

tube and a nutrient agar slant with the microorganisms from a typical colony or from not less than 2 subtypical colonies formed, and incubate between 35°C and 37°C. If gas is evolved in the lactose broth fermentation tube within 48 hours, apply Gram-staining to the colonies grown on the nutrient agar slant. Any Gram-negative, asporogenic bacillus indicates the presence of coliform bacilli.

Water for Injection

注射用水

Water for Injection is water prepared by distillation of Water or Purified Water, or by the Reverse Osmosis-Ultrafiltration of Purified Water, to be used for the preparation of injections, or preserved in containers and sterilized.

When Water for Injection is prepared by the Reverse Osmosis-Ultrafiltration, take precaution against microbial permeation through membrane.

Water for Injection for the preparation of injections must be used immediately after preparation. It may be stored overnight avoiding microbial contamination and growth.

Water for Injection preserved in containers and sterilized is used mainly as solvent for injections to be dissolved or suspended before use.

Water for Injection prepared by distillation may be labeled Distilled Water for Injection as commonly used Japanese name.

Purity (1) Acid or alkali—To 20 mL of Water for Injection add 0.1 mL of methyl red TS for acid or alkali test: a yellow to orange color develops. To 20 mL of Water for Injection add 0.05 mL of bromothymol blue TS: no blue color develops.

(2) Chloride—To 50 mL of Water for Injection add 3 drops of nitric acid and 0.5 mL of silver nitrate TS: no change occurs.

(3) Sulfate—To 50 mL of Water for Injection add 0.5 mL of barium chloride TS: no change occurs.

(4) Nitrogen from nitrate—Transfer 2.0 mL of Water for Injection to a 50-mL beaker, add 1 mL of sodium salicylate-sodium hydroxide TS, 1 mL of a solution of sodium chloride (1 in 500) and 1 mL of a solution of ammonium amidosulfate (1 in 1000), and evaporate on a water bath to dryness. Cool, dissolve in 2 mL of sulfuric acid, allow to stand for 10 minutes with occasional shaking, add 10 mL of water, and transfer to a Nessler tube. Cool, add 10 mL of a solution of sodium hydroxide (2 in 5) slowly, and add water to make 25 mL: no yellow color develops.

(5) Nitrogen from nitrite—Transfer 10 mL of Water for Injection to a Nessler tube, and add 1 mL of a solution of sulfanilamide in dilute hydrochloric acid (1 in 100) and 1 mL of *N*-(1-naphthyl)-*N'*-diethylethylenediamine oxalate TS: no pale red color develops.

(6) Ammonium—Perform the test as directed under the Ammonium Limit Test, using 30 mL of Water for Injection as the test solution. Prepare the control solution as follows: to 0.15 mL of Standard Ammonium Solution add purified water for ammonium limit test to make 30 mL, and proceed

in the same manner as the test solution (not more than 0.05 mg/L).

(7) Heavy metals—To 40 mL of Water for Injection add 2 mL of dilute acetic acid and 1 drop of sodium sulfide TS: no change occurs.

(8) Potassium permanganate-reducing substances—To 100 mL of Water for Injection add 10 mL of dilute sulfuric acid, boil, add 0.10 mL of 0.02 mol/L potassium permanganate VS, and boil again for 10 minutes: the red color does not disappear.

(9) Residue on evaporation—Evaporate 100 mL of Water for Injection on a water bath to dryness, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 1.0 mg.

For Water for Injection prepared by the Reverse Osmosis-Ultrafiltration for the preparation of injections, perform the test for (8) Total organic carbon described below, instead of (8) Potassium permanganate-reducing substances. For Water for Injection preserved in containers and sterilized, perform the test for (1) Acid or alkali, (2) Chloride, (6) Ammonium and (9) Residue on evaporation according to the following methods:

(1) Acid or alkali—Shake gently 20 mL of Distilled Water for Injection with 0.05 mL of phenol red TS and 0.13 mL of 0.01 mol/L sodium hydroxide VS, and allow to stand for 30 seconds: a red color develops. Shake gently 20 mL of Water for Injection with 0.05 mL of bromothymol blue TS and 0.13 mL of 0.01 mol/L hydrochloric acid VS, and allow to stand for 30 seconds: a yellow color develops.

