

# SLOVENSKI STANDARD

## SIST EN 1657:2016

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Nadomešča:

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**Kemična razkužila in antiseptiki - Kvantitativni suspenzijski preskus za vrednotenje fungicidnega delovanja ali delovanja kemičnih razkužil in antiseptikov na kvasovke v veterini - Preskusna metoda in zahteve (faza 2, stopnja 1)**

Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity of chemical disinfectants and antiseptics used in the veterinary area - Test method and requirements (phase 2, step 1)

Chemische Desinfektionsmittel und Antiseptika - Quantitativer Suspensionsversuch zur Bestimmung der fungiziden oder levuroziden Wirkung chemischer Desinfektionsmittel und Antiseptika für den Veterinärbereich - Prüfverfahren und Anforderungen (Phase 2, Stufe 1)

Antiseptiques et désinfectants chimiques - Essai quantitatif de suspension pour l'évaluation de l'activité fongicide ou levuricide des antiseptiques et des désinfectants chimiques utilisés dans le domaine vétérinaire - Méthode d'essai et prescriptions (phase 2, étape 1)

**Ta slovenski standard je istoveten z: EN 1657:2016**

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EUROPEAN STANDARD

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Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity of chemical disinfectants and antiseptics used in the veterinary area - Test method and requirements (phase 2, step 1)

Antiseptiques et désinfectants chimiques - Essai quantitatif de suspension pour l'évaluation de l'activité fongicide ou levuricide des antiseptiques et des désinfectants chimiques utilisés dans le domaine vétérinaire - Méthode d'essai et prescriptions (phase 2, étape 1)

Chemische Desinfektionsmittel und Antiseptika - Quantitativer Suspensionsversuch zur Bestimmung der fungiziden oder levuroziden Wirkung chemischer Desinfektionsmittel und Antiseptika für den Veterinärbereich - Prüfverfahren und Anforderungen (Phase 2, Stufe 1)

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

**CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels**

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**EN 1657:2016 (E)****European foreword**

This document (EN 1657:2016) 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 October 2016, and conflicting national standards shall be withdrawn at the latest by October 2016.

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

This document supersedes EN 1657:2005.

This European Standard was revised to harmonize the preparation of the fungal spore suspension with other fungicidal tests of CEN/TC 216 and to incorporate amendments applicable to all European Standards.

An additional requirement has been added for the *Aspergillus* spore suspension and therefore results obtained using EN 1657:2005 and not fulfilling this additional requirement will need to be confirmed by repeating the tests using EN 1657:2015.

The test conditions for teat disinfectants 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, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

## Introduction

This European Standard specifies a suspension test for establishing whether a chemical disinfectant or antiseptic has a fungicidal or yeasticidal 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 substances, 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.

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**EN 1657:2016 (E)****1 Scope**

This European Standard specifies a test method and the minimum requirements for fungicidal or yeasticidal 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 European Standard applies to products that are used in the veterinary area – i.e. in the breeding, husbandry, production, transport and disposal of all animals except when in the food chain following death and entry to the processing industry.

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 documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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*  
[standards.iteh.ai/catalog/standards/sist/636436f9-bd4c-4def-9aa4-640d62562af5/sist-en-1657-2016](https://standards.iteh.ai/catalog/standards/sist/636436f9-bd4c-4def-9aa4-640d62562af5/sist-en-1657-2016)

EN 14885, *Chemical disinfectants and antiseptics — Application of European Standards for chemical disinfectants and antiseptics*

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

**3 Terms and definitions**

For the purposes of this document, the terms and definitions given in EN 14885 apply.

**4 Requirements**

The product shall demonstrate at least a 4 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 Table 1 and Clause 5 under simulated low level soiling (3,0 g/l bovine albumin) or high level soiling (10 g/l yeast extract and 10 g/l bovine albumin) or 10 g/l skimmed milk for teat disinfectants or in additional test conditions.



