one another and to “use recommendations”.

NOTE 1 The method described is intended to determine the activity of commercial formulations or active substances under the conditions in which they are used.

NOTE 2 This method corresponds to a phase 2 step 1 test.

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 12353, *Chemical disinfectants and antiseptics - Preservation of test organisms used for the determination of bactericidal (including Legionella), mycobactericidal, sporicidal, fungicidal and virucidal (including bacteriophages) activity*

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

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

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

The product shall demonstrate a reduction of at least a 4 decimal log (lg) when diluted with hard water (**5.2.2.7**) or - in the case of ready-to-use products - with water (**5.2.2.2**) and tested in accordance with Clause **5** under simulated clean conditions (0,3 g/l bovine albumin solution - **5.2.2.8.2**) or simulated dirty conditions (3 g/l bovine albumin solution - **5.2.2.8.3**) according to its practical applications and under the other adopted test conditions as described in **5.5.1.1,**Tables 1 and 2 here below.

Table 1 — Experimental conditions for general purpose disinfection

Test Conditions	**Yeasticidal activity**	**Fungicidal activity**
Test organism (see 5.2.1) **obligatory**	*Candida albicans*	*Candida albicans* *Aspergillus brasiliensis (ex A. niger)*
Example of additional test microorganisms	*Saccharomyces cerevisiae* (for breweries) *Saccharomyces cerevisiae var. diastaticus* (for breweries)	any relevant test organism
Test temperature	in a range from 4 °C to 40 °C	in a range from 4 °C to 40 °C
Contact time	in a range from 1 min and between 5 andto to 60 min (from 1 min to 5 min at intervals of 1 min and from 5 min to 60 min at intervals of 5 min) at only with 5 min intervals of 5 min	in a range from 1 min to 60 min only with 5 min intervals
clean conditions	0,3 g/l Bovine Albumin for *C. albicans*	0,3 g/l Bovine Albumin for *C. albicans* and *A. brasiliensis*
Dirty conditions	3,0 g/l Bovine Albumin for *C. albicans*	3,0 g/l Bovine Albumin for *C. albicans* and *A. brasiliensis*
additional	any relevant substance	any relevant substance
Log reduction from a water control (decimal log)	≥ 4Log	≥ 4Log
The recommended contact time for the use of the product is within the responsibility of the manufacturer.		

Table 2 — Experimental conditions for hand hygiene

Test Conditions	Yeasticidal activity
Test organism (see 5.2.1) **obligatory**	*Candida albicans*
Test temperature	20 °C
Contact time	30 s or 60 s
clean conditions (for hygienic handrubs)	0,3 g/l Bovine
Dirty conditions (for hygienic handwashes)	3,0 g/l Bovine Albumin
Log reduction from a water control (decimal log)	≥ 4Lg for handrubs 2Lg for handwashes

Where indicated, additional specific fungicidal or yeasticidal activity shall be determined applying other interfering substances and test organisms (in accordance with 5.2.1, 5.2.2.8 and 5.5.1.1) in order to take into account intended specific use conditions.

5 Test method

5.1 Principle

5.1.1 A sample of the product as delivered and/or diluted with hard water (or water for ready to use products) is added to a test suspension of fungi (yeast cells or mould spores) in a solution of an interfering substance. The mixture is maintained at the chosen test temperature for the adopted contact time. At the end of this contact time, an aliquot is taken, and the fungicidal and/or the fungistatic activity in this portion is immediately neutralized or suppressed by a validated method. The method of choice is dilution-neutralization. If a suitable neutralizer cannot be found, membrane filtration is used. The numbers of surviving fungi in each sample are determined and the reduction is calculated.

5.1.2 The test is performed using the vegetative cells of *Candida albicans* and the spores of *Aspergillus brasiliensis* (fungicidal activity) or only the vegetative cells of *Candida albicans* (yeasticidal activity) as test organisms.

