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Chemical disinfectants and antiseptics — Quantitative test method for the evaluation of bactericidal and yeasticidal activity 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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National foreword

This British Standard is the UK implementation of EN 16615:2015.

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

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

Antiseptiques et désinfectants chimiques - Méthode d'essai quantitative pour l'évaluation de l'activité bactéricide et levuricide 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)

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

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Foreword

This document (EN 16615:2015) 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of an identical text or by endorsement, at the latest by October 2015 and conflicting national standards shall be withdrawn at the latest by October 2015.

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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 chemical disinfectant for use on surfaces administered with wipes has a bactericidal and yeasticidal 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 or need to be used.

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

1 Scope

This European Standard specifies a test method and the minimum requirements for bactericidal and yeasticidal 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 European Standard 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.

This European Standard includes ‘ready-to-use wipes‘ which are impregnated with a microbicidal solution.

This European Standard 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, 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 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.

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: four selected test organisms, temperature between 4 °C and 30 °C,

contact time min. 1 min and max. either 5 min or 60 min[1)] shall demonstrate at least a decimal log (lg) reduction in counts of 5 (*Staphylococcus aureus*, *Enterococcus hirae, Pseudomonas aeruginosa),*or 4 (*Candida albicans*) on test field 1. The mean of the cfus on the test fields 2 to 4 shall be equal or less than 50, the mean of the cfus of the water control shall be equal or more than 10.

The bactericidal activity shall be evaluated using the following test organisms: *Staphylococcus aureus*, *Enterococcus hirae, Pseudomonas aeruginosa.* The yeasticidal activity shall be evaluated using the following test organism: *Candida albicans.*

Where indicated, additional specific bactericidal and yeasticidal 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 bacteria or yeasts 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 immediately after test field 4 and wiped back to the starting point. In parallel a water control is performed: a wipe is soaked with water (5.5.2.2 e)) instead of the product.

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 cotton 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 *P. aeruginosa*, *S. aureus*, *E. hirae* and *C. albicans* as test organisms (minimum test conditions).

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[2)]:

— *Staphylococcus aureus* ATCC 6538;
— *Pseudomonas aeruginosa,* ATCC 15442;
— *Enterococcus hirae* ATCC 10541.

1) See 5.5.1.1 b).

2) 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.

The yeasticidal activity shall be evaluated using the following strain as test organism[3)]:

— *Candida albicans* ATCC 10231.

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). 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* is 30 °C ± 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 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 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.

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.

3) 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.

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 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 of the medium shall be equivalent to 5,6 ± 0,2 when measured at (20 ± 1) °C (5.3.2.4).

In case of an 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.

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.

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 (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.

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 ml 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 0,30 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 [4)]

and in particular, the following:

4) Disposable sterile equipment is an acceptable alternative to reusable glassware.

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 TSA 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 (bacteria) or 30 °C ± 1 °C (yeasts). The same temperature shall be used for all incubations of the bacteria 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

a) **Electromechanical agitator**, e.g. Vortex® mixer[5)].

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.

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

5.3.2.12 Volumetric flasks.

5.3.2.13 Centrifuge (800 g_N).

5.3.2.14 Rectangular glass spatula (4 cm edge length).

5.3.2.15 Loop (metal or plastic).

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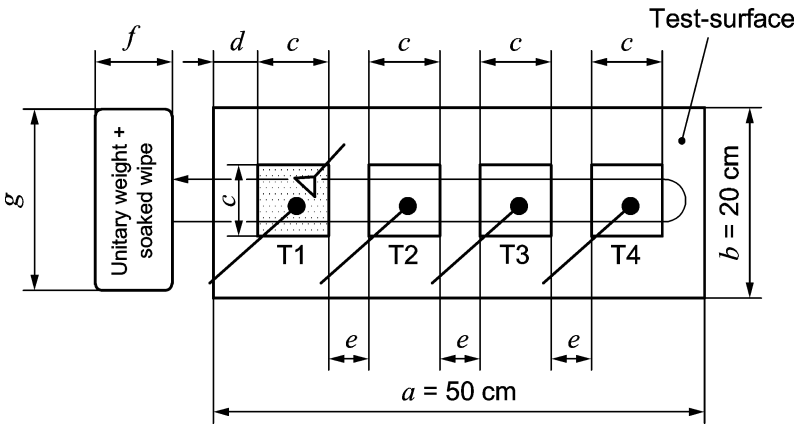
5) Vortex® in an example of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product.

5.3.2.16 Test-surface, PVC with PUR surface coating, thickness 2,5 mm; measuring 20 cm x 50 cm. Clean the test-surfaces before using with n-propanol 70 %. After drying mark with a pencil or a permanent marker four squares as test fields 1 to 4, each measuring 5 cm x 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 x 13 cm - marked with two squares of 5 cm x 5 cm. (Example for the test-surface: "DLW Solid Pur 521-029 2,0 mm, Art.Nr. 6235, manufacturer: "Armstrong", Stuttgarter Str. 75, 74321 Bietigheim Bissingen[6)].



Key

Schematic representation of the test-surface

a = 50 cm *b* = 20 cm

with four test areas T1 to T4 (5 cm x 5 cm) and a given range of wiper wipe.

c = 5 cm *d* = 10 cm *e* = 5 cm

Size of the unitary weight:

f = 8,6 cm *g* = 12,1 cm

Test field 1 is inoculated with 0,05 ml of the test organisms / interfering substance mixture (bacteria 1,5 to 5,0 × 10^9 cfu/ml, corresponding to a final number on test field 1 of 6,75 × 10^7 to 2,25 × 10^8 cfu; for *Candida albicans* 1,5 to 5,0 × 10^8 cfu/ml, corresponding to a final number on test field 1 of 6,75 × 10^6 to 2,25 × 10^7 cfu). The arrow shows the cleaning sweep with the wipe. The starting point is in front of test field 1 and the turn is immediately after test field 4. The end point of the wiping process is the starting point after passing test field 1 for the second time.

Figure 1 — Scheme of the markings and the wipe sweep over four test fields on the test-surface

5.3.2.17 Tools for mechanical action, wipe of 17,5 cm x 28 cm, Composition: 55 % pulp, 45 % Polyethylenterephthalat (PET); (example: "Tork Premium Spezial Tücher", art. nr. 90491, supplier: "SCA Tork"[7)] . Wipes are used only once. Other tools than described above are allowed to be used (see 5.9). The manufacturer is responsible to describe precisely how the wipes are to be used (e.g. the number of layers).

5.3.2.18 Unitary weight, granite block, 12,1 cm long, 8,6 cm wide and 8,6 cm high (the height may differ with other materials), weighing (2,3 to 2,5) kg. The use of the unitary weight standardizes the wiping procedure and simulates the average pressure when wiping is performed in practice.

6) This test-surface is 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.

7) This tool is 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.

5.3.2.19 Swabs (sterile for single use only), the soakable part shall be made from pure cotton, free from substances which might inhibit or promote the action of the product test solution and from substances inhibiting the test organisms.

5.3.2.20 Parafilm (for single use only), to protect the lower horizontal surface and the vertical surfaces of the unitary weight from any contamination during the wiping procedure a parafilm covering these parts is used. This parafilm shall be replaced by a new one after each wiping procedure.(example: Parafilm®[8)] M (100 mm) Art.Nr. 7016 05, BRAND GMBH + CO KG, Postfach 11 55, 97861 Wertheim, Germany).

5.4 Preparation of test organism suspensions and product test solutions

5.4.1 Test organism suspensions

5.4.1.1 General

For each test organism, two different suspensions shall be prepared: the “test suspension” to perform the test and the “validation suspension” to perform the controls and method validation.

5.4.1.2 Preservation and stock cultures of test organisms

The test organism and its stock cultures shall be prepared and kept in accordance with EN 12353.

5.4.1.3 Working culture of test organisms

In order to prepare the working culture of test organisms (5.2.1), prepare a subculture from the stock culture (5.4.1.2) by streaking onto TSA [5.2.2.3 a)] slopes or plates and incubate (5.3.2.3). After 18 h to 24 h prepare a second subculture from the first subculture in the same way and incubate for 18 h to 24 h. From this second subculture, a third subculture may be produced in the same way. The second and/or the third subculture are the working culture(s).

If it is not possible to prepare the second subculture on a particular day, a 48 h subculture may be used for subsequent sub-culturing, provided that the subculture has been kept in the incubator (5.3.2.3) during the 48 h period.

In the case of *Candida albicans* use MEA [5.2.2.3 b)] instead of TSA and incubate 42 h to 48 h instead of 18 h to 24h. The potential prolonged subculturing of 48 h for bacteria requires 72 h in the case of *Candida albicans*.

Never produce and use a fourth subculture.

5.4.1.4 Test suspension (*N*)

a) Take 10 ml of general diluent [(5.2.2.4 a)] and place in a 100 ml flask with 5 g of glass beads (5.3.2.11). Take the working culture (5.4.1.3) and transfer loopfuls (5.3.2.15) of the cells into the general diluent [(5.2.2.4 a)]. The cells should be suspended in the diluent by rubbing the loop against the wet wall of the flask to dislodge the cells before immersing in the diluent. Shake the flask for 3 min using a mechanical shaker [5.3.2.6 b)]. Aspirate the suspension from the glass beads and transfer to a tube.

