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Textiles — Determination of antibacterial activity of antibacterial finished products

Textiles — Détermination de l'activité antibactérienne des produits finis antibactériens

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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

ISO 20743 was prepared by Technical Committee ISO/TC 38, *Textiles*.

Introduction

These test methods were established in order to address the substantial need for an International Standard to determine antibacterial activity for antibacterial finished textile products.

Textiles — Determination of antibacterial activity of antibacterial finished products

1 Scope

This International Standard specifies quantitative test methods to determine the antibacterial activity of antibacterial finished textile products including nonwovens.

This International Standard is applicable to all textile products, including cloth, wadding, thread and material for clothing, home furnishings and miscellaneous goods regardless of the type of antibacterial agent used (organic, inorganic, natural or man-made) or the method of application (built-in, after-treatment or grafting).

Based on the intended application and on the environment in which the textile product is to be used, the user can select the most suitable of the following three methods on determination of antibacterial activity:

a) absorption method (an evaluation method in which test bacterial suspension is inoculated directly onto samples);

b) transfer method (an evaluation method in which test bacteria are placed on an agar plate and transferred onto samples);

c) printing method (an evaluation method in which test bacteria are placed on a filter and printed onto samples).

The colony plate count method and the ATP (ATP = Adenosine Tri-phosphate) luminescence method are also specified for measuring the enumeration of bacteria.

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.

ISO 6330, *Textiles — Domestic washing and drying procedures for textile testing*

3 Terms and definitions

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

3.1
textile
fabric
general term employed for designating textile surfaces, woven fabrics, knitted fabrics, etc., formed by the interlocking of textile materials having a certain cohesion and which are generally intended for clothing or furniture applications

NOTE Often includes certain types of nonwovens.

3.2
control fabric
fabric used to validate the growth condition of test bacteria

NOTE The same fabric as the fabric to be tested but without antibacterial treatment. If this is not possible, then 100 % cotton fabric without fluorescent brighteners or other finish.

3.3
antibacterial agent
product designed to prevent or mitigate the growth of bacteria, to reduce the number of bacteria or to kill bacteria

3.4
antibacterial finish
treatment designed to prevent or mitigate the growth of bacteria, to reduce the number of bacteria or to kill bacteria

3.5
antibacterial activity
activity of an antibacterial finish used to prevent or mitigate the growth of bacteria, to reduce the number of bacteria or to kill bacteria

3.6
plate count method
method in which the number of bacteria present after incubation is calculated by counting the number of colonies according to a ten-time dilution method

NOTE The results are expressed in “CFU (Colony Forming Unit)”.

3.7
luminescence method
method in which the amount of ATP contained in bacterial cells is measured

NOTE The results are expressed in “mol of ATP”.

3.8
neutralizer
chemical agents used to inactivate, neutralize, or quench the antibacterial properties of antibacterial agents

4 Safety precaution

Test methods specified herein require the use of bacteria.

These tests should be carried out by persons with training and experience in the use of microbiological techniques.

Appropriate safety precautions should be observed with due consideration given to country-specific regulations.

5 Apparatus

Usual laboratory apparatus and, in particular, the following.

5.1 Spectrophotometer, capable of measuring at a 620 nm to 660 nm wavelength, or **McFarland’s nephelometer**.

5.2 Incubator, capable of maintaining a constant temperature of 37 °C ± 2 °C.

5.3 Water baths, one capable of maintaining a constant temperature of 46 °C ± 2 °C and another capable of maintaining a temperature of 70 °C to 90 °C.

5.4 Mixer, producing a vortex shaking action.

5.5 Stomacher, capable of speeds of 6 blows/s to 8 blows/s, with the corresponding disposable containers.

5.6 Clean bench, for microbial test.

5.7 Washing machine, in accordance with the specifications of ISO 6330.

5.8 Humidity chamber, tropical chamber or other container capable of maintaining a high-humidity atmospheric condition.

5.9 Luminescence photometer, capable of measuring ATP of 10^{-13} mol/l to 10^{-7} mol/l at 300 nm to 650 nm with a luminescence-measuring reagent.

5.10 Printing apparatus, capable of applying a 4 N load to a test piece and rotating the piece 180° in one direction for a period of 3,0 s.

5.11 Refrigerator, capable of maintaining a temperature of between 2 °C and 8 °C.

5.12 Freezers, one adjustable to a temperature below −70 °C and another to a temperature below −20 °C.

5.13 Balance, which can be read to the nearest 0,01 g.

5.14 Filtering apparatus, consisting of an upper container equipped with a membrane filter and a lower container equipped with a suction opening.

5.15 Pipette, having the most suitable volume for each use, with a tip made of glass or plastic, and with a tolerance of 0,5 % or less.

5.16 Vials, 30 ml glass bottles, with screw openings, polytetrafluoroethylene or silicone packing and caps made of polypropylene, polycarbonate, or other suitable material.

5.17 Petri dishes, that have been sterilized, made of glass or plastic, in diameter sizes of 90 mm to 100 mm and 55 mm to 60 mm.

5.18 Glass rod, with a diameter of approximately 18 mm.

5.19 Anti-bumping granules (glass beads), with a diameter of 3 mm to 4 mm.

5.20 Erlenmeyer flask, capacity 100 ml.

5.21 Cutting template, made of a sterilizable material (stainless steel or glass) with a diameter of 3,8 cm ± 0,1 cm.

5.22 Disposable plastic bags, suitable for containing food products, to be used for storage of samples.

5.23 Tweezers, made of a material which can be sterilized.

5.24 Stainless steel cylinder, with a mass of 200 g ± 10 g and a diameter of 3,5 cm ± 0,1 cm.

5.25 Metal wire basket, for autoclaving.

5.26 Aluminium foil.

6 Reagents and culture media

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

Dehydrated products available on the commercial market are recommended for use in preparing the culture media. The manufacturer's instructions for the preparation of these products should be strictly followed.

6.1 **Water**.

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

6.2 **Tryptone soya broth (TSB)**.

This solution shall be used for the resuscitation of the freeze-dried bacterial strains.

Tryptone, pancreatic digest of casein	15 g
Soya peptone, papain digest of soya	5 g
Sodium chloride (NaCl)	5 g
Water	1 000 ml (final volume)
pH after sterilization	7,2 $\pm$ 0,2

6.3 **Tryptone soya agar (TSA)**.

Tryptone, pancreatic digest of casein	15 g
Soya peptone, papain digest of soya	5 g
Sodium chloride (NaCl)	5 g
Agar	15 g
Water	1 000 ml (final volume)
pH after sterilization	7,2 $\pm$ 0,2

6.4 **Agar for transfer**.

