SLOVENSKI STANDARD
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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)

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ICS

English Version

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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Foreword

This document (prEN 16616:2013) 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.

Introduction

This European Standard 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. The standard does not intend to validate the full laundry process from washing to folding.

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 practice.

The conditions are intended to cover general purposes and to allow reference between laboratories and product types. Each utilization concentration of the chemical disinfectant found by this test corresponds to the chosen experimental conditions. However, for some applications the instructions of use of a product may differ and therefore additional test conditions need to be investigated.

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1 Scope

This European Standard 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 5.3.2.17 and refers to the disinfection step without prewash. This procedure is not limited to certain types of textile. The standard should be able to be conduct fully according to the supplier recommendation (e.g. dosing disinfectant in whatever washing phase e.g. rinsing, disinfecting at 20 °C).

This European Standard applies to areas and situations where disinfection is indicated. Such indications occur in patient care, for example:

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

— in schools, kindergartens and of nursing homes;

— institutions where patients are accommodated, which could suffer from transmissible diseases;

— other applications where hygienic treatment of textile is necessary (e.g. food processing, hotels, workwear e. g. from the pharmaceutical industry, laboratories, foodstuffs area or similar institutions).

The method described is intended to determine the activity of a product or product combination under the conditions in which they are used.

NOTE This method corresponds to a phase 2, step 2 test (Annex F).

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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*

EN 13624: *Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of fungicidal and yeasticidal activity in the medical area - Test method and requirements (phase2, 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

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

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3.1
Liquor ratio
Ratio of the weight of dry textile in kg and volume of wash liquor in litre (w/v)

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3.2
Textile disinfection
Reduction of the number of microorganisms in or on textile, achieved by the irreversible action of a product-related process on their structure or metabolism, to a level judged to be appropriate for a defined purpose

3.3
Disinfection process
The process that takes into account practical conditions of application of the product including contact time, temperature, test organisms and interfering substances to disinfect the textile.

3.4
Treatment of contaminated textile
Handling the textile according the disinfection process to obtain disinfected textile.

4 Requirements

a) Processes with temperatures < 60°C

A chemical-thermal textile disinfection process, when tested in accordance with clause **5**, is considered effective when in three test runs (within a given time, with a suggested dosage, at a suggested temperature and liquor ratio) a reduction of bacteria in and on germ carriers of more than 7 log-units, a reduction of *Candida albicans* and *Aspergillus brasiliensis* of 6 lg-units, a reduction of mycobacteria (*M. avium and/or M. terrae*) of 7 lg-units will be achieved.

NOTE The implementation of spores and viruses was discussed. Further development is necessary to make it technical feasible.

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Further, no test organisms are to be detected in 100 ml washing/disinfection liquid.

As a minimum requirement the bactericidal activity shall be evaluated using the following test organisms: *Pseudomonas aeruginosa, Escherichia coli* (K12)*, Staphylococcus aureus, Enterococcus hirae* and *t*he yeasticidal activity shall be evaluated with *Candida albicans* as test organism.

If an additional fungicidal activity is claimed, also *Aspergillus brasiliensis* shall be used as test organism.

In case of an additional proof of tuberculocidal activity, *Mycobacterium terrae* is to be tested.

If a mycobactericidal effect should be confirmed, it is necessary to perform the test also with *Mycobacterium terrae* and *Mycobacterium avium.*

b) Processes with temperatures ≥ 60°C

A chemical-thermal textile disinfection process, when tested in accordance with clause 5, is considered as effective when in three test runs (within a given time, with a suggested dosage, at a process temperature ≥ 60°C and a defined liquor ratio) a reduction of *Enterococcus faecium* in and on germ carriers of more than 7 lg-units will be achieved.

Further, no test organisms are to be detected in 100 ml washing/disinfection liquid. This includes fungicidal, mycobactericidal / tuberculocidal activity.

5 Test methods

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5.1 Principle

Germ carriers made of cotton fabric are contaminated with a test suspension of microorganisms in defibrinated sheep blood. After drying the carriers are transferred into cotton bags and then the disinfection process in the washing machine is performed at temperatures below 60°C or at/above 60°C. The process refers to the disinfection step without prewash. At the end of the disinfection step of the procedure the bags with the carriers have to be taken out (see 5.3.2.18). Each carrier is transferred into a separate tube containing neutralizer and glass-beads. The bacteria should be recovered from the carriers by shaking. The number of surviving bacteria 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)	ATCC 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*	ATCC 16404

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

- *Mycobacterium avium*	ATCC 15769
- *Mycobacterium terrae*	ATCC 15755

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

The required incubation temperature for these test organisms is (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 control 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, 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

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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.

5.2.2.1.1 Water used for preparation of media

a) 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.

NOTE 3 See 5.2.2.2.2 for the procedure to prepare hard water.

5.2.2.1.2 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 (5.2.2.2.1) and dilute to 1000 ml. Sterilize by membrane filtration (5.3.2.7) or in the autoclave [5.3.2.1a)]. Autoclaving – if used - may cause a loss of liquid. In this case make up to 1000 ml with water

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(5.2.2.2.1) 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 (5.2.2.2.1) and dilute to 1000 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 (5.2.2.2.1) in a 1000 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 1000 ml with water (5.2.2.2.1). 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 hours.

NOTE When preparing the product test solutions (5.4.2), the addition of the product to the hard water produces different final water hardness in each test tube. In any case the final hardness is lower than 375 mg/l of calcium carbonate ($CaCO_3$) in the test tube.

5.2.2.2 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 (5.2.2.1)	to 1000,0 ml

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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.