   In the case of *Pseudomonas aeruginosa* use glycerol diluent [5.2.2.4 b)] instead of general diluent.

b) Adjust the number of cells in the suspension to 1,5 x 10^9 cfu/ml[9)] to 5,0 x 10^9 cfu/ml (*Candida albicans* 1,5 x 10^8 cfu/ml to 5,0 x 10^8 cfu/ml) using the general diluent [(5.2.2.4 a)] estimating the numbers of units

8) Parafilm is 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.

9) cfu/ml = colony forming unit(s) per millilitre.

by any suitable means. Maintain this test suspension in the water bath at 20 °C and use within 2 h. Adjust the temperature according to 5.5.1.1a) and 5.5.1.4 only immediately before the start of the test.

In the case of *Pseudomonas aeruginosa* use glycerol diluent [5.2.2.4 b)] instead of general diluent.

The use of a spectrophotometer for adjusting the number of cells is highly recommended (about 620 nm wavelength – cuvette 10 mm path length). Each laboratory should therefore produce a calibration curve for each test organism, knowing that suitable values of optical density are generally found between 0,150 and 0,460. To achieve reproducible results of this measurement it may be necessary to dilute the test suspension, e.g. 1+9.

NOTE A colourimeter is a suitable alternative.

c) For counting prepare 10^{-7} and 10^{-8} dilutions (for *Candida albicans* 10^{-6} and 10^{-7} dilutions) of the test suspension using general diluent [(5.2.2.4 a)]. Mix [5.3.2.6 a)].

d) Take a sample of 1,0 ml of each dilution in duplicate and inoculate using the pour plate or the spread plate technique.

   1) When using the pour plate technique, transfer each 1 ml sample into separate Petri dishes (i.e. in duplicate = two plates) and add 15 ml to 20 ml melted TSA [5.2.2.3 a)] for bacteria or melted MEA [5.2.2.3 b)] for *Candida albicans*, cooled to 45 °C ± 1 °C.

   2) When using the spread plate technique spread each 1,0 ml sample – divided in portions of approximately equal size – on an appropriate number (at least two – i.e. in duplicate at least four plates) of surface dried plates containing TSA (5.2.2.3 a) for bacteria or MEA for *Candida albicans* (5.2.2.3 b).

For incubation and counting see 5.4.1.6.

5.4.1.5 Validation suspension (N_V)

a) To prepare the validation suspension, dilute the test suspension (5.4.1.4) with the general diluent [(5.2.2.4 a)] to obtain $3{,}0 \times 10^2$ cfu/ml to $1{,}6 \times 10^3$ cfu/ml (about one fourth (1+3) of the 10^{-5} dilution).

NOTE The neutralizer control in this test is different from the neutralizer control N_{VB} in phase 2, step 1 suspension tests (EN 13727 and EN 13624) since the test organisms are not confronted with a higher concentration of neutralizer in the test N_a. Therefore, no 10-fold higher validation suspension for the neutralizer control is prepared.

b) Maintain and use the validation suspension the same way as the test suspension [5.4.1.4 b)].

c) For counting prepare a 10^{-1} dilution with the general diluent [(5.2.2.4 a)]. Mix [5.3.2.6 a)]. Take a sample of 1,0 ml in duplicate and inoculate using the pour plate or the spread plate technique [5.4.1.4 d1) or d2)].

For incubation and counting see 5.4.1.6.

5.4.1.6 Incubation and counting of the test suspensions

a) Incubate (5.3.2.3) the plates for 20 h to 24 h, for *Candida albicans* 40 h to 48 h. Discard any plates which are not countable (for any reason). Count the plates and determine the number of cfu. Incubate the plates for a further 20 h to 24 h (not with *Candida albicans*). Do not recount plates which no longer show well separated colonies. Recount the remaining plates. If the number has increased use only the higher number for further evaluation.

b) Note for each plate the exact number of colonies but record > 330 for any counts higher than 330 and determine the V_C-values according to 5.6.2.2.

c) Calculate the numbers of cfu/ml in the test suspension *N* and the validation suspension N_V using the method given in 5.6.2.3 and 5.6.2.5. Verify according to 5.7.

5.4.2 Product test solution

Product test solutions shall be prepared in hard water (5.2.2.7) at minimum two different concentrations to include one concentration in the active range and one concentration in the non-active range. In the case of ready-to-use products the product as received may be used as one of the product test solutions.

Dilutions of ready-to-use products, i.e. products which are not diluted when applied, shall be prepared in water (5.2.2.2) instead of hard water. The concentration of use (defined by the manufacturer) shall be tested, other concentrations are optional.

For solid products, dissolve the product as received by weighing at least 1 g ± 10 mg of the product in a volumetric flask and filling up with hard water. Subsequent dilutions (i.e. lower concentrations) shall be prepared in volumetric flasks (5.3.2.12) on a volume/volume basis in hard water (5.2.2.7).

For liquid products, dilutions of the product shall be prepared with hard water on a volume/volume basis using volumetric flasks.

The product test solutions shall be prepared freshly and used in the test within 2 h. They shall give a physically homogenous preparation, stable during the whole procedure. If during the procedure a visible inhomogeneity appears due to the formation of a precipitate or flocculate (for example through the addition of the interfering substance), it shall be recorded in the test report.

NOTE Counting microorganisms embedded in a precipitate or flocculate is difficult and unreliable.

Record the test concentration in terms of mass per volume or volume per volume and details of the product sample as received.

5.5 Procedure for assessing the bactericidal and yeasticidal activity of the product

5.5.1 General

5.5.1.1 Experimental conditions

Besides the temperature, contact time, interfering substance and test organism, additional experimental conditions may be selected according to the practical use considered for the product (Clause 4):

a) temperature θ (in °C):

   1) the temperature to be tested is between 4 °C and 30 °C; the deviation for each selected test temperature is ± 2,5 °C.

b) contact time *t* (in min):

   1) The contact times to be tested are those recommended by the manufacturer but not longer than 60 min; the allowed deviation is ± 1 min, for contact times of shorter than 15 min ± 15 s.

   2) The contact times for surface disinfectants are chosen on the basis of the practical conditions of the product. The recommended contact time for the use of the product is within the responsibility of the manufacturer. Products intended to disinfect surfaces that are likely to come into contact with the patient and / or the medical staff and surfaces, which are frequently touched by different people, leading to the transmission of microorganisms to the patient, shall be tested with a contact time of maximum 5 min. The same applies where the contact time of the product shall be limited for practical reasons. Products for other surfaces than stated above may be tested with a contact time of maximum 60 min.

3) The minimum contact time is 1 min (from the beginning of the application until the start of the re-covering process). During the recovery process the product might continue acting. Nevertheless, this time is disregarded and therefore limited to max. 1 min.

c) interfering substance:

1) The interfering substance to be tested is either 0,30 g/l bovine albumin (5.2.2.8.2) representing clean conditions or a mixture of 3 ml/l sheep erythrocytes and 3,0 g/l bovine albumin (5.2.2.8.3) representing dirty conditions – according to Clause 4 and practical applications. Additional interfering substances may be tested according to specific fields of application.

d) test organisms for bactericidal activity (5.2.1):

1) *Staphylococcus aureus;*

2) *Pseudomonas aeruginosa;*

3) *Enterococcus hirae.*

e) test organisms for yeasticidal activity (5.2.1):

1) *Candida albicans.*

5.5.1.2 Selection of neutralizer

The neutralization and/or removal of the bactericidal and yeasticidal and/or bacteriostatic and yeaststatic activity of the product shall be controlled and validated - only for the highest product test concentration - for each of the used test organisms. These procedures (neutralizer control and method validation) shall be performed *once before* the test 5.5.2.2.

To determine a suitable neutralizer carry out the validation of the dilution neutralization method (5.5.2.3 and 5.5.2.4 in connection with 5.5.2.5) using a neutralizer, chosen according to laboratory experience and published data.

If this neutralizer is not valid, repeat the validation test using an alternative neutralizer containing a combination of polysorbate 80 (30 g/l), saponin (30 g/l), L-histidine (1 g/l), lecithin (3 g/l), sodium thiosulphate (5 g/l) in either general diluent [5.2.2.4 a)] or in phosphate buffer 0,002 5 mol/l (see Annex B).

In special circumstances it may be necessary to add neutralizer to TSA [5.2.2.3 a)] or MEA [5.2.2.3 b)]. If neutralizer is added to TSA / MEA the same amount should be added to TSA / MEA used in the test procedure.

If because of problems with neutralization a neutralizer has been added to TSA or MEA (5.5.1.2) used for the validation and control procedures the TSA / MEA used for the test shall contain the same amount of this neutralizer as well.

5.5.1.3 General instruction for validation and control procedures

In the case of ready-to-use-products use water (5.2.2.2) instead of hard water.

5.5.1.4 Equilibration of temperature

Prior to testing, equilibrate all reagents (product test solutions (5.4.2), test suspension (5.4.1.4), diluent (5.2.2.4 a) and 5.2.2.4 b), hard water (5.2.2.7) and interfering substance (5.2.2.8) to the test temperature of θ [5.5.1.1 a)] using the water bath (5.3.2.2) controlled at θ. Observe the provisions laid down in 5.4.1.4 b). Check that the temperature of the reagents is stabilized at θ. In the case of ready-to-use-products, water (5.2.2.2) shall be additionally equilibrated to θ.

