International Standard

ISO 18184

Textiles — Determination of antiviral activity of textile products

Textiles — Détermination de l'activité virucide de produits textiles

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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 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).

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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 38, *Textiles*.

This third edition cancels and replaces the second edition (ISO 18184:2019), which has been technically revised.

The main changes are as follows:

— in 14.3.2, the calculation of antiviral activity has been updated;

— a new Annex B has been added for test method for SARS-CoV-2, and subsequent annexes have been moved;

— in Annex E, E.4 has been added for the composition of DMEM medium;

— a new Annex F has been added to give additional examples of the virus strain and host cells.

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.

Introduction

Recently, along with the global improvement in the level of living, consumers are showing the trend to seek healthcare or health protective products. Also, an increase in the people's interest for protection against epidemic diseases has been noted, as the overcrowded commuting train car where the commuters experience every day, the hospitals, nursing homes, etc.

Being supported by the processing technology of textile products to provide a high performance which has been highly developed recently, the health protective and hygiene relating products have been advancing into the market.

Because those products are relatively new and included the technical aspects out of textile technology, the testing methods have been developed by the individual producers to evaluate the product performance. That has resulted in inexistence of a unified test method, hindering for both consumers and producers a true explanation or understanding of those high functional products.

The antiviral textile product is one of those products and includes the technical fields of the textile technology and the biotechnology.

The demand to establish an international standard has been growing in the consumers, retailers, producers, etc. as the stakeholders in the market.

Antiviral textile products are textiles capable of reducing the number of infective virus particles that contact the surface of the textile. This document provides a quantitative test method to assess the antiviral performance of such products.

The data obtained in an objective manner by this document give the common knowledge to all the stake holders such as consumers, producers, retailers, etc. to understand the correct performance of the antiviral textile products.

There are two methods to quantify the number of infective virus, as infective virus titre in this document, which are the plaque method and the $TCID_{50}$ method. The method used can be selected by the experience and the convenience of each testing house. Any appropriate cellular system can be used and that the testing conditions when used should be reported.

See Annexes I, J and K for interlaboratory test results.

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WARNING — This document calls for use of the infectious viruses or substances/procedures that may be injurious to the health/environment if appropriate conditions are not observed. It refers only to technical suitability and does not absolve the user from legal obligations relating to health and safety/environment at any stage.

1 Scope

This document specifies testing methods for the determination of the antiviral activity of the textile products.

The textile products include woven and knitted fabrics and non-woven fabrics, cotton, fibres, yarns, braids, feathers, etc.

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

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 20743:2021, *Textiles — Determination of antibacterial activity of textile products*

3 Terms and definitions

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

ISO and IEC maintain terminological 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
virus
original biological entity which has a single type of nucleic acid (DNA or RNA), specific structure that opposes the virus to living organisms with a cellular structure (prokaryotes and eukaryotes), and reproduces from its genetic material by replication within the host cell, and leads to absolute intracellular parasitism

Note 1 to entry: The virion is the infectious viral particle.

3.2
virus activity
ability to replicate in the susceptible and permissive host cells

3.3
antiviral activity
property of any substance (chemical or otherwise) producing a modification of one of the elements of the virion structure which induces the latter's inability to replicate

Note 1 to entry: Property that reduces the viral activity, generally through morphological change or structural damage to the surface protein of the virus.

Note 2 to entry: It is not necessarily to imply that the change of antigenic response or the change of constituent element is the reduction of virus infectivity.

3.4
antiviral chemicals
inorganic or organic chemicals able to reduce *virus activity* (3.2)

Note 1 to entry: The organic antiviral chemicals give the change to the surface protein of virus by the chemical adsorption. The inorganic metallic antiviral substances destroy or change the morphology of the virus by the extraction of hydrogen atom in the virus protein by OH radicals which are generated by the radical reaction.

3.5
control fabric
fabric used to verify the stability of the test virus on a textile fabric

Note 1 to entry: The fabrics before the antivirus treatment should be used as a control fabric with the same condition described in 3.5.

Note 2 to entry: When the fabrics before the antivirus treatment is not applicable, in Note to entry 1, the 100 % cotton cloth described in ISO 105-F02 should be used without any chemical treatments such as the fluorescent bleach, etc.

3.6
control test
test to confirm that a test specimen does not affect the host cell

Note 1 to entry: This test is performed as same as actual test, but without virus.