Table 1 — Obligatory and additional test conditions

Test conditions	Fungicidal activity	Yeasticidal activity	Yeasticidal activity for teat disinfectants
<b>Test organisms</b> obligatory	<i>Aspergillus brasiliensis</i> <i>Candida albicans</i>	<i>Candida albicans</i>	<i>Candida albicans</i>
additional	any relevant test organism	any relevant test organism	any relevant test organism
<b>Test temperature</b> obligatory	10°C ± 1°C	10°C ± 1°C	30°C ± 1°C
additional	4°C ± 1°C; 20°C ± 1°C; 40°C ± 1°C;	4°C ± 1°C; 20°C ± 1°C; 40°C ± 1°C	20°C ± 1°C
<b>Contact time</b> obligatory	30 min ± 10 s	30 min ± 10 s	5 min ± 10 s for post-milking teat disinfectants 30 s ± 5 s for pre-milking teat disinfectants
additional	5 min ± 10 s; 60 min ± 10 s; 120 min ± 10 s <sup>a</sup>	5 min ± 10 s; 60 min ± 10 s; 120 min ± 10 s <sup>a</sup>	1 min ± 5 s
<b>Interfering substance</b> obligatory			
low level soiling	3,0 g/l bovine albumin	3,0 g/l bovine albumin	10,0 g/l of reconstituted skimmed milk
high level soiling	10 g/l yeast extract plus 10 g/l bovine albumin	10 g/l yeast extract plus 10 g/l bovine albumin	
additional	any relevant substance	any relevant substance	any relevant substance
The obligatory contact times for disinfectants stated in Table 1 were chosen to enable comparison of standard conditions.			
NOTE For the additional conditions, the concentration defined as a result can be lower than the one obtained under the obligatory test conditions.			
<sup>a</sup> The recommended contact time for the use of the product is within the responsibility of the manufacturer.			

Any additional specific fungicidal activity shall be determined in accordance with 5.2.1 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) is added to a test suspension of fungi (yeast cells or mould spores) in a solution of an interfering substance. The mixture is maintained at 10°C ± 1°C for 30 min ± 10 s or 30°C ± 1°C for 5 min ± 10 s or 30 s ± 5 s for teat disinfectants (obligatory test conditions). At the end of this contact time, an aliquot is taken, and the fungicidal/yeasticidal and/or the fungistatic/yeastistatic 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 fungi in each sample are determined and the reduction is calculated.

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**5.1.2** The test is performed using the vegetative cells of *Candida albicans* and the spores of *Aspergillus brasiliensis* (fungicidal activity) or only the vegetative cells of *Candida albicans* (yeasticidal activity) as test organisms (obligatory test conditions).

**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 fungicidal activity shall be evaluated using the following strains as test organisms: <sup>1)</sup>

- *Candida albicans* ATCC 10231;
- *Aspergillus brasiliensis* ATCC 16404.  
(formerly *A.niger*)

The yeasticidal activity shall be evaluated using only *Candida albicans*.

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

The required incubation temperature for these test organisms is  $(30 \pm 1)$  °C (see 5.3.2.3). The same temperature 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.

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.

For each culture medium and reagent, a limitation for use should be fixed.

**5.2.2.2 Water**

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

Sterilize in the autoclave [5.3.2.1 a)].

<sup>1)</sup> 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 European Standard and does not constitute an endorsement by CEN of the product named.

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 Malt extract agar (MEA)

Malt extract agar, consisting of:

Malt extract <sup>a</sup>	30,0 g
Agar	15,0 g
Water (5.2.2.2)	to 1 000,0 ml

<sup>a</sup> The malt extract should be of food grade (Cristomalt poudre from Difal is recommended) or equivalent that is not highly purified and not only based on maltose (Malt extract from OXOID is recommended <sup>2)</sup>).

Sterilize in the autoclave [5.3.2.1 a)]. After sterilization, the pH of the medium shall be equivalent to  $5,6 \pm 0,2$  when measured at  $20 \text{ }^\circ\text{C} \pm 1 \text{ }^\circ\text{C}$ .

In case of encountering problems with neutralization (5.5.1.2 and 5.5.1.3), it may be necessary to add neutralizer to the MEA. 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	<a href="https://standards.iteh.ai/catalog/standards/sist/636436f9-bd4c-4def-9aa4-640d62562af5/sist-en-1657-2016">SIST EN 1657:2016 https://standards.iteh.ai/catalog/standards/sist/636436f9-bd4c-4def-9aa4-640d62562af5/sist-en-1657-2016</a>
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  $7,0 \pm 0,2$  when measured at  $20 \text{ }^\circ\text{C} \pm 1 \text{ }^\circ\text{C}$ .