(2) Chloride—For Water for Injection in containers holding a volume not more than 10 mL, add 2.0 mL of dilute nitric acid to 15 mL of Distilled Water for Injection, and use this solution as the test solution. Separately, to 0.20 mL of 0.001 mol/L hydrochloric acid VS add water to make 15 mL, then add 2.0 mL of dilute nitric acid, and use this solution as the control solution. Mix the test solution and the control solution separately with 0.30 mL each of silver nitrate TS, allow to stand for 5 minutes under the protection from sunlight, and compare the turbidity of the solutions on a black background: the turbidity of the test solution is not thicker than that of the control solution (not more than 0.00005%). For Water for Injection in containers holding a volume exceeding 10 mL, add 3 drops of nitric acid and 0.5 mL of silver nitrate TS to 50 mL of Water for Injection: the solution remains unchanged.

(6) Ammonium—Perform the test as directed under the Ammonium Limit Test, using 30 mL of Water for Injection as the test solution. Prepare the control solution as follows: To 0.6 mL of Standard Ammonium Solution for Water for Injection in containers holding a volume not more than 10 mL, and 0.3 mL of Standard Ammonium Solution for Water for Injection in a volume exceeding 10 mL, add purified water for ammonium limit test to make 30 mL, and proceed in the same manner as the test solution (not more than 0.2 mg/L for Water for Injection in a volume not more than 10 mL, and not more than 0.1 mg/L for that exceeding 10 mL).

(8) Total organic carbon—Perform the test with Water for Injection prepared by the Reverse Osmosis-Ultrafiltration for the preparation of injections, using an apparatus for detection of total organic carbon: it contains not more than 0.50 mg/L of total organic carbon. Use an apparatus which is efficient enough to detect not more than 0.050 mg/L of

total organic carbon and, in determination of total organic carbon in a solution of sodium dodecylbenzenesulfonate (33.22 mg/L), not less than 1.7 mg/L. For the calibration of the apparatus use potassium hydrogen phthalate (standard reagent).

(9) Residue on evaporation—Evaporate 100 mL of Water for Injection, and dry the residue at 105°C for 1 hour: the residue weighs not more than 4.0 mg for Distilled Water for Injection in a volume not more than 10 mL, and not more than 3.0 mg for that exceeding 10 mL.

Bacterial endotoxins Less than 0.25 EU/mL.

Sterility test Perform the test with Water for Injection preserved in containers and sterilized: it meets the requirements of the Sterility Test. For sterilized Water for Injection in containers holding a volume exceeding 100 mL, perform the test according to the Membrane filtration method.

Containers and storage Containers—(1) For the preparation of injections, suitable containers, protected from microbial contamination.

(2) Hermetic containers for Water for Injection, previously sterilized in containers. Plastic containers for aqueous infusions may be used.

Purified Water

精製水

H₂O: 18.02

Purified Water is water purified by distillation, ion-exchange treatment, ultrafiltration or combination of these methods. In case the ion-exchange treatment is used at the end of the purification process, be careful to prevent bacterial contamination, and, if necessary, kill or remove bacteria by a suitable method.

Use immediately after purification. It may be stored in suitable containers preventing bacterial growth.

Description Purified Water is a clear, colorless liquid. It is odorless and tasteless.

Purity (1) Acid or alkali—To 20 mL of Purified Water add 0.1 mL of methyl red TS for acid or alkali test: a yellow to orange color develops. To 20 mL of Purified Water add 0.05 mL of bromothymol blue TS: no blue color develops.

