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APPENDIX I

MEDIA AND REAGENT FOR CELL CULTURE

1. Cell Growth Medium

RPMI 1640	200	ml
Fetal bovine serum (heat inactivated)	20	ml
Vancomycin (5 mg/ml)	4	ml
Gentamycin (0.5 mg/ml)	2	ml
Amphotericin B (0.5 mg/ml)	0.8	ml

Final pH 7.4

Store at 4°C

2. Cell Maintenance Medium with cycloheximide

RPMI 1640	200	ml
Fetal bovine serum (heat inactivated)	10	ml
Glucose (0.11 g/ml)	10	ml
Vancomycin (5 mg/ml)	4	ml
Gentamycin (0.5 mg/ml)	2	ml
Amphotericin B (0.25 mg/ml)	0.8	ml
Cycloheximide (0.1 mg/ml)	4	ml

Final pH 7.4

Store at 4°C

3. Glucose 0.11 g/ml

Dissolve glucose 10.76 g in 100 ml RPMI 1640 . Sterile the solution by filtration through membrane filter porc size 0.22 um. Dispense into aliquots of 5 ml each by aseptic technique and store at -20°C. It was used to prepare maintenance medium.

4. RPMI 1640

Dissolve 10.36 g of RPMI 1640 powder in 1000 ml double distilled water, sterile by filtration through membrane filter pore size 0.22 um and Store at 4°C. It was used to prepare growth medium , maintenance , and Glucose.

5. Phosphate Buffer Saline (PBS), pH 7.2

NaCl	10.0	g
KCl	0.25	g
$Na_2HPO_4.2H_2O$	1.78	g
KH_2PO_4	0.25	g
DDW	1000.00	ml

Dissolve the compositions and adjust pH to 7.2, Sterile by autoclave at 121°C for 15 min. This buffer was used for washing the McCoy cells culture in subpassage step.

6. 2SP transport medium

Preperation of 0.2 M Sucrose Phosphate Buffer (2SP)

Solution A : 68.46 g of sucrose in 700 ml DDW.

Solution B : 2.088 g of anhydrous K_2HPO_4 in 60 ml DDW

Solution C : 1.088 g of anhydrous KH_2PO_4 in 40 ml DDW

Combine solution A, B, and C. Adjust pH to 7.0 and adjust volumn to 1 liter. Sterile by autoclave at 115°C for 15 min and store at 4°C.

Preperation of 2SP Transport Medium.

2SP	200	ml
Fetal bovine serum	20	ml
Vancomycin (5 mg/ml)	4	ml
Gentamycin (0.5 mg/ml)	4	ml
Amphotericin B (0.25 mg/ml)	4	ml

Dispense the 2SP transport medium into sterile plastic centrifuge tube with approximately 1.5 ml per tube and store at -20°C.

7. 4SP Medium

Solution A : 136.92 g of sucrose in 600 ml DDW.

Solution B : 2.268 g of Na_2HPO_4 in 200 ml DDW.

Combine solution A and solution B. Add 2.0 ml of 0.5% phenol red and adjust volumn to 800 ml with DDW. Adjust pH to 7.0 and adjust volumn to 1 liter with DDW. Sterile by autoclave at 115°C 15 min and store at 4°C. It was used to store the prophgated *C. trachomatis* by using equal volumn of 4SP and *C. trachomatis* suspension in maintenance medium.

8. 1% Trypsin

Dissolve 1 g of trypsin in 100 ml of DDW and sterile by filtration through membrane filter pore size 0.22 um. Store at 4°C. It was usea to trypsinize McCoy cells when the cell culture was subpassage.

9. Jones' iodine (5% iodine solution)

KI	5	g
I_2	5	g
methanol or absolute ethanol	50	ml
DDW	50	ml

Mix them together and filter through whatman filter paper No.1, store at room temperature in a bottle protected from light. This solution was used to stain *C. trachomatis* inclusion bodies in cell culture technique.

