SLOVENSKI STANDARD
oSIST prEN 16777:2014

01-oktober-2014

Kemična razkužila in antiseptiki - Kvantitativni preskus na neporoznih površinah brez mehanskega delovanja za vrednotenje virucidnega delovanja kemičnih razkužil, ki se uporabljajo v humani medicini - Preskusna metoda in zahteve (faza 2/stopnja 2)

Chemical disinfectants and antiseptics - Quantitative non-porous surface test without mechanical action for the evaluation of virucidal activity of chemical disinfectants used in the medical area - Test method and requirements (phase 2/step 2)

Chemische Desinfektionsmittel und Antiseptika - Quantitativer Versuch auf nicht porösen Oberflächen ohne mechanische Einwirkung zur Bestimmung der viruziden Wirkung im humanmedizinischen Bereich - Prüfverfahren und Anforderungen (Phase 2, Stufe 2)

Antiseptiques et désinfectants chimiques - Essai quantitatif de surface non-poreuse sans action mécanique pour l'évaluation de l'activité virucide des désinfectants chimiques utilisés dans le domaine médical - Méthode d'essai et prescriptions (phase 2/étape 2)

Ta slovenski standard je istoveten z: **prEN 16777**

ICS:

11.080.20	Dezinfektanti in antiseptiki	Disinfectants and antiseptics

oSIST prEN 16777:2014 **en,fr,de**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

DRAFT
prEN 16777

July 2014

ICS 11.080.20

English Version

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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Foreword

This document (prEN 16777:2014) has been prepared by Technical Committee CEN/TC 216 “Chemical disinfectants and antiseptics”, the secretariat of which is held by AFNOR.

This document is currently submitted to the CEN Enquiry.

Introduction

This European Standard describes a surface test method for establishing whether a product proposed as a disinfectant in the fields described in clause 1 has or does not have virucidal activity on non-porous surfaces.

The laboratory test closely simulates practical conditions of application. Chosen conditions (contact time, temperature, organisms on surfaces etc.) reflect parameters which are found in practical situations including conditions which may influence the action of disinfectants. Each use concentration found from this test corresponds to defined experimental conditions.

The conditions are intended to cover general purposes and to allow reference between laboratories and product types. Each utilization concentration of the chemical disinfectant or antiseptic found by this test corresponds to defined experimental conditions.

However for special applications the recommendations of use of a product can differ and therefore additional test conditions might be needed, which cannot be covered by this European Standard.

1 Scope

This European Standard specifies a test method and the minimum requirements for virucidal activity of chemical disinfectants 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 without mechanical action.

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 1 The method described is intended to determine the activity of commercial formulations or active substances on viruses in the conditions in which they are used.

NOTE 2 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 14476, *Chemical disinfectants and antiseptics — Quantitative suspension test for the evaluation of virucidal activity in the medical area — Test method and requirements (Phase 2/Step 1).*

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.*

EN 10088-1, *Stainless steels - Part 1: List of stainless steels.*

EN 10088-2, *Stainless steels - Part 2: Technical delivery conditions for sheet/plate and strip of corrosion resisting steels for general purposes.*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN 14885 and EN 14476 apply.

4 Requirements for virucidal activity on surfaces

The product shall demonstrate at least a decimal log (lg) reduction of 4 in virus titre of the *Adenovirus* and *Murine Norovirus* test strains when tested in accordance with table 1 and Clauses 5, 6, 7, 8, and 9.

Table 1 — Minimum and additional test conditions

Test conditions	
Test virus	*Adenovirus* Type 5 *Murine Norovirus*
Test temperature	between 18 °C ± 1 °C and 25 °C ± 1 °C
Contact time	according to the manufacturer's recommendation, but not longer than 5 min or 60 min[a]
Interfering substances a) clean b) dirty	 0,3 g/l bovine serum albumin and/or 3,0 g/l bovine serum albumin plus 3,0 ml erythrocytes
Additional conditions[b]	Further contact time(s), interfering substance(s) or virus(es)

[a] The contact times for surface disinfectants stated in this table 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.

[b] Where appropriate (specific purposes), additional specific virucidal activity shall be determined under other conditions of time, temperature, and interfering substances (see 5.2.3.3) in accordance with 6.2 in order to take into account intended specific use conditions. Additional virus(es) can be tested, if relevant.

The determined virucidal concentration of the test product is suggested as being suitable for practical situations of use.

5 Test methods

5.1 Principle

5.1.1 A test suspension of viruses in a solution of interfering substances is inoculated onto a test stainless steel disc and dried. A prepared sample of the product under test is applied in a manner which covers the dried film.

The disc is maintained at a specified temperature for a defined period of time. The disc is transferred to cell maintenance medium so that the action of the disinfectant is immediately neutralised. The titre of the virus recovered from the disc is determined.

The titre of the virus on a disc treated with hard water in place of the disinfectant is also determined and the reduction in virus titre attributed to the product is calculated by difference.

