SLOVENSKI STANDARD
SIST EN 17430:2024

01-junij-2024

Kemična razkužila in antiseptiki - Higiensko razkuževanje rok z drgnjenjem z virucidnim sredstvom - Preskusna metoda in zahteve (faza 2, stopnja 2)

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

Chemische Desinfektionsmittel und Antiseptika - Viruzide hygienische Händedesinfektion - Prüfverfahren und Anforderungen (Phase 2, Stufe 2)

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

Ta slovenski standard je istoveten z: **EN 17430:2024**

ICS:

11.080.20	Dezinfektanti in antiseptiki	Disinfectants and antiseptics

SIST EN 17430:2024 **en,fr,de**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 17430

March 2024

ICS 11.080.20

English Version

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

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

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

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COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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European foreword

This document (EN 17430:2024) 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of an identical text or by endorsement, at the latest by September 2024, and conflicting national standards shall be withdrawn at the latest by September 2024.

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1 Scope

This document specifies a test method simulating practical conditions for establishing whether a product for hygienic handrub reduces the release of virus contamination 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 document is applicable 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 can occur in the workplace and in the home. It can 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 are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 12353, *Chemical disinfectants and antiseptics — Preservation of test organisms used for the determination of bactericidal (including Legionella), mycobactericidal, sporicidal, fungicidal and virucidal (including bacteriophages) activity*

EN 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.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at https://www.iso.org/obp
- IEC Electropedia: available at https://www.electropedia.org/

4 Requirements

When tested in accordance with Clause 5, the mean reduction of the test organism murine norovirus strain S99 Berlin 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 (70 % volume concentration of ethanol).

Table 1 — Minimum and additional test conditions

	Hygienic handrub
Test virus	**Virucidal activity** [a] Murine norovirus **Limited spectrum virucidal activity** [b] Murine norovirus
Test temperature	room temperature of 21,5 °C ± 3,5 °C
Contact time	according to the manufacturer's recommendation, but between 30 s and 60 s
Additional conditions	Further contact time(s)
[a] To claim the virucidal activity the product shall pass standards EN 14476 with *Poliovirus*, *Adenovirus* and *Murine Norovirus*. [b] To claim limited spectrum virucidal activity the product shall pass EN 14476 with *Adenovirus* and *Murine Norovirus.*	

5 Test methods

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 (virus titres) represents a measure for the virucidal 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.

5.2 Materials and reagents

5.2.1 Test organism

Murine norovirus strain S99 Berlin[1]

This test organism has been specifically chosen to meet health and safety guidance and ethical committee considerations. According to German Ordinance on Biological Substances (BiostoffV)/TRBA 462 Murine Norovirus is classified in risk group 1.

[1] Murine norovirus can be obtained from Friedrich-Loeffler-Institut Bundesinstitut für Tiergesundheit, Hauptsitz Insel Riems Südufer 10, 17493 Greifswald-Insel Riems; phone: +49 38351 7-0; fax: +49 38351 7-121. http://www.fli.de/. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of the product named.

5.2.2 Culture media and reagents

5.2.2.1 General

All weights of chemical substances given in this document refer to the anhydrous salts. Hydrated forms may be used as an alternative, but the weights required shall be adjusted to allow for consequent molecular weight differences.

The reagents shall be of analytical grade and/or appropriate for microbiological purposes. They shall be free from substances that are toxic or inhibitory to the test 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 demineralized water. If distilled water of adequate quality is not available, water for injections (see bibliographic reference [3]) 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.

If the water is sterilized during sterilization of the reagents, this is not necessary.

5.2.2.3 Phosphate buffered saline (PBS)

Sodium chloride (NaCl)	8,00 g
Potassium chloride (KCl)	0,20 g
Disodium hydrogen phosphate, 12-hydrate ($Na_2HPO_4 \cdot 12H_2O$)	2,89 g
Potassium phosphate, monobasic (KH_2PO_4)	0,20 g
Water (5.2.2.2)	to 1 000,0 ml

5.2.2.4 Neutral Red (1:1000 solution) or crystal violet solution

Prepare neutral red (e.g. Sigma N7005[2]) stock solution at 0,1 mg/ml in water (5.2.2.2).

Prepare 0,1 % crystal violet in 20 % ethanol.

Filter through a 0,40 µm pore size filter and store 4 °C in the dark.

5.2.2.5 Fetal calf serum (FCS)

FCS has to be certified free of viruses and mycoplasma. Extraneous viruses and mycoplasma may interfere with cell and virus growth resulting in false results.

For RAW 264.7 cells, special FCS has to be used due to the cells' high sensitivity to endotoxins.

5.2.2.6 Trichloroacetic acid (10 % solution) (TCA)

Dissolve 10 g of TCA crystals in 80 ml of water (5.2.2.2), then adjust the volume to 100 ml with water. Stir to complete solution.

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[2] Sigma N7005 is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product.

5.2.2.7 Hard water

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 (5.3.2.13) 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 the refrigerator (5.3.2.17) 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 (5.3.2.13). Store the solution in the refrigerator (5.3.2.17) 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 (5.3.2.10) and add 6,0 ml (5.3.2.9) of solution A, then 8,0 ml of solution B. Mix and dilute to 1 000 ml with water (5.2.2.2). The pH (5.3.2.4) of the hard water shall be 7,0 ± 0,2. If necessary, adjust the pH by using a solution of approximately 40 g/l (about 1 mol/l) of sodium hydroxide (NaOH) or approximately 36,5 g/l (about 1 mol/l) of hydrochloric acid (HCl).

