SLOVENSKI STANDARD
SIST EN 16615:2026

01-junij-2026

Nadomešča:
SIST EN 16615:2015

Kemična razkužila in antiseptiki - Kvantitativna preskusna metoda za vrednotenje baktericidnega delovanja in delovanja na kvasovke in/ali fungicidnega in/ali tuberkulocidnega in/ali mikobaktericidnega delovanja na neporoznih površinah z mehanskim delovanjem z uporabo robčkov ali krpic v humani medicini (4-področni preskus) - Preskusna metoda in zahteve (faza 2, stopnja 2)

Chemical disinfectants and antiseptics - Quantitative test method for the evaluation of bactericidal and yeasticidal and/or fungicidal and/or tuberculocidal and/or mycobactericidal activity on non-porous surfaces with mechanical action employing wipes or mops in the medical area (4- field test) - Test method and requirements (phase 2, step 2)

Chemische Desinfektionsmittel und Antiseptika - Quantitatives Prüfverfahren zur Bestimmung der bakteriziden und levuroziden und/oder fungiziden und/oder tuberkuloziden und/oder mykobakteriziden Wirkung auf nicht-porösen Oberflächen mit mechanischer Einwirkung mit Hilfe von Tüchern oder Mopps im humanmedizinischen Bereich (4-Felder-Test) - Prüfverfahren und Anforderungen (Phase 2, Stufe 2)

Antiseptiques et désinfectants chimiques - Méthode d’essai quantitative pour l’évaluation de l’activité bactéricide et levuricide et/ou fongicide et/ou tuberculocide et/ou mycobactéricide sur des surfaces non poreuses, avec action mécanique à l’aide de lingettes ou de serpillières dans le domaine médical (essai à 4 zones) - Méthode d'essai et exigences (phase 2, étape 2)

Ta slovenski standard je istoveten z: **EN 16615:2026**

ICS:

11.080.20	Dezinfektanti in antiseptiki	Disinfectants and antiseptics

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EUROPEAN STANDARD

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Chemical disinfectants and antiseptics - Quantitative test method for the evaluation of bactericidal and yeasticidal and/or fungicidal and/or tuberculocidal and/or mycobactericidal activity on non-porous surfaces with mechanical action employing wipes or mops in the medical area (4- field test) - Test method and requirements (phase 2, step 2)

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This European Standard was approved by CEN on 23 February 2026.

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

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European foreword

This document (EN 16615:2026) 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 2026, and conflicting national standards shall be withdrawn at the latest by October 2026.

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

This document supersedes EN 16615:2015.

The document was revised to adapt it to the latest state of science, to correct errors and ambiguities, to harmonize the structure and wording with other tests of CEN/TC 216 existing or in preparation and to improve the readability of the standard and thereby make it more understandable.

The following is a list of significant technical changes since the last edition:

— Annex ZA was deleted, the document was de-harmonized;

— N_0 was deleted;

— Harmonization of the text with EN 13727;

— Implementation of different applications forms;

— Implementation different swab material;

— Implementation of the definition of room temperature;

— Water control always be carried out with hard water (= process and method validation);

— Polysorbate 80 should be weighed 1 g instead 1 ml;

— Implementation of Apparatus Pre-cleaning of surfaces with 2-Propanol instead of n-Propanol;

— Correction of calculation and editorial mistakes;

— Implementation of additional test-surfaces;

— Implementation of standard wiping cloth (wipe) and specified wiping cloth;

— Implementation of fungicidal, tuberculocidal and mycobactericidal activity;

— Water control: Test field 1 to be taken into account for calculation of the water control;

— Implementation of Annex E and Annex G.

The changes of this revision have no impact on the test results obtained with reference to the version EN 16615:2015. Those results are still valid but additional data requirements concerning number of required test runs and test parallels per tested organisms/ claimed group of target organisms should be respected.

Any feedback and questions on this document should be directed to the users' national standards body. A complete listing of these bodies can be found on the CEN website.

