(2) After filtration of sample solution into the apparatus to which the membrane filters are fitted, 100 mL of each medium is added to the apparatus itself.

I-6. Culture and observation

Incubate thioglycollate medium I for sterility test at between 30°C and 35°C and soybean-casein digest medium at between 20°C and 25°C for not less than 14 days. Observe the test containers for growth of microorganisms at least once between the fifth and ninth day, two times in total. If the sample makes the medium turbid so that the presence or absence of microbial growth can not be determined readily or in other case of need, transfer suitable portions of the medium to fresh containers of the same medium, incubate the transfer containers at the same temperature for not less than 7 days and examine the medium for growth.

I-7. Interpretation

If no evidence of microbial growth is found as a result of the above-mentioned test, the product tested meets the requirement of the Sterility Test. If microbial growth is found, the product tested fails to meet the requirement of the Sterility Test. However, provided that various factors and/or properties of the contaminant(s) suggest that the sterility test itself was inadequate, the test is repeated. If no evidence of microbial growth is found in the repeat test the product complies with the Sterility Test. If microbial growth is found in the repeat test the product does not comply with the Sterility Test.

II. Direct transfer method

This is the method by which the entire content or a portion of the content of a sample container is transferred directly to the culture medium and incubated. Usually, this method is applied for medicines to which the membrane filtration method can not be applied or for which the application of the direct transfer method, rather than the Membrane filtration method, is rational.

II-1. Opening of containers

Usually, proceed as directed for the Membrane filtration method.

II-2. Preparation of sample solution

Usually, proceed as directed for the Membrane filtration method. In the case of an insoluble medicine, the product is suspended or crushed in a suitable manner and used as a sample.

II-3. Quantities of sample solution to be transferred

For a liquid medicine and for a solid medicine to be administered after dissolving or suspending, unless otherwise specified, take a quantity of the product specified in Table 3. The volume of the sample should be not more than 10% of the volume of the medium. For a hydrophobic medicine, transfer the quantity prescribed in Table 4 according to the amount stated on the label into 200 mL each of thioglycollate medium I for sterility test and soybean-casein digest medium.

II-4. Culture and observation

Proceed as directed for the Membrane filtration method.

II-5. Interpretation

Proceed as directed for the Membrane filtration method.

55. Sulfate Limit Test

The Sulfate Limit Test is a limit test for sulfate contained in drugs.

In each monograph, the permissible limit for sulfate (as SO_4) is described in terms of percentage (%) in parentheses.

Procedure

Unless otherwise specified, transfer the quantity of the sample, directed in the monograph, to a Nessler tube, dissolve it in sufficient water, and add water to make 40 mL. Add 1 mL of dilute hydrochloric acid and water to make 50 mL, and use this solution as the test solution. Transfer the volume of 0.005 mol/L sulfuric acid VS, directed in the monograph, to another Nessler tube, add 1 mL of dilute hydrochloric acid and water to make 50 mL, and use this solution as the control solution. When the test solution is not clear, filter both solutions according to the same procedure.

Add 2 mL of barium chloride TS to the test solution and to the control solution, mix well, and allow to stand for 10 minutes. Compare the white turbidity produced in both solutions against a black background by viewing downward or transversely.

The turbidity produced in the test solution is not thicker than that of the control solution.

56. Test for Acid-neutralizing Capacity of Gastrointestinal Medicines

The Test for Acid-neutralizing Capacity of Gastrointestinal Medicines is a test to determine the acid-neutralizing capacity of a medicine, as a crude material or preparation, which reacts with the stomach acid and exercises an acid control action in the stomach. When performing the test according to the following procedure, the acid-neutralizing capacity of a crude material is expressed in terms of the amount (mL) of 0.1 mol/L hydrochloric acid VS consumed per g of the material, and that of a preparation is expressed by the amount (mL) of 0.1 mol/L hydrochloric acid VS consumed per dose per day (when the daily dose varies, the minimum dose is used).

Preparation of sample

A crude material and a solid preparation which conforms to Powders in the General Rules for Preparations: may be used, without any treatment, as the sample. Preparations in dose-unit packages: weigh accurately the content of not less than 20 packages, calculate the average mass of the content for a daily dose, mix uniformly, and use the mixture as the sample. Granules in dose-unit packages and other solid preparations which do not conform to Powders in the General Rules for Preparations: weigh accurately the content of not less than 20 packages, calculate the average mass of the content for a daily dose, powder it, and use as the sample. Granules not in dose-unit packages and other solid preparations which do not conform to Powders in the General Rules for Preparations: take not less than 20 doses, powder it, and use as the sample. Capsules and tablets: take not less

than 20 doses, weigh accurately, calculate the average mass for a daily dose, powder it, and use as the sample. Liquid preparations: shake well, and use as the sample.

Procedure

Take an amount of the sample so that '*a*' in the equation falls between 20 mL and 30 mL, and perform the test.

