SLOVENSKI STANDARD
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Kemična razkužila in antiseptiki - Kvantitativni preskus brez mehanskega delovanja za vrednotenje razkužil za seske v veterini - Preskusna metoda in zahteve (faza 2, stopnja 2)

Chemical disinfectants and antiseptics - Quantitative surface test for the evaluation of teat disinfectants used in the veterinary area - Test method and requirements (phase 2 step 2)

Chemische Desinfektionsmittel und Antiseptika - Quantitativer Oberflächenversuch zur Beurteilung von Zitzendesinfektionsmittel für den Veterinärbereich - Prüfverfahren und Anforderungen (Phase 2, Stufe 2)

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

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Chemical disinfectants and antiseptics - Quantitative surface test for the evaluation of teat disinfectants used in the veterinary area - Test method and requirements (phase 2 step 2)

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EUROPÄISCHES KOMITEE FÜR NORMUNG

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European foreword

This document (prEN 17422:2019) 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 surface test for establishing whether a teat disinfectant, for use on teat skin without mechanical action, in the veterinary area, has or does not have bactericidal activity under the laboratory conditions defined by this document, which influence the action of disinfectants in practical use.

The laboratory test takes into account practical conditions of application of the product including applying test organisms and interfering substances on a synthetic skin surface, contact time and temperature, i.e. conditions which may influence its action in practical situations. The method is based on EN 16437 Chemical disinfectants and antiseptics - Quantitative surface test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in the veterinary area on porous surfaces without mechanical action - Test method and requirements (phase 2, step 2).

In this document synthetic human skin is used as the test surface.

1 Scope

This procedure specifies a test method and the minimum requirements for bactericidal activity of teat 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 method applies to teat disinfectants that are used on teat skin without mechanical action as pre-milking and/or post-milking teat disinfectants 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 into processing industry.

NOTE 1 The method described is intended to determine the activity of commercial formulations under the conditions in which they are used.

NOTE 2 This method corresponds to a phase 2 step 2 test.

NOTE 3 Two types of synthetic skin were assessed in a ring trial with no significant difference in performance. One has been chosen as the test surface because it is commercially available. Other synthetic skins can become available and can be used if it shown that they give comparable results to the one referenced in this standard.

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 1656, *Chemical disinfectants and antiseptics — Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in the veterinary area — Test method and requirements (phase 2, step 1)*

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 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/obpuirements

4 Requirements

The product shall demonstrate at least a 4 decimal log (lg) (post-milking disinfectant) or 3 decimal log (lg) (pre-milking disinfectant) reduction from a water control, when tested in accordance with Table 1 and Clause 5 under simulated soiling (10,0 g/l milk powder or 3,0 g/l bovine albumin).

Table 1 — Requirements

Test Conditions	**Bactericidal activity on synthetic skin without mechanical action**	
Test organism	- *Escherichia coli* - *Staphylococcus aureus*	
Test temperature	30°C ± 1°C	
Contact time	Minimum Up to 1 min at intervals of 30 s	Maximum For times > 1 min at intervals of 1 min
Post milking teat disinfectants Pre-milking teat disinfectants	1 min ± 5 s 30 s ± 5 s	5 min ± 10 s 3 min ± 10 s
Interfering substance Post milking teat disinfectants Pre-milking teat disinfectants	10,0 g/l milk powder 3,0 g/l bovine albumin	

5 Test method

5.1 Principle

A test suspension of bacteria mixed with interfering substance is inoculated onto a synthetic skin surface and maintained at 30 °C for a period of conditioning.

After this conditioning time, the skin surface is immersed in the product or dilutions of the product at 30 °C for a defined period of time specified in Table 1. At the end of that contact time, neutralizer is added so that the action of the disinfectant is immediately neutralized.

The bacteria are removed from the surface by ultrasound treatment. The numbers of surviving bacteria which can be recovered from the surface are determined quantitatively.

The number of bacteria on a surface treated with water in place of the disinfectant is also determined and the reduction is calculated.

5.2 Materials and reagents

5.2.1 Test organisms

The bactericidal activity shall be evaluated using the following strains as test organisms[1]:

- *Escherichia coli* ATCC 10536
- *Staphylococcus aureus* ATCC 6538

NOTE The ATCC numbers are the collection numbers of strains supplied by the American Type Culture Collections (ATCC).

Refer to Annex A for strain references in some other culture collections.

The required incubation temperature for these organisms is 36 °C ± 1 °C or 37 °C ± 1 °C (5.3.2.3).

[1] The ATCC numbers are the collection numbers of strains supplied by the American Type Culture Collection (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.

5.2.2 Culture media and reagents

5.2.2.1 General

All weights of chemical substances given in this method 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 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.

A ready-to-use medium may be used if it complies with the required specification.

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

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 [2]) 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.

5.2.2.3 Tryptone soya agar (TSA)

Tryptone soya agar, consisting of:

Tryptone, pancreatic digest of casein		15,0 g;
Soya peptone, papaic digest of soybean meal		5,0 g;
Sodium chloride (NaCl)		5,0 g;
Agar		15,0 g;
Distilled Water	to	1 000,0 ml.

Sterilize in the autoclave as above. After sterilization the pH of the medium shall be equivalent to 7,2 ± 0,2 when measured at (20 ± 1) °C.

