SLOVENSKI STANDARD
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Nadomešča:
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Kemična razkužila in antiseptiki - Higiensko razkuževanje rok - Preskusna metoda in zahteve (faza 2, stopnja 2)

Chemical disinfectants and antiseptics - Hygienic handrub - Test method and requirements (phase 2/step 2)

Chemische Desinfektionsmittel und Antiseptika - Hygienische Händedesinfektion - Prüfverfahren und Anforderungen (Phase 2/Stufe 2)

Antiseptiques et désinfectants chimiques - Traitement hygiénique de mains par frictions - Méthode d'essai et prescriptions (phase 2/étape 2)

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EUROPEAN STANDARD

NORME EUROPÉENNE

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EN 1500

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ICS 11.080.20; 71.100.35

Supersedes EN 1500:1997

English Version

Chemical disinfectants and antiseptics - Hygienic handrub - Test method and requirements (phase 2/step 2)

Antiseptiques et désinfectants chimiques - Traitement hygiénique de mains par frictions - Méthode d'essai et prescriptions (phase 2/étape 2)

Chemische Desinfektionsmittel und Antiseptika - Hygienische Händedesinfektion - Prüfverfahren und Anforderungen (Phase 2/Stufe 2)

This European Standard was approved by CEN on 1 March 2013.

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COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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Foreword

This document (EN 1500:2013) 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of an identical text or by endorsement, at the latest by October 2013, and conflicting national standards shall be withdrawn at the latest by October 2013.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN 1500:1997.

This document was revised to adapt it to the latest state of science, to correct errors and ambiguities, to harmonise the structure and wording with other tests of CEN/TC 216 existing or in preparation and to improve the readability of the standard and thereby make it more understandable.

The following technical changes have been made:

— Neutralization (5.5.1.2).

— The number of volunteers (5.5.1.4).

— The statistical evaluation (5.8).

— The annexes have been completely revised.

Data obtained using the former version of EN 1500 may still be used, if it is supplemented by data on neutralization, additional results from more volunteers and the new statistical evaluation of the “mixed” (old and new) set of data. The additional results will be obtained preferably in the same laboratory and with volunteers not having participated in the previous (“old”) study. If the neutralizer used in the test using the former version is not sufficiently neutralizing, a complete new test will be run. The changed procedure in Annex A is regarded as having no (or negligible) influence on the results.

According to the CEN-CENELEC Internal Regulations, the national standards organisations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

1 Scope

This European Standard specifies a test method simulating practical conditions for establishing whether a product for hygienic handrub reduces the release of transient microbial flora on hands when rubbed onto the artificially contaminated hands of volunteers.

NOTE 1 Attention is drawn to the fact that tests on human volunteers are the subject of legal provisions in certain European countries/regions.

This European Standard applies to products for hygienic handrub for use in 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 patient.

EN 14885 specifies in detail the relationship of the various tests to one another and to "use recommendations".

NOTE 2 This method corresponds to a phase 2, step 2 test.

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 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

When tested in accordance with Clause 5, the mean reduction of the release of the test organism *Escherichia coli K12* achieved by the hygienic handrub with the product under test shall be at least not inferior to that achieved by a specified reference hygienic handrub (60 % volume concentration of propan-2-ol).

5 Test method

5.1 Principle

Hands of volunteers are artificially contaminated with test organisms. The number of test organisms released from their fingertips into sampling fluids is assessed before and after the hygienic handrub. The ratio of the

two resulting values represents a measure for the antimicrobial activity of the product tested. The necessary precision is achieved by repeating the test on 18 to 22 volunteers. To compensate for extraneous influences it is compared with the reduction obtained by a reference handrub, which is performed with the same volunteers, on the same day and under comparable environmental conditions.

Prior to the test, a suitable neutralizer is validated. The neutralizer is used as a sampling fluid for recovering the test organisms after the hygienic handrub to ensure that the bactericidal and/or bacteriostatic activity in the sampling fluids is neutralized or suppressed.

5.2 Materials and reagents

5.2.1 Test organism

Escherichia coli K12 NCTC 10538; CIP 54.117; NCIMB 10083[1)]

NOTE This test organism has been specifically chosen to meet health and safety guidance and ethical committee considerations. It is a K12 strain of E. coli of normal flora origin internationally recognised as being non-pathogenic. According to the UK catalogue of the National Collections of Industrial & Marine Bacteria (see [2]), NCIMB strain 10083 is classified as a risk group 1 organism. The German Safety Ordinance on Gene Technology [3] also assigns the K12 strain to group 1. Directive 93/88/EEC [4] (Annex III to Directive 90/679/EEC [5]) explicitly states that non-pathogenic strains of Escherichia coli are excluded from the group 2 assignment.

