three endotoxin concentrations to generate the standard curve within the range of endotoxin concentrations indicated by the instructions for the lysate reagent used. Perform the test using at least three replicates of each standard endotoxin concentration according to the optimal conditions for the lysate reagent used (with regard to volume ratios, incubation time, temperature, pH, etc.). If the desired range is greater than two logs, additional standards should be included to bracket each log increase in the range of the standard curve.

The absolute value of the correlation coefficient, $|r|$, must be greater than or equal to 0.980 for the range of endotoxin concentrations set up.

If the test is not valid, repeat the test after verifying the test conditions.

The test for assurance of criteria for the standard curve must be repeated when any condition changes, which is likely to influence the result of the test.

(ii) Test for interfering factors

Prepare solutions A, B, C and D according to Table 4. Perform the test on these solutions following the optimal conditions for the lysate reagent used (with regard to volume of sample solution and lysate TS, volume ratio of sample solution to lysate TS, incubation time, etc.).

The test for interfering factors must be repeated when any condition changes, which is likely to influence the result of the test.

Table 4

Solution	Concentration of added endotoxin in each solution	Solution to which endotoxin is added	Number of test tubes or wells
A	0	Sample solution*1	Not less than 2
B	Middle concentration of the standard curve*2	Sample solution*1	Not less than 2
C	At least 3 concentrations*3	Water for BET	Each not less than 2
D	0	Water for BET	Not less than 2

*1 The sample solution may be diluted not to exceed the MVD.
*2 Add the Standard Endotoxin Solution to the sample solution to make an endotoxin concentration at or near the middle of the standard curve.
*3 The concentrations used in the test for the (i) Assurance of criteria for the standard curve of (3) Preparatory testing.

The test is not valid unless the following conditions are met.

1: The absolute value of the correlation coefficient of the standard curve generated using solution C is greater than or equal to 0.980.
2: The result with solution D does not exceed the limit of the blank value required in the description of the lysate reagent employed, or it is less than the endotoxin detection limit of the lysate reagent employed.

Calculate the recovery of the endotoxin added to solution B from the concentration found in solution B after subtracting the endotoxin concentration found in solution A.

When the recovery of the endotoxin added to solution B is within 50% to 200%, the sample solution under test is considered to be free of interfering factors.

When the endotoxin recovery is out of the specified range, the sample solution under test is considered to contain interfering factors. If the sample under test does not comply with the test, repeat the test using a greater dilution, not exceeding the MVD. Furthermore, interference of the sample solution or diluted sample solution not to exceed the MVD may be eliminated by suitable treatment, such as filtration, neutralization, dialysis or heat treatment.

(4) Assay

(i) Procedure

Prepare solutions A, B, C and D according to Table 4, and follow the procedure described in (ii) Test for interfering factors of (3) Preparatory testing.

(ii) Calculation of endotoxin concentration

Calculate the endotoxin concentration of solution A using the standard curve generated with solution C. The test is not valid unless all the following requirements are met.

1: The absolute value of the correlation coefficient of the standard curve generated using solution C is greater than or equal to 0.980.
2: The endotoxin recovery, calculated from the concentration found in solution B after subtracting the concentration of endotoxin found in solution A, is within the range of 50% to 200%.
3: The result with solution D does not exceed the limit of the blank value required in the description of the lysate reagent employed, or it is less than the endotoxin detection limit of the lysate reagent employed.

(iii) Interpretation

The sample complies with the Bacterial Endotoxins Test if the endotoxin concentration of the sample calculated from the endotoxin concentration of solution A meets the requirement of the endotoxin limit (in EU per mL, in EU per mg or mEq or in EU per Unit) specified in the individual monograph.

7. Boiling Point and Distilling Range Test

The Boiling Point and Distilling Range are determined by Method 1 or Method 2 as described herein, unless otherwise specified. Boiling point is the temperature shown between when the first 5 drops of distillate leave the tip of the condenser and when the last liquid evaporates from the bottom of the flask. Distilling range test is done to determine the volume of the distillate which has been collected in the range of temperature directed in the monograph.

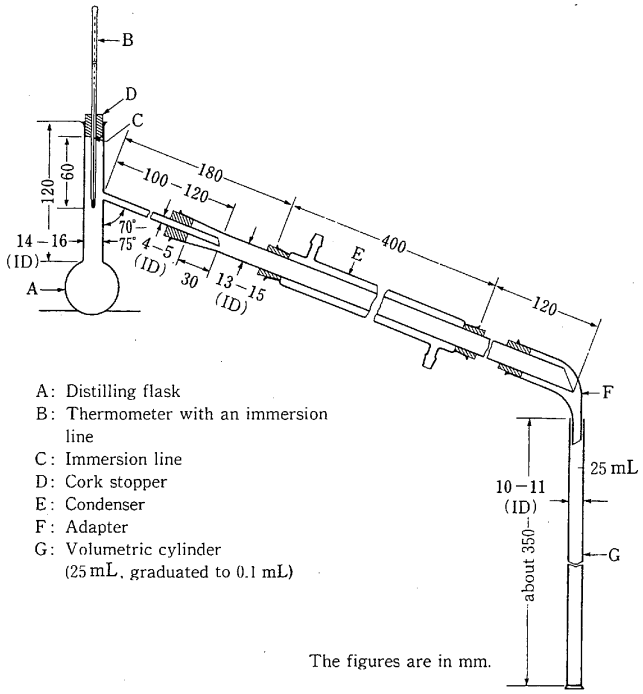
Method 1 This method is applied to a sample for which the permissible range of boiling temperature is smaller than 5 °C.