Tryptone, pancreatic digest of casein	0,75 g
Soya peptone, papain digest of soya	0,25 g
Sodium chloride (NaCl)	5 g
Agar	15 g
Water	1 000 ml (final volume)
pH after sterilization	7,2 $\pm$ 0,2

6.5 Nutrient broth (NB).

Beef extract	3 g
Peptone	5 g
Water	1 000 ml (final volume)
pH after sterilization	6,9 ± 0,2

6.6 Peptone-salt solution.

Tryptone, pancreatic digest of casein	1 g
Sodium chloride (NaCl)	8,5 g
Water	1 000 ml (final volume)
pH after sterilization	6,9 ± 0,2

6.7 Physiological saline.

Sodium chloride (NaCl)	8,5 g
Water	1 000 ml (final volume)

6.8 SCDLP medium.

Peptone, digest of casein	17 g
Peptone, digest of soybean	3 g
Sodium chloride (NaCl)	5 g
Potassium dihydrogenphosphate	2,5 g
Glucose	2,5 g
Lecithin	1 g
Polysorbate 80	7 g
Water	1 000 ml (final volume)
pH after sterilization	7,2 ± 0,2

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.

6.9 Dilution buffer for shake-out bacterial suspension.

This buffer solution consists of 0,005 mol/l sodium dihydrogenphosphate containing 0,037 % sucrose.

pH	7,2 ± 0,2

6.10 Neutralizing solution.

The composition of the standard neutralizing solution shall be as follows.

Polysorbate 80	30 g
Egg yolk lecithin	3 g
Histidine hydrochloride	1 g
Meat or casein peptone	1 g
Sodium chloride (NaCl)	4,3 g
Monopotassium phosphate	3,6 g
Disodium phosphate dihydrate	7,2 g
Water	1 000 ml (final volume)

When sufficient neutralizing power cannot be achieved, 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.

6.11 Enumeration agar (EA).

Dehydrated yeast extract	2,5 g
Casein tryptone	5,0 g
Glucose	1,0 g
Agar	12 g to 18 g (depending on the gel strength of the product)
Water	1 000 ml (final volume)
pH after sterilization	7,2 $\pm$ 0,2

6.12 Agar for printing.

Agar	20 g
Water	1 000 ml (final volume)

6.13 Cryoprotective solution for bacterial species.

For freezing, a cryoprotective solution containing 150 g/l of glycerol or 100 g/l of dimethylsulfoxide shall be used.

For solutions containing glycerol, prepare the nutritive solution and add 150 g of glycerol per litre prior to sterilizing.

For solutions containing dimethylsulfoxide, sterilize the dimethylsulfoxide by means of a filtering system equipped with a 0,22 µm membrane filter. Prepare the nutritive solution and, after sterilization, add 100 g of dimethylsulfoxide per litre.

NOTE Any commercially available product may be used as long as it is a cryoprotective solution or preserving system that contains glycerol or dimethylsulfoxide and allows preservation of the strains in the same manner as the specified solutions.

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

6.14 Stock solution of ATP standard reagent.

Adenosine-disodium 5'triphosphate trihydrate	59,7 mg
Water	1 000 ml (final volume)

After preparation, the solution shall be placed in a tightly sealed container and cryopreserved at a temperature of −20 °C or lower. The solution shall be used no later than 6 months from the date of preparation.

6.15 Buffer solution for ATP luminescence reagent.

Tris(hydroxymethyl)amino methane	760 mg
Ethylenediamine disodium tetraacetate dihydrate	370 mg
Magnesium acetate tetrahydrate	800 mg
DL-dithiothreitol	8 mg
Water	250 ml (final volume)
pH	7,5 ± 0,2

This solution shall be used no later than 8 h after preparation.

6.16 ATP luminescence reagent.

Luciferase (EC: 1.13.12.7)	10 mg
D-luciferin	15 mg
Bovine serum albumin	60 mg
Buffer solution of 6.15	30 ml (final volume)

After dissolving, the reagent shall be kept at room temperature for a minimum of 15 min before use and shall be used within 3 h of preparation.

6.17 ATP extracting reagent.

10 % aq. Benzalkonium chloride	1 ml
Water	9 ml

Use of any unspecified extracting agent and the recipe shall be recorded.

6.18 ATP eliminating agent.

An agent to reduce the ATP in nutrient broth to less than 10^{-13} mol/l within 15 min.

Apyrase (EC: 3.6.1.5)	5 international units
Adenosine deaminase (EC: 3.5.1.4)	5 international units
0,005 mol/l buffer solution of sodium dihydrogenphoshate containing 0,037 % sucrose	10 ml (final volume)
pH	7,2 ± 0,2

Use of any unspecified eliminating agent and the recipe shall be recorded.

6.19 SCDLP or other medium for preparing ATP reference solution.

SCDLP of 6.8 or other medium	10 ml
ATP eliminating agent of 6.18	1 ml

After mixing, maintain at 30 °C to 37 °C for 1 h to prevent microbiological contamination.

Next, transfer to a hot water bath at 70 °C to 90 °C for 1 h and cool down to room temperature.

Preserve the solution under refrigeration and use within 24 h.

An ATP reference solution should be prepared if the addition of neutralizing agents causes the ATP content in the shake-out solution to exceed 10^{-11} mol/l.

7 Reference strains

7.1 Strains

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

— *Staphylococcus aureus* ATCC 6538, CIP 4.83, DSM 799, NBRC 13276 or NCIMB 9518;

— *Klebsiella pneumoniae* ATCC 4352, CIP 104216, DSM 789, NBRC 13277 or NCIMB 10341.

NOTE ATCC is the American Type Culture Collection (USA); CIP is the Pasteur Institute Collection (France); DSM is the German Collection of Microorganism and Cell Cultures (Germany); NBRC is the NITE Biological Resource Center (Japan); and NCIMB is the National Collection of Industrial Bacteria (UK).

In lieu of the specified strains, equivalent bacteria strains obtained from agencies of the World Federation of Culture Collection (WFCC) may be used by agreement between the interested parties. The strains used in the test shall be stated in the test report together with their supply sources.

7.2 Storage of strains

7.2.1 General

The strains shall be stored in accordance with the supplier's recommendations or EN 12353.

The identification and origin (culture collection) of the strains as well as the laboratory storage method shall be recorded.

7.2.2 Ceramic bead method

Obtain a sample of the freeze-dried bacterial strain following the recommendations supplied with the culture and resuspend in 5 ml of TSB (6.2). Obtain a sample of the suspension and isolate it in a Petri dish (5.17) containing TSA (6.3). Incubate the cultures for 18 h to 24 h at 37 °C ± 2 °C.

After incubation, use the culture isolated in the Petri dish to verify the purity of the strain.

After verification, prepare the stock cultures.

Sample 0,7 ml of the broth culture and spread it over the surface of the Petri dish containing the TSA. Incubate the culture on plates for 18 h to 24 h under the conditions specified for the strain in the standard.

