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APPENDIX I

PREPARATION OF ANTIBIOTIC SOLUTIONS

1. Amphotericin B 0.25 mg/ml

1. Dissolve amphotericin B 50 mg in 200 ml sterile double distilled water with aseptic technique.

2. Dispense into aliquots of 0.8 ml and 4 ml each by aseptic technique.

3. Store at -20° C.

It was used to inhibit fungi in transport medium, maintenance medium and growth medium.

2. Cycloheximide 0.1 mg/ml

1. Dissolve cycloheximide 0.01 g in 0.5 ml acetone.

2. Aseptically add 100 ml sterile double distilled water.

3. Dispense into aliquots of 4 ml each by aseptic technique.

4. Store at -20° C.

It was used to inhibit growth of McCoy cells in maintenance medium.

3. Gentamycin 0.5 mg/ml

1. Dilute 2 ml of 80 mg gentamycin in 160 ml sterile double distilled water.

2. Dispense into aliquots of 2 ml by aseptic technique.

3. 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

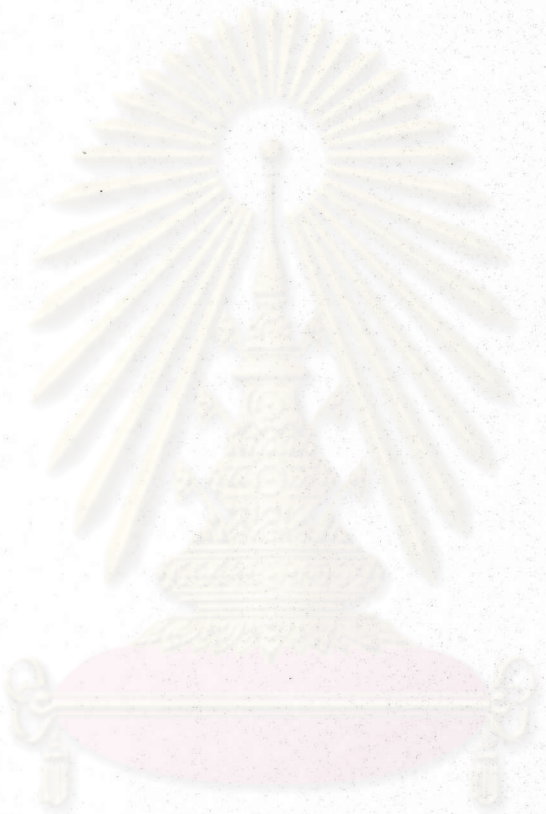
1. Dissolve vancomycin 500 mg in 100 ml sterile double

distilled water with aseptic technique.

2. Dispense into aliquots of 4 ml each.

3. Store at -20° C.

It was used to inhibit Gram positive bacteria in transport medium, maintenance medium and growth medium.



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APPENDIX II

BUFFER

1. 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
double distilled waster (DDW)	1000	ml

Dissolve the compositions, adjust pH to 7.2 and autoclave at 121° C.

This buffer was use for washing the McCoy cells culture.

2. Phosphate Buffer Saline (PBS), pH 7.2

NaCl	8.0	g
KCl	0.2	g
$Na_2HPO_4.2H_2O$	1.15	g
KH_2PO_4	0.196	g
double distilled waster (DDW)	1000	ml

Dissolve the compositions, adjust pH to 7.2 and store at 4° C.

This buffer was used to dilute serum and wash slides in micro-immunofluorescent test.

3. Phosphate Buffer Saline (PBS), pH 7.4

Solution A : 0.2 M NaH_2PO_4 ($NaH_2PO_4.H_2O$ 27.6 g/l)

Solution B : 0.2 M Na_2HPO_4 ($Na_2HPO_4.7H_2O$ 53.65 g/l)

or ($Na_2HPO_4.12H_2O$ 71.63 g/l)

Preparation of 0.01 M PBS, pH 7.4

Solution A	8	ml
Solution B	42	ml

NaCl	7.4	g

Then make to 1000 ml with DDW, adjust pH 7.4 then store at 4° C

This buffer was used to dilute serum and wash slides in rapid immunoperoxidase assay.