(1) Apparatus

Use the apparatus illustrated in the figure.

(2) Procedure

Measure 25 mL of the sample, whose temperature is previously noted, using a volumetric cylinder G graduated in 0.1 mL, and transfer it to a distilling flask A of 50- to 60-mL capacity. Use this cylinder as the receiver for the distillate without rinsing out any of the adhering liquid. Put boiling chips into the distilling flask A, insert a thermometer B with an immersion line so that its immersion line C is on a level with the lower end of cork stopper D and the upper end of its mercury bulb is located in the center of the delivery tube, and connect condenser E with the distilling flask A and adapter F with the condenser E. Insert the open end of F into the mouth of cylinder G (receiver) so that air can pass through slightly. Use a hood with a height sufficient to shield A, and heat A with a suitable heat source. When direct flame is applied as the heat source, put A on a hole of an asbestos board (a wire gauge of 150 mL square bonded to asbestos 6 mm



thick and having in its center a round hole 30 mm in diameter).

Unless otherwise specified, distil the liquid sample by the application of heat, at a rate of 4 to 5 mL per minute of distillate in the case of liquids whose boiling temperature to be determined is lower than 200°C and at a rate of 3 to 4 mL per minute in the case of liquids whose boiling temperature is 200°C or over, and read the boiling point. For the distilling range test, bring the temperature of distillate to the temperature at which the volume was originally measured, and measure the volume of distillate.

Liquids that begin to distil below 80°C are cooled to between 10°C and 15°C before measuring the volume, and the receiving cylinder is kept immersed in ice up to a point 25 mm from the top during the distillation.

Correct the observed temperature for any variation in the barometric pressure from the normal (101.3 kPa), by allowing 0.1 degree for each 0.36 kPa of variation, adding if the pressure is lower, or subtracting if higher than 101.3 kPa.

Method 2 This method is applied to the sample for which the permissible range of boiling temperature is 5°C or more.

(1) Apparatus

The same apparatus as described in Method 1 is used. However, use a 200-mL distilling flask A with a neck 18 to 24 mm in inside diameter having a delivery tube 5 to 6 mm in inside diameter. The asbestos board used for direct flame heating should have in its center a round hole 50 mm in diameter.

(2) Procedure

Measure 100 mL of the sample, whose temperature is previously noted, using a volumetric cylinder graduated in 1 mL, and carry out the distillation in the same manner as in Method 1.

8. Chloride Limit Test

The Chloride Limit Test is a limit test for chloride contained in drugs.

In each monograph, the permissible limit for chloride (as Cl) 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, and dissolve it in a proper volume of water to make 40 mL. Add 6 mL of dilute nitric acid and water to make 50 mL, and use this solution as the test solution. Transfer the volume of 0.01 mol/L hydrochloric acid VS, directed in the monograph, to another Nessler tube, add 6 mL of dilute nitric 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 by using the same procedure.

Add 1 mL of silver nitrate TS to the test solution and to the control solution, mix well, and allow to stand for 5 minutes protecting from direct sunlight. Compare the opalescence developed in both solutions against a black background by viewing downward or transversely.

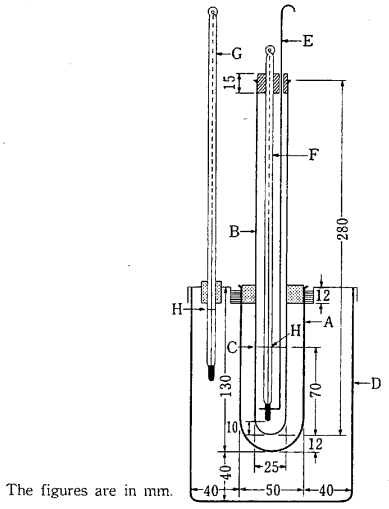
The opalescence developed in the test solution is not more than that of the control solution.

9. Congealing Point Determination

The Congealing Point is the temperature measured by the following method.

Apparatus

Use the apparatus illustrated in the figure.



A: Cylinder made of glass (the tube is painted with silicone oil on both sides of the wall to prevent clouding).

B: Sample container (a hard glass test tube, which is painted with silicone oil to prevent clouding, except at the region of the wall in contact with the sample; insert it into cylinder A, and fix with cork stopper).