SLOVENSKI STANDARD
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Antimikrobni sanitetni material - Zahteve in preskusne metode

Antimicrobial wound dressings - Requirements and test methods

Antimikrobielle Wundauflagen - Anforderungen und Prüfverfahren

Pansements antimicrobiens - Exigences et méthodes d'essai

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Antimicrobial wound dressings - Requirements and test methods

Pansements antimicrobiens - Exigences et méthodes d'essai

Antimikrobielle Wundauflagen - Anforderungen und Prüfverfahren

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COMITÉ EUROPÉEN DE NORMALISATION
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Contents

Page

Foreword

This document (prEN 16756:2014) has been prepared by Technical Committee CEN/TC 205 “Non-active medical devices”, the secretariat of which is held by DIN.

This document is currently submitted to the CEN Enquiry.

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association, and supports essential requirements of EU Directive(s).

For relationship with EU Directive(s), see informative Annex ZA, which is an integral part of this document.

Introduction

This standard describes a range of test methods for establishing whether a wound dressing exerts antimicrobial activity.

The laboratory tests attempt to simulate conditions of application, through the use of appropriate test fluids, temperature, organisms, and contact times reflecting the parameters found in clinical situations. Conditions which may influence the action of wound dressings having antimicrobial properties should be included.

The conditions are intended to cover general purposes and to allow comparison between laboratories and product types.

1 Scope

This standard specifies requirements and test methods for the antimicrobial activity of antimicrobial wound dressings. It is designed for microbicidal and microbistatic dressings.

Test methods specifically for microbial binding are not included in the standard.

2 Normative references

The following referenced documents are indispensable for the application 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.*

3 Terms and definitions

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

3.1
antimicrobial dressing
wound dressing which can be shown to exert antimicrobial activity when tested to the appropriate tests in Annex H of this standard

3.2
negative control dressing
wound dressing which is the same dressing as the dressing to be tested but without the antimicrobial treatment

Note 1 to entry: If this is not available, then use a non-medicated dressing from the same product group preferably from the same manufacturer. If no similar product is available, a sterile gauze swab of the same weight shall be used

3.3
plate count method
method in which the number of microorganisms present after incubation is calculated by counting the number of colonies according to a ten-fold dilution method

3.4
neutralizer
chemical agents used to inactivate, neutralize, or quench the antimicrobial properties of antimicrobial agents

3.5
test dressing
wound dressing which is to be tested to assess its antimicrobial activity

3.6
saturation volume
volume of fluid (SWF) absorbed by the dressing as determined by the method in H.4.3

Note 1 to entry: This volume of fluid is added to the dressing to prevent the dressing absorbing all of the challenge media (ISWF).

4 Classification

Classification of antimicrobial dressings is not presently standardized. The following two classes are thought to be useful in the context of this standard when selecting appropriate test methods as described in Table 1.

Microbicidal – capability of the dressing to produce at least a 3-log reduction in the number of viable cells from the challenge organisms when tested under the conditions in Annex H.

Microbistatic – capability of the dressing to prevent further growth of the initial inoculum but which does not achieve at least a 3 log reduction when tested under the conditions in Annex H.

If a manufacturer is claiming compliance with this standard, they must comply with the requirements in Monographs 6 and 7.

NOTE The editing panel recommend that the usefulness of these definitions be reviewed during the development of the standard.

5 Requirements

5.1 Microbicidal dressings

When tested using the relevant method in Annex H, antimicrobial dressings shall demonstrate at least a 3-log reduction in activity as determined by that particular test method over 24 hours and against all three test organisms.

5.2 Microbistatic dressings

When tested using the relevant method in Annex H, microbistatic dressings shall prevent further growth of the initial inoculum over 24 hours and against all three test organisms.

5.3 Performance Table

Table 1 shows the performance requirements for antimicrobial dressing pending on the two classifications.

Table 1 — Performance requirements for antimicrobial dressings

Performance requirements	
Microbicidal	**Microbistatic**
$A \geq 3$	$0 \leq A < 3$
A = Antimicrobial activity (see Annex H.10)	

6 Information to be supplied

The following information shall be supplied on request:

— the Annex number of the test method used;

— details of the test dressing, negative control dressing, and the mean values of C_0 C_t, T_0, and T_t;

— the antimicrobial activity A;

— the full test report (see Tables 2 and 3 as examples).

Table 2 — Example for a test report (blank)

Test dressing	_____
Control dressing	_____
Test method	Annex _____
Exposure period	_____

Organism	Dressing	Time 0 Log_{10} counts per sample (Ms)	Time XX Log_{10} counts per sample (Ms)	Antimicrobial Activity (A)
S. aureus	Control			
	Test			
P. aeruginosa	Control			
	Test			
C. albicans	Control			
	Test			

Result _____

NOTE The standard test time is (24 ± 1) h. Any additional exposure times should be provided in the same format.