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 organisms. To improve reproducibility, it is recommended that commercially available dehydrated material is used for the preparation of culture media. The manufacturer's instructions relating to the preparation of these products should be rigorously followed. For each culture medium and reagent, a time limitation for use should be fixed.

5.2.2.2 Water

The water shall be freshly glass-distilled water and not demineralised water. If distilled water of adequate quality is not available, water for injections (see bibliographic reference [1]) may be used.

Sterilise in the autoclave [5.3.2.1 a)]. Sterilisation is not necessary if the water is used e.g. for preparation of culture media and subsequently sterilised.

NOTE See 5.2.2.7 for the procedure to prepare hard water.

5.2.2.3 Tryptone soya agar and tryptone soya selective agar

a) Tryptone Soya Agar (TSA)

Tryptone soya agar, consisting of:

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1) The NCTC, CIP and NCIMB numbers are the collection numbers of this strain supplied by these cultures collections. 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.

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

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

NOTE 1 TSA is used for preparing and counting N, N_v and N_{vB} (5.4.1.4, 5.4.1.5).

b) Tryptone Soya Selective Agar (TSSA)

Tryptone soya selective agar, consisting of:

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

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

NOTE 2 TSSA is used for quantitative cultures of the sampling fluids and their dilutions (5.5.3.2, 5.5.3.3.4).

5.2.2.4 Tryptone Soya Broth (TSB)

Tryptone soya broth, consisting of:

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

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

5.2.2.5 Neutralizer

The neutralizer shall be chosen, controlled and validated for the product under test in accordance with 5.5.1.2, 5.5.2.1 and 5.5.2.2. Only neutralizers using TSB (5.2.2.4) as diluent are allowed. It shall be sterile. The reference product is neutralized by dilution only.

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

5.2.2.6 Diluted soft soap

Linseed oil	50,0 parts by weight
Potassium hydroxide [1]	9,5 parts by weight
Ethanol (min. 95 %) [1]	7,0 parts by weight
Hot distilled water (75 ± 5) °C	as needed

Prepare a solution of 9,5 parts potassium hydroxide in 15 parts water (5.2.2.2) and add 50 parts linseed oil. Heat up to approximately 70 °C while constantly stirring. Add the ethanol and continue heating while stirring until the saponification process is completed and a sample dissolves clearly in water and almost clearly in alcohol. The weight of the soft soap is then brought up to 100 parts by addition of water (5.2.2.2), and heated up to (75 ± 5) °C to dilute the soft soap. Take 200 g of the soft soap, fill up to 1 000 g with water (5.2.2.2) and sterilise in the autoclave (5.5.2.1). The pH of the final diluted soft soap shall range between 10,0 and 11,0.

For quality control of the soft soap, see Annex D.

5.2.2.7 Hard water for dilution of products

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. Sterilise 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 1000 ml with water (5.2.2.2) under aseptic conditions. Store the solution in the refrigerator (5.3.2.8) for no longer than one month;

— prepare solution B: dissolve 35,02 g sodium bicarbonate ($NaHCO_3$) in water (5.2.2.2) and dilute to 1000 ml. Sterilise by membrane filtration (5.3.2.7). Store the solution in the refrigerator (5.3.2.8) for no longer than one week;

— place 600 ml to 700 ml of water (5.2.2.2) in a 1 000 ml volumetric flask (5.3.2.12) and add 6,0 ml (5.3.2.9) of solution A, then 8,0 ml of solution B. Mix and dilute to 1000 ml with water (5.2.2.2). The pH of the hard water shall be 7,0 ± 0,2, when measured at 20 °C ± 1 °C (5.3.2.4). If necessary, adjust the pH by using a solution of approximately 40 g/l (about 1 mol/l) of sodium hydroxide (NaOH) or approximately 36,5 g/l (about 1 mol/l) of hydrochloric acid (HCl).

The hard water shall be freshly prepared under aseptic conditions and used within 12 h.

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

5.2.2.8 Propan-2-ol as reference handrub [52,3 % (weight concentration) corresponding to 60 % (volume concentration) at 20 °C]

Fill 471 g propan-2-ol [1] with a purity of min. 99,5 % *V/V* (determined by gas chromatography; density 0,785) in a 1000 ml flask equipped with a glass stopper on the weighing platform of a scale (precision 0,1 g). Add 429 g water (5.2.2.2). This will give a volume of approximately 1 000 ml. Close the flask with the matching glass stopper and shake the contents of the flask thoroughly.

