

JAPANESE
INDUSTRIAL
STANDARD

Translated and Published by
Japanese Standards Association

JIS L 1922 : 2016

(JTETC/JSA)

**Textiles—Determination of antiviral
activity of textile products**

ICS 59.080.01

Reference number : JIS L 1922 : 2016 (E)

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Date of Establishment: 2016-12-20

Date of Public Notice in Official Gazette: 2016-12-20

Investigated by: Japanese Industrial Standards Committee

Standards Board for ISO area

Technical Committee on Consumer Life Products

JIS L 1922:2016, First English edition published in 2017-06

Translated and published by: Japanese Standards Association
Mita MT Building, 3-13-12, Mita, Minato-ku, Tokyo, 108-0073 JAPAN

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Printed in Japan

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Foreword

This translation has been made based on the original Japanese Industrial Standard established by the Minister of Economy, Trade and Industry through deliberations at the Japanese Industrial Standards Committee according to the proposal for establishment of Japanese Industrial Standard submitted by Japan Textile Evaluation Technology Council (JTETC)/Japanese Standards Association (JSA) with the draft being attached, based on the provision of Article 12 Clause 1 of the Industrial Standardization Law.

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Textiles—Determination of antiviral activity of textile products

Introduction

This Japanese Industrial Standard has been prepared based on the first edition of **ISO 18184** published in 2014 with some modifications of the technical contents in order to correspond to the needs of users in Japan.

The antiviral product is one of high functional products available in market and includes the technical fields of the textile technology and the biotechnology, however, a unified test method has not existed.

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.

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 TCID₅₀ method. The method used can be selected by the experience and the convenience of each testing house. Annex E gives information about polio virus, Annex F about testing method using SPF embryonated hen's eggs which has been employed conventionally, and Annex G about antiviral efficacy. Annex H and Annex I describe round robin test results for reference.

The dotted underlines indicate changes from the corresponding International Standard. A list of modifications with the explanations is given in Annex JA.

1 Scope

This Standard specifies testing methods for the determination of antiviral activity of textile products. The textile products include woven and knitted fabrics, fibres, yarns, braids, etc. Viruses used in this Standard are as follows (see Annex A).

- **Influenza virus**: one of enveloped viruses, which is an infective virus in humans that causes respiratory tract infection
- **Feline calicivirus**: one of non-enveloped viruses, which is one of surrogates of noroviruses which are important enteric pathogens

NOTE : The International Standard corresponding to this Standard and the symbol of degree of correspondence are as follows.

ISO 18184:2014 *Textiles—Determination of antiviral activity of textile products* (MOD)

In addition, symbols which denote the degree of correspondence in the contents between the relevant International Standard and **JIS** are IDT (identical), MOD (modified), and NEQ (not equivalent) according to **ISO/IEC Guide 21-1**.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this Standard. The most recent editions of the standards (including amendments) indicated below shall be applied.

JIS K 3600 *Biotechnology—Vocabulary*

JIS K 8008 *General rules for biochemical reagents*

JIS L 0803 *Standard adjacent fabrics for staining of colour fastness test*

NOTE : Corresponding International Standard: ISO 105-F02:2009 *Textiles—Tests for colour fastness—Part F02: Specification for cotton and viscose adjacent fabrics* (MOD)

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

3 Terms and definitions

For the purposes of this Standard, the terms and definitions given in **JIS K 3600** and **JIS K 8008**, and the following apply.

3.1 virus

has no cell and consists of gene material enclosed by a shell of 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 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 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 : 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 The cotton 100 % adjacent fabrics 3-1, 3-2 or 3-3 specified in **JIS L 0803** should be used without any chemical treatments such as the fluorescent bleach, etc.

NOTE 2 The fabrics before the antiviral 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 : 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

NOTE : The lysis formed area is not dyed, therefore it can be observed as a transparent blotch.

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 TCID₅₀ method

method to determine the infectivity titre of virus from such dilution rate that a CPE is induced in 50 % of cell culture units

NOTE : TCID₅₀ is an abbreviation for median tissue culture infectious dose.