5.1.2 The test is performed using the test organisms as specified in clause 4, table 1.

5.1.3 Other contact times and temperatures within the limits specified in clause 4, table 1 may be used. Additional interfering substances and test organisms may be used.

5.2 Materials and reagents, including cell cultures

5.2.1 Test organisms

The virucidal activity shall be evaluated using the following strains as test organisms selected according to clause 4, table 1[1)]

a) Non-enveloped RNA virus[2)]

Murine norovirus, strain S99 Berlin

b) Non-enveloped DNA virus

Adenovirus type 5, strain Adenoid 75, ATCC VR-5*

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

If additional test organisms are used, they shall be kept and used under optimum growth conditions (temperature, time, atmosphere, media) noted in the test report. 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.

5.2.2 Culture media, reagents and cell cultures

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 – if appropriate the 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.

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

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 [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.

See 5.2.2.7 for the procedure to prepare hard water.

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1) The ATCC numbers are the collection numbers of strains supplied by these culture 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.

2) Virus strains may be obtained from a national or international culture collection. *Murine Norovirus* may be obtained from Friedrich-Loeffler-Insitut Bundesforschunsinstitut für Tiergesundheit, Hauptsitz Insel Riems Südufer 10, 17493, Greifswald-Insel Riems; phone: +49 38351 7-0, fax: +49 038351 7-121. http://www.fli.bund.de.

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 x $12H_2O$)	2,89 g
Potassium phosphate, monobasic (KH_2PO_4)	0,20 g
Water (5.2.2.2)	to 1000,0 ml

5.2.2.4 Neutral Red (1:1000 solution)

Prepare neutral red (Sigma N7005) stock solution at 0,1 mg/ml in water (5.2.2.2). Filter through a 0,40 µm pore size filter and store 4 °C in the dark.

5.2.2.5 Foetal 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), and then adjust the volume to 100 ml with water. Stir to complete solution.

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. 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 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 (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.

NOTE When preparing the product test solutions (5.4.2), 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 Interfering substance

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 and detergents) shall be defined.

“Diluent” is generally used in the other European Standards in the medical area to prepare the interfering substance. Since there is no experience in virucidal testing with diluent, water (5.2.2.2) is used instead.

NOTE The term “interfering substance” is used even if it contains more than one substance.

5.2.2.8.2 Clean conditions (bovine serum albumin)

Bovine serum albumin shall be used as commercially available product or shall be prepared as follows:

— dissolve 0,3 g of bovine albumin fraction V (suitable for microbiological purposes) in 100 ml of water (5.2.2.2);

— sterilize by membrane filtration;

— keep in a refrigerator and use within one month.

The final concentration of bovine serum albumin (BSA) in the test is 0,3 g BSA per litre.

5.2.2.8.3 Dirty conditions

a) bovine serum albumin:

Bovine serum albumin shall be used as commercially available product or shall be prepared as follows:

— dissolve 3 g of bovine albumin fraction V (suitable for microbiological purposes) in 100 ml of water (see 5.2.2.2);

— sterilize by membrane filtration;

— keep in a refrigerator and use within one month.

The final concentration of bovine serum albumin (BSA) in the control is 3 g BSA per litre (see 6.6.3).

b) sheep erthrocytes:

Prepare at least 8,0 ml fresh defibrinated sheep blood (5.2.2.9). Centrifuge the erythrocytes at 800 g_N for 10 min (5.3.2.13). After discarding the supernatant, resuspend erythrocytes in water (5.2.2.2). Repeat this procedure at least 3 times, until the supernatant is colourless.

c) bovine albumin and erythrocyte solution:

Resuspend 3 ml of packed erythrocytes with 97 ml of 3 % w/v of bovine albumin solution.

The final concentration of sheep erythrocytes and albumin in the test procedure is 3 ml/l and 3 g/l respectively. To avoid contamination this mixture shall be split in portions probably needed per day and stored in separate containers for a maximum of 7 days at 2 °C to 8 °C.

5.2.2.9 Defibrinated sheep blood

The defibrinated sheep blood shall be sterile (aseptic blood-letting and preparation). The defibrinated sheep blood can be pooled from more than one sheep and can be acquired from a commercial supplier.

5.2.2.10 Medium for cell cultures

Eagle's minimal essential medium (MEM) or equivalent, supplemented with FCS (5.2.2.5), antibiotics, and other growth factors as needed shall be used.

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

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 MEM.

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

See also bibliographic reference [2]. See EN 12353 for a detailed description.

5.2.2.11 Cell cultures

Cell monolayers shall be >90% confluent before inoculation. Cell lines are selected in accordance with their sensitivity to the test organisms (5.2.1). Cells for virus titration, if used as suspensions in quantal tests, shall be added to the dilutions of the test mixture (5.5.2) in such a density as to enable the formation of a monolayer in at least two days in the cell control. Cell cultures can be used as cell monolayers or in suspensions for quantal tests. For details of cell lines see 5.5.1.1e).

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:

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

d) 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)

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

f) 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 (**5.5.1**).

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