

·ISO 10993-10: Biological evaluation of medical devices—Tests for irritation and sensitization

Test Results to be Recorded per Production Unit

At the line production phase, it is required to establish the values of acceptable limits on at least the test items mentioned below and to record the test results of each production unit of plastic containers for pharmaceutical products. In addition, it is desirable to clarify the rationales for setting the values of limits. However, these requirements should not be applied to orally administered preparations except liquid ones.

- 1) Combustion Tests: Residue of ignition, heavy metals. If necessary, the amounts of the specified metals (lead, cadmium, etc.)
- 2) Extraction Tests: pH, ultraviolet absorption spectra, potassium permanganate-reducing substances, foaming, non-volatile residue
- 3) Cytotoxicity Test
- 4) Any other necessary tests for the specific container for aqueous infusions.

12. Preservatives-Effectiveness Tests

The purpose of the tests is to assess microbiologically the efficacy of preservatives in a product. The efficacy of the preservatives is assessed by direct inoculation and mixing of the test strains in the product, and further titration of survivals of the test strains with time.

The efficacy, either due to the action of the ingredients themselves or the additives, must be demonstrated for all injections packaged in multidose containers. The efficacy must also be demonstrated for other dosage forms such as otic, ophthalmic and nasal products. Furthermore, it is necessary to demonstrate the efficacy of oral products such as antacids for multiple dosing. Products are divided into two categories for purpose of testing: products made with aqueous bases, and other products. The first category is subdivided into four subtypes depending on the dosage form.

The microorganisms specified for use in the tests should be representatives of those that might be expected to be found in the environment in which the product is manufactured, used or stored. The designated microorganisms should be supplemented by other microorganisms which have contaminated or might contaminate the product and grown on it by their specific properties or by special manufacturing process. Preservatives must not be used solely to pass GMP for drugs or to reduce viable aerobic counts. The preservative itself is a toxic substance, and it must not be added to the product in amounts which might jeopardize the safety of human beings. Considerations must be given to minimizing the amounts of preservative used.

Products and their Categories

The products have been divided into two categories for these tests. Category I includes products made with aqueous bases, and Category II, those made with nonaqueous bases. Oil-in-water emulsions are considered Category I products, and water-in-oil emulsions Category II. Category I is further divided into four subtypes depending on the dosage forms.

Category IA: Injections and other parenterals including otic and ophthalmic products

Category IB: Topically used products applied to mucous surfaces, including liquids for nasal instillation and inhalants

Category IC: Oral products except for antacids made with aqueous bases

Category ID: Antacids (including solid forms of antacids intended for aqueous constitution)

Category II: All the dosage forms listed under Category I made with nonaqueous bases.

Test Microorganisms and Culture Media

The following strains or those considered to be equivalent are used as the test microorganisms.

Escherichia coli ATCC 8739, NCIMB 8545

Pseudomonas aeruginosa ATCC 9027, NCIMB 8626

Staphylococcus aureus ATCC 6538, NCTC 10788

Candida albicans ATCC 10231, NCPF 3179

Aspergillus niger ATCC 16404, IMI 149007

In addition to the five designated strains, strains which might contaminate products and grow on them during the manufacturing process may be used as test microorganisms. (For example, a suitable strain of the osmophilic yeast *Zygosaccharomyces rouxii* may be used with products containing high concentrations of sugars.) Single-strain challenges rather than mixed cultures should be used. The test strains can be harvested by growth on solid agar medium or liquid medium.

Cultures on agar plate media: Inoculate each of the five test strains on the surface of agar plates or agar slants. For growth of bacteria, use Soybean-Casein Digest Agar Medium, and for yeasts and moulds, use Sabouraud Agar, Glucose-Peptone (GP) Agar or Potato Dextrose Agar Medium. Incubate bacterial cultures at 30°C to 35°C for 18 to 24 hours, the culture of *C. albicans* at 20°C to 25°C for 48 hours and the culture of *A. niger* at 20°C to 25°C for one week. Harvest the cells aseptically using a platinum loop, etc. Suspend in sterile physiological saline or 0.1% peptone water and adjust the viable cell count to about 10⁸ microorganisms per mL. To harvest *A. niger*, suspend in sterile physiological saline or 0.1% peptone water containing 0.05 w/v% of polysorbate 80 with the viable cell count adjusted to about 10⁸ microorganisms per mL. Use these suspensions as the inocula.

Liquid cultures: After culturing each of the four strains except for *A. niger* in a suitable medium, remove the medium by centrifugation. Wash the cells in sterile physiological saline or 0.1% peptone water and resuspend them in the same solution with the vegetative cell or spore count of the inoculum adjusted to about 10⁸ per mL.