4 Principal

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 i.e. plaque assay (**3.11**) and TCID₅₀ method (**3.12**). 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 are described in Annex A. Moreover, the host cells corresponding to viruses are described in Annex A. One or both viruses are chosen for test according to the end use of 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 used in this Standard shall be the one of biotechnology safety level Class II classified by the directives of WHO as stated. The responsible parties of test shall have enough knowledge and experience of the biotechnology. Moreover, they shall comply strictly to the safety standard of the manufacturers and Japan's regulation.

7 Apparatus

The apparatus used for determination of antiviral activity shall be as follows.

7.1 High pressure steam sterilizer: autoclave, capable of operating at a temperature of $(121 \pm 2) ^\circ\text{C}$ and a pressure of $(103 \pm 5) \text{ kPa}$.

7.2 Dry heat sterilizer: ovens, capable of operating at a temperature of $(180 \pm 2) ^\circ\text{C}$ and $(160 \pm 2) ^\circ\text{C}$.

7.3 Measuring flask, with capacity of 1 L.

7.4 Scale, with the available range of $\pm 0.01 \text{ g}$.

7.5 Glass pipette, with capacities of $50 \text{ ml} \pm 0.5 \text{ ml}$, $25 \text{ ml} \pm 0.25 \text{ ml}$, $10 \text{ ml} \pm 0.1 \text{ ml}$ and $5 \text{ ml} \pm 0.05 \text{ ml}$.

7.6 Plastic pipette, with capacities of $50 \text{ ml} \pm 0.5 \text{ ml}$, $25 \text{ ml} \pm 0.25 \text{ ml}$, $10 \text{ ml} \pm 0.1 \text{ ml}$ and $5 \text{ ml} \pm 0.05 \text{ ml}$.

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 $(37 \pm 2) ^\circ\text{C}$, $(50 \pm 2) ^\circ\text{C}$ or $(56 \pm 2) ^\circ\text{C}$.

7.10 Vortex-type mixer, used for microbial testing.

7.11 Freezer, capable of operating at a temperature of $-80 ^\circ\text{C}$ or $-20 ^\circ\text{C}$.

7.12 Liquid nitrogen bath, for the preservation at $-196 ^\circ\text{C}$.

7.13 Membrane filter, with a pore size of $0.22 \mu\text{m}$.

7.14 Refrigerator, capable of operating at a temperature between $2 ^\circ\text{C}$ and $8 ^\circ\text{C}$.

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 $4 ^\circ\text{C}$, and relative centrifugal force of approximately $9\,800 \text{ m/s}^2$ ($1\,000 \text{ g}$).

7.19 Biological safety cabinet, capable of being used for microbial test, and of Class II.

NOTE : The cabinet should correspond to **JIS K 3800** or have the equal performance given in that standard.

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

7.21 96 wells microplate, for TCID₅₀ method with gamma radiation sterilization finish¹⁾ (see Figure 1).

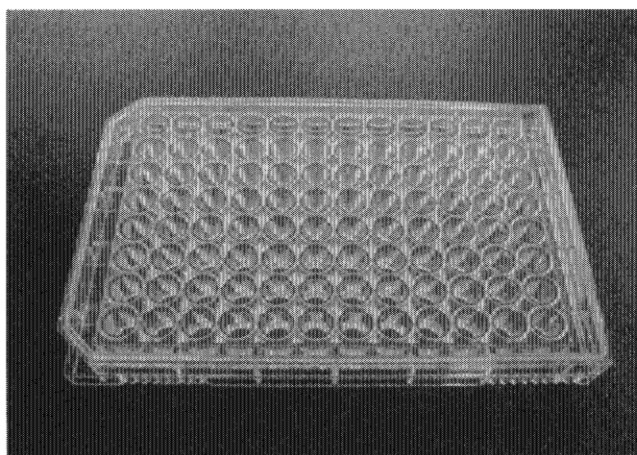


Figure 1 96 wells microplate for TCID₅₀ method

7.22 6 wells plastic plate, for plaque assay with gamma radiation sterilization finish¹⁾ (see Figure 2).

