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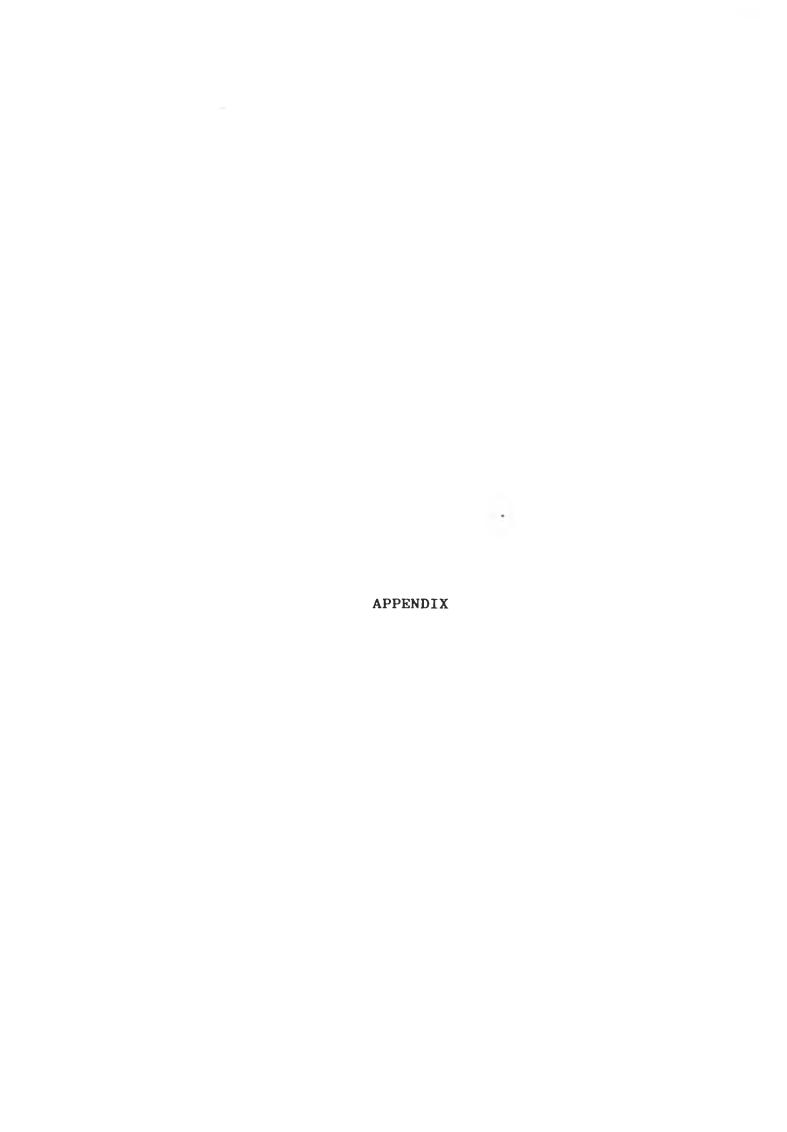
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APPENDIX I



1. Yeast nitrogen base (YNB) agar.

Formula

Yeast nitrogen base (Difco)	0.67 gm
Noble's agar	20.00 gm
Bromcresol purple (0.1% solution)	20.00 m]
Distilled water	1000 m]

- 1.1 Suspend YNB and Noble's agar in distilled water, heat to boiling with frequent agitation until the medium is completely dissolved and allow to cool at 60 $^{\circ}$ C, add bromcresol purple solution, and adjust pH to 7.2 + 0.5 by 0.1 N NaOH.
- 1.2 Dispense 15 ml in test tube and cotton plug.
- 1.3 Sterilize by autoclaveing at 121 $^{\circ}\text{C}$ for 15 minutes.
- 1.4 Cool to room temperature before keeping in cold room (0 4 $^{\circ}$ C).