Add 10 ml of cryoprotective solution (6.13) to the surface of the TSA plate culture and resuspend the cells in the solution using a sterile glass spreader. Sample the suspended cells from the surface of the agar, dilute them in 100 ml of cryoprotective solution and incubate for 30 min at 20 °C.

Using a pipette (5.15), sample 1 ml of the suspension and transfer it to a cryogenic vial (5.16) containing the beads (5.19). Shake the vial in order to spread the suspended cells around the beads.

— Where a cryoprotective solution containing dimethylsulfoxide is used, do not let it stand longer than 1 min at ambient temperature.

— Where a cryoprotective solution containing glycerol is used, let it stand for 30 min at 20 °C.

— Withdraw the excess cryoprotective solution with a sterile pipette. Place the cryogenic vials in a freezer (5.12) set at −70 °C or lower.

Prepare 10^{-6} and 10^{-7} dilutions of the suspension using the serial dilution method. Take a 1,0 ml sample of each dilution and transfer it to separate Petri dishes. Add 12 ml to 15 ml of nutritive solution, cooled down to 45 °C ± 1 °C. Incubate for 18 h to 24 h under the conditions specified for the strain. Enumerate the plate cultures and confirm that the suspension contains less than 5×10^8 CFU/ml.

Store the cryogenic vials in a freezer at a temperature below −70 °C.

7.2.3 Glycerol suspension method

Inoculate a 15 ml culture tube containing 5 ml of appropriate medium with a freshly grown isolated colony. Incubate at 37 °C until the culture is in the late log or stationary phase (usually 5 h to overnight).

For each strain to be stored below −70 °C, for the archives, prepare a sterile, labelled cryogenic vial. Place 225 µl of sterile 80 % glycerol in a cryogenic vial. Add 1,0 ml of the bacterial culture (frozen stock shall be 15 % glycerol). Mix well using the vortex mixer (5.4) and store in a tube at −70 °C or lower.

For each strain to be stored at −20 °C, as liquid glycerol working stock, pipette equal volumes of 80 % glycerol and bacterial culture into a labelled polypropylene tube. Mix the contents well to avoid formation of ice crystals that will decrease the viability of the cells. Place the tube in a freezer at −20 °C. Check the viability of the cells after 1 week if possible.

To recover a strain from the below −70 °C glycerol stock, use a sterile toothpick to scrape pieces of the solid substance, then streak the cells onto the appropriate medium. Do not thaw the frozen stock because each freeze-thaw cycle will result in a 50 % loss in cell viability.

To use the −20 °C working stock, pipette 50 µl to 100 µl as inoculum for a 5 ml overnight culture.

7.2.4 Remarks

The identity of the strain shall be verified in accordance with commonly accepted identification methods.

For each microbial strain, the following information shall be recorded:

a) the name of the national culture collection from which the freeze-dried strain was obtained;

b) the taxonomic name and reference number of the freeze-dried strain;

c) the batch number of the freeze-dried sample obtained from the culture collection;

d) the date of the resuscitated freeze-dried sample;

e) the date of prepared stock culture;

f) the stock culture laboratory code.

Details of the methods used for verifying the purity and identity of the strain and dates on which the verifications were conducted, and the results obtained, shall also be recorded.

8 Quantitative measurement

8.1 Plate count method

8.1.1 Take 1 ml of the inoculum using a pipette (5.15), add it to a test tube containing 9,0 ml $\pm$ 0,1 ml of the nutrient broth (6.5) or the peptone-salt solution (6.6) and shake well.

8.1.2 Take 1 ml of this solution using a new pipette, add it to a separate test tube containing 9,0 ml $\pm$ 0,1 ml of the medium and shake well. Repeat the procedure successively and prepare a dilution series so that the dilutions are undertaken ten times in total. Ensure that 1 ml of each dilution is pipetted into two Petri dishes each.

8.1.3 Warm approximately 15 ml of EA (6.11) to a temperature of 45 °C to 46 °C using a water bath (5.3), add to the dishes and mix well. Maintain at room temperature and, when the medium solidifies, turn the dishes upside down and incubate at 37 °C $\pm$ 2 °C for 24 h to 48 h.

8.1.4 After incubation, count the number of colonies on the Petri dishes of dilution series on which 30 CFU to 300 CFU have appeared, and obtain the bacteria concentration in the solution according to the following formula:

$$c_B = Z \cdot R$$

where

c_B is the bacteria concentration, in Colony Forming Units per millilitre (CFU/ml);

Z is the average value, in Colony Forming Units (CFU), in the two Petri dishes;

R is the dilution rate.

8.2 Luminescence method

8.2.1 Calibration curve formula

8.2.1.1 Take the standard ATP reagent (6.14), and the physiological saline (6.7), the SCDLP medium (6.8) or another suitable medium, and prepare three separate solutions diluted by 2×10^{-8} mol/l, 2×10^{-9} mol/l and 2×10^{-10} mol/l, respectively.

8.2.1.2 Pour 0,1 ml of each solution specified above into three separate test tubes. Add 0,9 ml of the dilution buffer (6.9) to each tube and shake amply. Pour 0,1 ml of each into three separate test tubes and designate them as samples for measuring the diluted standard reagent.

8.2.1.3 Pour 0,1 ml of physiological saline (6.7), SCDLP (6.8) or other suitable medium, 0,8 ml of the dilution buffer (6.9), and 0,1 ml of ATP eliminating agent (6.18) into a plastic test tube. Shake amply and pour 0,1 ml portions into three separate test tubes. Let them stand for 5 min to 30 min and designate them as samples for measuring the blank.

8.2.1.4 Add to a test tube containing the sample for measuring the blank, 0,1 ml of ATP extracting reagent (6.17) and shake. Add 0,1 ml of ATP luminescence reagent (6.16), shake again and immediately apply a luminescence photometer (5.9) to determine the quantities of luminescence.

8.2.1.5 Add to the samples for measuring the diluted standard reagent, 0,1 ml portions of ATP extracting reagent (6.17) in order, starting from the lowest concentration, and shake. Then add 0,1 ml of ATP luminescence reagent (6.16), shake again and immediately apply a luminescence photometer (5.9) to determine the quantities of luminescence.

8.2.1.6 Divide the ATP concentration by the average of the quantity of luminescence obtained from the measurement of diluted standard reagent (2×10^{-8} mol/l, 2×10^{-9} mol/l and 2×10^{-10} mol/l), and record this value as the average value coefficient A.

8.2.1.7 Obtain coefficient B by substituting coefficient A, and $c_{ATP} = 0$ into the following calibration curve formula:

$$c_{ATP} = AX + B$$

where

c_{ATP} is the ATP concentration, in moles per litre (mol/l);

X is the quantity of luminescence, in Relative Light Units (RLU).

NOTE The correlation coefficient between c_{ATP} and X is $R^2 \geqslant 0{,}9$ and the confidence level is $> 0{,}95$.

