# Appendices

## Appendix A

### Medium and chemicals

**1. Mckeen medium**

| | | |
|---|---|---|
| Glucose | 20.0 | g |
| DL-glutamic acid | 5.0 | g |
| $MgSO_4.7H_2O$ | 1.02 | g |
| $K_2HPO_4$ | 1.0 | g |
| KCl | 0.5 | g |
| Distilled water | 1,000 | ml |
| Trace elements | 1.0 | ml |
| pH | 7.0 | |

**Method:** dissolved all ingredients in 1000 ml distilled water and autoclaved at 15 pond/inch$^2$ at 121 $^o$C for 15 minutes.

**Note:** Trace elements containing (g/100 ml distilled water)

| | | |
|---|---|---|
| $MgSO_4.4H_2O$ | 0.5 | g |
| Cu $SO_4.5H_2O$ | 0.16 | g |
| Fe $SO_4.7H_2O$ | 0.015 | g |

**2. Nutrient broth**

| | | |
|---|---|---|
| Beef extract | 3.0 | g |
| Peptone | 5.0 | g |
| pH | 7.0 | |

**Method:** dissolved all ingredients in 1000 ml distilled water and autoclaved at 15 pond/inch$^2$ at 121$^o$C for 15 minutes.

**3. Nutrient agar**

| | | |
|---|---|---|
| Beef extract | 3.0 | g |
| Peptone | 5.0 | g |
| Agar | 15.0 | g |
| pH | 7.0 | |

**Method:** dissolved all ingredients in 1000 ml distilled water, then mixed well and boiled. Autoclaved at 15 pond/inch$^2$ at 121 $^o$C for 15 minutes.

**4. Mueller Hinton agar**

| | | |
|---|---|---|
| Beef extract powder | 2.0 | g |
| Acid digest of casein | 17.5 | g |
| Soluble starch | 1.5 | g |
| Agar | 15.0 | g |
| pH | 7.3 | |

**Method:** dissolved all ingredients in 1000 ml distilled water, then mixed well and boiled. Autoclaved at 15 pond/inch$^2$ at 121 $^o$C for 15 minutes.

**5. Method and reagents for the detection of biochemical compounds on thin layer chromatograms (Dawson *et al*., 1968)**

4.1 Amino acids

4.1.1 Ninhydrin

For general use as a locating agent use 0.25% w/v ninhydrin in acetone. The spots may be developed by heating at 100$^o$C for 5 min or for a lighter background, by leaving at room temperature for few hours. The amino acid give purple spots except for histidine and glycine (red-gray); phenylalanine, tyrosine and aspartic acid (blue); tryptophane (brown); asparagine (dirty yellow); proline (yellow).

4.1.2 Ultraviolet light

The amino group can react with free aldehyde groups in the paper. The resulting Schiff's bases fluoresces blue in ultraviolet light. Heat the paper at 100 $^{o}$C for 30 min N-substituted amino acids give dark spots.

4.2 Carbohydrates

4.2.1 Alkaline potassium permanganate

Spray with 1.0% aq. $KMnO_4$ containing 2.0% $Na_2CO_3$. Dry at room temperature or rapidly at 100 $^{o}$C. Yellow spots on a purple ground, then gray spots on brown ground are given by sugar alcohols, glycosides, reducing and non-reducing sugars. Not given by methyl or acetyl sugars.

4.2.2 Iodine vapour

Expose for 15 min to iodine vapour. Brown spots are given by sugar mercaptals and alcohols, glycosides, N.acylamino sugars, neutral and acidic polysaccharides.

4.2.3 Anisaldehyde

Anisaldehyde 0.5 ml in 20 ml of methanol and 1 ml of sulfuric acid were used as anisaldehyde reagent for detecting sugar. Gray spot are given by the sugar.

4.3 Lipids

4.3.1 Rhodamine 6G

0.001% aq. rhodamine 6G in 0.25 M $K_2HPO_4$. View wet under UV light. Purple, blue and yellow spots against rose background.

4.3.2 Iodine vapour

Detect all lipids, nitrogenous compounds, non-reducing carbohydrates. Expose for 15 min to iodine vapour. Brown spots are given by all lipids.

6. **Tris-HCl buffer was prepared by the method of Bates and Bower** (1956, cited by Stoll and Blanchard, 1990)

   A: 0.02 M Tris (hydroxymethyl) aminomethane

   B: 0.02 M HCl

   50 ml of A + X ml of B

| B solution (ml) | pH |
|---|---|
| 46.6 | 7.0 |
| 45.7 | 7.1 |
| 44.7 | 7.2 |
| 43.4 | 7.3 |
| 42.0 | 7.4 |
| 40.3 | 7.5 |
| 38.5 | 7.6 |
| 36.6 | 7.7 |
| 34.5 | 7.8 |
| 32.0 | 7.9 |
| 29.2 | 8.0 |
| 26.2 | 8.1 |
| 22.9 | 8.2 |
| 19.9 | 8.3 |
| 17.2 | 8.4 |
| 14.7 | 8.5 |
| 12.4 | 8.6 |
| 10.3 | 8.7 |
| 8.5 | 8.8 |
| 7.0 | 8.9 |