When strains other than the five listed above are cultured, select a culture medium suitable for growth of the strain concerned. The cell suspension may also be prepared by a method suitable for that strain. Immediately before use titrate the viable cell count of the inocula obtained by the agar plate or liquid culture method. Calculate the viable cell count per mL (g) of the product immediately after inoculation from the viable cell count obtained. Do not allow the suspension to stand at room temperature for more than one hour.

Test Procedure**(1) Category I products**

Inject each of the cell suspension aseptically into five containers containing the product and mix uniformly. When it is difficult to inject the cell suspension into the container aseptically, transfer 10 to 20 mL of the product aseptically into each of five sterilized original containers or containers made of the same materials, and mix the inoculum. When the product is not sterile, use two additional containers containing the product only as uninoculated controls. A sterile syringe, spatula or glass rod may be used to mix the cell suspension uniformly in the product. The volume of the suspension mixed in the product must not exceed 1/100 of the volume of the product. Generally, the cell suspension is inoculated and mixed so that the concentration of viable cells is 10^5 to 10^6 cells per mL of product. For antacids, the concentration of viable cells in the test preparation is 10^3 to 10^4 cells per mL of product. Incubate these containers between 20°C and 25°C and calculate the viable cell counts at 7, 14, 21 and 28 days. Record any marked changes, such as changes in color or the appearance of a bad odor, when observed in the mixed samples during this time. Express sequential changes in the viable counts as percentages with the count at the start of the test taken as 100. Titration of the viable cell counts is based, in principle, on Pour Plate Methods in "Microbial Limit Tests". Where a specific inactivator of the preservative is available, add a suitable amount of it to the plate. However, it is necessary to confirm that the inactivator has no effect on growth of the microorganism. When the occurrence of the preservative or the product itself affects titration of the viable cell count and there is no suitable inactivator available, titrate the viable cell counts by Membrane Filtration Method in "Microbial Limit Tests".

(2) Category II products

The procedures are the same as those described in Category I products, but special procedures and considerations are required for uniform dispersion of the test microorganism in the product and titration of viable cell counts in the samples.

For semisolid ointment bases, heat the sample to 45°C to 50°C until it becomes oily, add the cell suspension after lowering the temperature and disperse the inoculum uniformly with a sterile glass rod or spatula. To confirm uniform dispersion visually, an indicator (e.g., phenol red) may be incorporated in the mixture which is known to have no bad effect on survival or growth of the test microorganisms. Surfactants may also be added to achieve uniform dispersion, but it is necessary to confirm that the surfactant added has no effect on survival or growth of the test microorganisms and that it does not potentiate the preservative efficacy of the product. For titration of the viable cell count, a surfactant or emulsifier may be added to disperse the test sample uniformly in the recovery medium. Sorbitan monooleate, polysorbate 80 or lecithin may be added to improve miscibility between the liquid medium and semisolid ointments or oils in which test microorganisms were inoculated. These agents serve to inactivate or neutralize many commonly used preservatives.

Interpretation

The criteria for interpretation is determined according to the category to which the product belongs and the type of the dosage form. When the following results are obtained,

the product is considered to be effectively preserved. There is a strong possibility of massive microbial contamination occurring when microorganisms other than the inoculated ones are found in sterile products, and caution is required for the test procedures and the control of manufacturing process of the product.

Category IA: Reduction of more than 0.1% of the viable bacterial count in mixed samples within 14 days from the initial inoculum and the viable cell count remaining at the same level or decreasing further until completion of the test at 28 days after challenge; and the viable count of yeasts and moulds in mixed samples at the same level or less than the inoculum at 14 and 28 days after inoculation.

Category IB: Reduction of more than 1.0% of the viable bacterial count in mixed samples within 14 days from the initial inoculum and the viable count remaining at the same level or decreasing further until completion of the test at 28 days after challenge; and the viable count of yeasts and moulds in mixed samples at the same level or less than the inoculum at 14 and 28 days after inoculation.

Category IC: Reduction of more than 10% of the viable bacterial count in mixed samples within 14 days from the initial inoculum and the viable count remaining at the same level or decreasing further until completion of the test at 28 days after challenge; and the viable count of yeasts and moulds in mixed samples at the same level or less than the inoculum at 14 and 28 days after inoculation.

Category ID: The viable count of bacteria or yeasts and moulds in mixed samples at the same level or less than the inoculum at 14 and 28 days after inoculation.

