# SLOVENSKI STANDARD
# SIST EN 17111:2019

**01-januar-2019**

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**Kemična razkužila in antiseptiki - Kvantitativni preskus s steklenim nosilcem za vrednotenje virucidnega delovanja kemičnih razkužil in antiseptikov za instrumente, ki se uporabljajo v humani medicini - Preskusna metoda in zahteve (faza 2, stopnja 2)**

Chemical disinfectants and antiseptics - Quantitative carrier test for the evaluation of virucidal activity for instruments used in the medical area - Test method and requirements (phase 2, step 2)



Chemische Desinfektionsmittel und Antiseptika - Quantitativer Keimträgerversuch zur Prüfung der viruziden Wirkung für Instrumente im humanmedizinischen Bereich - Prüfverfahren und Anforderungen (Phase 2, Stufe 2)



Désinfectants chimiques et antiseptiques - Essai quantitatif de porte germe pour l'évaluation de l'activité virucide pour instruments utilisés en médecine - Méthode d'essai et prescriptions (phase 2, étape 2)

**Ta slovenski standard je istoveten z:** **EN 17111:2018**

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**SIST EN 17111:2019** **en,fr,de**

EUROPEAN STANDARD

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# EN 17111

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English Version

# Chemical disinfectants and antiseptics - Quantitative carrier test for the evaluation of virucidal activity for instruments used in the medical area - Test method and requirements (phase 2, step 2)

Désinfectants chimiques et antiseptiques - Essai quantitatif de porte-germe pour l'évaluation de l'activité virucide pour instruments utilisés en médecine - Méthode d'essai et exigences (phase 2, étape 2)

Chemische Desinfektionsmittel und Antiseptika - Quantitativer Keimträgerversuch zur Prüfung der viruziden Wirkung für Instrumente im humanmedizinischen Bereich - Prüfverfahren und Anforderungen (Phase 2, Stufe 2)

This European Standard was approved by CEN on 18 June 2018.



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COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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# Contents

Page

## European foreword

This document (EN 17111:2018) has been prepared by Technical Committee CEN/TC 216 “Chemical desinfectants 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 2019, and conflicting national standards shall be withdrawn at the latest by April 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 European Standard specifies a carrier test for establishing whether a chemical disinfectant for use on instruments (surgical instruments, anaesthesia material, endoscopes etc.) has a virucidal activity in the fields described in the scope.

The laboratory test closely simulates practical conditions of application including pre-drying viruses on a carrier, contact time, temperature, test organisms and interfering substances, i.e. conditions which may influence the action of chemical disinfectants in practical situations. Each utilization concentration of the chemical disinfectant found by this test corresponds to defined experimental conditions.

# 1 Scope

This document specifies a test method and the minimum requirements for virucidal activity of chemical disinfectant products 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 instruments by immersion.

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 under 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 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 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

The product, when diluted with hard water or – in the case of ready-to-use products – with water, and tested in accordance with Clause 5 under simulated clean conditions (0,3 g/l bovine albumin solution) or simulated dirty conditions (3 g/l bovine albumin solution, plus 3 ml/l washed sheep erythrocytes)

according to its practical applications and under the minimum test conditions shall demonstrate at least a 4 decimal log (lg) reduction.

**Table 1 — Minimum and additional test conditions**

| **Test Conditions** | **Virucidal activity against enveloped viruses**[b] (**Pre-cleaning products** with a combined cleaner/disinfectant) | **Virucidal activity**[a] (**Instrument disinfection** when temperature is < 40 °C) | **Virucidal activity** (**Instrument disinfection** when temperature is ≥ 40 °C) |
|---|---|---|---|
| **Minimum spectrum of test organisms** | modified vaccinia virus Ankara or vaccinia virus strain Elstree | Adenovirus and murine norovirus | murine parvovirus |
| **additional** | Any relevant test organism | | |
| **Test temperature** | according to the manufacturer's recommendation, but at / between | | |
| | 20 °C | 20 °C and < 40 °C | ≥ 40 °C and 70 °C |
| **Contact time** | according to the manufacturer's recommendation, but no longer than | | |
| | 60 min | 60 min | 60 min |
| **Interfering substance** | | | |
| clean conditions | 0,3 g/l bovine albumin solution and/or | 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 washed sheep erythrocytes | 3,0 g/l bovine albumin solution plus 3,0 ml/l washed sheep erythrocytes | 3,0 g/l bovine albumin solution plus 3,0 ml/l washed sheep erythrocytes |
| Additional conditions[c] | any relevant substance | any relevant substance | any relevant substance |

[a] Poliovirus (as used in the corresponding suspension test) cannot be used for surfaces, because of drying problems. To claim the virucidal activity the product shall pass standards EN 14476 with polio-, adeno- and murine norovirus.

