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APPENDIX I

PRIMARY CELL CULLURE

Preparation of chicken and duck embryo fibroblast (CEF and DEF)

CEF is used for antigen preparation and in the serum-neutralization test. Embryos are used on day 9 to 11 of incubation.

DEF is used for duck plague virus isolation. The cells are prepared from 15-day-old embryos.

Cell cultures are prepared using method described (2, 80)

1. Candle a group of embryonated eggs for viability

2. Clean the shell surface by swabbing with alcohol and tincture of iodine.

3. Cut around the shell just below the air sac, and flip open the shell. Remove the embryo by the forcep and lift the embryo out from its yolk sac and place in a petridish.

4. The head is cut off. The limbs and viscera can be removed and the rest can be minced with scissors. The minced tissues are then transferred to a trypsinization flask which contains sterite PBS.

5. Wash embryos by gentle stirring . Pour out the PBS and add fresh PBS. Repeat washing until the PBS is clear and free of blood and then pour out as much PBS as possible.

6. Trypsinization can be carried out by adding 5-10 ml of 0.25 % prewarmed trypsin solution per embryo and allow it to act for 15 minutes while stirring slowly at 25 ^{o}C. Place the flask on a slant. After the tissues have settled out, collect the supernatant containing single cells and small clusters of cells in a container with calf serum. Again, add a similar volume of trypsin to the rest of tissues

in the trypsinization flask and repeat the procedure 2 times until only the white fibrous tissues are left and no more cells disperse.

7. Filter the cell suspension through four layers of gauze into a suitable centrifuge tube.

8. Centrifuge for 15 minutes at about 500 g, preferably in a refrigerated centrifuge. Remove and discard the trypsin.

9. Add small amount of growth media to the packed cells and break up the cell pellet by gentle repeated pipetting.

10. Determine the number of viable cells in the suspension by mixing the cell with trypan blue and count in the hemocytometer.

11. The desired number of cells can be diluted by growth media and distribute to the containers, depending on the main objectives.

APPENDIX II

MEDIA AND REAGENTS

Growth medium (GM)

MEM	100	ml
Calf serum (heat inactivated)	10	ml
Penicillin and Streptomycin (10^4 unit/ml, 10^4 ug/ml)	1	ml
Fungizone (2.5 mg/ml)	0.1	ml

Final pH. 7.2

Store at 4 °C

Maintainance medium (MM)

The constituents of MM per 100 ml. medium is the same as GM. except need calf serum only 5 ml.

MEM

MEM	10.6	g
$NaHCO_3$	0.35	g
Distilled water	1000	ml

Suspend MEM powder in double distilled water and sterile by filter through 0.22 um millipore filter paper. Store at 4 °C.

0.25 % trypsin

Trypsin	0.25	g
PBS	100	ml

Suspend trypsin in PBS and sterile by filter through 0.22 um millipore filter paper. Store at 4 °C

Phosphate Buffer Saline (PBS), pH 7.2

NaCl	8.0	g
KCl	0.2	g
Na_2HPO_4	1.15	g
KH_2PO_4	0.20	g
Distilled water	1,000	ml

Final pH 7.2

Store at 4 °C

Penicillin 10^4 unit/ml and Streptomycin 10^4 ug/ml

1 Dissolve penicillin 5×10^6 unit and streptomycin sulfate 5 g in 500 ml double distilled water.

2 Sterile by filter through 0.22 um millipore fiter paper

3 Devide each 10 ml of penicillin and streptomycin by aseptic technic into sterile vial.

Store at -20 °C

Fungizone 2.5 mg/ml

1 Dissolve fungizone 50 mg in 25 ml sterile double distilled water with aseptic technic.

2 Devide each 2 ml of fungizone by aseptic technic into sterile vial.

Alsever's solution

Trisodium citrate, $2H_2O$	0.8	g
Sodium Chloride	0.42	g
Dextrose	2.05	g
Distilled water	100	ml

7.5 % formalin in normal saline

Full strength formalin (37-40 %)	18.75	ml
0.9 % normal saline	81.25	ml

1 : 40,000 tannic acid in normal saline

tannic acid	1	g
0.9 % normal saline	40	ml

After making 1,000 fold dilution from this solution, it is ready to use.

20 % Bromoethlamoniumbromide

Bromoethylamoniumbromide	20	g
Distilled water	100	ml

20 % Sodium thiosulfate

Sodium thiosulfate	20	g
Distilled water	100	ml

BIOGRAPHY

Mr. Taveesak Utamathanakorn was born on May 2, 1960 in Bangkok, Thailand. He graduated with the Bachelor degree of Science (second class honours) in Medical Technology from Faculty of Medicine, Chulalongkorn University in 1982.