The neutralizer (5.2.2.5) shall be equilibrated at a temperature of 20 °C ± 1 °C.

5.5.1.5 Precautions for manipulation of test organisms

Do not touch the upper part of the test tube sides when adding the test- or the validation suspensions (5.4.1).

5.5.1.6 Inoculation of the test-surface

Per contact time, interfering substance and test organism prepare one test-surface (5.3.2.16) for each concentration of the product test solution plus one for the water control (N_W) and one for the drying control. The test-surface for the drying control can be smaller (5.3.2.16). Eight “normal” (5.3.2.16) plus four small test-surfaces are needed for the minimum spectrum of test organisms tested with two concentrations of the product (5.4.2). For the water control minimum 4 “normal” test surfaces are needed.

Pipette 1,0 ml of interfering substance (5.2.2.8) into a tube. Add 9,0 ml of the test suspension (5.4.1.4). Mix [5.3.2.6 a)] and pipette 0,05 ml of this mixture as a spot on each test field 1, marked on the test-surfaces (5.3.2.16) and on the two test fields D_{C0}, i.e. “drying control contact time zero” and D_{Ct}, i.e. “drying control contact time “t” “(small test-surface). The rectangular glass spatula (5.3.2.14) used for distribution of the test suspension (one single for $D_{C0,}$ D_{Ct} and field 1) should be used initially on a blank sample to ensure that field 1 is contaminated with sufficient test suspension. Distribute the spots with one rectangular glass spatula (5.3.2.14) – beginning with D_{C0} - equally within the marked areas. Let the inoculum dry at the test temperature until visible dryness (maximum 60 min). Use the test-surfaces immediately at the end of the drying time.

Note the exact drying time in the test report.

5.5.2 Method[10)]

5.5.2.1 General

a) The test, the controls and validation procedures (see 5.5.2.2 through 5.5.2.6) shall be carried out at the same test arrangement. If the work is performed by more than one person, each procedure (inoculation, spreading or recovery of the test organisms from the different test fields) shall be done by the same person.

b) The water control and the drying control may be performed only once if different concentrations of a product and/ or different interfering substances are tested in parallel / at the same time. For each temperature, contact time and test organism a separate water control and drying control shall be performed.

c) For each test-surface prepared according to 5.5.1.6 – except the drying control test-surface - one wipe as tool for mechanical action (5.3.2.17) shall be prepared.

d) The soaked wipes shall be weighed immediately before and immediately after the wiping procedure – see 5.5.2.2 - to measure the amount of released liquid onto the test-surface.

e) Prepare for each test field of a “normal” test-surface (5.3.2.16) one tube containing 5 ml neutralizer (5.2.2.5), i.e. four tubes for one “normal” test-surface.

f) The wipe (5.3.2.17) should be folded once or if recommended by the manufacturer more than once.

5.5.2.2 Test N_a (Determination of bactericidal and yeasticidal concentrations), water control N_W

a) Soak the wipe (5.3.2.17) with 16 ml of the product test solution in a Petri dish (5.3.2.10) approximately 30 min as minimum before the wiping procedure, i.e. before the expected end of the drying time (5.5.1.6). Other volumes of the product and longer soaking times are allowed according to manufacturer's

10) For a graphical representation of this method, see Annex C.

instruction if these deviation(s) are clearly mentioned in the test report especially in its conclusion. Place the wipe on the unitary weight and start the wiping process (5.5.2.2 c).

b) In case of ready-to-use pre-moistened wipes prepare the wipes according to the manufacturers' instructions before starting the wiping process (5.5.2.2 c). In such cases the water control shall be performed with the wipe (5.3.2.17) according 5.5.2.2 f.

c) Use the unitary weight (5.3.2.18) and protect the base with parafilm (5.3.2.20). Cover the paraffin-protected site of the unitary weight with the wipe and fix it with the fingers of the hand, which hold the unitary weight. Begin the wiping-process in front of test field 1, then wipe one second in direction of field 4, turn immediately after test field 4 on that position and wipe back for another second to test field 1 (Figure 1). Having passed the second time test field 1 after two seconds start the stopwatch. Leave the test-surface for the chosen contact time *t* [5.5.1.1 b)].

d) At the end of t soak a swab (5.3.2.19) with neutralizer (5.2.2.5) from the relevant neutralizer tube [(5.5.2.1 e)]. Rub the whole surface of test field 1 with the swab (one swab per test field) and wash out in the neutralizer tube. Repeat the recovery procedure with the washed out swab, then cut the swab's lower half (or less) and put the soaked part into the neutralizer tube [(5.5.2.1 e)]. Afterwards repeat the recovery procedure with a second (dry) swab till the test field is visually dry. Cut this swab's lower half as well and put it also into the neutralizer tube. Mix [5.3.2.6 a)]. The whole recovery procedure with the two swabs shall not be longer than 1 min per test field. Repeat this procedure with the test fields 2, 3 and 4, i.e. 8 swabs per "normal" test-surface are needed. Never touch the swab's parts which are later transferred into the neutralizer tube.

e) After a neutralization time of 5 min (15 s for contact times < 15 min), mix and immediately take a sample of 1,0 ml of the neutralized test mixture N_a in duplicate and inoculate using the pour plate or the spread plate technique [5.4.1.4 d)].

f) Perform the procedure a) to e) using the other product test solution(s) at the same contact time.

g) Water control N_W: Perform the procedure a) to e), but instead of the product test solution, use hard water (5.2.2.7 a)) with the addition of 0,1 % Polysorbate 80 or – in the case of ready-to-use products – water (5.2.2.2) with the addition of 0,1 % Polysorbate 80. For counting prepare a 10^{-1} dilution with general diluent [(5.2.2.4 a)]. Deviations from the volume and soaking time requested in a) are not allowed. Mix [5.3.2.6 a)]. Take a sample of 1,0 ml in duplicate of the undiluted sample and the 10^{-1} dilution in diluent [5.2.2.4 a)] and inoculate using the pour plate or the spread plate technique [5.4.1.4 d) 1) or 2)].

h) Drying control: The recovery procedure from the test fields D_{C0} and D_{Ct} is the same as described in [5.5.2.2 d)]. The recovery from D_{C0} starts immediately after the drying procedure according to 5.5.1.6. However, the recovery from D_{Ct} starts immediately after the contact time t has elapsed. Perform the recovery of D_{Ct} after recovering N_a from the test fields 1 to 4. For counting prepare a 10^{-4} and 10^{-5} dilution with diluent [5.2.2.4 a)]. Mix [5.3.2.6 a)]. Take a sample of 1,0 ml in duplicate and inoculate using the pour plate or the spread plate technique [5.4.1.4 d) 1) or 2)].

i) For incubation and counting, see 5.5.2.5.

j) Perform the procedure a) to f) applying the other minimum and – if appropriate – other additional experimental conditions (5.5.1.1).

5.5.2.3 Neutralizer control *B* – verification of the absence of toxicity of the neutralizer

The following test may be omitted if a valid Neutralizer control B has been performed according to EN 13727 and/or EN 13624. If there are no results the test shall be done only once before the test itself (5.5.2.2).

Pipette 8,0 ml of the neutralizer – used in the test (5.5.2.2) – and 1,0 ml water (5.2.2.2) into a tube. Add 1,0 ml of the validation suspension (N_V) (5.4.1.5). Start the stopwatch at the beginning of the addition, mix [5.3.2.6 a)]

and place the tube in a water bath controlled at 20 °C ± 1 °C for 5 min ± 10 s. Just before the end of this time, mix [5.3.2.6 a)].

At the end of this time, take a sample of 1,0 ml of this mixture *B* in duplicate and inoculate using the pour plate or the spread plate technique [5.5.2.2 e)].

For incubation and counting, see 5.5.2.5.

5.5.2.4 Method validation *C* – dilution-neutralization validation

This test shall be performed according to 5.5.1.2 for each test organism only once before the test itself (5.5.2.2).

Pipette 8,8 ml of the neutralizer – used in the test (5.5.2.2) into a tube and add 0,2 ml of the product test solution only of the highest concentration used in the test (5.5.2.2). Start the stopwatch at the beginning of the addition, mix [5.3.2.6 a)] and place the tube in a water bath controlled at 20 °C ± 1 °C for 5 min ± 10 s. For products with contact times of 10 min or shorter the neutralization time is 10s ± 1s instead of 5 min ± 10 s. Add 1,0 ml of the validation suspension (N_V) (5.4.1.5). Start a stopwatch at the beginning of the addition and mix [5.3.2.6 a)]. Place the tube in a water bath controlled at (20 ± 1) °C for 30 min ± 1 min. Just before the end of this time, mix [5.3.2.6 a)] again.

At the end of this time, take a sample of 1,0 ml of the mixture *C* in duplicate and inoculate using the pour plate or the spread plate technique [5.5.2.2 e)].

For incubation and counting, see 5.5.2.5.

