SLOVENSKI STANDARD
oSIST prEN 13697:2022

01-september-2022

Kemična razkužila in antiseptiki - Kvantitativni preskus na neporoznih površinah brez mehanskega delovanja za vrednotenje baktericidnega in/ali fungicidnega delovanja ter delovanja kemičnih razkužil na kvasovke v živilski in drugih industrijah, gospodinjstvu in javnih ustanovah - Preskusna metoda in zahteve brez mehanskega delovanja (faza 2, stopnja 2) (vključno z dopolnilom A1)

Chemical disinfectants and antiseptics - Quantitative non-porous surface test for the evaluation of bactericidal and yeasticidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas without mechanical action - Test method and requirements without mechanical action (phase 2, step 2)

Chemische Desinfektionsmittel und Antiseptika - Quantitativer Oberflächen-Versuch zur Bestimmung der bakteriziden und levuroziden und/oder fungiziden Wirkung chemischer Desinfektionsmittel auf nicht porösen Oberflächen in den Bereichen Lebensmittel, Industrie, Haushalt und öffentliche Einrichtungen ohne mechanische Einwirkung - Prüfverfahren und Anforderungen ohne mechanische Behandlung (Phase 2, Stufe 2)

Antiseptiques et désinfectants chimiques - Essai quantitatif de surface non-poreuse pour l'évaluation de l'activité bactéricide et levurocide et/ou fongicide des désinfectants chimiques utilisés dans le domaine de l'agro-alimentaire, dans l'industrie, dans les domaines domestiques et en collectivité sans action mecanique - Méthode d'essai sans action mécanique et prescriptions (phase 2/étape 2)

Ta slovenski standard je istoveten z: **prEN 13697**

ICS:

71.100.35	Kemikalije za dezinfekcijo v industriji in doma	Chemicals for industrial and domestic disinfection purposes

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EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

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English Version

Chemical disinfectants and antiseptics - Quantitative non-porous surface test for the evaluation of bactericidal and yeasticidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas without mechanical action - Test method and requirements without mechanical action (phase 2, step 2)

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

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

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European foreword

This document (prEN 13697:2022) 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 supersedes EN 13697:2015+A1:2019.

In comparison with the previous version EN 13697:2015+A1:2019 of edition EN 13697:2015, the following modifications have been made:

— the inoculum under clean conditions for *Pseudomonas aeruginosa* and *Candida albicans* has been reduced to the previous level as in EN 13697:2015;

— a more exact monitoring of the drying process has been included as Annex F (informative) in order to support achieving sufficient levels of surviving cells for valid results;

— inclusion of yeasticidal activity in the title of the document;

— clarification that 1 % reconstituted milk can be used as sole soiling in dairy industry (no need to test BSA);

— the designation of variables Nd, Nc, NC and NT have been renamed to Na, A, B and C for harmonization with other CEN TC 216 standards;

— clarification of the counting procedure in 5.5.3;

— correction of the Formulae (2) and (3) in 5.5.3.

The following changes in version EN 13697:2015+A1:2019 as compared to edition EN 13697:2015 were maintained:

— deletion of obligatory and additional conditions (see Table 1 and 5.5.1);

— update of Bovine albumin and skimmed solutions preparations (see 5.2.2.8.2);

— add of instruction for using vacuum desiccator;

— clarification to the determination of microbicidal concentrations by updating 5.5.2.1 b) and adding pictures of carriers.

Data obtained from EN 13697:2015 and from EN 13697:2015+A1:2019 are still valid.

Introduction

This document describes a surface test method for establishing whether a product proposed as a disinfectant in the fields described in Clause 1 has or does not have bactericidal and/or fungicidal or yeasticidal activity on non-porous surfaces.

This document has been revised in order to modify the test parameters under “clean conditions” adopted for *P. aeruginosa* and *C. albicans*; in order to harmonize the designation of test variables N, B, C, A, Na with the other recent CEN TC 216 standards and to clarify and/or correct details in colony counting and calculation.

The laboratory test closely simulates practical conditions of application. Chosen conditions (contact time, temperature, organisms on surfaces ...) reflect parameters which are found in practical situations including conditions which may influence the action of disinfectants. Each use concentration found from this test corresponds to defined experimental conditions.

The conditions are intended to cover general purposes and to allow reference between laboratories and product types.

However, for some applications the recommendations of use of a product can differ and therefore additional test conditions need to be used.

1 Scope

This document specifies a test method (phase 2/step 2) and the minimum requirements for bactericidal and/or fungicidal or yeasticidal activity of chemical disinfectants that form a homogeneous physically stable preparation in hard water or – in the case of ready-to-use products – with water in food, industrial, domestic and institutional areas, excluding areas and situations where disinfection is medically indicated and excluding products used on living tissues.

The scope of this document applies at least to the following:

a) processing, distribution and retailing of:

   1) food of animal origin:

      i) milk and milk products;

      ii) meat and meat products;

      iii) fish, seafood and products;

      iv) eggs and egg products;

      v) animal feeds;

      vi) etc.

