### 11. ANNEXURE

**Chemical Parameters - Reagents**

**Dissolved Oxygen**
- **Conc. Sulphuric acid**
- Sodium thiosulphate (0.025N) - 6.205g / 1000 mL distilled water
- Alkali iodide - azide reagent - 175 g KOH + 37.5g KI in 250ml distilled water
- Manganese sulphate solution 91 g / 250 ml distilled water.
- Starch indicator solution

**Chlorides**
- Silver nitrate (0.02N) - 4.71g / 1000 ml distilled water
- Potassium chromate 5%
- Methyl red indicator
- Hydrochloride acid solution (1+1)
- Barium chloride solution 5 %
- Silver nitrate-nitric acid reagent - 25g/ 500 ml distilled water

**Sulphates**
- Methyl red indicator
- Hydrochloride acid solution (1+1)
- Barium chloride solution 5 %
- Silver nitrate-nitric acid reagent - 8.5g silver nitrate and 0.5ml Concentrated HNO₃ in 500 ml distilled water

**Biochemical Oxygen Demand (BOD)**
- Sodium thiosulphate (0.1N)
- Sodium thiosulphate (0.025N)
- Alkali iodide - azide reagent - 175 g KOH + 37.5g KI / 250ml D.W
- Manganese sulphate solution - 40g / 100ml distilled water.
- Starch indicator solution

**Reagents for the dilution water**
- Phosphate buffer solution - Dissolve 8.5g KH₂PO₄; 21.5 g K₂HPO₄; 33.4 g Na₂HPO₄·7H₂O 1.7g NH₄Cl in 500 ml distilled water and make up to 1000 mL
- Magnesium sulphate solution - 25 g / 1000ml distilled water
- Calcium chloride solution - 27.5 g /1000ml distilled water
- Ferric chloride solution - 0.25 g / 1000 ml distilled water

**Total Chemical Oxygen Demand (TCOD)**
- Potassium dichromate solution (0.025N) - 6.129 in 500 ml distilled water
- Silver sulphate reagent - 1g of silver sulphate in 100ml Conc. sulphuric acid.

**Ferroin Indicator**
- Ferrous Ammonium sulphate solution (0.1N) - Dissolve 39.29 g ferrous ammonium sulphate in distilled water. Add 20 ml conc. Sulphuric acid. Cool and make up to 100 ml in a volumetric flask.

**Total Nitrogen (TN)**
- Potassium sulphate crystals
- Sodium hydroxide (50 %)
- Phenolphthalein indicator solution
- Sulphuric acid (0.02N)
- Boric acid solution (2 %)
- Mixed indicator solution - 100g / 200 ml distilled water
- .56 ml / 1000ml distilled water
- 10 g in 500 ml distilled water
Cone. Sulphuric acid
Copper sulphate solution 10 % - 10 g / 100 distilled water

Ammoniacal Nitrogen (NH₄N)
HCL(0.01N)
Boric acid cum indicator solution

Nitrate nitrogen (NO₃N)
Brucine sulphanilic acid solution: Dissolve 1 g brucine sulphate and 100 mg sulphanilic acid in 70 ml hot distilled water. Add 3 ml conc. HCl, cool and dilute with 1000 ml distilled water.

Sulphuric acid solution - 500 ml / 75 ml distilled water
Nitrate stock solution - 722 mg / 1000 ml distilled water
Nitrate standard solution - 100 ml nitrate stock solution in 1000 ml distilled water
Sodium arsenite solution - 1.83 g / 100 distilled water
Aluminium hydroxide suspension

Total Phosphorus and Phosphate Phosphorus
Perchloric acid (70%)
Phenolphthalein indicator
Sodium hydroxide solution (1N)
Ammonium molybdate solution - 4 g 100 ml distilled water

Add 280 ml of concentrated sulphuric acid to 400 ml distilled water and cool. Meanwhile dissolve 25 g of ammonium molybdate in about 200 ml distilled water. Add the molybdate solution to the diluted acid and dilute the mixture to 1000 ml with distilled water.