NOTE 1 The amount of the product test solution is calculated as the maximum amount per test field (25 cm^2 out of 540 cm^2 wiped surface is 4,6 %). 4,6 % of 2,0 ml product test solution (which remains at maximum on the surface after wiping) is about 0,1 ml and per 10 ml neutralizer (double the figure as in the test only 5 ml neutralizer are used). Since the release of any product is maximum 12,5 % the maximum amount per test field and 10 ml neutralizer is 0,2 ml.

NOTE 2 The interfering substance has not been taken into account as the amount per test field is maximum 0,000 23 ml (4,6 % of 0,005 ml which is brought onto test field 1 and then distributed over 540 cm^2) and therefore negligible. Furthermore it is expected that the interfering substance is supporting the neutralizer's action. Since the interfering substance is not used in the validation *C* the contact time t is not relevant either.

If a neutralizer capable of working within the specified time of 5 min (or 10 s for certain products) cannot be found, the time for neutralization may be increased. The additional time required for neutralization should be included in the contact time in 5.5.2.2.

5.5.2.5 Incubation and counting of the test mixture

a) Incubate (5.3.2.3) the plates for 20 h to 24 h, for *Candida albicans* 40 h to 48 h. Discard any plates which are not countable (for any reason). Count the plates and determine the number of cfu. Incubate the plates for a further 20 h to 24 h (not with *Candida albicans*). Do not recount plates which no longer show well separated colonies. Recount the remaining plates. If the number has increased use only the higher number for further evaluation.

b) Note for each plate the exact number of colonies but record > 330 for any counts higher than 330 and determine the V_C-values according to 5.6.2.2.

c) Calculate the numbers of cfu/ml in the test mixtures of N_a and N_W using the methods given in 5.6.2.3, 5.6.2.4 and 5.6.2.6. Verify according to 5.7.

5.6 Experimental data and calculation

5.6.1 Explanation of terms and abbreviations

5.6.1.1 Overview of the different suspensions and test mixtures

N and N_V represent the bacterial or yeastal suspensions, N_a represents the bactericidal or yeasticidal test mixture, N_W represents the test mixture in the water control. B (neutralizer control) and C (method validation) represent the different control test mixtures.

N_0, N_{V0}, N_a and B and C represent the number of cells counted per ml in the different test mixtures in accordance with Table 1.

Table 1 — Number of cells counted per ml or per cm^2 in the different test mixtures

	Number of cells per ml in the bacterial and yeastal suspensions	Number of cells per 25 cm^2 on the test field 1, D_{C0} after inoculation (time 0) and D_{Ct} at the end of the drying time; For N_0 and N_{V0} number of cells per ml in the test mixture at the beginning of the contact time (time 0)	Number of survivors on the test fields 1 to 4 per 25 cm^2 at the end of the contact time *t* and per ml after 5 min (B) or 30 min (C)
Test	*N* Test suspension	D_{C0} (= N/22,22) D_{Ct} (= N/22,22) N_0 (= N/11)	N_a, N_W (before neutralization)
Controls	N_V Validation suspension	N_{V0} (= N_V/10)	B, C

5.6.1.2 V_C-values

All experimental data are reported as V_C-values:

— in the dilution-neutralization method (test and controls), a V_C-values is the number of cfu counted per 1,0 ml sample.

5.6.2 Calculation

5.6.2.1 General

The first step in the calculation is the determination of the V_C-values, the second the calculation of N, N_0, N_a, N_W, N_V, N_{V0}, B and C. The third step is the calculation of the reduction R (5.8).

5.6.2.2 Determination of V_C-values

The V_C-values are determined as follows.

a) The usual limits for counting colonies on agar plates are between 15 and 300 colonies for bacteria and 15 and 150 for yeasts. In this European Standard, a deviation of 10 % is accepted, so the limits are 14 and 330 for bacteria and yeasts.

NOTE The lower limit (14) is based on the fact that the variability is increasing the smaller the number counted in the sample (1 ml or 0,1 ml) is, and therefore subsequent calculations may lead to wrong results. The lower limit refers only to the sample (and not necessarily to the counting on one plate), e.g. three plates per 1 ml sample with 3 cfu, 8 cfu and 5 colony-forming units give a V_C-value of 16. The upper limit (330) reflects the imprecision of counting confluent colonies and growth inhibition due to nutriment depletion. They refer only to the counting on one plate, and not necessarily to the sample.

b) For counting the test suspension *N* (5.4.1.6*)*, the validation suspensions N_V (5.4.1.6) and for all countings of the method (5.5.2), determine and record the V_C-values according to the number of plates used per 1 ml sample (5.6.1.2).

If more than one plate per 1 ml sample has been used to determine the V_C-value, the countings per plate should be noted.

If the count on one plate is higher than 330, report the number as " > 330". If more than one plate per 1 ml sample has been used and at least one of them shows a number higher than 330, report this V_C-values as "more than sum of the counts," e.g. for ">330, 310, 302", report "> 942".

If a V_C-value is lower than 14, report the number.

c) Only V_C-values within the counting limits are taken into account for further calculation, except in the case of N_a (5.6.2.4)

5.6.2.3 Calculation of *N* and N_W

N is the number of cells per ml in the test suspension (5.4.1.4; 5.6.1.1).

Since two dilutions of the test suspension (5.4.1.4 in connection with 5.4.1.6) are evaluated, calculate the number of cfu/ml as the weighted mean count using the following formula:

$$N = \frac{c}{(n_1 + 0{,}1\, n_2)\, 10^{-5}} \tag{1}$$

where

c is the sum of V_C-values taken into account;

n_1 is the number of V_C-values taken into account in the lower dilution, i.e. 10^{-5};

n_2 is the number of V_C-values taken into account in the higher dilution, i.e. 10^{-6};

10^{-5} is the dilution factor corresponding to the lower dilution.

Round off the results calculated to two significant figures. For this, if the last figure is below 5, the preceding figure is not modified; if the last figure is more than 5, the preceding figure is increased by one unit; if the last figure is equal to 5, round off the preceding figure to the next nearest even figure. Proceed stepwise until two significant figures are obtained. As a result, the number of cfu/ml is expressed by a number between 1,0 and 9,9 multiplied by the appropriate power of 10.

EXAMPLE

$$N = \frac{168 + 213 + 20 + 25}{(2 + 0{,}1 \times 2)\, 10^{-5}} = \frac{426}{2{,}2 \times 10^{-5}} = 1{,}9363 \times 10^{7} = 1{,}9 \times 10^{7}\ (cfu/ml)$$

N_0 is the number of cells per ml in the test mixture [5.5.2.2 a)] at the beginning of the contact time (time "zero" = 0). It is one-tenth of the weighted mean of N due to the tenfold dilution by the addition of the product and interfering substance.

5.6.2.4 Calculation of N_a

N_a is the number of survivors per ml in the test mixture [5.5.2.2 a)] at the end of the contact time and before neutralization. It is fivefold higher than the V_C-values due to the addition of neutralizer [5.5.2.2 b)].

a) Calculate the mean for each dilution step N_a^0, N_a^{-1} using the following formula:

$$N_a^0, N_a^{-1}, N_a^{-2} = 5 \times \frac{c}{n} \quad (2)$$

where

c is the sum of V_C-values taken into account;

n is the number of V_C-values taken into account.

If one or both of the duplicate V_C-values are either below the lower or above the upper limit, express the results as “less than” or “more than”.

EXAMPLE 1

duplicate V_C-values N_a: 2, 16

$$N_a = \frac{(<14+16)\times 5}{2} = <75$$

duplicate V_C-values N_a: 150, 160

$$N_a = \frac{(150+160)\times 5}{2} = 775 = 7{,}75\times 10^2$$

duplicate V_C-values N_a^{-1}: 2, 16

$$N_a^{-1} = \frac{(<14+16)\times 5}{2} = <75\times 10^1 = <750 = <7{,}5\times 10^2$$

duplicate V_C-values N_a^{-2}: > 330, 290

$$N_a^{-2} = \frac{(>330+290)\times 5}{2} = >1\,550\times 10^2 = >155\,000 = >1{,}55\times 10^5$$

duplicate V_C-values N_a^0, > 990, > 870 (3 plates per 1,0 ml sample)

$$N_a^0 = \frac{(>990+870)\times 5}{2} = >4\,650 = >4{,}65\times 10^3$$

b) For calculation of N_a use only N_a^0, N_a^{-1}, N_a^{-2}, N_a^{-3} results where one or both V_C-values are within the counting limits. Exceptions and rules for special cases:

If all subsequent dilutions of N_a show mean values of “more than”, take only the highest dilution (10^{-3}) as result for N_a.

EXAMPLE 2

	V_{C1}	V_{C2}		mean x 5	
N_a^0	> 330	> 330	=	> 1 650	
N_a^{-1}	> 330	> 330	=	> 1 650	N_a = > 1 650 × 10^3 = > 1,65 × 10^6
N_a^{-2}	> 330	> 330	=	> 1 650	
N_a^{-3}	> 330	> 330	=	> 1 650	

If all subsequent dilutions of N_a^0, N_a^{-1}, N_a^{-2}, N_a^{-3} show mean values of ”less than”, take only the lowest dilution (10^0) as result for N_a.

