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Standards of Accuracy—The capacity tolerances for volumetric flasks, transfer pipets, and burets are those accepted by the National Institute of Standards and Technology (Class A),[1] as indicated in the *accompanying tables*. Use Class A volumetric apparatus unless otherwise specified in the individual monograph. For plastic volumetric apparatus the accepted capacity tolerances are Class B.[2]

The capacity tolerances for measuring (i.e., "graduated") pipets of up to and including 10-mL capacity are somewhat larger than those for the corresponding sizes of transfer pipets, namely, 10, 20, and 30 µL for the 2-, 5-, and 10-mL sizes, respectively.

Transfer and measuring pipets calibrated "to deliver" should be drained in a vertical position and then touched against the wall of the receiving vessel to drain the tips. Volume readings on burets should be estimated to the nearest 0.01 mL for 25- and 50-mL burets, and to the nearest 0.005 mL for 5- and 10-mL burets. Pipets calibrated "to contain" are called for in special cases, generally for measuring viscous fluids like syrups; however, a volumetric flask may be substituted for a "to contain" pipet. In such cases, the pipet or flask should be washed clean, after draining, and the washings added to the measured portion.

Volumetric Flasks

Designated volume, mL	10	25	50	100	250	500	1000
Limit of error, mL	0.02	0.03	0.05	0.08	0.12	0.15	0.30
Limit of error, %	0.20	0.12	0.10	0.08	0.05	0.03	0.0

Transfer Pipets

Designated volume, mL	1	2	5	10	25	50	100
Limit of error, mL	0.006	0.006	0.01	0.02	0.03	0.05	0.08
Limit of error, %	0.60	0.30	0.20	0.20	0.12	0.10	0.08

Burets

Designated volume, mL	10 ("micro" type)	25	50
Subdivisions, mL	0.02	0.10	0.10
Limit of error, mL	0.02	0.03	0.05

⟨41⟩ WEIGHTS AND BALANCES

The intent of this section is to bring the requirements for weights into conformity with American National Standard ANSI/ASTM E617, "Laboratory Weights and Precision Mass Standards." This standard is incorporated by reference and should be consulted for full descriptions and information on the tolerances and construction of weights.[1]

Pharmacopeial tests and assays require balances that vary in capacity, sensitivity, and reproducibility. Unless otherwise specified, when substances are to be "accurately weighed" for *Assay* the weighing is to be performed with a weighing device whose measurement uncertainty (random plus systematic error) does not exceed 0.1% of the reading. Measurement uncertainty is satisfactory if three times the standard deviation of not less than ten replicate weighings divided by the amount weighed, does not exceed 0.001. Unless otherwise specified, for titrimetric limits tests, the weighing shall be performed to provide the number of significant figures in the weight of the analyte that corresponds to the number of significant figures in the concentration of the titrant.

The class designations below are in order of increasing tolerances.

Class 1.1 weights are used for calibration of low-capacity, high-sensitivity balances. They are available in various denominations from 1 to 500 mg. The tolerance for any denomination in this class is 5 µg. They are recommended for calibration of balances using optical or electrical methods for accurately weighing quantities below 20 mg.

Class 1 weights are designated as high-precision standards for calibration. They may be used for weighing accurately quantities below 20 mg. (For weights of 10 g or less, the requirements of class 1 are met by *USP XXI* class M.)

Class 2 weights are used as working standards for calibration, built-in weights for analytical balances, and laboratory weights for routine analytical work. (The requirements of class 2 are met by *USP XXI* class S.)[2]

Class 3 and class 4 weights are used with moderate-precision laboratory balances. (Class 3 requirements are met by *USP XXI class S-1; class 4 requirements are met by USP XXI* class P.)[2]

A weight class is chosen so that the tolerance of the weights used does not exceed 0.1% of the amount weighed. Generally, class 2 may be used for quantities greater than 20 mg, class 3 for quantities of greater than 50 mg, and class 4 for quantities of greater than 100 mg. Weights should be calibrated periodically, preferably against an absolute standard weight.

[1] See "Testing of Glass Volumetric Apparatus," N.B.S. Circ. 602, April 1, 1959, and NTIS COM-73-10504, National Technical Information Service.

[2] See ASTM E 288, Fed. Spec. NNN-F-289, and ISO Standard 384.

[1] Copies of ASTM Standard E 617-81 (Reapproved 1985) may be obtained from the American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103.

[2] Note that the designations S and P no longer designate weight classes but rather weight grades, that is, design limitations such as range of density of materials, surface area, surface finish, corrosion resistance, and hardness.