Table 1. Interpretation criteria by product

Preparation	Micro-organisms	Interpretation criteria	
		After 14 days	After 28 days
Category IA	Bacteria	0.1% of inoculum count or less	Same or less than level after 14 days
	Yeasts/moulds	Same or less than inoculum count	Same or less than level inoculum count
Category IB	Bacteria	1% of inoculum count or less	Same or less than level after 14 days
	Yeasts/moulds	Same or less than inoculum count	Same or less than inoculum count
Category IC	Bacteria	10% of inoculum count or less	Same or less than level after 14 days
	Yeasts/moulds	Same or less than inoculum count	Same or less than inoculum count
Category ID or Category II	Bacteria	Same or less than inoculum count	Same or less than inoculum count
	Yeasts/moulds	Same or less than inoculum count	Same or less than inoculum count

Category II: The viable count of bacteria or yeasts and moulds in mixed samples at the same level or less than the inoculum at 14 and 28 days after inoculation.

The above interpretation criteria are summarized in Table 1. In addition, the bacterial count and the yeast and mould counts for uninoculated controls at 14 and 28 days should be at the same level or less than the count at the initial inoculation.

Culture Media

Culture media and buffer solution used for Preservatives-Effectiveness Tests are described below. Other media may be used if they have similar nutritive ingredients and selective and growth-promoting properties for the microorganisms to be tested.

Soybean Casein Digest Agar Medium

Casein peptone	15.0 g
Soybean peptone	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Water	1,000 mL

Mix all of the components and sterilize at 121°C for 15 – 20 minutes in an autoclave.

pH after sterilization: 7.1 – 7.5.

Sabouraud Glucose Agar Medium

Peptone (animal tissue and casein)	10.0 g
Glucose	40.0 g
Agar	15.0 g
Water	1,000 mL

Mix all of the components and sterilize at 121°C for 15 – 20 minutes in an autoclave.

pH after sterilization: 5.4 – 5.8. Addition of antibiotics not required.

Glucose Peptone (GP) Agar Medium

Glucose	20.0 g
Yeast extract	2.0 g
Magnesium sulfate	0.5 g
Peptone	5.0 g
Monobasic potassium phosphate	1.0 g
Agar	15.0 g
Water	1,000 mL

Mix all of the components and sterilize at 121°C for 15 – 20 minutes in an autoclave.

pH after sterilization: 5.6 – 5.8. Addition of antibiotics not required.

Potato Dextrose Agar Medium

Potato extract	4.0 g
Glucose	20.0 g
Agar	15.0 g
Water	1,000 mL

Mix all of the components and sterilize at 121°C for 15 – 20 minutes in an autoclave.

pH after sterilization: 5.4 – 5.8. Addition antibiotics not required.

0.1% Peptone Water

Peptone	1.0 g
Sodium chloride	8.9 g
Water	1,000 mL

Mix all of the components and sterilize at 121°C for 15 – 20 minutes in an autoclave.

pH after sterilization: 7.2 – 7.4.

13. Sterility Assurance for Terminally Sterilized Pharmaceutical Products

As indicated in the “Terminal Sterilization and Sterilization Indicators”, the pharmaceuticals to which terminal sterilization can be applied, generally must be sterilized so that a sterility assurance level of 10^{-6} or less is obtained. The sterility assurance level of 10^{-6} or less can be proven by using a sterilization process validation based on physical and microbiological methods, but cannot be proven by sterility tests of the sterilized products. This chapter deals with the necessary requirements for the appropriate management of the important control points of the sterilization process for the parametric release of products, without performing sterility tests on products which have been subjected to terminal sterilization (in the case of radiation sterilization, called dosimetric release). Parametric release is a method that can be applied in cases where the sterilization system is clearly defined, important control points are clearly specified, and the sterilization system process can be validated by microbiological methods using appropriate biological indicators.

1. Definitions

The definitions of the terminology used in this chapter are provided below.

1.1 Terminal sterilization

A process whereby a product is sterilized in its final container or packaging, and which permits the measurement and evaluation of quantifiable microbial lethality.

1.2 Validation

A documented procedure for obtaining, recording and interpreting the results needed to show that a process will consistently yield a product complying with predetermined specifications.

1.3 Periodic re-validation

Validation that is regularly performed to reconfirm that a process is consistently yielding a product complying with predetermined specifications. It should confirm that variables and the acceptable ranges are permissible to yield a product consistently of the required quality.

1.4 Facility/equipment qualification

This is to provide evidence that the manufacturing facilities/equipment, measuring equipment, and manufacturing environment control facilities, etc. have been properly selected, correctly installed, and are operated in conformity with the specifications at the time of installation and during operation.

1.5 Operation qualification

This is to provide evidence to confirm physically, chemically and microbiologically that equipment, operated in accordance with its operational instructions, operates as specified and affords a product meeting the specifications.

1.6 Support system for sterilization process

This refers to the facility/equipment that is associated with the sterilization devices, such as the preconditioning and aeration for ethylene oxide sterilization, the steam supply equipment for moist heat sterilization, and the loading devices for radiation sterilization.