EXAMPLE 3

	V_{C1}	V_{C2}		mean x 5	
N_a^0	< 14	18	=	< 80	
N_a^{-1}	< 14	< 14	=	< 70	N_a = < 80 = < 8 × 10^1
N_a^{-2}	< 14	< 14	=	< 70	
N_a^{-3}	< 14	< 14	=	< 70	

If one or both duplicate V_C-values in only one dilution of N_a are within the counting limits, use this result as N_a.

EXAMPLE 4 (2 plates per 1 ml sample):

	V_{C1}	V_{C2}		mean x 5	
N_a^0	> 660	> 660	=	> 3 300	
N_a^{-1}	96	107	=	508	N_a = 508 × 10^1 = 5,08 × 10^3
N_a^{-2}	< 14	< 14	=	< 70	
N_a^{-3}	< 14	< 14	=	< 70	

If the higher dilution in two subsequent dilutions of N_a shows a mean value of “less than” and the lower dilution shows a mean value of “more than”, take only the lower dilution as N_a value.

EXAMPLE 5 (2 plates per 1 ml sample):

	V_{C1}	V_{C2}		mean x 5	
N_a^0	> 660	> 660	=	> 3 300	
N_a^{-1}	> 660	> 625	=	> 3 212	N_a = > 3 213 × 10^1 = 3,2 × 10^4
N_a^{-2}	< 14	29	=	< 108	
N_a^{-3}	< 14	< 14	=	< 70	

c) Use maximum 2 subsequent dilutions for calculating N_a as a weighted mean. Rules for special cases:

If one or both duplicate V_C-values in three or more subsequent dilutions of N_a (including N_a^0) are within the counting limits (e.g. N_a^{-2} : 17, 23; N_a^{-1} : 120, 135; N_a^0 : 308, > 330) the whole test is invalid (5.7).

If two subsequent dilutions of N_a show duplicate V_C-values within the counting limits calculate N_a as the weighted mean using the Formula (3):

$$N_a = \frac{c \times 5}{2{,}2 \times 10^z} \quad (3)$$

where

- c is the sum of V_C-values taken into account;
- z is the dilution factor corresponding to the lower dilution, e.g. N_a^{-2} is the lower dilution in comparison with N_a^{-3}.

If in two subsequent dilutions of N_a both V_C-values of the higher dilution are within the counting limits and one V_C-value of the lower dilution is "more than", calculate N_a as the weighted mean, using the Formula (3).

EXAMPLE 6 (2 plates per 1 ml sample):

	V_{C1}	V_{C2}		mean x 5
N_a^0	> 330	300	=	> 1 575
N_a^{-1}	46	57	=	258
N_a^{-2}	< 14	< 14	=	< 70
N_a^{-3}	< 14	< 14	=	< 70

$$N_a = \frac{(> 330 + 300 + 46 + 57) \text{ x } 5}{2{,}2 \text{ x } 10^0} =$$

> 1 666 = > 1,66 × 10^3

If in two subsequent dilutions of N_a one of the higher dilution duplicate values shows „ < 14", take only the lower dilution as result for N_a.

EXAMPLE 7

	V_{C1}	V_{C2}		mean x 5
N_a^0	> 330	> 330	=	> 1 650
N_a^{-1}	> 330	> 330	=	> 1 650
N_a^{-2}	301	> 330	=	> 1 578
N_a^{-3}	< 14	26	=	< 100

N_a = > 1 578 × 10^2 = > 1,6 × 10^5

5.6.2.5 Calculation of N_V and N_{V0}

N_V is the number of cells per ml in the validation suspension [5.4.1.5 a)]. It is tenfold higher than the counts in terms of V_C-values due to the dilution step of 10^{-1} [5.4.1.5 b)].

N_{V0} is the number of cells per ml in the mixtures B and C at the beginning of the contact time (time 0) (5.6.1.1). N_{V0} is one-tenth of the mean of the V_C-values of N_V [5.4.1.6 c)] taken into account.

Calculate N_V and N_{V0} using the following formulae:

$$Nv = 10\ c / n \tag{4}$$

$$Nv_0 = c / n \tag{5}$$

where

c is the sum of V_C-values taken into account;

n is the number of V_C-values taken into account

5.6.2.6 Calculation of *B* and *C*

B and C are the numbers of survivors in the neutralizer control B (5.5.2.3) and method validation C (5.5.2.4) at the end of the defined times 5 min (B) and 30 min (C). They correspond to the mean of the V_C-values of the mixtures B and C taken into account.

Calculate *B* and C using the following formula:

$$B = c / n \tag{6}$$

$$C = c / n \tag{7}$$

where

c is the sum of V_C-values taken into account;

n is the number of V_C-values taken into account

5.7 Verification of methodology

5.7.1 General

A test is valid if:

— all results meet the criteria of 5.7.3 and;

— the requirements of 5.8.2 are fulfilled and;

— it is not invalidated by a result described under 5.6.2.4 c) first special case.

5.7.2 Control of weighted mean counts

For results calculated by weighted mean of two subsequent dilutions (e.g. *N*), the quotient of the means of the two results shall be not higher than 15 and not lower than 5. Results below the lower limit are taken as the lower limit number (14). Results above the respective upper limit [5.6.2.2 b)] are taken as the upper limit number.

EXAMPLE For *N*: 10^{-5} dilution: 168 + 215 cfu/ml, 10^{-6} dilution: 20 + < 14 cfu/ml; (168 + 215) / (20 + 14) = 383/34 = 11, 26 = between 5 and 15.

When the counts obtained on plates are out of limits fixed for the determination of V_C-values [5.6.2.2 b)], check for the weighted mean as mentioned above but use only the V_C-values within the counting limits for the calculation of N.

5.7.3 Basic limits

5.7.3.1 Basic limits for test microorganisms for bactericidal activity:

a) N is between $1,5 \times 10^9$ cfu/ml and $5,0 \times 10^9$ cfu/ml ($9,17 \leq \lg N \leq 9,70$)

b) N_0 is between $7,5 \times 10^7$ cfu/ml and $2,5 \times 10^8$ cfu/ml ($7,88 \leq \lg N \leq 8,40$)

c) N_V is between 300 cfu/ml and 1600 cfu/ml ($3,0 \times 10^2$ cfu/ml and $1,6 \times 10^3$ cfu/ml)

d) N_{V0} is between 30 cfu/ml and 160 cfu/ml ($3,0 \times 10^1$ and $1,6 \times 10^2$)

e) B, C are equal to or greater than 0,5 x N_{V0}

f) Control of weighted mean counts (5.7.2): Quotient is not lower than 5 and not higher than 15.

g) D_{C0} is between $7,5 \times 10^6$ cfu/ml and $2,5 \times 10^8$ cfu/ml ($6,88 \leq \lg N \leq 8,40$)

h) D_{Ct} is between $7,5 \times 10^6$ cfu/ml and $2,5 \times 10^8$ cfu/ml ($6,88 \leq \lg N \leq 8,40$)

i) N_W is on average > 10 cfu/25 cm^2 on test fields 2 to 4

5.7.3.2 Basic limits for test microorganisms for yeasticidal activity:

a) N is between $1,5 \times 10^8$ cfu/ml and $5,0 \times 10^8$ cfu/ml ($8,17 \leq \lg N \leq 8,70$)

b) N_0 Is between $7,5 \times 10^6$ cfuml and $2,5 \times 10^7$ cfu/ml ($6,88 \leq \lg N \leq 7,40$)

c) N_V is between 300 cfu/ml and 1600 cfu/ml ($3,0 \times 10^2$ cfu/ml and $1,6 \times 10^3$ cfu/ml)

d) N_{V0} is between 30 cfu/ml and 160 cfu/ml ($3,0 \times 10^1$ and $1,6 \times 10^2$)

e) B, C are equal to or greater than 0,5 x N_{V0}

f) Control of weighted mean counts (5.7.2): Quotient is not lower than 5 and not higher than 15.

g) D_{C0} is between $7,5 \times 10^5$ cfu/ml and $2,5 \times 10^7$ cfu/ml ($5,88 \leq \lg N \leq 7,40$)

h) D_{Ct} is between $7,5 \times 10^5$ cfu/ml and $2,5 \times 10^7$ cfu/ml ($5,88 \leq \lg N \leq 7,40$)

i) N_W is on average > 10 cfu/25 cm^2 on test fields 2 to 4

5.8 Expression of results and precision

5.8.1 Overview of the different suspensions / test mixtures

— N represent the bacterial and yeastal suspensions, N_a represents the bactericidal and yeasticidal test mixture from the surface, N_W represents the test mixture in the water control from the surface.

— D_{C0} represents the recovery immediately after drying.

— D_{Ct} represents the recovery after drying and the contact time t.

5.8.2 V_C-values

a) Reduction on test field 1

The reduction ($R = D_{Ct} / N_a$) is expressed in logarithm.

For each test organism record the number of cfu/ml in the drying control D_{Ct} [5.5.2.2 f)] and of the results of the test N_a (5.6.2.4).