4. Tris Buffer, 0.05 M, pH 7.6

1. Dissolve 6.1 g Tris (Trishydroxymethyl aminomethane) base in 50 ml distilled water.
2. Add 37 ml of 1 N HCl.
3. Dilute to a total volume of 1000 ml with distilled water. The pH should be 7.60±0.2 at 25° C.

This Tris buffer was used in substrate solution preparation when rapid immunoperoxidase assay was performed.

5. Tris Buffer, pH 8.0

Tris	1.2114	g
EDTA	0.2922	g
NaCl	5.544	g
DDW	1000	ml

Dissolve the compositions, adjust pH to 8.0 and store at 4° C.

This buffer was mixed with glycerene volume by volume to make mounting fluid in micro-immunofluorescent test.

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APPENDIX III

MEDIA AND REAGENTS FOR CELL CULTURE

1. Cell Growth Medium

RPMI 1640	200	ml
Fetal bovine serum (heat inactivated)	20	ml
Vancoycin (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

1. Dissolve glucose 10.76 g in 100 ml RPMI medium.
2. Sterile the solution by filtration through membrane filter pore size 0.22 u.
3. Dispense into aliquots of 5 ml each by aseptic technique.

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4. Store at -20° C.

It was used to prepare maintenance medium.

4. RPMI 1640 Medium

RPMI 1640 powder	10.36	g
DDW	1000	ml

1. Suspend RPMI 1640 powder in double distilled water.

2. Sterile by filtration through membrane filter pore size 0.22 u.

3. Store at 4° C.

It was used to prepare growth medium and maintenance medium.

5. 2SP Transport medium

5.1 Preparation of 0.2 M Sucrose Phosphate Buffer (2SP)

1.1 Solution A: 68.46 g of sucrose in 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.

1.2 combine solution A, B and C; bring to close up 1000 ml with DDW.

1.3 Adjust pH to 7.0.

1.4 Bring the volume to 1000 ml with DDW.

1.5 Sterile by autoclave at 115° C, 15 minutes.

1.6 Store at 4° C.

5.2 Preparation of 2SP Transport Medium

2SP (from 1)	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 ml per tube. Store at -20° C.

6. 4SP Medium

1. Solution A: 136.92 g of sucrose in 600 ml DDW.
   Solution B: 2.268 g of Na_2HPO_4 in 200 ml DDW.
2. Combine solution A and Solution B.
3. Add 2.0 ml of 0.5% phenol red.
4. Bring the volume close up 1000 ml, adjust pH to 7.0.
5. Bring the volume to 1000 ml with DDW.
6. Sterile by autoclave at 115° C, 15 minutes.
7. Store at 4° C.

4SP medium (0.4 M Sucrose Phosphate Buffer) was used to store the propagated C. trachomatis by using equal volume of 4SP and C. trachomatis suspension in maintenance medium.

7. 1% Trypsin

Trypsin	1	g
DDW	1000	ml

1. Suspend trypsin in DDW.
2. Sterile by filtration through membrane filter pore size 0.22 u.
3. Store at 4° C.

It was used to trypsinize McCoy cells when the cell culture was sppassage.

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APPENDIX IV

STAIN AND CHROMATOGENIC SOLUTION

1. Alcohol-Formalin

Formalin or formaldehyde 37%	100	ml
Methanol or Absolute ethanol	900	ml

Mix together and store at room temperature.

This solution was used to fix the McCoy cells to the coverslip in iodine staining technique for C. trachomatis inclusion bodies.

2. 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 culture technique.

3. Jones' iodine-glycerine

An equal volume of Jones' iodine and glycerine was mixed together and store at room temperature in a bottle protected from light.

This solution was used as mounting fluid after stain with Jones' iodine solution.

