SLOVENSKI STANDARD
SIST EN 1275:2001

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Kemična razkužila in antiseptiki - Osnovno fungicidno delovanje - Preskusna metoda in zahteve (faza 1)

Chemical disinfectants and antiseptics - Basic fungicidal activity - Test method and requirements (Phase 1)

Chemische Desinfektionsmittel und Antiseptika - Fungizide Wirkung (Basistest) - Prüfverfahren und Anforderungen (Phase 1)

Antiseptiques et désinfectants chimiques - Activité fongicide de base - Méthode d'essai et prescription (phase 1)

Ta slovenski standard je istoveten z: **EN 1275:1997**

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English version

Chemical disinfectants and antiseptics - Basic fungicidal activity - Test method and requirements (Phase 1)

Antiseptiques et désinfectants chimiques - Activité fongicide de base - Méthode d'essai et prescriptions (phase 1)

Chemische Desinfektionsmittel und Antiseptika - Fungizide Wirkung (Basistest) - Prüfverfahren und Anforderungen (Phase 1)

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CEN

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

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Contents

Foreword

This European Standard has been prepared by CEN Technical Committee 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.

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

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of an identical text or by endorsement, at the latest by September 1997, and conflicting national standards shall be withdrawn at the latest by September 1997.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, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

Annex A is normativ, annexes B, C, D, E and F are informative.

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 under the laboratory conditions defined by this European Standard. If a product complies with the test requirements, it can be considered as possessing a fungicidal activity. The acceptibility of a product as a chemical disinfectant or antiseptic for a defined purpose cannot be determined from this test method. Chemical disinfectants and antiseptics are subjected to further testing by relevant tests according to European Standards to evaluate their activity under conditions appropriate to their intended use[1)].

There is no evidence that the strains used in this standard are virulent.

1 Scope

This European Standard specifies a test method (phase 1) and the minimum requirements for fungicidal activity of chemical disinfectant and antiseptic products that form a homogeneous physically stable preparation in water. This European standard is applicable to products for use in agricultural (but not crop protection), domestic service, food hygiene and other industrial fields, institutional, medical and veterinary applications.

NOTE 1 : Using this European Standard it is not possible to determine the fungicidal activity of the undiluted product as some dilution is always produced by adding the inoculum.

NOTE 2 : This European Standard does not evaluate the activity of a product for an intended use. More specific test methods described in European Standards (see introduction) are used for further assessment of the efficacy of chemical disinfectants and antiseptics for a defined purpose.

NOTE 3 : This method corresponds to a phase 1 test (see Annex F).

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
ISO 4793	Laboratory sintered (fritted) filters - Porosity grading, classification and designation

1) CEN/TC 216 will develop relevant tests.

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.

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

3.3 fungicidal activity (EN 1275): Capability of a product to produce a reduction in the number of viable vegetative yeast cells and mould spores of relevant organisms under conditions defined by this European Standard.

4 Requirements

The product, when tested in accordance with clause 5, shall demonstrate at least a 10^4 logarithmic reduction in viable counts when the test organisms are vegetative cells of *Candida albicans* and the spores of *Aspergillus niger*.

5 Test method

5.1 Principle

5.1.1 A test suspension of yeast cells or mould spores is added to a prepared sample of the product under test. The mixture is maintained at 20 °C. At a specified contact time chosen from one of the following, 5 min ± 10 s, 15 min ± 10 s, 30 min ± 10 s or 60 min ± 10 s, 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 calulated.

5.1.2 The test is performed using vegetative cells of *Candida albicans* and spores of *Aspergillus niger*.

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 [2)]

Aspergillus niger ATCC 16404 [2)]

NOTE : See annex E for corresponding strain numbers in some other culture collections.

[2)] 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.

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 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 preparations (see European Pharmacopeia) can be used.

5.2.2.3 Malt extract agar (MEA)

Malt extract	30,0 g
Soya peptone	3,0 g
Agar	15,0 g
Water (see 5.2.2.2)	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 (see 5.2.2.2)	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.

