SLOVENSKI STANDARD
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Kemična razkužila in antiseptiki - Kvantitativni preskus na neporoznih površinah za vrednotenje virucidnega delovanja kemičnih razkužil in antiseptikov v veterini - Preskusna metoda in zahteve (faza 2, stopnja 2)

Chemical disinfectants and antiseptics - Quantitative non-porous surface test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in the veterinary area - Test method and requirements (phase 2, step 2)

Chemische Desinfektionsmittel und Antiseptika - Quantitativer Oberflächenversuch zur Bestimmung der viruziden Wirkung chemischer Desinfektionsmittel und Antiseptika für den Veterinärbereich auf nicht-porösen Oberflächen - Prüfverfahren und Anforderungen - Phase 2, Stufe 2

Antiseptiques et désinfectants chimiques - Essai quantitatif de surfaces non poreuses pour l'évaluation de l'activité virucide des désinfectants chimiques utilisés dans le domaine vétérinaire - Méthode d'essai et prescriptions - Phase 2, étape 2

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Chemical disinfectants and antiseptics - Quantitative non-porous surface test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in the veterinary area - Test method and requirements - Phase2, step2

Antiseptiques et désinfectants chimiques - Essai quantitatif de surfaces non poreuses pour l'évaluation de l'activité virucide des désinfectants et antiseptiques chimiques utilisés dans le domaine vétérinaire - Méthode d'essai et prescriptions - Phase 2, étape 2

Chemische Desinfektionsmittel und Antiseptika - Quantitativer Oberflächenversuch zur Bestimmung der viruziden Wirkung chemischer Desinfektionsmittel und Antiseptika für den Veterinärbereich auf nicht-porösen Oberflächen - Prüfverfahren und Anforderungen - Phase 2, Stufe 2

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European Foreword

This document (EN 17122:2019) 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of an identical text or by endorsement, at the latest by May 2020, and conflicting national standards shall be withdrawn at the latest by May 2020.

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 document describes a surface test method for establishing whether a product proposed as a disinfectant in the fields described in Clause 1 has or does not have virucidal activity on non-porous surfaces.

The laboratory test closely simulates practical conditions of application. Chosen conditions (contact time, temperature, organisms on surfaces etc.) reflect parameters which are found in practical situations including conditions which may influence the action of disinfectants. Each use concentration found from this test corresponds to defined experimental conditions.

The conditions are intended to cover general purposes and to allow reference between laboratories and product types. Each utilization concentration of the chemical disinfectant found by this test corresponds to defined experimental conditions.

However, for special applications the recommendations for use of a product can differ and therefore additional test conditions might be needed, which cannot be covered by this document.

1 Scope

This document specifies a test method and the minimum requirements for virucidal activity of chemical disinfectants 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 for disinfecting without mechanical action non-porous surfaces in the veterinary area - i.e. in the breeding, husbandry, production, veterinary care facilities, transport and disposal of all animals except when in the food chain following death and entry to the processing industry.

EN 14885 specifies in detail the relationship of the various tests to one another and to "use recommendations".

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.

NOTE 3 Using this document, it is possible to determine the virucidal activity of the undiluted product.

NOTE 4 This document uses Porcine Parvovirus because Bovine Enterovirus E (former Bovine Enterovirus Type 1 (ECBO)) virus used in the suspension test EN 14675 cannot be used for surface testing because of its loss of titre during drying. Porcine Parvovirus has comparable resistance to ECBO virus.

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 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 14675, *Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in the veterinary area – Test method and requirements (Phase 2, step 1)*

EN 14885, *Chemical disinfectants and antiseptics — Application of European Standards for chemical disinfectants and antiseptics*

EN 10088-2, *Stainless steels — Part 2: Technical delivery conditions for sheet/plate and strip of corrosion resisting steels for general purposes*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN 14675 and 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 https://www.iso.org/obp

4 Requirements for virucidal activity on surfaces

The product shall demonstrate at least a decimal log (lg) reduction of 3 in virus titre of the parvovirus and coronavirus test strains when tested in accordance with Table 1 and Clause 5. To claim virucidal activity against enveloped viruses the product shall pass this standard with the coronavirus test strain and to claim full virucidal activity the product shall pass both EN 14675 with the bovine enterovirus test strain and this standard with the porcine parvovirus test strain.

NOTE See Annex E for further information on the appropriateness of claims of virucidal activity against enveloped viruses and full virucidal activity

Table 1 — Minimum and additional test conditions

Minimum spectrum of test organisms	**Full virucidal activity** Porcine Parvovirus, Strain NADL2 **Virucidal activity against enveloped viruses** Feline Coronavirus, Strain Munich
Test temperature	10 °C ± 1 °C
Additional temperatures	4 °C ± 1 °C; 20 °C ± 1 °C; 40 °C ± 1 °C
Contact time	The contact time(s) shall be selected from the values given below [a]
Minimum contact time	1min ± 5 s
Other contact times	5min ± 10 s, 15 min ± 10 s, 30 min ± 10 s, 60 min ± 10 s
Maximum contact time	120 min ± 10 s
Interfering substances - low level soiling	3,0 g/l bovine albumin
Interfering substances - high level soiling	10 g/l yeast extract and 10 g/l bovine albumin
Additional conditions[b]	Further contact time(s), interfering substance(s) or virus(es)

[a] 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.

[b] Where appropriate (specific purposes), additional specific virucidal activity shall be determined under other conditions of time, temperature, and interfering substances (see 5.2.2.8) 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.