### 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.

2) This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of the product named. Equivalent products may be used if they can be shown to lead to the same results.

**EN 1657:2016 (E)****5.2.2.7 Hard water for dilution of products**

For the preparation of 1 l of hard water, the procedure is as follows:

- prepare solution A: dissolve 19,84 g magnesium chloride ( $\text{MgCl}_2$ ) and 46,24 g calcium chloride ( $\text{CaCl}_2$ ) 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 ( $\text{NaHCO}_3$ ) 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  $7,0 \pm 0,2$ , when measured at  $20 \text{ }^\circ\text{C} \pm 1 \text{ }^\circ\text{C}$  (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 ( $\text{NaOH}$ ) or approximately 36,5 g/l (about 1 mol/l) of hydrochloric acid ( $\text{HCl}$ ).

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 ( $\text{CaCO}_3$ ) in the test tube.

**5.2.2.8 Interfering substance**

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**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 Low-level soiling (bovine albumin solution)**

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.

**5.2.2.8.3 High-level soiling (mixture of bovine albumin solution with yeast extract)**

Dissolve 50,0 g yeast extract powder in 150 ml of water (5.2.2.2) in a 250 ml volumetric flask (5.3.2.12) and allow foam to collapse. Make up to the mark with water (5.2.2.2). Transfer to a clean dry bottle and sterilize in an autoclave [5.3.2.1 a)]. Allow to cool to  $20 \text{ }^\circ\text{C} \pm 1 \text{ }^\circ\text{C}$ .

Pipette 25 ml of this solution into a 50 ml volumetric flask (5.3.2.12) and add 10 ml of water (5.2.2.2). Dissolve 5,0 g of bovine albumin fraction V (suitable for microbiological purposes) in the solution with

shaking and allow foam to collapse. Make up to the mark with 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 in the test procedure (5.5) is 10,0 g/l yeast extract and 10,0 g/l bovine albumin.

#### 5.2.2.8.4 Milk for teat disinfectants

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  $105^{\circ}\text{C} \pm 3^{\circ}\text{C}$  or 5 min at  $121^{\circ}\text{C} \pm 3^{\circ}\text{C}$ .

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

### 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) by moist heat, in the autoclave [5.3.2.1 a)];
- b) by dry heat, in the hot air oven [5.3.2.1 b)].

#### 5.3.2 Usual microbiological laboratory equipment<sup>3)</sup> and, in particular, the following

##### 5.3.2.1 Apparatus for sterilization

- a) for moist heat sterilization, an autoclave capable of being maintained at  $(121^{+3})^{\circ}\text{C}$  for a minimum holding time of 15 min;
- b) for dry heat sterilization, a hot air oven capable of being maintained at  $(180^{+5})^{\circ}\text{C}$  for a minimum holding time of 30 min, at  $(170^{+5})^{\circ}\text{C}$  for a minimum holding time of 1 h or at  $(160^{+5})^{\circ}\text{C}$  for a minimum holding time of 2 h.

**5.3.2.2 Water baths**, capable of being controlled at  $4^{\circ}\text{C} \pm 1^{\circ}\text{C}$ ,  $10^{\circ}\text{C} \pm 1^{\circ}\text{C}$ , at  $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$ , at  $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$ ,  $40^{\circ}\text{C} \pm 1^{\circ}\text{C}$  at  $45^{\circ}\text{C} \pm 1^{\circ}\text{C}$  (to maintain melted MEA in case of pour plate technique) and at additional test temperatures  $\pm 1^{\circ}\text{C}$  (5.5.1).

**5.3.2.3 Incubator**, capable of being controlled at  $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$ .

**5.3.2.4 pH-meter**, having an inaccuracy of calibration of no more than  $\pm 0,1$  pH units at  $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$ . A puncture electrode or a flat membrane electrode should be used for measuring the pH of the agar media (5.2.2.3).

##### 5.3.2.5 Stopwatch

<sup>3)</sup> Disposable sterile equipment is an acceptable alternative to reusable glassware.