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Textiles — Determination of antifungal activity of textile products —

Part 2: Plate count method

Textiles — Détermination de l'activité antifongique des produits textiles —

Partie 2: Méthode par dénombrement sur plaque de gélose

Reference number
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Foreword

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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 documents 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).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 38, *Textiles*.

ISO 13629 consists of the following parts, under the general title *Textiles — Determination of antifungal activity of textile products*:

— *Part 1: Luminescence method*

— *Part 2: Plate count method*

Introduction

This part of ISO 13629 adopts the plate count method as a basis of quantitative determination of antifungal activity.

The following are characteristics of the plate count method:

— conventional method which is easy to operate in bacteriological laboratories;

— no need to use special apparatus such as a lumino photometer;

— long history and common procedure.

Textiles — Determination of antifungal activity of textile products —

Part 2:
Plate count method

1 Scope

This part of ISO 13629 specifies a test method for quantitative determination of antifungal activity by plate count method.

This part of ISO 13629 is applicable to various kinds of textile products such as fibres, yarns, fabrics, clothing, bedclothes, home furnishings, and other miscellaneous goods.

2 Normative reference

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 105-F02, *Textiles — Tests for colour fastness — Part F02: Specification for cotton and viscose adjacent fabrics*

ISO 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

3 Terms and definitions

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

3.1
control fabric
fabric used to validate the growth condition of test fungi

Note 1 to entry: Control specimens are sampled from the control fabric.

Note 2 to entry: The control fabric may be the same fabric as the fabric to be tested but without antifungal treatment. If this is not available, a 100 % cotton fabric without fluorescent brighteners or other finish, complying with the requirements of ISO 105-F02, is used as control fabric, after washing at the temperature of 60 °C without detergents or any brighteners, with mechanical agitation and rinsing.

3.2
antifungal agent
chemical agent to prevent or mitigate the growth of fungi or to reduce the number of fungi

3.3
antifungal treatment
treatment to prevent or mitigate the growth of fungi or to reduce the number of fungi

3.4
spore suspension
liquid with evenly dispersed fungal spores in sterilized water containing an anionic surfactant

3.5
plate count method
method in which the number of fungi present after incubation is calculated by counting the number of colonies according to a ten-time dilution method

Note 1 to entry: The results are expressed in CFU (Colony Forming Unit).

3.6
neutralizer
chemical agent used to inactivate, neutralize, or quench the antifungal properties of antifungal agents

4 Principle

A test specimen and a control specimen are inoculated with spore suspension of reference fungi and incubated at 30 °C for 48 h.

In this part of ISO 13629, fungal growth is quantitatively determined by the visual counting of colonies on the agar plate as CFU and the fungal activity is calculated by CFU.

In case the test specimen absorbs water, the absorption method is recommended. In case the test specimen does not absorb water, the transfer method is recommended.

5 Safety precaution

The test method specified herein requires use of fungi.

According to ISO 7218, this test shall be performed only by personnel with training and experience in microbiological techniques.

All regulations, rules, and recommendations regarding appropriate safety precautions in the country concerned may be consulted and followed.

6 Reference fungi

The fungi to be used shall be selected from Annex A, Table A.1.

The equivalent fungi types obtained from other agencies of the World Federation for Culture Collection (WFCC) shall be used as agreed upon between interested parties.

The strain number and supply source of the fungi used shall be stated in the test report.

7 Apparatus

Usual laboratory apparatuses and, in particular, the following apparatuses are used. When relevant, the items have to be sterilized before using.

7.1 **Gauze**, sterilized.

7.2 **Petri dish**, made of glass or plastic, with a diameter of about 60 mm or 90 mm.

7.3 **Autoclave**, capable of maintaining the temperature of (121 ± 2) °C (equivalent to 103 kPa).

7.4 **Platinum loop**, with a loop of 2 mm to 4 mm in diameter (or plastic equivalent).

7.5 **L-shaped platinum colony hook** (or plastic equivalent).