For each product concentration and each experimental condition calculate and record the decimal log reduction separately using the Formula (8):

$$R = \frac{D_{Ct}}{N_a} \quad \text{or} \quad \lg R = \lg D_{Ct} - \lg N_a \tag{8}$$

For the controls and validation record N_{V0} (5.6.2.5), the results of B and C (5.6.2.6) and their comparison with N_{V0} [5.7.3 d)].

b) Accumulation on fields 2 to 4

The accumulation is expressed in real counts.

For each product concentration, each experimental condition and each test organism record the number of cfu/test field 2, 3 and 4 are counted and the mean $\overline{x}$ or $\overline{x}_{wm}$ of V_{T2to4} are calculated separately using the Formula (9):

$$\overline{x} \quad \text{or} \quad \overline{\overline{x_{wm}}} = \frac{(V_{T2} + V_{T3} + V_{T4}) \times 5}{3} \tag{9}$$

5.8.3 Limiting test organism and bactericidal and yeasticidal concentration

5.8.3.1 Bactericidal concentration

For each test organism, record the lowest concentration of the product which passes the test on field 1 ($\lg R \geq 5$ for *Staphylococcus aureus*, *Enterococcus hirae* and *Pseudomonas aeruginosa*). For each test organism, record the lowest concentration of the product which passes the test on fields 2 to 4 (on average < 50 cfu per 25 cm^2). Record as the limiting test organism the test organism requiring the highest of these concentrations (it is the least susceptible to the product in the chosen experimental conditions). For each test organism, record if the water control passes the test on fields 2 to 4 (on average > 10 cfu per 25 cm^2).

The lowest concentration of the product active on the limiting test organism is the bactericidal concentration determined according to this European Standard.

5.8.3.2 Yeasticidal concentration

Record the lowest concentration of the product which passes the test with *Candida albicans* on field 1 ($\lg R \geq 4$). Record the lowest concentration of the product which passes the test on fields 2 to 4 (on average ≤ 50 cfu per 25 cm^2). The lowest concentration of the product active on *Candida albicans* is the yeasticidal concentration determined according to this European Standard. Record if the water control passes the test on fields 2 to 4 (on average ≥ 10 cfu per 25 cm^2).

5.8.4 Precision, repetitions

Details on the precision and repetitions are given in EN 14885.

5.9 Interpretation of results – conclusion

The product shall be deemed to have passed the EN 16615 standard (bactericidal and yeasticidal activity) if it demonstrates in a valid test under the conditions defined by this standard within maximum 60 min contact time:

5.9.1 Bactericidal interpretation of results:

— a 5 lg reduction for *Staphylococcus aureus*, *Enterococcus hirae, Pseudomonas aeruginosa* on test field 1; and

— an average of equal or less than 50 cfu on test fields 2 to 4 for each test organism.

5.9.2 Yeasticidal interpretation of results:

— a 4 lg reduction for *Candida albicans* on test field 1; and

— an average of equal or less than 50 cfu on test fields 2 to 4 for each test organism.

The water control shall demonstrate in a valid test under the conditions defined by this standard for each test organism (bacterial and fungal) an average of equal or more than 10 cfu on the test fields 2 to 4.

5.9.3 If the test were done with other wipes as described in 5.3.2.17, the results should only be used for the tested wipe and this should be clearly expressed.

For further fields of application, see EN 14885.

5.10 Test report

The test report shall refer to this European Standard (EN 16615).

The test report shall state, at least, the following information:

a) identification of the testing laboratory;

b) identification of the client;

c) identification of the sample:

   1) name of the product;

   2) batch number and — if available — expiry date;

   3) manufacturer – if not known: supplier;

   4) date of delivery;

   5) storage conditions;

   6) product diluent recommended by the manufacturer for use;

   7) active substance(s) and its/their concentration(s) (optional);

   8) appearance of the product;

d) test method and its validation:

   For the dilution-neutralization method full details of the test for validation of the neutralizer shall be given;

e) experimental conditions:

   1) date(s) of test (period of analysis);

2) diluent used for product test solution (hard water or distilled water);

3) product test concentrations (= *desired* test concentrations according to 5.4.2);

4) appearance of the product dilutions;

5) contact time(s);

6) interfering substance;

7) stability and appearance of the mixture during the procedure (note the formation of any precipitate or flocculant);

8) temperature of incubation;

9) neutralizer or rinsing liquid;

10) identification of the test strains used;

11) the type of wipe used if deviating from the one described in the standard;

f) test results:

1) controls and validation;

2) evaluation of bactericidal and yeasticidal activity;

3) number of repetitions per test organism;

g) special remarks;

h) conclusion;

i) locality, date and identified signature.

Annex A
(informative)

Referenced strains in national collections

—	*Staphylococcus aureus:*	ATCC	6538
		CIP	4.83
		DSM	799
		NCTC	10788
		NCIMB	9518
—	*Pseudomonas aeruginosa:*	ATCC	15442
		CIP	103467
		DSM	939
		NCIMB	10421
—	*Enterococcus hirae:*	ATCC	10541
		CIP	58.55
		DSM	3320
		NCIMB	8192
—	*Candida albicans:*	ATCC	10231
		CIP	4872
		DSM	1386
		CBS	6431
		NCTC	3179

Annex B
(informative)

Neutralizers

Examples of neutralizers of the residual antimicrobial activity of chemical disinfectants and antiseptics.

Important! Neutralizers of the residual antimicrobial activity of chemical disinfectants and antiseptics shall be validated according to the prescriptions of the standard.

Antimicrobial agent	Chemical compounds able to neutralize residual antimicrobial activity	Examples of suitable neutralizers [a]
Quaternary ammonium compounds and fatty amines Amphoteric compounds	Lecithin, Saponin, Polysorbate 80, Sodium dodecyl sulfate, Ethylene oxide condensate of fatty alcohol [b] (non-ionic surfactants)	— Polysorbate 80, 30 g/l + saponin, 30 g/l + lecithin, 3 g/l. — Polysorbate 80, 30 g/l + sodium dodecyl sulfate, 4 g/l + lecithin, 3 g/l. — Ethylene oxide condensate of fatty alcohol, 3 g/l + lecithin, 20 g/l + polysorbate 80, 5 g/l.
Biguanides and similar compounds	Lecithin[c], Saponin, Polysorbate 80	— Polysorbate 80, 30 g/l + saponin, 30 g/l + lecithin, 3 g/l.
Oxidizing compounds (Chlorine, iodine, hydrogen peroxide, peracetic acid, hypochlorites, etc…)	Sodium thiosulphate [d] Catalase [for hydrogen peroxide or products releasing hydrogen peroxide]	— Sodium thiosulphate, 3 g/l to 20 g/l + polysorbate 80, 30 g/l + lecithin, 3 g/l. — Polysorbate 80, 50 g/l + catalase 0,25 g/l + lecithin 10 g/l.
Aldehydes	L – histidine Glycine	— Polysorbate 80, 30 g/l + lecithin, 3 g/l + L-histidine, 1 g/l (or + glycine, 1 g/l). — Polysorbate 80, 30 g/l + saponin, 30 g/l + L-histidine, 1 g/l (or + glycine, 1 g/l).
Phenolic and related compounds: orthophenylphenol, phenoxyethanol, triclosan, phenylethanol, etc…. Anilides	Lecithin Polysorbate 80 Ethylene oxide condensate of fatty alcohol [b]	— Polysorbate 80, 30 g/l + lecithin, 3 g/l. — Ethylene oxide condensate of fatty alcohol, 7 g/l + lecithin, 20 g/l, + polysorbate 80, 4 g/l.
Alcohols	Lecithin, Saponin, Polysorbate 80 [e]	— Polysorbate 80, 30 g/l + saponin, 30 g/l + lecithin, 3 g/l.

[a] According to the pH of the tested product, the pH of the neutralizer may be adjusted at a suitable value or prepared in phosphate buffer [ex: phosphate buffer 0,25 mol/l: potassium dihydrogen phosphate (KH2PO4) 34 g; distilled water (500 ml); adjusted to pH 7,2 ± 0,2 with sodium hydroxide (NaOH) 1 mol/l; distilled water up to 1 000 ml].

[b] The carbon chain-length varies from C_{12} to C_{18} carbon atoms.

[c] Egg and soya; egg is preferable.

[d] The toxic effect of sodium thiosulphate differs from one test organism to another.

