APPENDIX

REAGENTS

Barritt’s reagent (for detection of acetylmethyl-carbinol in VP test)

Solution A
Alpha naphthol - 5.0g
Ethanol absolute - 95.0ml
Dissolve the alpha-naphthol in the ethanol with constant stirring.

Solution B
Potassium hydroxide - 40.0g
Creatine - 0.3g
Distilled water - 100.0ml
Dissolve the potassium hydroxide in 75ml of distilled water. The solution will become warm. Allow to cool to room temperature. Add the creatine and stir to dissolve. Add the remaining water. Store in a refrigerator.

Gram Staining reagents

Crystal violet (Hucker’s)

Solution A
Crystal violet (90% dye content) - 2.0 g
Ethyl alcohol (95%) - 20.0 ml

Solution B
Ammonium oxalate - 0.8 g
Distilled water - 80.0 ml
Note: Mix solutions A and B.

Gram’s iodine
Iodine - 1.0 g
Potassium iodide - 2.0 g
Distilled water - 300.0 ml

Ethyl alcohol (95%)
Ethyl alcohol (100%) - 95.0 ml
Distilled water - 5.0 ml

Safranin
Safranin O - 0.25 ml
Ethyl alcohol (95%) - 10.0 ml
Distilled water - 100.0 ml
Hanks balanced salt solution
Distilled water - 8lt.
Potassium phosphate -0.44mM
Potassium chloride -5.37mM
Sodium phosphate, dibasic -0.34mM
Sodium chloride -136.89mM
D-Glucose -5.55mM

Hydrogen peroxide, 3%, for detection of catalase activity
Note: Refrigerate when not in use.

Kovac’s reagent (for detection of indole)

p-Dimethylaminobenzaldehyde-5.0 g
Amyl alcohol -75.0 ml
Hydrochloric acid (concentrated)-25.0 ml

Note: Dissolve the p-dimethylaminobenzaldehyde in the amyl alcohol. Add the hydrochloric acid.

MacFarland’s standard

- Prepare 1% sulphuric acid solution.
- Prepare 1.175% solution of barium chloride by dissolving 2.35g of dehydrate barium chloride (BaCl₂.2H₂O) in 200ml of distilled water.
- To make the turbidity standard, add 0.5ml of barium chloride solution to 99.5ml of sulphuric acid solution and mix.
  The standard can be stored in dark at room temperature for upto 6 months.

Methyl red solution (for detection of acid)

Methyl red -0.1 g
Ethyl alcohol -300.0 ml
Distilled water -200.0 ml

Note: Dissolve the methyl red in the 95% ethyl alcohol. Dilute to 500 ml with distilled water.

Oxidase reagent (for detection of oxidase activity)

p-Aminodimethylanaline oxalate-0.5 g
Distilled water -50.0 ml
Note: To dissolve fully, gently warm the solution.
PBS (1x) (1lt)

Distilled water - 800ml
NaCl - 8g
KCl - 0.2 g
Na$_2$HPO$_4$ - 1.44 g
KH$_2$PO$_4$ - 0.24 g

Adjust the pH to 7.4 with HCl.
Add distilled water to a total volume of 1 liter.

Dispense the solution into aliquots and sterilize them by autoclaving (20 min, 121°C). Store at RT.

Protein estimation reagents- Lowry's method

Solution:A
2% of sodium carbonate in 0.1N NaOH

Solution:B
0.5% copper sulphate solution in 1% sodium potassium tartarate (to be prepared fresh)

Solution:C
Mix 50ml of solution A with 1ml of solution B, just prior to use.

Solution:D
Folin-ciocatteau reagent in the ratio 1:1 with water.

Agarose gel electrophoresis- reagents

TAE buffer (50X)- Stock solution

Tris base - 242 gm
Glacial acetic acid - 57.1 ml
0.5M EDTA - 100 ml
pH - 8.0
Distilled water - 1000ml

TAE buffer (1X)- working solution

Tris acetate - 40mM
EDTA - 1mM
pH - 8.0

Ethidium Bromide Solution

Stock - 10mg/ml
Dissolve 0.2g in 20ml of distilled water, mixed well and stored in dark bottles.
Working solution - 0.5μg/ml in gel.
Sample Buffer/ Gel Loading Buffer (6X)
  Sucrose -40.0%
  Bromophenol blue -0.25%

SDS- PAGE- reagents

Stock Acrylamide mix (solution in warm deionised water)
  Acrylamide -29.0%
  N,N’-methylene Bisacrylamide-1.0%
(Stored at 4°C in dark)
stored at RT in dark bottles.

Separating gel buffer (12%)- 30ml.
  Water -9.9ml
  30% acrylamide mix - 12.0ml
  1.5M Tris(pH 8.8) - 7.5ml
  10%SDS - 0.3ml
  10%APS - 0.3ml
  TEMED - 0.012ml.

Stacking gel buffer 5% (10ml)
  Water -6.8ml
  30% acrylamide mix - 1.7ml
  1.0M Tris(pH 6.8) - 1.25ml
  10%SDS - 0.1ml
  10%APS - 0.1ml
  TEMED - 0.01ml.