7.6 Incubator, capable of maintaining a temperature in a range from 25 °C to 37 °C with a tolerance of ±2 °C.

7.7 Vial, capacity of 30 ml screw-top glass vial with polytetrafluoroethylene or silicone gasket and polypropylene cap. It shall be carefully washed in alkaline or neutral detergent, rinsed, and dried.

7.8 Glass funnel.

7.9 Pipettes, capacity of 0,2 ml, 1 ml, 5 ml, and 10 ml with a tolerance of 0,5 % or less and with a tip made of glass or plastic.

7.10 Pasteur pipette, for microbiological testing (or plastic equivalent).

7.11 Conical flask, capacity of 100 ml to 500 ml.

7.12 Tweezers, made of material which can be sterilized.

7.13 Centrifuge, with centrifugal acceleration of approximate 2 000 × *g*.

7.14 Centrifuge tube, used for centrifuge.

7.15 Hemacytometer, capable of measuring 1×10^6 cells/ml to 3×10^6 cells/ml.

7.16 Microscope, capable of 200x magnification.

7.17 Ultrasonic cleaner, compact for experiment tools, with frequency of approximately 30 kHz to 50 kHz.

7.18 pH meter, with glass electrodes for biochemical testing or equivalent pH paper.

7.19 Erlenmeyer flask, capacity of 100 ml.

7.20 Cutting template, made of stainless steel with a diameter of (3,8 ± 0,1) cm.

7.21 Stainless steel cylinder, with a weight of (200 ± 10) g and a diameter of (3,5 ± 0,1) cm.

7.22 Shaker, capable of producing a Vortex shaking action.

7.23 Paddle blender [Stomacher-type], capable of speed 6 blows/s to 8 blows/s with the corresponding disposable containers.

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

7.25 Refrigerator, capable of maintaining a temperature of between 2 °C and 8 °C with a tolerance of ±2 °C.

7.26 Freezers, adjustable to a temperature below −70 °C and below −20 °C with a tolerance of ±2 °C.

7.27 Balance, capable of measuring 0,01 g as the readability.

7.28 Disposable plastic bags, suitable for containing food products to be used for the shake-out of sample.

7.29 Microbiological safety cabinet (MSC Type II), designed for microbiological tests use, or other system with equivalent performances.

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

8 Reagents and culture media

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

Dehydrated products available on the commercial market are recommended for use in preparing the culture media strictly in accordance with the manufacturer's instructions.

8.1 Pure water

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 fungi inhibitory substances.

8.2 Anionic surfactant

Dioctyl sodium sulfosuccinate to prepare spore suspension. The concentration of the anionic surfactant in pure water (8.1) is 50 mg/l. Sterilize this solution by an autoclave (7.3) at 121 °C for 20 min.

8.3 Culture medium

Use a culture medium prepared as described below. Commercially prepared items may be used after appropriate validation.

Culture media which will not be used immediately after preparation shall be stored at 5 °C to 10 °C and discarded after one month.

8.3.1 Sabouraud dextrose broth (SDB)

Peptone	10 g
Dextrose	20 g
Pure water	1 000 ml
pH after sterilization	5,6 ± 0,2

8.3.2 Potato dextrose agar (PDA)

Potato infusion from	200 g
Dextrose	20 g
Agar	15 g
Pure water	1 000 ml
pH after sterilization	5,6 ± 0,2

This medium encourages mould sporulation.

8.3.3 Sabouraud dextrose agar (SDA)

Pepsic meat peptone	10 g
Dextrose	40 g
Agar	15 g
Pure water	1 000 ml (final volume)

Follow the indications of the supplier.

pH after sterilization	5,6 ± 0,2

NOTE This medium will be used for the transfer method.

8.3.4 Slant culture

8.3.4.1 Pour approximately 10 ml of pre-heated and fully dissolved PDA (described in 8.3.2) into a sterilized test tube.

8.3.4.2 Put a cotton plug on and sterilize it with steam after sterilization.

8.3.4.3 Place the test tube at an approximately 15° angle against a level surface on a clean laboratory table, and leave the contents to solidify.