5.3 Apparatus and glassware

5.3.1 General

Sterilize all glassware and parts of the apparatus that will come into contact with the culture media and reagents or the sample, except those which are supplied sterile, by one of the following methods :

a) in the autoclave (see 5.3.2.1) by maintaining it at (121 $^{+3}_{0}$)°C for a minimum holding time of 15 min ;

b) in the dry heat sterilizer (see 5.3.2.1) by maintaining it at 180°C for a minimum holding time of 30 min, at 170°C for a minimum holding time of 1 h or at 160°C for a minimum holding time of 2 h.

5.3.2 Usual microbiological laboratory equipment [3] and in particular, the following :

5.3.2.1 Apparatus for sterilization :

a) For moist heat sterilization, an autoclave capable of being maintained at (121 $^{+3}_{0}$) °C for a minimum holding time of 15 min ;

b) For dry heat sterilization, a hot air oven capable of being maintained at 180 °C for a minimum holding time of 30 min, at 170 °C for a minimum holding time of 1 h or at 160 °C for a minimum holding time of 2 h.

5.3.2.2 Water baths capable of being controlled at 20 °C ± 1 °C and 45 °C ± 1 °C.

5.3.2.3 Incubator, capable of being controlled at 30 °C ± 1 °C.

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[3] Disposable equipment is an acceptable alternative to reusable glassware.

5.3.2.4 **pH-meter**, having an accuracy of calibration of ± 0,1 pH units at 25 °C.

5.3.2.5 **Fritted filter :** Porosity of 40 µm to 100 µm (see ISO 4793).

5.3.2.6 **Stopwatch**

5.3.2.7 **Vortex mixer (electromechanical agitator, i.e. Vortex® mixer[4])**

5.3.2.8 **Membrane filtration apparatus** (if this method is used), constructed of a material compatible with the product under test, with a filter holder which shall have a usable volume of 50 ml minimum, and suitable for use with filters of diameter 47 mm to 50 mm, of 0,45 µm pore size.

The vacuum source used shall give an even filtration flow rate. In order to obtain a uniform distribution of the microorganisms over the membrane and in order to prevent overlong filtration, the device shall be set so as to obtain the filtration of 100 ml of rinsing liquid in 20 s to 40 s.

5.3.2.9 **Container :** Test tubes or flasks of suitable capacity.

5.3.2.10 **Graduated pipettes**, of nominal capacities 10 ml and 1 ml and 0,1 ml. Calibrated automatic pipettes may be used.

5.3.2.11 **Petri dishes** of size 90 mm to 100 mm and Roux bottles.

5.3.2.12 **Glass beads** (Diameter : 3 mm to 4 mm).

5.3.2.13 **Volumetric flasks** calibrated at 20 °C.

5.3.2.14 **Centrifuge**

5.3.2.15 **Mechanical shaker**

5.4 Preparation of fungal suspensions and test solutions

5.4.1 Fungal suspensions

5.4.1.1 Stock cultures of test organisms

Stock cultures shall be kept in accordance with the requirements of prEN 12353.

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[4] Vortex® is an example of a suitable product available commercially. This information is given for the convenience of users of this standard and does not constitute an endorsement by CEN of this product.

5.4.1.2 Working culture of test organisms

In order to prepare the working culture of *Candida albicans,* subculture from the stock culture (see 5.4.1.1) by streaking onto MEA slopes (see 5.2.2.3) and incubate (see 5.3.2.3). After 42 h to 48 h prepare a second subculture from the first subculture in the same way and incubate for 42 h to 48 h. From this second subculture, a third subculture may be produced in the same way.

NOTE : The second and/or third subculture are the working culture(s).

If it is not possible to prepare the second subculture on a particular day, a 72 h subculture may be used for subsequent subculturing, provided that the subculture has been kept in the incubator during the 72 h period. In these circumstances, prepare a further 48 h subculture after proceeding. Do not take a fourth subculture.

For *Aspergillus niger*, use a subculture grown on MEA (see 5.2.2.3) in Roux bottles and incubate at 30 °C ± 1 °C for 7 to 9 days.

5.4.1.3 Fungal test suspensions

Prepare two cell suspensions, one using *Candida albicans* vegetative cells and the other using spores of *Aspergillus niger* (see 5.2.1).