(2) Chloride—To 50 mL of Purified Water add 3 drops of nitric acid and 0.5 mL of silver nitrate TS: no change occurs.

(3) Sulfate—To 50 mL of Purified Water add 0.5 mL of barium chloride TS: no change occurs.

(4) Nitrogen from nitrate—Transfer 2.0 mL of Purified Water to a 50-mL beaker, add 1 mL of sodium salicylate-sodium hydroxide TS, 1 mL of a solution of sodium chloride (1 in 500) and 1 mL of a solution of ammonium amidosulfate (1 in 1000), and evaporate on a water bath to dryness. Cool, dissolve in 2 mL of sulfuric acid, allow to stand for 10 minutes with occasional shaking, add 10 mL of water, and transfer to a Nessler tube. Cool, add 10 mL of a solution of sodium hydroxide (2 in 5) slowly, and add water to make 25 mL: no yellow color develops.

(5) Nitrogen from nitrite—Transfer 10 mL of Purified Water to a Nessler tube, and add 1 mL of a solution of sulfanilamide in dilute hydrochloric acid (1 in 100) and 1 mL of *N*-(1-naphthyl)-*N'*-diethylethylenediamine oxalate TS: no pale red color develops.

(6) Ammonium—Perform the test as directed under the Ammonium Limit Test, using 30 mL of Purified Water as the test solution. Prepare the control solution as follows: to 0.15 mL of Standard Ammonium Solution add purified water for ammonium limit test to make 30 mL, and proceed in the same manner as the test solution (not more than 0.05 mg/L).

(7) Heavy metals—The 40 mL of Purified Water add 2 mL of dilute acetic acid and 1 drop of sodium sulfide TS: no change occurs.

(8) Potassium permanganate-reducing substances—To 100 mL of Purified Water add 10 mL of dilute sulfuric acid, boil, add 0.10 mL of 0.02 mol/L potassium permanganate VS, and boil again for 10 minutes: the red color does not disappear.

(9) Residue on evaporation—Evaporate 100 mL of Purified Water on a water bath to dryness, and dry the residue at 105°C for 1 hour: the amount of the residue is not more than 1.0 mg.

Containers and storage Containers—Tight containers.

Sterile Purified Water

滅菌精製水

Sterile Purified Water is sterilized Purified Water. It is not to be used for preparation of injections.

Description Sterile Purified Water is a clear, colorless liquid. It is odorless and tasteless.

Purity (1) Acid or alkali—To 20 mL of Sterile Purified Water add 0.1 mL of methyl red TS for acid or alkali test: a yellow to orange color develops. To 20 mL of Sterile Purified Water add 0.05 mL of bromothymol blue TS: no blue color develops.

(2) Chloride—To 50 mL of Sterile Purified Water add 3 drops of nitric acid and 0.5 mL of silver nitrate TS: no change occurs.

(3) Sulfate—To 50 mL of Sterile Purified Water add 0.5 mL of barium chloride TS: no change occurs.

(4) Nitrogen from nitrate—Transfer 2.0 mL of Sterile Purified Water to a 50-mL beaker, add 1 mL of sodium salicylate-sodium hydroxide TS, 1 mL of a solution of sodium chloride (1 in 500) and 1 mL of a solution of ammonium amidosulfate (1 in 1000), and evaporate on a water bath to dryness. Cool, dissolve in 2 mL of sulfuric acid, allow to stand for 10 minutes, with occasional shaking, add 10 mL of water, and transfer to a Nessler tube. Cool, add 10 mL of a solution of sodium hydroxide (2 in 5) slowly, and add water to make 25 mL: no yellow color develops.

(5) Nitrogen from nitrite—Transfer 10 mL of Sterile Purified Water to a Nessler tube, and add 1 mL of a solution of sulfanilamide in dilute hydrochloric acid (1 in 100) and 1 mL of *N*-(1-Naphthyl)-*N'*-diethylethylenediamine oxalate TS: no pale red color develops.