SLOVENSKI STANDARD
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Chemical disinfectants and antiseptics - Quantitative test method for the evaluation of sporicidal activity against Clostridioides difficile on non-porous surfaces with mechanical action employing wipes in the medical area (4- field test) - Test method and requirements (phase 2, step 2)

Chemische Desinfektion und Antiseptika - Quantitativer Prüfversuch zur Bestimmung der sporiziden Wirkung gegen Clostridioides difficile auf nicht-porösen Oberflächen mit mechanischer Einwirkung mit Hilfe von Tüchern 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 sporicide contre Clostridioides difficile sur des surfaces non poreuses, avec action mécanique à l'aide de lingettes dans le domaine médical (essai à 4 zones) - Méthode d'essai et prescriptions (phase 2, étape 2)

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Chemical disinfectants and antiseptics - Quantitative test method for the evaluation of sporicidal activity against *Clostridioides difficile* on non-porous surfaces with mechanical action employing wipes in the medical area (4-field test) - Test method and requirements (phase 2, step 2)

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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 17846:2022) has been prepared by Technical Committee CEN/TC 216 “Chemical disinfectants and antiseptics”, the secretariat of which is held by AFNOR.

This document is a working document.

Introduction

This document specifies a carrier test for establishing whether a chemical disinfectant for use on surfaces administered with wipes has a sporicidal activity against *Clostridioides difficile* 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 or 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 sporicidal activity against spores of *Clostridioides difficile* 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 applies to products that are used in the medical area for disinfecting non-porous surfaces including surfaces of medical devices by wiping – regardless if they are covered by the 93/42/EEC Directive on Medical Devices 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 method.

The document is applicable for four method 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) preimpregnation of specified wipes or mops by the manufacturer as ready-to-use wipes or mops.

In all types of application the water control has to be done with the standard wipe [5.3.2.17 a)], because it is a process or method control.

This document does not apply to products that are sprayed on or flooding surfaces, then left until the contact application phase 2, step 2 standards without mechanical action should be used and their methods performed.

The test surface (5.3.2.16) was selected as standard surface and should cover all non-porous surfaces. It was not intended to cover the influence of each different surface.

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 may occur in the workplace and in the home. It may 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, *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 17126, *Chemical disinfectants and antiseptics — Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectants in the medical area — Test method 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 apply.

3.1
pre-impregnated wipe
ready-to-use 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 wipe soaked in disinfectant, a wipe sprayed with disinfectant.

4 Requirements

The product, when diluted with hard water or – in the case of ready-to-use products – with water, and tested in accordance with Clause 5 under simulated clean conditions (0,3 g/l bovine albumin) or simulated dirty conditions (3,0 g/l bovine albumin + 3,0 ml/l sheep erythrocytes) according to its practical applications and under the following test conditions: temperature between 4 °C and 30 °C, contact time min. 1 min and max. either 30 min or 60 min[1)] shall demonstrate at least a decimal log (lg) reduction in counts of 4 on test field 1. The mean of the number of cfu per 25 cm^2 on the test fields 2 to 4 shall be equal or less than 50, the mean of the number of cfu on 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.

The sporicidal activity against *Clostridioides difficile* spores shall be evaluated using the following test organisms: *Clostridioide difficile* R027.

Where indicated, additional specific sporicidal activity shall be determined applying other contact times and test organisms in order to take into account intended specific use conditions.

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

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 *Clostridioides difficile* (*C. difficile*) spores 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 (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

[1)] See 5.5.1.1 b).

immediately after test field 4 and wiped back to the starting point. In parallel a water control is performed: a wipe is soaked with hard water [5.5.2.2 e)] instead of the product.

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. 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. In parallel to the test with the product water is applied in the same way to ensure that the test organisms are spread on the 4 fields and their number reaches a certain level. The test is performed using *C. difficile* as test organism (minimum test conditions).

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

5.2 Materials and reagents

5.2.1 Test organism

The sporicidal activity against *C. difficile* shall be evaluated using the following strain as test organism[2]:

— Clostridioides difficile R027 NCTC 13366

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.

The required incubation temperature for these test bacteria is 36 °C ± 1 °C or 37 °C ± 1 °C (5.3.2.3) under anaerobic conditions. The same temperature (36 °C or 37 °C) and anaerobic conditions shall be used for all incubations performed during its control and validation.

