SLOVENSKI STANDARD
SIST EN 12791:2016+A1:2018

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Nadomešča:
SIST EN 12791:2016

Kemična razkužila in antiseptiki - Razkužila za roke v kirurgiji - Preskusna metoda in zahteve (faza 2, stopnja 2) (vključuje dopolnilo A1)

Chemical disinfectants and antiseptics - Surgical hand disinfection - Test method and requirements (phase 2, step 2)

Chemische Desinfektionsmittel und Antiseptika - Chirurgische Händedesinfektionsmittel - Prüfverfahren und Anforderungen (Phase 2, Stufe 2)

Antiseptiques et désinfectants chimiques - Désinfection chirurgicale des mains - Méthodes d'essai et prescriptions (phase 2/étape 2)

Ta slovenski standard je istoveten z: **EN 12791:2016+A1:2017**

ICS:

11.080.20	Dezinfektanti in antiseptiki	Disinfectants and antiseptics

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EUROPEAN STANDARD

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English Version

Chemical disinfectants and antiseptics - Surgical hand disinfection - Test method and requirements (phase 2, step 2)

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This European Standard was approved by CEN on 13 December 2015 and includes Amendment 1 approved by CEN on 20 July 2017.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
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Contents

Page

European foreword

This document (EN 12791:2016+A1:2017) 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of an identical text or by endorsement, at the latest by May 2018, and conflicting national standards shall be withdrawn at the latest by May 2018.

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 includes Amendment 1 approved by CEN on 2017-07-20.

This document supersedes A1 EN 12791:2016 A1.

The start and finish of text introduced or altered by amendment is indicated in the text by tags A1 A1.

A1 *deleted text* A1

Data obtained using the former version of EN 12791 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 should 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 should be run.

According to the CEN/CENELEC Internal Regulations, the national standards organizations 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, Serbia, 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 surgical handrub and handwash reduces the release of resident and eventually present transient microbial flora on hands when used for the treatment of clean hands of volunteers.

This European Standard applies to products for surgical handrub or handwash 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 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 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 13727:2012+A2:2015, *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 mean reduction for immediate effect and 3 h effect of a product shall - when tested in accordance with Clause 5 - at least be not inferior to that achieved by a specified reference product (60 % volume concentration of propan-1-ol).

To demonstrate additionally a “sustained effect”, the mean reduction for the 3 h effect of a product shall be superior to that achieved by the reference product.

5 Test methods

5.1 Principle

A specified preparatory handwash (pre-wash) is carried-out on volunteers in order to remove most of the transient flora and foreign material, which could otherwise influence the “prevalues”, i.e. the number of microorganisms on the hands before treatment. The following samples from the hands are taken for bacterial counts:

— immediately after the pre-wash (before treatment with a product or the reference handrub);

— immediately after the surgical handrub or –wash procedure;

— 3 h after the surgical handrub or –wash procedure.

The ratio of the resulting values before and after treatment (reduction in numbers) represents a measure for the antimicrobial activity of the product under test. The immediate effect is characterized by the reduction achieved immediately after the procedure with the product. The 3 h effect is characterized by the reduction achieved three hours after the procedure with a product. To compensate for extraneous influences, these reductions from surgical handrub or surgical handwash procedures are compared individually with the corresponding reductions of a reference surgical handrub performed in parallel on the same volunteers.

Prior to the test a suitable neutralizer is validated. The neutralizer is used as sampling fluids for recovering the test organisms (5.2.1) after the surgical handrub or handwash procedure 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

The test is performed on the resident microbial flora of volunteers' hands and not on specified test organisms.

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.

All specified pH values are measured at (20 ± 1) ° C.

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 [1]) may be used.

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

NOTE See 5.2.2.7 for the procedure to prepare hard water.

5.2.2.3 Tryptone Soya Agar (TSA)

Tryptone soya 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
Agar	15,0 g
Water (5.2.2.2)	to 1000,0 ml

Sterilize in the autoclave [5.3.2.1a)]. After sterilization the pH of the medium shall be equivalent to 7,2 ± 0,2.

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 1000,0 ml

Sterilize in the autoclave [5.3.2.1a)]. After sterilization the pH of the medium shall be equivalent to 7,0 ± 0,2.

5.2.2.5 Neutralizer

The neutralizer shall be chosen, controlled and validated for the product under test in accordance with EN 13727 and EN 13624 (only yeasticidal activity). See 5.5.1.2 for more details.

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 °C ± 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), heated up to 75 °C ± 5 °C to dilute the soft soap. Take 200 g of the soft soap, fill up to 1000 g with water (5.2.2.2) and sterilize in the autoclave [5.3.2.1a)]. 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 B.

