APPENDIX

Preparation of Sabouraud Dextrose Agar (SDA):

1. Dextrose - 40g
2. Agar - 20g
3. Peptone - 10g

The above ingredients were dissolved in 1000ml distilled water and autoclaved at 121°C for 15 min. The final pH was adjusted to 5.6.

Preparation of Sabouraud Dextrose Broth (SDB):

1. Dextrose - 20g
2. Peptone - 10g

The above ingredients were dissolved in 1000ml distilled water and autoclaved at 121°C for 15 min. The final pH was adjusted to 5.6.

Vero Cell Line

a) Requirements for the maintenance of Vero cell line

1. Vero cell monolayer bottles (National Centre for Cell Science, India)
2. Eagle’s minimum essential medium (MEM) containing Earle’s salts, L-Glutamine, Non-essential amino acids (HiMedia, India)
3. Sodium bicarbonate (HiMedia, India)
4. Newborn calf serum (Sterile, virus free. Sigma, USA)
5. Antibiotics (Penicillin; Streptomycin; Gentamycin. HiMedia, India)
6. Trypsin (Sigma, USA)

7. Ethylene diamine tetra acetic acid (EDTA, Stratagene GmbH, Heidelberg, FRG)

8. Glucose (Sigma, USA)

b) Preparation of Medium for Vero Cells:

Growth medium (for 1 litre)

Dehydrated MEM - 9.6 gm
Triple distilled water - 940 ml
Sodium bicarbonate (7.8% solution) - 10 ml
Newborn Calf serum - 50 ml

Antibiotics

- Penicillin - 0.1 ml
- Streptomycin - 0.1 ml
- Gentamycin - 0.1 ml

All the ingredients were dissolved, and the pH of the medium was adjusted to 7.1. It was then sterilised by passing through 0.2 μm membrane filter (Sartorius AG, Goettingen, Germany). The filtrate was distributed in small quantities and stored at 4°C till use. Sterility testing was done for each batch of medium.
Maintenance medium (for 1 litre)

1. Dehydrated MEM - 9.6 gm
2. Triple distilled water - 930 ml
3. Sodium bicarbonate (7.8% solution) - 50 ml
4. Newborn Calf serum - 20 ml
5. Antibiotics
   - Penicillin - 0.1 ml
   - Streptomycin - 0.1 ml
   - Gentamycin - 0.1 ml

All the ingredients were dissolved, filtered, sterilised and stored at 4°C until use.

Trypsin Versene Glucose (TPVG) for 100 ml:

1. Phosphate buffered saline, pH 7.2 - 100 ml
2. Trypsin - 0.1 gm
3. EDTA - 0.2 gm
4. Glucose - 0.5 gm

Sterilised by filtration through 0.2 µm membrane filter (Sartorius AG, Germany), aliquoted and stored at -20°C. Sterility testing was done on all the batches.
McCoy Cell Line

a) Requirements for the maintenance of McCoy cell line

1. McCoy cell monolayer bottles (National Centre for Cell Science, India)

2. Eagle’s minimum essential medium (MEM) containing Earle’s salts, L-Glutamine, Non-essential amino acids (HiMedia, India)

3. Sodium bicarbonate (HiMedia, India)

4. Newborn calf serum (Sterile, virus free, Hyclone, USA)

5. Antibiotics (Gentamycin, Vancomycin, Amphotericin B, HiMedia, India)

6. Trypsin (Sigma, USA)

7. Ethylene diamine tetra acetic acid (EDTA, Stratagene GmbH, Heidelberg, FRG)

8. Glucose (Sigma, USA)

b) Preparation of Medium for McCoy Cells

Growth Medium (for 1 litre)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehydrated MEM</td>
<td>9.6 gm</td>
</tr>
<tr>
<td>Triple distilled water</td>
<td>940 ml</td>
</tr>
<tr>
<td>Sodium bicarbonate (7.8% solution)</td>
<td>10 ml</td>
</tr>
<tr>
<td>Newborn Calf serum</td>
<td>80 ml</td>
</tr>
</tbody>
</table>
Antibiotics in final concentration

- Gentamycin - 2 ml
- Vancomycin - 2.5 ml
- Amphotericin B - 0.5 ml

All the ingredients were dissolved, and the pH of the medium was adjusted to 7.2. It was then sterilised by passing through 0.2 µm membrane filter (Sartorius AG, Goettingen, Germany). The filtrate was distributed in small quantities and stored at 4°C till use. Sterility testing was done for each batch of medium.

Maintenance medium (for 1 litre)

1. Dehydrated MEM - 9.6 gm
2. Triple distilled water - 930 ml
3. Sodium bicarbonate
   (7.8% solution) - 50 ml
4. Newborn Calf serum - 40 ml
5. Antibiotics
   - Gentamycin - 2 ml
   - Vancomycin - 2.5 ml
   - Amphotericin B - 0.5 ml
All the ingredients were dissolved, sterilised and stored vis-a-vis growth medium.

**Trypsin Versene Glucose (TPVG) for 100 ml:**

1. Phosphate buffered saline, pH 7.2 - 100 ml
2. Trypsin - 0.1 gm
3. EDTA - 0.2 gm
4. Glucose - 0.5 gm

Sterilised by filtration through 0.2 μm membrane filter (Sartorius AG, Germany), aliquoted and stored at -20°C. Sterility testing was done on all the batches.