SLOVENSKI STANDARD
SIST EN 14476:2013+A1:2015

01-november-2015

Kemična razkužila in antiseptiki - Kvantitativni suspenzijski preskus za vrednotenje virucidnega delovanja v medicini - Preskusna metoda in zahteve (faza 2, stopnja 1)

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)

Chemische Desinfektionsmittel und Antiseptika - Quantitativer Suspensionsversuch zur Bestimmung der viruziden Wirkung im humanmedizinischen Bereich - Prüfverfahren und Anforderungen (Phase 2, Stufe 1)

Antiseptiques et désinfectants chimiques - Essai quantitatif de suspension pour l□évaluation de l□activité virucide dans le domaine médical - Méthode d□essai et prescriptions (Phase 2/Étape 1)

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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)

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European foreword

This document (EN 14476:2013+A1:2015) 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of an identical text or by endorsement, at the latest by March 2016 and conflicting national standards shall be withdrawn at the latest by March 2016.

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 supersedes [A1] EN 14476:2013 [/A1].

This document includes Amendment 1 approved by CEN on 2015-07-27.

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

[A1] This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association, and supports essential requirements of EU Directive(s).

For relationship with EU Directive(s), see informative Annex ZA, which is an integral part of this document. [/A1]

The document was revised to adapt it to the latest state of science, to correct errors and ambiguities, to harmonise the structure and wording with other existing tests of CEN/TC 216 or in preparation and to improve the readability of the standard and thereby make it more understandable. The following list is a list of significant technical changes since the last edition:

- The scope was expanded for the following fields of application within the medical area, i.e. products for textile disinfection.
- “Obligatory test conditions” were replaced by “minimum test conditions” (test temperatures and contact times can be chosen within limits) that have to be performed to pass the test.
- An additional modified method is described to test ready-to-use products in a higher concentration than 80 %, i.e. 9 7%;

[A1]

- For the hygienic handrub and handwash method a test for virucidal activity against enveloped viruses with *Vacciniavirus* was added.
- The relationship between this European Standard and the MDD was added (Foreword and Annex ZA).
- The value of v_n in C.1 was corrected (0,001 instead of 0,0001). [/A1]

Data obtained using the former version of EN 14476 may still be used.

Other methods to evaluate the efficacy of chemical disinfectants and antiseptics for different applications in the medical area 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, 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, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Introduction

This European Standard specifies a suspension test for establishing whether a chemical disinfectant or an antiseptic has a virucidal activity in the area and fields described in the scope.

This laboratory test takes into account practical conditions of application of the product including contact time, temperature, test organisms and interfering substances, i.e. conditions which may influence its action in practical situations. Each utilisation concentration of the chemical disinfectant or antiseptic found by this test corresponds to the chosen experimental conditions.

1 Scope

This European Standard specifies a test method and the minimum requirements for virucidal activity of chemical disinfectant and antiseptic products that form a homogeneous physically stable preparation when diluted with hard water – or in the case of ready-to-use products, i. e, products that are not diluted when applied,– with water. Products can only be tested at a concentration of 80 % (97 %, with a modified method for special cases) as some dilution is always produced by adding the test organisms and interfering substance.

This European Standard applies to products that are used in the medical area in the fields of hygienic handrub, hygienic handwash, instrument disinfection by immersion, surface disinfection by wiping, spraying, flooding or other means and textile disinfection.

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 under the conditions in which they are used.

NOTE 2 This method corresponds to a phase 2, step 1 test.

NOTE 3 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 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 and the following apply.

3.1
cytotoxicity
morphological alteration of cells and/or their destruction or their reduced sensitivity to virus multiplication caused by the product

3.2
plaque forming units
PFU
number of infectious virus particles per unit volume (ml)

3.3
reference test for virus inactivation
test with a defined product (e.g. formaldehyde) in parallel with a product under test for the internal control of the test

3.4
$TCID_{50}$
50 % infecting dose of a virus suspension or that dilution of the virus suspension that induce a CPE (3.5) in 50 % of cell culture units

3.5
viral cytopathic effect
CPE
morphological alteration of cells and/or their destruction as a consequence of virus multiplication

3.6
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

3.7
virus titre
amount of infectious virus per unit volume present in a cell culture lysate or in a solution

4 Requirements

The product shall demonstrate at least a decimal log (lg) reduction of 4 in virus titre when tested in accordance with Table 1 and Clause 5.