8.2.2 ATP concentration of the bacterial suspension

8.2.2.1 Prepare one test tube for the ATP eliminating treatment and three test tubes for measuring.

8.2.2.2 Pour 0,1 ml of the shake-out bacterial suspension, 0,8 ml of the dilution buffer (6.9) and 0,1 ml of the ATP eliminating agent (6.18) into the test tube designated for the ATP eliminating treatment and shake amply. Pour 0,1 ml portions of the solution into the three test tubes designated for measuring and let them stand for 5 min to 30 min.

8.2.2.3 Add 0,1 ml of the ATP extracting reagent (6.17) to each measuring test tube and shake amply. Add 0,1 ml of the ATP luminescence reagent (6.16), shake again and immediately apply a luminescence photometer (5.9) to determine the quantities of luminescence.

8.2.2.4 Obtain the ATP concentration, c_{ATP}, according to the formula specified in 8.2.1.7 and obtain the concentration of ATP in the shake-out bacterial suspension according to the following formula:

$$c_{ATP'} = c_{ATP}/1\ 000$$

where $c_{ATP'}$ is the ATP concentration, in moles per millitre (mol/ml), in the shake-out bacterial suspension.

9 Shaking method

9.1 Shaking by vortex mixer

Press the bottom portion of the test tubes or bottles on the plate or rubber holder of the vortex mixer (5.4) and mix for 5 s × 5 cycles.

9.2 Shaking by hand

Take the test tube or bottle by hand and shake in an arc of approximately 30 cm for 30 s.

9.3 Shaking by Stomacher

Place the designated disposable bag (5.22) in the Stomacher machine (5.5) and run the machine for 1 min on each face of the bag:

10 Test procedures

10.1 Absorption method

10.1.1 Incubation and preparation of test inoculum

10.1.1.1 Incubation A

Pick up the preserved stock bacteria from the storage container using an inoculating loop. Streak onto the plate of EA (6.11) and incubate at 37 °C ± 2 °C for 24 h to 48 h.

NOTE The plate is kept at 5 °C to 10 °C and used within 1 week from the date of preparation.

10.1.1.2 Incubation B

Place 20 ml of the nutrient broth (6.5) or the TSB (6.2) into a 100 ml Erlenmeyer flask. Apply an inoculating loop to pick one colony up from the incubation A as specified in 10.1.1.1 and inoculate it in the broth. Incubate under the following conditions:

Temperature: 37 °C ± 2 °C

Rate of shaking: 110 min^{-1} and 3 cm width

Incubation time: 18 h to 24 h

10.1.1.3 Incubation C

Place 20 ml of the nutrient broth (6.5) or the TSB (6.2) into a 100 ml Erlenmeyer flask. Add 0,4 ml of the inoculum from the incubation B as specified in 10.1.1.2 that contains 1×10^8 CFU/ml to 3×10^8 CFU/ml in

bacteria concentration or an ATP concentration of 1×10^{-6} mol/l to 3×10^{-6} mol/l to the flask and incubate under the following conditions:

Temperature: 37 °C ± 2 °C

Rate of shaking: 110 min^{-1} and 3 cm width

Incubation time: 3 h ± 1 h

Target CFU or ATP concentration after incubation: 10^7 CFU/ml or 10^{-7} mol/l.

NOTE The prepared inoculum is preserved by ice-cooling and used within 8 h.

10.1.1.4 Preparation of test inoculum

Adjust the bacteria to a concentration of 1×10^5 CFU/ml to 3×10^5 CFU/ml by spectrophotometer or McFarland's nephelometer (5.1) or adjust the ATP to a concentration of 1×10^{-9} mol/l to 3×10^{-9} mol/l by the luminescence method using the nutrient broth (6.5) after it has been diluted 20 times with water at room temperature.

NOTE The prepared inoculum is preserved by ice-cooling and used within 4 h.

10.1.2 Preparation of test samples

10.1.2.1 Mass and shape of test pieces

Obtain test pieces with a mass of 0,40 g ± 0,05 g and cut to a size suitable for test samples. Obtain six test samples of control fabric and six test samples of antibacterial treatment.

NOTE Three of the control fabric samples and three of the antibacterial-treated samples are used for zero time, immediately after inoculation. The remaining samples are used for contact time, after 18 h to 24 h incubation.

10.1.2.2 Setting the test sample

Place each of the test samples into separate vials by selecting the following method appropriate to the nature of the test sample.

a) If the test sample is a fabric that tends to curl easily, or if it contains wadding or down, place a glass rod (5.18) onto the sample in the vial. Alternatively, secure both ends of the test sample with thread.

b) If the test sample is yarn, arrange the yarn in a bundle and place a glass rod onto the sample in the vial.

c) If the test sample is a carpet or of similar construction, cut the pile and place a glass rod onto the sample in the vial.

When necessary, test samples may be washed in accordance with ISO 6330 or another suitable method, and after the final washing, the samples are rinsed with water to eliminate the washing detergent. Use of an unspecified method shall be recorded.

When necessary, sterilize test samples by autoclave according to the following procedure.

1) Cover the upper portion of the vials containing the test samples with aluminium foil (5.26).

2) Place the covered vials in a metal wire basket (5.25) for autoclaving.

3) Wrap the vial caps with aluminium foil and place them into the wire basket.

4) Sterilize the caps and the vials containing the test samples by autoclave at 121 °C and 103 kPa for 15 min to 20 min.

5) After sterilization, remove the aluminium foil and allow the test samples in the vials to dry for 60 min or more by placing them on a clean bench (5.6) or any other place where there is no risk of airborne contamination.

6) Securely tighten the vial caps.

When autoclaving is not possible, sterilization may be accomplished by ethylene oxide gas, γ-ray or another suitable method. Use of alternative methods shall be recorded.

10.1.3 Test operation

10.1.3.1 Inoculation of test samples

Accurately pipette 0,2 ml of the inoculum prepared in 10.1.1.4 at several points on each test sample prepared in 10.1.2.2 to ensure that no inoculum touches the surface of the vial and tighten the cap.

10.1.3.2 Shake-out after inoculation

Immediately after the inoculation of 10.1.3.1, add 20 ml of SCDLP medium (6.8) into each of the six vials in which a control fabric sample and an antibacterial-treated test sample have been placed, tighten the caps, and shake-out as specified in Clause 9.

10.1.3.3 Incubation

Incubate the vials (three test samples of control fabric, three treated test samples) at 37 °C $\pm$ 2 °C for 18 h to 24 h.

10.1.3.4 Shake-out after incubation

After the incubation of 10.1.3.3, add 20 ml of SCDLP medium (6.8) into each of the vials, tightly secure the caps, and shake-out as specified in Clause 9.

