

# Rapid Microbiological Methods for Sterility Testing of Regenerative Medical Products in Japan

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# Outline

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- Testing Methods
- RMMs for Regenerative Medical Products

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- Testing Methods
- RMMs for Regenerative Medical Products

# Japanese Pharmacopoeia

## ■ General test

- ✓ 4.06 is Sterility Test


## ■ General information

- ✓ Rapid Microbiological Methods
- ✓ Rapid Counting of Microbes using Fluorescent Staining
- ✓ Rapid Identification of Microorganisms Based on Molecular Biological Method
- ✓ Mycoplasma Testing for Cell Substrates used for the Production of Biotechnological/Biological Products

Japanese Pharmacopoeia 17th Edition

Japanese Pharmacopoeia 17th Edition

- JP17th Edition

[March 7, 2016, the MHLW Ministerial Notification No.64 ([Provisional translation](#) )

<https://www.pmda.go.jp/english/rs-sb-std/standards-development/jp/0019.html>

# 4.06 Sterility Test

- ✓ is harmonized by Pharmacopoeial discussion group (PDG)
- ✓ is Interchangeable in the ICH regions (discussed ICH-Q4B EWG)

## 4.06 Sterility Test

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (♦ ♦).

The test is applied to active pharmaceutical ingredients, preparations or articles which, according to the Pharmacopoeia, are required to be sterile. However, a satisfactory result only indicates that no contaminating micro-organism has been found in the sample examined in the conditions of the test.

### 1. Precautions against microbial contamination

The test for sterility is carried out under aseptic conditions. In order to achieve such conditions, the test environment has to be adapted to the way in which the sterility test is performed. The precautions taken to avoid contamination are such that they do not affect any micro-organisms which are to be revealed in the test. The working conditions in

pink color, the medium may be restored once by heating the containers in a water-bath or in free-flowing steam until the pink color disappears and cooling quickly, taking care to prevent the introduction of non-sterile air into the container. Do not use the medium for a longer storage period than has been validated.

Fluid thioglycollate medium is to be incubated at 30 – 35°C. For products containing a mercurial preservative that cannot be tested by the membrane-filtration method, fluid thioglycollate medium incubated at 20 – 25°C may be used instead of soya-bean casein digest medium provided that it has been validated as described in growth promotion test.

Where prescribed or justified and authorized, the following alternative thioglycollate medium might be used. Prepare a mixture having the same composition as that of the fluid thioglycollate medium, but omitting the agar and the resazurin sodium solution (1 in 1000), sterilize as directed above. The pH after sterilization is  $7.1 \pm 0.2$ . Heat in a water bath prior to use and incubate at 30 – 35°C under anaerobic conditions.

# Rapid Microbiological Methods (1)

## 1. Direct method

Name	Target	Example of detection/measurement device
Solid phase cytometry	Microorganism	Fluorescence microscope, Laser scanning cytometer, etc.
Flow cytometry	Microorganism	Flow cytometer, etc.

## 2. Indirect method

Name	Target	Example of detection/measurement device
Immunological methods	Antigen	Immunochemistry, Micro plate reader, etc.
Nucleic acid amplification	Nucleic acid	Electrophoresis apparatus, Quantitative PCR

# Rapid Microbiological Methods (2)

## 2. Indirect method (Cont.)

Name	Target	Example of detection/measurement device
Bioluminescence	ATP, etc.	luminescence detector, Fluorescence detector, etc.
Micro colony method	Growth (Micro colony)	Fluorescence microscopy etc.
Impedance method	Growth (Electrical characteristic)	Electrodes
Gas measuring method	Growth (Gas production, etc.)	Gas measuring instrument Color change of medium
Fatty acid profiles	Fatty acid	Gas chromatography
Infrared spectroscopy	Cell component	Fourier transformation infrared spectroscope
Mass spectrometry	Cell component	Mass spectrometry
Genetic fingerprinting method	DNA	Electrophoresis apparatus
High throughput sequencing	Nucleic acid	Sequencer, etc.

