International Standard

ISO 16187

Second edition
2025-02

# Footwear and footwear components — Test method to assess antibacterial activity

*Chaussure et composants de chaussure — Méthode d'essai pour évaluer l'activité antibactérienne*



Reference number
ISO 16187:2025(en)



ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

# Contents

Page

# Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

ISO draws attention to the possibility that the implementation of this document may involve the use of (a) patent(s). ISO takes no position concerning the evidence, validity or applicability of any claimed patent rights in respect thereof. As of the date of publication of this document, ISO had not received notice of (a) patent(s) which may be required to implement this document. However, implementers are cautioned that this may not represent the latest information, which may be obtained from the patent database available at www.iso.org/patents. ISO shall not be held responsible for identifying any or all such patent rights.

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 216, *Footwear*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 309, *Footwear*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This second edition cancels and replaces the first edition (ISO 16187:2013), which has been technically revised.

The main changes are as follows:

— a new term “neutralizer” and its definition have been added;

— a new Clause 4 has been added;

— AS No. has been revised to CGMCC No.;

— the light intensity of UV lamp has been added;

— the normative references and bibliography have been revised and updated;

— TSA and TSB have been added as alternative culture medium if NA and NB are not available;

— Annex D has been deleted.

Any feedback or questions on this document should be directed to the user’s national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

# Footwear and footwear components — Test method to assess antibacterial activity

**CAUTION — Test methods specified in this document require the use of bacteria. These tests shall only be carried out in facilities with containment techniques for handling microorganisms and by persons with training and experience in the use of microbiological techniques.**

## 1 Scope

This document specifies quantitative test methods to evaluate the antibacterial activity of footwear and footwear components.

This document is applicable to all types of footwear and footwear components employing non-diffusing antibacterial treatments.

## 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.

ISO 7218, *Microbiology of the food chain — General requirements and guidance for microbiological examinations*

ISO 19952, *Footwear — Vocabulary*

## 3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 19952 and the following apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

**3.1**
**antibacterial activity**
efficacy of a material or finish used to prevent or mitigate the growth of bacteria, to reduce the number of bacteria or to kill bacteria

**3.2**
**control sample**
material identical to the test material but without antibacterial treatment

**3.3**
**neutralizer**
chemical agent used to inactivate, neutralize, or quench the antibacterial properties of antibacterial agents

## 4 Principle

The test specimens and control specimens are inoculated with a bacterial suspension of a selected test strain specified or claimed in independent tests with one Gram-positive and one Gram-negative bacterial test organism.

Three test methods are available to assess antibacterial activity in a challenge test procedure under static or under dynamic conditions.

Antibacterial performance is quantitatively determined by counting the number of viable cells and calculating the antibacterial activity ratio.

## 5 Safety

Handling of microorganisms which are potentially hazardous requires a high degree of technical competence and can be subject to current national legislation and regulations. Only personnel trained in microbiological techniques should carry out such tests.

NOTE Refer to country-specific codes of practice for personal hygiene, disinfection and sterilization.

Persons who perform the test should consult IEC 60068-2-10:2005/AMD1:2018, Annex A, and ISO 7218.

## 6 Apparatus

Disposable apparatus is an acceptable alternative to re-usable glassware and plastic if it has the suitable specifications.

Apparatus includes usual microbiological laboratory equipment in accordance with ISO 7218 and, in particular, the following.

**6.1 Biological safety cabinet.**

**6.2 Incubator**, capable of maintaining a temperature of (37 ± 2) °C.

**6.3 Autoclave**, capable of maintaining a temperature of (121 ± 2) °C and a pressure of (103 ± 5) kPa, for wet sterilization, used in accordance with ISO 7218.

**6.4 Humidity chamber**, capable of maintaining a temperature of (37 ± 2) °C and a relative humidity of (85 ± 5) %.

**6.5 Ultraviolet lamp**, capable of emitting light intensity of 100 000 μWs/cm$^2$.

**6.6 Wide mouth jars**, with cap, 100 ml, capable of being used with an autoclave (6.3).

**6.7 Cover film**, that does not affect bacterial growth or absorb water, which can be made of either polyethylene, polypropylene or polyester [poly (ethylene terephthalate)]. Film that is 0,05 mm to 0,10 mm thick should be used. For example, disposal bag suitable for use with an autoclave (6.3).