8.3.4.4 When there is no bleed water on the solidified agar, dissolve, and solidify it again for use.

8.3.5 Neutralizing solution, SCDLP medium

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 dehydrate	7,2 g
Water	1 000 ml (final volume)
pH after sterilization	7,2 ± 0,2

When sufficient neutralizing power cannot be achieved, the content of polysorbate 80 or lecithin may be adjusted or another neutralizing agent may be added. Commercial solutions of neutralizer can be used after having tested their efficacy (see Annex B). The use of any unspecified neutralizer shall be recorded along with the name and concentration.

9 Fungi preservation and use

9.1 Subculture the reference fungi and spores and handle the fungi and spores inside a safety cabinet (7.29) or other equivalent system.

9.2 Perform either flame sterilization or chemical sterilization on the cotton plugs and necks of the test tubes before and after subculture.

9.3 Scrape off a small amount of fungi from the original fungi, spread the spores over the bleed water at the bottom of the slant culture (8.3.4) and smear on to the top end of the slant culture either in a straight or wavy line (see Figure 1).

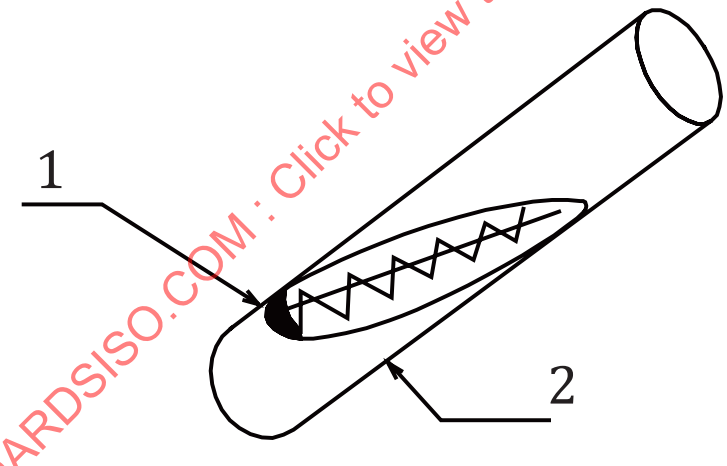
9.4 Use flame-sterilized platinum colony loop (7.4) and hook (7.5) every time when different types of fungi are subcultured.

9.5 Place the subcultured slant cultures in an incubator (7.6) at (25 ± 2) °C for at least 8 d, and confirm that sufficient spores have been grown before preserving them at 5 °C to 10 °C.

9.6 Transplant the subcultured fungi to new slant cultures for further incubation and preservation within three months.

Do not repeat more than five subcultures at intervals of up to three months. Do not use fungi over three months old for further subculture.

NOTE Long term preservation might be possible by freeze drying at –80 °C.



Key

1 bleed water

2 slant culture

Figure 1 — Subculture to a slant culture

10 Spore suspension

10.1 General

See Figure 2.