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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 TSA. Annex B gives guidance on the neutralizers that may be used.

5.2.2.3 Tryptonr Soy Broth (TSB)

Tryptone, pancreatic digest of casein	17,0 g
Soy peptone, papaic digest of soybean meal	3,0 g
Sodium chloride (NaCl)	3,0 g
Dipotassium phosphate (K2HPO4)	2,5 g
Glucose	2,5 g
Water (5.2.2.2)	to 1000,0 ml

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

5.2.2.4 Brain Heart Infusion Agar (BHI)

Brain heart infusion	12,0 g
Beef heart infusion	5,0 g

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Proteose-Peptone	10,0 g
Glucose	2,0 g
Sodium chloride (NaCl)	5,0 g
Dinatriumhydrogenphosphate	2,5 g
Agar	10,0 g
Water (5.2.2.2)	to 1000,0 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.

5.2.2.5 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 1000,0 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.

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5.2.2.6 Middlebrook & 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

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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 (25 ± 1) °C.

5.2.2.7 Diluent

Tryptone, pancreatic digest of casein	1,0 g
Sodium chloride (NaCl)	8,5 ml
Water (5.2.2.2)	to 1000,0 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.

5.2.2.8 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.

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5.2.2.9 Sterile defibrinated sheep blood

The sterile defibrinated sheep blood 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:

5.3.2.1 Apparatus for sterilization (moist and dry heat)

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

b) for dry heat sterilization, a hot air oven capable of being maintained at $(180\,^{+5}_{0})$°C for a minimum contact time of 30 min, at $(170\,^{+5}_{0})$°C for a minimum contact time of 1 h or at $(160\,^{+5}_{0})$°C for a minimum contact time of 2 h.

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5.3.2.2 Water baths, capable of being controlled at (20 ± 1) °C, at (45 ± 1) °C (to maintain melted medium in case of pour plate technique) and at additional test temperatures ± 1 °C.

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5.3.2.3 Incubator, capable of being controlled either at (30 ± 1) °C or (36 ± 1) °C. The same temperature shall be used for incubations performed during a test and its control and validation.

5.3.2.4 pH-meter, having an inaccuracy of calibration of no more than ± 0.1 pH units at (20 ± 1) °C.

NOTE A puncture electrode or a flat membrane electrode should be used for measuring the pH of the agar media (**5.2.2.3** to **5.2.2.7**).

5.3.2.5 Stopwatch, a digital stopwatch is recommended

5.3.2.6 Shakers

a) Electromechanical agitator, e.g. Vortex® mixer[3)]

b) Orbital shaker

5.3.2.7 Membrane filtration apparatus, constructed of a material compatible with the substances to be filtered, with a filter holder of at least 50 ml volume, and suitable for use of filters of diameter 47 mm to 50 mm and 0,45 µm pore size for sterilization of hard water (**5.2.2.10**).

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

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

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The vacuum source used shall give an even filtration flow rate. In order to obtain a uniform distribution of microorganisms over the membrane and 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.

5.3.2.8 **Refrigerator**, capable of being controlled at 2 °C to 8 °C

5.3.2.9 **Graduated pipettes,** of nominal capacities 10 ml and 1 ml and 0,1 ml, or calibrated automatic pipettes

5.3.2.10 **Petri dishes,** (plates) of size 90 mm to 100 mm

5.3.2.11 **Glass beads (diameter 3 mm to 4 mm)**

5.3.2.12 **Volumetric flasks**

5.3.2.13 **Centrifuge** (3000 g_N)

5.3.2.14 **Coned bottom screw cap tubes** (contents of 50 ml, diameter: about 28 mm)

5.3.2.15 **Cylindrical screw vial** (contents about 15 ml to 50 ml) for recovery of the test organisms from the carriers

5.3.2.16 **Cotton carriers,** 1 cm2 (The germ carriers are prepared by using standard cotton fabric thoroughly cooked in double-distilled water three times. The fabric is cut into 1 cm^2 cotton carriers and autoclaved (see 5.3.2.1). For practical reasons the 1 cm² carriers are put in the pockets of a bigger cotton towel.

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NOTE The incorporation of the test suspension can be improved if the carriers are not dried after autoclaving.

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Mass per unit area:	(170 ± 10) g/m² (real 160 g/m²)
Fibrous material (wrap and weft):	cotton, double carded
Fibre length (wrap and weft):	at least 27 mm
Yarn linear density (wrap and weft):	(295 ± 10) dtex
Yarn twist (wrap and weft):	Z-twist (700 ± 25) t/m
Weave:	plain wave 1
Threads per unit length:	270 threads/dm each

The cotton control cloth shall be bleached, unfinished and not brightened.
After three washes the maximum tensile strength, wet, in the warp should be (63 ± 5) daN

Note: Cotton proofed in accordance with DIN 53919 *[6]* fulfils the requirements.

5.3.2.17 **Machine ballast load and preparation of ballast load**

Textile of poly cotton (65 % polyester / 35 % cotton) shall be used. For special purposes textile of cotton (100 %) can be used.

NOTE The bounded liquor is higher in pure cotton than in poly cotton.

Amount of ballast load: 80 % ± 5 % of the max. capacity.

The ballast load should be washed for 30 min at 80 °C to 90 °C without additives following at least 3 rinsing steps and sterilized in the autoclave prior to first use.

The ballast load should be used for not more than 100 washing cycles (incl. preparation and disinfection cycles).

After disinfection testing the ballast load should be washed for 30 min at 80 °C to 90 °C following at least 5 rinsing steps (at least 5 min each) and sterilized in the autoclave prior to use.