SLOVENSKI STANDARD
SIST EN 14476:2013+A1:2015/oprA2:2017

01-januar-2017

Kemična razkužila in antiseptiki - Kvantitativni suspenzijski preskus za vrednotenje virucidnega delovanja v medicini - Preskusna metoda in zahteve (faza 2, stopnja 1) - Dopolnilo A2

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)

Ta slovenski standard je istoveten z: **EN 14476:2013+A1:2015/prA2**

ICS:

11.080.20	Dezinfektanti in antiseptiki	Disinfectants and antiseptics

SIST EN 14476:2013+A1:2015/oprA2:2017 **en,fr,de**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

DRAFT
EN 14476:2013+A1:2015

prA2

November 2016

ICS 11.080.20

English Version

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)

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)

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

This draft amendment is submitted to CEN members for enquiry. It has been drawn up by the Technical Committee CEN/TC 216.

This draft amendment A2, if approved, will modify the European Standard EN 14476:2013+A1:2015. If this draft becomes an amendment, CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for inclusion of this amendment into the relevant national standard without any alteration.

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Recipients of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

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

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European foreword

This document (EN 14476:2013+A1:2015/prA2:2016) 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.

This document will supersede EN 14476:2013+A1:2015.

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).

1 Modifications to the Foreword

Add the following to the existing list of modifications: "

- For the surface disinfection a test for virucidal activity against enveloped viruses with *Vaccinia Virus* was added.
- The spelling of *Vaccinavirus* is corrected to *Vaccinia Virus* (Table 1).
- The *Vaccinia Virus* Elstree was added as alternative strain (5.2.1 c) 1)), (5.5.1.1 e)).
- For dirty conditions (5.2.2.8.3) the resuspension shall be done in PBS and not in water.
- The dilution in ice-cold medium for the control of efficiency of suppression of products activity (5.5.5.1) was clarified.
- Addition of the large-volume-platting method (5.5.4.3, B.3).".

2 Modifications to Clause 4, Table 1

In Table 1, 4th column, add "Limited spectrum virucidal activity [a]", "*Adenovirus, Murine Norovirus*", "Virucidal activity against enveloped viruses [b]" *and* "*Vaccinia Virus*" *to read as follows*:

"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* Limited spectrum virucidal activity [a] *Adenovirus* *Murine Norovirus* **Virucidal activity against enveloped viruses** [b] *Vaccinia Virus*	*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				

Test Conditions	Hygienic handrub and handwash	Instrument disinfection	Surface disinfection	Textile disinfection
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.

".

3 Modification to 5.2.1 c) 1)

*Replace "*Vacciniavirus, strain Ankara (MVA), ATCC VR-1508." *with*

"*Vaccinia virus,* strain Modified *Vaccinia Virus* Ankara (MVA), ATCC VR-1508 or strain Elstree, ATCC VR-1549".

4 Modification to 5.2.2.8.3

Replace "water (5.2.2.2)" *in the 3rd paragraph with* "PBS (5.2.2.3)" *to read:*

"Prepare at least 8,0 ml fresh defibrinated sheep blood (5.2.2.9). Centrifuge the erythrocytes at 800 g_N for 10 min (5.3.1.13). After discarding the supernatant, resuspend erythrocytes in PBS (5.2.2.3). Repeat this procedure at least 3 times, until the supernatant is colourless.".

5 Modification to 5.5.1.1 e)

Replace "⟨A1⟩ *Vacciniavirus* is multiplied in BHK-21cells (ATCC CCL-10) or other cell lines of appropriate sensitivity. ⟨A1⟩" *with*

"Modified *Vaccinia Virus* Ankara is multiplied in BHK-21cells (ATCC CCL-10) or other cell lines of appropriate susceptibility. *Vaccinia Virus* Elstree is multiplied in Vero cells (ATCC CCL-81), CV-1 cells (ATCC CCL-70) or other cell lines of appropriate susceptibility.".

6 Modification of 5.5.4.3

Add a new paragraph c):

"c) determination of the residual virus titre by the large-volume-plating (LVP) method (B.3).".