Electrophoresis buffer(1x)
  Tris Base -25mM
  Glycine -250mM
  SDS -0.1%
  pH -8.3

1x SDS Gel loading buffer
  Tris HCl(pH 6.8) - 50mM
  Dithiothreitol - 100mM
  SDS - 2%
  Bromophenol blue - 0.1%
  Glycerol - 10%
1x SDS gel loading buffer lacking dithiothreitol is stored at room temperature. Dithiothreitol is added just before the buffer is used.

**TEMED** (N,N,N’,N’- tetramethylethylenediamine): TEMED accelerates the polymerization of acrylamide and bis acrylamide by catalyzing the formation of free radicals from APS.

**APS**: APS provides the free radicals that drive polymerization of acrylamide and bis acrylamide. 10% stock solution should be prepared in deionized water and stored at 40°C. APS decomposes slowly, so fresh solutions should be used.

**Staining solution**

0.25 mg of Coomassie brilliant blue R 250 per 100ml of methanol: acetic acid solution. Filter the solution through a whatman no.1. filter to remove any particulate matter.

Methanol: acetic acid solution

- Methanol: 500ml
- Water: 400ml
- Glacial acetic acid: 100ml

Immerse the gel in at least 5 volumes of staining solution and place on a slowly rotating platform for a minimum of 4 hrs at room temperature.

**Destaining solution**

- Methanol: 500ml
- Acetic acid: 100ml
- Distilled water: 400ml

Destain the gel by soaking in destaining solution on a slowly rocking platform for 4-8 hrs, changing the destaining solution 3 or 4 times.

**MEDIA COMPOSITION**

(g/l unless otherwise specified)

**Carbohydrate Fermentation Broth**

- Trypticase: 10.0
- Sodium chloride: 5.0
- Phenol red: 0.018
- Desired sugar (Salicin, raffinose, Sucrose): 5.0g

**Eagle’s Minimum Essential Medium (MEM)**

**Inorganic Salts**

- CaCl₂·2H₂O: 0.2
MgSO$_4$ (anhydrous) -0.09767
KCl -0.4
NaCl -6.8
Na$_2$HPO$_4$ (anhydrous) -0.122
Sodium Succinate . 6H$_2$O - 0.1
Succinic Acid (free acid) -0.075

**Amino acids**
- L-Arginine . HCl -0.126
- L-Cystine . 2HCl - 0.0313
- L-Histidine . HCl . H$_2$O -0.042
- L-Isoleucine -0.052
- L-Leucine -0.052
- L-Lysine .HCl -0.0725
- L-Methionine -0.015
- L-Phenylalanine -0.032
- L-Threonine - 0.048
- L-Tryptophan -0.01
- L-Tyrosine . 2Na . 2H$_2$O - 0.036
- L-Valine - 0.046

**Vitamins**
- Choline Bitartrate -0.0018
- Folic Acid -0.001
- myo-Inositol -0.002
- Niacinamide -0.001
- D-Panthothenic acid . ½Ca - 0.001
- Pyridoxal . HCl -0.001
- Riboflavin -0.0001
- Thiamine . HCl -0.001

**Other**
- Glucose -1
- Phenol Red . Na -0.0064

**Add**
- L-Glutamine - 0.292
- NaHCO$_3$ -2.2

**Enrichment lactose broth**
- Beef extract -3.0
- Peptone -5.0
- Lactose -5.0
- pH -6.9

**LB Broth**
- Tryptone - 10g
- Yeast extract - 5g
- NaCl - 10g
- pH -7.0
**Macconkey Agar**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto peptone</td>
<td>-17.0</td>
</tr>
<tr>
<td>Proteose or polypeptone</td>
<td>-3.0</td>
</tr>
<tr>
<td>Lactose</td>
<td>-10.0</td>
</tr>
<tr>
<td>Bile salts</td>
<td>-1.5</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>-5.0</td>
</tr>
<tr>
<td>Agar</td>
<td>-15.0</td>
</tr>
<tr>
<td>Neutral red</td>
<td>-0.030</td>
</tr>
<tr>
<td>Crystal violet</td>
<td>-0.001</td>
</tr>
<tr>
<td>pH</td>
<td>-7.1</td>
</tr>
</tbody>
</table>

**MR-VP broth (pH 6.9)**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>-7.0</td>
</tr>
<tr>
<td>Dextrose</td>
<td>-5.0</td>
</tr>
<tr>
<td>Potassium phosphate</td>
<td>-5.0</td>
</tr>
</tbody>
</table>

**MUG EC O157 agar**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptic digest of animal tissue</td>
<td>-20.00</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>-5.0</td>
</tr>
<tr>
<td>Bile salt</td>
<td>-1.12</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>-20.00</td>
</tr>
<tr>
<td>4-methylumbelliferyl b-D-glucuronide</td>
<td>0.05</td>
</tr>
<tr>
<td>Bromocresol purple</td>
<td>-0.015</td>
</tr>
<tr>
<td>Agar</td>
<td>- 12.00</td>
</tr>
<tr>
<td>pH</td>
<td>-7.2±0.2</td>
</tr>
</tbody>
</table>