Microbiological Tests

⟨51⟩ ANTIMICROBIAL EFFECTIVENESS TESTING

Antimicrobial preservatives are substances added to nonsterile dosage forms to protect them from microbiological growth or from microorganisms that are introduced inadvertently during or subsequent to the manufacturing process. In the case of sterile articles packaged in multiple-dose containers, antimicrobial preservatives are added to inhibit the growth of microorganisms that may be introduced from repeatedly withdrawing individual doses.

Antimicrobial preservatives should not be used as a substitute for good manufacturing practices or solely to reduce the viable microbial population of a nonsterile product or control the presterilization bioburden of multidose formulations during manufacturing. Antimicrobial preservatives in compendial dosage forms meet the requirements for *Added Substances* under *Ingredients and Processes* in the *General Notices*.

All useful antimicrobial agents are toxic substances. For maximum protection of patients, the concentration of the preservative shown to be effective in the final packaged product should be below a level that may be toxic to human beings.

The concentration of an added antimicrobial preservative can be kept at a minimum if the active ingredients of the formulation possess an intrinsic antimicrobial activity. Antimicrobial effectiveness, whether inherent in the product or whether produced because of the addition of an antimicrobial preservative, must be demonstrated for all injections packaged in multiple-dose containers or for other products containing antimicrobial preservatives. Antimicrobial effectiveness must be demonstrated for multiple-dose topical and oral dosage forms and for other dosage forms such as ophthalmic, otic, nasal, irrigation, and dialysis fluids (see *Pharmaceutical Dosage Forms* ⟨1151⟩).

This chapter provides tests to demonstrate the effectiveness of antimicrobial protection. Added antimicrobial preservatives must be declared on the label. The tests and criteria for effectiveness apply to a product in the original, unopened container in which it was distributed by the manufacturer.

Change to read:

PRODUCT CATEGORIES

For the purpose of testing, compendial articles have been divided into ▲four▲USP25 two categories (see *Table 1*). ▲▲USP25 The criteria of antimicrobial effectiveness for these products are a function of the route of administration.▲ ▲USP25

Table 1. Compendial Product Categories.

Category	Product Description
▲1▲USP25	Injections, other parenterals including emulsions, otic, sterile nasal products, and ophthalmic products made with aqueous bases or vehicles.
▲2▲USP25	Topically used products made with aqueous bases or vehicles, nonsterile nasal products, and emulsions, including those applied to mucous membranes.
▲3▲USP25	Oral products ▲other than antacids,▲USP25 made with aqueous bases or vehicles.
▲4▲USP25	▲Antacids made with an aqueous base.▲ USP25

TEST ORGANISMS

Use cultures of the following microorganisms[1]: *Candida albicans* (ATCC No. 10231), *Aspergillus niger* (ATCC No. 16404), *Escherichia coli* (ATCC No. 8739), *Pseudomonas aeruginosa* (ATCC No. 9027), and *Staphylococcus aureus* (ATCC No. 6538). The viable microorganisms used in the test must not be more than five passages removed from the original ATCC culture. For purposes of the test, one passage is defined as the transfer of organisms from an established culture to fresh medium. All transfers are counted. In the case of organisms maintained by seed lot techniques, each cycle of freezing, thawing, and revival in fresh medium is taken as one transfer. A seed stock technique should be used for long-term storage of cultures. Cultures received from the ATCC should be resuscitated according to directions. If grown in broth, the cells are pelleted by centrifugation. Resuspend in 1/20th the volume of fresh maintenance broth, and add an equal volume of 20% (v/v in water) sterile glycerol. Cells grown on agar may be scraped from the surface into the 10% glycerol broth. Dispense small aliquots of the suspension into sterile vials. Store the vials in liquid nitrogen or in a mechanical freezer at no more than -50°. When a fresh seed stock vial is required, it may be removed and used to inoculate a series of working cultures. These working cultures may then be used periodically (each day in the case of bacteria and yeast) to start the inoculum culture.

Change to read:[1] Available from American Type Culture Collection, ▲10801 University Boulevard, Manassas, VA 20110-2209 (http://www.atcc.org).▲USP25

MEDIA

All media used in the test must be tested for growth promotion. Use the microorganisms indicated above under *Test Organisms.*

PREPARATION OF INOCULUM

Preparatory to the test, inoculate the surface of a suitable volume of solid agar medium from a recently revived stock culture of each of the specified microorganisms. The culture conditions for the inoculum culture are described in Table 2 in which the suitable media are Soybean-Casein Digest or Sabouraud Dextrose Agar Medium (see *Microbial Limits Testing* ⟨61⟩).

