BS EN 16616:2015

BSI Standards Publication

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

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National foreword

This British Standard is the UK implementation of EN 16616:2015.

BSI, as a member of CEN, is obliged to publish EN 16616 as a British Standard. However, attention is drawn to the fact that during the development of this European Standard, the UK committee voted against its approval as a European Standard.

The UK committee believes that this method is limited to washer-extractors. It is also considered that some of the reduction in microbial numbers achieved in the test conditions (in which a very small proportion of the load is contaminated) may be due to redistribution of the contamination amongst the load. Hence the antimicrobial efficacy measured by this test method may be significantly higher, and therefore potentially misleading, than that obtained in practice if a greater proportion of the load is contaminated.

The UK participation in its preparation was entrusted to Technical Committee CH/216, Chemical disinfectants and antiseptics.

A list of organizations represented on this committee can be obtained on request to its secretary.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

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ISBN 978 0 580 82797 6

ICS 11.080.20

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This British Standard was published under the authority of the Standards Policy and Strategy Committee on 31 August 2015.

Amendments/corrigenda issued since publication

Date	Text affected

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EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 16616

August 2015

ICS 11.080.20

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)

This European Standard was approved by CEN on 3 July 2015.

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European foreword

This document (EN 16616:2015) 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 February 2016, and conflicting national standards shall be withdrawn at the latest by February 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 has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association, and supports essential requirements of EU Directive(s).

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

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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 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 may 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 shall be needed to determine the effective dosage. Instructions for use are the responsibility of manufactures of detergents or disinfectants.

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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 5.3.2.18 and refers to the disinfection step without prewash. This procedure is not limited to certain types of textile. The suppliers instructions shall be sufficient to allow the method in the standard to be carried out fully (e.g. dosing disinfectant in whatever washing phase e.g. rinsing, disinfecting at 40 °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 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. 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).

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 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.

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3.1
liquor ratio
ratio of the weight of dry textile in kilogram and volume of wash liquor in litre (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).

The following phase 2, step 1 test shall be passed in addition to this test: EN 13727, EN 13624 and EN 14348 under the following test conditions:

— temperature as recommended by the manufacturer;

— contact time recommended by the manufacturer;

— dirty conditions and

— reduction as recommended for instrument disinfection.

For products used > 60 °C EN 13624 and EN 14348 should be passed with *Aspergillus brasiliensis* and *M. avium*.

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 is reached.

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

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

As a minimum requirement the bactericidal and yeasticidal activity shall be evaluated using the following test organisms: for bactericidal activity *Pseudomonas aeruginosa, Escherichia coli* (K12), *Staphylococcus aureus, Enterococcus hirae* and for yeasticidal activity *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.*

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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

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 test temperatures either t < 60°C or ≥ 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* (formerly *Aspergillus Niger* ATCC 16404) ATCC 16404

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.

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1) The ATCC numbers are the collection numbers of strains supplied by the American Type Culture Collections (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.

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The required incubation temperature for these test organisms is (36 ± 1) °C or (37 ± 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/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.

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 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

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 5.2.2.3 for the procedure to prepare hard water.

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 - 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) at 2 °C 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 °C 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

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(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.

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.

5.2.2.4 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 may be necessary to add neutralizer to the TSA. Annex B gives guidance on the neutralizers that may be used.

5.2.2.5 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.

5.2.2.6 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
Dinatriumhydrogenphosphate	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.

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5.2.2.7 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.

5.2.2.8 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 (25 ± 1) °C.

5.2.2.9 Diluent

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

5.2.2.10 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.

5.2.2.11 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)].

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