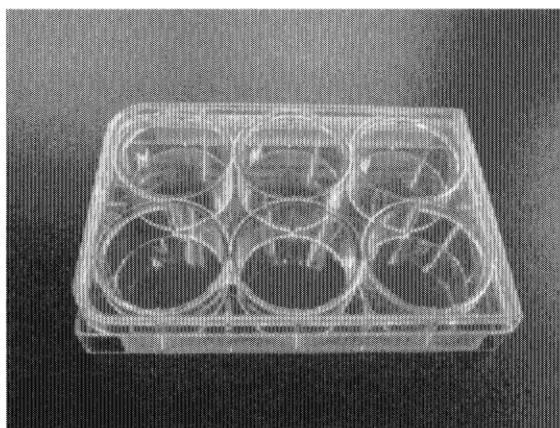


Figure 2 6 wells plastic plate for plaque assay

7.23 Flasks, for cell culture use with an adherent type, a cell culture area of 75 cm² and vent cap. The vent cap can be exchanged abacterial air through 0.2 µm filter and with gamma radiation sterilization finish¹⁾ (see Figure 3).

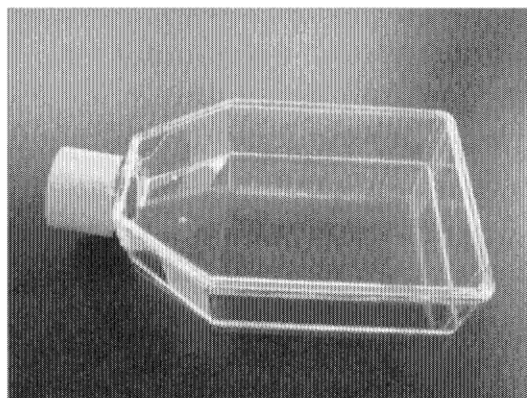


Figure 3 Flask for cell culture use

Note ¹⁾ Flasks with ethylene oxide sterilization finish or sterile flasks may also be used.

7.24 CO₂ incubator, capable of maintaining an atmosphere with 5 % CO₂, at a temperature of $(34 \pm 2) ^\circ\text{C}$ and $(37 \pm 2) ^\circ\text{C}$.

7.25 Incubator, capable of maintaining at a temperature of $(25 \pm 2) ^\circ\text{C}$, $(34 \pm 2) ^\circ\text{C}$ or $(37 \pm 2) ^\circ\text{C}$.

7.26 Centrifuge tube: glass or plastic container used for centrifugal separation.

7.27 Culture container

7.28 Test tube

7.29 Beaker

8 Sterilization of apparatus

Sterilize all glass and plastic apparatus²⁾ which come in contact with cells, chemicals, or test specimen. The sterilization shall be performed by high pressure steam or dry heat.

- High pressure steam sterilization: by an autoclave (**7.1**) at a temperature of $121 ^\circ\text{C}$ and a pressure of 103 kPa for 15 min.
- Dry-heat sterilization: by a dry heat sterilizer (**7.2**) at a temperature of $180 ^\circ\text{C}$ for 30 min or $160 ^\circ\text{C}$ for 2 h.

Note ²⁾ Heat-resistant or sterile plastic apparatus may also be used.

9 Reagent and medium

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

9.1 Water, of grade 3 according to **ISO 3696**, or water which has been refined by one or combination of ion exchange, distillation, reverse osmosis, ultrafiltration etc. that has the quality suitable for analytical needs and can be used for preparation of microbiological medium.

9.2 Eagle's minimum essential medium (EMEM), is available in market. The composition is described in Annex D. If there are any components missing from the composition specified in Annex D, 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 Water (9.1) of 1 000 ml is also sterilized by autoclave.