4. Chromogen/Substrate Solution

1. Dissolve 3 mg of 4-chloro-1-naphthol in 0.1 ml absolute

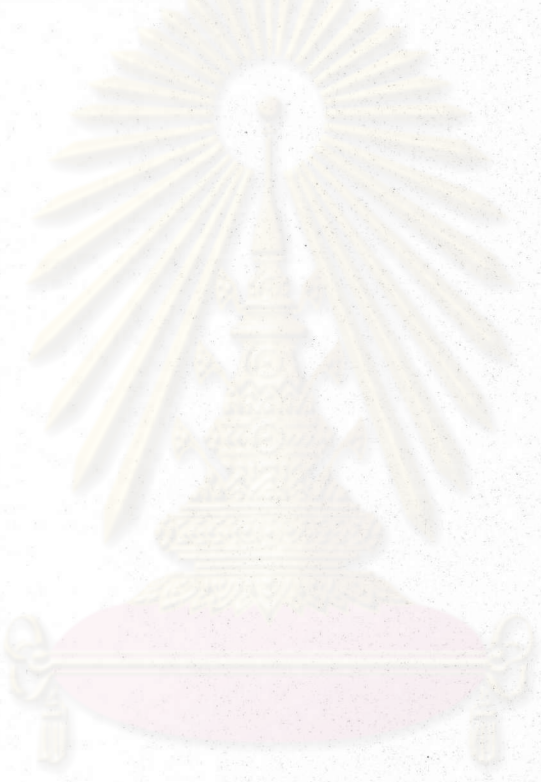
ethanol.

2. While stirring, add this to 10 ml 0.05 M Tris buffer, pH 7.6.

3. Add 0.1 ml of 3% hydrogen peroxide.

4. Filter out a white precipitate before use.

This solution was used as substrate/chromogen in rapid immunoperoxidase assay. It must be freashy prepared before used.



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APPENDIX V

Table 1A Titration of C. trachomatis serotype L_2 (from 100% infection) against degree of infection in McCoy Cells

dilution	% infected cells
1:2	100
1:5	100
1:10	100
1:20	100
1:50	100
10^{-2}	100
10^{-3}	86
10^{-4}	38
10^{-5}	3

Table 2A Appropriate amount of C. trachomatis serotype L_2 infected McCoy cells for slide coating

concentration (cells/ml)	coating results
$5x10^5$	confluent monolayer
$2x10^5$	optimum, good distribution
$1x10^5$	fair
$1x10^4$	very thin
$1x10^3$	non detectable

Optimum conditions for rapid immunoperoxidase assay.

Suitable dilution of rabbit anti-human immunoglobulin peroxidase conjugate

Table 3A Suitable dilution of rabbit anti-human IgG/peroxidase conjugate

conjugate dilutions	control dilutions							C-*	NHS**
	1:50	1:100	1:150	1:200	1:250	1:300	1:500		
1:20	3	3	3	3	2	1	-	-	-
1:40	3	2	2	2	1	-	-	-	-
1:60	1	1	1	1	-	-	-	-	-
1:80	1	1	-	-	-	-	-	-	-

* Negative control sera

** Normal human sera (1:8 dilution)

- negative, cells having no color
1 light blue inclusion
2 blue inclusion
3 dark blue inclusion

Table 4A Suitable dilution of rabbit anti-human IgM/peroxidase conjugate

conjugate dilutions	control dilutions						C-	NHS
	1:100	1:200	1:250	1:300	1:350	1:400		
1:20	1	1	1	1	1	-	-	-
1:40	1	-	-	-	-	-	-	-
1:60	-	-	-	-	-	-	-	-
1:80	-	-	-	-	-	-	-	-

Table 5A Suitable dilution of rabbit anti-human IgA/peroxidase conjugate

conjugate dilutions	control dilutions						C-	NHS
	1:4	1:8	1:16	1:32	1:64	1:128		
1:20	1	1	1	1	-	-	-	-
1:40	-	-	-	-	-	-	-	-
1:60	-	-	-	-	-	-	-	-
1:80	-	-	-	-	-	-	-	-