According to the CEN-CENELEC Internal Regulations, the national standards organisations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Türkiye and the United Kingdom.

Introduction

This document specifies a carrier test for establishing whether a chemical disinfectant for use on surfaces administered with wipes has a bactericidal and yeasticidal and/or fungicidal and/or tuberculocidal and/or mycobactericidal activity in the fields described in the scope.

The laboratory test closely simulates practical conditions of application such as contact time, temperature and interfering substances, including pre-drying specified test organisms on a test-surface as carrier and wiping the product on the test-surface with a wipe. The conditions are intended to cover general purposes. However, if for some applications the recommendations of use of a product differ additional test conditions may be used or may need to be used.

Each utilization concentration of the product found by this test corresponds to defined experimental conditions.

1 Scope

This document specifies a test method and the minimum requirements for bactericidal and yeasticidal and/or fungicidal and/or tuberculocidal and/or mycobactericidal activity of chemical disinfectant products that form a homogeneous, physically stable preparation when diluted with hard water – or in the case of ready-to-use products – with water.

This document is applicable to products that are used in the medical area for disinfecting non-porous surfaces including surfaces of medical devices by wiping or mopping – regardless if they are covered by the Medical Device Regulation [7] or not.

Due to the new methods of application of surface disinfectants like pre-impregnated wipes this document was established to cover the different application methods.

This document is applicable for four methods of application of products for wiping and/or mopping:

a) soaking any non-specified wipe or mop with product;

b) spraying the product on any non-specified wipe and / or mop or a specified wipe or mop;

c) impregnation of specified wipes or mops by the user with the product according to the manufacturer's recommendation;

d) pre-impregnation of specified wipes or mop by the manufacturer as ready-to-use wipes or mops.

In all types of application, the water control is done with the standard wipe (5.3.2.17.1), because it is a process or method control.

This document does not apply to products that are sprayed on or flooding surfaces, without wiping in the contact time. In this case, the methods of phase 2/ stage 2 without mechanical action apply.

The test-surface (5.3.2.16) was selected as standard surface to cover all non-porous surfaces. This document does not apply to the testing of the influence of different surfaces.

This document is applicable to areas and situations where disinfection is medically indicated. Such indications occur in patient care, for example:

— in hospitals, in community medical facilities and in dental institutions;

— in clinics of schools, of kindergartens and of nursing homes;

and can occur in the workplace and in the home. It can also include services such as laundries and kitchens supplying products directly for the patients.

NOTE This method corresponds to a phase 2, step 2 test.

EN 14885 specifies in detail the relationship of the various tests to one another and to "use recommendations".

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 12353:2021, *Chemical disinfectants and antiseptics - Preservation of test organisms used for the determination of bactericidal (including Legionella), mycobactericidal, sporicidal, fungicidal and virucidal (including bacteriophages) 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 terms and definitions given in EN 14885 and the following apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
ready-to-use wipe
pre-impregnated wipe
wipe containing disinfectant added by the wipe manufacturer at the manufacturing site

3.2
impregnated wipe
wipe containing disinfectant added by the user

Note 1 to entry: Examples include a dry wipe soaked in disinfectant by the user, a dry wipe sprayed with disinfectant.

4 Requirements

The product, when diluted with hard water or – in the case of ready-to-use products – with water, shall demonstrate at least a 5 decimal log (lg) reduction for bacteria and at least a 4 decimal log (lg) reduction for fungi and mycobacteria on test field 1, when tested in accordance with Table 1 and Clause 5.

The mean of the number of cfu per 25 cm^2 on the test fields 2 to 4 of the product under test shall be equal or less than 50, the mean of the number of cfu per 25 cm^2 on the test fields 1 to 4 of the water control shall be equal or more than 10. Details on the precision and repetition are given in 5.8.4 and EN 14885.