Stannous chloride solution - 2.5 g / 100 ml glycerol
Phosphate stock solution - 439 mg KH₂PO₄ / 1000 ml distilled water
Phosphate standard solution - 10 ml of phosphate stock solution in 1000 ml distilled water

SEDIMENTS
Organic matter
Potassium dichromate solution 1N
Sulphuric acid (sp. gr.1.84)
Phosphoric acid
Ferrous ammonium sulphate 0.4N
Diphenyl amine indicator

Phosphorus
Ammonium molybdate
Stannous chloride solution (conc.)
Stannous chloride solution (diluted)
0.5m sodium bicarbonate solution

**Nitrogen**
- potassium permanganate: 0.32%
- sodium hydroxide: 2.5%
- Boric acid: 2%
- Double indicator (Mauzazuga indicator)

**Medium Composition**

<table>
<thead>
<tr>
<th>Medium Composition</th>
<th>Starch agar</th>
<th>Milk agar medium</th>
<th>Nutrient gelatin</th>
<th>Carbohydrate fermentation medium</th>
<th>Litmus milk reaction</th>
<th>SIM Medium</th>
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<tbody>
<tr>
<td></td>
<td>Peptone</td>
<td>Skim milk powder</td>
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**Biological Parameters**
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<tr>
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<tr>
<td>Distilled water</td>
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<td>Glucose 10% solution</td>
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<td>Magnesium sulphate</td>
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<tr>
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<tr>
<td>Agar</td>
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<td>Bromothymol blue (0.2%)</td>
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<td>Yeast extract</td>
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<td>Sucrose</td>
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<td>Ferrous ammonium sulphate</td>
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<td>Distilled water</td>
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<td>pH</td>
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<td>pH</td>
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<tr>
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<td>Yeast extract</td>
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<td>Recipe</td>
<td>Volume</td>
<td>Peptone (g)</td>
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<td><strong>Nutrient agar</strong></td>
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<td><strong>Peptone water</strong></td>
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<td>- 5g</td>
</tr>
<tr>
<td><strong>Mannitol salt agar</strong></td>
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<td>- 1g</td>
</tr>
<tr>
<td><strong>SS agar</strong></td>
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<td>- 5g</td>
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<td><strong>Mac conkey agar</strong></td>
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<tr>
<td><strong>Thiosulfate Citrate Bile salts sucrose Agar (TCBS)</strong></td>
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<td>- 5.0g</td>
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251
Sodium chelate
Feeric citrate
Sodium chloride
Bromothymol blue
(0.2% solution)
Thymol blue
(1% solution)
Agar
Distilled water

**Eosin Methylene Blue agar (EMB)**
Peptone
Lactose
Dipotassium hydrogen phosphate
Agar
Eosin
Methylene blue
Distilled water
pH

**EC medium**
Tryptose
Lactose
Bile salts No.3
Dipotassium hydrogen phosphate
Potassium dihydrogen phosphate
Sodium chloride
Agar
Distilled water
pH

**Lauryl sulphate tryptose broth**
Tryptose
Lactose
Dipotassium hydrogen phosphate
Potassium dihydrogen phosphate
Sodium chloride
Sodium lauryl sulphate
Distilled water
pH

**Starch casein – nitrate agar**
Soluble starch
Casein (vitamin free)
Potassium nitrate
Sodium nitrate
Dipotassium hydrogen phosphate
Magnesium sulphate
Calcium Carbonate
Ferrous sulphate
Agar
Distilled water
pH

**Potato dextrose agar**
Infusion from potatoes
Dextrose - 20g
Agar - 15g
Distilled water - 1000ml

Czapek dox agar
Sucrose - 30g
Sodium Nitrate - 3g
Dipotassium phosphate - 1g
Magnesium sulphate - 0.5g
Potassium chloride - 0.01g
Agar - 15g
pH - 7.3

Muller hinton agar
Beef infusion - 300g
Casamino acids - 17.5g
Starch - 1.5g
Agar - 17g
pH - 7.4

