SLOVENSKI STANDARD
oSIST prEN 16616:2021

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Kemična razkužila in antiseptiki - Termokemično razkuževanje tekstila - Preskusna metoda in zahteve (faza 2, stopnja 2)

Chemical disinfectants and antiseptics - Chemical-thermal textile disinfection - Test method and requirements (phase 2, step 2)

Chemisches Desinfektionsmittel und Antiseptika - Chemothermische Wäschedesinfektion - Prüfverfahren und Anforderungen (Phase 2, Stufe 2)

Désinfectants chimiques et antiseptiques - Désinfection thermochimique du textile - Méthode d'essai et prescriptions (phase 2, étape 2)

Ta slovenski standard je istoveten z: **prEN 16616**

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Chemical disinfectants and antiseptics - Chemical-thermal textile disinfection - Test method and requirements (phase 2, step 2)

Désinfectants chimiques et antiseptiques - Désinfection thermochimique du textile - Méthode d'essai et prescriptions (phase 2, étape 2)

Chemisches Desinfektionsmittel und Antiseptika - Chemothermische Wäschedesinfektion - Prüfverfahren und Anforderungen (Phase 2, Stufe 2)

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If this draft becomes a European Standard, 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.

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

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European foreword

This document (prEN 16616:2020) has been prepared by Technical Committee CEN/TC 216 “Chemical disinfectants and antiseptics”, the secretariat of which is held by AFNOR.

This document is currently submitted to the CEN Enquiry.

This document will supersede EN 16616:2015.

This document has been prepared under a standardization request given to CEN by the European Commission and the European Free Trade Association, and supports essential requirements of EU Directive.

For relationship with EU Directive, see informative Annex ZA, which is an integral part of this document.

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:

— the scope is adapted to the scope of the WG 1;

— in Clause 4 the requirements for phase 2, step 1 tests were deleted;

— in 5.1. some editorial changes were done;

— in 5.2.2.4 water for the test and reference control were added;

— old 5.2.2.6 was deleted, because it was not used in the standard;

— the example for washing machines in 5.3.2.18 of the previous version was switched to Annex D;

— Potter S1 apparatus was added (5.3.2.21);

— 5.4.3.1 to 5.4.3.3 were adapted to other current standards;

— 5.4.4 was reviewed (editorial changes and better description);

— the description of the neutralization (5.5.1.2) was re-worded, a reference to phase 2, step 1 tests was added;

— in 5.4.3.1 and 5.4.3.2 an information on using a spectrophotometer for counting cell numbers of mycobacteria was added (NOTE);

— in 5.4.5 some editorial changes were done;

— 5.4.2 (old version) was deleted;

— the choice of the neutralizer shall be documented and justified in the test report (5.5.1.2);

— 5.5.1.4 was deleted, no longer necessary;

— in 5.5.2.1 a new NOTE was added;

— in 5.5.2.2 a NOTE and the reference to Annex G was added, c) is deleted, no longer necessary;

— the reference control was added (5.5.2.3);

— *R*II was deleted (5.5.2.5);

— 5.5.2.6 to 5.5.2.8 were deleted, a reference to phase 2, step 1 tests was added;

— in 5.5.2.9 some editorial changes were done;

— in 5.6.1.1 the table was corrected and adapted to the current tests;

— in 5.6.2.1 two paragraphs are added (former 5.6.2.3);

— in 5.6.2.2 the V_c-values will be expressed per carrier (former per ml);

— In 5.6.2.3 the calculation of N and N_0 was added, the formula was corrected;

— in 5.6.2.4 the calculation was changed to values per carrier, the formula was corrected and the weighted mean was added in all calculations;

— in 5.6.2.4 the requirements for N_w were corrected;

— 5.6.2.5 was deleted, a reference to phase 2, step 1 test was added;

— 5.6.2.6 calculation was changed: only N_w will be calculated, *R*I is not counted and *R*II, *B* and *C* are no longer used in the standard;

— the example in 5.7.2 was corrected;

— 5.7.3 was adapted to the tests in the current version;

— in 5.8.1 some editorial changes were done;

— in 5.10 a new test report was added;

— Annex A was adapted to EN 12353;

— Annex C was corrected;

— addition of a new Annex D “Example of a washing machine”;

— addition of a new Annex E “Preparation of hard water for using in the test and reference procedure”;

— addition of a new Annex F “Example of a test report”;

— addition of a new Annex G “Example for loading the washing machine”.

The changes of this revision have no impact on the test results obtained with reference to the version EN 16616:2015. Those results are still valid except the reduction of the reference control N_W was higher than 3 lg and/or the calculation of the results followed the wrong way of version 2015.

Introduction

This document specifies a carrier test for establishing whether a single-wash disinfecting product or combination of products for the treatment of contaminated textile has or does not have necessary microbicidal activity. This document only intends to validate the disinfection part of the laundry process.

