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Textiles — Determination of antiviral activity of textile products

Textiles — Détermination de l'activité virucide de produits textiles

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Contents

Page

Foreword

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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.*

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

The demand to establish the 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 standard provides a quantitative test method to assess the antiviral performance of such products.

The data obtained in objective manner by this standard 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 standard, 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.

Textiles — Determination of antiviral activity of textile products

1 Scope

This International Standard specifies testing methods for the determination of the antiviral activity of the textile products. The textile products include woven and knitted fabrics, fibres, yarns, braids, etc.

Viruses used in this International Standard are as follows:

— one of enveloped viruses, an influenza virus, which is an infective virus in humans that causes respiratory tract infection;

— one of non-enveloped viruses, a feline calicivirus, which is one of surrogates of noroviruses which are important enteric pathogens.

2 Normative references

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

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
virus
has no cell and consists of the gene material enclosed by the shell of the protein, it can replicate in the specific host cells

3.2
virus activity
ability to replicate in the specific host cells

3.3
antiviral property
property to give the morphological change or structural damage to the surface protein of virus

Note 1 to entry: As the result, the damaged virus loses the fitting to the receptor of host cell and reduces the virus activity. Depending on the type of molecules the property can also be an alteration of nucleic acids. In addition to enveloped viruses there is an alteration of envelope as well.

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

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
reference cloth
cloth used to verify the stability of the test virus on a textile fabric

Note 1 to entry: The 100 % cotton cloth described in ISO 105-F02 should be used without any chemical treatments such as the fluorescent bleach, etc.

Note 2 to entry: The fabrics before the antivirus treatment may be used as a reference cloth with the same condition described in 3.5.

3.6
control test of specimen
test to confirm that a 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 (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 a solution

3.9
plaque
lysis formed area in a cell monolayer under semisolid medium due to infection by and multiplication of a single infectious virus

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

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

3.12
$TCID_{50}$ method
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.

4 Principle

The viruses are inoculated to a specimen. After specific contacting time, the remaining infectious virus is counted and the reduction rate is calculated by the comparison between the antiviral product test specimen and the reference specimen by common logarithm. There are two methods to quantify the infectious virus titre. One method is the plaque assay (3.11) and the other is the $TCID_{50}$ method (3.12)

as explained. The selection of the method depends on the convenience and experience of the testing organization.

5 Virus and host cell

Viruses used in this standard are **an** Influenza virus and **a** feline calicivirus which is described in Annex A. Moreover, the host cells are described corresponding to the viruses in Annex A. One or both viruses are chosen for the test depended on the end use of the textile products.

6 Warning

This standard 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.

The warning is extended as the following. The virus in the standard shall be the one of biotechnology safety level class II classified by the directives of WHO as stated. The user of this standard shall have enough knowledge and experience of the biotechnology. Moreover, users shall comply strictly to the safety standard of the manufacturers and the domestic regulation.

7 Apparatus

7.1 High pressure steam sterilizer: Autoclave, capable of operating at a temperature of (121 ± 2) °C and a pressure of (103 ± 5) kPa.

7.2 Dry heat sterilizer: 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 Scale, with the available range of 100 g ± 0,1 g to 0,01 g ± 0,000 1 g.

7.5 Glass pipette, with capacities of 50 ml ± 0,5 ml, 25 ml ± 0,25 ml, 10 ml ± 0,1 ml and 5 ml ± 0,05 ml.

7.6 Plastic pipette, with capacities of 50 ml ± 0,5 ml, 25 ml ± 0,25 ml, 10 ml ± 0,1 ml and 5 ml ± 0,05 ml.

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

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 ± 2) °C, (50 ± 2) °C or (56 ± 2) °C.

7.10 Vortex-type mixer, used for microbial testing.

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 approximately at –196 °C.

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

7.14 Refrigerator, capable of operating at a temperature between (2 ± 2) °C and (8 ± 2) °C.

7.15 pH meter, with a glass electrode detector.

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.

