SLOVENSKI STANDARD
SIST EN 14476:2005+A1:2007

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Kemiļ na razkuģila in antiseptiki - Kvantitativni suspenzijski preskus virucidnosti kemiļ nih razkuģil in antiseptikov, ki se uporabljajo v humani medicini - Preskusna metoda in zahteve (faza 2, stopnja 1)

Chemical disinfectants and antiseptics - Virucidal quantitative suspension test for chemical disinfectants and antiseptics used in human medicine - Test method and requirements (phase 2, step 1)

Chemische Desinfektionsmittel und Antiseptika - Quantitativer Suspensionsversuch Viruzidie für in der Humanmedizin verwendete chemische Desinfektionsmittel und Antiseptika - Prüfverfahren und Anforderungen (Phase 2, Stufe 1)

Antiseptiques et désinfectants chimiques - Essai virucide quantitatif de suspension pour les antiseptiques et désinfectants chimiques utilisés en médecine humaine - Méthode d'essai et prescriptions (phase 2, étape 1)

Ta slovenski standard je istoveten z: **EN 14476:2005+A1:2006**

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11.080.20	Dezinfektanti in antiseptiki	Disinfectants and antiseptics

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EUROPEAN STANDARD

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Chemische Desinfektionsmittel und Antiseptika - Quantitativer Suspensionsversuch Viruzidie für in der Humanmedizin verwendete chemische Desinfektionsmittel und Antiseptika - Prüfverfahren und Anforderungen (Phase 2, Stufe 1)

This European Standard was approved by CEN on 3 March 2005 and includes Amendment 1 approved by CEN on 28 September 2006.

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This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

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COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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Contents

Foreword

This document (EN 14476:2005+A1:2006) 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 April 2007, and conflicting national standards shall be withdrawn at the latest by April 2007.

This document includes Amendment 1, approved by CEN on 2006-09-28.

This document supersedes EN 14476:2005.

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

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.

Other methods to evaluate the efficacy of chemical disinfectants and antiseptics for different applications in the medical field are in preparation.

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

1 Scope

This document specifies a test method and the minimum requirements for virucidal activity of chemical disinfectants or antiseptic products for instruments, surfaces or hands 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 is applicable to a broad spectrum of viruses (Annex B) and 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.

It is impossible to determine the virucidal activity of the undiluted product as some dilution is always produced by adding the inoculum and interfering substance. However, a disinfectant or antiseptic which is used in undiluted form is tested in 80% concentration and shall pass this test prior to further assessment.

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

NOTE 2 This method corresponds to a phase 2, step 1 test (see Annex F).

2 Normative references

The following referenced documents are indispensable for the application 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 14820, *Single-use containers for human venous blood specimen collection*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
clean conditions
conditions representative of surfaces which have received a satisfactory cleaning programme and/or are known to contain minimal levels of organic and/or inorganic substances

3.2
cytotoxicity
morphological alteration of cells and/or their destruction or their reduced sensitivity to virus multiplication caused by the product

3.3
dirty conditions
conditions representative of surfaces which are known to or may contain organic and/or inorganic substances

3.4
inactivation of viruses
reduction of infectivity of a virus by a product

NOTE Alteration of antigenic reactivity or of any viral component does not necessarily mean reduction of infectivity of a virus.

3.5
interfering substances
protein solutions and erythrocytes that are added to a test virus suspension before addition of the product test solution to demonstrate any influence of protein and erythrocytes on the virucidal activity of the product test solution

3.6
plaque forming units (PFU)
number of infectious virus particles per unit volume (ml)

3.7
product
chemical agent or formulation used as a chemical disinfectant or antiseptic

3.8
reference virus inactivation test
test with a defined product (e.g. formaldehyde) in parallel with a product under test for the internal control of the test

3.9
$TCID_{50}$
50 % infecting dose of a virus suspension or that dilution of the virus suspension that induce a CPE (see 3.10) in 50 % of cell culture units

3.10
viral cytopathic effect (CPE)
morphological alteration of cells and/or their destruction as a consequence of virus multiplication

3.11
viral infectivity
ability of a virus to produce infectious progeny in a sensitive cell strain

NOTE Viral infectivity may also include the expression of at least part of the virus' genetic information in cells

3.12
viral plaque
area of lysis formed in a cell monolayer under semisolid medium due to infection by and multiplication of a single infectious virus particle

3.13
virucide
product that inactivates viruses under defined conditions

NOTE The adjective derived from "virucide" is "virucidal".

