SLOVENSKI STANDARD
SIST EN 14675:2006

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Kemiļ na razkuģila in antiseptiki - Kvantitativni suspenzijski preskus za vrednotenje virucidnega delovanja kemiļ nih razkuģil in antiseptikov v veterini - Preskusna metoda in zahteve (faza 2, stopnja 1)

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)

Chemische Desinfektionsmittel und Antiseptika - Quantitativer Suspensionsversuch zur Bestimmung der viruziden Wirkung chemischer Desinfektionsmittel und Antiseptika für den Veterinärbereich - Prüfverfahren und Anforderungen (Phase 2, Stufe 1)

Antiseptiques et désinfectants chimiques - Essai quantitatif de suspension pour l'évaluation de l'activité virucide des antiseptiques et des désinfectants chimiques utilisés dans le domaine vétérinaire - Méthode d'essai et prescriptions (phase 2, étape 1)

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Foreword

This European Standard (EN 14675:2006) 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of an identical text or by endorsement, at the latest by August 2006, and conflicting national standards shall be withdrawn at the latest by August 2006.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

Introduction

This European Standard specifies a suspension test for establishing whether a chemical disinfectant or antiseptic has or does not have a virucidal activity in the fields described in the scope.

The laboratory test closely simulates practical conditions of application. Chosen conditions e.g. contact time, temperature, test organisms, interfering substances, reflect parameters which are found in practical situations including conditions which may influence the action of antiseptics or chemical disinfectants.

The conditions are intended to cover general purposes and to allow reference between laboratories and product types. Each utilization concentration of the chemical disinfectant or antiseptic found by this test corresponds to defined experimental conditions. However, for some applications the recommendations of use of a product may differ and therefore additional test conditions need to be used.

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 – with water. Products can only be tested at a concentration of 80 % or less 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 veterinary area i.e. in the breeding, husbandry, production, transport and disposal of all animals except when in the food chain following death and entry to the processing industry.

NOTE 1 The method described is intended to determine the virucidal 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 (Annex E).

2 Normative references

Not applicable.

3 Terms and definitions

For the purposes of this European Standard, the following terms and definitions apply.

3.1
product
chemical agent or formulation used as chemical disinfectant or antiseptic

3.2
virucide
product which inactivates viruses under defined conditions

NOTE The adjective derived from ‘virucide' is ‘'virucidal''.

3.3
virucidal activity
capability of a product to produce a reduction in the number of infectious virus particles of relevant test organisms under defined conditions

3.4
viral infectivity
ability of a virus to express in cells its genetic information and/or multiply in them to infectious progeny

3.5
inactivation of viruses
reduction of infectivity of a virus by a product

NOTE Alteration of antigenic reactivity or of any viral component does not necessarily mean reduction of infectivity of a virus.

3.6
reference virus suspension
virus suspension of a defined virus strain which is not passaged more than 10 times, is maintained in national culture collection centres and kept in small volumes (less than 1 ml) at a temperature of –196 °C over liquid nitrogen

NOTE Stock virus suspensions are prepared from reference virus suspensions.

3.7
stock virus suspension
virus suspension of a defined strain that is multiplied on a large scale to obtain a virus suspension of the same characteristics as the reference virus suspension and kept in a small volume at a temperature of below –70 °C or preferably at –196 °C over liquid nitrogen

3.8
test virus suspension
virus suspension that is used in the virucidal testing of the disinfectant

3.9
virus Titre
amount of infectious virus per unit volume present in a cell culture lysate

3.10
reference virus inactivation test
test with a defined reagent (e.g. formalin) instead of a product for the internal control of the test

Results of reference virus inactivation test shall be within limits for validating the method.