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 could influence its action in practice.

The conditions are intended to cover general purposes and to allow reference between microbiological laboratories and types of detergents and disinfectants. Each effective dosage of the chemical disinfectant found by this test corresponds only to the chosen experimental conditions. Where actual conditions vary additional testing in microbiological laboratories is needed to determine the effective dosage. Instructions for use are the responsibility of manufactures of detergents or disinfectants.

1 Scope

This document specifies a test method and the minimum requirements for the microbicidal activity of a defined disinfection process for the treatment of contaminated textile. This procedure is carried out by using a washing machine as defined in 5.3.2.18 and refers to the disinfection step without prewash. This procedure is not limited to certain types of textile. The suppliers' instructions are expected to be sufficient to allow the method in this document to be carried out fully (e.g. dosing disinfectant in whatever washing phase e.g. main wash, rinsing, disinfecting at 40 °C).

This document applies 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 could occur in the workplace and in the home.

It could also include services such as laundries and kitchens supplying products directly for the patients.

The method described is intended to determine the activity of a product or product combination under the conditions in which they are used. This is a phase 2, step 2 laboratory test that simulates the conditions of application of the product.

NOTE This method corresponds to a phase 2, step 2 test (see EN 14885).

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, *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 13624, *Chemical disinfectants and antiseptics — Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity in the medical area — Test method and requirements (phase 2, step 1)*

EN 13727, *Chemical disinfectants and antiseptics — Quantitative suspension test for the evaluation of bactericidal activity in the medical area — Test method and requirements (phase 2, step 1)*

EN 14348, *Chemical disinfectants and antiseptics — Quantitative suspension test for the evaluation of mycobactericidal activity of chemical disinfectants in the medical area including instrument disinfectants - Test methods and requirements (phase 2, step 1)*

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 terminological databases for use in standardization at the following addresses:

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

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

3.1
liquor ratio
ratio of the weight of dry textile in kilograms and volume of wash liquor in litres (w/v)

3.2
disinfection process
process taking into account practical conditions of application of the product including contact time, temperature, test organisms and interfering substances to disinfect the textile

3.3
treatment of contaminated textile
handling the textile according the disinfection process to obtain disinfected textile

4 Requirements

The test results shall fulfil the basic limits (see 5.7.3).

Table 1 — Minimum and additional test conditions

Test conditions	Textile disinfection temperature < 60°C	Textile disinfection temperature ≥ 60°C
Test organism	*E. coli* K12 *E. hirae* *P. aeruginosa* *S. aureus* *C. albicans*	*E. faecium*[b]
Additional test organism Fungicidal activity Tuberculocidal activity Mycobactericidal activity	*A. brasiliensis* *M. terrae* *M. terrae* and *M. avium*	
Temperature	As recommended by the manufacturer and < 60°C[a]	As recommended by the manufacturer and ≥ 60°C[a]
Contact time	As recommended by the manufacturer	As recommended by the manufacturer
Interfering substance	Sheep blood	Sheep blood
NOTE The implementation of bacterial spores and viruses was discussed. Further development is necessary to make it technically feasible.		

[a] The temperature and the contact time shall be chosen on the basis of the practical conditions of the product application and within the responsibility of the manufacturer.

[b] This includes bactericidal, yeasticidal, fungicidal, tuberculocidal and mycobactericidal activity.

5 Test methods

5.1 Principle

Carriers made of cotton fabric (5.3.2.16) are contaminated with a test suspension of microorganisms in defibrinated sheep blood (5.2.2.12). After drying the carriers are transferred into cotton towels and then the disinfection process in the washing machine is performed at test temperatures either $t < 60$ °C or $t \geq 60$ °C. The process refers to the disinfection step without prewash. At the end of the disinfection step of the procedure, the towels with the carriers have to be taken out. Each carrier is transferred into a separate tube containing neutralizer (5.2.2.11) and glass-beads (5.3.2.11). The microorganisms should be recovered from the carriers by shaking. The number of surviving microorganisms in each sample is determined and the reduction rate is calculated.

5.2 Materials and reagents

5.2.1 Test organisms

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

— *Pseudomonas aeruginosa* ATCC 15442
— *Escherichia coli* (K12) NCTC 10538
— *Staphylococcus aureus* ATCC 6538
— *Enterococcus hirae* ATCC 10541
— *Enterococcus faecium* ATCC 6057

The yeasticidal/fungicidal activity shall be evaluated using the following test organisms:

— *Candida albicans* ATCC 10231
— *Aspergillus brasiliensis* (formerly *Aspergillus niger* ATCC 16404) ATCC 16404

The tuberculocidal/mycobactericidal activity shall be evaluated using the following test organisms:

— *Mycobacterium terrae* ATCC 15755
— *Mycobacterium avium* ATCC 15769

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

The required incubation temperatures for these test organisms are (36 ± 1) °C (5.3.2.3) [*C. albicans* and *A. brasiliensis*: (30 ± 1) °C]. The same temperature shall be used for all incubations performed during a test and its controls and validation.