   2) food of vegetable origin:

      i) beverages;

      ii) fruits, vegetables and derivatives (including sugar distillery);

      iii) flour, milling and backing;

      iv) animal feeds;

      v) etc.

b) institutional and domestic areas:

   1) catering establishments;

   2) public areas;

   3) public transports;

   4) schools;

   5) nurseries;

   6) shops;

   7) sports rooms;

   8) waste container (bins);

9) hotels;

10) dwellings;

11) clinically non sensitive areas of hospitals;

12) offices;

13) etc.

c) other industrial areas:

1) packaging material;

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

3) pharmaceutical;

4) cosmetics and toiletries;

5) textiles;

6) space industry, computer industry;

7) etc.

Using this document, it is possible to determine the bactericidal or fungicidal or yeasticidal activity of the undiluted product. As three concentrations are tested, in the active to non-active range, dilution of the product is required and, therefore, the product forms a homogeneous stable preparation in hard water.

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 on bacteria and/or fungi in the conditions in which they are used.

NOTE 2 This method cannot be used to evaluate the activity of products against mycobacteria.

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.

4 Requirements

The product shall demonstrate at least a 4 decimal log (lg) reduction for bacteria and at least a 3 decimal log (lg) reduction for fungi, when tested in accordance with Table 1 and 5.5.1.

Table 1 — Experimental conditions

Test conditions	**Bactericidal activity on non-porous surfaces without mechanical action**	**Yeasticidal activity on non-porous surfaces without mechanical action**	**Fungicidal activity on non-porous surfaces without mechanical action**
Test organisms (see 5.2.1) **minimum spectrum of test organisms**	*Enterococcus hirae* *Escherichia coli* *Pseudomonas aeruginosa* *Staphylococcus aureus*	*Candida albicans*	*Candida albicans* *Aspergillus brasiliensis*
Test organisms **additional** (examples)	*Salmonella typhimurium* *Lactobacillus brevis* *Enterobacter cloacae*	*Saccharomyces cerevisiae* (for breweries) *Saccharomyces cerevisiae* var. *diastaticus* (for breweries)	any relevant test organism
Test temperature	In a range from (4 ± 1) °C to (40 ± 1) °C For tests performed at room temperature, the range shall be between 18 °C and 25 °C	In a range from (4 ± 1) °C to (40 ± 1) °C For tests performed at room temperature, the range shall be between 18 °C and 25 °C	In a range from (4 ± 1) °C to (40 ± 1) °C For tests performed at room temperature, the range shall be between 18 °C and 25 °C
Contact time	in a range from 1 min 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)	in a range from 1 min 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)	in a range from 1 min 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)
Interfering substance clean conditions	0,3 g/l bovine albumin for *Staphylococcus aureus*, *Enterococcus hirae*, *Escherichia coli* and *Pseudomonas aeruginosa*	0,3 g/l bovine albumin for *C. albicans*	0,3 g/l bovine albumin for *C. albicans* and *A. brasiliensis*

Test conditions	Bactericidal activity on non-porous surfaces without mechanical action	Yeasticidal activity on non-porous surfaces without mechanical action	Fungicidal activity on non-porous surfaces without mechanical action
Interfering substance dirty conditions	3,0 g/l bovine albumin for *Staphylococcus aureus*, *Enterococcus hirae*, *Pseudomonas aeruginosa* and *Escherichia coli*	3,0 g/l bovine albumin for *C. albicans*	3,0 g/l bovine albumin for *C. albicans* and *A. brasiliensis*
Interfering substance in dairy industry	1,0 % reconstituted milk (= 1,0 g/l milk powder)	1,0 % reconstituted milk (= 1,0 g/l milk powder)	1,0 % reconstituted milk (= 1,0 g/l milk powder)
Interfering substance **additional**	any relevant substance	any relevant substance	any relevant substance
Log reduction from a water control (decimal lg)	≥ 4 lg	≥ 3 lg	≥ 3 lg
The referenced test conditions (General purposes) are by no means intended as requirements for the use of a product, nor as requirements for the evaluation and acceptance of products by regulatory authorities. The application time for the product is specified by the manufacturer. If specific applications have to be considered, the bactericidal/yeasticidal/fungicidal activity has to be determined additionally under relevant conditions concerning application time, temperature, strains and Interfering Substances.			

5 Test methods

5.1 Principle

A test suspension of bacteria or fungi in a solution of interfering substances is inoculated onto a test stainless steel surface and dried. A prepared sample of the product under test is applied in a manner which covers the dried film. The surface is maintained at a specified temperature for a defined period of time. The surface is transferred to a previously validated neutralization medium so that the action of the disinfectant is immediately neutralized. The number of surviving organisms which can be recovered from the surface is determined quantitatively.

The number of bacteria or fungi on a surface treated with hard water in place of the disinfectant is also determined and the reduction in viable counts attributed to the product is calculated by difference.