3.7
cytopathic effect
cytopathic effect (CPE) caused by virus
effect appears as morphological change or destruction of the host cells as a result of the virus multiplication

3.8
infectivity titre of virus
number of infectious viral particles present per unit volume in a cell lysate or in viral suspension

3.9
plaque
area of lysed cells in a monolayer cell culture

3.10
plaque forming units
PFU
unit expressed as the concentration of the infectious virus per unit volume

3.11
plaque assay
assay to determine the infectivity *titre of virus* (3.8) from PFU by using the series of dilution

3.12
$TCID_{50}$
50 % infectious dose of a wash-out virus suspension or the dilution of the virus suspension that induces a CPE in 50 % of cell culture units

Note 1 to entry: See 3.7.

3.13
$TCID_{50}$ method
assay to determine the *infectivity titre of virus* (3.8) from $TCID_{50}$ by using the series of dilution

3.14
cytotoxicity
morphological alteration of cells and/or their destruction or the reduction of their sensitivity to the multiplication of viruses induced by a product

3.15
antiviral textile product
fabric treated with antiviral chemicals

4 Principle

The viruses are deposited onto an antiviral test specimen and control test specimen. After specific contact time, the remaining infectious virus is counted, and the reduction rate is calculated by the comparison between the antiviral test specimen and the control test specimen by common logarithm. There are two methods to quantify the infectious virus titre. One method is the plaque assay and the other is the $TCID_{50}$ method. The selection of the method depends on the convenience and experience of the testing organization.

Due to the individual sensitivities, the results of one test virus cannot be transposed to other viruses.

5 Example of virus and host cell

Examples of species of viruses and host cells are shown in Annex A, Annex B and Annex F.

Other species of viruses and host cells can be used after appropriate validations, as the important virus may differ depending on target application. If the other species are used, the name of the species and the specific reason for their use shall be included in the test report.

NOTE Reference viruses are listed in EN 14476 and EN 14675.

6 Warning

Handling and manipulation of viruses and host cells which are potentially hazardous requires a high degree of technical competence and can be subject to current national legislation and regulations. Appropriate safety precautions should be observed with due consideration given to country-specific regulations. Only personnel trained in biological techniques should carry out such tests. Appropriate practices for disinfection, sterilization and personal hygiene shall be strictly observed.

7 Apparatus

7.1 High pressure steam sterilizer, such as autoclave, capable of operating at a temperature of (121 ± 2) °C, in accordance with ISO 20743:2021, 5.28.

7.2 Dry heat sterilizer, such as ovens, capable of operating at a temperature of (180 ± 2) °C and (160 ± 2) °C.

7.3 Measuring flask, with capacity of 1 l.

7.4 Balance, with the available range of 0,01 g to 100 g with accuracy of 1,0 % in accordance with ISO 20743:2021, 5.13.

7.5 Pipette, of various capacities with accuracy of 10 % of the nominal volume.

7.6 Washing machine.

7.7 Pipetter, capable of mounting the glass or plastic pipettes.

7.8 Micropipette, 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.

7.9 Water bath, capable of maintaining at a temperature of (37 ± 1) °C, (50 ± 1) °C and (56 ± 1) °C.

7.10 Mixer, producing a vortex shaking action in accordance with ISO 20743:2021, 5.4.

7.11 Freezer, capable of operating at a temperature of (–80 ± 2) °C or (–20 ± 2) °C.

7.12 Liquid nitrogen bath, for the preservation at approximately –196 °C.

7.13 Membrane filtration device, with a pore size of 0,22 µm.

7.14 Refrigerator, capable of operating at a temperature between [2 °C, 8 °C].

7.15 pH meter, having an inaccuracy of calibration ±0,1 pH units at (20 ± 1) °C in accordance with ISO 20743.

7.16 Inverted microscope, capable of being used for cultured cells observation.

7.17 Tweezers, capable of being sterilized.

7.18 Centrifuge, capable of being operated at a temperature of (4 ± 2) °C, and relative centrifugal force of approximately 1 000 g.

7.19 Biological safety cabinet, class II.

7.20 Vial container, with a capacity of 30 ml and closed with the screw cap. The gasket is made of perfluoroethylene or silicone and the cap is made of polypropylene.

7.21 96 wells microplate with the gamma radiation sterilization, for $TCID_{50}$ method.

