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Handläggande organ	Fastställd	Utgåva	Sida
Hälso- och sjukvårdsstandardiseringen, HSS	1997-11-21	1	1 (1+35)

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)

Kemiska desinfektionsmedel och antiseptiska medel – Kvantitativt suspensionsprov för utvärdering av den baktericida verkan hos kemiska desinfektionsmedel och antiseptiska medel för användning i livsmedels-, industri-, hem- och institutionsmiljöer – Provningsmetod och krav (Fas 2, steg 1)

The European Standard EN 1276:1997 has the status of a Swedish Standard. This document contains the official English version of EN 1276:1997.

Swedish Standards corresponding to documents referred to in this Standard are listed in ”Catalogue of Swedish Standards”, issued by SIS. The Catalogue lists, with reference number and year of Swedish approval, International and European Standards approved as Swedish Standards as well as other Swedish Standards.

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ICS 11.080

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Descriptors: desinfectants, chemical compounds, tests, determination, antibacterial activity, culture media, dilution, neutralizing, preparation, counting, bacteria, filtration analysis, tests results

English version

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)

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CEN

European Committee for Standardization
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Europäisches Komitee für Normung

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Contents

Foreword

This European Standard has been prepared by Technical Committee CEN/TC 216 "Chemical disinfectants and antiseptics" the secretariat of which is held by AFNOR.

A collaborative trial is currently being undertaken and will be used to provide a precision annex to this standard.

In this standard, annex A is normative and annex B, annex C, annex D, annex E and annex F are informative.

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 December 1997, and conflicting national standards shall be withdrawn at the latest by December 1997.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

0 Introduction

This European Standard describes a suspension test method for establishing whether a chemical disinfectant or antiseptic has or does not have a bactericidal activity in the fields described in clause 1.

The laboratory test closely simulates practical conditions of application. Chosen conditions (contact time, temperature, organisms in suspension, ...) reflect parameters which are found in practical situations including conditions which may influence the action of antiseptics or disinfectants. Each utilization 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 may differ and therefore additional test conditions need to be used.

1 Scope

This European Standard specifies a test method (phase 2/step 1) and the minimum requirements for bactericidal activity of chemical disinfectant and antiseptic products that form a homogeneous, physically stable preparation in hard water and 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.

This European Standard applies at least to the following :

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 transport ;
- 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.

Using this European Standard, it is not possible to determine the bactericidal activity of undiluted product as some dilution is always produced by adding the inoculum and interfering substance. Products can only be tested at a concentration of 80 % or less.

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

2 Normative references

This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

prEN 12353	Chemical disinfectants and antiseptics - Preservation of microbial strains used for the determination of bactericidal and fungicidal activity
EN 1040	Chemical disinfectants and antiseptics - Basic bactericidal activity - Test method and requirements

3 Definitions

For the purposes of this European Standard, the following definitions apply :

3.1 product (for chemical disinfection and/or antisepsis)

Chemical agent or formulation used as a chemical disinfectant or antiseptic [EN 1040].

3.2 bactericide

Product which kills vegetative bacteria under defined conditions [EN 1040].

NOTE : The adjective derived from "bactericide" is "bactericidal".

3.3 bactericidal activity (EN 1276)

Capability of the product to produce at least a 10^5 reduction in the number of viable bacterial cells belonging to reference strains of *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus hirae* under conditions defined by this European Standard.

3.4 clean conditions

Conditions representative of surfaces which have received a satisfactory cleaning programme and/or are known to contain minimal levels of organic and/or inorganic materials.

3.5 dirty conditions

Conditions representative of surfaces which are known to or may contain, organic and/or inorganic materials.

4 Requirements

The product, when diluted in hard water and tested in accordance with clause 5 under simulated clean conditions (0,3 g/l bovine albumin see 3.4) or dirty conditions (3 g/l bovine albumin see 3.5) according to its practical applications and under the required test conditions (20°C, 5 min, 4 selected referenced strains), shall demonstrate at least a 10^5 log reduction in viable counts.

The bactericidal activity shall be evaluated using the following four test organisms : *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus hirae*.

The determined bactericidal concentration of the test product is suggested as being suitable for practical situations of use.

Where appropriate, additional specific bactericidal activity shall be determined under other conditions of time, temperature, additional strains and interfering substances (see 5.2.1 and 5.5.1) in accordance with 5.5.1 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 initial test conditions of 20 °C, 5 min, 4 selected reference strains.

5 Test method

5.1 Principle

5.1.1 A test suspension of bacteria in a solution of interfering substances is added to a prepared sample of the product under test diluted in hard water. The mixture is maintained at 20 °C ± 1 °C for 5 min ± 10 s (required test conditions).

At this contact time, an aliquot is taken ; the bactericidal and/or the bacteriostatic action 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, menbrane filtration is used. The number of surviving bacteria in each sample are determined and the reduction in viable counts is calculated.

5.1.2 The test is performed using *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus hirae*. Additional and optional exposure times, temperatures, strains, and interfering substances are specified.

5.2 Materials and reagents

5.2.1 Test organisms

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

- *Pseudomonas aeruginosa* ATCC 15442 [1] ;
- *Escherichia coli* ATCC 10536 ;
- *Staphylococcus aureus* ATCC 6538 ;
- *Enterococcus hirae* ATCC 10541.

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

- *Salmonella typhimurium* ATCC 13311 ;
- *Lactobacillus brevis* DSM 6235 ;
- *Enterobacter cloacae* DSM 6234.

NOTE : See annex E 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 center, 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.

NOTE : 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.

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[1] ATCC 15442, ATCC 10536, ATCC 6538 and ATCC 8043 are the collection numbers of strains supplied by the American Type Culture Collections. This information is given for the convenience of users of this standard and does not constitute an endorsement by CEN of the product named. Corresponding strains supplied by other culture collections may be used if they can be shown to lead to the same results.

5.2.2.2 Water

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

Sterilize in the autoclave (see 5.3.1).

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

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

5.2.2.3 Tryptone Soya Agar (TSA)

For maintainance 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)	to 1000,0 ml

Sterilize in the autoclave (see 5.3.1). After sterilization the pH of the medium shall be equivalent to 7,2 ± 0,2 when measured at 20 °C.

5.2.2.4 Diluent

Tryptone Sodium Chloride Solution :

Tryptone, pancreatic digest of casein	1,0 g
NaCl	8,5 g
Water (see 5.2.2.2)	to 1000,0 ml

Sterilize in the autoclave (see 5.3.1). After sterilization the pH of the medium shall be equivalent to 7,0 ± 0,2 when measured at 20 °C.

5.2.2.5 Neutralizer

The neutralizer shall be validated for the product under test in accordance with annex A. 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.

5.2.2.6 Rinsing liquid (for membrane filtration)

The liquid shall be sterile, compatible with the filter membrane and capable of filtration through the filter membrane under the test conditions described in annex A.