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APPENDICES

APPENDIX I

MEDIAS, REAGENTS, MATERIALS AND INSTRUMENTS

A. MEDIA AND REAGENTS

Absolute ethanol	(Scharlau, Spain)
Agarose (ultrapure)	(GIBCO BRL, USA)
Ethidium bromide	(USB, UK)
Ethylenediaminetetraacetic acid	(Bio – Rad, Canada)
λ DNA / *Hind III* Fragments	(GIBCO BRL, USA)
Maleic acid	(Merck, Germany)
McFarland	(bioM' Erieux)
Phenol, Equilibrated	(USB, UK)
RPMI 1640	(Angus, USA)
Sabouraud Dextrose Broth	(Difco, USA)
Sodium acetate	(USB,UK)
Sodium chloride	(Merck, Germany)
Sodium citrate	(Sigma, USA)
Sodium dodecyl sulphate	(Pharmacia Biotech, Sweden)
Sodium Hydroxide	(Sigma, USA)
Tris Base	(Promega, USA)
Tween 20	(USB, UK)
8-hydroxyquinolene	(Sigma, USA)
Chloroform	(USB,UK)
Phenol, crystal	(Merck, Germany)
Taq DNA polymerase	(Promega, USA)
Dig – High Prime starter and detection kit	(Roche, Germany)

B. MATERIALS

Eppendrof

Gelblock

Micropipett

Test tube

Tip

Centrifuge tube

C. INSTRUMENTS

Autoclave (model SS-325)	(Tomy seiko, Japan)
Cooling system	(Bio – Rad, Canada)
Electrophoresis chamber	(CBS, USA)
Freezer	(Sunyo, Japan)
Hybridization oven	(Thermo hybraid, USA)
Incubator	(Contherm, New Zealand)
Microcentrifuge	(Hanil, Korea)
Microwave	(Sharp, Japan)
pH meter	(Orion, USA)
Power supply	(CBS, USA)
Pulse – Field Gel box	(Bio – Rad, Canada)
Pump, Gel molds	(Bio – Rad, Canada)
Refrigerator centrifuge	(Kubota, Japan)
Rotary shaker	(Bellco Glass, USA)
Vacuum blotter model 780	(Bio – Rad, Canada)
Vortex mixer	(Scientific, USA)
Water bath	(Yamato, Japan)
UV transilluminator	(Bio – Rad, Canada)
Amplify nucleic acid membrane	(Hybaid, Canada)

APPENDIX II

MEDIAS AND REAGENTS PREPARATION

A. MEDIA FOR YEAST CULTURE AND IDENTIFICATION

1. Sabouraud Dextrose Broth

Sabouraud Dextrose Broth powder	30	g
Distilled water	1000	ml

This media was prepared by dissolve the powder in distilled water and mix well. The suspension steriled by autoclave at 121°C, 15 pound/inch^2 pressure, for 15 minutes

2. RPMI 1640 (Sigma, USA)

RPMI 1640 powder	10.4	g
MOPS	34.53	g
Glutamine	0.3	g

The media was prepared by dissolve the powder and MIPS in DW 800 ml. Adjust to pH 7.0 and add the DW to 990 ml. The autoclave at 121°C, 15 pound/inch^2 pressure, for 15 minutes. Glutamine was dissolve in 10 ml sterile DW and terile by filtered. Finally, add filtered glutamine to RPMI 1640 base medium.

B. REAGENT FOR PLUG PREPARATION

1. 0.5 M EDTA

Ethylene diaminetetraacetic acid	186.5	g
NaOH	30	g
Deionized water	1000	ml

The reagent was made by dissolve 186.5 g of ethylene diaminetetraacetic acid in 1000 ml of deionized water. The stock reagent steriled by autoclave at 121°C, 15 pound/inch^2 pressure, for 15 minutes. The stock reagent was stored at room temperature.

2. 1M Tris – HCl (pH 9.0)

Tris base	121.14 g
30% HCl	30 – 40 ml
Deionized water	1000 ml

This stock reagent was prepared by dissolve 121.14 g of Tris base in 700 ml of deionized water, then pH was adjusted to 9.0 with conc. HCl. The final volume was bought up to 1000 ml with deionized water. The stock reagent steriled by autoclave at 121°C, 15 pound/inch^2 pressure, for 15 minutes. The stock reagent was stored at room temperature.

C. REAGENT AND MEDIA FOR ANTIFUNGAL SUSCEPTIBILITY TEST

1. RPMI 1640

RPMI 1640	46.19 g
(Angus, contains 0.165 MOPS and L – glutamine)	
Glucose	20 g
Agar	15 g
Distilled water	1000 ml

1.1 Dissolve the RPMI powder in 500 ml deionized water. Adjust the pH to 7.0 with 1 N NaHO

1.2 Filter sterilise with a 0.2 μm filter.

1.3 Dissolve the glucose and agar in 500 ml deionized water, autoclave at 121°C, 15 pound/inch^2 pressure, for 15 minutes and then cool to appox. 50°C.

1.4 Gently warm the sterile RPMI + MOPS solution to approximately 45°C and mis it with the cooled glucose – agar solution.

