APPENDIX-1

A. Media

1. Blood agar medium (g l\(^{-1}\)):

The nutrient agar medium (Section 3.2.1) was supplemented with sterile 5.0% (v/v) sheep blood before pouring into the Petri plates.

2. Cetyltrimethylammonium bromide -methylene blue (CTAB-MB) agar medium:

The mineral salt medium (MSM, Section 3.2.2) containing 0.2 g l\(^{-1}\) of CTAB was supplemented with 2.0% (w/v) glucose, 0.25% (w/v) yeast, and 0.005 g l\(^{-1}\) of methylene blue from their sterilized stock solutions.

B. Chloride-ion estimation by mercuric thiocyanate method

Reagents: 1. 0.25 M Ferric ammonium sulphate in 9 M HNO\(_3\)

2. Saturated solution of mercuric thiocyanate in absolute alcohol

Chloride-ion in the culture filtrate was measured by reaction with mercuric thiocyanate. 5 ml of the cell-free supernatant was diluted with 15 ml distilled water. To this 2.0 ml of Reagent 1 was added followed by addition of 2.0 ml of Reagent 2. The solutions were mixed and volume was made up to 25.0 ml using distilled water and mixed again. The blank was also treated in the same manner. The reaction mixture was allowed incubation at ambient temperature for 10 min followed by recording absorbance at 460 nm. The amount of chloride ions in sample was calculated from standard curve prepared with NaCl (10-100 µg ml\(^{-1}\)).

C. Dinitrosalicylic acid (DNS, g l\(^{-1}\)) solution:

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNS</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Phenol</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Sodium sulfite</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Sodium potassium tartarate</td>
<td>200.0 g</td>
</tr>
</tbody>
</table>
All the ingredients are dissolved in 1.0% (w/v) NaOH and the volume is made up using distilled water.

**D. Spraying reagents:**

1. **Anthrone method (For carbohydrates):**
   
   Pour about 10 ml sulphuric acid in a 50 ml beaker and add some anthrone to it to get yellow colour. Spray the plate, air dry and then heat at 105°C for 10 min. Sugars produce blue/violet spots. Carbohydrates are dehydrated by conc. H₂SO₄ to form furfural. Furfural condenses with anthrone to form a blue colored complex.

2. **α-Naphthol sulphuric acid (For carbohydrates):**
   
   Spray the plate with 0.5% α-naphthol (in 5% H₂SO₄), air dry and heat at 105°C for 5 min. Carbohydrates form dull-violet coloration immediately.

3. **Diphenylamine reagent (For carbohydrates):**
   
   Prepare the following solutions:
   - **Reagent A:** 4 g diphenylamine in 80 ml of acetone and make up to 100 ml with acetone
   - **Reagent B:** add 4 ml of aniline to 96 ml of acetone and mix well
   - **Reagent C:** 85% ortho-phosphoric acid
   
   For the spray reagent, mix 100 ml of reagent A, 100 ml of reagent B and 20 ml of reagent C just prior to use. Spray the plate, air dry and heat at 105°C for 10 min. The color appears in 2-4 min. This spray reagent can be used for aldoses, ketoses, deoxysugars, oligosaccharides and uronic acids. It gives a wide variation of colors with different carbohydrates, aldoses producing blue grey spots, whereas ketoses give light red spots.

4. **Rhodamine-6G method (For lipids):**
   
   Spray the plate with 0.1 g l⁻¹ of rhodamine-6G in water and observe under UV light. The dye dissolves in lipids and shows a spot of pink/purple/red fluorescence.
5. **Hydroxylamine-ferric chloride reagent (For esterified fatty acids):**

**Alkaline hydroxylamine solution (freshly prepared):** Mix 100 ml of a 10% ethanolic solution of hydroxylamine hydrochloride (10 g NH$_2$OH.HCl in 25 ml of water; dilute to 100 ml with ethanol) with 200 ml of 12% ethanolic sodium hydroxide solution (24 g of NaOH in 25 ml of water; dilute to 200 ml with ethanol); centrifuge down the precipitate of sodium chloride and use the clear supernatant.

**Ferric chloride reagent:** grind 10 g of FeCl$_3$.6H$_2$O and 20 ml of conc. HCl in a mortar and shake the solution with 300 ml of ethyl ether to form an ethereal ferric chloride solution.

**Procedure:** Spray the plate with alkaline hydroxylamine solution; dry the plate briefly and spray it with ethereal ferric chloride solution. Esters appear as purple spots on a yellow background.

6. **Ninhydrin method (for amino acids):**

A 250 mg of ninhydrin is dissolved in 100 ml of acetone, spray the plate and purple/violet spots appear in about 8 h or overnight.