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/species, 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 stored under a reference for five years.

[1] 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.

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 growth of 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 used for preparation of media

The water shall be fresh distilled water and not just demineralized water. Sterilize in the autoclave [5.3.2.1a)].

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.

5.2.2.3 Hard water for dilution of products for validation tests

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 [5.3.2.1a)]. Autoclaving, if used, could 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) at (2 to 8) °C for no longer than one month.

— Prepare solution B: Dissolve 35,02 g sodium bicarbonate ($NaHCO_3$) 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) at (2 to 8) °C 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 with the use of a pipette (5.3.2.9) 6,0 ml 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 ± 0,2, when measured at (20 ± 1) °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 (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.

5.2.2.4 Water for the test and reference control

Water with potable water quality is necessary (the water should contain less than 100 cfu/ml of bacteria at 36 °C and 22 °C). Water hardness shall be logged and mentioned in the laboratory protocol and shall be documented in the test report. The final hardness shall be equal or higher than 4 mmol/l alkaline earth ions (Mg^{2+} and Ca^{2+}). The temperature of the water influx should be between (12 and 20) °C.

NOTE 1 Annex E indicates how to adjust the water hardness for the test and reference procedure using solutions A and B from 5.2.2.3. The water hardness could be measured using commercial available test kits.

NOTE 2 Water supplier data can be used to document water hardness.

5.2.2.5 Tryptone Soy Agar (TSA)

Tryptone, pancreatic digest of casein	15,0 g
Soy 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 [5.3.2.1a)]. After sterilization the pH of the medium shall be equivalent to 7,2 ± 0,2 when measured at (20 ± 1) °C.

In case of encountering problems with neutralization (5.5.1.2 and 5.5.1.3) it could be necessary to add neutralizer to the TSA. Annex B gives guidance on the neutralizers that may be used.

5.2.2.6 Tryptone Soy Broth (TSB)

Tryptone, pancreatic digest of casein	15,0 g
Soy peptone, papaic digest of soybean meal	5,0 g
Sodium chloride (NaCl)	5,0 g
Water (5.2.2.2)	to 1 000,0 ml

Sterilize in the autoclave [5.3.2.1a)]. After sterilization the pH of the medium shall be equivalent to 7,2 ± 0,2 when measured at (20 ± 1) °C.

5.2.2.7 Brain Heart Infusion Agar (BHI)

EXAMPLE

Brain heart infusion	12,5 g
Beef heart infusion	5,0 g
Proteose-Peptone	10,0 g
Glucose	2,0 g
Sodium chloride (NaCl)	5,0 g
Disodiumhydrogen phosphate	2,5 g
Agar	10,0 g
Water (5.2.2.2)	to 1 000,0 ml

Sterilize in the autoclave [5.3.2.1a)]. After sterilization the pH of the medium shall be equivalent to 7,2 ± 0,2 when measured at (20 ± 1) °C.

5.2.2.8 Malt Extract Agar (MEA)

Malt extract	30,0 g
Soy peptone, papaic digest of soybean meal	3,0 g

Agar	15,0 g
Water (5.2.2.2)	to 1 000,0 ml

Sterilize in the autoclave [5.3.2.1a)]. After sterilization the pH of the medium shall be equivalent to 5,6 ± 0,2 when measured at (20 ± 1) °C.

5.2.2.9 Middlebrook and Cohn 7H10 medium incl. 10 % OADC (7H10)

Middlebrook 7H10 agar	19,0 g
Glycerol	5,0 ml
Water (5.2.2.2)	to 900,0 ml

Heat to boiling to dissolve completely. Sterilize for 10 min in the autoclave [5.3.2.1a)] and cool to 50 °C to 55 °C. Add 100 ml Middlebrook OADC enrichment under aseptic conditions. The final pH of the medium shall be equivalent 6,6 ± 0,2 when measured at (20 ± 1) °C.

5.2.2.10 Diluent

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

Sterilize in the autoclave [5.3.2.1a)]. After sterilization, the pH of the diluent shall be equivalent to 7,0 ± 0,2 when measured at (20 ± 1) °C.

5.2.2.11 Neutralizer

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

5.2.2.12 Sterile defibrinated sheep blood

The sterile defibrinated sheep blood (aseptic blood-letting and preparation) pooled from more than one sheep can be acquired from a commercial supplier.

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 [5.3.2.1a)];

b) by dry heat, in the hot air oven [5.3.2.1b)].

5.3.2 Usual microbiological laboratory equipment[2]

and, in particular, the following:

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