**6.8 Vortex mixer**.

**6.9 Dimensional shaker**, two dimensional or three dimensional, capable of adjusting to 50 r/min.

**6.10 Shaking incubator**, capable of maintaining a temperature of (37 ± 2) °C and a rotational frequency of (120 ± 10) r/min.

## 7 Reagents and culture medium

### 7.1 General

The preparation and test shall be freshly prepared in order to ensure the culture quality.

This can be done according to ISO 11133, or according to national standards, in case no national regulations apply.

Dehydrated products available on the commercial market can be used for preparing the culture media. The manufacture user's instructions for the preparation of these products should be strictly followed.

Reagents used in tests shall be of analytical grade and/or suited for microbiological purposes.

## 7.2 Water

Water used in tests shall be free from all toxic or microorganism inhibitory substances and be analytical-grade water for microbiological media preparation, which is freshly distilled and/or ion-exchanged and/or ultra-filtered and/or filtered with reverse osmosis (RO).

NOTE Water grade 3 according to ISO 3696 can be used.

## 7.3 Nutrient broth (NB)

### 7.3.1 Composition

| | |
|---|---|
| Beef extract | 3,0 g |
| Peptone | 5,0 g |
| Sodium chloride, NaCl | 5,0 g |
| Water | 1 000 ml |

### 7.3.2 Preparation

Stir and adjust pH to (7,2 ± 0,2) (at room temperature). Heat with stirring on a hotplate or in a boiling-water bath until the components are completely dissolved. Sterilize with autoclave (6.3) at (121 ± 2) °C for 15 min.

## 7.4 Nutrient agar (NA)

### 7.4.1 Composition

| | |
|---|---|
| Beef extract | 5,0 g |
| Peptone | 10,0 g |
| Sodium chloride, NaCl | 5,0 g |
| Agar | 15,0 g |
| Water | 1 000 ml |

NOTE If solidification is insufficient, 15 g to 18 g of agar can be used.

### 7.4.2 Preparation

Stir and adjust pH to (7,2 ± 0,2) (at room temperature). Heat with stirring on a hotplate or in a boiling-water bath until the components are completely dissolved. Sterilize with autoclave (6.3) at (121 ± 2) °C for 15 min. Cool and shake solution well, then pour into the Petri dishes.

## 7.5 Tryptic soy broth (TSB)

### 7.5.1 Composition

| | |
|---|---|
| Tryptone, pancreatic digest of casein | 17,0 g |
| Soya peptone, papain digest of soya | 3,0 g |
| Sodium chloride, NaCl | 5,0 g |
| Glucose | 2,5 g |
| Dipotassium hydrogen phosphate, $K_2HPO_4$ | 2,5 g |
| Water | 1 000 ml |

### 7.5.2 Preparation

Stir and adjust pH to (7,2 ± 0,2) (at room temperature). Heat with stirring on a hotplate or in a boiling-water bath until the components are completely dissolved. Sterilize with autoclave (6.3) at (121 ± 2) °C for 15 min.

NOTE TSB can be used as alternative culture medium if NB is not available.

## 7.6 Tryptone soy agar (TSA)

### 7.6.1 Composition

| | |
|---|---|
| Tryptone, pancreatic digest of casein | 15,0 g |
| Soya peptone, papain digest of soya | 5,0 g |
| Sodium chloride, NaCl | 5,0 g |
| Agar | 15,0 g |
| Water | 1 000 ml |

### 7.6.2 Preparation

Stir and adjust pH to (7,2 ± 0,2) (at room temperature). Heat with stirring on a hotplate or in a boiling-water bath until the components are completely dissolved. Sterilize with autoclave (6.3) at (121 ± 2) °C for 15 min. Cool and shake solution well, then pour into the Petri dishes.

