SLOVENSKI STANDARD
SIST EN 1276:2019

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Nadomešča:
SIST EN 1276:2010
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Kemična razkužila in antiseptiki - Kvantitativni suspenzijski preskus za vrednotenje baktericidnega delovanja kemičnih razkužil in antiseptikov 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 bactericidal 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 bakteriziden 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 1276:2016

Antiseptiques et désinfectants chimiques - Essai quantitatif de suspension pour lévaluation de lactivité bactéricide des antiseptiques et des désinfectants chimiques utilisés dans le domaine de lagro-alimentaire, dans lindustrie, dans les domaines domestiques et en collectivité - Méthode dessai et prescriptions (phase 2, étape 1)

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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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EN 1276

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

Antiseptiques et désinfectants chimiques - Essai quantitatif de suspension pour l'évaluation de l'activité bactéricide 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 (phase 2, étape 1)

Chemische Desinfektionsmittel und Antiseptika - Quantitativer Suspensionsversuch zur Bestimmung der bakteriziden Wirkung chemischer Desinfektionsmittel und Antiseptika in den Bereichen Lebensmittel, Industrie, Haushalt und öffentliche Einrichtungen - Prüfverfahren und Anforderungen (Phase 2, Stufe 1)

This European Standard was approved by CEN on 17 June 2019.

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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.

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

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European foreword

This document (EN 1276:2019) 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 February 2020 and conflicting national standards shall be withdrawn at the latest by February 2020.

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

This document supersedes EN 1276:2009.

Data obtained by using the latest version of EN 1276 are still valid.

The main changes in relation to EN 1276:2009 are:

— handrub and handwash test conditions and test requirements have been harmonized with EN 13727;

— 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 13727 keeping specified time intervals and temperature steps;

— test conditions for temperatures ≥ 40 °C have been added.

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

Introduction

This document describes a suspension test for establishing whether a chemical disinfectant or antiseptic has or does not have bactericidal 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 test conditions. 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 and the minimum requirements for bactericidal 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, etc.);

      — 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, etc.);

- hotels;
- dwellings;
- clinically non sensitive areas of hospitals;
- offices;
- etc.

c) other industrial areas:

- packaging material;
- biotechnology (yeast, proteins, enzymes, etc.);
- 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".

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 14885:2018, *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 14885:2018 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 https://www.iso.org/obp

4 Requirements

The product shall demonstrate at least a 5 decimal logarithm (lg) reduction (3 lg for handwashes) 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 suitable test conditions as described in 5.5.1.1, Tables 1 and 2 here below.

Table 1 — Test conditions for general purpose disinfection

Test Conditions	**Bactericidal activity**
Test organism (see 5.2.1) **obligatory**	*Enterococcus hirae* *Escherichia coli* *Pseudomonas aeruginosa* *Staphylococcus aureus* *E. faecium* (for temperatures ≥ 40 °C)
Example of additional test microorganisms	*Salmonella Typhimurium* *Lactobacillus brevis* *Enterobacter cloacae*
Test temperature	in a range from 4 °C to 60 °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)
Clean conditions	0,3 g/l Bovine Albumin for *Staphylococcus aureus*, *Enterococcus hirae*, *Escherichia coli* and *Pseudomonas aeruginosa*
Dirty conditions	3,0 g/l Bovine Albumin for *Staphylococcus aureus*, *Enterococcus hirae*, *Pseudomonas aeruginosa* and *Escherichia coli*
additional	any relevant substance
Log reduction (decimal lg)	≥ 5 lg
The recommended contact time for the use of the product is within the responsibility of the manufacturer.	

Table 2 — Test conditions for hand hygiene

Test Conditions	Bactericidal activity
Test organisms (see 5.2.1) **obligatory**	*Enterococcus hirae* *Escherichia coli K12* (NCTC 10538) *Pseudomonas aeruginosa* *Staphylococcus aureus*
Test temperature	20 °C
Contact time	30 s or 60 s
clean conditions (for hygienic handrubs)	0,3 g/l Bovine Albumin
Dirty conditions (for hygienic handwashes)	3,0 g/l Bovine Albumin
Log reduction (decimal lg)	≥ 5 lg for handrubs ≥ 3 lg for handwashes

Where indicated, additional specific bactericidal 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 with the exception of handwash products whose first dilution is done in hard water (5.4.2)) is added to a test suspension of bacteria 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 bactericidal and/or the bacteriostatic 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 bacteria in each sample are determined and the reduction is calculated.

5.1.2 The test is performed using *Pseudomonas aeruginosa, Escherichia coli*, *Staphylococcus aureus and Enterococcus hirae* as test organisms. For temperatures ≥ 40 °C only *Enterococcus faecium* shall be used.

For testing of hand hygiene products, *Pseudomonas aeruginosa, Escherichia coli K12*, *Staphylococcus aureus and Enterococcus hirae* are used as test organisms.

5.1.3 Additional test organisms can be used.

5.2 Materials and reagents

5.2.1 Test organisms

The bactericidal activity shall be evaluated using the following strains as test organisms:

— *Pseudomonas aeruginosa* ATCC 15442;

— *Escherichia coli* ATCC 10536;

— *Staphylococcus aureus* ATCC 6538;

— *Enterococcus hirae* ATCC 10541;

— *Escherichia coli K12* NCTC 10538;

— *Enterococcus faecium* ATCC 6057.

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

— *Salmonella Typhimurium* ATCC 13311;

— *Lactobacillus brevis* DSM 6235;

— *Enterobacter cloacae* DSM 6234.

Refer to Annex A for strain references in some other culture collections.

The required temperature for growing these test organisms is (36 ± 1) °C or (37 ± 1) °C (5.3.2.3). The same temperature (either 36 °C or 37 °C) shall be used for all incubations for growing microorganisms performed during a test and its control and validation.

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 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.

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.

5.2.2.2 Water

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

Sterilize in the autoclave (see 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 can be used.

See 5.2.2.7 for the procedure to prepare hard water.

5.2.2.3 Tryptone Soya Agar (TSA)

Tryptone soya agar, consisting of:

Tryptone, pancreatic digest of casein	15,0 g
Soya peptone, papaic digest of soybean meal	5,0 g
Sodium chloride (NaCl)	5,0 g
Agar	15,0 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 7,2 ± 0,2 when measured at (20 ± 1) °C.

NOTE In the case of encountering problems with neutralization (5.5.1.2 and 5.5.1.3), it can be necessary to add neutralizer to the TSA. 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.

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.

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 (CaCO3) 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.