5.4.1.3.1 Preparation of yeast test suspension

Take 10 ml of diluent (see 5.2.2.4) and place in a 100 ml flask with 10 g of glass beads (see 5.3.2.12). Take the working cultures (see 5.4.1.2) and transfer loopfuls of the cells into the diluent. The cells should be suspended in the diluent by immersing the loop in the diluent and rubbing it against the side of the flask to dislodge the cells. Shake the flask for 3 min using a mechanical shaker (see 5.3.2.15). Aspirate the suspension from the glass beads and transfer to another tube. Adjust the number of cells in the suspension to 1,5 x 10^7 cfu/ml [5] to 5 x 10^7 cfu/ml using the diluent, estimating the numbers of units by any suitable means. Maintain this suspension in the water bath at 20 °C ± 1 °C and use within 2 h.

5.4.1.3.2 Preparation of *Aspergillus niger* spores test suspension

Take the working culture (see 5.4.1.2) and suspend the cells in 10 ml of sterile 0,05 % w/v polysorbate 80 solution in water (see 5.2.2.2). Using a sterile glass spatula detach the conidiospores from the culture surface. The suspension is transferred into a conical flask and gently shaken for one minute together with glass beads (see 5.3.2.12). The suspension is filtered through a fritted filter (see 5.3.2.5).

Microscopic examination under 400 magnification shall be carried out immediately after the preparation and just before the test, to show the absence of mycelial fragments and spore germination (less than one in ten fields of view should show either).

If germinated spores are present, the suspension shall be discarded.

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[5] cfu/ml : Colony Forming Unit per ml.

If mycelia are present, the washing process shall be set up :

Centrifugation procedure (washing process) : Transfer the filtered suspension to centrifuge tubes. The filtered suspension is centrifuged at 2000 g for 20 min. The conidiospores are washed at least two times by resuspension and centrifugation in diluent (see 5.2.2.4). If mycelia are still present, the washing process should be repeated.

Adjust the number of spores in the suspension to 1,5 x 10^7 cfu/ml to 5 x 10^7 cfu/ml using the diluent (see 5.2.2.4), estimating the number of units by any suitable means.

This suspension shall not be stored more than 2 days at 2 °C to 8 °C.

The test suspension shall be mixed (see 5.3.2.7) immediately before use to resuspend the spores.

5.4.1.4 Counting of fungal test suspensions

For counting of the fungal test suspension prepare 10^{-5} and 10^{-6} dilutions of the test suspension (see 5.4.1.3) using diluent (see 5.2.2.4). Mix (see 5.3.2.7). Take a sample of 1,0 ml of each dilution in duplicate and transfer each 1,0 ml sample into separate Petri dishes (see 5.3.2.11) and add 12 ml to 15 ml melted MEA (see 5.2.2.3), cooled to 45 °C ± 1 °C.

a) *Candida albicans*

Incubate the plates at 30 °C ± 1 °C for 24 h. Discard any plates which are not countable (for any reason). Count the plates and determine the number of colony forming units for each plate. Incubate the plates for a further 24 h.

b) *Aspergillus niger*

Incubate the plates at 30 °C ± 1 °C for 42 h to 48 h. Discard any plates which are not countable (for any reason). Count the plates and determine the number of colony forming units for each plate. Incubate the plates for a further 24 h and if necessary an additional 20 h to 24 h, provided the number of countable colonies (discreet colonies) is increasing.

For both strains, do not recount plates which no longer show well-separated colonies. Recount the remaining plates. Determine the higher number of colonies for each 1,0 ml sample. Calculate the number of cfu/ml in the test suspension (N) using the method given in 5.6.1.1.

5.4.2 Product test solution

Details of samples of the product as received shall be recorded.

Product test solutions shall be prepared at three different concentrations to include at least two concentrations in the active range. The concentrations shall decrease in a geometrical progression with a factor of at least 2 (i.e. at least doubling the dilutions). The concentration of the product test solution shall be 1,25 times the required test concentration.

NOTE : The product as received may be used as one of the product test solutions.

For solid products, dissolve the product as received by weighing at least 1g ± 10 mg of the product in a volumetric flask (see 5.3.2.13) and filling up with water (see 5.2.2.2). Subsequent dilutions shall be prepared in volumetric flasks on a volume/volume basis.