96 wells microplates with other sterilization finish may be used after appropriate validation for the growth of cells. See Figure 1.

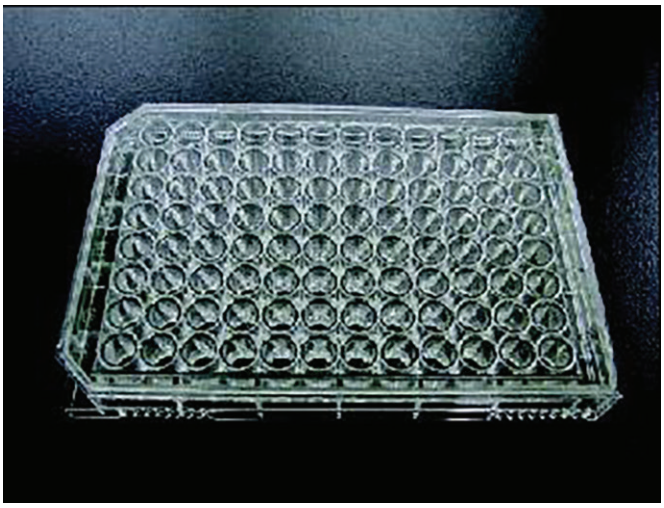

Figure 1 — 96 wells microplate for $TCID_{50}$ method

7.22 6 wells plastic plate with the gamma radiation sterilization, for plaque assay.

Six wells plates with other sterilization finish may be used after appropriate validation for the growth of cells. See Figure 2.

Figure 2 — 6 wells plastic plate for plaque assay

7.23 Flask, for cell culture use with the gamma radiation sterilization finish, with an adherent type, a cell culture area of 75 cm^2 and with the vent cap. The vent cap can exchange bacterial air through 0,2 µm filter. See Figure 3.

Flask with other sterilization finish may be used after appropriate validation for the growth of cells.

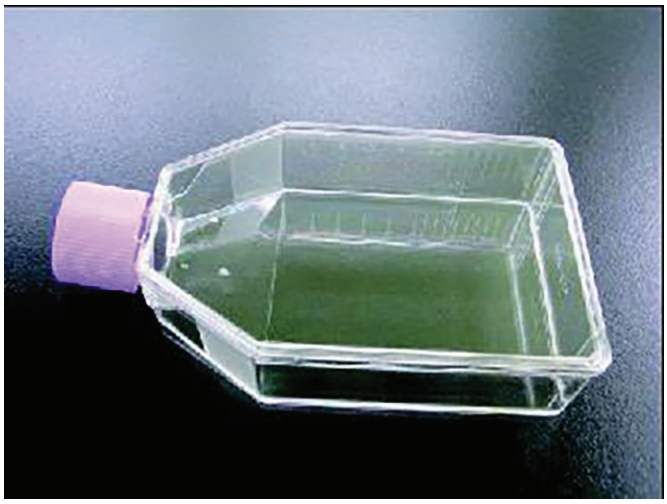

Figure 3 — Flask for cell culture use

7.24 CO_2 incubator, capable of maintaining an atmosphere with 5 % CO_2, at a temperature of (34 ± 1) °C and (37 ± 1) °C.

7.25 Incubator, capable of maintaining at a temperature of (25 ± 1) °C, (35 ± 1) °C and (37 ± 1) °C.

7.26 Centrifuge tube.

7.27 Culture container.

7.28 Test tube.

7.29 Beaker.

7.30 Glass rod, with approximately 18 mm in diameter.

7.31 Mixer, producing spinning action by a stir bar with a rotating magnetic field.

8 Sterilization of apparatus

Sterilize all apparatus which come in contact with the cells, the chemicals, or test specimen. The sterilization method shall be used by high pressure steam or dry heat method.

— High pressure steam sterilization: by an autoclave (7.1) at a temperature of 121 °C and a pressure of 103 kPa for 15 min.

— Dry-heat sterilization: by a dry heat sterilizer (7.2) at a temperature of 180 °C for 30 min or 160 °C for 2 h.

In case of plastics products, heat-resistant plastics products or sterilization finish plastics products may be used.

9 Reagents and materials

All reagents shall have the quality suitable for virological needs, i.e. free of toxic substances for testing microorganisms. Some of the media are available in the market.