1.5 Cool the autoclaved agar solution to approx. 45 – 50 °C before pouring.

1.6 Generally, 60 ml agar solution to is required for a 150 mm petri dish and 25 ml for a 90 mm petridish.

1.7 Perform quality control for yeast and molds as relevant.

2. 0.85% Normal Saline

NaCl	0.85	g
Distilled water	100	ml

Suspended NaCl 0.85 g in 100 ml distilled water. The stock reagent steriled by autoclave at 121°C, 15 pound/inch^2 pressure, for 15 minutes. This solution was stored at room temperature.

D.REAGENTS FOR NOTHERN HYBRIDIZATION

3. 10X SSC buffer

NaCl	262.5	g
Trisodium citrate	132.3	g
Distilled water	3000	ml

Add all ingredients in the distilled water and mix will. The solution was stored in room temperature until use.

4. 2X SSC buffer

10 X SSC buffer	100	ml
Distilled water	400	ml

This solution was prepared by diluted 100 ml of 10X SSC buffer in 400 ml of distilled water. The solution was stored in roomtemperature until use.

5. 2X SSC buffer + 0.1%SDS (100 ml)

NaCl	17.5	g
Sodium citrate	8.8	g
Distilled water	990	ml

This solution is prepared by mix all ingredient in 990 ml of distilled water. The sterilization was made by autoclave at 121°C, 15 pound/inch^2 pressure, for 15 minutes. After this solution is cool down add 10% SDS 10 ml to this solution.

6. 0.5X SSC buffer + 0.1% SDS

NaCl	4.735	g
Sodium citrate	2.2	g

Distilled water	990	ml

This solution is prepared by mix all ingradient in 990 ml of distilled water. The sterilization was made by autoclave at 121°C, 15 pound/inch^2 pressure, for 15 minutes. After this olution is cool down add 10% SDS 10 ml to this solution.

7. Washing buffer

Maleic acid	11.067	g
NaCl	8.766	g
Distilled water	1000	ml

Dissolve all ingradient in 800 ml of deionized water, then the pH was adjusted to 7.5 with 1N NaHO. The final volume was bought up to 997 ml with deionized water and add Tween 20 (v/v) 3 ml into this reagent. The stock reagent steriled by autoclave at 121°C, 15 pound/inch^2 pressure, for 15 minutes. The stock reagent was stored at 15 - 25°C.

8. Maleic acid

Maleic acid	11.607	g
NaCl	8.766	g
Distilled water	900	ml

Dissolve all ingradient in 800 ml of deionized water, then the pH was adjusted to 7.5 with 1N NaHO. The final volume was bought up to 1000 ml. The stock reagent steriled by autoclave at 121°C, 15 pound/inch^2 pressure, for 15 minutes. The stock reagent was stored at 15 - 25°C.

9. Detection buffer

1 M Tris HCl	100	ml
5 M NaCl	20	ml
Distilled water made up to	1000	ml

The reagent was prepared by mix all solution together afterthat add the distilled water made up to 1000 ml. the reagent was sterilization by autoclaving at 121°C, 15 pound/inch^2 pressure, for 15 minutes. The stock reagent was stored at 15 - 25°C.

10. TE buffer

Tris	1.2114 g
EDTA	0.372 g
Distilled water	900 ml

Dissolve all ingradient in 900 ml of deionized water, then the pH was adjusted to 8.0 with HCl. The final volume was grought up to 1000 ml with deionzied water. The stock reagent steriled by by autoclave at 121°C, 15 pound/inch^2 pressure, for 15 minutes. The stock reagent was stored at 15 - 25°C.

11. 1M Tris HCl (pH 9.5)

Tris base	121.14 g
30% HCl	30 – 40 ml
Dionized water	1000 ml

This stock reagent was prepared by dissolve 121.14 g of Tris base in 700 ml of dionized water, then the pH was adjusted to 9.5. The final volume was bought up to 1000 ml with deionized water. The stock reagent was steriled by by autoclave at 121°C, 15 pound/inch^2 pressure, for 15 minutes. The stock reagent was stored at room temperature.

12. 1M NaCl

NaCl	58.44 g
Distilled water	1000 ml

The solution was prepared by add 58.44 g of NaCl in distilled water 1000 ml and mix well. The solutoin was steriled by by autoclave at 121°C, 15 pound/inch^2 pressure, for 15 minutes. The stock reagent was stored at room temperature.

13. 1X Blocking solution (Fresh solution)

10X blocking solution	10 ml
Maleic acid	90 ml

The reagent was prepared by mix all solution together and mix well. No sterilization.

14.	Antibody solution (150 Mu/ml) (Freshly prepare)		
	1X blocking solution	5	ml
	Antibody (750 mU/ml)	0.001	ml
15.	Color substrate solution (Freshly prepare)		
	Detection buffer	10	ml
	NBT/BCIP	0.2	ml

BIOGRAPHY

Miss Sirada Kaocharoen was born on August 23, 1979 in Chonburi, Thailand. She graduated with the Bachelor degree of Science in Microbilogy from Faculty of Science, Burapha University in 2000.

TRAINING AND EXPERIENCES:

1. Attended the molecular methods workshop "From Cells to ATGC in 7 Days" at the Gibthai Training Center, October 19-25, 2002.

2. Teaching Assistant in Medical Microbiology, Department of Microbiology, Faculty of Medicine, Chulalongkorn University, 2001.

CONFERENCES:

Meeting in "1st Asian Congress of Pediatric Infectious Diseases (ACPID) (Towards Holistic Approach to the prevention of Pediatric Infectious Diseases), Nov. 10-13, 2002.