The determined virucidal concentration of the test product is suggested as being suitable for practical situations of use.

5 Test method

5.1 Principle

5.1.1 Outline

A test suspension of viruses in a solution of interfering substances is inoculated onto a test surface and dried. A prepared sample of the product under test is applied in a manner which covers the dried film.

The test surface is maintained at a specified temperature for a defined period of time. The test surface is transferred to cell maintenance medium so that the action of the disinfectant is immediately neutralized. The titre of the virus recovered from the test surface is determined.

The titre of the inoculum on a test surface treated with hard water in place of the disinfectant is also determined and the reduction in virus titre attributed to the product is calculated by difference.

5.1.2 Test Organisms

The test is performed using the test organisms as specified in Clause 4, Table 1.

5.1.3 Variations

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

a) Non-enveloped DNA virus

Porcine Parvovirus strain NADL2 (PPV)

b) Enveloped RNA virus

Feline Coronavirus, strain Munich (FeCoV)

NOTE Virus strains can be obtained from a national or international culture collection. PPV and FeCoV can be obtained from the Friedrich-Loeffler-Insitut, Bundesforschungsinstitut für Tiergesundheit, Hauptsitz Insel Riems Südufer 10, 17493, Greifswald-Insel Riems[1].

The required incubation temperature for these test organisms is (36 ± 1) °C or (37 ± 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 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 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 organisms.

[1] This information is given for the convenience of users of this standard and does not constitute an endorsement by CEN of this institute.

To improve reproducibility, it is recommended that commercially available – dehydrated if appropriate - 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 ± 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 [5.3.2.1 a]. 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)[2] stock solution at 0,1 mg/ml in water (5.2.2.2). Filter through a 0,44 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.

5.2.2.6 Trichloroacetic acid (10 % 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 (5.3.2.1 c) 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;

[2] Sigma N 7005 is an example of a suitable product available commercially. This information is given for the convenience of users of this standard and does not constitute an endorsement by CEN of this product.

— 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 (5.3.2.1 c). 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 (5.3.2.4) 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 (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 and detergents) shall be defined.

“Diluent” is generally used in the other European Standards in the veterinary area to prepare the interfering substance. Since there is no experience in virucidal testing with diluent, water (5.2.2.2) is used instead.

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

5.2.2.8.2 Low level soiling (Bovine albumin solution)

Bovine serum albumin shall be used as commercially available or shall be prepared as follows:

Dissolve 3 g of bovine albumin fraction V (suitable for microbiological purposes) in 90 ml of water (5.2.2.2) in a 100 ml volumetric flask (5.3.2.9). Make up to the mark with water (5.2.2.2).

Sterilize by membrane filtration (5.3.2.1 c). Keep in a refrigerator (5.3.2.6) and use within one month.

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

5.2.2.8.3 High level soiling (mixture of bovine albumin solution with yeast extract)

Dissolve 50 g yeast extract powder in 150 ml of water (5.2.2.2) in a 250 ml volumetric flask (5.3.2.9) and allow foam to collapse. Make up to the mark with water (5.2.2.2). Transfer to a clean dry bottle and sterilize in the autoclave (5.3.2.1 a). Allow to cool to 20 °C ± 1 °C.

Pipette 25 ml of this solution into a 50 ml volumetric flask (5.3.2.9) and add 10 ml of water (5.2.2.2). Dissolve 5 g of bovine albumin fraction V (suitable for microbiological purposes) in the solution with shaking and allow foam to collapse. Make up to the mark with water (5.2.2.2), sterilize by membrane filtration (5.3.2.1 c), keep in a refrigerator (at 2 °C to 8 °C) (5.3.2.6) and use within one month.

The final concentration in the test procedure (5.5) is 10 g/l yeast extract and 10 g/l bovine albumin.

5.2.2.9 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], EN 14675 and EN 12353 for more detailed descriptions.

5.2.2.10 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) in such a density as to enable the formation of a monolayer no longer than 2 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 e).

5.3 Apparatus and glassware

5.3.1 General

Sterilize all glassware and parts of the apparatus that will come into contact with the culture media and reagents or the sample, except those which are supplied sterile, by one of the following methods:

a) by moist heat, in the autoclave [5.3.2.1 a];

b) by dry heat, in the hot air oven [5.3.2.1 b].

5.3.2 Usual microbiological laboratory equipment [3]

And, in particular, the following:

5.3.2.1 Apparatus for sterilization (moist and dry heat)

a) For moist heat sterilization, an autoclave capable of being maintained at (121_{0}^{+3}) °C for a minimum holding time of 15 min;

b) for dry heat sterilization, a hot air oven capable of being maintained at (180_{0}^{+5}) °C for a minimum holding time of 30 min, at (170_{0}^{+5}) °C for a minimum holding time of 1 h or at (160_{0}^{+5}) °C for a minimum holding time of 2 h.

c) for media sterilization, use suitable membrane filtration apparatus with filters of diameter 47 mm to 50 mm and membranes with 0,22 μm pore size.

5.3.2.2 Water baths, capable of being controlled at (20 ± 1) °C, and at additional test temperatures ± 1 °C (5.5.1).

5.3.2.3 Inverted microscope for reading cell cultures microscopically

5.3.2.4 pH meter, having an accuracy of calibration of 0,1 pH units at 20 °C.

5.3.2.5 Stopwatch

[3] Disposable sterile equipment is an acceptable alternative to reusable glassware.