
**Measurement of antiviral activity
on plastics and other non-porous
surfaces**

*Mesure de l'activité antivirale sur les matières plastiques et autres
surfaces non poreuses*

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Published in Switzerland

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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.

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 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 the following URL: www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 61, *Plastics*, Subcommittee SC 6, *Ageing, chemical and environmental resistance*.

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.

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Introduction

Antibacterial-treated porous and non-porous products have been widely accepted and used among general consumers as their new choices to purchase for the additional function, which are different from what traditional materials had in terms of material protection.

Recently, antiviral-treated porous and non-porous products have been also in the market.

The measuring test method of antibacterial activity on non-porous products is described in ISO 22196.

The measuring test method of antibacterial activity on porous products (textiles) is described in ISO 20743.

The measuring test method of antiviral activity on porous products (textiles) is described in ISO 18184.

This document is the test method of antiviral activity on non-porous products. It is written based on ISO 22196 and ISO 18184.

In ISO 22196, the scope has been expanded to include surfaces made of plastics and other non-porous materials, thus this document is intended to be applicable to products such as plastics, coating materials, ceramics, natural and artificial leathers, stainless, rubbers, etc.

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Measurement of antiviral activity on plastics and other non-porous surfaces

WARNING — Handling and manipulation of viruses and host cells which are potentially hazardous requires a high degree of technical competence and may be subject to current national legislation and regulations. Only personnel trained in biological techniques should carry out such tests. Appropriate practices for disinfection, sterilization and personal hygiene must be strictly observed.

1 Scope

This document specifies proper methods for measuring antiviral activity on plastics and other non-porous surfaces of antiviral-treated products against specified viruses. Due to the individual sensitivities, the results of one test virus might not be applicable for other viruses.

2 Normative references

There are no normative references in this document.

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 <http://www.electropedia.org/>

3.1

antiviral

state where the number of infectious virus particles on surfaces of products is reduced

3.2

antiviral agent

agent that reduces the number of infectious virus on surface of products

3.3

antiviral activity

difference in the logarithm of the infectivity titer of virus found on an antiviral-treated product and an untreated product after inoculation with and contact to virus

3.4

cytopathic effect

morphological change or destruction of the host cells as a result of the virus multiplication

3.5

infectivity titer of virus

number of infectious viral particles present per unit volume in a suspension

3.6

plaque

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

**3.7
plaque forming units
PFU**

unit expressed as the concentration of the infectious viral particle per unit volume (ml)

**3.8
plaque assay**

assay to determine the *infectivity titer of virus* (3.5) from PFU by using the series of dilution

4 Materials

4.1 Virus and host cell to be used for the tests

The example species of virus and host cell to be used are shown in [Table 1](#).

Other species of virus and host cells can be used after appropriate validations, as the important virus may differ depending on the 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.

Table 1 — Examples of viruses, virus strains, host cells and media to be used

Virus name	<i>Influenza virus</i>	<i>Feline calicivirus</i>
Virus strain	<i>Influenza A virus</i> (H3N2): A/Hong Kong/8/68: TC adapted ATCC VR-1679	<i>Feline calicivirus</i> ; Strain: F-9 ATCC VR-782
Host cell ^a	MDCK cell (dog kidney cell origin) ATCC CCL-34	CRFK cell (cat kidney cell origin) ATCC CCL-94
Growth medium ^b	EMEM (4.3.8)	RPMI 1640 (4.3.8)
^a The other host cells can be used after appropriate validations regarding the sensitivity against each virus.		
^b The other media can be used after appropriate validations for the growth of cells.		

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4.2 Reagents

Any water used shall be deionized and/or distilled and/or ultra-filtered and/or filtered with RO [reverse osmosis] and have a conductivity of <1 ìS/cm.

All reagents shall be of analytical grade and/or of a grade appropriate for microbiological purposes.

4.3 Culture medium and solutions

4.3.1 General

The culture medium specified below shall be used. The medium may be obtained from commercial suppliers which shall be prepared for use in accordance with the manufacturer's instructions.

4.3.2 Eagle's minimum essential medium (EMEM)

The composition is described in [Annex A](#). If there are any components missing from the composition, they can be added according to [Table A.1](#).

4.3.3 RPMI 1640 medium

The composition is described in [Annex A](#). If there are any components missing from the composition, they can be added according to [Table A.2](#).

4.3.4 7,5 % sodium bicarbonate solution

Select and prepare the solution using one of the two following options:

- Option 1: Prepare a 7,5 % sodium bicarbonate solution by dissolving 75 g of sodium bicarbonate in 1 000 ml of water. Sterilize the solution by using a 0,22 µm membrane filter.
- Option 2: Prepare a 7,5 % sodium bicarbonate solution by sterilizing 75 g of sodium bicarbonate in a culture container with a cap closed tightly in an autoclave. Sterilize 1 000 ml of water in the autoclave. Dissolve the sterilized sodium bicarbonate in the sterilized water well.

If the solution is not intended to be used immediately after preparation, then preserve it at 5 °C to 10 °C. Never use 7,5 % sodium bicarbonate solution that has been kept for longer than one month after preparation.

4.3.5 Formaldehyde solution

Prepare the formaldehyde solution by adding 100 ml of 37 % formaldehyde solution to 900 ml of water. If it is not intended to be used immediately after preparation, preserve it at 20 °C to 25 °C. Never use the formalin solution that has been kept for longer than one month after preparation.

