Appendix VIII

MEDIA

GROWTH MEDIUM (Not in routine use)

500 mL Eagles' MEM with Hanks' salt/without glutamine
10 mL Vitamins
5 mL L-glutamine (200 mM)
50 mL Inactivated fetal calf serum

Store at 4°C. Stable for 2 weeks.

MAINTENANCE MEDIUM

500 mL Eagles' MEM with Hanks' salt/without glutamine (stored at 4°C)
5 mL L-glutamine (200 mM, stored frozen at -20 °C in clean room freezer)
10 mL Inactivated fetal calf serum (stored frozen at -20 °C in clean room freezer)
5 mL Fungizone (250 µg/mL, stored frozen at -20 °C in clean room freezer)
*5 mL Gentamicin (1 mg/mL, stored frozen at -20 °C in clean room freezer)
**5 mL Vancomycin (10 mg/mL, stored frozen at -20 °C in clean room freezer)

Store at 4°C. Stable for 2 weeks.

* **Gentamicin Solution (1 mg/mL):**
Dilute entire contents (10 mL) of 10 mg/mL Gentamicin Sulphate vial into 90 mL of d. H_2O to achieve 1 mg/mL. Filter sterilize, then dispense into 5 mL aliquots (stored frozen at -20 °C in clean room freezer).

** **Vancomycin Solution (10 mg/mL):**
Add contents of 1 gram vial of Vancomycin to 100 mL of distilled H_2O. Filter sterilize, then dispense into 5 mL aliquots (stored frozen at -20 °C in clean room freezer.

Media Quality Control:

a. A 2 mL aliquot of maintenance media is to be placed into each of CMK, HFF, HEp-2 and RD tubes for a check on sterility/toxicity before the media is used.
b. Maintenance Media is to be registered into the LIS (micqc) using the date made as the lot number. The individual components and their lot numbers are recorded at this time.
c. QC results are all entered into the LIS when reading tube culture QC on Mondays, Wednesdays and Fridays.

Failed Media QC:

a. Any cell lines showing microbial contamination or toxicity is considered abnormal.
b. Do not release media lot for use pending resolution of the QC failure.
c. Inform charge/senior technologist to discuss further actions including the following.
d. Record in Incident Report (if necessary).
e. Repeat QC/re-make media to evaluate if it is the cell media, the supplement(s) or other materials causing the problem.

CELL WASHING MEDIUM (Purchased)

Hanks' Balanced Salt solution without $CaCL_2$, $MgCL_2$ and $MgSO_4.7H_2O$

CELL FREEZING MEDIUM (SIGMA C6164) (Purchased)

MEM supplemented with a mixture of fetal bovine serum and calf serum containing DMSO.

VIRAL FREEZING MEDIUM (SIGMA C6039) (Purchased)

MEM supplemented with a mixture of fetal bovine serum and calf serum containing 10% glycerol.

TRANSPORT MEDIA:

BARTEL'S VIRAL TRANSPORT MEDIUM (B1029-36A) (Purchased)

Eagles' MEM with nonessential amino-acid and L-glutamine in HBSS.
2% FBS
10 µg/mL Gentamicin
50 µg/mL Streptomycin
50 µg/mL Penicillin
4 µg/mL Amphotericin B
15 mM HEPES
9.5 mM Sodium bicarbonate

CHLAMYDIA TRANSPORT MEDIUM (Not in routine use)

500 mL Eagles' MEM with Hank's salt/without glutamine.
50 mL Fetal Calf Serum (normal)
5 mL 3M Glucose
5 mL Gentamicin (1000 µg/mL)
5 mL Vancomycin (1000 µg/mL)
5 mL Fungizone (250 µg/mL)

Combine all components aseptically. Dispense 1.5 mL transport medium into sterile screw-cap vials containing 3 glass beads. Store at -20^{o}C.