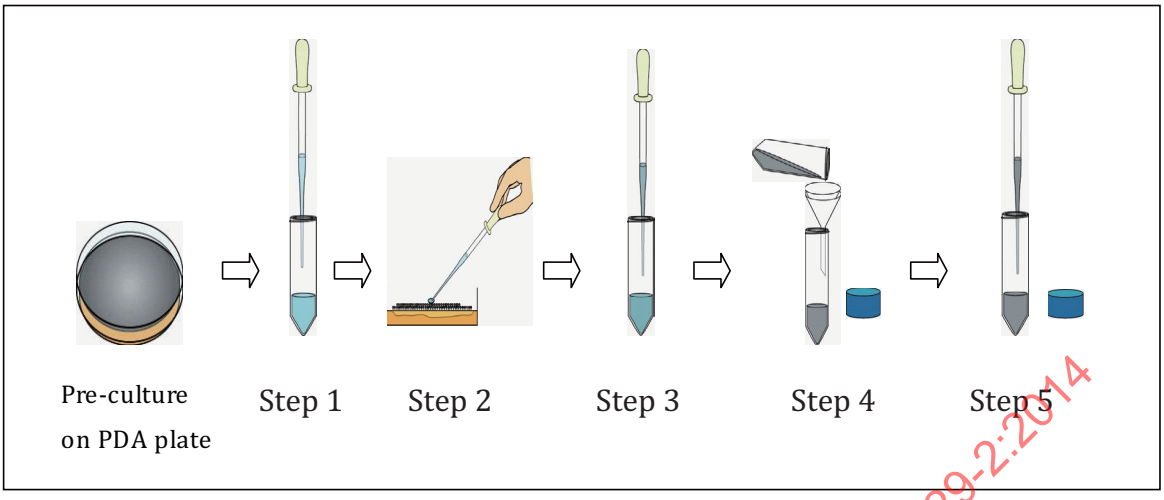


Figure 2 — Steps for adjusting spore suspension

10.2 Suspending spores in culture media

10.2.1 Use a short Pasteur pipette (7.10) or similar apparatus to take 0,5 ml of sterilized water containing anionic surfactant (8.2) (Step 1).

10.2.2 Release it slowly around five times on the spores in the centre of the pre-culture PDA plate to wash off the surface gently (Step 2).

NOTE Such minor modification as increasing the amount of washing water can be acceptable. Keep a record of all conditions in such cases.

10.3 Collection and dispersion of spore suspension from a culture medium

10.3.1 Take the spore suspension in 10.2 by using a short Pasteur pipette (7.10) or similar apparatus.

10.3.2 Transfer it to approximate 5 ml of sterilized water containing anionic surfactant (8.2).

10.3.3 Pipette around 100 times or agitate the suspension with a shaker (7.22), three times for 20 s, or apply light ultrasonic cleaning for around 5 min so that spores can be sufficiently dispersed.

10.3.4 Check visually that the suspension looks slightly cloudy (Step 3).

10.4 Filtering to remove hyphae and spore thread

Use a funnel (7.8) or other apparatus with gauze (7.1) or glass wool for filtration (Step 4).

The gauze or glass wool may consist of a ply of three to five sheets of 5 cm × 5 cm squares.

10.5 Using centrifuge and re-suspension to remove supernatant

10.5.1 After filtration, carry out centrifuge at approximately 2 000 × *g* at least for 5 min at (25 ± 2) °C or at room temperature if the centrifuge (7.13) has no temperature control device.

10.5.2 Remove the supernatant.

10.5.3 Add 5 ml of anionic surfactant (8.2) to 10.5.2.

10.5.4 Pipette thoroughly to disperse spores or agitate the suspension with a shaker (7.22), three times for 20 s, or apply light ultrasonic cleaning for around 5 min so that spores can be sufficiently dispersed (Step 5).

10.6 Confirming the concentration of spore suspension

Check the following items with a hemocytometer.

10.6.1 Confirm that spore count is 1×10^6 ml to 3×10^6 ml and over 90 % of the spores are single spores free from hyphae.

10.6.2 If there are too many spores, dilute the suspension with sterilized water containing anionic surfactant (8.2) to reduce the spore count to 1×10^6 ml to 3×10^6 ml, and check the spore count again.

10.6.3 If there are not enough spores, repeat centrifuge to remove supernatant, and use sterilized water containing anionic surfactant (8.2) to adjust spore count to 1×10^6 ml to 3×10^6 ml, and check the spore count again.

10.7 Adjusting spore suspension for testing

10.7.1 For absorption method, use 1/20 SDB in anionic surfactant (8.2) to adjust the concentration of spore suspension between from 1×10^5 ml to 3×10^5 ml.

NOTE To prepare 100 ml of 1/20 SDB in anionic surfactant, add 5 ml of SDB (8.3.1) in 95 ml of anionic surfactant (8.2).

