Appendix

- **Penicillin (100 IU) and Streptomycin (100 µg)**
  The antibiotics were dissolved in millipore distilled water so as to give a final concentration of 100 IU of penicillin and 100 µg of streptomycin, mixed well and distributed in 1 mL aliquots and stored at −20°C.

- **Kanamycin (20 µg/mL)**
  20 µg of Kanamycin was dissolved in 1mL millipore distilled water and stored in refrigerator at −20°C.

- **Fungizone (20 µg/mL)**
  20 µg of fungizone was dissolved in 1 mL millipore distilled water and stored in refrigerator at −20°C.

- **NaHCO₃ (7.5%)**
  7.5 g of NaHCO₃ were dissolved in 100 mL of millipore water, filtered through Whatmann No: 1 filter paper and autoclaved at 15 lbs/15 minutes.

- **Dulbecco’s Modified Eagle Medium (DMEM ) media (10%)**
  Dissolve 2.5 g of DMEM in 250 mL of water. DMEM contains low glucose, L-glutamine, 110 ng/mL sodium pyruvate, pyridoxine hydrochloride. It does not contain sodium bicarbonate.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin (100 IU)</td>
<td>125 µL</td>
</tr>
<tr>
<td>Streptomycin (100 µg)</td>
<td>125 µL</td>
</tr>
<tr>
<td>Kanamycin (20 µg/mL)</td>
<td>250 µL</td>
</tr>
<tr>
<td>Amphotericin B. (20 µg/mL)</td>
<td>250 µL</td>
</tr>
<tr>
<td>3% L-glutamine (2 mM)</td>
<td>2.5 mL</td>
</tr>
</tbody>
</table>
7.5% sodium bicarbonate - 5 mL
1M HEPES - 1.25 mL
Fetal calf or bovine serum. - 25 mL

Except FCS/FBS, all the ingredients are added in the order to prepare the media. The pH was adjusted to 7.2-7.4 using CO₂ and then FCS/FBS was added.

❖ **Phosphate buffer solution**

Sodium chloride (NaCl) - 2 g
Potassium chloride - 50 mg
Disodium hydrogen phosphate - 720 mg
Potassium dihydrogen phosphate - 50 mg

All the above chemicals weighed and make it up to 250 mL using distilled water. Adjust the pH to 7.4.

❖ **RPMI 1640 (Rosewell Park Memorial Institute) media**

RPMI - 16.2 g
Streptomycin - 100 µg/mL
Kanamycin - 20 µg/mL
Penicillin - 100 IU/mL
FCS - 10%

16.2 g of RPMI weighed and dissolved in 1000 mL of water. The media was autoclaved and antibiotics were added.

To prepare complete RPMI 1640 media (c RPMI 1640), 100 mL of FCS was added to the prepared RPMI 1640 media.
❖ **Trypan Blue (0.1%)**

0.1g of trypan blue was dissolved in 100 mL of PBS, filtered through Whatmann No: 4 filter paper and autoclaved, stored at 4°C.

❖ **Bromophenol blue**

0.0125 g Bromophenol blue was diluted with 2g sucrose in 5mL distilled