# Rapid Microbiological Methods (3)

## Validation

- To qualify introduced equipment, a standard component or strain, which represents the target of each method, should be utilized.
  - ✓ Direct measurement; standard strains
  - ✓ Indirect measurement; target bacteria
- To validate a protocol/procedure, it is required to demonstrate that the detection target is a suitable index/indicator for bacterial number or quantity.



# Rapid Counting of Microbes using Fluorescent Staining

This chapter provides rapid methods using fluorescence staining for quantitative estimation of viable microorganisms.

- Fluorescence staining method (ex. CFDA (carboxyfluorescein diacetate)-DAPI (4',6-diamidino-2-phenylindole) double staining)
  - ✓ Microorganisms are stained with fluorescence dye and counted by fluorescence microscope or flow cytometer.
- Microcolony method
  - ✓ Microcolonies in the early stage of colony formation are counted.

# Rapid Identification of Microorganisms Based on Molecular Biological Method

- This chapter provides the methods for the identification or estimation of microorganisms (bacteria and fungi), found in in-process control testes or lot release tests of pharmaceutical products, at the species or genus level based on DNA sequence homology.
- ✓ Rapid methods to identify or estimate microorganisms based on partial sequences or divergent regions of the 16S rRNA gene for bacteria and of the internal transcribed spacer 1 (ITS1) region located between 18S rRNA and 5.8S rRNA for fungi, followed by comparison of the sequences with those in the database.

# Mycoplasma Testing

This chapter describes the currently available methods of mycoplasma testing that should be performed for cell substrates that are used in the manufacture of biotechnological /biological products.

A. Culture methods

B. Indicator cell culture methods

C. Nucleic Acid Amplification test (NAT)

## Validation of NAT for the detection of mycoplasma

- ✓ Specificity
- ✓ Robustness
- ✓ Limit of detection

Acholeplasma laidlawii (ATCC 23206, NBRC 14400)

Mycoplasma arginini (ATCC 23838)

Mycoplasma fermentans (ATCC 19989, NBRC 14854)

Mycoplasma hyorhinis (ATCC 17981, NBRC 14858)

Mycoplasma orale (ATCC 23714, NBRC 14477)

Mycoplasma pneumoniae (ATCC 15531, NBRC 14401)

Mycoplasma salivarium (ATCC 23064, NBRC 14478)

# Outline

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- Testing Methods
- RMMs for Regenerative Medical Products

# Related Guidelines for Regenerative Medical Products

Standard for Biological Raw Materials  
MHLW Public Notice No.210 (2003)

General Principles for the Handling and Use of Cells/Tissue-Based Products  
PFSB/MHLW Notification No.1314 Appendix1 (2000)

Guidelines on Ensuring Quality and Safety of Products Derived from Processed Cell/Tissue

Autologous (2008)

Allogeneic (2008)

Autologous Somatic Stem Cells (2012)  
Autologous iPS-like Cells (2012)

Allogeneic Somatic Stem Cells (2012)  
Allogeneic iPS-like Cells (2012)  
Embryonic Stem Cells (2012)

Points to Consider for the Evaluation of Specific Products

- Implant-type tissue-engineered cartilage for severe nasal deformity in orofacial cleft (2015)
- Allogeneic induced pluripotent stem cells-derived retinal pigment epithelial cells (2014)
- Autologous induced pluripotent stem cells-derived retinal pigment epithelial cells (2013)
- Articular cartilage repair (2010) ▪ Cell sheet for periodontal tissue regeneration (2011)
- Cell sheet for heart failure (2010) ▪ Corneal epithelial cell sheet (2010)
- Corneal endothelial cell sheet (2010)