APPENDIX II

PREPARATION OF ANTIBIOTIC SOLUTION

1. Amphotericin B 0.25 mg/ml

Dissolve 50 mg of amphotericin B in 200 ml sterile DDW with aseptic technique and dispense into aliquots of 0.8 ml and 4 ml each by aseptic technique. Store at -20°C. It was used to inhibit fungi in transport medium, maintenance medium,and growth medium.

2. Cycloheximide 0.1 mg/ml

Dissolve 0.01 g of cycloheximide in 0.5 ml of acetone and aseptically add 100 ml sterile DDW. Dispense into aliquots of 4 ml each by aseptic technique and store at -20°C. It was used to prepare the maintenance medium in order to inhibit growth of McCoy cells.

3. Gentamycin 0.5 mg/ml

Dilute 2 ml of 80 mg gentamycin in 160 ml sterile DDW and dispense into aliquotes of 2 ml by aseptic technique. Store at -20°C. It was used to inhibit Gram negative bacteria in transport medium, maintenance medium, and growth medium.

4. Vancomycin 5 mg/ml

Dissolve 500 mg of vancomycin in 100 ml sterile DDW and dispense into aliquots of 4 ml each with aseptic technique. Store at -20°C. It was used to inhibit Gram positive bacteria in transport medium, maintenance medium, and growth medium.

APPENDIX III

REAGENTS FOR PLASMID ISOLATION

1) 1 M Tris-HCl (pH 8.0)

Dissolve 121.1 g Tris base in 800 ml of DDW. ADjust the pH to 8.0 by adding 42 ml of concentrated HCl. Allow the solution to cool at room temperature before making the final adjustments to the pH. Make up the volume of the solution to 1 liter. Dispense into aliquots and sterilize by autoclaving. If the 1 M solution has a yellow color, discard it and obtain better-quality Tris.

2) 0.5 mM EDTA (PH 8.0)

Add 186.1 g of disodium ethylene diamine tetraacetate.$2H_2O$ to 800 ml of DDW. Stir vigorously on a magnetic stirrer. Adjust the pH to 8.0 with NaOH (20 g of NaOH pellets). Dispense into aliquots and sterilize by autoclaving. The disodium salt of EDTA will not go into solution until the pH of the solution is adjusted to approximately 8.0 by the addition of NaOH.

3) 5 M NaCl

Dissolve 292.2 g of NaCl in 800 ml of DDW. Adjust volume to 1 liter. Dispense into aliquots and sterilize by autoclaving.

4) 25% Sodium dodecyl sulfate (SDS)

Dissolve 500 g of SDS in 800 ml of DDW. Heat to 68°C to assist dissolution. Adjust the volume to 1 liter. Dispense into aliquots. Wear a mask when weighing SDS. There is no need to sterilize 25% SDS.

5) 2M Tris-HCl (pH 7.0)

Dissolve 242.2 g Tris base in 800 ml of DDW. Adjust the pH to 7.0 by adding concentrated HCl. Allow the solution to cool to room temperature before making the final adjustments to the pH. Make up the volume of the solution to 1 liter. Dispense into aliquots and sterilize by autoclaving.

6) TE buffer (pH 8.0)

50 mM Tris-HCl (pH 8.0)

10 mM EDTA (pH 8.0)

preperation (10 ml)

1 M Tris-HCl, pH 8.0	0.5 ml
0.5 M EDTA, pH 8.0	0.2 ml
DDW	9.3 ml

7) Lysis buffer

TE buffer

4% SDS

pH 12.4

preperation (10 ml)

TE buffer	8.4 ml
25 % SDS	1.6 ml

adjust pH to 12.0 with 10 N NaOH and then adjust pH to 12.4 with 3 N NaOH

APPENDIX IV

REAGENTS FOR AGAROSE GEL ELECTROPHORESIS

1. 50 x Tris-acetate buffer (TAE)

Tris base	424.0	g
glacial acetic acid	57.1	ml
0.5 M EDTA pH 8.0	100.0	ml

Adjust the volume to 1 liter with DDW and sterilize by autoclaving at 121°C for 15 min.