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

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

# 5 Test method

## 5.1 Principle

**5.1.1** A test suspension of test viruses in a solution of interfering substances is spread on a glass carrier (5.3.2.23). After drying the carrier is immersed into a sample of the product as delivered and/or diluted with hard water (for ready to use products: water). In parallel, a second carrier is treated with (hard) water instead of the product applying the same test conditions (water control). The carriers are maintained at one of the temperatures and contact times specified in Clause 4 and 5.5.1.1. At the end of this contact time, the carriers are transferred into a maintenance medium containing glass beads. The viruses are to be severed from the surface by shaking (5.3.2.17). The numbers of surviving viruses in each sample are determined and the reduction is calculated by comparing the results of the product and the water control.

**5.1.2** The test is performed using modified vaccinia virus Ankara or vaccinia virus strain Elstree (pre-cleaning products with a combined cleaner/disinfectant), adenovirus type 5 and murine norovirus as test-organisms (minimum spectrum of test organisms); in case of test temperatures of 40 °C or higher only murine parvovirus shall be used.

**5.1.3** Other contact times and temperatures within the limits specified in Clause 4 may be used. Additional interfering substances may be used.

## 5.2 Materials and reagents



### 5.2.1 Test organisms

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



a) Enveloped DNA virus

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

b) Non-enveloped RNA virus[2)]

Murine norovirus, strain S99 Berlin

c) Non-enveloped DNA virus

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

Murine parvovirus, minute virus of mice, strain Crawford, ATCC VR-1346

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1) The ATCC numbers are the collection numbers of strains supplied by the American Type Culture Collections (ATCC). 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-Institut Bundesforschungsinstitut für Tiergesundheit, Hauptsitz Insel Riems Südufer 10, 17493, Greifswald-Insel Riems; phone: +49 (0) 38351 7-0, fax: +49 (0) 38351 7-1219. https://www.fli.de/en/home/.

The required incubation temperature for these test organisms is 36 °C ± 1 °C or 37 °C ± 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 incubated under optimum growth conditions (temperature, time, atmosphere, media) noted in the test report. If the additional test organisms selected do not correspond to the specified strains, their suitability for supplying the required inocula shall be verified. 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. The source of the strains shall be indicated.

### 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 (5.3.2.4) 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.

#### 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 0,45 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 % w/V 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 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, detergents) shall be defined.

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

##### 5.2.2.8.2 Clean conditions (bovine albumin solution – low concentration)

Dissolve 0,30 g of bovine albumin fraction V (suitable for microbiological purposes) in 100 ml of water (5.2.2.2).

Sterilize by membrane filtration (5.3.2.11), keep in a refrigerator (5.3.2.6) and use within one month.

The final concentration of the bovine albumin in the test procedure (5.5) shall be 0,3 g/l.

#### 5.2.2.8.3 Dirty conditions (Mixture of bovine albumin solutions – high concentration with sheep erythrocytes)

Dissolve 3,00 g of bovine albumin fraction V (suitable for microbiological purposes) in 97 ml of water (5.2.2.2).

Sterilize by membrane filtration (5.3.2.11).

Prepare at least 8,0 ml fresh sterile defibrinated sheep blood (5.2.2.9). Centrifuge the sheep blood at 800 $g_N$ for 10 min (5.3.2.18). After discarding the supernatant, resuspend erythrocytes in PBS (5.2.2.3).

Repeat this procedure at least 3 times, until the supernatant is colourless.

Resuspend 3 ml of the packed sheep erythrocytes in the 97 ml of sterilized bovine albumin solution (see above). To avoid contamination this mixture should be split in portions probably needed per day and kept in separate containers (5.3.2.8) for a maximum of 7 days in a refrigerator (5.3.2.6).

The final concentration of bovine albumin and sheep erythrocytes in the test procedure (5.5) shall be 3 g/l and 3 ml/l respectively.

### 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). The density shall 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).

### 5.2.2.12 Reference glutardialdehyde (Glutaral, 1,5-Pentanedial) CAS Number 111-30-8

Required chemical and physical parameters for use as reference standard for testing disinfectant preparations are defined in Table 2.