SLOVENSKI STANDARD
kSIST FprEN 1276:2009

01-april-2009

Kemiļ na razkuģila in antiseptiki - Kvantitativni suspenzijski preskus za ocenjevanje baktericidnega delovanja kemiļ nih razkuģil in antiseptikov v ģivilski 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)

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)

Ta slovenski standard je istoveten z: **FprEN 1276**

ICS:

11.080.20	Dezinfektanti in antiseptiki	Disinfectants and antiseptics
71.100.35	Kemikalije za dezinfekcijo v industriji in doma	Chemicals for industrial and domestic disinfection purposes

kSIST FprEN 1276:2009 **en**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

FINAL DRAFT
FprEN 1276

March 2009

ICS 11.080

Will supersede EN 1276:1997

English Version

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This draft European Standard is submitted to CEN members for unique acceptance procedure. 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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Foreword

This document (FprEN 1276:2009) 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 Unique Acceptance Procedure.

This document will supersede EN 1276:1997.

It was revised to include the results of a collaborative trial (ANDISTAND), to correct obvious errors and ambiguities, to harmonize the structure and wording with other quantitative suspension tests of CEN/TC 216 (existing or in preparation) and to improve the readability of the standard and thereby make it more understandable.

Introduction

This European Standard specifies 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 experimental conditions. However, for some applications, the recommendations of use of a product may 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”.

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 referenced documents are indispensable for the application 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, mycobactericidal, sporicidal and fungicidal activity*

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 following terms and definitions apply.

3.1
product
chemical agent or formulation used as a chemical disinfectant or antiseptic

3.2
bactericide
product that kills vegetative bacteria under defined conditions

NOTE The adjective derived from “bactericide” is “bactericidal”.

3.3
bactericidal activity
capability of a product to produce a reduction in the number of viable bacterial cells of relevant test organisms under defined conditions

3.4
bacteriostatic activity
capability of a product to inhibit the growth of bacteria under defined conditions

4 Requirements

The product shall demonstrate at least a 5 decimal log (lg) reduction 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 obligatory test conditions (four selected test organisms, 20 °C, 5 min).

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

— *Pseudomonas aeruginosa*;

— *Escherichia coli*;

— *Staphylococcus aureus*;

— *Enterococcus hirae*.

Where indicated, additional specific bactericidal activity shall be determined applying other contact times, temperatures, 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.

NOTE For these additional conditions, the concentration defined as a result can be lower than the one obtained under the obligatory test 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 bacteria in a solution of an interfering substance. The mixture is maintained at (20 ± 1) °C for 5 min ± 10 s (obligatory test conditions). 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.

5.1.3 Additional and optional contact times and temperatures are specified. 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[1)]:

— *Pseudomonas aeruginosa* ATCC 15442;

— *Escherichia coli* ATCC 10536;

— *Staphylococcus aureus* ATCC 6538;

— *Enterococcus hirae* ATCC 10541.

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.

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

The required incubation temperature for 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 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.

NOTE 1 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 should be rigorously followed.

NOTE 2 For each culture medium and reagent, a limitation for use should 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 (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 (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 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 may be necessary to add neutralizer to the TSA. Annex B gives guidance on the neutralizers that may 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.

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 300 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, etc.)

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.

5.2.2.8.5 Yeast extract (breweries, etc.)

Dehydrated yeast extract for bacteriology, shall be prepared as follows:

— prepare a 100 g/l solution in water (5.2.2.2), adjust to pH 7,0 ± 0,2 with sodium hydroxide (NaOH);

— sterilize in the autoclave (5.3.2.1 a).

The final concentration of yeast extract in the test procedure (5.5) is 10,0 g/l.

5.2.2.8.6 Sucrose (beverage, soft drink industries)

Prepare a 100 g/l solution of sucrose in water (5.2.2.2), sterilize by membrane filtration (5.3.2.7).

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

5.2.2.8.7 pH 5,0 and pH 9,0 buffer solutions (cleaning in place, etc.)

The buffer solution used shall be described in the test report and pH values shall be recorded. The final pH in the test tubes (together with test organisms and product) shall be controlled and found equal to 5,0 ± 0,2 or 9,0 ± 0,2.

5.2.2.8.8 Sodium dodecyl sulphate (cosmetic area, etc.)

Prepare a 50 g/l solution of sodium dodecyl sulphate ($C_{12}H_{25}NaO_4S$) in water (5.2.2.2). Sterilize in the autoclave (5.3.2.1 a).

The final concentration of sodium dodecyl sulphate in the test procedure (5.5) is 5,0 g/l.