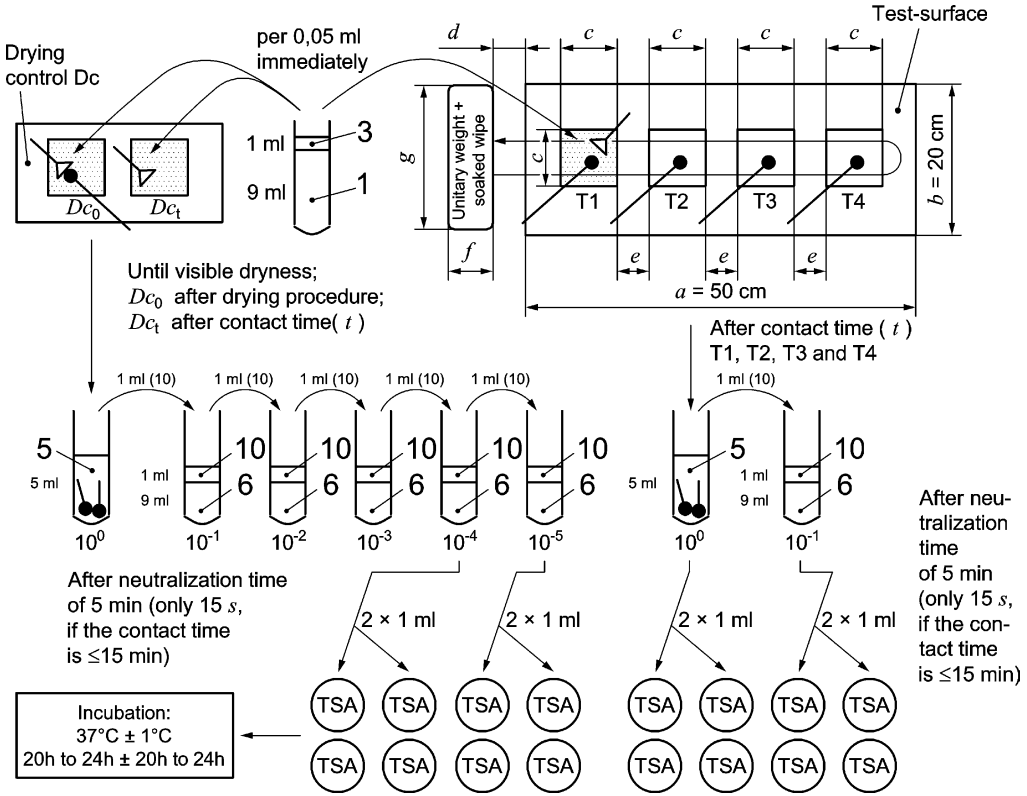
[e] For the neutralization of short chain alcohols (less than C_5), simple dilution may be appropriate. Care should be taken if the alcohol-based - products contain additional antimicrobial agents.

Other neutralizer mixtures may be required for products containing more than one antimicrobial agent.

NOTE The concentrations of the various neutralizing compounds or of the neutralizer as such may not be adequate to neutralize high concentrations of the products.

Annex C
(informative)

Graphical representations of the test method

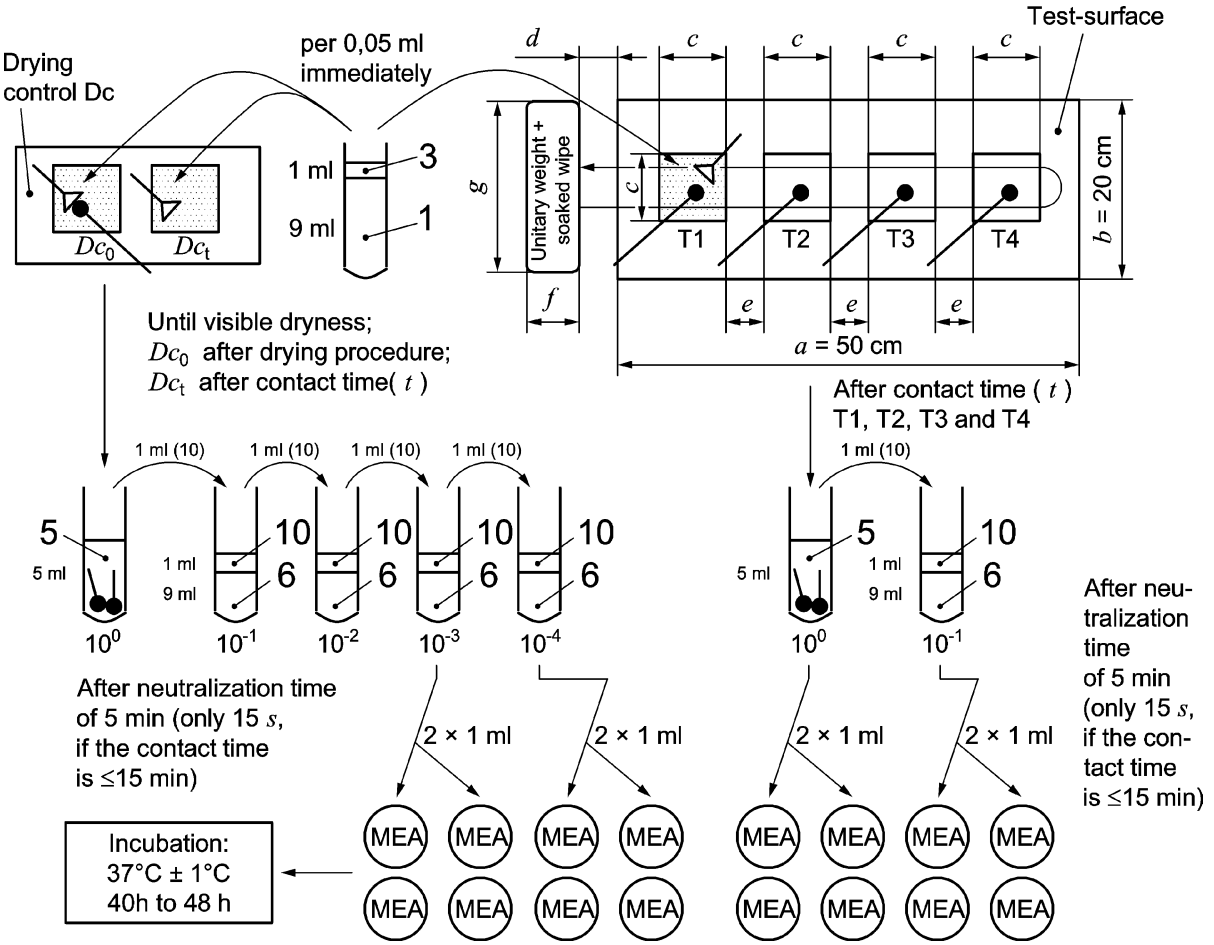


Key

Schematic representation of the test-surface:

a = 50 cm	*c* = 5 cm	*e* = 5 cm	*g* = 12,1 cm
b = 20 cm	*d* = 10 cm	*f* = 8,6 cm	

Figure C.1 — Graphical representations of the test method - bactericidal activity



Key

Schematic representation of the test-surface:

a = 50 cm	*c* = 5 cm	*e* = 5 cm	*g* = 12,1 cm
b = 20 cm	*d* = 10 cm	*f* = 8,6 cm	

Figure C.2 — Graphical representations of the test method - yeasticidal activity

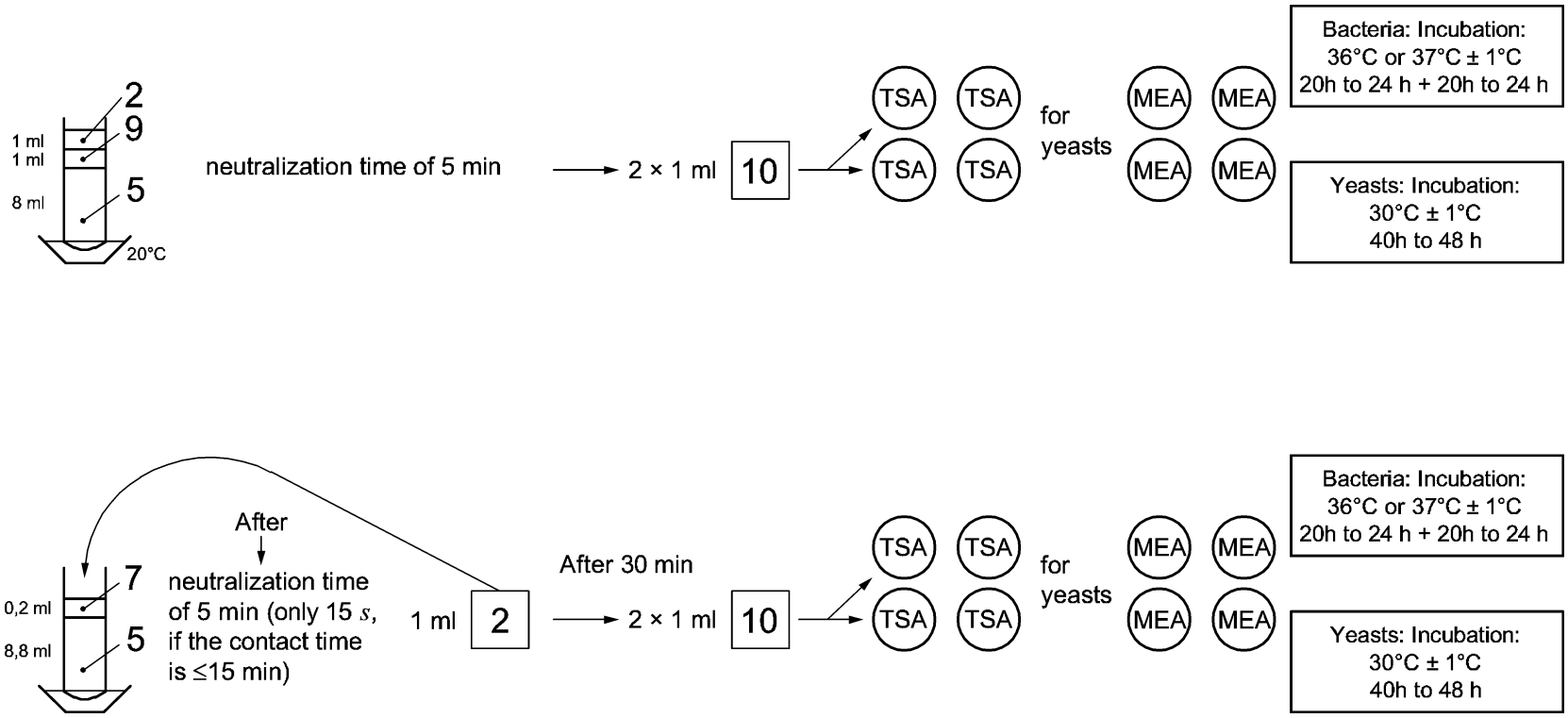


Figure C.3 — Validation

Annex D
(informative)

Example of a typical test report

NOTE 1 All names and examples in Annex D are fictitious apart from those used in this European Standard.