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 1000 ml. Sterilize by membrane filtration (5.3.2.7) or in the autoclave [5.3.2.1a)]. Autoclaving – if used - may cause a loss of liquid. In this case make up to 1000 ml with water (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. Sterilize 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 1000 ml volumetric flask (5.3.2.12) and add 6,0 ml (5.3.2.9) of solution A, then 8,0 ml of solution B. Mix and dilute to 1000 ml with water (5.2.2.2). The pH 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.

NOTE When preparing the product test solutions (5.4), the addition of the product to the hard water produces a different final water hardness in each test tube. In any case the final hardness, expressed as calcium carbonate ($CaCO_3$), is in the test tube lower than 375 mg/l.

5.2.2.8 Propan-1-ol as reference product [48,3 % w/w (weight concentration) corresponding to 60 % v/v (volume concentration) at 20 °C]

Fill 483 g propan-1-ol with a purity of min 99,5 % V/V (determined by gas chromatography; density 0,804) in a 1000 ml graduated flask equipped with a glass stopper on the weighing platform of a scale (precision 0,1 g). Add 420 g water (5.2.2.2). This will give a volume of approximately 1000 ml. Close the flask with the matching glass stopper and shake the contents of the flask thoroughly.

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

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[1)]

and, in particular, the following:

5.3.2.1 Apparatus for sterilization (moist and dry heat):

a) For moist heat sterilization, an autoclave capable of being maintained at (121^{+3}_{0}) °C for a minimum 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 additional test temperatures ± 1 °C.

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

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

5.3.2.4 **pH-meter,** having an inaccuracy of calibration of no more than ± 0,1 pH units at (20 ± 0,1) °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 **Electromechanical agitator,** e.g. Vortex® mixer[2)]

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 0,22 µm pore size for sterilization 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.

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 **Surgical gloves**, unpowdered glove for use in invasive surgery, sterile and free of antimicrobial activity.

If the manufacturer does not certify the freedom of any antimicrobial activity the following agar diffusion test may be used:

Prepare a suspension of 10^8 per ml of *Bacillus subtilis* spores (commercially available e.g. as strain ATCC 6633). This suspension can be stored at 4 °C for months.

Mueller-Hinton agar commercially available as powered medium is prepared and autoclaved according to the manufacturer's instructions and – while still liquid – temperature-equilibrated at 50 °C in a water bath. A quantity of the spore suspension equalling 1 % of the medium's volume is added, carefully mixed with the agar, and poured into Petri dishes (5.3.2.10) (20 ml per plate). After the agar has solidified these plates can be stored at 4 °C for 2 to 4 weeks.

A round test piece of 6 mm to 8 mm diameter is punched out with the cork borer (diameter 6 mm to 8 mm) from the surgical glove under test and placed onto the surface of a seeded Mueller-Hinton-plate. After incubation (5.3.2.3) for 48 h the plate is inspected for the presence of any inhibition zone around the test piece.

5.3.2.15 **Towels**, fresh and clean, e.g. made of paper or sterile cotton.

5.4 Product test solutions

The product as received shall be used as product test solution if recommended by the manufacturer. Product test solutions of products recommended by the manufacturer to be diluted shall be prepared in hard water (5.2.2.7).

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2) 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.

For solid products, dissolve the product as received by weighing at least 1,0 g ± 10 mg of the product in a volumetric flask and filling up with hard water (5.2.2.7). Subsequent dilutions (= 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 in volumetric flasks (5.3.2.12) on a volume/volume basis.

The product test solutions shall be prepared freshly and used in the test within 3 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, it shall be recorded in the test report.

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 microbicidal activity of the product on volunteers' hands

5.5.1 General

5.5.1.1 Experimental conditions

Contact time t (in min):

The contact time to be tested is to be chosen according to the manufacturer's recommendation, but not shorter than 60 s and not longer than 5 min. The contact time for the reference surgical handrub [propan-1-ol (5.2.2.8)] is 3 min.

The allowed deviation for each chosen contact time is ± 5 s.

NOTE The minimum contact time of 60 s takes into account the time needed to treat the hands the prescribed way, see 5.5.3.2.2.