7 Modification to 5.5.5.1

Replace

"Immediately after preparation of the test mixture (5.5.2) mix [5.3.1.6 a)] and pipette 0,5 ml of the test mixture into 4,5 ml of ice-cold MEM + 2 % FCS (5.2.2.5). Mix again and start the clock. Incubate the mixture in the ice bath (5.3.1.11) for 30 min ± 10 s. Immediately prepare dilutions up to 10^{-8}, and titrate the virus [5.5.2 a) or b) applies]. This control is performed in parallel to the test (5.5.2).

The difference of titre with the test suspension shall be ≤ 0,5 lg."

with

"Pipette 1 part of interfering substance (5.2.2.8) into a container of suitable capacity for appropriate mixing. Add 1 part of MEM + 2 % FCS (5.2.2.5) to the container. Add 8 parts of the product test solution (5.4.2) to the container. Mix (5.3.1.6 a)).

If the modified method for ready-to-use products (5.5.3) is used, 9,7 parts of the product test solution shall be mixed with 0,2 parts of the interfering substance(s), prepared in fivefold higher concentration, and 0,1 part MEM + 2 % FCS (5.2.2.5).

Pipette 0,5 ml of the mix into 4,0 ml of ice-cold MEM + 2 % FCS (5.2.2.5). Add 0,5 ml of the virus test suspension (5.4.1) to the container, carefully avoiding the upper part of the sides. Mix again and start the clock. Incubate the mixture in the ice bath (5.3.1.11) for 30 min ± 10 s. Immediately after incubation prepare serial tenfold dilutions and titrate the virus (5.5.2 a) or b)) applies. This control is performed in parallel to the test (5.5.2).

The difference of titre with the test suspension shall be ≤ 0,5 lg.".

8 Modification of Annex B

Add the following new B.3 in Annex B: "

B.3 Determination of the residual virus titre by the large-volume-plating (LVP) method

B.3.1 General

Using the LVP, the lowest apparently non-cytotoxic dilution of the test mixture is added to ice-cold medium after the specified contact time and this test mixture has to be added to a defined number of wells containing the indicator cells in 100 µl cell culture medium.

NOTE The detection of residual virus can be improved by the testing of a large sample volume.

The cells are cultivated for a specified incubation period. Then, the cells are inspected for virus-induced changes in cell morphology. The viral titre has to be calculated as follows:

If no virus is observed, the number infectious virus particles are determined by the Poisson distribution using the following formula:

$$c = \frac{\ln p}{-v} \quad \text{(B.1)}$$

where

c is the concentration of virus particles in the test mixture;

p is denoting the 95 % probability to detect virus;

v is the plated volume and shall be < < V in ml;

V is the total test volume in ml.

If low amount of viruses is detected the most probable average number of TCID50 can be calculated by the use of the following formula which is derived from the Taylor series:

$$c = \frac{D}{V_w} \times \left(-\ln \frac{n - n_p}{n} \right) \quad \text{(B.2)}$$

where

c is the concentration of virus particles in the test mixture;

D is the dilution factor of pre-diluted sample;

V_W is the plated volume per well;

n is the number of inoculated wells;

n_p is the number of successfully infected wells.

B.3.2 Example for the calculation of titres and the reduction according to the large-volume-plating method

If the LVP method is applied, there are two possibilities for the calculation of the titres which are used for the calculation of the reduction.

a) Calculation referred to the Taylor formula

If low amount of viruses is detected the most probable average number of $TCID_{50}$ can be calculated by the use of the Formula (B.2) which is derived from the Taylor series with the following values:

V_W = 0,1 ml

$n = 96$

$n_p = 9$

D = 1 000

According to the Taylor formula the result is 984 infectious particles per ml.

However, one $TCID_{50}$ is equivalent to 0,69 infectious virus particles because the natural logarithm of a 50 % likelihood (P = 0,5) is 0,69 or ln(0,5) = 0,69. This implies that at least 69 trials are necessary to infect successfully 50 % of 100 wells. Therefore this factor is needed to calculate the $TCID_{50}$ /ml as:

984/ 0,69 = 1 426 $TCID_{50}$ /ml