SLOVENSKI STANDARD
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Kemična razkužila in antiseptiki - Kvantitativna preskusna metoda za vrednotenje virucidnega delovanja na neporoznih površinah z mehanskim delovanjem z odvzemom brisa v humani medicini (4-področni preskus) - Preskusna metoda in zahteve (faza 2, stopnja 2)

Chemical disinfectants and antiseptics - Quantitative test method for the evaluation of virucidal activity on nonporous surfaces with mechanical action employing wipes in the medical area (4-field test) - Test method and requirements (phase 2, step 2)

Chemische Desinfektionsmittel und Antiseptika - Quantitatives Prüfverfahren zur Bestimmung der viruziden Wirkung auf nicht-porösen Oberflächen mit mechanischer Einwirkung mit Hilfe von Tüchern im humanmedizinischen Bereich (4-Felder-Test) - Prüfverfahren und Anforderungen (Phase 2, Stufe 2)

Antiseptiques et désinfectants chimiques - Méthode d´essai quantitative pour l ´évaluation de l´activité virucide sur des surfaces non poreuses, avec action mécanique à l´aide de lingettes dans le domaine médical (essai à 4 zones) - Méthode d'essai et prescriptions (phase 2, étape 2)

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Chemical disinfectants and antiseptics - Quantitative test method for the evaluation of virucidal activity on nonporous surfaces with mechanical action employing wipes in the medical area (4-field test) - Test method and requirements (phase 2, step 2)

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

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European foreword

This document (prEN 18368:2026) 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.

Introduction

This document specifies a carrier test for establishing whether a chemical disinfectant for use on surfaces administered with wipes has a virucidal activity in the fields described in the scope.

The laboratory test closely simulates practical conditions of application such as contact time, temperature and interfering substances, including pre-drying specified test organisms on a test-surface as a carrier and wiping the product on the test-surface with a wipe. The conditions are intended to cover general purposes. However, if for some applications the recommendations of use of a product differ additional test conditions may be or need to be used.

Each use concentration of the product identified by this test relates solely to the 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 is applicable to products that are used in the medical area for disinfecting non-porous surfaces including surfaces of medical devices by wiping or mopping – regardless if they are covered by the Medical Device Regulation [1] or not.

Due to the new methods of application of surface disinfectants like pre-impregnated wipes this document was established to cover the different application methods.

This document is applicable for four methods of application of products for wiping and/or mopping:

a) soaking any non-specified wipe or mop with product;

b) spraying the product on any non-specified wipe and / or mop or a specified wipe or mop;

c) impregnation of specified wipes or mops by the user with the product according to the manufacturer's recommendation;

d) pre-impregnation of specified wipes or mops by the manufacturer as ready-to-use wipes or mops.

In all types of application the water control has to be conducted with the standard wipe (5.3.2.17 a)), because it is process or method control. The contact time for the water control is no longer than 5 min.

This document does not apply to products that are sprayed on or used for flooding of surfaces, without wiping or mopping during the contact time. In this case, the methods of phase 2/ step 2 without mechanical action apply.

The test-surface (5.3.2.16) was selected as a standard surface and should cover all non-porous surfaces. It was not intended to cover the influence of each different surface.

This document is applicable 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 can occur in the workplace and in the home. It can also include services such as laundries and kitchens supplying products directly for the patients.

NOTE 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, 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 14476:2025, *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 and the following apply.

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

— IEC Electropedia: available at https://www.electropedia.org/

— ISO Online browsing platform: available at https://www.iso.org/obp

3.1
ready-to-use wipe
pre-impregnated wipe
wipe containing disinfectant added by the wipe manufacturer at the manufacturing site

3.2
impregnated wipe
wipe containing disinfectant added by the user

Note 1 to entry: Examples include a dry wipe soaked in disinfectant by the user, a dry wipe sprayed with disinfectant.

3.3
cytotoxicity

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

3.4
reference test for virus inactivation
test with a defined product (e.g. formaldehyde or glutaraldehyde) in parallel with a product under test (suspension test or test under simulated use conditions) for the internal control of the test and the quality / stability of the test virus

Note 1 to entry: A defined product e.g. glutaraldehyde or formaldehyde could be used.