3.11
cytotoxicity
morphological alteration of cells and/or their destruction or their reduced sensitivity to virus multiplication caused by the product

3.12
viral cytopathic effect (CPE)
morphological alteration of cells and/or their destruction as a consequence of virus multiplication

3.13
tissue culture infecting dose ($TCID_{50}$)
virus dose that gives rise to cytopathic change (CPE) in 50 % of the inoculated cell cultures

3.14
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

3.15
plaque forming units (PFU)
number of infectious virus particles per unit volume (ml)

4 Requirements

The product when diluted with hard water (**5.2.2.3**) or – in the case of ready-to-use products – with water (**5.2.2.2**) and tested in accordance with **Clause 5** under simulated low level soiling (3 g/l bovine albumin solution) (**5.2.2.4.2**) or simulated high level soiling (10 g/l bovine albumin solution plus 10 g/l yeast extract) (**5.2.2.4.3**) according to its practical applications and under the other obligatory test conditions: 1 selected test organism, 10 °C, 30 min shall demonstrate at least a lg reduction in virus titre of 4. It is possible to test also the product as delivered (highest test concentration is 80 %).

The virucidal activity shall be evaluated using the following test organism: *Bovine enterovirus* Type 1 (ECBO).

Where indicated, additional specific virucidal activity shall be determined applying other contact times, temperatures and test organisms in accordance with **5.2.1** and **5.5.1.1** in order to take into account intended specific use conditions.

NOTE For these additional conditions, the concentration defined as a result can be lower than the one obtained under the obligatory test conditions.

5 Test method

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 virus in a solution of an interfering substance. The mixture is maintained at 10 °C ± 1 °C for 30 min ± 10 s (obligatory test conditions).

At the end of the contact time, 0,5 ml of virus/disinfectant mixture is taken. The virucidal activity is immediately suppressed by dilution in ice-cold diluent. A dilution series with a factor of ten is prepared in an ice-cold medium held in an ice bath for 10 min. Dilutions shall be prepared in glass tubes (**5.3.2.9**) or microtitre plates (**5.3.2.8**). Pipettes shall be changed after each dilution to avoid carry-over of virus.

The dilutions are transferred into cell culture units (wells of microtitre plates) containing suspended cells. Eight units shall be inoculated with each dilution. After incubation, the titre of infectivity is calculated. The titration results of quantal tests shall show dilution steps with the percentage of positive results (presence of CPE or plaques) lying between 100 % and 0 %. The values are calculated according to Spearman and Kärber (see Annex C).

Values of virus inactivation are calculated from differences of virus titres before and after treatment with the product.

5.1.2 Additional and optional contact times and temperatures are specified. Additional test organisms can be used.

5.2 Materials and reagents

5.2.1 Test virus

The virucidal activity shall be evaluated using the following strain:

— *Bovine enterovirus* Type 1 (Enteric Cytopathogenic Bovine Orphan Virus – ECBO) ATCC VR-248[1)]

1) ATCC VR-248, is a strain supplied by the American Type 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. A corresponding strain supplied by other culture collections may be used if they can be shown to lead to the same results.

NOTE 1 *Bovine enterovirus* Type 1, strain ECBO, is selected as the model virus for the large *Genus Picornavirus*. The *Genus Picornavirus* includes many clinically important virus species, for example Coxsackie A and B, and enteric cytopathogenic human orphan (ECHO). Some of these viruses are of primary importance and therefore a constant risk for animals in the veterinary area. Moreover, they have a high resistance to chemicals, are acid-stable (except rhinoviruses) and are unaffected by lipid solvents such as ether, and most detergents or quaternary ammonium products.

NOTE 2 It is the model virus for all applications namely for disinfection of instruments and surfaces and post-contamination treatment of post-mortem rooms, kennels and for animal accommodation.

NOTE 3 Due to large differences of resistance against physical and chemical influences between and within different virus groups, the testing of all viruses against any particular chemical disinfectant or antiseptic is financially impossible. Therefore, in this European Standard, testing is restricted to only one so called 'model virus' that has been selected on the basis of the present knowledge as a representative example of virus tenacity and of important clinical relevance in the veterinary area. If a chemical disinfectant or antiseptic shows virucidal activity according to the requirements of this European Standard, it may be considered for a phase 2 step 2 test (see Annex E).

NOTE 4 If improvements in the methodology of virus multiplication, virus infectivity or cytoxicity reduction of products are elaborated, they may be used in parallel with the methodology described in this method to show the improvement.