Accurately weigh the sample of the crude material or preparation, and place it in a glass-stoppered, 200-mL flask. Add exactly 100 mL of 0.1 mol/L hydrochloric acid VS, stopper tightly, shake at 37 ± 2°C for 1 hour, and filter. Take precaution against gas to be generated on the addition of 0.1 mol/L hydrochloric acid VS, and stopper tightly. After cooling, filter the solution again, if necessary. Pipet 50 mL of the filtrate, and titrate the excess hydrochloric acid with 0.1 mol/L sodium hydroxide VS (pH Determination, end point: pH 3.5). Perform a blank determination.

For liquid preparations, pipet the sample in a 100-mL volumetric flask, add water to make 45 mL, then add exactly 50 mL of 0.1 mol/L hydrochloric acid VS while shaking. Add water again to make the solution 100 mL. Transfer the solution to a glass-stoppered, 200-mL flask, wash the residue with 20.0 mL of water, stopper tightly, shake at 37 ± 2°C for 1 hour, and filter. Pipet 60 mL of the filtrate, and titrate the excess hydrochloric acid with 0.1 mol/L sodium hydroxide VS (pH Determination, end point: pH 3.5). Perform a blank determination.

Acid-neutralizing capacity (amount of 0.1 mol/L hydrochloric acid VS consumed per g or daily dose) (mL)

$$= (b - a)f \times 2 \times \frac{t}{s}$$

a: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed
b: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed in the blank determination
f: The molarity coefficient of 0.1 mol/L sodium hydroxide VS
t: 1000 mg of crude material or daily dose of preparation (in mg of solid preparation, mL of liquid preparation)
s: Amount of the sample (in mg of crude material and solid preparation, mL of liquid preparation)

57. Test for Glass Containers for Injections

The glass containers for injections do not interact physically or chemically with the contained medicament to alter any property or quality, can protect the contained medicament from the invasion of microbes by means of perfect sealing or other suitable process, and meet the following requirements. The surface-treated container for aqueous infusion is made from glass which meets the requirements for the soluble alkali test for a container not to be fused under method 1.

(1) The containers are colorless or light brown and transparent, and have no bubbles which interfere with the test for foreign material specified in General Rules for Preparations, Injections (12).

(2) Multiple-dose containers are closed by rubber stoppers or any other suitable stoppers. The stoppers permit penetration of an injection needle without detachment of fragments, and upon withdrawal of the needle, they reclose the containers immediately to prevent external contamination, and also do not interact physically or chemically with the contained medicaments.

Containers intended for aqueous infusions are closed by rubber stoppers meeting the requirements for Rubber Closure for Aqueous Infusions.

(3) Soluble alkali test—The testing methods may be divided into the following two methods according to the type of container or the dosage form of the medicament.

(i) Method 1: This method is applied to containers to be fused, or containers not to be fused except containers for aqueous infusions with a capacity exceeding 100 mL.

Rinse thoroughly the inside and outside of the containers to be tested with water, dry, and roughly crush, if necessary. Transfer 30 to 40 g of the glass to a steel mortar, and crush. Sieve the crushed glass through a No. 12 (1400 μm) sieve. Transfer the portion retained on the sieve again to the steel mortar, and repeat this crushing procedure until 2/3 of the amount of powdered glass has passed through a No. 12 (1400 μm) sieve. Combine all portions of the glass powder passed through a No. 12 (1400 μm) sieve, shake the sieve in a horizontal direction for 5 minutes with slight tapping at intervals using No. 18 (850 μm) and No. 50 (300 μm) sieves. Transfer 7 g of the powder, which has passed through a No. 18 (850 μm) sieve but not through a No. 50 (300 μm) sieve to a No. 50 (300 μm) sieve, immerse it in a suitable container filled with water, and wash the contents with gentle shaking for 1 minute. Rinse again with ethanol (95) for 1 minute, dry the washed glass powder at 100°C for 30 minutes, and allow to cool in a desiccator (silica gel). Transfer exactly 5.0 g of the powder thus prepared to a 200-mL conical flask of hard glass, add 50 mL of water, and gently shake the flask so that the powder disperses on the bottom of the flask evenly. Cover the flask with a small beaker of hard glass or a watch glass of hard glass, then heat it in boiling water for 2 hours, and immediately cool to room temperature. Decant the water from the flask into a 250-mL conical flask of hard glass, wash well the residual powdered glass with three 20-mL portions of water, and add the washings to the decanted water. Add 5 drops of bromocresol green-methyl red TS and titrate with 0.01 mol/L sulfuric acid VS until the color of the solution changes from green through slightly grayish blue to slightly grayish red purple. Perform a blank determination in the same manner, and make any necessary correction.

The quantity of 0.01 mol/L sulfuric acid VS consumed does not exceed the following quantity, according to the type of containers.

Containers to be fused	0.30 mL
Containers not to be fused (including injection syringes used as containers)	2.00 mL

(ii) Method 2: This method is applied to containers not to be fused for aqueous infusions with a capacity exceeding 100 mL.

Rinse thoroughly the inside and outside of the containers to be tested with water, and dry. Add a volume of water equivalent to 90% of the overflow capacity of the container, cover it with a small beaker of hard glass or close tightly with a suitable stopper, heat in an autoclave at 121°C for 1 hour, and allow to stand until the temperature falls to room