3.5
$TCID_{50}$ (Tissue Culture Infectious Dose 50 %)
50 % infecting dose of a virus suspension or that dilution of the virus suspension that induce a CPE (3.6) in 50 % of cell culture units

3.6
viral cytopathic effect
CPE
morphological alteration of cells and/or their destruction as a consequence of virus multiplication

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

4 Requirements

The product, when diluted with hard water or – in the case of ready-to-use products – with water, shall demonstrate at least a decimal log (lg) reduction of 4 in virus titre (in justified cases at least a 3 lg reduction) on test field 1, 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 document with vaccinia virus;

— to claim limited spectrum virucidal activity the product shall pass both EN 14476 and this document with adenovirus and murine norovirus;

— to claim the spectrum virucidal activity the product shall pass standards EN 14476 with poliovirus, adenovirus and murine norovirus and this document with adenovirus and murine norovirus, because poliovirus is not resistant to drying.

All data of test fields 2 to 4 shall be noted for N_a and N_W. Requirements for test fields 2 to 4 for N_a and N_W are given in 5.7.2. Details on the precision and repetition are given in 5.8.2 and EN 14885.

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	Room temperature, the range shall be (21,5 ± 3,5) °C; further test temperature(s) are allowed for example 4°C and 10°C according to the manufacturer's recommendation [d] [e]
Contact time	according to the manufacturer's recommendation, but at minimum 1 min and no longer than 60 min [f] based on the following rules: from 1 min to 5 min at intervals of 1 min and from 5 min to 60 min at intervals of 5 min
Interfering substances **a) clean** **b) dirty**	a) 0,3 g/l bovine serum albumin and/or b) 3,0 g/l bovine serum albumin plus 3,0 ml erythrocytes
Additional conditions [g]	Any relevant 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] At temperatures higher than room temperature, the test surface needs to be placed in a temperature chamber (prior and after the wiping procedure) and thus there is a higher safety risk of exposure to virus particles in the laboratory and to the laboratory staff.

[e] Where a different test temperature than room temperature is to be used for testing, all materials (test surface, wipe, unitary weight etc.) and all used media (product test solution etc.) must be at test temperature prior to testing.

[f] For further information regarding validity at elevated contact times see EN 14885.

[g] Where appropriate (specific purposes), additional specific virucidal activity shall be determined under other conditions of time, temperature and interfering substances (see 5.2.2.7) 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.

5 Test methods

5.1 Principle

5.1.1 A test-surface is marked with 4 squares of 5 × 5 cm, the “test fields”, in a row. Test field 1 on the test-surface is inoculated with a test suspension of virus in a solution of interfering substances. The inoculum is dried. A wipe is soaked with a sample of the product as delivered and/or diluted with hard water (5.2.2.6) (for ready to use products: water (5.2.2.2)). The test-surface is wiped with the soaked wipe across the four marked test fields, starting in front of test field 1, turning immediately after test field 4 and wiped back to the starting point. In parallel a water control [5.5.2.2 h)] is performed with the standard wipe which is soaked with hard water (5.2.2.6)) instead of the product in the same way to ensure that the test organisms are spread on the 4 fields and their number reaches a certain level (contact time limit for water control 5 min).

NOTE For the purposes of this document, references to wiping, wipe and wiped can be equated to mopping, mop and mopped when the standard method is used to test a mopping application.

Temperature, interfering substance and contact time are employed as recommended by the manufacturer. At the end of the contact time, the test organisms are recovered from each test field with moistened swabs (5.3.2.19). The swabs are brought into a tube containing medium and the test organisms are to be extracted from the swab by shaking. The numbers of infectious test organisms in each sample are determined, and the reduction is calculated by comparing the results of the drying control D_{Ct} and the results obtained with the product N_a. The test is performed using modified vaccinia virus Ankara as the test organism for virucidal activity against enveloped viruses and murine norovirus (MNV) and adenovirus for limited spectrum virucidal activity or virucidal activity.

5.1.2 Additional test virus strains, contact times and interfering substances can be used.

5.2 Materials and reagents

5.2.1 Test organism (test viruses)

The virucidal activity shall be evaluated using the following strains as test organisms selected according to Clause 4, Table 1. Virus strains shall be obtained from the national or international culture collection[1,]

The virucidal activity shall be evaluated using the following strains as test organisms:

a) Non-enveloped RNA virus

Murine norovirus (MNV), strain S99 Berlin[2)]

a) Non-enveloped DNA virus

Adenovirus type 5 strain Adenoid 75, ATCC[3)] VR-5

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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 document and does not constitute an endorsement by CEN of the product named.