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

NOTE When preparing the product test solutions (5.6.3), the addition of the product to the hard water produces different final water hardness in each test tube. In any case, the final hardness in the test tube expressed as calcium carbonate ($CaCO_3$) is lower than 375 mg/l.

5.2.2.8 Growth and maintenance media

Dulbecco's Modified Eagle's Medium (DMEM) or equivalent, supplemented with appropriate concentration of heat inactivated (e.g. 56 °C for 30 min) and mycoplasma-free fetal calf serum FCS (5.2.2.5), antibiotics, and other growth factors as needed shall be used. Additional supplements like glucose, glutamine, and sodium pyruvate shall be indicated in the test report.

a) A *growth medium* for cell multiplication is supplemented with 10 % FCS (5.2.2.5). Add 10 parts of FCS to 90 parts of DMEM.

b) A *maintenance medium* to maintain the cell culture metabolism without stimulation of cell proliferation is supplemented with 2 % FCS. Add 2 parts of FCS (5.2.2.5) to 98 parts of DMEM.

Other media may be used if appropriate for certain cell lines.

See also bibliographic reference [5]. See EN 12353 for a detailed description.

5.2.2.9 Diluted soft soap

Linseed oil	50 parts by weight
Potassium hydroxide [3]	9,5 parts by weight
Ethanol (min. 95 %) [3]	7 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 dissolve 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 1 000 g

with water (5.2.2.2) and sterilize in the autoclave [5.3.2.1 a)]. 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 C.

5.2.2.10 Ethanol as reference handrub [volume fraction of 70 % at 21,5 °C ± 3,5 °C]

Fill 547,2 g ethanol with a purity of min volume fraction of 99,5 % (determined by gas chromatography; density 0,790) in a 1 000 ml flask equipped with a glass stopper on the weighing platform of a scale (precision 0,1 g). Add 322,7 g water (5.2.2.2). Close the flask with the matching glass stopper and shake the contents of the flask thoroughly. During mixing the solution warms up about to 30 °C. After cooling down to 21,5 °C ± 3,5 °C, it will give a volume of approximately 980 ml.

NOTE This solution can be kept indefinitely at approximately room temperature if protected from light, provided that the flask is closed with a ground-in glass stopper to avoid any evaporation.

5.3 Apparatus and glassware

5.3.1 General

Sterilize all glassware and parts of the apparatus that will come into contact with the culture media and reagents or the sample, except those which are supplied sterile, by one of the following methods:

a) by moist heat, in the autoclave [5.3.2.1 a)];

b) by dry heat, in the hot air oven [5.3.2.1 b)].

5.3.2 Usual microbiological laboratory equipment[3] and, in particular, the following:

5.3.2.1 Apparatus for sterilization (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 21,5 °C ± 3,5 °C.

5.3.2.3 Inverted microscope for reading cell cultures microscopically.

5.3.2.4 pH meter, having an inaccuracy of calibration of 0,1 pH units at 21,5 °C ± 3,5 °C.

5.3.2.5 Stopwatch.

5.3.2.6 Deep freezer (−20 °C and −70 °C or less).

[3] Disposable sterile equipment is an acceptable alternative to reusable glassware.

5.3.2.7 Electromechanical agitator e.g. Vortex® mixer[4].

5.3.2.8 Containers: sterile test tubes, culture bottles or flasks of suitable capacity.

5.3.2.9 Graduated pipettes of nominal capacities 10 ml, 1 ml and 0,1 ml. Calibrated automatic pipettes, with disposable tips, may be used.

5.3.2.10 Volumetric flasks, calibrated at 21,5 °C ± 3,5 °C.

5.3.2.11 Sterile microtitre plates, six or eight well plates for cell cultures.

5.3.2.12 Flasks for cell cultures.

5.3.2.13 Membrane filtration apparatus for filtration of media, 0,2 µm pore size.

5.3.2.14 CO_2 incubator (95 % air, 5 % CO_2), capable of being controlled at either 36 °C ± 1 °C, or at 37°C ± 1 °C for incubation of cell cultures.

5.3.2.15 Biological safety cabinet, class II.

5.3.2.16 Centrifuge (400 g_N to 1000 g_N).

5.3.2.17 Refrigerator, capable of being controlled at 2 °C to 8 °C.

5.4 Preparation of test organism suspensions and product test solutions

5.4.1 Test virus suspension (test organisms suspensions, contamination fluid)

The test organisms and their stock cultures shall be prepared and kept in accordance with EN 12353.

The stock virus suspension is multiplied in an appropriate cell line that produces high titres of infectious viruses. The cell debris is separated by centrifugation (5.3.2.16) (400 g_N for 15 min). This preparation is called “test virus suspension”.

It is suggested that the minimum titre of the virus suspension – determined by a quantal test (5.6.1) or by plaque test (5.6.2) – is at least 10^8 $TCID_{50}$/ml. In any case, it shall be sufficiently high to at least 10^4 $TCID_{50}$/ml may be recovered from the hands.

In exceptional cases the test virus suspension may be concentrated by appropriate methods (e.g. ultracentrifugation).

The test virus suspension is kept in small volumes below −70 °C or preferably at −196 °C under nitrogen. Due to safety reasons, and – in some cases – to limit the possibility of genetic mutations, only 10 passages from the original seed (e.g. virus from culture collection) are allowed.

The test virus suspension is used undiluted for the test procedure (5.7).

The titre of the test virus suspension is determined as described (see 6.2 and 6.3).

[4] Vortex® is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product.