Deoxycholate Citrate Agar (DCA)
Malt extract broth - 1000 ml
Proteose peptone - 10.0g
Lactose - 10.0 g
Neutral red 1 % solution - 2.5 ml
Agar - 17.0 g

Solution 1:
Sodium Citrate - 17.0 g
Sodium thiosulphate - 17.0 g
Ferric ammonium citrate - 2g
Distilled water - 100 ml

Solution 2:
Sodium deoxycholate - 10 g
Distilled water - 100g
Melt 200 ml of the base medium and cool to about 80°C. Add aseptically 10 ml of solution 1 and the appropriate volume of the solution using two different pipettes, mix well after each dilution. Distribute into sterile petridishes.

Xylose Lysine Deoxycholate Agar (XLD agar)
Xylose - 3.7g
L-Lysine HCl - 5.0g
Lactose - 7.5g
Sucrose - 7.5 g
Sodium chloride - 5.0 g
Yeast extract - 3.0 g
Phenol red (0.2 % solution) - 40.0 ml
Agar - 15.0 g
Distilled water - 960 ml

Cary Blair Medium
Sodium thioglycolate - 1.5g
Disodium hydrogen phosphate - 1.1g
Sodium chloride - 5.0 g
Agar - 5.0 g
Distilled water - 991.0 ml
Dissolve the ingredients in the water while heating in a water bath until the solution is clear. After cooling to 50° C, add 9.0 ml of freshly prepared 1% calcium chloride solution and adjust the pH to 8.4

Bismuth Sulphite Agar
Beef Extract - 5.0g
Peptone -10.0g
Glucose - 5.0 g
Sodium monohydrogen phosphate - 4.0g
Ferrous sulphate - 0.3g
Bismuth sulphite indicator - 8.0 g
Brilliant Green - 5ml
Agar - 20.0 g
Distilled water - 1000ml

Reagents
Crystal violet
Solution: A
Crystal violet - 2g
Ethanol 95% (v/v) - 20ml
Solution: B
Ammonium oxalate - 0.8g
Distilled water - 80ml
Mix A and B obtain the crystal violet staining reagent store for 24 hrs and filter through paper before using.

Mordant
Iodine - 1g
Potassium iodide - 2g
Distilled water - 300ml
Grind the iodine and potassium in a mortar and add water slowly with continuous grinding until the iodine is dissolved. Store amber colored bottles.

Decolorizing Agent:
Ethanol 95% (v/v)

Counter stain:
Safranin 2.5% (w/v) in
(v/v) ethanol - 10ml
Distilled water - 100ml

Nitrate Reduction Test.
Solution: A
Dissolve 8.0g sulphanilic acid in 1 litre of acetic acid (5mol / lit).

Solution: B
Dissolve 5.0g of alpha naphthalamine in 1 litre of acetic acid (5mol/lit).

Immediately before use, mix equal volumes of solution A and B to give the test reagent.

Kovac's Reagent
Amyl or isoamyl alcohol - 150ml
P-Dimethyl amino benzal dehyde - 10g
Conc. Hydrochloric acid - 50ml
Dissolve the aldehyde in the alcohol at 50-55°C. Cool and add the acid.

**Methyl red indicator solution**

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<th>Component</th>
<th>Quantity</th>
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<tr>
<td>Ethanol</td>
<td>300ml</td>
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<td>Distilled water</td>
<td>200ml</td>
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**VP reagent: A**

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<tr>
<td>Alpha naphthol</td>
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<tr>
<td>Ethyl alcohol</td>
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Dissolve alpha naphthol in small amount of alcohol first and then add the remaining alcohol to 100ml.

**VP reagent: B**

<table>
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<td>3% H₂O₂</td>
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<tr>
<td>Hydrogen peroxide (H₂O₂)</td>
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**Oxidase Disc**

Disc impregnated with the N, N tetra methyl para phenylene diamine dihydroxide.

**Lactophenol Cotton Blue Solution**

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<td>Phenol</td>
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<td>Glycerol</td>
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<td>Distilled water</td>
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<tr>
<td>Aniline blue</td>
<td>0.05g</td>
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