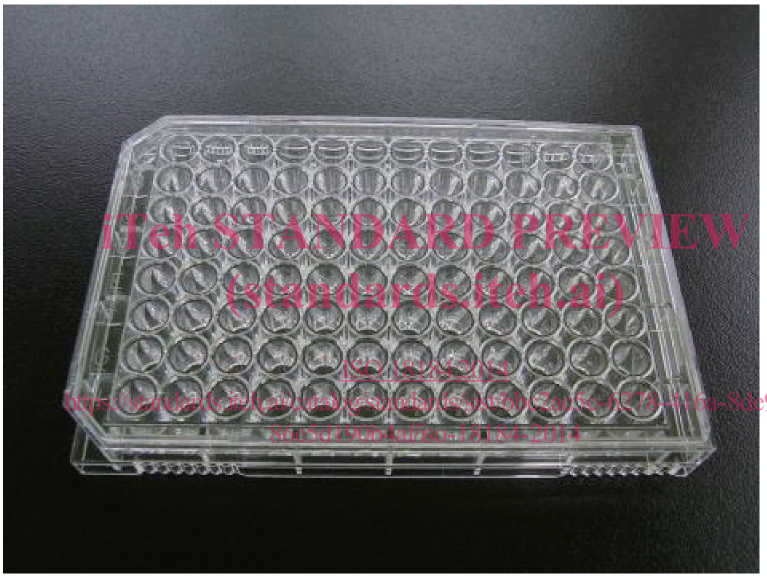

Figure 1 — 96 wells microplate for $TCID_{50}$ method

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

Figure 2 — 6 wells plastic plate for plaque assay

7.23 Flasks, 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, and the tight closed cap. The vent cap can be exchanged abacterial air through 0,2 µm filter.

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 ± 2) °C and (37 ± 2) °C.

7.25 Incubator, capable of maintaining at a temperature of (25 ± 2) °C, (34 ± 2) °C or (37 ± 2) °C.

7.26 Centrifuge tube.

7.27 Culture container.

7.28 Test tube.

7.29 Beaker.

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.

9 Reagent and medium

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

9.1 Water, which must be of grade 3 according to ISO 3696.

9.2 Eagle's minimum essential medium (EMEM), is available in the market. The composition is described in Annex D. If there are any components missing from the composition, add them according to the composition table.

9.3 7,5 % sodium bicarbonate solution.

9.3.1 Sterilize sodium bicarbonate 75 g in autoclave in a culture container with a cap closed tightly.

9.3.2 Grade 3 water is also sterilized by autoclave.

9.3.3 Dissolve sodium bicarbonate in the sterized water of 1 000 ml well.

9.4 Formalin solution.

9.4.1 Use for cell fixation.

9.4.2 Prepare 37 % formaldehyde solution of 100 ml,

9.4.3 Add grade 3 water of 900 ml to 9.4.2.

9.5 Methylene blue solution, use for the cells dyeing.

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

— Grade 3 water, 1 000 ml;

— Methylene blue, 0,375 g;

— 1 N 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 freezed 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, put it in the water bath at a temperature of 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.

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, used for cell culture.

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

— Grade 3 water, 800 ml;

— Kanamycin sulfate, 60 mg;

— Eagle's minimum essential medium, 9,53 g, or RPMI 1640 medium, 10,4 g.

9.7.2 Dissolve and mix well and make up whole solution to 1 000 ml by grade 3 water.

NOTE RPMI stands for Roswell Park Memorial Institute.

9.7.3 Sterilize the mixed solution of 9.7.2 by using 0,22 µm filter (7.13).

9.7.4 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 of 9.7.3.

NOTE When L-glutamine is not included in the EMEM purchased in the market, add it according to the composition of Annex C before use.

9.8 Maintenance medium, used for cell culture.

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

— Grade 3 water, 800 ml;

— Kanamycin sulfate, 60 mg;

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

9.8.2 Dissolve them well, then, make up the whole amount to 1 000 ml by adding grade 3 water.

9.8.3 Sterilize the mixed solution 9.8.2 by using the filter (7.13) with a pore size of 0,22 µm.

9.8.4 Add 15 ml of 7,5 % sodium bicorbonate solution (9.3) in the solution 9.8.3.

NOTE When L-glutamine is not included in the EMEM purchased in the market, mix it according to the composition of Annex C before use.

