

APPENDIX A

Media and reagents for parasite culture

1. RPMI solution (serum-free RPMI)

The RPMI medium was prepared as a stock solution by mixing the following ingredients 1 L of distilled water (DW):

RPMI 1640 (Biochrom, Germany)	10.43 g
Sodium hydrogen carbonate	2.00 g

This mixture was filtrated through 0.22 μm acrylic membrane of bottle top filter set (Corning, U.S.A.) for sterilizing and then stored at 4°C until used.

2. 1M HEPES buffer

This buffer was prepared by dissolving 23.831 g of N-2- Hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) (Sigma, U.S.A.) in 100 ml of DW. The solution was adjusted to pH 7.4 with 1 N HCl and sterilized by filtering through a sterile 0.2 μm acrylic membrane (Ultra pure, AquaPor LM, U.S.A.) and stored at 4°C until used.

3. Complete medium

To prepare complete medium, 5 ml of HEPES, 10% of AB or B group of serum and 300 μl of 10 mg/ml Gentamicin were mixed with 200 ml of serum free RPMI. The complete medium was stored at 4°C until used.

4. Phosphate buffer saline (PBS buffer pH 7.2)

This buffer was prepared by dissolving 1 tablet of PBS (Zymed, U.S.A.) in 100 ml of DW. The solution was then sterilized by filtration through a sterile 0.2 μm acrylic membrane (Ultra pure, AquaPor LM, U.S.A.) and stored at 4°C until used.

5. 0.9% Sodium chloride (NaCl)

The solution was prepared by dissolving 4.5 g NaCl in 500 ml DW, and filtered through a sterile 0.2 μm acrylic membrane (Ultra pure, AquaPor LM, U.S.A.) and stored at 4°C until used.

6. Freezing solution

The freezing solution consisted of 7.56 g sorbital, 180 ml of 0.9% NaCl and 70 ml of 99% glycerol. The solution was sterilized by filtering through a 0.45 µm Millipore membrane and kept at 4°C until used.

7. 3.5% Sodium Chloride (NaCl)

The solution was prepared by dissolving 3.5 g NaCl in 100 ml DW. The solution was sterilized by filtering through a sterile 0.2 µm acrylic membrane (Ultra pure, AquaPor LM, U.S.A.) and stored at 4°C until used.

8. 5% Sorbital

The solution was prepared by dissolving 5 g of sorbital (Sigma, U.S.A.) in 100 ml DW and sterilized by filtering through a sterile 0.2 µm acrylic membrane (Ultra pure, AquaPor LM, U.S.A.) and stored at 4°C until used.

9. Non-infected erythrocytes

Preparing pack red cell group O from blood bank by transferred to 15 ml centrifuge tube and added with serum-free RPMI or PBS and centrifuge at 2000x g, 10 minutes, and then removed supernatant. This pack red cell was washed until clear of buffy coat or white blood cell and kept 4°C until used.

10. [³H] Hypoxanthine

This solution was prepared by made up 500 µl into 10,000 µl of complete medium that was twenty-fold dilution.

11. Preparation of drug solution for *in vitro* sensitivity tests

Each drug test was prepared as a stock drug solution of 10⁻² nM

11.1 Chloroquine (CQ)

To prepared a stock CQ concentration of 10^{-2} nM, 51.59 mg of CQ diphosphate salt (Sigma, U.S.A.; MW = 515.9) was dissolved in 10 ml of 50% ethalnlol (HPLC grade).

11.2 Quinine (QN)

To prepared a stock QN concentration of 10^{-2} nM, 36.09 mg of QN hydrochloride (Sigma, U.S.A.; MW = 360.9) was dissolved in 10 ml of 50% ethalnlol (HPLC grade).

11.3 Mefloquine (MQ)

To prepared a stock MQ concentration of 10^{-2} nM, 42.48 mg of MQ hydrochloride (ARENCO Phramaceutica, Belgium; MW = 423.8) was dissolved in 10 ml of 50% ethalnlol (HPLC grade).

11.4 Artesunate (ARS)

To prepared a stock ARS concentration of 10^{-2} nM, 38.44 mg of ARS (Dafra Pharma NV; MW= 384.4) was dissolved in 10 ml of 50% ethalnlol (HPLC grade).