3.14
virucidal activity
capability of a product to produce a reduction in the number of infectious virus particles of relevant test organisms under defined conditions

3.15
virus titre
amount of infectious virus per unit volume present in a cell culture lysate

4 Requirements

A product, when tested in accordance with Clause 6 and Clause 7, shall demonstrate at least a decimal log (lg) reduction of 4 in virus titre of the test strains when tested under the test conditions described in Table 1.

Table 1 — Required test conditions

Test conditions	Instrument and surface disinfectants	Hygienic handrub and handwash	Chemothermal disinfection procedure
Test virus	*Poliovirus* and *Adenovirus*	*Poliovirus* and *Adenovirus*	*Parvovirus*
Test temperature	20 °C ± 1 °C (except for chemothermal disinfection)	20 °C ± 1 °C (except for chemothermal disinfection)	according to the recommendation of the manufacturer, but not higher than 60°C
Contact time a) obligatory	60 min	1 min or 30 s, if manufacturer recommends < 1 min	according to the contact time recommended by the manufacturer, but not longer than 60 min
b) additional	5 min, 15 min, 30 min	3 min	-
Interfering substances a) clean	0,3 g/l bovine serum albumin	PBS[a]	0,3 g/l bovine serum albumin
and/or b) dirty	and/or 3,0 g/l bovine serum albumin plus 3,0 ml erythrocytes	and/or -	and/or 3,0 g/l bovine serum albumin plus 3,0 ml erythrocytes
a) Phosphate Buffered Saline			

NOTE The human immunodeficiency virus (HIV) is not considered a virus which requires testing, because it is a highly fragile virus. Therefore testing of the virucidal activity of chemical disinfectants against HIV is not necessary within the framework of this European Standard, if the product is active against poliovirus. In fact, poliovirus is selected as test virus because it has a high resistance to chemicals, is acid-stable and is unaffected by lipid solvents such as ether, and most detergents or quaternary products.

5 Materials and reagents

5.1 Apparatus and glassware

5.1.1 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 that are supplied sterile, by one of the methods described in 5.1.2.2.

5.1.2 Usual microbiological laboratory equipment[1)]

5.1.2.1 General

and, in particular, the following:

5.1.2.2 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.

5.1.2.3 **Water bath** capable of being controlled at 20 °C ± 1 °C.

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

5.1.2.5 **pH-meter,** having an accuracy of calibration of 0,1 pH units at 25 °C.

5.1.2.6 **Inverted microscope** for reading cell cultures microscopically

5.1.2.7 **Stopwatch**

5.1.2.8 **Electromechanical agitator**, e.g. Vortex ® mixer[2)].

5.1.2.9 **Membrane filtration apparatus** for filtration of media (0,22 µm pore size).

5.1.2.10 **Microtitre plates or tubes**, **petri dishes and flasks** for cell culture use.

5.1.2.11 **Magnetic stirrer** for keeping cells in suspension before seeding.

5.1.2.12 **CO_2 incubator** (95% air, 5% CO_2), capable of being controlled at 36 °C ± 1 °C, for incubation of cell cultures. An incubator at 37 °C ± 1 °C may be used if an incubator at 36 °C ± 1 °C is not available.

5.1.2.13 **Ice producing machine or commercially available ice** to cool the cell maintenance medium and the reaction mixtures during the test (see 6.1, 6.6.3, and 6.6.6.1).

5.1.2.14 **Basin as ice bath** with ice and water.

5.1.2.15 **Container**: sterile test tubes, culture bottles or flasks of suitable capacity.

5.1.2.16 **Graduated sterile pipettes**, of nominal capacities 10 ml and 1 ml and 0,1 ml.

NOTE Calibrated automatic pipettes may be used.

5.1.2.17 **Volumetric flasks** calibrated at 20 °C.

5.1.2.18 **Mechanical shaker**

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

2) 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.

5.1.2.19 Centrifuge

5.1.2.20 Biological safety cabinet, class II

5.1.2.21 Freezer, -70 °C

5.2 Culture media and reagents

5.2.1 General

The reagents shall be of analytical grade and/or appropriate for virological purposes.

NOTE To improve reproducibility, it is recommended that commercially available dehydrated material be used for the preparation of culture media. The manufacturer's instructions relating to the preparation of these products should be rigorously followed.

5.2.2 Water

5.2.2.1 General requirements on water

The water shall be free from substances that are toxic to cells. It shall be freshly glass double-distilled or demineralised water.

Sterilise in the autoclave (5.1.2.2).

NOTE 1 If the water is sterilised during the sterilisation of the reagents, this is not necessary.

NOTE 2 If distilled water of adequate quality is not available, water for injectable preparations (see bibliographic reference [1]) can be used.