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Table 6A Optimum reaction time for chlamydial antigen and serum

antibody class	control dilution	time (minutes)				
		15	30	60	90	120
IgG	1:128	3	2	2	3	3
	1:256	2	2	2	2	2
	1:512	1	2	2	2	2
	1:1024	–	1	1	1	1
	1:2048	–	–	–	–	1
	C–	–	–	–	–	–
	NHS	–	–	–	–	–
IgM	1:32	2	3	3	3	3
	1:64	1	2	2	3	3
	1:128	1	1	1	2	2
	1:256	–	–	–	1	1
	1:512	–	–	–	–	–
	C–	–	–	–	–	–
	NHS	–	–	–	–	–
IgA	1:4	1	2	1	1	2
	1:8	1	2	1	1	2
	1:16	1	1	1	1	1
	1:32	–	1	1	1	1
	1:64	–	–	–	–	1
	C–	–	–	–	–	–
	NHS	–	–	–	–	–

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Table 7A Incubation time of anti-human Ig peroxidase conjugate

antibody class	control dilution	time (minutes)			
		30	60	90	120
IgG	1:128	-	1	2	2
	1:256	-	1	1	1
	1:512	-	-	-	-
	1:1024	-	-	-	-
	1:2048	-	-	-	-
	C-	-	-	-	-
	NHS	-	-	-	-
IgM	1:8	-	-	1	1
	1:16	-	-	-	-
	1:32	-	-	-	-
	1:64	-	-	-	-
	1:128	-	-	-	-
	C-	-	-	-	-
	NHS	-	-	-	-
IgA	1:4	-	-	1	1
	1:8	-	-	-	-
	1:16	-	-	-	-
	1:32	-	-	-	-
	1:64	-	-	-	-
	C-	-	-	-	-
	NHS	-	-	-	-

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Table 8A Optimum temperature

antibody class	control dilution	temperature (° C)			
		37*/37**	37*/RT**	RT*/37**	RT*/RT**
IgG	1:32	2	2	2	2
	1:64	2	2	2	2
	1:128	1	1	1	1
	1:256	1	–	–	–
	1:512	–	–	–	–
	C–	–	–	–	–
	NHS	–	–	–	–
IgM	1:4	1	1	1	–
	1:8	1	1	–	–
	1:16	–	–	–	–
	1:32	–	–	–	–
	1:64	–	–	–	–
	C–	–	–	–	–
	NHS	–	–	–	–
IgA	undil.	3	3	3	3
	1:4	2	2	2	2
	1:8	1	1	1	1
	1:16	–	–	–	–
	1:32	–	–	–	–
	C–	–	–	–	–
	NHS	–	–	–	–

* = serum and Ag step

** = conjugate reaction step

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Table 9A Optimum condition for substrate/chromogen reaction

antibody class	control dilution	condition		
		RT,30 min	37°C,15 min	37°C,30 min
IgG	1:32	2	2	2
	1:64	2	1	1
	1:128	1	1	1
	1:256	–	–	–
	1:512	–	–	–
	C–	–	–	–
	NHS	–	–	–
IgM	1:4	1	1	1
	1:8	1	–	–
	1:16	–	–	–
	1:32	–	–	–
	1:64	–	–	–
	C–	–	–	–
	NHS	–	–	–
IgA	undil	2	2	2
	1:4	2	2	2
	1:8	1	1	1
	1:16	1	1	–
	1:32	–	–	–
	C–	–	–	–
	NHS	–	–	–

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Final suitable dilution of rabbit anti-human immunoglobulin peroxidase conjugate

Table 10A Final suitable dilution of rabbit anti-human IgG/peroxidase conjugate

conjugate dilutions	control dilutions					C-	NHS
	1:50	1:100	1:150	1:200	1:250		
1:20	2	1	1	-	-	-	-
1:40	2	1	-	-	-	-	-
1:60	1	-	-	-	-	-	-
1:80	1	-	-	-	-	-	-

Table 11A Final suitable dilution of rabbit anti-human IgM/peroxidase conjugate

conjugate dilutions	control dilutions			C-	NHS
	1:4	1:8	1:16		
1:20	1	-	-	-	-
1:40	-	-	-	-	-
1:60	-	-	-	-	-
1:80	-	-	-	-	-