To harvest the bacterial and *C. albicans* cultures, use sterile saline TS, washing the surface growth, collecting it in a suitable vessel, and adding sufficient sterile saline TS to obtain a microbial count of about 1×10^8 colony-forming units (cfu) per mL. To harvest the cells of *A. niger*, use sterile saline TS containing 0.05% of polysorbate 80, and add sufficient sterile saline TS to obtain a count of about 1×10^8 cfu per mL.

Alternatively, the stock culture organisms may be grown in a suitable liquid medium (i.e., Soybean-Casein Digest Broth or Sabouraud Dextrose Broth) and the cells harvested by centrifugation, then washed and resuspended in sterile saline TS to obtain a microbial count of about 1×10^8 cfu per mL.

[NOTE—The estimate of inoculum concentration may be performed by turbidimetric measurements for the challenge microorganisms. Refrigerate the suspension if it is not used within 2 hours.]

Determine the number of cfu per mL in each suspension, using the conditions of media and microbial recovery incubation times listed in Table 2 to confirm the initial cfu per mL estimate. This value serves to calibrate the size of inoculum used in the test. The bacterial and yeast suspensions are to be used within 24 hours of harvest, but the fungal preparation may be stored under refrigeration for up to seven days.

Change to read:

PROCEDURE

▲ ▲USP25 The test can be conducted either in five original containers if sufficient volume of product is available in each container and the product container can be entered aseptically (i.e., needle and syringe through an elastomeric rubber stopper), or in five sterile, capped bacteriological containers of suitable size into which a sufficient volume of product has been transferred. Inoculate each container with one of the prepared and standardized inoculum, and mix. The volume of the suspension inoculum used is between 0.5% and 1.0% of the volume of the product. The concentration of test microorganisms that is added to the product ▲(Categories 1, 2, and 3)▲USP25 are such that the final concentration of the test preparation after inoculation is between 1×10^5 and 1×10^6 cfu per mL of the product. ▲For Category 4 products (antacids) the final concentration of the test preparation after inoculation is between 1×10^3 and 1×10^4 cfu per mL of the product.▲USP25

The initial concentration of viable microorganisms in each test preparation is estimated based on the concentration of microorganisms in each of the standardized inoculum as determined by the plate-count method.

Incubate the inoculated containers at 22.5 ± 2.5°. Sample each container at the appropriate intervals specified in Table 3. Record any changes observed in appearance at these intervals. Determine by the plate-count procedure the number of cfu present in each test preparation for the applicable intervals (see *Procedure* under *Microbial Limit Tests* ⟨61⟩). Incorporate an inactivator (neutralizer) of the specific antimicrobial in the plate count or in the appropriate dilution prepared for plating. These conditions are determined in the validation study for that sample based upon the conditions of media and microbial recovery incubation times listed in *Table 2*. Using the calculated concentrations of cfu per mL present at the start of the test, calculate the change in $\log_{10}$ values of the concentration of cfu per mL for each microorganism at the applicable test intervals, and express the changes in terms of log reductions.

▲ ▲USP25

Table 2. Culture Conditions for Inoculum Preparation

Organism	Suitable Medium	Incubation Temperature	Inoculum Incubation Time	Microbial Recovery Incubation Time
Escherichia coli (ATCC No. 8739)	Soybean-Casein Digest Broth; Soybean-Casein Digest Agar	32.5 ± 2.5°	18 to 24 hours	3 to 5 days
Pseudomonas aeruginosa (ATCC No. 9027)	Soybean-Casein Digest Broth; Soybean-Casein Digest Agar	32.5 ± 2.5°	18 to 24 hours	3 to 5 days
Staphylococcus aureus (ATCC No. 6538)	Soybean-Casein Digest Broth; Soybean-Casein Digest Agar	32.5 ± 2.5°	18 to 24 hours	3 to 5 days
Candida albicans (ATCC No. 10231)	Sabouraud Dextrose Agar; Sabouraud Dextrose Broth	22.5 ± 2.5°	44 to 52 hours	3 to 5 days
Aspergillus niger (ATCC No. 16404)	Sabouraud Dextrose Agar; Sabouraud Dextrose Broth	22.5 ± 2.5°	6 to 10 days	3 to 7 days

CRITERIA FOR ANTIMICROBIAL EFFECTIVENESS

The requirements for antimicrobial effectiveness are met if the criteria specified under *Table 3* are met (see *Significant Figures and Tolerances* under *General Notices*). No increase is defined as not more than 0.5 $\log_{10}$ unit higher than the previous value measured.