NOTE 2 Only the test results of one replicate for *Staphylococcus aureus* is given as an example.

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TEST REPORT

EN 16615**, BACTERICIDAL ACTIVITY**

(minimum and additional conditions)

Client: Cult Formulations Inc., Mannheim/Euroland

Disinfectant-sample

Name of the product: JN (instruments and surfaces)

Batch number: 10-10-76

Manufacturer or – if not known – **supplier**: MDI Formulations Inc. (manufacturer)

Storage conditions (temp. and other): Room temperature, darkness

Appearance of the product: Liquid, clear, yellowish

Active substance(s) and their concentration(s): Not indicated

Product diluent recommended by the manufacturer for use: Potable water

Period of testing
Date of delivery of the product: 2012-10-20 **Dates of tests:** see “Test results” (attached)

Experimental conditions
Amount of released liquid (analogous to 5.5.2.1 d)

Product diluent: hard water**; concentrations of the product tested:** see “Test results” (attached)

Test-organisms: *Staphylococcus aureus* ATCC 6538, *Enterococcus hirae* ATCC 10541, *Pseudomonas aeruginosa* ATCC 15442, *Candida albicans* ATCC 10231

Test temperature: 20 °C; contact time: 60 min

Interfering substance: 0,3 g/l bovine albumin = clean conditions; (+ 3,0 g/l bovine albumin plus 3,0 ml/l erythrocytes = dirty conditions)

Incubation temperature: 36 °C

Test results: see attached sheets

Special remarks regarding the results:

1. All controls and validation were within the basic limits.
2. At least one concentration of the product demonstrated a lg reduction of less than 5 lg.
3. No precipitate during the test procedure (test mixtures were homogeneous).

Conclusion:

For the product JN (batch 10-10-76), the bactericidal concentration for surface disinfection determined according to the EN 16615 standard under clean conditions at 20 °C within 60 min is:

1,0 % (v/v)

(the mean reduction of six repetitions with the limiting test organism *Staphylococcus aureus* was $1,2 \times 10^5$. *Enterococcous hirae* and *Pseudomonas aeruginosa* were tested once and showed a 5 lg reduction or more at a lower concentration than *Staphylococcus aureus*).

Antiseptville, 2012-11-20

Alexander May, MD, PhD, Scientific Director

Test results (bactericidal quantitative carrier test)

EN....*XXXXX*….(Phase 2, step 2) Product-name: ..*JN..(Instruments and surfaces*)...Batch No.: .. *10-10-76*.

Remarks:

Dilution neutralization method [X] Pour plate [ ] Spread plate [x] Number of plates .....2 / ml
Neutralizer:.....*Lecithin 3,0 g/l* in diluent.....

Membrane filtration method □ Rinsing liquid:.....

Test temperature: *20* °C interfering substance:….bovine albumin: 0,3 g/l ……

Test organism: .... *Staphylococcus aureus* ATCC 6538.....Incubation temperature: 36°C

Internal lab. No:...QS 68*/00* .... Date of test:.*2012-10-20* Responsible person: ...*Fang*..... Signature: Fang.
Diluent used for product test solutions: ...*water*..Appearance of the product test solutions: .....*clear*.....

Validation and controls

Validation suspension (N_{V0})						Neutralizer control (*B*)			Method validation (*C*) Product conc.: 10 ml/l		
V_{C1}	86 (40 + 46)	$\bar{x}$ = 89				V_{C1}	86 (42 + 44)	$\bar{x}$ = 88,5	V_{C1}	75 (35 + 40)	$\bar{x}$ = 81
V_{C2}	92 (47 + 45)					V_{C2}	91 (43 + 48)		V_{C2}	87 (41 + 46)	
30 ≤ $\bar{x}$ of N_{V0} ≤ 160? ☒ *yes* ☐ *no*						$\bar{x}$ of *B* is ≥ 0,5 × $\bar{x}$ of N_{V0}? (or N_V /1 000) ☒*yes* ☐*no*			$\bar{x}$ of *C* is ≥ 0,5 × $\bar{x}$ of N_{V0}? ☒ *yes* ☐ *no*		

Test suspension and Test

Test-suspension (*N* and N_0):	*N*	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = *193,64* × 10^7 ; lg N = 9,29
	10^{-7}	*168*	*213*	N_0 = N /20 ; lg N_0 = 7,98
	10^{-8}	*20*	*25*	**7,88 ≤ lg N_{V0} ≤ 8,40? ☒ yes ☐ no**

Drying controls

Drying control (D_{C0}):	T_0	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = *190,45* × 10^5 ; lg T_0 = 7,28
	10^{-5}	*190*	*191*	
	10^{-6}	*19*	*19*	**6,88 ≤ lg T_0 ≤ 8,40? ☒ yes ☐ no**

Drying controls

Drying control (D_{Ct}):	T_0	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = *190,45* × 10^5; lg T_t = 7,28
	10^{-5}	*190*	*191*	
	10^{-6}	*19*	*19*	**6,88 ≤ lg T_t ≤ 8,40? ☒ yes ☐ no**

Test field 1 (reduction)

Real conc. of the product %	Dilution step	V_{C1}	V_{C2}	N_a (= $\overline{x}$ or $\overline{x}_{wm}$)	**lg** N_a	**lg** R (lgT_t - lgN_a)	Contact time (min)
0,50	10^0	> 660	> 660	> 660	>2,81	<4,47	60
1,00	10^0	14	17	15,5	1,19	6,09	60

Test fields 2 to 4 (cfu/25 cm^2)

Real conc. of the product %	Dilution step	V_C T_2	V_C T_3	V_C T_4	V_{T2to4} (= $\overline{x}$ or $\overline{x}_{wm}$ × 5) **cfu/25 cm^2**	Contact time (min)
0,50	10^0	50	50	50	250	60
1,00	10^0	1	3	0	6,66	60

N_W Test fields 2 to 4 (cfu/25 cm^2)

Real conc. of the product %	Dilution step	V_C T_2	V_C T_3	V_C T_4	$V_{NW\,T2to4}$ (= $\overline{x}$ or $\overline{x}_{wm}$ × 5) **cfu/25 cm^2**	Contact time (min)
0,00	10^0	100	100	100	500	60
	10^{-1}	10	10	10		
0,00	10^0	100	100	100	500	60
	10^{-1}	10	10	10		

Countings per plate for

N = 10^{-7}: 80+88; 105+108 10^{-8}: 9+11; 15+10

N_a = **0,50 %** 10^0: >330+>330: >330+330

1,00 % 10^0 : 7+7; 10+7

Explanations:

V_C = count per ml (one plate or more)

$\overline{x}$ = average of V_{C1} and V_{C2} (1. + 2. duplicate)

$\overline{x}_{wm}$ = weighted mean of $\overline{x}$

R = reduction (lg R = lg N_0 – lg N_a)

Annex ZA
(informative)

Relationship between this European Standard and the Essential Requirements of EU Directive 93/42/EEC

This European Standard has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association to provide a means of conforming to Essential Requirements of the New Approach Directive 93/42/EEC.

Once this standard is cited in the Official Journal of the European Union under that Directive and has been implemented as a national standard in at least one Member State, compliance with the clauses of this standard given in Table ZA.1 confers, within the limits of the scope of this standard, a presumption of conformity with the corresponding Essential Requirements of that Directive and associated EFTA regulations.

Table ZA.1 — Correspondence between this European Standard and Directive 93/42/EEC

Clauses of this EN	**Essential Requirements (ERs) of Directive 93/42/EEC**	**Qualifying remarks/Notes**
1, 2, 3 and 4	6a (in connection with Annex X, 1.1d)	This standard is intended for products whose main efficacy claim is of a microbicidal nature, e.g. chemical disinfectants and antiseptics. Complying with the requirements of this standard demonstrates the bactericidal and yeasticidal activity of the product. Generally at least one additional European Standard (phase 2, step 1) shall be complied with to demonstrate a sufficient performance evaluation of such products.

WARNING — Other requirements and other EU Directives may be applicable to the product(s) falling within the scope of this standard.

Bibliography

[1] EUROPEAN PHARMACOPOEIA (EP). Edition 1997 supplement 2000, *Water for injections, glycerol*

[2] DIN 53919 (all parts), *Test cotton fabrics for laundering process control*

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