10.1.3.5 Calculation of number of bacteria or amount of ATP

10.1.3.5.1 General

Obtain the number of bacteria or amount of the ATP as specified in 10.1.3.2 and 10.1.3.4 from the bacteria concentration or the ATP concentration obtained in Clause 8 according to the following formula:

10.1.3.5.2 Number of bacteria

$$M = c_B \times 20$$

where

M is the number of bacteria per specimen;

c_B is the bacteria concentration obtained in 8.1;

20 is the volume of the shake-out solution, in millitres (ml).

10.1.3.5.3 Amount of ATP

$$M' = c_{ATP'} \times 20$$

where

M' is the amount of ATP per specimen;

$c_{ATP'}$ is the ATP concentration obtained in 8.2;

20 is the volume of the shake-out solution, in millilitres (ml).

10.1.4 Test results

10.1.4.1 Judgement of test effectiveness

When a), b) and c) or a), b) and d) are satisfied, the test is judged to be effective. When the test is judged to be ineffective, a retest shall be carried out.

a) The test inoculum of 10.1.1.4 shall be 1×10^5 CFU/ml to 3×10^5 CFU/ml or the ATP concentration shall be 1×10^{-9} mol/l to 3×10^{-9} mol/l.

b) The difference in extremes for the three control fabrics immediately after inoculation and after incubation respectively shall be lg $\leqslant$ 1.

c) The growth value obtained according to the following formula shall be $\geqslant$ 1,0 in the plate count method.

d) The growth value obtained according to the following formula shall be $\geqslant$ 0,5 in the luminescence method.

$$F = \lg C_t - \lg C_o$$

where

F is the growth value on the control fabric;

$\lg C_t$ is the average common logarithm for the number of bacteria, or the average amount of ATP, obtained from three test samples of control fabric after an 18 h to 24 h incubation;

$\lg C_o$ is the average common logarithm for the number of bacteria, or the average amount of ATP, obtained from three test samples of control fabric immediately after inoculation.

10.1.4.2 Calculation of antibacterial activity value

When the test has been judged to be effective, obtain the antibacterial activity value according to the following formula:

$$A = (\lg C_t - \lg C_o) - (\lg T_t - \lg T_o) = F - G$$

where

A is the antibacterial activity value;

F is the growth value on the control fabric ($F = \lg C_t - \lg C_o$);

G is the growth value on the antibacterial-treated sample ($G = \lg T_t - \lg T_o$);

lg T_t is the average common logarithm for the number of bacteria, or the average amount of ATP, obtained from three antibacterial-treated test samples after an 18 h to 24 h incubation;

lg T_o is the average common logarithm for the number of bacteria, or the average amount of ATP, obtained from three antibacterial-treated test samples immediately after inoculation.

10.1.4.3 Record of test results

Records shall include the name of the test bacteria, strain number, concentration of inoculum, antibacterial activity value, method for measuring the number of bacteria and type of sample (refer to the examples in Tables 1 and 2).

Also record the names and concentration of any special neutralizers used for the shake-out solution.

Table 1 — Example 1: Test results (antibacterial activity value by absorption method)

Name of test bacteria (strain number)	***Staphylococcus aureus*** (ATCC 6538)		***Klebsiella pneumoniae*** (ATCC 4352)	
Concentration of inoculum (CFU/ml)	$1{,}2 \times 10^5$		$1{,}1 \times 10^5$	
Difference of extremes for three control fabrics (lg)	0 h	20 h	0 h	20 h
	0,4	0,3	0,5	0,4
Growth value of F (F = lg C_t – lg C_o)	+2,7 (lg C_t: +7,0, lg C_o: +4,3)		+3,2 (lg C_t: +7,5, lg C_o: +4,3)	
Growth value of G (G = lg T_t – lg T_o)	–1,0 (lg T_t:+3,2, lg T_o: +4,2)		+0,7 (lg T_t: +4,9, lg T_o: +4,2)	
Antibacterial activity value (A = $F - G$)	3,7		2,5	
Measuring method	Plate count method			
Type of sample material	Socks Cotton 100 %			
Sterilization method	Autoclave			
Incubation time	20 h			

Table 2 — Example 2: Test results (antibacterial activity value by absorption method)

Name of test bacteria (strain number)	***Staphylococcus aureus*** (ATCC 6538)		***Klebsiella pneumoniae*** (ATCC 4352)	
Concentration of inoculum (mol/l)	$1{,}2 \times 10^{-9}$		$1{,}9 \times 10^{-9}$	
Difference of extremes for three control fabrics (lg)	0 h	20 h	0 h	20 h
	0,5	0,6	0,5	0,4
Growth value of F (F = lg C_t – lg C_o)	+1,7 (lg C_t: –11,0, lg C_o: –12,7)		+2,1 (lg C_t: –10,4, lg C_o: –12,5)	
Growth value of G (G = lg T_t – lg T_o)	–0,8 (lg T_t: –13,6, lg T_o: –12,8)		–0,1 (lg T_t: –12,6, lg T_o: –12,5)	
Antibacterial activity value ($A = F - G$)	2,5		2,2	
Measuring method	Luminescence method			
Type of sample material	Curtain Polyester 100 %			
Sterilization method	Ethylene oxide gas			
Incubation time	20 h			

10.2 Transfer method

10.2.1 Preparation of test inoculum

10.2.1.1 Incubation of test strain

Obtain the strain preserved as stock culture using an inoculating loop, streak onto the plate of TSA (6.3) and incubate at 37 °C ± 2 °C for 18 h to 24 h. After incubation, extract a colony from the plate, streak onto another plate of TSA and incubate at 37 °C ± 2 °C for 18 h to 24 h.

NOTE The second transfer constitutes the working culture(s).

When inoculation cannot be completed within a single day, a 48-h culture may be used for the subsequent inoculation, provided that the culture is stored in an incubator (5.2) for 48 h. In this event, a new 24-h subculture shall be prepared prior to performing the test. A fourth subculture shall not be used.

10.2.1.2 Preparation of test inoculum

Obtain a colony from the second transferred TSA using an inoculating loop, place in the Peptone-salt solution (6.6) and mix well by vortex mixer (5.4). Adjust the number of bacteria to a concentration of 1×10^8 CFU/ml to 3×10^8 CFU/ml or an ATP concentration of 2×10^{-7} mol/l to 6×10^{-7} mol/l using the Peptone-salt solution (6.6) by the absorbance or luminescence method. Dilute the inoculum to a concentration of 1×10^6 CFU/ml to 3×10^6 CFU/ml or an ATP concentration of 2×10^{-9} mol/l to 6×10^{-9} mol/l using the Peptone-salt solution (6.6). The final number of bacteria should be checked by the method as specified in Clause 8.

10.2.2 Preparation of test samples

Using a template (5.21), cut test samples with 3,8 cm diameters.

The test samples should not contain any seams, selvedges, embroidery, fasteners, etc.

There should be a sufficient number of test samples to allow for repeat tests, be a minimum of 0,5 m^2, be from the same batch and be free from selvedges or stemming.