Table 3 — Example for a test report (filled out)

Test dressing	Silver foam_____
Control dressing	Silver-free foam_____
Test method	Annex H.7_____
Exposure period	24 hours_____

Organism	Dressing	Time 0 Log_{10} counts per sample (Ms)	Time 24 h Log_{10} counts per sample (Ms)	Antimicrobial Activity (A)
S. aureus	Control	6.3	6.7	- 0.4
	Test	6.3	2.1	4.2
P. aeruginosa	Control	6.4	7.0	- 0.6
	Test	6.1	1.2	4.9
C. albicans	Control	6.1	6.2	- 0.1
	Test	6.1	< 1.6	> 4.5

Result Microbicidal_____

Annex A
(normative)

Validation of dilution-neutralisation

A.1 Principle

The effectiveness of antimicrobial agents incorporated into wound dressings is measured by their ability to kill microorganisms within a specified contact time. Consequently, accurate determination of antimicrobial activity requires complete and immediate neutralisation of the antimicrobial agent. Inefficient or incomplete neutralisation will permit killing or inactivation of microorganisms to continue beyond the experimental exposure time, resulting in an overestimation of antimicrobial activity. A neutralizer is therefore required for each antimicrobial dressing to inactivate or quench the microbicidal properties of the antimicrobial agent.

Neutralising agents may also be incorporated into agar to increase the neutralising capacity for the test methods detailed in Annex H. Agar containing neutralising agents may be used for the enumeration of microbial test suspensions providing its neutralisation effectiveness has been validated and it does not have an inhibitory effect on organism viability.

A.2 Neutralizer selection

Refer to Annex B for examples of suitable neutralizers for certain antimicrobial agents. ASTM E1054-08 also documents examples of appropriate neutralizers for antimicrobial agents.

The neutralizer selected should, where possible, minimize the neutralizer volume to allow the use of 1ml pour plates to be used from the neat dilution, therefore maximising the sensitivity of the test.

A.3 Preparation of microbial suspension

NOTE The reference strains detailed in Annex F should be used in the neutralisation assay.

a) Prepare the microbial suspensions in accordance with Annex G.

b) Separately dilute each microbial suspension in MRD such that when a 100 µl volume is inoculated, the final concentration within each neutralizer validation suspension contains 30 to 100 cfu/ml (see Table A.1).

c) Using standard microbiological techniques perform an inoculum count on each suspension to confirm the inoculum concentration.

A.4 Test validation

The neutralisation evaluation consists of three experimental tests (i.e. neutralizer toxicity, test organism viability and neutralisation effectiveness test) for each test strain. At least three replicates must be performed for each experimental condition. Table A.1 details the specific requirements of each experimental test which are dependent on the chosen test method.

NOTE Each experimental test must simulate the test conditions as those detailed in Annex H (e.g. sample volumes, dilution ratios and incubation conditions must replicate those in the chosen test method).

Table A.1 — Requirements for the neutralisation validation

	Test Method		
Requirements	**Direct Contact Method (see H.6)**	**Shaking Method (see H.7**	**Two Compartment Method (see H.8)**
Neutralizer toxicity	8.9 ml Neutralizer + 100 µl inoculum + 1 ml MRD	4.4 ml Neutralizer + 100 µl inoculum + 0.5 ml SWF	4.4 ml Neutralizer + 100 µl inoculum + 0.5 ml SWF
Test organism viability	8.9 ml MRD + 100 µl inoculum + 1 ml MRD	4.4ml MRD + 100µl inoculum + 0.5ml SWF	4.4 ml MRD + 100 µl inoculum + 0.5 ml SWF
Neutralisation effectiveness	Dressing sample as per H.5 + 5 x 0.1 ml SWF as per H.6.3 Incubate (32 ± 2) °C for desired contact time 8.9 ml Neutralizer + 100 µl inoculum + Dressing sample	Dressing sample as per H.5 + 5 ml SWF + the determined SV as per H.4.3 Incubate (32 ± 2) °C for desired contact time 4.4 ml Neutralizer + 100 µl inoculum + 0.5 ml SWF sample	Dressing sample as per H.5 + 2.5 ml SWF + the determined SV as per H.4.3 Incubate (32 ± 2) °C for desired contact time 4.4 ml Neutralizer + 100 µl inoculum + 0.5 ml SWF sample

A.5 Neutralizer toxicity

This is performed to demonstrate that the chosen neutralizer does not have any inhibitory effect on the challenge organisms.

a) Transfer the required volume of suitable neutralizer (see E.7) to a test tube.

b) Inoculate a 100 µl volume of microbial suspension prepared in A.3.

c) Add the appropriate volume of MRD or SWF to the neutralizer/challenge organism suspension such that the resulting suspension contains 30 to 100 cfu/ml of the challenge organism. Mix contents.

d) Allow the suspension to stand for an appropriate time (see note below).

e) After the exposure time, transfer 1ml volumes of the suspension to duplicate agar pour plates; agar spread plates may be used by transferring a 0.1 ml volume onto duplicate plates, taking into account the extra 10-fold dilution. If neutralizers are incorporated into agar for the test method, then use the same medium for plating the suspension.

f) Repeat this procedure an additional two times, for a total of three replicates.

g) Incubate the agar plates under the same conditions as those used in the chosen test method.

h) Following incubation, count the number of colonies on each agar plate and determine the cfu/ml as detailed in A.8.

NOTE Allow the resulting suspension to stand for the longest exposure period representative of that used in chosen test method. Unless prior knowledge and experience in the use of the chosen neutralizer is known, a validation exercise may be performed to determine optimal standing time for effective neutralisation and absence of toxicity.