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Table 12A Final suitable dilution of rabbit anti-human IgA/peroxidase conjugate

conjugate dilutions	control dilutions					C-	NHS
	undil	1:4	1:8	1:16	1:32		
1:20	2	1	1	-	-	-	-
1:40	2	1	-	-	-	-	-
1:60	2	-	-	-	-	-	-
1:80	1	-	-	-	-	-	-

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APPENDIX VI

Table 15 Serological manifestation of 69 patients with positive chlamydial isolation

method	serum			secretion		
	IgG	IgM	IgA	IgG	IgM	IgA
m-IF	65	5	3	54	2	41
IP	67	1	5	36	0	39
avidin-biotin IP	ND	ND	ND	ND	ND	55

ND = Not done

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Table 16 Serum IgG chlamydial antibody by micro-immunofluorescence (m-IF) versus isolation of C. trachomatis

m-IF titer	Isolation of C. trachomatis		Percent of culture positive
	positive	negative	
< 1:8	4	23	14.8
1:8	8	19	29.6
1:16	16	30	34.7
1:32	20	29	40.8
1:64	13	10	56.5
1:128	8	10	44.0
1:256	-	7	0
1:512	-	2	0
1:1024	-	1	0

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Table 17 Serum IgG chlamydial antibody by rapid immunoperoxidase (IP) versus isolation of C. trachomatis

IP titer	Isolation of C. trachomatis		Percent of culture positive
	positive	negative	
< 1:8	2	12	14.3
1:8	10	24	29.4
1:16	24	40	37.5
1:32	17	29	36.9
1:64	13	12	52.0
1:128	3	7	30.0
1:256	-	6	0
1:512	-	-	0
1:1024	-	1	0

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Table 18 Comparison of IgG antibody detection in secretion by rapid immunoperoxidase (IP) and micro-immunofluorescence (m-IF)

Test		m-IF		Total
		Positive	Negative	
IP	positive	46	1	47
	negative	8	145	153
Total		54	146	200

Sensitivity 85.19% Positive predictive value 97.87%

Specificity 99.32% Negative predictive value 94.77%

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Table 19 Comparison of secretory IgA antibody detection by rapid immunoperoxidase (IP) and micro-immunofluorescence (m-IF)

Test		m-IF		Total
		Positive	Negative	
IP	positive	46	7	53
	negative	12	135	147
Total		58	142	200

Sensitivity 79.31%
Specificity 95.07%
Positive predictive value 86.79%
Negative predictive value 91.84%

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Table 20 Comparison of secretory IgA antibody detection by avidin-biotin immunoperoxidase and micro-immunofluorescence (m-IF)

Test		m-IF		Total
		Positive	Negative	
avidin-biotin IP	positive	55	29	84
	negative	3	113	116
Total		58	142	200

Sensitivity 94.83% Positive predictive value 65.48%

Specificity 79.58% Negative predictive value 97.41%

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Table 21 Comparison of IgG antibody in secretion demonstrated by rapid immunoperoxidase (IP) and isolation of C. trachomatis

Test	Isolation of C. trachomatis		Total
	Positive	Negative	
IgG positive	36	11	47
IgG negative	33	120	153
Total	69	131	200

Sensitivity 52.17%

Positive predictive value 76.60%

Specificity 91.60%

Negative predictive value 78.43%

X^2 = 48.18

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Table 22 Comparison of secretory IgA demonstrated by rapid immunoperoxidase (IP) and isolation of C. trachomatis

IP	Isolation of C. trachomatis		Total
	Positive	Negative	
Secretory IgA positive	39	14	53
Secretory IgA negative	30	117	147
Total	69	131	200

Sensitivity 56.52%

Positive predictive value 73.58%

Specificity 89.31%

Negative predictive value 79.59%

X^2 = 48.75

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BIOGRAPHY

Miss Wimon Chanchaem was born on September 17, 1961 in Bangkok, Thailand. She graduated with the Bachelor degree of Science in Microbiology from the Faculty of Science, Chulalongkorn University. Now she works as a scientist at Department of Microbiology, Faculty of Medicine, Chulalongkorn university.

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