9.1 Water, which shall be analytical-grade water for microbiological media preparation, which is ion-exchanged and/or freshly distilled and/or ultra-filtered and/or filtered with RO (reverse osmosis) or grade 3 water in accordance with ISO 3696.

9.2 Eagle's minimum essential medium (EMEM) or **Roswell Park Memorial Institute medium (RPMI)**, available in the market. The composition is described in Annex E. If there are any components missing from the composition, add them according to the composition table.

9.3 sodium bicarbonate solution

Prepare the solution at the concentration of 7,5 % according to the method 1 or method 2, and mix well just before using.

9.3.1 Method 1

9.3.1.1 Sterilize sodium bicarbonate, 75 g in autoclave in a culture container (7.27) with a cap closed tightly.

9.3.1.2 Water (9.1) is also sterilized by autoclave.

9.3.1.3 Dissolve sodium bicarbonate in the sterilized water (9.3.1.2) of 1 000 ml well.

9.3.2 Method 2

9.3.2.1 Prepare 7,5 % sodium bicarbonate solution by dissolving 75 g of sodium bicarbonate in 1 000 ml of water (9.1).

9.3.2.2 Sterilize the solution by using 0,22 µm membrane filter (7.13).

9.4 Formaldehyde solution

Prepare a formaldehyde solution at the concentration of 3,7 % in water as follows.

9.4.1 Prepare 100 ml of a 37 % formaldehyde solution.

9.4.2 Add 900 ml of water (9.1) into the solution of 9.4.1.

The other solution for cell fixation may be used after appropriate validation for the cell fixation.

9.5 Methylene blue solution

9.5.1 Prepare a measuring flask (7.3) of 1 l, then, put the following materials in the flask (7.3):

— Water (9.1), 1 000 ml;

— Methylene blue, 0,375 g;

— 1 mol/l sodium hydroxide solutions 62,5 µl.

9.5.2 Dissolve and mix well.

9.6 Inactivated fetal bovine serum (FBS)

9.6.1 Put the frozen cryopreserved fetal bovine serum in a package in the water bath (7.9) at a temperature of 37 °C and keep it until defrosting.

9.6.2 Then, raise the temperature of the water bath to 56 °C and keep it for 30 min to inactivate.

9.6.3 Divide it into several tubes. Put them in the freezer (7.11) at a temperature lower than –20 °C until use for testing.

9.6.4 Just before use, put it in the water bath at a temperature of 37 °C and keep it until defrosting.

9.7 Growth medium.

9.7.1 Influenza virus and feline calicivirus

9.7.1.1 Prepare a measuring flask (7.3) of 1 l, and put the following materials into the flask (7.3):

— water (9.1), 800 ml;

— kanamycin sulfate, 60 mg;

— Eagle's minimum essential medium (EMEM) (E.2), 9,53 g, or RPMI 1640 medium (E.3), 10,4 g.

9.7.1.2 Dissolve and mix well and make up whole solution to 1 000 ml by water (9.1).

9.7.1.3 Sterilize the mixed solution from 9.7.2 by using filter (7.13).

9.7.1.4 Add 15 ml of 7,5 % sodium bicarbonate solution (9.3) and 100 ml of the inactivated fetal bovine serum (9.6) in the solution from 9.7.1.3.

9.7.2 Other viruses

In case of SARS-CoV-2, prepare the growth medium according to Annex B. In case of additional example of viruses described in Annex F, select the appropriate growth medium for host cells of each virus.

9.8 Maintenance medium.

9.8.1 Influenza virus and feline calicivirus

9.8.1.1 Prepare a measuring flask (7.3) of 1 l, then, put the following materials in the flask:

— water (9.1), 800 ml;

— kanamycin sulfate, 60 mg;

— Eagle's minimum essential medium, 9,53 g.

9.8.1.2 Dissolve them well, then, make up the whole amount to 1 000 ml by adding water (9.1).

9.8.1.3 Sterilize the mix solution from 9.8.1.2 by using the filter (7.13) with a pore size of 0,22 µm.

9.8.1.4 Add 15 ml of 7,5 % sodium bicarbonate solution (9.3) in the mix solution of 9.8.1.3.

9.8.2 Other viruses

In case of SARS-CoV-2, prepare the maintenance medium according to Annex B. In case of additional example of viruses described in Annex F, select the appropriate maintenance medium for host cells of each virus.