5.2 Materials and reagents

5.2.1 Test organisms

The bactericidal activity shall be evaluated using the following four strains:

— *Pseudomonas aeruginosa* ATCC 15 442[1)];

— *Staphylococcus aureus* ATCC 6 538;

— *Enterococcus hirae* ATCC 10 541;

— *Escherichia coli* ATCC 10 536.

The fungicidal or yeasticidal activity shall be evaluated using the following two strains:

— *Candida albicans* ATCC 10 231;

— *Aspergillus brasiliensis (ex A. niger)* ATCC 16 404.

If required for specific applications, additional strains may be chosen from, for example:

— *Salmonella typhimurium* ATCC 13 311;

— *Lactobacillus brevis* DSM 6 235;

— *Enterobacter cloacae* DSM 6 234;

— *Saccharomyces cerevisiae* (for breweries) or ATCC 9 763 or DSM 1 333;

— *Saccharomyces cerevisiae* var. *diastaticus* (for breweries) DSM 70 487.

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

If additional strains are used, they shall be incubated under optimum growth conditions (temperature, time, atmosphere) and noted in the test report.

If the additional strains selected do not correspond to the specified strains, their suitability for supplying inocula of sufficient concentration shall be verified. If the additional strains tested 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 under a reference for 5 years.

5.2.2 Culture media and reagents

5.2.2.1 General

The reagents shall be of analytical grade and/or appropriate for microbiological purposes.

5.2.2.2 Water

The water shall be free from substances that are toxic or inhibiting to bacteria and fungi. It shall be freshly glass distilled and not demineralized water.

Sterilize in the autoclave (see 5.3.2.1).

NOTE 1 If the water is sterilized during sterilization of the reagents, this is not necessary.

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1) ATCC 15 442, ATCC 6 538, ATCC 10 541, ATCC 10 536, ATCC 10 231, ATCC 16 404 and ATCC 13311 are the collection numbers of strains supplied by the American Type Culture Collections. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of the product named. Equivalent products can be used if they can be shown to lead to the same results.

NOTE 2 If distilled water of adequate quality is not available, water for injectable preparation (see European Pharmacopoeia) can be used.

5.2.2.3 Tryptone Soya Agar (TSA)

For maintenance of bacterial strains and performance of viable counts.

Tryptone, pancreatic digest of casein	15,0 g
Soya peptone, papaic digest of Soybean meal	5,0 g
NaCl	5,0 g
Agar	15,0 g
Water (see 5.2.2.2)	1 000,0 ml

Sterilize in the autoclave (see 5.3.2.1). After sterilization, the pH of the medium shall be equivalent to 7,2 ± 0,2 when measured at 20 ° C.

5.2.2.4 Malt extract agar (MEA)

For maintenance of fungal strains, sporulation and performance of viable counts.

Malt extract (food grade, e.g. Cristomalt powder from Difal)	30,0 g
Agar	15,0 g
Water (see 5.2.2.2)	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.

In case of encountering problems with neutralization (5.5.2.3 and 5.5.2.4), it may be necessary to add neutralizer to the MEA. Annex B gives guidance on the neutralizers that may be used.

5.2.2.5 Diluent

Tryptone sodium chloride solution:

Tryptone, pancreatic digest of casein	1,0 g
NaCl	8,5 g
Water (see 5.2.2.2)	1 000,0 ml

[2)] This information is given for the convenience of users of this document 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.

Sterilize in the autoclave (see 5.3.2.1). After sterilization the pH shall be equivalent to 7,0 ± 0,2 when measured at 20 °C.

5.2.2.6 Neutralizer

The neutralizer shall be validated for the product under test in accordance with 5.5.2.3 and 5.5.2.4. The neutralizer shall be sterile.

NOTE Information on neutralizers 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 the products

Hard water for dilution of products shall be prepared as follows:

— solution A: Dissolve 19,84 g anhydrous $MgCl_2$ and 46,24 g anhydrous $CaCl_2$ in water (see 5.2.2.2) and dilute to 1 000 ml. Sterilize by membrane filtration (5.3.2.19) 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 (5.3.2.15) for no longer than one month;

— solution B: Dissolve 35,02 g $NaHC0_3$ in water (see 5.2.2.2) and dilute to 1 000 ml. Sterilize by membrane filtration (5.3.2.19). Store the solution in the refrigerator (5.3.2.15) for no longer than one week.

Add at least 600 ml water (see 5.2.2.2) to 6,0 ml of solution A in a 1 000 ml volumetric flask, then add 8,0 ml solution B. Mix and dilute to 1 000 ml with water (see 5.2.2.2).

Sterilize by passing through a filter with a maximum effective pore size of 0,45 µm.

The pH of the hard water shall be 7,0 ± 0,2, when measured at (20 ± 1) °C (5.3.2.6). 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 test product solutions (5.4.2), the addition of the product to the hard water produces a different final water hardness expressed as calcium carbonate ($CaCO_3$) in each test tube. In any case, the final hardness is lower than 375 mg/l of calcium carbonate.

5.2.2.8 Interfering substances

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 2 times its final concentration in the test.

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

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

The method of preparation and sterilization together with the composition shall be noted in the test report (see 5.7).