10.7.2 For transfer method, prepare the inoculum in anionic surfactant only (8.2) (SDB 1/20 is not necessary).

10.7.3 Agitate the suspension well.

10.7.4 Cool the suspension in ice and use within 4 h.

10.8 Enumeration of inoculum

The dilution of 10^{-2} to 10^{-3} by anionic surfactant will be enumerated as described in 11.2.4.

11 Testing procedure

11.1 Inoculation and preparation of specimens

11.1.1 General

For the inoculation method, absorption method or transfer method shall be used. The specimen for each method is prepared as follows. The transfer method is applicable to the textile product sample which does not absorb water.

11.1.2 Absorption method

11.1.2.1 Washing of sample

When necessary, such as to assess durability of the treatment for example, test specimens may be washed in accordance with ISO 6330 or another suitable method, and after the final washing, the specimens are rinsed with water to eliminate the washing detergent. Use of an unspecified method shall be recorded.

11.1.2.2 Mass and shape of specimens

Obtain specimens with a mass of (0,40 ± 0,05) g and cut it in a suitable size for the test. A single piece of 0,4 g is preferable. Obtain six control specimens from the control fabric and six test specimens from the test fabric of antifungal treatment.

NOTE Three of the control specimens and three of the antifungal-treated test specimens are used for the time zero of incubation, immediately after inoculation. The remaining specimens are used for after incubation.

11.1.2.3 Setting specimens

11.1.2.3.1 Placement of specimen

Place each specimen into separate vials (7.7) by selecting the following method appropriate to the nature of the test specimen.

— If a test sample is a fabric that tends to curl easily, or if it contains wadding or down, place a glass rod on to the specimen in the vial (7.7). Alternatively, secure both ends of the specimen with thread.

— If a test sample is yarn, arrange the yarn in a bundle as a specimen and place a glass rod on to the specimen in the vial (7.7).

— If a test sample is a carpet or similar construction textile product, cut the pile as a specimen and place a glass rod on to the specimen in the vial (7.7).

11.1.2.3.2 Sterilization

When necessary, such as if contamination of specimens is suspected for example, sterilize the specimens by autoclave (7.3) according to the following procedure.

Cover the upper portion of the vials (7.7) containing the specimens with aluminium foil.

Place the covered vials (7.7) in a metal wire basket for autoclaving.

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

Sterilize the caps and the vials (7.7) containing the specimens by autoclave (7.3) at 121 °C and 103 kPa for 20 min.

After sterilization, remove the aluminium foil and allow the specimens in the vials (7.7) to dry for 60 min or more by placing them in a safety cabinet or any other place where there is no risk of airborne contamination.

NOTE 1 When autoclaving is not possible, sterilization can be accomplished by ethylene oxide gas, γ - ray, or another suitable method. Use of alternative methods shall be recorded.

NOTE 2 Autoclaving can deactivate or increase the release of certain antimicrobial agents and hence give false results.

NOTE 3 The control fabric can be sterilized by the above method.

11.1.2.3.3 Close of the vial cap

Tighten securely the vial caps after any treatment to keep in clean condition before inoculation.

11.1.2.4 Inoculation on specimens

11.1.2.4.1 Open vial cap.

11.1.2.4.2 Pipette accurately 0,2 ml of the inoculum prepared in 10.7 (1×10^5 ml to 3×10^5 ml) at several points on each specimen prepared in 11.1.2.3.

If inoculum deposits are not absorbed well into the specimen, tamp down them with, for example, a glass rod.

11.1.2.4.3 Make sure that the suspension is well mixed prior to application and is well absorbed by the specimen, and close the vial, being sure that no inoculum touches its surface.

11.1.3 Transfer method

11.1.3.1 Preparation of test specimen

Using a template (7.20), cut six specimens with a diameter of (3,8 ± 0,1) cm.

The specimens shall not contain any seams, selvedges, embroidery, fasteners, etc.