5.2.2.4 Diluent

Tryptone sodium chloride solution, consisting of:

Tryptone, pancreatic digest of casein		1,0 g;
Sodium chloride (NaCl)		8,5 g;
Distilled Water	to	1 000,0 ml

Dispense in 9 ml portions and sterilize in the autoclave as above. After sterilization, the pH of the diluent shall be equivalent to 7,0 ± 0,2 when measured at (20 ± 1) °C.

5.2.2.5 Neutralizer

The neutralizer shall be validated for the product being tested in accordance with 5.5.1.2. It shall be sterile.

NOTE Information on neutralizers that have been found to be suitable for some categories of products is given in Annex B.

5.2.2.6 Standardized hard water

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 distilled water and dilute to 1 000 ml. Sterilize by membrane filtration or in the autoclave. Autoclaving - if used - may cause a loss of liquid. In this case make up to 1 000 ml with water under aseptic conditions. Store the solution in the refrigerator (5.3.2.8) at 2-8 °C for no longer than one month.

Prepare solution B:

Dissolve 35,02 g sodium bicarbonate (NaHCO3) in distilled water and dilute to 1 000 ml. Sterilize by membrane filtration. Store the solution in the refrigerator (5.3.2.8) at 2-8 °C for no longer than one week.

Place 600 ml to 700 ml of sterile distilled water in a sterile 1 000 ml volumetric flask and add 6,0 ml of solution A, then 8,0 ml of solution B. Mix and dilute to 1 000 ml with sterile distilled water. The pH of the hard water shall be 7,0 ± 0,2 when measured at (20 ± 1) °C. 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.

5.2.2.7 Interfering substances, shall be sterile and prepared at 2 times its final concentration in the test.

5.2.2.7.1 Skimmed milk, guaranteed free of antibiotics and additives and prepared as follows:

— prepare a solution of 20 g milk-powder in 1 000 ml water (5.2.2.2).

— Heat for 30 min at 105^{+3}_{0} °C (or 5 min at 121^{+3}_{0} °C).

The final concentration in the test procedure (5.5) is 10g/l milk powder.

5.2.2.7.2 Bovine albumin solution

Dissolve 0,6 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. Make up to the mark with water (5.2.2.2).

Sterilize by membrane filtration (5.3.2.7). Keep in the refrigerator (5.3.2.8) at 2-8 °C and use within one month.

The final concentration of the bovine albumin in the test procedure (5.5.2) is 3 g/l.

5.2.3 Test surface - synthetic skin

5.2.3.1 Vitro skin[2]

Handle skin with sterile tweezers/implements. Eight pieces are used for each test: 3 for the test dilutions, 1 for the water control, 1 for control B, 2 for control C (1 inoculated and 1 un-inoculated) and 1 for a sterility check.

Vitroskin: Mark with a pencil and ruler on the outside of the sealed skin packet eight 2 × 2 cm squares (per test). Cut using sterile scissors and re-hydrate for 16 to 24 h before use (follow instructions provided from the supplier). When ready to start the test, place each piece in a sterile wide necked vessel, with lid on.

[2] Vitro skin may be obtained from IMS Inc., 110 Marginal Way, PMB 155, Portland ME 04101-2497 USA. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of the product named.

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 an autoclave;

b) by dry heat, in a hot air oven

5.3.2 Usual microbiological equipment[3],and in particular, the following:

5.3.2.1 Apparatus for sterilization (dry and moist heat):

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

b) for dry heat sterilisation, a hot air oven capable of being maintained at (180 $^{+3}_{0}$) °C for a minimum holding time of 30 min, at (170 $^{+5}_{0}$) °C for a minimum holding time of 1 h or at (160 $^{+5}_{0}$) °C for a minimum holding time of 2 h.

5.3.2.2 Water bath, capable of being controlled at (20 ± 1) °C, (30 ± 1) °C, and (45 ± 1) °C.

5.3.2.3 Incubator, capable of being controlled at (30 ± 1) °C, (36 ± 1) °C or (37 ± 1) °C.

5.3.2.4 pH-meter, having an inaccuracy of calibration of no more than ± 0,1 pH units at (20 ± 1) °C. A puncture electrode or a flat membrane electrode should be used for measuring the pH of the agar-media.

5.3.2.5 Stopwatch

5.3.2.6 Shakers

a) Electromechanical agitator, e.g. Vortex® mixer[4]

b) Mechanical shaker.

5.3.2.7 Membrane filtration apparatus, constructed of a material compatible with the substances to be filtered with a filter holder of at least 50 ml volume and suitable for use with filters of diameter 47 mm to 50 mm and 0,45 µm pore size for sterilization of hard water (5.2.2.6) and bovine albumin (5.2.2.7.2).

5.3.2.8 Refrigerator, capable of being controlled at 2 °C to 8 °C.

5.3.2.9 Graduated pipettes of nominal capacities 10 ml, 1 ml, 0,1 ml and 0,05 ml or calibrated automatic pipettes.

5.3.2.10 Petri dishes, (plates) of size 90 mm to 100 mm.

5.3.2.11 Glass beads (diameter 3 mm to 4 mm).

5.3.2.12 Volumetric flasks

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

[4] Vortex® 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.