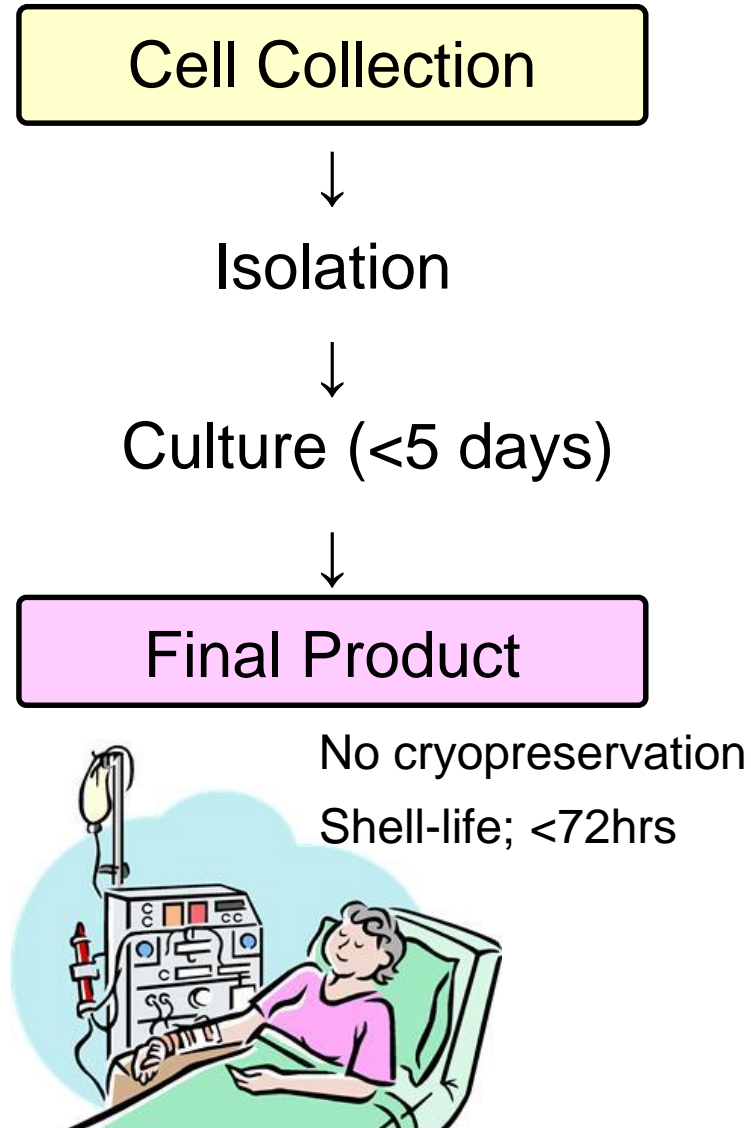
# Q/S GLs: Sterility Test (1)

- Sterility should be ensured throughout the entire manufacturing process by evaluating test samples.
- The sterility (negative results of tests for common bacteria and fungi) of the final product should be demonstrated before its use in a patient.
- If the results of sterility and other tests on the final product can be obtained only after the product is administered to the patient, methods for dealing with the lack of sterility detected after administration should be established beforehand.

## Q/S GLs: Sterility Test (2)

- In such cases, demonstrate by testing that the intermediate products are sterile and that sterility has been strictly maintained in all processes leading to the final product.
- If the test results can be obtained only after administration to the patient, the decision to administer the product will be based on the most recent data.
- However, even in such cases, **the final product shall be tested.**

# RMMs for Regenerative Medical Products



## ■ Limitations

- ✓ Short shelf-life
- ✓ Short manufacturing process

## ■ RMMs advantages

- ✓ Reduce products loss
- ✓ Reduce release time

## ■ RMMs used for

- ✓ In-process control testes
- ✓ Lot release tests



Thank you for  
your attention!

Please visit the PMDA website

<http://www.pmda.go.jp>

<http://www.pmda.go.jp/english/index.html>