BS EN 14675:2015

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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)

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The UK participation in its preparation was entrusted to Technical Committee CH/216, Chemical disinfectants and antiseptics.

A list of organizations represented on this committee can be obtained on request to its secretary.

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ISBN 978 0 580 79145 1

ICS 11.080.20; 11.220

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This British Standard was published under the authority of the Standards Policy and Strategy Committee on 30 April 2015.

Amendments issued since publication

Date	Text affected

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EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 14675

April 2015

ICS 71.100.35

Supersedes EN 14675:2006

English Version

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)

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)

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)

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Ref. No. EN 14675:2015 E

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Foreword

This document (EN 14675:2015) 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of an identical text or by endorsement, at the latest by October 2015 and conflicting national standards shall be withdrawn at the latest by October 2015.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN 14675:2006.

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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 areas described in the scope.

This laboratory test takes into account practical conditions of application of the product including contact time, temperature, test organisms and interfering substances, i.e. conditions which may influence its action in practical situations.

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.

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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.

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 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:

3.1
cytotoxicity
morphological alteration of cells and/or their destruction or their reduced sensitivity to virus multiplication caused by the product

3.2
plaque forming units
PFU
number of infectious virus particles per unit volume (ml)

3.3
reference test for virus inactivation
test with a defined reagent (e.g. formalin) instead of a product for the internal control of the test

Note 1 to entry: Results of reference virus inactivation test should be within limits for validating the method.

3.4
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 -70°C or preferably at about -196 °C under liquid nitrogen

Note 1 to entry: Stock virus suspensions are prepared from reference virus suspensions.

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3.5
stock virus suspension
virus suspension of a defined strain that is multiplied in a suitable cell line which produces high virus titers, 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 about -196 °C over liquid nitrogen

3.6
test virus suspension
virus suspension that is used in the virucidal testing of the disinfectant

3.7
tissue culture infectious dose
$TCID_{50}$
viral dose that induces a cytopathic effect (CPE) (3.8) in 50 % of inoculated cell culture

3.8
viral cytopathic effect
CPE
morphological alteration of cells and/or their destruction as a consequence of virus multiplication

3.9
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

3.10
virus titre
amount of infectious virus per unit volume present in a cell culture lysate or in a solution

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 Table 1 and Clause 5 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 %).

Table 1 — Obligatory and additional test conditions

	Obligatory conditions	Additional conditions
Test organism	*Bovine enterovirus* Type 1 (ECBO)	
Test temperature [a]	10 °C	4 °C, 20 °C or 40 °C
Contact time [b]	30 min	1 min, 5 min and 60 min
Interfering substance–low level soiling [c]	3,0 g/l bovine albumin	
Interfering substance-high level soiling [c]	10 g/l bovine albumin plus 10 g/l yeast extract	

[a] Allowed deviation ± 1 °C.

[b] The allowed deviation for each chosen contact time is ± 10 s, except for ≤1 min for which it is ± 5 s.

[c] To be chosen according to practical applications.

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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. 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 series 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.

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)].

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 inter alia rhinovirus, aphtovirus) 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 can be considered for a phase 2 step 2 test.

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.

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.

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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.

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 shall be rigorously followed.

For each culture medium and reagent a time limitation for use shall be fixed.

Commercial ready-to-use products and culture media can be purchased, if they comply with the required specifications.

5.2.2.2 Water

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

Sterilize in the autoclave [5.3.2.1 a)].

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

NOTE 2 See 5.2.2.3 for the procedure to prepare hard water.

If distilled water of adequate quality is not available, water for injections (see bibliographic reference [3]) can be used.

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 [5.3.2.1 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 (5.3.2.16) 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 [5.3.2.1 c)]. 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.

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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 375 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 [5.3.2.1 c)]. 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 [5.3.2.1 a)]. Allow to cool to 20 °C ± 1 °C.

Pipette 25 ml of this solution into a 50 ml volumetric flask (5.3.2.13) 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 [5.3.2.1 c)], 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 591502 [2)])
Water (5.2.2.2) to 2,5 l.	

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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