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APPENDIX

Culture Media

1. Sabouraud Dextrose Agar (SDA)

Dehydrated SDA agar (Becton Dickinson)	65.0	gm
Distilled water	1000	ml

Suspend 65 gm of the powder in 1 liter of distilled water. Mix thoroughly, heat with frequent agitation and boil for 1 min to completely dissolve the powder. Autoclave at 121 °C 15lbs for 15 min.

2. Chaskes and Tyndall medium (L-Dopa agar)

L-asparagine	1.0	gm
Glucose	1.0	gm
KH ₂ PO ₄	3.0	gm
MgSO ₄ ·7H ₂ O	0.25	gm
Thiamine HCl	1.0	mg
Biotin	5.0	µg
L-dopa	100.0	mg
Agar	20.0	gm
Distilled water	1000	ml

Add agar to 900 ml of distilled water and autoclave at 121 °C 15 lbs for 15 min. Dissolve remaining ingredients except thiamin HCl and biotin in 100 ml of distilled water and adjust to pH 5.6. Add the vitamins after the pH adjustment; filter sterilize, and add to the cooled (50°C) agar solution.

3. Assimilation and fermentation

Yeast nitrogen base	6.7	gm
Neopeptone	1.0	gm
Carbohydrate	10.0	gm
If using raffinose	20.0	gm
Agar	3.0	gm
Bromcresol purple (1.6%)	2.0	ml
Distilled water	1000	ml

Mix the powder into distilled water (gently heat if necessary). Mix well.

Adjust to pH 7.0. Add the Carbohydrate and mix well. Dispense in 5 ml amount in 16x125 mm. screw cap tubes. Sterilize by autoclaving at 115° C 10 lbs for 10 min.

Allow to solidify in slanted position. Store in refrigerator at 4°C.

4. Urea agar

Solution A

Urea agar base	29.0	gm
Distilled water	100.0	ml

Dissolve powder in water and sterilize by filtration

Solution B

Agar	15.0	g
Distilled water	900.0	ml

Dissolve agar in water and sterilize by autoclaving at 121°C 15lbs for 15 min.

Cool agar to approximately 50°C. Add the 100 ml of sterilize urea agar base. Mix

well; dispense aseptically into sterilize tubes. Allow to cool in slanted position to form butt about 1 inch deep and slant approximately 1.5 inches long.

Reagents and buffers

1. Phosphate buffer saline (PBS) pH 7.2

Sodium Chloride	8.0	gm
Potassium chloride	0.2	gm
Na ₂ HPO ₄	1.15	gm
KH ₂ PO ₄	0.2	gm
Distilled water	1000	ml
Adjust pH to 7.2 with 1 N HCl		

2. Lysis buffer

KCl	7.45	gm
MgCl ₂	3.15	gm
Gelatin	0.2	gm
Tween 80	0.1	ml
Distilled water	1000	ml

3. 500 mM Ethylene diaminetetra acetic acid (EDTA)

EDTA (sodium salt, dehydrate)	18.61	gm
Distilled water	100	ml

4. Phenol-chloroform-isoamyl mixture

Phenol	25.0	ml
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Chloroform	24.0	ml
Amyl alcohol	1.0	ml

Mix thoroughly and store at 4 °C in the dark glass bottle.

5. 10X Tris EDTA (TE) buffer

1M Tris-Cl (pH 8.0)	100	ml
0.5M EDTA	20	ml
Distilled water	1000	ml

6. 50X Tris-Acetate-EDTA (TAE) electrophoresis buffer

2M Tris-base	243.0	gm
0.1M EDTA	37.2	gm
1M Acetic acid	60.0	ml
Distilled water	1000	ml

Dissolve and adjust the pH to 8.0

7. Agarose gel (1.5%)

Agarose gel powder	1.5	gm
0.5X TAE buffer	100	ml

CURRICULUM VITAE



Name Miss Treepradab Norkaew

Date of birth September 20, 1983

Institution attended

2002 Certificate in Mathayom VI, Phayaopittayakhom School, Phayao, Thailand

2006 Bachelor of Science (Microbiology), Faculty of Science, Chiang Mai University, Chiang Mai, Thailand

Experience

2010 Research Poster Presentation in 12th Western Pacific Congress on Chemotherapy and Infectious Diseases, 2nd - 5th December 2010, Singapore

2011 Research Oral Presentation in Burapha University National Conference 2011, 6th-7th July 2011, Thailand