9.9 Double consentration of the maintenance medium 9.8.

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

— Grade 3 water, 800 ml;

— Kanamycin sulfate, 120 mg;

— Eagle's minimum essential medium, 19,06 g.

9.9.2 Dissolve them well, then, make up the whole amount to 1 000 ml by adding grade 3 water.

9.9.3 Sterilize the mixed solution 9.9.2 by using the filter (7.13) with a pore size of 0,22 µm.

9.10 0,01 mol/l phosphate buffered saline PBS (-).

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

— Sodium chloride, 8 g;

— Potassium chloride, 0,2 g;

— Phosphoric acid hydrogen 2 sodium 12 hydrate, 2,9 g;

— Phosphoric acid 2 hydrogen potassium, 0,2 g.

9.10.2 Add grade 3 water by making up whole amount to 1 000 ml and dissolve well, then,

9.10.3 Sterilize the solution 9.10.2 by using autoclave (7.1) at a temperature of 121 °C and a pressure of 103 kPa for 15 min.

9.11 Trypsin derived from beef pancreas and PBS (-) solution.

9.11.1 Prepare a beaker, then, put the following materials in the beaker:

— 0,01 mol/l phosphate buffered saline PBS (-) (9.10), 100 ml;

— Trypsin derived from beef pancreas, 1,0 g.

9.11.2 Dissolve and mix well by using mixer for 2 h.

9.11.3 Then, sterilize the solution 9.11.2 by using the filter (7.13) with a pore size of 0,22 µm.

The divided solution tubes that are not used immediately are preserved in the freezer at a temperature of lower than −80 °C.

9.11.4 Prepare a test tube and put the following solutions in the test tube:

— 0,01 mol/l phosphate buffered saline PBS (-) (9.10), 9 ml;

— Trypsin derived from beef pancreas and PBS (-) mixed solution 9.11.3, 1ml.

9.11.5 Dissolve and mix them well.

9.11.6 Divide the solution in test tubes and preserve in the freezer at a temperature of lower than −20 °C.

9.11.7 Just before using, put it in the water bath (7.9) at a temperature of 37 °C and keep it until defrosting.

9.12 Trypsin EDTA solution.

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

— 0,01 mol/l phosphate buffered saline PBS (-) (9.10), 1 000 ml;

— Trypsin, 2,5 g;

— Kanamycin sulfate, 0,1 g;

— Streptomycin sulfate, 0,1 g;

— Amphotericin B, 2 mg;

— EDTA, 0,014 mol.

9.12.2 Dissolve and mix well.

9.12.3 Then, sterilize the solution 9.12.2 by using the filter (7.13) with a pore size of 0,22 µm.

9.12.4 Divide the solution in test tubes and preserve in the freezer at a temperature of lower than - 20 °C.

9.12.5 Just before using, put it in the water bath (7.9) at a temperature of 37 °C and keep it until defrosting.

NOTE Trypsin EDTA solution is available in market. The products with the different components from 9.12.1, could be used after proper validation.

9.13 DEAE-dextran solution.

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

— Grade 3 water, 1 000 ml;

— DEAE-dextran, 20 g.

9.13.2 Dissolve and mix well.

9.13.3 Sterilize the solution 9.13.2 by using the filter (7.13) with a pore size of 0,22 µm.

9.14 Agar medium, used for the plaque assay. This is prepared with A liquid and B liquid as follows and mixed well just before using.

9.14.1 A liquid.

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

— Double concentration of maintenance medium (9.9), 1 000 ml;

— DEAE-dextran solution (9.13), 10 ml;

— 7,5 % sodium bicorbonate solution (9.3), 40 ml.

Mix well.

9.14.1.2 Only for the influenza virus test and for the plaque assay, add 3,0 ml of the Trypsin from beef pancreas and PBS (-) solution (9.11).

