

Add the sodium chloride 70.0 g for (ii) Fluid Soybean-Casein Digest Medium (containing 5 g of sodium chloride), mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 7.1 - 7.5.

(xix) Vogel-Johnson Agar Medium

Casein peptone	10.0 g
Yeast extract	5.0 g
D-Mannitol	10.0 g
Dipotassium hydrogenphosphate	5.0 g
Lithium chloride	5.0 g
Glycine	10.0 g
Phenol red	0.025 g
Agar	16.0 g
Water	1000 mL

Mix all the components, and boil for 1 minute to effect solution. Sterilize by heating in an autoclave at 121°C for 15 to 20 minutes, and cool to between 45°C and 50°C. pH after sterilization: 7.0 - 7.4. To this solution add 20 mL of sterile potassium tellurite solution (1 in 100), and mix.

(xx) Baird-Parker Agar Medium

Casein peptone	10.0 g
Meat extract	5.0 g
Yeast extract	1.0 g
Lithium chloride	5.0 g
Glycine	12.0 g
Sodium pyruvate	10.0 g
Agar	20.0 g
Water	950 mL

Mix all the components. Heat the mixture with frequent agitation, and boil for 1 minute. Sterilize by heating in an autoclave at 121°C for 15 to 20 minutes, and cool to between 45°C and 50°C. pH after sterilization: 6.6 - 7.0. To this solution add 10 mL of sterile potassium tellurite solution (1 in 100) and 50 mL of egg-yolk emulsion. Mix gently, and pour into petri dishes. Prepare the egg-yolk emulsion by mixing egg-yolk and sterile saline with the ratio of about 30% to 70%.

(xxi) Mannitol-Salt Agar Medium

Casein peptone	5.0 g
Animal tissue peptone	5.0 g
Meat extract	1.0 g
D-Mannitol	10.0 g
Sodium chloride	75.0 g
Phenol red	0.025 g
Agar	15.0 g
Water	1000 mL

Mix all the components. Heat with frequent agitation, and boil for 1 minute. Sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 7.2 - 7.6.

(3) Reagent-Test solution

Amphotericin B powder Amphotericin B added sodium deoxycholic acid, sterilized by γ -ray.

Amphotericin B TS Dissolve 22.5 mg of amphotericin B powder in 9 mL of sterile purified water.

Bile salts Yellow-brown powder made from dried bile of animal, consist of sodium taurocholic acid and sodium glycocholic acid, and containing not less than 45% of cholic acid. pH of 5% solution: 5.5 - 7.5.

Rose bengal $C_{20}H_2Cl_4Na_2O_5$ [Special class] Red-brown powder, purple-red solution in water.

Rose bengal TS Dissolve 1 g of rose bengal in water to make 100 mL.

2,3,5-Triphenyl-2H-tetrazolium chloride TS (TTC TS)

Prepare a 0.8% TTC solution (dissolve 0.8 g of TTC in water to make 100 mL), distribute in small tubes and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. Store in light-resistant containers.

(4) Preparation

Preparation of agar medium with TTC

Just prior to use, add 2.5 to 5 mL of TTC TS per liter of sterile medium and mix.

Preparation of agar medium with amphotericin B

Dissolve 22.5 mg of amphotericin B sterilized by γ -ray in 9 mL of sterile purified water. Just prior to use, add 2 mL of this solution in a liter of sterile medium and mix.

Preparation of agar medium with rose bengal

Add 5 mL of rose bengal TS in a liter of medium and mix. Sterilize in an autoclave at 121°C for 15 to 20 minutes.

37. Mineral Oil Test

The Mineral Oil Test is a method to test mineral oil in nonaqueous solvents for injections and for eye drops.

Procedure

Pour 10 mL of the sample into a 100-mL flask, and add 15 mL of sodium hydroxide solution (1 in 6) and 30 mL of ethanol (95). Put a short-stemmed, small funnel on the neck of the flask, and heat on a water bath to make clear, with frequent shaking. Then transfer the solution to a shallow porcelain dish, evaporate the ethanol on a water bath, add 100 mL of water to the residue, and heat on a water bath: no turbidity is produced in the solution.

38. Nitrogen Determination (Semimicro-Kjeldahl Method)

The Nitrogen Determination is a method to determine ammonia in ammonium sulfate obtained by decomposition of organic substances containing nitrogen with sulfuric acid.

Apparatus

Use the apparatus illustrated in the figure. It is thoroughly constructed of hard glass, and ground glass surfaces may be used for joints. All rubber parts used in the apparatus should be boiled for 10 to 30 minutes in sodium hydroxide TS and for 30 to 60 minutes in water, and finally washed thoroughly with water before use.

Procedure

Unless otherwise specified, proceed by the following method. Weigh accurately or pipet a quantity of the sample corresponding to 2 to 3 mg of nitrogen (N:14.01), and place in the Kjeldahl flask A. Add 1 g of a powdered mixture of 10 g of potassium sulfate and 1 g of copper (II) sulfate pentahydrate. Wash down any adhering sample from the neck of the flask with a small quantity of water. Add 7 mL of sulfuric acid, allowing it to flow down the inside wall of the flask.