5.2.2.2 Hard water

Hard water for dilution of products is prepared as follows:

— solution A:

dissolve 19,84 g anhydrous magnesium chloride ($MgCl_2$) or an equivalent of hydrated magnesium chloride and 46,24 g anhydrous calcium chloride ($CaCl_2$) or an equivalent of hydrated calcium chloride in water (5.2.2.1) and dilute to 1 000 ml;

sterilize in the autoclave (5.1.2.2). Store the solution at 2 °C to 8 °C for no longer than one month;

— solution B:

dissolve 35,02 g sodium bicarbonate ($NaHCO_3$) in water (see 5.2.2.1) and dilute to 1 000 ml. Sterilize by membrane filtration. Store the solution at 2 °C to 8 °C for no longer than one week.

Hard Water:

— for the preparation of 1 l, place at least 600 ml water (see 5.2.2.1) in a 1 000 ml volumetric flask (5.1.2.17) and add 6,0 ml of solution A, then 8,0 ml of solution B;

— mix and dilute to 1 000 ml with water (see 5.2.2.1);

— the pH 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 the addition of the product to this hard water produces a different final water hardness in each test tube. In any case the final hardness is lower than 300 mg/l of calcium carbonate ($CaCO_3$) in the test tube.

5.2.3 Composition and preparation of cell culture media and reagents

5.2.3.1 General

Chemicals should be of analytical reagent grade. New or cleaned and dried spatulas should be used between each weighing to prevent cross-contamination of reagents. As each batch of medium is prepared, the lot number of each reagent should be recorded as it is weighed out.

5.2.3.2 Growth and maintenance media

Eagle's minimal essential medium (MEM) or equivalent, supplemented with appropriate concentration of inactivated and mycoplasma-free foetal calf serum (FCS), antibiotics, and other growth factors as needed shall be used. (For reference see bibliographic reference [2]).

NOTE Materials and reagents for cell culture may be purchased from biological supply companies.

5.2.3.3 Interfering substances

5.2.3.3.1 General

The interfering substance(s) shall be chosen according to the conditions of use laid down for the product (see Table 1).

The interfering substance(s) shall be sterile and prepared at 10 times the final concentration in the test (except the fresh defibrinated sheep blood).

The method of preparation and sterilisation together with the composition shall be noted in the test report (9.2).

5.2.3.3.2 Clean conditions (bovine serum albumin)

Bovine serum albumin should 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 (see 5.2.2);

— sterilise by membrane filtration.

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

5.2.3.3.3 Dirty conditions

a) bovine serum albumin:

Bovine serum albumin should 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);

— sterilise by membrane filtration.

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

b) sheep erthrocytes:

Sterile defibrinated sheep blood should be used as commercially available product or shall be prepared according to EN 14820.

Centrifuge erythrocytes from at least 8 ml fresh defibrinated sheep blood at 800 g (5.1.2.19) for 10 min. After discarding the supernatant, resuspend the erythrocytes in sterile phosphate buffered saline (PBS). Repeat this procedure at least three times (the supernatant should be 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 should 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.

5.3 Viruses

5.3.1 Test viruses

The virucidal activity shall be evaluated using the test viruses listed in Table 2 depending on the intended use of the product.

Table 2 — Viruses for virucidal testing

Intended use of disinfectant	Test viruses (group, virustype, strain)
Instruments and surface disinfectants Hygienic handrub and handwash	Non-enveloped RNA virus Picornavirus group - *poliovirus* type 1, LSc-2ab[a] Non-enveloped DNA virus Adenovirus group - *adenovirus* type 5, strain Adenoid 75, ATCC VR-5
Chemothermal disinfection procedure	*Bovine Parvovirus*, strain Haden, ATCC VR-767

[a] Only virus material that passed the requirements for the production of oral polio vaccine of the World Health Organisation (WHO) must be used. (Other stocks derived from LSc-2ab cannot be used any longer).

5.3.2 Reference virus suspension

Virus suspension of a defined virus strain is maintained in reference centres.

Virus suspension of a defined strain may be obtained from a national or international collections (e.g. American Type Culture Collection (ATCC)).

In the case of polio virus LSc-2ab only virus material that passed the requirements for the production of oral polio vaccine of the World Health Organisation (WHO) must be used. (Other laboratory strains with the name “LSc-2ab” but that do not fulfil these requirements cannot be used any longer).

The virus suspension is kept in small volumes at temperatures below –70 °C or preferably at -196 °C under nitrogen.

NOTE Stock virus suspensions are prepared from reference virus suspensions.

5.3.3 Stock virus suspension

The virus has to be multiplied on a large scale to obtain a virus suspension of the same characteristics as the reference virus suspension. Due to safety reasons, only 10 passages from the original seed virus are allowed in case of the polio virus vaccine strain.