5.2.2 Culture media and reagents

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 virological purposes. They shall be free from substances that are toxic or inhibitory to the test organism.

NOTE 1 To improve reproducibility, it is recommended that commercially available dehydrated material is used for the preparation of culture media. The manufacturer's instructions relating to the preparation of these products should be rigorously followed.

NOTE 2 For each culture medium and reagent a limitation for use should be fixed.

5.2.2.2 Water

The water shall be freshly glass distilled water and not demineralised water.

Sterilize in the autoclave [**5.3.2.1a)**].

NOTE 1 Sterilization is not necessary if the water is used, e.g. for preparation of culture media, and subsequently sterilized.

NOTE 2 If distilled water of adequate quality is not available, water for injections (see bibliographic reference [2]) can be used.

NOTE 3 See **5.2.2.3** for the procedure to prepare hard water.

5.2.2.3 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 [**5.3.2.1c)**] or in the autoclave [**5.3.2.1a)**]. 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 (**5.3.2.16**) 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 [**5.3.2.1c)**]. Store the solution in the refrigerator (**5.3.2.16**) 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 (**5.3.2.13**) and add 6,0 ml of solution A, then 8,0 ml of solution B. Mix and dilute to 1 000 ml with water (**5.2.2.2**). The pH of the hard water shall be 7,0 ± 0,2, when measured at 20 °C ± 1°C (**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**), the addition of the product to the hard water produces a different final water hardness in each test tube. In any case the final hardness is lower than 300 mg/l of calcium carbonate ($CaCO_3$) in the test tube.

5.2.2.4 Interfering substance

5.2.2.4.1 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.

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

5.2.2.4.2 Low level soiling (Bovine albumin solution)

Dissolve 3 g of bovine albumin (Cohn fraction V for Dubos Medium) in 90 ml of water (**5.2.2.2**) in a 100 ml volumetric flask (**5.3.2.13**). Make up to the mark with water (**5.2.2.2**).

Sterilize by membrane filtration [**5.3.2.1c)**]. Keep in a refrigerator (**5.3.2.16**) and use within one month.

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

5.2.2.4.3 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 (**5.3.2.13**) 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 [**5.3.2.1a)**]. Allow to cool to 20 °C ± 1 °C.

Pipette 25 ml of this solution into a 50 ml volumetric flask (**5.3.2.13**) and add 10 ml of water (**5.2.2.2**). Dissolve 5 g of the bovine albumin fraction V 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 [**5.3.2.1c)**], keep in a refrigerator (at 2 °C to 8 °C) (**5.3.2.16**) 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.

5.2.2.5 Antibiotic suspension

Chemicals

50 million units penicillin-G	(e.g. Sigma PEN-K[2)])
50 g streptomycin sulphate (approx. equal to 750 i.u./mg)	(e.g. Sigma Cat: 56501[2)])
500,000 units mycostatin	(e.g. Nystatin: E R Squibb 59150[2)])

Water (**5.2.2.2**) to 2,5 l.

Preparation

Dissolve vial contents in water (**5.2.2.2**) and make up to 2,5 l.

Dispense aseptically into 50 ml and 5 ml aliquots.

Store at –20 °C. Shake the bottle after thawing.

Use 5 ml per litre of medium to give a final concentration of:

Penicillin	100 units/ml
Streptomycin	100 µg /ml
Mycostatin	25 units/ml

5.2.2.6 Antibiotics-Trypsin-Versene (ATV) 10 × Concentrate

Chemicals

Sodium chloride (NaCl)	80 g
Potassium chloride (KCl)	4 g
Glucose	10 g
Trypsin	5 g (e.g. Difco 1:250 Cat No: 0152-15-9[3)])
Versene (EDTA)	2 g (e.g. Koch-Light Cat No: 0012424-/B[3)])
0,2 % Phenol Red solution	
in water (**5.2.2.2**)	1 000 ml

2) This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of the products named. Corresponding products supplied by other manufacturers may be used if they can be shown to lead to the same results.

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