NOTE The other solution for cell fixation can be used after appropriate validation for cell fixation.

4.3.6 Methylene blue solution

Prepare the methylene blue solution by dissolving 0,375 g of the methylene blue and 62,5 µl of 1 N sodium hydroxide solutions in 1 000 ml of water. If it is not intended to be used immediately after preparation, then preserve it at 20 °C to 25 °C. Never use the methylene blue solution that has been kept for longer than one month after preparation.

4.3.7 Inactivated fetal bovine serum (FBS)

Put a frozen cryopreserved fetal bovine serum in a package into a water bath at 37 °C and keep it until it defrosts. Then, raise the temperature of the water bath to 56 °C and keep it for 30 min to inactivate. Divide it into several tubes. Put them in a freezer at a temperature less than -20 °C. Just before using, put it in the water bath at 37 °C and keep it until it defrosts.

4.3.8 Growth medium

Dissolve 9,53 g of the Eagle's minimum essential medium or 10,4 g of RPMI 1640 medium and 60 mg of kanamycin sulfate in 800 ml of water. Add water to make 1 000 ml of solution in total. Sterilize the solution by using a 0,22 µm membrane filter.

When L-glutamine is not included in the EMEM or RPMI 1640 medium purchased in the market, sterilizing in the autoclave may be applied. Then, before use, add L-glutamine as listed in the example of composition in [Annex A](#).

Add 15 ml of 7,5 % sodium bicarbonate solution and 100 ml of the inactivated fetal bovine serum in the solution and mix well. If it is not intended to be used immediately after preparation, then preserve it at 5 °C to 10 °C. Never use a growth medium that has been kept for longer than one month after preparation.

4.3.9 Maintenance medium

Dissolve 9,53 g of the Eagle's minimum essential medium and 60 mg of kanamycin sulfate in 800 ml of water. Add water to make 1 000 ml of solution in total. Sterilize the solution by using a 0,22 µm membrane filter.

When L-glutamine is not included in the EMEM purchased in the market, sterilizing by autoclaving may be applied. Then, before use, add L-glutamine as listed in the example of composition in [Annex A](#).

Add 15 ml of 7,5 % sodium bicarbonate solution in the solution and mix well. If it is not intended to be used immediately after preparation, then preserve it at 5 °C to 10 °C. Never use a maintenance medium that has been kept for longer than one month after preparation.

4.3.10 Double concentration of the maintenance medium

Dissolve 19,06 g of the Eagle's minimum essential medium and 120 mg of kanamycin sulfate in 800 ml of water. Add water till there are 1 000 ml of solution in total. Sterilize the solution by using a 0,22 µm membrane filter. If it is not intended to be used immediately after preparation, then preserve it at 5 °C to 10 °C. Never use a growth medium that has been kept for longer than one month after preparation. When L-glutamine is not included in the EMEM purchased on market, sterilizing by autoclaving could be applied. Then, before use, add L-glutamine as listed in the example of composition in [Annex A](#).

4.3.11 Phosphate buffered saline [PBS (-)]

Prepare PBS (-) by dissolving 8,0 g of sodium chloride, 0,2 g of potassium chloride, 2,9 g of phosphoric acid hydrogen 2 sodium 12 hydrate and 0,2 g of phosphoric acid 2 hydrogen potassium in 1 000 ml of water. Sterilize by autoclaving (see [6.2](#)). If it is not intended to be used immediately after preparation, preserve it at 5 °C to 10 °C. Never use a PBS (-) that has been kept for longer than one month after preparation.

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

4.3.12.1 Dissolve 1,0 g of trypsin derived from pancreas in 100 ml of PBS (-) and mix well for 2 h by using a mixer. Sterilize the solution by using 0,22 µm membrane filter. If it is not intended to be used immediately after preparation, divide the solution in test tubes and preserve them in the freezer at a temperature less than -80 °C. Just before using, put it in the water bath at 37 °C and keep it until it defrosts.

4.3.12.2 Add 1,0 ml of the solution of [4.3.12.1](#) to 9,0 ml of PBS (-) and mix well. Divide the solution in test tubes and preserve them in the freezer at a temperature less than -20 °C. Just before using, put it in the water bath at 37 °C and keep it until it defrosts.

4.3.13 Trypsin EDTA solution¹⁾

Prepare Trypsin EDTA solution by dissolving 2,5 g of trypsin, 0,1 g of kanamycin sulfate, 0,1 g of streptomycin sulfate, 2 mg of amphotericin B and 0,014 mol of EDTA in 1 000 ml of PBS (-). Sterilize the solution by using a 0,22 µm membrane filter. Divide the solution in test tubes and preserve them in the freezer at a temperature less than -20 °C. Just before using, put it in the water bath at 37 °C and keep it until it defrosts.

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

4.3.14 DEAE-dextran solution

Prepare DEAE-dextran solution by dissolving 20 g of DEAE-dextran in 1 000 ml of water. Sterilize the solution by using 0,22 µm membrane filter. If it is not intended to be used immediately after preparation, then preserve it at 5 °C to 10 °C. Never use a DEAE-dextran solution that has been kept for longer than one month after preparation.

1) Trypsin EDTA solution is an example of a product available in the market. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.