5.5.1.2 Neutralization

The product under test has to be neutralized during the test. A suitable neutralizer (5.2.2.5) has to be found before the test procedure (5.5.3) is performed. For that purpose carry out the validation of the neutralization according to EN 13727 and EN 13624 (only yeasticidal activity). Pay special attention to the following points of these norms: 5.2.2.5 ("Neutralizer"), 5.5.1.2 ("Choice of test method"), 5.5.1.3 ("General instructions for validation and control procedures"), 5.5.2.3 ("Control A…"), 5.5.2.4 ("Neutralizer control B"), 5.5.2.5 ("Method validation "C"…") in connection with 5.5.2.6 ("Incubation and counting …"). If the membrane filtration method is used follow 5.5.3, 5.5.3.1, 5.5.3.3, 5.5.3.4, 5.5.3.5 in connection with 5.5.3.6). Calculation and verification shall be performed according to 5.6.2.4, 5.6.2.6 and 5.7.

The selected neutralizer shall work on all test-organisms and with the relevant interfering substances to be tested according to EN 13727 and EN 13624 (only yeasticidal activity) for surgical handrub and surgical handwash (EN 13727:2012+A2:2015, Clause 4 and EN 13624).

5.5.1.3 Equilibration of temperature

Prior to testing, equilibrate all reagents [product test solutions (5.4), propan-1-ol (5.2.2.8), diluted soft soap (5.2.2.6), TSB (5.2.2.4), the neutralizer (5.2.2.5) and – if necessary - hard water (5.2.2.7)] to the test temperature of 20 °C using the water bath (5.3.2.2) controlled at 20 °C. Check that the temperature of the reagents is stabilized at 20 °C.

5.5.1.4 Selection of volunteers

The test shall be performed on 23 to 28 healthy persons who have hands with healthy skin, without cuts or abrasions, and with short and clean fingernails. Starting from three days prior to test, they should not

use substances with antimicrobial activity, e.g. medicated soaps or hand creams. During a period of 10 days prior to the test they should not take antibiotics and products with similar efficacy. Furthermore they should not wear any jewellery or other items on the hands including the wrists on the actual test day.

Although, in general, age is not a limiting factor, volunteers should be at least 18 years of age.

As it may happen that values of volunteers cannot be used for calculation (for example volunteers drop out after the first test round, 5.5.1.5), it is recommended to do the test rather with more than 23 volunteers.

5.5.1.5 Experimental design

For testing a single product a crossover design is used. The volunteers are randomly divided into two groups of approximately the same size in a first run. Group 1 uses the reference surgical handrub ("RP", 5.5.3.2.2), group 2 the product under test ("PP", 5.5.3.2.3 or 5.5.3.2.4). After at least one week, allowing reconstitution of the normal skin flora, the test is repeated with changed roles in a second run.

Half of the volunteers are randomly chosen that their right hand is used for the "immediate postvalue" and the left hand for the "3-hour postvalue". The other half of the volunteers is treated the other way round.

For testing more than one product at a time, a Latin-square design is used with as many groups of volunteers and as many experimental runs as there are products (including the reference propan-1-ol). Only products can be simultaneously tested for which either no neutralization is necessary or for which the same neutralizer can be used for the assessment of post-values. In each run all disinfection procedures are employed in parallel. At least one week is required between the individual experimental runs, allowing reconstitution of the normal skin flora. At the end of the whole series every volunteer shall have used each product, including propan-1-ol, once.

5.5.2 Preparatory handwash

The volunteers' hands are prepared by washing for 1 min with 5 ml diluted soft soap (5.2.2.6). After having been rinsed with running tap water, they are thoroughly dried with towels (5.3.2.15).

5.5.3 Test procedure with volunteers

5.5.3.1 Sampling of the resident skin flora before treatment ("Prevalue")

Immediately after drying (5.5.2), rub all 10 fingertips for 1 min on the base of two Petri dishes (5.3.2.10) – one for each hand – each containing 10 ml of TSB (5.2.2.4) as sampling fluids in order to assess the release of the skin microorganisms before treatment of the hands (prevalues).

Dilutions of 10^{-1} and 10^{-2} of these sampling fluids are prepared with the sampling fluid, i.e. TSB (5.2.2.4). For each dilution, 0,1 ml is spread on surface dried plates containing TSA (5.2.2.3) using spreaders (5.3.2.13). The interval between sampling and plating shall not exceed 30 min. As an alternative technique to the spread plate technique the pour plate technique may be used by transferring each 0,1 ml sample into separate Petri dishes and adding 15 ml to 20 ml melted TSA (5.2.2.3), cooled to 45 °C ± 1 °C.

The sampling fluid for the prevalues should not contain neutralizer (5.2.2.5) as this may influence the performance of the product under test. The different sampling procedures for pre- and postvalues will not influence the evaluation of the product since the reference product is treated the same way.

For incubation and counting, see 5.5.4.