2. 10 mg/ml Ethidium bromide

ethidium bromide	1	g
DDW	100	ml

Stir on a magnetic stirrer for several hours to ensure that the dye has dissolved. Wrap the container in aluminum foil or transter to a dark bottle and store at 4°C.

3. 0.7 % Agarose gel

agarose ultrapure (Amresco, U.S.A.)	0.14	g
1x TAE	20.0	ml
10 mg/ml ethidium bromide	1.0	ul

4. 1.5 % Agarose gel

agarose ultrapure (Amresco, U.S.A.)	0.3	g
1x TAE	20.0	ml
10 mg/ml ethidium bromide	1.0	ul

APPENDIX VI

REAGENTS FOR DOT BLOT HYBRIDIZATION

1. 20X SSC

NaCl	175.3	g
sodium citrate	88.2	g
DDW	800.0	ml

Dissolved these components and adjust pH to 7.0 with NaOH (6.5 ml of a 10 N solution). Adjust volume to 1 liter. Dispense into a aliquots. Sterilize by autoclaving

2. Prehybridization solution (100 ml)

Component	Final concentration	Amount
Formamide	50% (V/V)	50 ml
NaCl	0.9 M	5.29 g
$NaH_2PO_4.H_2O$	0.06 M	0.83 g
$Na_2EDTA.H_2O$	0.006 M	0.22 g
Ficoll	0.1%(W/V)	0.1 g
Polyvinylpyrrolidone (PVP)	0.1%(W/V)	0.1 g
Bovine serum albumin (BSA)	0.1%(W/V)	0.1 g
Sodium dodecyl sulfate (SDS)	1.0%(W/V)	1.0 g
Sheared, denatured Salmon sperm DNA	200 ug/ml	20 mg

Dissolve solid components except DNA in 40 ml DDW. Adjust pH to 7.4 with 4 M NaOH. Add 2.0 ml of 10 mg/ml sheared,denatured salmon sperm DNA. Adjust volume to 50 ml with DDW. Add 50 ml formamide. Store at -20°C.

3. 2 x Hybridization solution (50 ml)

Component	Final concentration	Amount
NaCl	1.8 M	5.29 g
$NaH_2PO_4.H_2O$	0.12 M	0.83 g
$Na_2EDTA.H_2O$	0.012 M	0.22 g
Ficoll	0.2%(W/V)	0.1 g
Polyvinylpyrrolidone (PVP)	0.2%(W/V)	0.1 g
Bovine serum albumin (BSA)	0.2%(W/V)	0.1 g
Sodium dodecyl sulfate (SDS)	2.0%(W/V)	1.0 g
Sheared, denatured Salmon sperm DNA	240 ug/ml	20 mg

Dissolve solid components except DNA in 40 ml DDW. Adjust pH to 7.4 with 4 M NaOH. Add 2.0 ml of 10 mg/ml sheared,denatured salmon sperm DNA. Adjust volume to 50 ml with DDW. Store at -20°C.

4. TBS-Tween 20 (1 lit)

Component	Final concentration	Amount
Tris base	100 mM	12.1 g
NaCl	150 mM	8.77 g
Tween 20	0.05 % (V/V)	0.5 ml

Adjust pH to 7.5 with 4 M HCl. Filter through a sterile 0.2 um membrane. Store as a sterile solution at 4°C.

5. Blocking solution (100 ml)

Dissolve 3 g Bovine serum albumin in 100 ml TBS-Tween 20. Adjust pH to 7.5. Filter through a sterile 0.45 um membrane. Store at 4°C.

Note: Bovine serum albumin contains phosphatase that may interfere with the assay. Heat-treated BSA from pasteurized serum has reduced levels of phosphatase activity, and has been used successfully with this system.

BIOGRAPHY

Miss Karnjana Hrimpeng was born on January 25, 1963 in Chacherngsao, Thailand. She graduated with the Bachelor degree of Science in biology (microbiology) from Faculty of Science, Kasetsart University in 1985. Now she works as a scientist at Department of Microbiology, Chulalongkorn Hospital, Thai red cross society.