NOTE This solution can be kept indefinitely at approximately room temperature if protected from light.

5.3 Apparatus and glassware

5.3.1 General

Sterilise 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[2)] and, in particular, the following:

5.3.2.1 Apparatus for sterilisation

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

b) for dry heat sterilisation, 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 and TSSA in case of pour plate technique).

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

5.3.2.4 pH-meter, having an inaccuracy of calibration of no more than ± 0,1 pH units at 20 °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[3)];

b) mechanical shaker.

5.3.2.7 Membrane filtration apparatus, constructed of a material compatible with the substances to be filtered

The apparatus shall have a filter holder of at least 50 ml volume. It shall be suitable for use with filters of diameter 47 mm to 50 mm and 0,45 µm pore size for sterilisation of hard water (5.2.2.7).

The vacuum source used shall give an even filtration flow rate. To prevent overlong filtration, the device shall be set so as to obtain the filtration of 100 ml of rinsing liquid in 20 s to 40 s.

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

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

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

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

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

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

5.3.2.12 **Volumetric flasks**

5.3.2.13 **Spreader**, made of glass or other material

5.3.2.14 **Container** of sufficient capacity to immerse two hands vertically up to the mid-metacarpals simultaneously in 2 l of contamination fluid

5.3.2.15 **Two bottles of at least 1 l capacity**

5.4 Preparation of test organism suspensions and product test solutions

5.4.1 Test organism suspensions (test and validation suspension)

5.4.1.1 General

For the test organism, two different suspensions have to be prepared: the "test suspension", i.e. contamination fluid 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 the test organism (5.2.1), prepare a first 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. The second subculture is used to prepare the test suspension.

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

5.4.1.4 Test suspension ("*N*")/ contamination fluid

a) Take loopfuls of the cells from the working culture (5.4.1.3) in two tubes, each containing 5 ml of TSB (5.2.2.4), and incubate (5.3.2.3) for 18 h to 24 h. Inoculate these cultures into two bottles (5.3.2.15) with maximum 1 l TSB (5.2.2.4) each and incubate again (5.3.2.3) for 18 h to 24 h. Pool the resulting bacterial suspensions in a container (5.3.2.14).

b) Adjust the number of cells in the suspension to 1,5 x 10^8 cfu/ml[4)] to 5,0 x 10^8 cfu/ml using TSB (5.2.2.4), estimating the number of cfu by any suitable means. Maintain this test suspension in the water bath at 20 °C and use within 4 h as contamination fluid and to prepare the validation suspensions (5.4.1.5). The use of a spectrophotometer for adjusting the number of cells is highly recommended (about

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

620 nm wavelength - cuvette 10 mm path length). Each laboratory should therefore produce calibration data 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 colorimeter is a suitable alternative.

c) For counting, prepare 10-6 and 10-7 dilutions of the test suspension using TSB (5.2.2.4). Mix [5.3.2.6 a)]. 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 and add 15 ml to 20 ml melted TSA [5.2.2.3 a)], cooled to 45 °C ± 1 °C.

2) When using the spread plate technique, spread each 1,0 ml sample – divided into portions of approximately equal size – on an appropriate number (at least two) of surface dried plates containing TSA [5.2.2.3 a)].

For incubation and counting, see 5.4.1.6.

5.4.1.5 Validation suspension ("*N*v","*N*vB")

a) To prepare the validation suspension (*N*v), dilute the test suspension (5.4.1.4) with TSB (5.2.2.4) to obtain 3,0 x 102 cfu/ml to 1,6 x 10^3 cfu/ml [about one-fourth (1+3) of the 10-5 dilution].

b) To prepare the validation suspension (*N*vB) for the neutralizer control (5.5.2.1), dilute the test suspension (5.4.1.4) with TSB (5.2.2.4) to obtain 3,0 x 104 cfu/ml to 1,6 x 105 cfu/ml [about one-fourth (1+3) of the 10-3 dilution] (*N*vB).

c) Maintain and use these validation suspensions (*N*v and *N*vB) the same way as the test suspension [5.4.1.4 b)].

d) For counting, prepare a 10-1 dilution with TSB (5.2.2.4), but prepare a 10-3 dilution of the validation suspension to count the neutralizer control [see b)].

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 c)].

For incubation and counting, see 5.4.1.6.

5.4.1.6 Incubation and counting of the test and the validation suspensions

a) Incubate (5.3.2.3) the plates for 20 h to 24 h. Discard any plates that 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. Do not recount plates that 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 in the validation suspensions "*N*v" and "*N*vB" using the methods given in 5.6.2.3 and 5.6.2.5. Verify according to 5.7.