When necessary, test samples may be washed in accordance with ISO 6330 or another suitable method, and after the final washing, the samples are rinsed with water to eliminate the washing detergent. Use of an unspecified method shall be recorded.

When necessary, test samples may be sterilized by autoclave, ethylene oxide gas, *γ*-ray or any other suitable method. Use of an unspecified method shall be recorded.

10.2.3 Test operation

10.2.3.1 Inoculation to agar plates

Prepare 12 plates of the agar for transfer (6.4). Inoculate 1 ml of the test inoculum of 10.2.1.2 on the agar, inclining the plate in several directions so as to completely flood the surface of the plate. Suck up as much of the excess liquid as possible. Let stand for 300 s ± 30 s.

10.2.3.2 Transfer to samples

Prepare three test samples of control fabric and three test samples of antibacterial treatment for use immediately after transfer and after incubation respectively. Set each sample on the agar surface of 10.2.3.1 and weigh down with a 200 g stainless steel cylinder (5.24) for 60 s ± 5 s. Place each sample in a 55 mm to 60 mm diameter Petri dish (5.17) with the transferred surface face up. Incubate in a humidity chamber (5.8) for 18 h to 24 h at 37 °C ± 2 °C.

10.2.3.3 Shake-out after transfer

Immediately after transfer, place each sample in a sterile bag or a vial containing 20 ml of the neutralizing solution (6.10) and shake out as specified in Clause 9.

10.2.3.4 Shake-out after incubation

After incubation, place each sample in a sterile bag or a vial containing 20 ml of the neutralizing solution (6.10) and shake out as specified in Clause 9.

10.2.3.5 Calculation of number of bacteria or amount of ATP

10.2.3.5.1 General

Obtain the number of bacteria or the amount of ATP from 10.2.3.3 and 10.2.3.4 from the bacteria concentration or ATP concentration obtained in Clause 8 according to the following formulae.

10.2.3.5.2 Number of bacteria

$$M = c_B \times 20$$

where

M is the number of bacteria per specimen;

c_B is the bacteria concentration obtained in 8.1;

20 is the volume of the shake-out solution, in millitres (ml).

10.2.3.5.3 Amount of ATP

$$M' = c_{ATP'} \times 20$$

where

M' is the amount of ATP per specimen;

$c_{ATP'}$ is the ATP concentration obtained in 8.2;

20 is the volume of the shake-out solution, in millilitres (ml).

10.2.4 Test results

10.2.4.1 Judgement of test effectiveness

When a), b) and c) or a), b) and d) are satisfied, the test is judged to be effective. When the test is judged to be ineffective, a retest shall be carried out.

a) The test inoculum of 10.2.1.2 shall be a concentration of 1×10^6 CFU/ml to 3×10^6 CFU/ml or the ATP concentration shall be 2×10^{-9} mol/l to 6×10^{-9} mol/l.

b) The difference in extremes for the three control fabrics immediately after transfer and after incubation respectively shall be lg $\leqslant$1.

c) The growth value obtained according to the following formula shall be $\geqslant$ 1,0 in the plate count method.

d) The growth value obtained according to the following formula shall be $\geqslant$ 0,5 in the luminescence method.

$$F = \lg C_t - \lg C_o$$

where

F is the growth value on the control fabric;

$\lg C_t$ is the average common logarithm for the number of bacteria or for the average amount of ATP, obtained from three test samples of control fabric after an 18 h to 24 h incubation;

$\lg C_o$ is the average common logarithm for the number of bacteria or for the average amount of ATP, obtained from three test samples of control fabric immediately after transfer to the control fabric.

10.2.4.2 Calculation of antibacterial activity value

When the test has been judged to be effective, obtain the antibacterial activity value according to the following formula:

$$A = (\lg C_t - \lg C_o) - (\lg T_t - \lg T_o) = F - G$$

where

A is the antibacterial activity value;

F is the growth value on the control fabric ($F = \lg C_t - \lg C_o$);

G is the growth value on the antibacterial-treated sample ($G = \lg T_t - \lg T_o$);

lg T_t is the average common logarithm for the number of bacteria or for the average amount of ATP, obtained from three antibacterial-treated test samples after an 18 h to 24 h incubation;

lg T_o is the average common logarithm for the number of bacteria or for the average amount of ATP, obtained from three antibacterial-treated test samples immediately after transfer.

10.2.4.3 Record of test results

Records shall include the name of the test bacteria, strain number, transferred bacteria number, antibacterial activity value, method for measuring the number of bacteria and type of sample (refer to the examples in Tables 3 and 4).

The names and concentration of any special neutralizers used for the shake-out solution are also recorded.

Table 3 — Example 1: Test result (antibacterial activity value by transfer method)

Name of test bacteria (strain number)	***Staphylococcus aureus*** (ATCC 6538)		***Klebsiella pneumoniae*** (ATCC 4352)	
Concentration of inoculum (CFU/ml)	$2{,}2 \times 10^6$		$1{,}1 \times 10^6$	
Difference of extremes for three control fabrics (lg)	0 h	22 h	0 h	22 h
	0,3	0,4	0,2	0,3
Growth value of F (F = lg C_t – lg C_o)	+2,9 (lg C_t: +8,5, lg C_o: +5,6)		+2,7 (lg C_t: +8,1, lg C_o: +5,4)	
Growth value of G (G = lg T_t – lg T_o)	–1,7 (lg T_t: +3,8, lg T_o: +5,5)		–2,5 (lg T_t: +2,8, lg T_o: +5,3)	
Antibacterial activity value ($A = F - G$)	4,6		5,2	
Measuring method	Plate count method			
Type of sample material	Socks Cotton 100 %			
Sterilization method	Autoclave			
Incubation time	**22 h**			

Table 4 — Example 2: Test results (antibacterial activity value by transfer method)

Number of test bacteria (strain number)	***Staphylococcus aureus*** (ATCC 6538)		***Klebsiella pneumoniae*** (ATCC 4352)	
Concentration of inoculum (mol/l)	$3{,}2 \times 10^{-9}$		$4{,}9 \times 10^{-9}$	
Difference of extremes for three control fabrics (lg)	0 h	22 h	0 h	22 h
	0,5	0,6	0,4	0,3
Growth value of F (F = lg C_t – lg C_o)	+3,1 (lg C_t: –9,1, lg C_o: –12,2)		+2,6 (lg C_t: –9,4, lg C_o: –12,0)	
Growth value of G (G = lg T_t – lg T_o)	–2,2 (lg T_t: –14,5, lg T_o: –12,3)		–2,2 (lg T_t: –14,1, lg T_o: –11,9)	
Antibacterial activity value (A = $F - G$)	5,3		4,8	
Measuring method	Luminescence method			
Type of sample material	Curtain Polyester 100 %			
Type of neutralizing agent added in 6.10 Concentration	Egg yolk lecithin 0,5 % of the total volume			
Sterilization method	*γ*-ray			
Incubation time	**22 h**			

10.3 Printing method

10.3.1 Incubation and preparation of test inoculum

10.3.1.1 Incubation A

Obtain the strain preserved as stock culture using an inoculating loop, streak onto the plate of the EA (6.11) and incubate at 37 °C ± 2 °C for 24 h to 48 h.