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 formulas 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.

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[2] The NCTC numbers are the collection numbers of strains supplied by the National Collection of Type Cultures (NCTC). This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of the product named.

All specified pH values are measured at 20 °C ± 1 °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) BHIYT-L Agar

—	Brain heart infusion	37,0 g
—	Yeast extract	5,0 g
—	L-Cysteine	1,0 g
—	Sodium taurocholate	1,0 g
—	Agar	15,0 g
—	Water (5.2.2.2)	to 1 000,0 ml

Sterilize in the autoclave [5.3.2.1 a)]. After sterilization the pH (5.3.2.4) of the medium shall be equivalent to 7,0 ± 0,2. Let the medium cool down to 48 °C ± 2 °C. Dissolve 200 000 units of lysozyme in 10 ml water (5.2.2.2). Sterilize the enzymatic solution by membrane filtration (5.3.2.7).

In case of encountering problems with neutralization (5.5.1.2 and 5.5.1.3) it may be necessary to add neutralizer to BHIYT-L. 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.

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

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.

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.

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 0,30 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 3,00 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 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 g/l and 3 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)].

5.3.2 Usual microbiological laboratory equipment [3)]

and in particular, the following:

5.3.2.1 Apparatus for sterilization:

a) For moist heat sterilization, an autoclave capable of being maintained at $\left(121\ ^{+3}_{0}\right)$ °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}_{0}) °C for a minimum holding time of 30 min, at (170^{+5}_{0}) °C for a minimum holding time of 1 h or at (160^{+5}_{0}) °C for a minimum holding time of 2 h.

5.3.2.2 Water baths, capable of being controlled at 20° C ± 1 °C and at 45 °C ± 1 °C [to maintain melted agar in case of pour plate technique and at additional test temperatures ± 1 °C (5.5.1)].

5.3.2.3 Incubator, capable of being controlled at either 36 °C ± 1 °C or at 37 °C ± 1 °C (5.2.1). The same temperature shall be used for all incubations of the *C. difficile* spores performed during a test and its controls and validation.

5.3.2.4 pH-meter, having an inaccuracy of calibration of no more than ± 0,1 pH units at 20 °C ± 1 °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.

5.3.2.6 Shakers.

Error! Bookmark not defined.) Disposable sterile equipment is an acceptable alternative to reusable glassware.

a) **Electromechanical agitator**, e.g. Vortex® mixer[4)].

b) **Mechanical 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 (5.2.2.7) and bovine albumin (5.2.2.8.2 and 5.2.2.8.3).

The vacuum source used shall give an even filtration flow rate.

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. Calibrated automatic pipettes may be used.

5.3.2.10 Petri dishes (plates) of size 90 mm to 100 mm. (The Petri dishes are needed for pour plate technique and for pre-moistening the standard wipes cloth with 16 ml product test solution or hard water)

5.3.2.11 Glass beads (diameter: 3 mm to 4 mm).

5.3.2.12 Volumetric flasks.

5.3.2.13 Centrifuge capable to being controlled at 2 °C to 8 °C (4 000 g_N).

5.3.2.14 Rectangular glass spatula (4 cm edge length).

5.3.2.15 Loop (metal or plastic).

5.3.2.16 Test-surface, PVC plate free foam, thickness 2 mm; measuring 20 cm × 50 cm. Clean the test-surfaces before using with 2-propanol 70 %. To ensure uniform precleaning a standard wiping cloth is impregnated with 16 ml 2-propanol (v/v) and, using the unitary weight, is wiped once back and forth over the test surface. After drying mark with a pencil or a permanent marker four squares as test fields 1 to 4, each measuring 5 cm × 5 cm, figuring a row at a distance of 5 cm from one another. The row should be approximately in the middle of the test-surface (see Figure 1). The drying controls D_{C0} and D_{Ct} are performed on a smaller test-surface measuring minimum 7 cm × 13 cm - marked with two squares of 5 cm × 5 cm.

Example for the test-surface:

a) PVC plate free foam (20 cm × 50 cm, thickness 2 mm) FOREX classic, white matt finished, one side with foil, art. nr. SFSFOXC020RW1F, thyssenkrupp Plastics, Widdersdorfer Str. 158, 50825 Cologne, Germany[5)].

[4)] Vortex® in an example 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.

[5] This test-surface is an example 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.