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

When necessary, test specimens may be sterilized by autoclave (7.3), ethylene oxide gas, γ - ray, or any other suitable method. Use of an unspecified method shall be recorded.

11.1.3.2 Inoculation to agar plates

Prepare 12 Petri dishes (7.2) with a diameter of 60 mm or six Petri dishes (7.2) with a diameter of 90 mm with SDA (8.3.3) for transfer.

Inoculate 1 ml of the initial spores of 10.7, a range of concentrations between 1×10^5 ml to 3×10^5 ml. Flood completely on the bottom of the dishes by gently inclining the dishes, and remove the excess liquid as much as possible. Then keep them still for (300 ± 30) s.

11.1.3.3 Transfer to specimens

11.1.3.3.1 Prepare six control specimens from a control fabric and six test specimens of antifungal treated product respectively.

11.1.3.3.2 Select two control specimens: one is for time zero and another is for time 48 h. Set each specimen on the agar surface (11.1.3.2) in 60 mm in diameter Petri dish (7.2) or a couple of specimens in 90 mm in diameter Petri dish (7.2) (so as to eliminate overlap with both specimens), and weigh down with a 200 g stainless-steel cylinder (7.21) for (60 ± 5) s. Take off the cylinder and each specimen.

Place one specimen 60-mm-diameter Petri dish (7.2) with the transferred surface face up for the incubation.

Place the other specimen in a sterile bag or a vial to be tested according to 11.2.

11.1.3.3.3 Repeat 11.1.3.3.2 for another four control specimens. Finally, prepare three control specimens for time zero and three control specimens for time 48 h incubation.

11.1.3.3.4 Repeat 11.1.3.3.2 for six test specimens to obtain three test specimens for time zero and three test specimens for time 48 h incubation.

11.2 Plate count method procedure

11.2.1 Shake-out for zero hour inoculation

In case of absorption method, immediately after the inoculation of specimens, add 20 ml of SCDLP (8.3.5) medium into each of the six vials (7.7) in which a control specimen and a test specimen have been placed, tighten caps, and shake-out as specified in a) or b).

In case of transfer method, immediately after transfer, place each specimen in a sterile bag or a vial containing 20 ml of SCDLP medium and shake-out as specified in a), b), or c).

a) Shaking by vortex mixer

Mix by using the vortex mixer for 1 min × 5 cycles.

b) Shaking by hand

Take the test tube or bottle by hand and shake in an arc of approximately 30 cm for 1 min × 5 cycles.

c) Shaking by paddle blender (7.23)

Place the designated disposable bag (7.28) in the Stomacher-type paddle blender (7.23) machine and run the machine for 1 min on each face of the bag.

11.2.2 Incubation for 48 h incubation

In case of absorption method, incubate the vials (7.7) (three control specimens and three test specimens) at a temperature of (30 ± 2) °C for (48 ± 2) h.

In case of the transfer method, incubate the Petri dishes (7.2) with specimens in a humidity chamber (7.24) at a temperature of (30 ± 2) °C and a relative humidity of higher than 95 % for (48 ± 2) h.

11.2.3 Shake-out after 48 h incubation

In case of absorption method, after the incubation, add 20 ml of SCDLP medium into each of the vials, tightly secure the caps, and shake-out as specified in 11.2.1.

In case of transfer method, after the incubation, place each specimen in a sterile bag or vial containing 20 ml of SCDLP (8.3.5) and shake-out as specified in 11.2.1.

11.2.4 Enumeration by using plate count method

11.2.4.1 Prepare dilution series

11.2.4.1.1 Prepare the suspension 11.2.1 or 11.2.3 which is zero dilution, 10^0.

11.2.4.1.2 Take 1 ml of the suspension 11.2.1 or 11.2.3 using a pipette (7.9), add it to a test tube containing (9,0 ± 0,1) ml anionic surfactant (8.2), and shake well. These become dilution, 10^{-1}.