5.1.3 Additional test organisms can be used.

5.2 Materials and reagents

5.2.1 Test organisms

The fungicidal activity shall be evaluated using the following strains as test organisms: [1)]

— *Candida albicans* ATCC 10231

— *Aspergillus brasiliensis* ATCC 16404

The yeasticidal activity shall be evaluated using only *Candida albicans*.

NOTE See Annex A for strain references in some other culture collections.

The required incubation temperature for these test organisms is (30 ± 1) °C (**5.3.2.3**).

If required for specific applications, additional strains may be chosen from, e.g. for breweries:

— *Saccharomyces cerevisiae* DSM 1333

— *Saccharomyces cerevisiae var. diastaticus* DSM 70487

If additional test organisms are used, they shall be incubated under optimum growth conditions (temperature, time, atmosphere, media) noted in the test report. If the additional test organisms selected do not correspond to the specified strains, their suitability for supplying the required inocula shall be verified. If these additional test organisms are not classified at a reference centre, their identification characteristics shall be stated. In addition, they shall be held by the testing laboratory or national culture collection under a reference for five years.

5.2.2 Culture media and reagents

5.2.2.1 General

All weights of chemical substances given in this 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.

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 are to be rigorously followed.

NOTE For each culture medium and reagent, a limitation for use is to be fixed.

[1)] The ATCC numbers are the collection numbers of strains supplied by the American Type Culture Collection (ATCC). This information is given for the convenience of users of this standard and does not constitute an endorsement by CEN of the product named.

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 e.g. for 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.

NOTE 3 See **5.2.2.7** for the procedure to prepare hard water.

5.2.2.3 Malt extract agar (MEA)

Malt extract agar, consisting of:

Malt extract	30,0 g
Agar	15,0 g
Water (**5.2.2.2**)	to 1 000,0 ml

The malt extract should be food grade (e.g. Cristomalt powder from Difal) or equivalent that is not highly purified and not only based on maltose (e.g. Malt extract from OXOID)[2)] However if there are problems producing at least 75 % spiny spores see 5.4.1.4.2.

Sterilize in the autoclave [**5.3.2.1**a)]. After sterilization, the pH of the medium shall be equivalent to 5,6 ± 0,2 when measured at (20 ± 1) °C.

NOTE In case of encountering problems with neutralization (5.5.1.2 and 5.5.1.3), it can be necessary to add neutralizer to the MEA. Annex B gives guidance on the neutralizers that can be used.

5.2.2.4 Diluent

Tryptone sodium chloride solution, consisting of:

Tryptone, pancreatic digest of casein	1,0 g
Sodium chloride (NaCl)	8,5 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 diluent shall be equivalent to 7,0 ± 0,2 when measured at (20 ± 1) °C.

5.2.2.5 Neutralizer

The neutralizer shall be validated for the product being tested in accordance with **5.5.1.2, 5.5.1.3** and **5.5.2**. It shall be sterile.

NOTE Information on neutralizers that have been found to be suitable for some categories of products is given in Annex **B**.

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2) This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of the product named. Equivalent products may be used if they can be shown to lead to the same results.

5.2.2.6 Rinsing liquid (for membrane filtration)

The rinsing liquid shall be validated for the product being tested in accordance with **5.5.1.2**, **5.5.1.3** and **5.5.3**. It shall be sterile, compatible with the filter membrane and capable of filtration through the filter membrane under the test conditions described in **5.5.3**.