Table 1 — Experimental conditions

Test conditions	**Bactericidal activity**	**Yeasticidal activity**	**Fungicidal activity**	**Tuberculocidal activity**	**Mycobactericidal activity**
Minimum spectrum of test organisms	*Staphylococcus aureus* *Enterococcus hirae* *Pseudomonas aeruginosa*	*Candida albicans*	*Aspergillus brasiliensis* *Candida albicans*	*Mycobacterium terrae*	*Mycobacterium avium* *Mycobacterium terrae*
additional	any relevant test organism				
Test temperature	according to the manufacturer's recommendation, but between (4 ± 1) °C to (30 ± 1) °C For tests performed at room temperature, the range shall be (21,5 ± 3,5) °C				
Contact time	according to the manufacturer's recommendation, but at minimum 1 min and no longer than 60 min[a], based on the following rules: from 1 min to 5 min at intervals of 1 min and from 5 min to 60 min at intervals of 5 min				
Interfering substance					
clean conditions	0,3 g/l bovine albumin				
dirty conditions	3,0 g/l bovine albumin plus 3,0 ml/l erythrocytes				
additional	any relevant substance				

NOTE For the additional conditions, the concentration defined as a result can be lower than the one obtained under the minimum test conditions.

[a] The contact times for surface disinfectants stated in this table are chosen on the basis of the practical conditions of the product. For further information see EN 14885.

5 Test methods

5.1 Principle

5.1.1 A test-surface is marked with 4 squares of (5 × 5) cm, the "test fields", in a row. Test field 1 on the test-surface is inoculated with a test suspension of bacteria, yeasts, fungal spores or mycobacteria in a solution of interfering substances. The inoculum is dried. A wipe is soaked with a sample of the product as delivered and/or diluted with hard water (5.2.2.7) (for ready to use products: water). The test-surface is wiped with the soaked wipe across the four marked test fields, starting in front of test field 1, turning immediately after test field 4 and wiped back to the starting point. In parallel a water control [5.5.2.2 h)] is performed: a wipe is soaked with hard water (5.2.2.7) instead of the product in the same way to ensure that the test organisms are spread on the 4 fields and their number reaches a certain level.

NOTE For the purposes of this document, references to wiping, wipe and wiped can be equated to mopping, mop and mopped when the standard method is used to test a mopping application.

Temperature, soiling and contact time are employed as recommended by the manufacturer. At the end of the contact time, the test organisms are recovered from each test field with moistened swabs (5.3.2.19). The swabs are brought into a tube containing broth and neutralizer and the test organisms are to be severed from the swab by shaking. The numbers of surviving test organisms in each sample are determined, and the reduction is calculated by comparing the results of the drying control D_{ct} and the results obtained with the product. The test is performed using *P. aeruginosa*, *S. aureus*, *E. hirae* and *C. albicans* (minimum test condition) and/or *A. brasiliensis* and/or *M. terrae* and/or *M. avium* (optional test condition) as test organisms.

5.1.2 Additional test organisms (only bacterial or fungal strains), contact times and interfering substances can be used.

5.2 Materials and reagents

5.2.1 Test organism

The bactericidal activity shall be evaluated using the following strains as test organisms[1]:

— *Staphylococcus aureus* ATCC 6538;

— *Pseudomonas aeruginosa,* ATCC 15442;

— *Enterococcus hirae* ATCC 10541.

The yeasticidal activity shall be evaluated using the following strain as test organism[1]:

— *Candida albicans* ATCC 10231.

The fungicidal activity shall be evaluated using the following strains as test organisms[1]:

— *Aspergillus brasiliensis* ATCC 16404;

— *Candida albicans* ATCC 10231.

The tuberculocidal activity shall be evaluated using the following strain as test organism[1]:

— *Mycobacterium terrae* ATCC 15755.

The mycobactericidal activity shall be evaluated using the following strains as test organisms[1]:

— *Mycobacterium terrae* ATCC 15755;

— *Mycobacterium avium* ATCC 15769.