**Muller Hinton Agar**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef infusion</td>
<td>-300ml</td>
</tr>
<tr>
<td>Casein hydrolysate</td>
<td>-17.5</td>
</tr>
<tr>
<td>Starch</td>
<td>-1.5</td>
</tr>
<tr>
<td>Agar</td>
<td>-15.0</td>
</tr>
</tbody>
</table>

**M9 minimal medium**

**M9 Salts**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$_2$HPO$_4$-7H$_2$O</td>
<td>64g</td>
</tr>
<tr>
<td>KH$_2$PO$_4$</td>
<td>15g</td>
</tr>
<tr>
<td>NaCl</td>
<td>2.5g</td>
</tr>
<tr>
<td>NH$_4$Cl</td>
<td>5.0g</td>
</tr>
<tr>
<td>H$_2$O</td>
<td>800 ml</td>
</tr>
</tbody>
</table>

Stir until dissolved
Adjust to 1000ml with distilled H$_2$O
Sterilize by autoclaving
Medium

Distilled H₂O (sterile) - 700ml
M9 salts - 200ml
1M MgSO₄ (sterile) - 2ml
20% glucose (or other carbon source) - 20 ml
1M CaCl₂ (sterile) - 100μl
Thiamine - 1 mg
Lactate - 4g
Casamino Acids - 0.1g.
Adjust to 1000ml with distilled H₂O

Nutrient Agar

Peptone - 5.0
Beef extract - 3.0
Agar - 15.0
pH - 6.8

Nutrient broth

Peptone - 5.0
Beef extract - 3.0
pH - 7.0

Peptone broth

Peptone - 10.0 g
Sodium chloride - 5.0 g
pH - 7.2

Simmons Citrate Agar

Sodium citrate - 2.0
Sodium chloride - 5.0
Magnesium sulphate - 0.20
Ammonium dihydrogen phosphate - 1.0
Dipotassium phosphate - 1.0
Agar - 20.0
Bromothymol blue - 0.08
pH - 6.9

Sorbitol Mac Conkey agar

Peptone - 20.00
Sorbitol - 10.0
Synthetic detergent - 1.5
Sodium chloride - 5.0
Crystal violet - 0.001
<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar</td>
<td>-13.5</td>
</tr>
<tr>
<td>pH</td>
<td>-7.1±0.2</td>
</tr>
</tbody>
</table>

**Sorbitol Iron agar**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>-15.00</td>
</tr>
<tr>
<td>Beef extract</td>
<td>-3.0</td>
</tr>
<tr>
<td>D-Sorbitol</td>
<td>-2.0</td>
</tr>
<tr>
<td>Sodiumchloride</td>
<td>-5.0</td>
</tr>
<tr>
<td>Ferrous ammonium citrate</td>
<td>-0.5</td>
</tr>
<tr>
<td>Sodium thiosulphate</td>
<td>-0.5</td>
</tr>
<tr>
<td>Phenol red</td>
<td>-0.03</td>
</tr>
<tr>
<td>Agar</td>
<td>-20.00</td>
</tr>
<tr>
<td>pH</td>
<td>-7.6±0.2</td>
</tr>
</tbody>
</table>

**Starch agar (pH 7.0)**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>-5.0</td>
</tr>
<tr>
<td>Beef extract</td>
<td>-3.0</td>
</tr>
<tr>
<td>Starch (soluble)</td>
<td>-2.0</td>
</tr>
<tr>
<td>Agar</td>
<td>-15.0</td>
</tr>
</tbody>
</table>

**TSI Agar**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>-20.0</td>
</tr>
<tr>
<td>Lactose</td>
<td>-10.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>-10.0</td>
</tr>
<tr>
<td>Glucose</td>
<td>-1.0</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>-5.0</td>
</tr>
<tr>
<td>Beef extract</td>
<td>-3.0</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>-3.0</td>
</tr>
<tr>
<td>Ferric citrate</td>
<td>-0.3</td>
</tr>
<tr>
<td>Sodium thiosulphate</td>
<td>-0.3</td>
</tr>
<tr>
<td>Phenol red</td>
<td>-0.025</td>
</tr>
<tr>
<td>Agar</td>
<td>-20.0</td>
</tr>
<tr>
<td>pH</td>
<td>-7.4±0.2</td>
</tr>
</tbody>
</table>

**Urea broth**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea broth concentrate</td>
<td></td>
</tr>
<tr>
<td>(filter-sterilized solution)</td>
<td>10.0 ml</td>
</tr>
<tr>
<td>Sterile distilled water</td>
<td>90.0 ml</td>
</tr>
</tbody>
</table>

Note: Aseptically add the urea broth concentrate to the sterilized and cooled distilled water. Under aseptic conditions, dispense 3-ml amounts into sterile tube.