9.3.3 Dissolve sodium bicarbonate in the sterilized water (9.3.2) of 1 000 ml well.

9.4 Formalin solution, used for cell fixation

9.4.1 Prepare 37 % formaldehyde solution of 100 ml.

9.4.2 Add water (9.1) of 900 ml to 9.4.1.

NOTE : Other solution having the equal performance may be used.

9.5 Methylene blue solution used for cells dyeing

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

- Water (9.1), 800 ml;
- Methylene blue, 0.375 g;
- 1 mol/L sodium hydroxide solutions, 62.5 µl.

9.5.2 Dissolve, mix well, and add water (9.1) to make 1 000 ml.

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 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, put the following materials into the flask:

- Water (9.1), 800 ml;
- Kanamycin sulfate, 60 mg;
- Eagle's minimum essential medium (EMEM), 9.53 g, or RPMI 1640 medium, 10.4 g.

NOTE : RPMI stands for Roswell Park Memorial Institute.

9.7.2 Dissolve, mix well and add water (9.1) to make 1 000 ml.

9.7.3 Sterilize the mixed solution 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 market, add it according to the composition of Annex D 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:

- Water (9.1), 800 ml;
- Kanamycin sulfate, 60 mg;
- Eagle's minimum essential medium, 9.53 g.

9.8.2 Dissolve, mix well and add water (9.1) to make 1 000 ml.

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 bicarbonate solution (9.3) in the solution 9.8.3.

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

9.9 Double concentration 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:

- Water (9.1), 800 ml;
- Kanamycin sulfate, 120 mg;
- Eagle's minimum essential medium, 19.06 g.

9.9.2 Dissolve, mix well and add water (9.1) to make 1 000 ml.

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:

- Water (9.1), 800 ml;
- 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 Dissolve, mix well and add water (9.1) to make 1 000 ml.

9.10.3 Place the solution 9.10.2 into a culture container (7.27), and sterilize by using autoclave (7.1) at a temperature of 121 °C and a pressure of 103 kPa.

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

9.11.1 Prepare a beaker, 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 Sterilize the solution 9.11.2 by using the filter (7.13) with a pore size of 0.22 µm. Divide the solution in test tubes, preserve the solution tubes that are not used immediately in the freezer (7.11) 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), 1 ml.

9.11.5 Dissolve and mix them well.

9.11.6 Divide the solution in test tubes and preserve in the freezer (7.11) 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, put the following materials in the measuring flask:

- 0.01 mol/L phosphate buffered saline [PBS (-)] (9.10), 800 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, mix well, and add 0.01 mol/L phosphate buffered saline [PBS (-)] (9.10) to make 1 000 ml.

9.12.3 Then, sterilize the solution 9.12.2 by using the filter with a pore size of 0.22 µm (7.13).

9.12.4 Divide the solution in test tubes and preserve in the freezer (7.11) 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, put the following materials in the measuring flask:

- Water (9.1), 800 ml;
- DEAE-dextran, 20 g.

9.13.2 Dissolve, mix well and add water (9.1) to make 1 000 ml.

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

This is used for plaque assay, and 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 culture container (7.27) of 1 L, then, put the following materials in the culture container and mix well:

- Double concentration of maintenance medium (9.9), 1 000 ml;
- DEAE-dextran solution (9.13), 10 ml;
- 7.5 % sodium bicarbonate solution (9.3), 40 ml.

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).

9.14.1.3 Put the solution 9.14.1.1 or 9.14.1.2 in the water bath (7.9) at a temperature of 37 °C and keep it until using.

9.14.2 B liquid

9.14.2.1 Prepare a culture container (7.27) of 2 L, then, put the following materials in the culture container and mix well:

- Water (9.1), 1 000 ml;
- Cell culture agar, 15 g.

9.14.2.2 Sterilize mixed solution 9.14.2.1 by using autoclave (7.1) at a temperature of 121 °C and a pressure of 103 kPa for 15 min.

9.14.2.3 Put the solution 9.14.2.2 in the water bath (7.9) at a temperature of 50 °C, and keep it until using.

9.15 SCDLP medium

Use for wash-out and for suppression of agent activity of the treated textile product.