Appendix 1

**Martin’s medium (g/l)**

Dextrose 5; potassium dihydrogen ortho-phosphate 1; magnesium sulphate 0.5; streptomycin 0.006; rose Bengal 2 part in 3000 part of medium. (1g of chloramphenicol/nalidixic acid was dissolved in 100 ml of sterile water. 0.3 ml of this solution was added to 100 ml of rose Bengal medium after it cooled to 45°C).

Appendix 2

**Pikovskaya medium (g/l)**

Glucose 10; Ca₃(PO₄)₂ 5; (NH₄)₂ SO₄ 0.5; NaCl 0.2; MgSO₄.7H₂O 0.1; KCl 0.1; yeast extract 0.5; MnSO₄ and FeSO₄ trace; pH 7

Appendix 3

**Yeast extract mannitol medium (g/l)**

Mannitol 10; K₂HPO₄ 0.5; MgSO₄.7H₂O 0.2; NaCl 0.1; yeast extract 1.0; CaCO₃ 2; pH 7

Appendix 4

**Gram staining**

**Primary stain**

**Solution A**

Crystal violet (90% dye content) 2 g; Ethyl alcohol (95%) 20 ml

**Solution B**

Ammonium oxalate 0.8 g; distilled water 80 ml

**Gram’s iodine**

Iodine 1 g; potassium iodide 2 g; distilled water 300 ml

**Decolorizer**

Ethyl alcohol 95 ml; distilled water 5 ml

**Counter stain**

Safranin (2.5% solution in 95% ethyl alcohol) 10 ml; distilled water 100 ml

Appendix 5

**Kovac’s reagent**

p-dimethyl amino benzaldehyde 10 g; Iso-amyl alcohol 15 ml

(Dilute 10 times in distilled water before use)

Appendix 6

**Methyl red solution (g/l)**
Methyl red 0.1; ethyl alcohol 300 ml; distilled water 200 ml

Appendix 7

**Barrit's reagent (g/l)**

**Solution A**
A- naphthol 5; ethanol 95 ml

**Solution B**
Creatine 0.30; potassium hydroxide 40

Appendix 8

**Simmons citrate agar (pH 7.0 ± 0.2) (g/l)**
Ammonium dihydrogen phosphate 1; dipotassium phosphate 1; magnesium sulfate 0.2; sodium chloride 5; sodium citrate 2; bromothymol blue 0.08

Appendix 9

**Trypticase nitrate broth (g/l)**
Trypticase 20; disodium phosphate 2; dextrose 1; potassium nitrate 1; agar 20; Ph 7

Appendix 10

**Solution A (g/l)**
Sulfanilic acid 8; acetic acid 5N 1000 ml
(5N: 1 part glacial acetic acid to 2-5 parts distilled water)

Appendix 11

**Solution B (g/l)**
Dimethyl amine 1- napthylamine 5; acetic acid 1000 ml

Appendix 12

**Fermentation broth (g/l)**
Beef extract 1; peptone 10; phenol red 0.018; pH 7.4

Appendix 13

**Starch agar (g/l)**
Peptone 5; beef extract 3; starch 2; agar 20; pH 7.0

Appendix 14

**Chrome Azurol S (CAS) agar medium**
CAS agar is prepared from four solutions

**Solution 1:** Fe-CAS indicator solution
Mix 10 ml of 1 mM FeCl₃·6H₂O [in 10 Mm HCl} with 50 ml of an aqueous solution of CAS (1.21 mg/ml). The above solution was then added to 40 ml of HDTMA (1.82 mg/ml) and cooled to 50 °C.

**Solution 2: Buffer solution**

Dissolve 30.24 g of PIPES in 750 ml of a salt solution containing 0.3 g KH₂PO₄, 0.5 g NaCl and 1 g NH₄Cl, pH 6.8 with 50% KOH and water was added to bring the volume to 800 ml.

**Solution 3:** in 70 ml water

2 g glucose, 2 g mannitol, 493 mg MgSO₄. 7H₂O, 11 mg CaCl₂, 1.17 mg MnSO₄. H₂O, 1.4 mg H₃BO₃, 0.04 mg CuSO₄. 5H₂O, 1.2 mg ZnSO₄. 7H₂O and 1 mg Na₂MoO₄. 2H₂O. Autoclaved, cooled to 50 °C, then added to the buffer solution along with 30 ml filter-sterilized 10% (W: V) casamino acids (solution 4). The indicator solution was added last with sufficient stirring to mix the ingredients without forming bubbles.

**Appendix 15**

**Chloromolybdic acid**

Ammonium molybdate 15 g; distilled water 400 ml; 10 N HCl 400 ml  
The above described materials were mixed slowly with rapid stirring, cool and make the volume to 1 liter with distilled water.

**Appendix 16**

**Chlorostannous acid**

Stannous chloride 10 g; concentrated hydrogen chloride 25 ml  
The stock solution was kept in airtight bottle. 1 ml of stock solution is mixed in 132 ml of distilled water at the time of experiment.

**Appendix 17**

**Nessler’s reagent**

Potassium iodide 50 g; distilled water (ammonia free) 35 ml  
Add saturated aqueous solution of mercuric chloride until a slight precipitate persists  
Potassium hydroxide 400 ml  
Dilute the solution to 1000 ml with ammonia free distilled water. Allow to stand for one week, decant supernatant liquid and store in a tightly capped amber bottle.

**Appendix 18**

**Phosphate buffer 1% (pH 7.2-7.4)**

*Solution A*
Disodium phosphate 1.4 g; distilled water 100 ml

Solution B

Sodium dihydrogen phosphate 1.4 g; distilled water 100 ml

(84.1 ml of solution A to 15.9 ml of solution B and 8.5 g of sodium chloride and volume was made upto one liter)

Appendix 19

Pyridine reagent

Sodium hydroxide 0.8 g (dissolved in 50 ml), pyridine 33.8 ml.

The volume was made upto 100 ml

Appendix 20

Copper solution

Solution A: Sodium carbonate 2g (mixed with 0.1 N sodium hydroxide)

Solution B: Copper sulphate 0.5 g, potassium sodium tartrate 1g, distilled water 100 ml

Copper solution was prepared by mixing 50 ml solution A with 1 ml of solution B

Appendix 21

Folins reagent

Sodium tungstate 100 g, sodium molybdate 25 g, distilled water 700 ml, 85% orthophosphoric acid 50 ml, HCl 100 ml, bromine water few drops

(Reflux the above given mixture for 10 h)

Boil the solution without condenser for 15 min. to remove excess bromine, cool and dilute it to 1 liter