NOTE TSA can be used as alternative culture medium if NA is not available.

## 7.7 Soybean casein digest broth with lecithin and polyoxyethylene medium (SCDLP)

### 7.7.1 Composition

| | |
|---|---|
| Peptone, digest of casein | 17,0 g |
| Peptone, digest of soybean | 3,0 g |
| Sodium chloride, NaCl | 5,0 g |
| Potassium dihydrogen phosphate, $KH_2PO_4$ | 2,5 g |
| Glucose | 2,5 g |

| | |
|---|---|
| Lecithin | 1,0 g |
| Polysorbate 80 | 7,0 g |
| Water | 1 000 ml |

If the neutralizing power is insufficient, the content of polysorbate 80 or lecithin may be adjusted, or another neutralizing agent may be added. The use of any unspecified neutralizer shall be recorded along with the name and concentration.

NOTE Information about selection and evaluation of alternative antibacterial neutralizing agents can be found in ASTM E 1054 and EN 1040.

#### 7.7.2 Preparation

After mixing well, adjust pH to (7,2 ± 0,2) (at room temperature) and sterilize with autoclave (6.3) at (121 ± 2) °C for 15 min.

### 7.8 Sodium chloride solution (physiological saline)

#### 7.8.1 Composition

| | |
|---|---|
| Sodium chloride, NaCl | 8,5 g |
| Water | 1 000 ml |

#### 7.8.2 Preparation

After mixing well, adjust pH to (6,9 ± 0,2) (at room temperature) and sterilize at (121 ± 2) °C for 15 min.

## 8 Test microorganisms

### 8.1 Test strains

The following strains shall be used in all antibacterial activity tests.

a) *Staphylococcus aureus* CGMCC[1)] 1.89 or ATCC® 6538™[2)] or WDCM 00032.

b) *Klebsiella pneumoniae* CGMCC 1.1736 or ATCC® 4352™[2)] or WDCM 00192.

If required, other strains or other species can be used. However, the selected organisms shall contain at least one Gram-positive and one Gram-negative organism as the antibacterial agents can have different activities.

Test strains shall be obtained from agencies of the World Federation of Culture Collection (WFCC).

The bacteria strains and their supply sources shall be included in the test report.

### 8.2 Storage of strains

Inoculate the strains to the nutrient agar (NA) (7.4) or tryptone soy agar (TSA) (7.6) and incubate at (37 ± 2) °C for 24 h. Store at (5 ± 3) °C (maximum one month) and keep it as stock culture of the strains. Transfer and incubate one time each month.

Strains can be preserved in accordance with the supplier's direction or EN 12353.

1) CGMCC refers to the China General Microbiological Culture Collection Centre, ATCC® is the American Type Culture Collection, WDCM is World Data Centre for Microorganisms (refer to WDCM and website: http://refs.wdcm.org).

2) ATCC® 6538™ and ATCC® 4352™ are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

## 9 Preparation of test inoculums

Using a sterile inoculating loop, transfer one colony (8.2) into 20 ml of nutrient broth (NB) (7.3) or tryptic soy broth (TSB) (7.5) and incubate in the shaking incubator (6.10) at (37 ± 2) °C for approximately 16 h (overnight culture). Estimate the number of bacteria with microscopic observation or other methods. Prepare physiological saline (7.8) with 1 % nutrient broth (NB) (7.3) or tryptic soy broth (TSB) (7.5). Use this media to prepare a suspension with a bacterial concentration of $2,5 \times 10^5$ CFU/ml to $10 \times 10^5$ CFU/ml as test inoculum.

If necessary, store the test inoculum on ice and use it within 4 h.

If required, the inoculum may be > $1,0 \times 10^6$ CFU/ml.

## 10 Preparation of test samples

### 10.1 General

Test only the components or material which are claimed to be antibacterial. If the whole footwear is claimed as antibacterial, major components, including upper, lining, insole, insock and outsole, shall be tested separately.