The plate is kept at 5 °C to 10 °C and should be used within 1 week from the date of preparation.

10.3.1.2 Incubation B

Place 20 ml of the nutrient broth (6.5) into a 100 ml Erlenmeyer flask. Apply an inoculating loop to pick one colony up from the incubation A as specified in 10.3.1.1 and inoculate it in the broth. Incubate under the following conditions:

Temperature: 37 °C ± 2 °C

Rate of shaking: 110 min^{-1} and 3 cm width

Incubation time: 18 h to 24 h

10.3.1.3 Incubation C

Place 20 ml of the nutrient broth (6.5) into a 100 ml Erlenmeyer flask. Add 0,4 ml of the inoculum from the incubation B as specified in 10.3.1.2 that contains 1×10^8 CFU/ml to 3×10^8 CFU/ml in bacteria concentration or an ATP concentration of 1×10^{-6} mol/l to 3×10^{-6} mol/l to the flask and incubate under the following conditions:

Temperature: 37 °C ± 2°C

Rate of shaking: 110 min^{-1} and 3 cm width

Incubation time: 3 h ± 1 h

Target CFU or ATP concentration after incubation: 10^7 CFU/ml or 10^{-7} mol/l

NOTE The prepared inoculum is preserved by ice cooling and used within 8 h.

10.3.1.4 Preparation of test inoculum

Adjust the inoculum to a concentration of 1×10^7 CFU/ml to 3×10^7 CFU/ml by the absorbance method or an ATP concentration of 1×10^{-7} mol/l to 3×10^{-7} mol/l by the luminescence method using a 20 times dilution with the nutrient broth (6.5) at room temperature.

NOTE The prepared inoculum is preserved by ice cooling and used within 4 h.

10.3.2 Pretreatment of test sample

10.3.2.1 Sampling

Obtain six test samples each of control fabric and antibacterial treatment cut to 60 mm squares.

When necessary, test samples may be washed in accordance with ISO 6330 or another suitable method, and after the final washing, the samples are rinsed with water to eliminate the washing detergent. Use of an unspecified method shall be recorded.

NOTE Three of the control fabric samples and three of the antibacterial-treated samples are used to measure the number of bacteria immediately after the printing of test bacteria. The remaining samples are used to measure the number of bacteria after the incubation test.

10.3.2.2 Sterilization of test samples

Place the samples in a Petri dish (5.17), cover with aluminium foil and sterilize by autoclave at 121 °C and 103 kPa for 15 min to 20 min. After sterilizing, remove the foil, place on a clean bench (5.6) or any other places where there is no risk of airborne contamination and dry for 60 min or more.

When autoclaving is not possible, sterilization may be accomplished by ethylene oxide gas, γ-ray or another suitable method. Use of alternative methods shall be recorded.

10.3.2.3 Humidity regulation of test samples

Pour 10 ml of the agar for printing (6.12) into a Petri dish (5.17). Place the uncovered dish on a clean bench (5.6) to cool and solidify. Cool to room temperature avoiding dew formation. When the agar solidifies, turn the dish upside down. Place the sample on the inside of the dish lid and regulate the humidity of the sample for 18 h to 24 h.

10.3.3 Test operation

10.3.3.1 Filtering of test bacteria

Set a membrane filter on a filtering apparatus sterilized by autoclave on a clean bench (5.6).

Pour 5 ml of the nutrient broth (6.5) after it has been diluted 20 times on the membrane filter, add 2 ml of the test inoculum prepared in 10.3.1.4, and filtrate under aspiration. Continue the aspiration for approximately 1 min after the liquid on the membrane filter disappears.

NOTE 1 The sterilization for the membrane filter is not carried out because the pore size is changed by sterilization.

NOTE 2 A sintered glass or polytetrafluoroethylene-coated net is placed under the membrane filter when using the filtering apparatus. An aspirator, small air pump or other simple apparatus is used for aspiration.

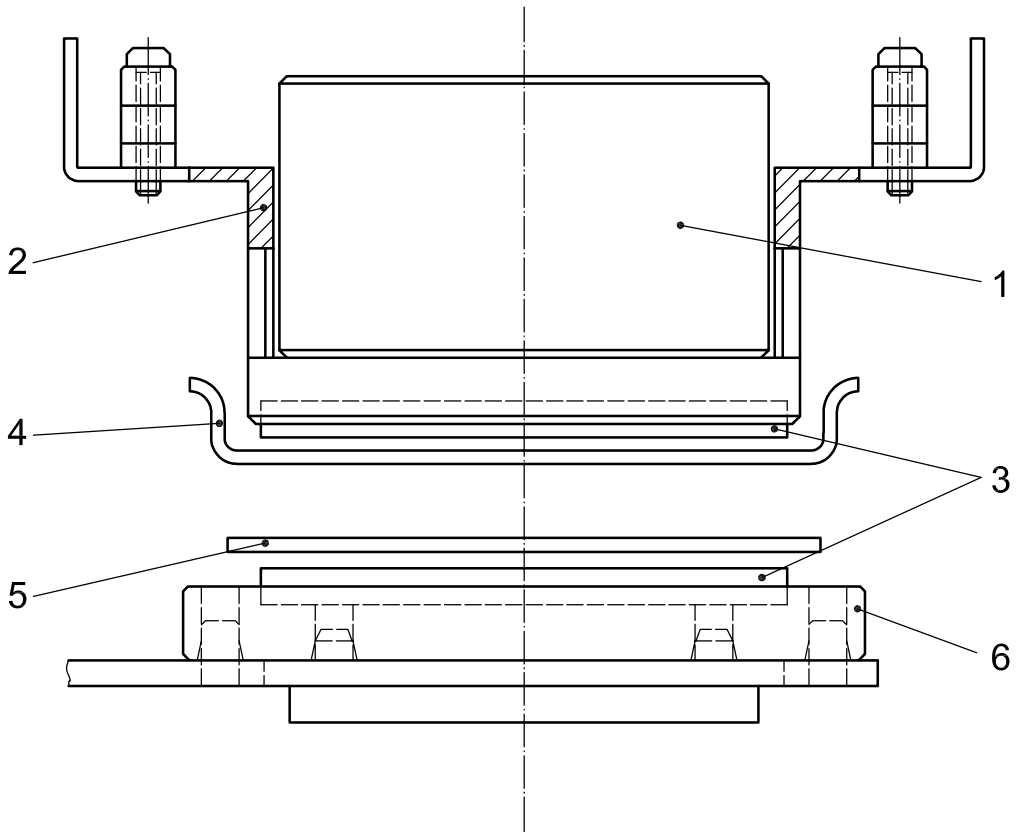
10.3.3.2 Printing of test bacteria

10.3.3.2.1 Remove the membrane filter collecting the test bacteria from the filtering apparatus using sterilized tweezers (5.23), place on the rotating table of the printing apparatus (5.10) with the bacteria facing upwards as shown in Figure 1.

