BIBLIOGRAPHY


xvi


OIE, (2012). *Laboratory methodologies for bacterial Antimicrobial susceptibility testing.* p1-11


XXIV


## APPENDIX

### I. Media Composition

**Blood agar base (per litre)**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar</td>
<td>15.0g</td>
</tr>
<tr>
<td>Beef extract</td>
<td>10.0g</td>
</tr>
<tr>
<td>Peptone</td>
<td>10.0g</td>
</tr>
<tr>
<td>NaCl</td>
<td>5.0g</td>
</tr>
<tr>
<td>pH</td>
<td>7.3±0.2 at 25°C</td>
</tr>
<tr>
<td>Sterile defibrinated blood</td>
<td>50.0ml</td>
</tr>
</tbody>
</table>

**Eosin Methylene Blue agar (per litre)**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar</td>
<td>13.5g</td>
</tr>
<tr>
<td>Pancreatic digest of caesin</td>
<td>10.0 g</td>
</tr>
<tr>
<td>K$_2$HPO$_4$</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Lactose</td>
<td>5.0g</td>
</tr>
<tr>
<td>Sucrose</td>
<td>5.0g</td>
</tr>
<tr>
<td>Eosin</td>
<td>0.4 g</td>
</tr>
<tr>
<td>Methylene blue</td>
<td>0.065 g</td>
</tr>
<tr>
<td>pH</td>
<td>7.2±0.2 at 25°C</td>
</tr>
</tbody>
</table>

**Luria Bertani Agar (Per litre)**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptone</td>
<td>10g</td>
</tr>
<tr>
<td>Yeast Extract</td>
<td>5g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5g</td>
</tr>
<tr>
<td>Agar</td>
<td>10g</td>
</tr>
<tr>
<td>pH</td>
<td>7.2 at 37°C</td>
</tr>
</tbody>
</table>

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XXVIII
### MacConkey Agar (per litre)

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>-20.0g</td>
</tr>
<tr>
<td>Agar</td>
<td>-12.0g</td>
</tr>
<tr>
<td>Lactose</td>
<td>-10.0g</td>
</tr>
<tr>
<td>Bile salts</td>
<td>-5.0g</td>
</tr>
<tr>
<td>NaCl</td>
<td>-5g</td>
</tr>
<tr>
<td>Neutral Red</td>
<td>-0.075g</td>
</tr>
<tr>
<td>pH</td>
<td>7.4±0.2 at 25°C</td>
</tr>
</tbody>
</table>

### Mannitol Salt Agar (per litre)

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>-75.0g</td>
</tr>
<tr>
<td>Agar</td>
<td>-15g</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>-10.0g</td>
</tr>
<tr>
<td>Pancreatic digest of Caesin</td>
<td>-5.0g</td>
</tr>
<tr>
<td>Peptic digest of animal tissue</td>
<td>-5.0g</td>
</tr>
<tr>
<td>Beef extract</td>
<td>-1.0g</td>
</tr>
<tr>
<td>Phenol Red</td>
<td>-0.025 g</td>
</tr>
<tr>
<td>pH</td>
<td>7.4±0.2 at 25°C</td>
</tr>
</tbody>
</table>

### MRVP Broth (Methyl Red- Voges Proskauer Broth) (per litre)

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic digest of casein</td>
<td>-3.5g</td>
</tr>
<tr>
<td>Peptic digest of animal tissue</td>
<td>-3.5g</td>
</tr>
</tbody>
</table>
KH$_2$PO$_4$ - 5.0g
Glucose - 5.0g

**Mueller-Hinton Agar (per litre)**

Acid Hydrolysate of casein - 17.5g
Agar - 17.0g
Beef extract - 2g
Starch - 1.5g
pH - 7.3±0.1 at 25ºC

**Nutrient Agar (per litre)**

Agar - 15
Peptone - 5.0g
NaCl - 5.0g
Yeast extract - 2.0g
Beef extract - 2.0g
pH - 7.4±0.2 at 25ºC

**Nutrient Broth (per litre)**

Peptone - 5.0g
NaCl - 5.0g
Yeast extract - 2.0g
Beef extract - 1.0g
pH - 7.4±0.2 at 25ºC

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**Peptone Water (Per litre)**