In the case where only one material of a component is claimed to be antibacterial, it shall be tested separately, if possible. Otherwise, the whole component shall be tested.

Each test sample shall be at least 80 % of the surface area of the component or material. If a single material accounts for less than 80 %, take two main materials used in the composition of the component.

The test samples can be obtained directly from the footwear raw materials.

### 10.2 Test specimen

The area of test specimen should be approximately 500 $mm^2$. For the static challenge test method described in Annex A, the area of test specimen shall have a thickness of less than 2,0 mm. The area and the weight shall be reported in the test report. If a larger test specimen is used, then the volume of bacterial suspension should be increased proportionally.

If it is impossible to lower the thickness of the test specimen (for example, if components are thicker and cannot be separated or cut without changing critical properties such as surface morphology which can affect how the bacteria interact with the surface), the thickness shall be indicated in the test report.

At least six test specimens shall be taken for each material or component and for each test strain.

### 10.3 Pre-treatment of the test specimen

Pre-treatment of the test specimen is optional and should only be conducted if necessary due to high bioburden (contamination, etc.).

If sterilization methods are applied, they shall be reported in detail, and shall not affect the antibacterial properties or the material itself.

NOTE The test and control sample can be sterilized with the autoclave (6.3) at (121 ± 2) °C and 103 kPa for 15 min, or with ultraviolet rays [ultraviolet lamp (6.5), 100 000 µWs/$cm^2$, 300 mm away from the sample, each side for one hour respectively] or by immersion in 70 % ethanol (analytical grade) for 10 minutes and drying overnight in the workbench, or other suitable sterilizing methods.

## 11 Test procedure

Table 1 lists the circumstances under which each test method should be applied.

The static challenge test method described in Annex A shall be used only for absorbent single material, and does not include materials that cannot be fully eluted. The film contact method described in Annex B shall be used only for non-absorbent single materials. The dynamic challenge test method described in Annex C shall be applied to both absorbent and non-absorbent materials or combinations.

For single material, the static challenge test method and the film contact method are preferred.

**Table 1 — List of test methods**

| No. | Type of material | Test method | Remarks |
|---|---|---|---|
| 1 | Absorbent | Static challenge test in Annex A | Textile and leather |
| 2 | Non-absorbent | Film contact method in Annex B | Micropore, (i.e. coated or heavy leather, synthetic/artificial materials, EVA foaming material, PU foaming material) and compacted material (i.e. plastic or coated material) |
| 3 | Absorbent and non-absorbent | Dynamic challenge test (shake flask test) in Annex C | Components of different materials, shaped material, materials with fixed antibacterial agent |

## 12 Expression of results

### 12.1 Calculation of the number of viable bacteria

For each test specimen, determine the number of viable bacteria recovered in accordance with Formula (1):

$$M = Z \times B \times E \times I \quad (1)$$

where

$M$ is the number of viable bacteria of each test specimen (expressed as CFU/ml);

$Z$ is the average number of viable bacteria in the two Petri dishes;

$B$ is the dilution ratio of plate counting;

$E$ is the dilution ratio of neutralization (20 for static challenge test method; 10 for film contact method and dynamic challenge test method);

$I$ is the dilution factor (1 for static challenge test method and dynamic challenge test method; for film contact method: 10 for inoculum of 0,1 ml, 5 for inoculum of 0,2 ml).

### 12.2 Judgement of test effectiveness

a) The extreme difference value of the three control specimens after inoculation and incubation shall be $\Delta(\lg C) \leq 1$, where $C$ is the number of viable bacteria of control specimen obtained from 12.1.

b) The average number of colonies of the controls immediately after inoculation shall be at least $1 \times 10^5$ CFU for the static challenge test method and the dynamic challenge test method and $1 \times 10^4$ CFU for the film contact method, and it shall not be reduced more than one order of magnitude compared to the suspension of inoculation.