Table 3. Criteria for Tested Microorganisms

For Category ▲1▲USP25 Products	
Bacteria:	Not less than 1.0 log reduction from the initial calculated count at 7 days, not less than 3.0 log reduction from the initial count at 14 days, and no increase from the 14 days' count at 28 days.
Yeast and Molds:	No increase from the initial calculated count at 7, 14, and 28 days.
For Category ▲2▲USP25 Products	
Bacteria:	Not less than 2.0 log reduction from the initial count at 14 days, and no increase from the 14 days' count at 28 days.
Yeast and Molds:	No increase from the initial calculated count at 14 and 28 days.
For Category ▲3▲USP25 Products	
Bacteria:	Not less than 1.0 log reduction from the initial count at 14 days, and no increase from the 14 days' count at 28 days.
Yeast and Molds:	No increase from the initial calculated count at 14 and 28 days.
For Category ▲4▲USP25 Products	
Bacteria, Yeast, and Molds:	No increase from the initial calculated count at 14 and 28 days.

⟨55⟩ BIOLOGICAL INDICATORS—RESISTANCE PERFORMANCE TESTS

Total Viable Spore Count—Remove three specimens of the relevant biological indicator from their original individual containers. Pulp the paper into component fibers by placing the test specimens in a sterile 250-mL cup of a suitable blender containing 100 mL of chilled sterilized Purified Water and blending for 3 to 5 minutes to achieve a homogeneous suspension. Transfer a 10-mL aliquot of the suspension to a sterile, screw-capped 16- × 125-mm tube. For *Biological Indicator for Steam Sterilization, Paper Carrier*, heat the tube containing the suspension in a water bath at 95° to 100° for 15 minutes, starting the timing when the temperature reaches 95°. For *Biological Indicator for Dry-Heat Sterilization, Paper Carrier*, and for *Biological Indicator for Ethylene Oxide Sterilization, Paper Carrier*, heat the tube containing the suspension in a water bath at 80° to 85° for 10 minutes, starting the timing when the temperature reaches 80°. Cool rapidly in an ice water bath at 0° to 4°. Transfer two 1-mL aliquots to suitable tubes, and make appropriate serial dilutions in sterilized Purified Water, the dilutions being selected as calculated to yield preferably 30 to 300 colonies, but not less than 6, on each of a pair of plates when treated as described below. Where the biological indicator has a low spore concentration, it may be necessary to modify the dilution series and to use more plates at each dilution. Prepare a separate series of plates for each aliquot. Place 1.0 mL of each selected dilution in each of two 15- × 100-mm Petri dishes. Within 20 minutes, add to each plate 20 mL of *Soybean-Casein Digest Agar Medium* (see *Microbial Limit Tests* ⟨61⟩) that has been melted and cooled to 45° to 50°. Swirl to attain a homogeneous suspension, and allow to solidify. Incubate the plates in an inverted position at 55° to 60° for *Biological Indicator for Steam Sterilization, Paper Carrier*, and at 30° to 35° for *Biological Indicator for Ethylene Oxide Sterilization, Paper Carrier*, and for *Biological Indicator for Dry-Heat Sterilization, Paper Carrier*, or at the optimal recovery temperature specified by the manufacturer, and examine the plates after 24 and 48 hours, recording for each plate the number of colonies, and using the number of colonies after 48 hours to calculate the results. Calculate the average number of spores per specimen from the results, using the appropriate dilution factor. The test is valid if the log number of spores per Carrier at 48 hours is equal to or greater than the log number after 24 hours in each case. For *Biological Indicators for Steam Sterilization, Self-Contained*, aseptically remove the spore strip from the container, and proceed as directed for *Biological Indicator for Steam Sterilization, Paper Carrier*.

D Value Determination—For all tests described in this section, handle each test specimen with aseptic precautions, using sterilized equipment where applicable.

Apparatus—For *Biological Indicator for Dry-Heat Sterilization, Paper Carrier*, use an apparatus of known thermodynamic characteristics that has been validated for compliance with the requirements for safety[1] and performance,[2] that consists of a sterilizing chamber equipped with a means of heating the contained air, preferably electrically rather than gas fired, and that has adequate movement of the

[1] Safety includes design to prevent electric shock or gas exposition and burns, where operators can wear protective clothing and gloves against burns from touching hot surfaces.

[2] Descriptions of different types of dry-heat sterilizing equipment and detailed guidelines for determining, monitoring, and controlling the operating parameters have been published by the Health Industry Manufacturers Association (HIMA) in Report No. 78-1.7, *Operator Training for Dry Heat Sterilizing Equipment*, and by the Parenteral Drug Association, Inc., (PDA) in Technical Report No. 3, *Validation of Dry Heat Processes used for Sterilization and Depyrogenation*.