11.2.4.1.3 Repeat the procedure successively and prepare a dilution series 10^0 and 10^{-1} dilutions for zero hour incubation and 10^0 to 10^{-4} for 48 h incubation.

11.2.4.2 Incubation of the dilution series suspensions

The numeration is made on the surface of SDA (8.3.3) medium poured in Petri dishes (7.2) with a diameter of 90 mm.

11.2.4.2.1 Numeration carried out on the surface of SDA medium plate. Spread 0,1 ml of the suspensions and 0,1 ml of their dilution series on the surface of the SDA medium (one dish per one numeration) using a plastic or heat-sterilized spreader.

11.2.4.2.2 Numeration carried out in the depth of SDA medium plate. Transfer 1 ml of each dilution into two Petri dishes (7.2). Warm approximately 15 ml of SDA medium to a temperature of 45 °C to 46 °C using a water bath (7.30), add to the dishes, and mix well. Maintain at room temperature and let the medium solidify.

11.2.4.2.3 Turn the dishes upside down and incubate at (28 ± 2) °C for 24 h to 48 h; observe the fungal colony growth.

11.2.4.3 Numeration of colonies

11.2.4.3.1 After incubation, count the number of colonies on the Petri dishes (7.2) of dilution series on which 1 CFU to 300 CFU have appeared.

11.2.4.3.2 Obtain the fungal concentration in the solution using the following formula according to ISO 7218:2007:

$$N = \frac{\sum C}{V \times 1{,}1 \times d} \tag{1}$$

where

N is the fungi concentration in colony-forming units per millimetre (CFU/ml);

ΣC is the sum of the CFU counted on two dishes retained from two successive dilutions, at least one of which contains a minimum of 5 CFU;

d is the first dilution step above two successive dilutions;

1,1 is a coefficient to combine the two successive dilutions, from ISO 7218:2007;

V is the volume of inoculum placed in each dish, in millimiter (0,1 ml).

In the case when no Petri dish contains a minimum of 5 CFU, the test is judged to be ineffective.

EXAMPLE:

At dilution 10^{-2} 168 CFU

At dilution 10^{-3} 14 CFU

$$N = \frac{168 + 14}{0{,}1 \times 1{,}1 \times 10^{-2}} = \frac{182}{0{,}001\ \ 1} = 165\ \ 454\ \ \text{CFU/ml} = 1{,}65 \times 10^{5}\ \ \text{CFU/ml}$$

12 Test results

12.1 Judgment of test effectiveness

When the conditions of the following a), b), and c) 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 shall be 1×10^5 ml to 3×10^5 ml.

b) The difference in common logarithm in the extremes of the number of fungi, for the three control fabrics immediately after inoculation, shall be less than one. This difference immediately after incubation shall also be less than one.

c) The growth value obtained according to Formula (2) shall be more than 1,0.

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

where

F is the growth value on the control fabric;

C_t is the arithmetic average of the number of fungi obtained from three test samples of control fabric after an (48 ± 2) h incubation;

C_0 is the arithmetic average of the number of fungi obtained from three test samples of control fabric immediately after inoculation.

F is a difference between the common logarithm of C_t and C_0.

12.2 Calculation of antifungal activity value

When the condition of 12.1 is satisfied, the test is judged to be effective. When the test is judged to be ineffective, a retest shall be carried out.

When the test has been judged to be effective, obtain the antifungal activity value according to Formula (3):

$$A = (\lg C_t - \lg C_0) - (\lg T_t - \lg T_0) = F - G \quad (3)$$

where

A is the antifungal activity value;

F is the growth value on the control fabric, $F = \lg C_t - \lg C_0$;

G is the growth value on the antifungal-treated samples, $G = \lg T_t - \lg T_0$;

T_t is the arithmetic average of the number of fungi obtained from three antifungal-treated test samples after (48 ± 2) h incubation;

T_0 is the arithmetic average number of the number of fungi obtained from three antifungal-treated test samples immediately after inoculation.