In case of inoculum > $1{,}0 \times 10^6$ CFU/ml, the average number of colonies of the controls immediately after inoculation shall be at a minimum $1{,}0 \times 10^6$ CFU for the static challenge test method and for the dynamic challenge test method, and $1 \times 10^5$ CFU for the film contact method.

c) In the plate counting, calculate the growth value ($F$) in accordance with Formula (2), and F shall be ≥ 0.

$$F = \lg C_t - \lg C_0 \quad (2)$$

where

$F$ is the bacteria growth value of the controls;

$C_t$ is the average number of colonies of the three control specimens after incubation, expressed as CFU/ml;

$C_0$ is the average number of colonies of the three controls immediately after inoculation, expressed as CFU/ml.

When the conditions of a), b) and c) are satisfied, the test is judged to be effective. If one or more conditions are not met, the test is not valid, and the samples shall be retested.

### 12.3 Calculation of antibacterial activity ratio

Antibacterial performance of footwear or footwear components shall be reported separately based on the antibacterial activity ratio.

Calculate the antibacterial activity ratio ($R$) in accordance with Formula (3), or $R^*$ in accordance with Formula (4). Record the result in percentage with three significant figures.

$$R = \frac{C_t - T_t}{C_t} \times 100\ \% \quad (3)$$

where

$C_t$ is the average number of colonies of three control samples after 24 h or the specified incubation period, expressed as CFU/ml;

$T_t$ is the average number of colonies of three test samples after 24 h or the specified incubation period, expressed as CFU/ml.

In cases where there is no control sample available, calculate $R^*$ by replacing $C_t$ on Formula (3) by $T_0$ using Formula (4).

$$R^* = \frac{T_0 - T_t}{T_0} \times 100\ \% \quad (4)$$

where $T_0$ is the average number of colonies of three samples immediately after inoculation, expressed as CFU/ml.

## 13 Test report

The test report shall include at least the following information:

a) a reference to this document, i.e. ISO 16187:2025;

b) all information necessary for the complete identification of the treated test components, e.g. positions, areas, weights;

c) test methods for different materials;

d) preparation of the test specimen, including pre-treatment methods, if applied, for different samples (i.e. sterilization method);

e) species, serial number, culture medium used and number of viable cells of test strains for different materials;

f) surfactant and its concentration added into the test inoculums;

g) judgement of test effectiveness;

h) antibacterial activity ratio of different materials or components;

i) the date of the test;

j) any deviations from this method.

# Annex A
(normative)

# Static challenge test

## A.1 Test procedure

### A.1.1 Inoculation

Place each of the six test samples and the six control samples into separate sterilized wide mouth jars (6.6). Pipette (1,0 ± 0,1) ml of the inoculum prepared in Clause 9 to each test sample and tightly close the screw cap. The number of swatches to be used is dependent on the sample type.

If no control samples are available, inoculate wide mouth jars without samples as control to determine the test effectiveness.

### A.1.2 Elution after inoculation (time zero)

Add 20 ml SCDLP medium (7.7) to each of three inoculated test and control samples (if available). Tighten the caps and shake them in an arc of approximately 30 cm for 30 s, or mix for 5 s × 5 cycles using the vortex mixer (6.8) in order to elute out bacteria into the medium.

### A.1.3 Incubating

Culture the three inoculated test and control samples (if available) at (37 ± 2) °C for (24 ± 1) h.

### A.1.4 Elution after incubating (time 24 h)

Proceed as in A.1.2.

### A.1.5 Determination of the number of viable bacteria — Surface culture

Take 1 ml elution from A.1.2 or elution from A.1.4 with a sterile pipette and add it into a test tube with (9,0 ± 0,1) ml physiological saline (7.8) and shake it well. Dilute the elution with physiological saline (7.8) and obtain 10-fold serial dilutions.

Inoculate 100 µl of each dilution onto nutrient agar (NA) (7.4) or tryptone soy agar (TSA) (7.6) twice, turn the agar upside down and incubate it for 24 h to 48 h.

After incubation, count the number of colonies in the Petri dishes containing 30 to 300 colonies. If the minimum number of colonies is less than 30, then count and record the number of colonies in these plates. If there are no colonies recovered in the plate, record the number of colonies as < 1.