NOTE Information on rinsing liquids that have been found to be suitable for some categories of products is given in Annex **B.**

5.2.2.7 Hard water for dilution of products

For the preparation of 1 000 ml of hard water, the procedure is as follows:

— prepare solution A: dissolve 19,84 g magnesium chloride ($MgCl_2$) and 46,24 g calcium chloride ($CaCl_2$) in water (**5.2.2.2**) and dilute to 1 000 ml. Sterilize by membrane filtration (**5.3.2.7**) or in the autoclave [**5.3.2.1**a)]. Autoclaving - if used - may cause a loss of liquid. In this case make up to 1 000 ml with water (**5.2.2.2**) under aseptic conditions. Store the solution in the refrigerator (**5.3.2.8**) for no longer than one month;

— prepare solution B: dissolve 35,02 g sodium bicarbonate ($NaHCO_3$) in water (**5.2.2.2**) and dilute to 1 000 ml. Sterilize by membrane filtration (**5.3.2.7**). Store the solution in the refrigerator (**5.3.2.8**) for no longer than one week;

— place 600 ml to 700 ml of water (**5.2.2.2**) in a 1 000 ml volumetric flask (**5.3.2.12**) and add 6,0 ml (**5.3.2.9**) of solution A, then 8,0 ml of solution B. Mix and dilute to 1 000 ml with water (**5.2.2.2**). The pH of the hard water shall be 7,0 ± 0,2, when measured at (20 ± 1) °C (**5.3.2.4**). If necessary, adjust the pH by using a solution of approximately 40 g/l (about 1 mol/l) of sodium hydroxide (NaOH) or approximately 36,5 g/l (about 1 mol/l) of hydrochloric acid (HCl).

The hard water shall be freshly prepared under aseptic conditions and used within 12 h.

NOTE When preparing the product test solutions (**5.4.2**), the addition of the product to the hard water produces a different final water hardness in each test tube. In any case the final hardness is lower than 375 mg/l of calcium carbonate ($CaCO_3$) in the test tube.

5.2.2.8 Interfering substance

5.2.2.8.1 General

The interfering substance shall be chosen according to the conditions of use laid down for the product.

The interfering substance shall be sterile and prepared at 10 times its final concentration in the test.

The ionic composition (e.g. pH, calcium and/or magnesium hardness) and chemical composition (e.g. mineral substances, protein, carbohydrates, lipids and detergents) shall be defined.

NOTE The term “interfering substance” is used even if it contains more than one substance.

5.2.2.8.2 Clean conditions (bovine albumin solution – low concentration)

Dissolve 0,3 g of bovine albumin fraction V (suitable for microbiological purposes) in 100 ml of water (**5.2.2.2**).

Sterilize by membrane filtration (**5.3.2.7**), keep in the refrigerator (**5.3.2.8**) and use within one month.

The final concentration of bovine albumin in the test procedure (**5.5**) is 0,3 g/l.

5.2.2.8.3 Dirty conditions (bovine albumin solution – high concentration)

Dissolve 3,0 g of bovine albumin fraction V (suitable for microbiological purposes) in 100 ml of water (**5.2.2.2**).

Sterilize by membrane filtration (**5.3.2.7**), keep in the refrigerator (**5.3.2.8**) and use within one month.

The final concentration of bovine albumin in the test procedure (**5.5)** is 3,0 g/l.

5.2.2.8.4 Milk (dairies ...)

Skimmed milk, guaranteed free of antibiotics and additives and reconstituted at a rate of 100 g powder per litre of water (**5.2.2.2**), shall be prepared as follows :

— prepare a solution of 100 g milk-powder in 1 000 ml water (**5.2.2.2**). Heat for 30 min at (105 ± 3) °C [or 5 min at (121 ± 3 °C)].

The final concentration of reconstituted milk in the test procedure (**5.5**) is 10,0 g/l of reconstituted milk.

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 [3] and in particular, the following:

5.3.2.1 Apparatus for sterilization

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

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

5.3.2.2 Water baths, capable of being controlled at (20 ± 1) °C, at (45 ± 1) °C (to maintain melted MEA in case of pour plate technique) and at additional test temperatures ± 1 °C (5.5.1).

5.3.2.3 Incubator, capable of being controlled at (30 ± 1) °C.

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

A puncture electrode or a flat membrane electrode should be used for measuring the pH of the agar media (5.2.2.3).

5.3.2.5 Stopwatch

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3) Disposable sterile equipment is an acceptable alternative to reusable glassware.