10.3.3.2.2 Remove the sample of 10.3.2.3 from the Petri dish (5.17) using sterilized tweezers (5.23). Place on the membrane filter facing downwards.

10.3.3.2.3 Place the weight on the weighting plate, and print the bacteria on the membrane filter by rotating the table by 180° in one direction for 3,0 s.

10.3.3.2.4 Immediately after printing, transfer the sample to the lid for the Petri dish containing the agar of 10.3.2.3 with the printed surface facing upwards and let it stand for 1 h to 4 h in an incubator (5.2).



Key

1	weight (17 kg)	4	sample, (∅ 60 mm)
2	weighting plate	5	membrane filter, (∅ 47 mm)
3	silicone rubber, (∅ 42 mm)	6	turn plate, (∅ 54 mm) (anticlockwise rotation)

NOTE This figure illustrates the side of the printing apparatus (5.10).

External dimensions (height 170 mm, width 160 mm, depth 150 mm).

Figure 1 — Printing apparatus[1)]

1) This apparatus is available from e.g. Aloka Co., Ltd., 6-22-1, Mure, Mitaka-shi, Tokyo 181-8622, Japan. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

10.3.3.3 Incubation test

Incubate the printed sample in the Petri dish of 10.3.3.2.4 in an incubator (5.2) conditioned at 20 °C ± 2 °C and 70 % RH or more for a period of 1,0 h ± 0,1 h, 2,0 h ± 0,1 h, 3,0 h ± 0,1 h, or 4,0 h ± 0,1 h. (The incubation time is determined by specific test condition requirements.) Record the incubation time.

10.3.3.4 Shake-out after printing

Immediately after printing, transfer each sample of the control fabric of 10.3.3.2 to a vial containing 20 ml of the SCDLP medium (6.8), and shake out the bacteria from each sample as specified in Clause 9.

10.3.3.5 Shake-out after incubation

After incubation, transfer each sample of 10.3.3.3 to a vial containing 20 ml of the SCDLP medium (6.8), and shake out the bacteria from each sample as specified in Clause 9.

10.3.3.6 Calculation of number of bacteria or amount of ATP

10.3.3.6.1 General

Obtain the number of bacteria or amount of ATP from 10.3.3.4 and 10.3.3.5 from the bacteria concentration or ATP concentration obtained in Clause 8 according to the following formulae.

10.3.3.6.2 Number of bacteria

$$M = c_B \times 20$$

where

M is the number of bacteria per specimen;

c_B is the bacteria concentration obtained in 8.1;

20 is the volume of the shake-out solution, in millilitres (ml).

10.3.3.6.3 Amount of ATP

$$M' = c_{ATP'} \times 20$$

where

M' is the amount of ATP per specimen;

$c_{ATP'}$ is the ATP concentration obtained in 8.2;

20 is the volume of the shake-out solution, in millilitres (ml).

10.3.4 Test results

10.3.4.1 Judgement of test effectiveness

When the following two items are satisfied, the test is judged to be effective. When the test is judged to be ineffective, a retest shall be carried out.

a) The number of test bacteria or amount of ATP printed on the control fabric shall not be less than $1{,}0 \times 10^6$ CFU or $1{,}0 \times 10^{-11}$ mol.

b) The amount of increase or decrease on the control fabric, F, obtained according to the following formula, shall be +0,5 to −0,5.

$$F = \lg C_t - \lg C_o$$

where

F is the value of increase or decrease on the control fabric;

$\lg C_t$ is the average common logarithm for the number of bacteria, or the average amount of ATP, obtained from three test samples of control fabric after a 1 h to 4 h incubation;

$\lg C_o$ is the average common logarithm for the number of bacteria, or the average amount of ATP, obtained from three test samples of control fabric immediately after printing.

10.3.4.2 Calculation of antibacterial activity value

When the test is determined to be effective, obtain the antibacterial activity value according to the following formula:

$$A = \lg C_t - \lg T_t$$

where

A is the antibacterial activity value;

$\lg C_t$ is the average common logarithm for the number of bacteria or for the average amount of ATP, obtained from three test samples of control fabric after a 1 h to 4 h incubation;

$\lg T_t$ is the average common logarithm for the number of bacteria or for the average amount of ATP, obtained from three antibacterial-treated test samples after a 1 h to 4 h incubation.

10.3.4.3 Record of test results

Records shall include the name of the test bacteria, strain number, transferred bacteria number, antibacterial activity value, method for measuring the number of bacteria and type of sample (refer to the examples in Tables 5 and 6).

The names and concentration of any special neutralizers used for the shake-out solution are also recorded.

Table 5 — Example 1: Test results (antibacterial activity value by printing method)

Name of test bacteria (strain number)	***Staphylococcus aureus*** (ATCC 6538)	***Klebsiella pneumoniae*** (ATCC 4352)
Number of bacteria on filter (CFU)	$3{,}0 \times 10^7$	$3{,}6 \times 10^7$
Number of bacteria on control fabric immediately after printing (CFU)	$4{,}2 \times 10^6$	$6{,}4 \times 10^6$
Antibacterial activity value ($A = \lg C_t - \lg T_t$)	1,7 ($\lg C_t$: +6,7, $\lg T_t$: +5,0)	1,1 ($\lg C_t$: +6,7, $\lg T_t$: +5,6)
Measuring method	Plate count method	
Type of sample material	Curtain Polyester 100 %	
Sterilization method	Autoclave	
Incubation time	4 h	

Table 6 — Example 2: Test results (antibacterial activity value by printing method)

Name of test bacteria (strain number)	***Staphylococcus aureus*** (ATCC 6538)	***Klebsiella pneumoniae*** (ATCC 4352)
Amount of ATP on filter (mol)	$2{,}9 \times 10^{-10}$	$1{,}7 \times 10^{-10}$
Amount of ATP on control fabric immediately after printing (mol)	$4{,}0 \times 10^{-11}$	$3{,}0 \times 10^{-11}$
Antibacterial activity value ($A = \lg C_t - \lg T_t$)	1,7 ($\lg C_t$: −10,4, $\lg T_t$: −12,1)	1,1 ($\lg C_t$: −10,6, $\lg T_t$: −11,7)
Measuring method	Luminescence method	
Type of sample material	Curtain Polyester 100 %	
Sterilization method	Ethylene oxide gas	
Incubation time	2 h	

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2) Corrected and reprinted in 1995.

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