Table 1 — Minimum and additional test conditions

Test Conditions	Hygienic handrub and handwash	Instrument disinfection	Surface disinfection	Textile disinfection
Minimum spectrum of test organisms	*Poliovirus* *Adenovirus* *Murine Norovirus* Limited spectrum virucidal activity [a] *Adenovirus* *Murine Norovirus* [A1] **Virucidal activity against enveloped viruses** [b] *Vaccinia virus* [A1]	*Poliovirus* *Adenovirus* *Murine Norovirus* when temperature is 40 °C or higher: only *Parvovirus*	*Poliovirus* *Adenovirus* *Murine Norovirus*	*Parvovirus*
additional	Any relevant test organism			
Test temperature	according to the manufacturer's recommendation, but at / between			
	20 °C	20 °C and 70 °C	4 °C and 30 °C	30 °C and 70 °C
Contact time	according to the manufacturer's recommendation			
	but between 30 s and 120 s	but no longer than 60 min	but no longer than 5 min or 60 min [c]	but no longer than 20 min
Interfering substance				
clean conditions	0,3 g/l bovine albumin solution (hygienic handrub) [d]	0,3 g/l bovine albumin solution **and/or**	0,3 g/l bovine albumin solution **and/or**	
dirty conditions	3,0 g/l bovine albumin solution plus 3,0 ml/l erythrocytes (hygienic handwash) [e]	3,0 g/l bovine albumin solution plus 3,0 ml/l erythrocytes	3,0 g/l bovine albumin solution plus 3,0 ml/l erythrocytes	3,0 g/l bovine albumin solution plus 3,0 ml/l erythrocytes
Additional conditions [f]	clean or dirty [d, e]; any relevant substance	any relevant substance	any relevant substance	any relevant substance

[a] The test for limited spectrum virucidal activity will cover all enveloped viruses (Annex A) and the specified test organisms .

[b] [A1] The test for virucidal activity against enveloped virus will cover all enveloped viruses only (Annex A). [A1]

[c] 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.

[d] Hygienic handrub shall be tested as a minimum under clean conditions.

[e] Hygienic handwash shall be tested as a minimum under dirty conditions.

[f] For the additional conditions, the concentration defined as a result can be lower than the one obtained under the minimum test conditions.

5 Test methods

5.1 Principle

5.1.1 A sample of the product as delivered and/or diluted with hard water (or water for ready to use products) is added to a test suspension of viruses in a solution of an interfering substance. The mixture is maintained at one of the temperatures and the contact times specified in Clause 4 and 5.5.1.1. At the end of this contact time, an aliquot is taken; the virucidal action in this portion is immediately suppressed by a validated method (dilution of the sample in ice-cold cell maintenance medium). The dilutions are transferred into cell culture units (petri dishes, tubes or wells of microtitre plates) either using monolayer or cell suspension. Infectivity tests are done either by plaque test or quantal tests. After incubation, the titres of infectivity are calculated according to Spearman and Kärber (quantal tests, C.1) or by plaque counting (plaque test, C.2) and evaluated. Reduction of virus infectivity is calculated from differences of lg virus titres before (virus control) and after treatment with the product.

NOTE Handwash products are always prediluted with hard water (5.2.2.7). The resulting solution is regarded as a ready-to-use product (5.4.2).

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)]

   1) *Poliovirus type 1*, LSc 2ab (Picornavirus)

   2) *Murine norovirus,* strain S99 Berlin

b) Non-enveloped DNA virus

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

   2) *Murine Parvovirus,* minute virus of mice, strain Crawford, ATCC VR-1346

[A1]

c) Enveloped DNA virus

   1) Vacciniavirus, strain Ankara (MVA), ATCC VR-1508. [A1]

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. Regarding Poliovirus only virus material that passed the requirements for the production of oral polio vaccine of the World Health Organisation (WHO) shall be used (Other stocks derived from LSc-2ab cannot be used any longer). LSc-2ab can be obtained from NIBSC (www.nibsc.ac.uk: contact Dr. Javier Martin) or from Eurovir Hygiene Institut (www.eurovir.de: contact Dr. Jursch).

Murine Norovirus may be obtained from Friedrich-Loeffler-Insitut [A1] Bundesforschungsinstitut [A1] 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.

The required incubation temperature for these test organisms is 36 °C ± 1 °C or 37 °C ± 1 °C (5.3.1.3). 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 demineralised water. If distilled water of adequate quality is not available, water for injections (see bibliographic reference [1]) may be used.

Sterilise in the autoclave [5.3.1.1 a)]. Sterilisation is not necessary if the water is used e.g. for preparation of culture media and subsequently sterilised.

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 (Na2HPO4 x 12H2O)	2,89 g
Potassium phosphate, monobasic (KH2PO4)	0,20 g
Water (5.2.2.2)	to 1 000,0 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.