- Peptone: 10g
- Sodium chloride: 5g
- pH: 7.2 ± 0.2

**Simmons’ Citrate Agar (Per litre)**

- Agar: 15.0g
- NaCl: 5.0g
- Sodium citrate: 2.0g
- K$_2$HPO$_4$: 1.0 g
- (NH$_4$) H$_2$PO$_4$: 1.0 g
- MgSO$_4$ 7H$_2$O: 0.2g
- Bromothymol Blue: 0.08g
- pH: 06.9±0.2 at 25°C

**Triple sugar Iron Agar (Per litre)**

- Peptone: 20.0g
- Beef extract: 3.0g
- Yeast extract: 3.0g
- Lactose: 10.0g
- Sucrose: 10.0g
- NaCl: 5.0g
- Glucose: 1.0g
| Ferric citrate | -0.3g |
| Na₂S₂O₃     | -0.3g |
| Agar        | -12g |
| Phenol Red  | -0.025g |
| pH          | -7.4±0.2 at 25°C |

**Tryptophan Broth (Per litre)**

| L-Tryptophan | -0.5g |
| NaCl         | -0.5g |
| KH₃PO₄      | -0.25g |

**Urea Agar base (Per litre)**

| Agar        | -15g |
| NaCl        | -5.0g |
| Na₂HPO₄     | -12g |
| Peptone     | -1.0g |
| Glucose     | 1.0g |
| KH₂PO₄      | -0.8g |
| Phenol Red  | -0.012g |
| Urea solution | -50.0ml |
II REAGENT AND SOLUTIONS

Barritt's reagent

Solution A- 5 % alpha-naphthol in absolute ethanol.
Solution B- 40% KOH (aqueous)

Beta-lactam solution

(i) Benzyl Penicillin - 0.5mM
(Molecular weight of the benzyl penicillin- 356.4)
0.017g in 100ml of 0.1M phosphate buffer.
(ii) Ceftazidime - 0.5mM
(Molecular weight of the Ceftazidime- 636.6)
0.003g in 100ml of 0.1M phosphate buffer.

Crystal violet solution

Solution A
Crystal violet - 2g
Ethyl alcohol - 20 ml.
Solution B
Ammonium Oxalate - 0.8 g.
Distilled Water - 80 ml.

Grams Iodine

Iodine - 1.0 g
Potassium Iodide - 2.0 g
Distilled Water - 300 ml

Kovac's reagent

5.0 g para dimethylaminobenzaldehyde
75 ml amyl alcohol and 25 ml concentrated Hydrochloric acid

0.5g of para dimethylaminobenzaldehyde was dissolved in 75ml of amyl alcohol and heated to 50ºC. The solution was cooled acid was added, Hydrochloric acid was added carefully and stored in brown bottle.
Lowry’s reagent (Alkaline copper solution)

Solution A - 4% Na$_2$CO$_3$
Solution B - 0.5% CuSO$_4$. 5H$_2$O in 1% potassium sodium tartrate
Mixed 50 ml of reagent A with 2 ml of reagent B.

Mc Farlands standard (0.5N)
1.175% Barium chloride - 0.5ml
1% Sulphuric acid - 99.5ml

Phosphate buffer (0.1M)

Solution A (Monobasic sodium phosphate)
27.8g in 1000ml
Solution B (Dibasic sodium phosphate)
53.65g in 1000ml
39ml of solution A and 61ml of solution B
pH adjusted to 7.0

Safranin
Safranin - 10 ml
Distilled Water - 100 ml

SET buffer
75mM NaCl
25mM EDTA pH 8
20mM Tris HCl pH 7.5

TE buffer
10mM Tris
1mM EDTA

TAE buffer
Tris-acetate buffer (0.04M, pH 8.0), containing 0.00 M EDTA;
Sodium phosphate buffer (0.2M, pH 7.4)
Sodium dihydrogen phosphate 0.2 M - (13.8g/100ml)
ANNEXURE

List of Publications:


List Seminar / Conferences

1. Presented Paper entitled “Antibiogram and molecular characterisation of *staphylococcus*…..” in National level seminar on Recent advances and challenges in clinical microbiology and biomedical engineering organized by JJ college of Arts and science, Pudukottai on 23rd & 24th September 2010.