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

If additional test organisms are used, they shall be incubated under optimum growth conditions (temperature, time, atmosphere and 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.

[1] The ATCC numbers are the collection numbers of strains supplied by these culture collections. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product.

The required incubation temperature for these test bacteria is (36 ± 1) °C or (37 ± 1) °C (5.3.2.3). The same temperature (36 °C or 37 °C) shall be used for all incubations performed during its control and validation. The required incubation temperature for *Candida albicans* and *Aspergillus brasiliensis* is (30 ± 1) °C (5.3.2.3).

5.2.2 Culture media and reagents

5.2.2.1 General

All weights of chemical substances given in this document 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 organism.

To improve reproducibility, it is recommended that commercially available dehydrated material is used for the preparation of culture media if it complies with the formulae given below. 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.

All specified pH values are measured at (21,5 ± 3,5)°C (5.3.2.4).

5.2.2.2 Water

The water shall be freshly glass-distilled or deionized and demineralized water. If distilled water or deionized and demineralized water of adequate quality is not available, water for injections (see [1]) may be used.

Sterilize in the autoclave [5.3.2.1 a)]. Sterilization is not necessary if the water is used, e.g. for preparation of culture media and subsequently sterilized.

See 5.2.2.7 for the procedure to prepare hard water.

5.2.2.3 Medium

a) Soya Agar (TSA)

—	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 (5.3.1). After sterilization the pH (5.3.2.4) of the medium shall be equivalent to 7,2 ± 0,2.

In case of encountering problems with neutralization (5.5.1.2) it may be necessary to add neutralizer to TSA. Annex B gives guidance on the neutralizers that may be used. It is recommended not to use a neutralizer that causes opalescence in the agar.

b) Malt Extract Agar (MEA)

Malt extract agar, consisting of:

— Malt extract [food grade (e.g. Christomalt powder from Difal) or an equivalent extract that is not highly purified and not only based on maltose (e.g. Malt extract from OXOID)[2]	30,0 g
— Agar	15,0 g
— Water (5.2.2.2)	to 1 000,0 ml

Sterilize in the autoclave (5.3.1). After sterilization, the pH (5.3.2.4) of the medium shall be equivalent to 5,6 ± 0,2.

In case of encountering problems with neutralization (5.5.1.2) it may be necessary to add neutralizer to MEA. Annex B gives guidance on the neutralizers that may be used. It is recommended not to use neutralizer that causes opalescence in the agar.

If there are problems with producing at least 75 % spiny conidiospores, see 5.4.1.4 b 2).

c) Middlebrook and Cohn 7H10 medium with 10 % OADC enrichment (MCO)

— Middlebrook 7H10 agar-powder	19,0 g
— Glycerol [bibliographic reference 1]	5,0 ml
— Water (5.2.2.2)	to 900,0 ml

Heat to boiling to dissolve completely. Sterilize in the autoclave (5.3.1) and cool to 50 °C to 55 °C. Add 100 ml Middlebrook OAOC enrichment under aseptic conditions. Fill 18 ml to 20 ml per plate (5.3.2.10). The pH of the medium shall be equivalent to 6,6 ± 0,2 (5.3.2.4).

In case of encountering problems with neutralization (5.5.1.2) it may be necessary to add neutralizer to MCO. Annex B gives guidance on the neutralizers that may be used. It is recommended not to use neutralizer that causes opalescence in the agar.

d) Middlebrook 7H10 broth with 10 % ADC enrichment (MADC-broth)

— Middlebrook 7H10 broth-powder	4,7 g
— Glycerol [bibliographic reference 1]	100,0 ml
— Water (5.2.2.2)	750,0 ml

Sterilize in the autoclave (5.3.1) and cool to 45 °C. Add 100 ml Middlebrook ADC enrichment under aseptic conditions and sterilized water (5.2.2.2) to 1 000,0 ml. The pH of the medium shall be equivalent to 6,6 ± 0,2 (5.3.2.4).

