SLOVENSKI STANDARD
SIST EN 1650:2001

01-februar-2001

Kemična razkužila in antiseptiki - Kvantitativni suspenzijski preskus za ocenjevanje fungicidnega 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 fungicidal 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 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 fongicide 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: **EN 1650:1997**

ICS:

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

SIST EN 1650:2001 **en**

EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN 1650

December 1997

ICS 07.100.01; 11.080

Descriptors: disinfectant, chemical compounds, tests, estimation, fungicides, culture media, dilution, neutralizing, preparation, filtration analysis

English version

Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of fungicidal 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 fongicide 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 fungiziden 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 9 November 1997.

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. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

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 Central Secretariat has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and United Kingdom.

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

Central Secretariat: rue de Stassart, 36 B-1050 Brussels

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.

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 June 1998, and conflicting national standards shall be withdrawn at the latest by June 1998.

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

Other methods to evaluate the efficacy of chemical disinfectants and antiseptics for different fields of application are in preparation.

Annex A is normative.

Annexes B, C, D, E and F are informative.

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 fungicidal 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 conditons.

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 fungicidal 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 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.

Using this European Standard, it is not possible to determine the fungicidal 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 fungi 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 draft European Standard only when incorporated in it by amendment or revision. For undated references the lastest edition of the publication referred to applies.

EN 1275	Chemical disinfectants and antiseptics - Basic fungicidal activity - Test method and requirements (phase 1)
prEN 12353	Chemical disinfectants and antiseptics - Preservation of microbial strains used for the determination of bactericidal and fungicidal activity
ISO 4793	Laboratory sintered (fritted) filters - Porosity grating, classification and designation

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 fungicide : Product which kills fungi including their spores under defined conditions [EN 1275].

NOTE : The adjective derived from "fungicide" is "fungicidal".

3.3 fungicidal activity (EN 1650) : Capability of a product to produce at least a 10^4 reduction in the number of viable vegetative yeast cells and mould spores belonging to reference strains of *Candida albicans* and *Aspergillus niger* 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, 15 min, two selected referenced strains), shall demonstrate at least a 10^4 log reduction in viable counts.

The fungicidal activity shall be evaluated using the following two strains *Candida albicans* and *Aspergillus niger*.

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

Where appropriate, additional specific fungicidal 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, 15 min and two selected reference strains.

5 Test method

5.1 Principle

5.1.1 A test suspension of yeast cells or mould spores 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 15 min ± 10 s (required test conditions).

After this contact time, an aliquot is taken ; the fungicidal 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, membrane filtration is used. The number of surviving yeast cells or mould spores in each sample is determined and the reduction in viable counts is calculated.

5.1.2 The test is performed using vegetative cells of *Candida albicans* and spores of *Aspergillus niger*. Additional and optional exposure times, temperatures, strains, and interfering substances are specified.

5.2 Materials and reagents

5.2.1 Test organisms

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

- *Candida albicans* ATCC 10231 [1)]
- *Aspergillus niger* ATCC 16404

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

- *Saccharomyces cerevisiae* ATCC 9763 or DSM 1333
(for breweries) or

[1)] ATCC 10231 and ATCC 16404 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.

- *Saccharomyces cerevisiae* var. *diastaticus* (for breweries) DSM 70487

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 collection 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.

5.2.2.2 Water

The water shall be free from substances that are toxic or inhibiting to the yeast cells and the fungus spores. 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 Pharmacopoeia) can be used.

5.2.2.3 Malt extract Agar (MEA)

Malt extract (technical grade)	30,0 g
Soya peptone	3,0 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 5,6 ± 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 (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.

NOTE : Information on rinsing liquids that have been found to be suitable for some categories of products is given in annex C.

5.2.2.7 Hard water for dilution of 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 1000 ml ;

- Solution B : Dissolve 35,02 g $NaHC0_3$ in water (see 5.2.2.2) and dilute to 1000 ml.

Add at least 600 ml water (see 5.2.2.2) to 6,0 ml of solution A in a 1000 ml volumetric flask, then add 8,0 ml solution B and dilute to 1000 ml with water (see 5.2.2.2).

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

After adjustment, the pH of the solution shall be 7,0 ± 0,2 before use.

The solution shall be stored at 4 °C to 8 °C for a maximum holding time of one month.

NOTE : When preparing the three concentrations of product test solutions (see 5.4.2) the addition of the product in this hard water solution yields to different final water hardness in each test tube. In any case the final hardness is lower than 300 mg/kg of $CaCO_3$ in the test tube.

5.2.2.8 Interfering substances

5.2.2.8.1 General

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

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 method of preparation and sterilization together with the composition shall be noted in the test report (see 5.8).

5.2.2.8.2 Bovin albumin solutions

Bovine albumin solutions for the test conditions shall be prepared as follows :

a) preparation for clean conditions :

- dissolve 0,30 g of bovine albumin (Cohn fraction V for Dubos medium) in 100 ml of water (see 5.2.2.2) ;

- sterilize by membrane filtration ;

The final concentration in the test procedure (see 5.5.2) is 0,3 g/l for the bovine albumin.

b) preparation for dirty conditions :

- dissolve 3,00 g of bovine albumin (Cohn fraction V for Dubos medium) in 100 ml of water (see 5.2.2.2) ;

- sterilize by membrane filtration ;

The final concentration of bovine albumin in the test procedure (see 5.5.2) is 3 g/l.

In addition, other interfering substances can be chosen from :

5.2.2.8.3 Milk (dairies...)

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

- prepare a solution of 10,0 % (V/V) in water (see 5.2.2.2) of reconstituted milk. Sterilize for 30 min at 105 °C ± 1 °C (or 5 min at 121 °C ± 1 °C).

The final concentration of reconstituted milk in the test procedure (see 5.5.2) shall be 1,0 % (V/V) of reconstitued milk.

5.2.2.8.4 Yeast extract (breweries...)

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

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

- sterilize in an autoclave (see 5.3.1).

The final concentration of yeast extract in the test procedure (see 5.5.2) shall be 10 g/l.