2) Murine norovirus may be obtained from Friedrich Loeffler Institute Federal Research Institute for Animal Health, Headquarters Riems Island South Bank 10, 17493 Greifswald-Insel Riems, Germany; phone: +49 38351 7-0; fax: +49 38351 7-121. http: www.fli.bund.de

3) 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 document and does not constitute an endorsement by CEN of the product named.

b) Enveloped DNA virus

Modified vaccinia virus, strain Ankara (MVA), ATCC[4)] VR-1508

If additional test organisms are used, they shall be kept and used under optimum growth conditions (temperature, time, atmosphere, media, susceptible cell line) noted in the test report. If these additional test organisms (viruses and cell line) 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 required incubation temperature for these test organisms is 36 °C ± 1 °C or 37 °C ± 1 °C (5.3.2.3). The same temperature (either 36 °C or 37 °C) shall be used for all incubations performed during a test and its control and validation.

5.2.2 Culture media and reagents

5.2.2.1 General

All weights of chemical substances given in this document 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 organism.

To improve reproducibility, it is recommended that commercially available dehydrated material – if appropriate- is used for the preparation of culture media if it complies with the formulas given below. 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 21,5 °C ± 3,5 °C (5.3.2.4).

5.2.2.2 Water

The water shall be freshly glass-distilled water or demineralized water, which meets the requirements of ASTM D 1193 (Type I or II, see Table 2) or use water for injections (see [2]).

Sterilize in the autoclave [5.3.2.1]. Sterilization is not necessary if the water is used e.g. for preparation of culture media and subsequently sterilized. Alternatively, cell culture grade water is suitable for preparation of cell culture media and other solutions used in cell culture.

4) 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 document and does not constitute an endorsement by CEN of the product named.

Table 2 — Consolidated classification of water qualities

Characteristic	Unit	Type I (ultrapure water)	Type II (purified water)
Resistance	(M/cm)Ω	> 18	> 1
Conductivity	µS/cm	< 0,056	< 1
Silicates (SiO_4)	ppb or µg/L	3	3
TOC	ppb or µg/l	50	50
Bacteria	CFU/ml	< 1	< 100
Endotoxin	EU/ml	< 0,001	0,03
Type I	Required for critical laboratory applications (e.g. for HPLC, GC; MS etc.). Also for the production of buffers for cell culture, as well as for applications in molecular biology.		
Type II	Used for buffers and microbiological culture media. Used as system water for laboratory automats and for dissolving controls and reagents		

See 5.2.2.6 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 12*H_2O)2,89 g

Potassium phosphate, monobasic (KH_2PO_4) 0,20 g

Water (5.2.2.2) to 1 000,0 ml

5.2.2.4 Neutral Red (1:1 000 solution)

Prepare neutral red (e.g. Sigma N7005[5]) stock solution at 0,1 mg/ml in water (5.2.2.2). Filter through a 0,40 µm pore size filter and store at 4 °C in the dark.

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, low endotoxin FCS shall be used due to the cells' high sensitivity to endotoxins.

5.2.2.6 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 (5.3.2.7) or in the autoclave [5.3.2.1 a)]. Autoclaving – if used - may cause a loss of liquid. In this case make up to

5) 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.

1 000 ml with water (5.2.2.2) under aseptic conditions. Store the solution in the refrigerator (5.3.2.8) 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 1 000 ml. Sterilize by membrane filtration (5.3.2.7). Store the solution in the refrigerator (5.3.2.8) for no longer than one week;

— place 600 ml to 700 ml of water (5.2.2.2) in a 1 000 ml volumetric flask (5.3.2.12) and add 6,0 ml of solution A (5.2.2.6), then 8,0 ml of solution B (5.2.2.6). Mix and dilute to 1 000 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 (approximately 1 mol/l) of sodium hydroxide (NaOH) or approximately 36,5 g/l (approximately 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.

5.2.2.7 Interfering substances

5.2.2.7.1 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 specified.

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

5.2.2.7.2 Clean conditions (bovine serum albumin solution – low concentration)

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

Sterilize by membrane filtration (5.3.2.7), keep in a refrigerator (5.3.2.8) and use within 1 month.

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

5.2.2.7.3 Dirty conditions (mixture of bovine serum albumin solutions – high concentration with sheep erythrocytes)

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

Sterilize by membrane filtration (5.3.2.7).

Prepare at least 8,0 ml fresh sterile defibrinated sheep blood (5.2.2.8). Centrifuge the erythrocytes at 800 g_N for 10 min (5.3.2.13). After discarding the supernatant, resuspend erythrocytes in sterile phosphate buffer (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 serum albumin solution (see above). To avoid contamination this mixture should be split in portions aligned with the expected need per day and kept in separate containers for a maximum of 7 days in a refrigerator at 2 °C to 8 °C.