## A.2 Expression of results

Calculate the number of viable bacteria according to 12.1, judge the test effectiveness according to 12.2, calculate the antibacterial activity ratio according to 12.3.

# Annex B
(normative)

# Film contact method

## B.1 Preparation of the sample

### B.1.1 Test specimen

If available, a larger sample size should be used instead of the size mentioned in the procedure (10.2). The size of the test specimen shall be indicated in the test report.

NOTE The inoculum can spread over onto the Petri dish when placing the film on top of the small sample depending on the sample composition.

### B.1.2 Cover film

Match the size and shape of the cover film to the test or control samples. The test inoculum shall not leak beyond the edges of the film. The film shall be smaller than the sample. The actual size and shape used shall be stated in the test report.

The film should be made of PE, PP, PET which have no effect on the growing of bacteria. It should have a thickness of approximately 0,05 mm to 0,1 mm.

### B.1.3 Sterilization of samples (optional)

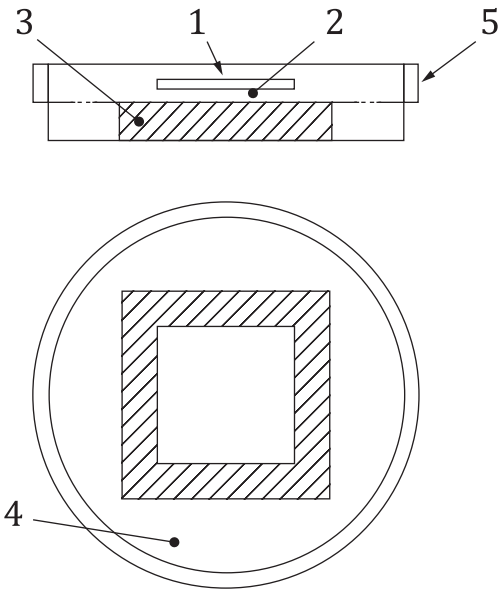
As an alternative to the procedure given in 10.3, the following procedure can be used. Wipe the surface of the control samples and test samples with 70 % ethanol (volume fraction, analytical grade). After 5 min, wash them with sterile distilled water and dry naturally. Samples which cannot be treated with ethanol (analytical grade) can be washed directly with sterile distilled water or sterilized by other methods without affecting the antibacterial activity and the experiment results.

## B.2 Test procedure

### B.2.1 Inoculation

Put six test samples and the six controls into the Petri dishes separately and keep the test surface facing upwards. Accurately pipette 0,1 ml to 0,2 ml of the bacteria suspension prepared in Clause 9 and drip it slowly onto the test surface. Grip the cover film (6.7) with a pair of sterile tongs and spread it on the test surface without any bubbles so that the inoculum can contact the sample evenly. Cover the dish. See Figure B.1.

If no control samples are available, inoculate the Petri dish without samples as a control to determine the test effectiveness.




**Key**

1 cover film
2 test inoculum
3 test sample
4 petri dish
5 cover of the dish

**Figure B.1 — Inoculation and the placement of the cover film**

### B.2.2 Elution after inoculation (time zero)

After the inoculation of the three test samples or controls, add 10 ml of SCDLP medium (7.7) into each Petri dish. Shake at 50 r/min by two-dimensional or three-dimensional shaker (6.9) for 5 min to elute out the bacteria from the test samples and controls.

### B.2.3 Incubation

Incubate three test samples and three controls at a temperature of (37 ± 2) °C and a relative humidity of (85 ± 5) % for (24 ± 1) h.

### B.2.4 Elution after incubation (time 24 h)

Proceed as in B.2.2.

### B.2.5 Determination of the number of viable bacteria

Determine the number of viable bacteria of each sample elution from B.2.2 or B.2.4 according to A.1.5.

## B.3 Expression of results

Calculate the number of viable bacteria according to 12.1, judge the test effectiveness according to 12.2, calculate the antibacterial activity ratio according to 12.3.