5.2.2.4 Diluent

a) General Diluent

Tryptone Sodium Chloride Solution:

— Tryptone, pancreatic digest of casein	1,0 g
— Sodium chloride (NaCl)	8,5 g
— Water (5.2.2.2)	to 1 000,0 ml

[2] This Malt extracts from Difal and OXOID are examples of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product.

Sterilize in the autoclave (5.3.1). After sterilization the pH (5.3.2.4) of the general diluent shall be equivalent to 7,0 ± 0,2.

b) Glycerol Diluent (for *Pseudomonas aeruginosa* only)

Tryptone Sodium Chloride Glycerol Solution:

—	Tryptone, pancreatic digest of casein	1,0 g
—	Sodium chloride (NaCl)	8,5 g
—	Glycerol [bibliographic reference 1]	2,0 g
—	Water (5.2.2.2)	to 1 000,0 ml

Sterilize in the autoclave (5.3.1). After sterilization the pH (5.3.2.4) of the diluent shall be equivalent to 7,0 ± 0,2.

This modified diluent [5.2.2.4 b)] should be only used for the preparation of the test suspension of *Pseudomonas aeruginosa* (5.4.1.4). All further dilutions should be done with the general diluent [5.2.2.4 a)].

5.2.2.5 Neutralizer

The neutralizer shall be validated for the product being tested in accordance with 5.5.1.2 and 5.5.2. It shall be sterile.

Information on neutralizer that has been found to be suitable for some categories of products is given in Annex B.

5.2.2.6 Sterile defibrinated sheep blood

The sterile defibrinated sheep blood can be acquired from a commercial supplier.

5.2.2.7 Hard water for dilution of products

a) Hard water general

For the preparation of 1 l 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 a refrigerator (5.3.2.8) for no longer than one month.

— Prepare solution B: dissolve 35,02 g sodium bicarbonate ($NaHCO_3$) in water and dilute to 1 000 ml. Sterilize by membrane filtration (5.3.2.7). Store the solution in a refrigerator (5.3.2.8) for no longer than one week.

— Place 600 ml to 700 ml 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 (5.3.2.4) of the hard water shall be 7,0 ± 0,2. 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.

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 expressed as calcium carbonate ($CaCO_3$) is in the test tube lower than 375 mg/l.

b) Hard water with the addition of polysorbate 80

Use the procedure described in 5.2.2.7 a). At the end add 1 g of polysorbate 80 per litre. Sterilized by membrane filtration. The hard water with the addition of polysorbate 80 shall be freshly prepared under aseptic conditions and used within 12 h.

5.2.2.8 Interfering substances

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, 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 general diluent [5.2.2.4 a)]

Sterilize by membrane filtration (5.3.2.7), keep in a refrigerator (5.3.2.8) and use within 1 month.

The final concentration of the bovine albumin in the test procedure (5.5) is 0,3 g/l.

5.2.2.8.3 Dirty conditions (mixture of bovine albumin solutions – high concentration with sheep erythrocytes)

Dissolve 3,0 g of bovine albumin fraction V (suitable for microbiological purposes) in 97 ml of general diluent [5.2.2.4 a)].

Sterilize by membrane filtration (5.3.2.7).

Prepare at least 8,0 ml fresh sterile defibrinated sheep blood (5.2.2.6). Centrifuge the sheep blood at 800 g_N for 10 min. After discarding the supernatant, resuspend erythrocytes in general diluent [5.2.2.4 a)]. Repeat this procedure at least 3 times, until the supernatant is colourless. Resuspend 3,0 ml of the packed sheep erythrocytes in the 97 ml of sterilized bovine albumin solution (see above). To avoid contamination this mixture should be split in portions probably needed per day and kept in separate containers for a maximum of 7 days in a refrigerator at 2 °C to 8 °C.

The final concentration of bovine albumin and sheep erythrocytes in the test procedure (5.5) shall be 3,0 g/l and 3,0 ml/l respectively.

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)].