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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)

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National foreword

This British Standard is the UK implementation of EN 16777:2018.

The UK participation in its preparation was entrusted to Technical Committee CH/216, Chemical disinfectants and antiseptics.

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 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)

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 exigences (phase 2/étape 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)

This European Standard was approved by CEN on 24 September 2018.

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European foreword

This document (EN 16777:2018) 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of an identical text or by endorsement, at the latest by June 2019, and conflicting national standards shall be withdrawn at the latest by June 2019.

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

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Introduction

This document 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 document.

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1 Scope

This document 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 document applies to products that are used in the medical area for disinfecting non-porous surfaces including surfaces of medical devices without mechanical action.

This document 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 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 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*

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3 Terms and definitions

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

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

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

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 Clause 5. As described in EN 14885, to claim virucidal activity against enveloped virus the product shall pass both EN 14476 and this standard with vaccinia virus and to claim limited spectrum virucidal activity the product shall pass both EN 14476 and this standard with adenovirus and murine norovirus. However, to claim the virucidal activity the product shall pass standards EN 14476 with poliovirus, adenovirus and murine norovirus and this standard with adenovirus and murine norovirus, because poliovirus is not resistant to drying.

Table 1 — Minimum and additional test conditions

Minimum spectrum of test organisms	**Virucidal activity**[a] adenovirus murine norovirus **Limited spectrum virucidal activity**[b] adenovirus murine norovirus **Virucidal activity against enveloped viruses**[c] vaccinia virus
Test temperature	between 18 °C and 25 °C
Additional temperature	between 4 °C and 30 °C
Contact time	according to the manufacturer's recommendation, but not longer than 5 min or 60 min[d]
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[e]	Further contact time(s), interfering substance(s) or virus(es)

[a] Poliovirus (as used in the corresponding suspension test) cannot be used for surfaces, because of drying problems.

[b] The test for "limited spectrum virucidal activity" will cover all enveloped viruses (Annex A) and norovirus, rotavirus and adenovirus.

[c] The test for "virucidal activity against enveloped viruses" will cover all enveloped viruses only (Annex A).

[d] The contact times for surface disinfectants stated in this table are chosen on the basis of the practical

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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.

[e] Where appropriate (specific purposes), additional specific virucidal activity shall be determined under other conditions of time, temperature, and interfering substances (see 5.2.2.8) in accordance with 5.5, in order to take into account intended specific use conditions. Additional virus(es) can be tested, if relevant. For the additional conditions, the concentration defined as a result can be lower than the one obtained under the minimum test conditions.

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 surface and dried. A prepared sample of the product under test is applied in a manner which covers the dried film.

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

The titre of the inoculum on a test surface 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

Murine norovirus, strain S99 Berlin

NOTE Virus strains can be obtained from a national or international culture collection. Murine norovirus can be obtained from Friedrich-Loeffler-Insitut Bundesforschungsinstitut 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.

b) Non-enveloped DNA virus

[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.

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

c) Enveloped DNA virus

Vaccinia virus, strain modified vaccinia virus Ankara (MVA), ATCC VR-1508 or vaccinia virus strain Elstree, ATCC VR-1549

The required incubation temperature for these test organisms is (36 ± 1) °C or (37 ± 1) °C (5.3.2.12). 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 ± 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.

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 [2] stock solution at 0,1 mg/ml in water (5.2.2.2). Filter through a 0,44 µm pore size filter and store at 4 °C in the dark.

[2] Sigma N 7005 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.

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5.2.2.5 Foetal calf serum (FCS)

FCS shall 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 shall 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 (5.3.2.11) 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 (5.3.2.6) 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 (5.3.2.11). Store the solution in the refrigerator (5.3.2.6) for no longer than one week;

— place 600 ml to 700 ml of water (5.2.2.2) in a 1000 ml volumetric flask (5.3.2.9) and add 6,0 ml 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:

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