2. Carbohydrate discs

Formula

Carbohydrate 10.0 gm (except for raffinose in 20.0 gm)

Distilled water 100.0 ml

Preparation

- 2.1 Dissolve carbohydrate powder in distilled water and sterilize by filtration (0.45 micron, millipore membrane filter).
- 2.2 50 microliters of 10% carbohydrate solution was dropped on 8 mm sterile paper disc (Tokyo, Japan) by aseptic technique.
- 2.3 Allow the carbohydrate disc to dry at room temperature before keeping in cold room (0 4 $^{\circ}$ C).

3. Carbohydrate fermentation medium

Formula

Beef extract	3.0	gm
Peptone	10.0	gm
NaCl	5.0	gm
Distilled water	100.0	ml
Bromcresol purple (stock solution)	1.0	ml

Preparation

- 3.1 Suspend all ingredients in distilled water.
 - 3.2 Adjust pH to 7.2 by 0.1 N NaOH.
- 3.3 Dispense 9 ml aliquotes in test tube with Durham tube.
- 3.4 Sterilize by autoclaving at 121 $^{\circ}\text{C}$ for 15 minutes.

4. Bromcresol purple (stock solution)

Bromcresol purple powder 1.6 ml 95% ethyl alcohol 100.0 ml

5. Stock carbohydrate solution

Sterilize 10% aqueous solutions of dextrose, maltose, sucrose, lactose, galactose, and trehalose by filtration. Add 1 ml of carbohydrate to one tube containing 9 ml of sterile fermentation broth.

6. Christensen's urea agar

Formula

Urea agar base 29 gm Distilled water 1000 ml Agar powder 17 gm

Preparation

- 6.1 Dissolve 29 gm of urea agar base in 100 ml distilled water and sterilize by filtration.
- 6.2 Melt agar powder in 900 ml distilled water, sterilize by autoclaving and cool to 50 $^{\circ}\text{C}.$
- 6.3 Mix urea agar base and melted sterile agar thoroughly.
- 6.4 Aseptically dispense in sterile test tube and cool as slants.

7. Nitrate assimilation test medium

Formula

Yeast carbon base	11.7	gm
Noble agar	20.0	gm
Distilled water	1000.0	ml

- 7.1 Mix all ingredients in distilled water and heat to boiling until the medium is completely dissloved.
- 7.2 Dispense 15.0 ml aliquote into 25 125 mm test tube and sterilize by autoclaving at 121 $^{\rm c}$ C for 15 minutes.

1 gm

 $^{7.3}$ Cool to room temperature and keep in cold room (0 - 4 $^{\circ}\text{C}).$

8. Peptone disc

Formula

Peptone 1.0 gm

Distilled water 100.0 ml

Preparation

- 8.1 Dissolve peptone in distilled water and sterilize by autoclaving at 121 °C for 15 minutes.
- 8.2 Aseptically dispense 50 microliters of peptone solution on sterile disc (Tokyo, Japan).
- 8.3 Allow disc to dry at room temperature and keep in cold room.

9. Potassium nitrate disc

Formula

Potassium nitrate

Distilled water 100 ml

Preparation: The same as peptone disc.

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10. Sabouraud Dextrose Broth (Difco)

Formula

Neopeptone	10	gm
Bacto-dextrose	20	gm
Distilled water	1000	m1

Preparation

- 10.1 Suspend 30 gm of the powder Sabouraud Broth in 1000 ml distilled water.
- 10.2 Mix thoroughly until medium is completely dissolved.
- 10.3 Dispense 5 ml aliquotes in screw-capped tubes.
- 10.4 Sterilize by autoclaving at 121 °C for 15 minutes and keep in cold room.

11. Carbohydrate solution

Ten per cent of 15 different kinds of carbohydrate aqueous solution except for raffinose (20%) were sterilized by filtration (0.45 micron, millipore membrane filter).

Combination of carbohydrate solution with yeast nitrogen base.

- 11.1 Add 50 ml sterilized carbohydrate solution to 450 ml sterilized yeast nitrogen base. Final concentration is 1% sugar.
- 11.2 Aseptically dispense 5 ml aliquotes insterile test tube.
- 11.3 Keep in cold room (0.4 $^{\circ}$ C, warm to room temperature before use)

12. Glutinous rice agar for chlamydoconidia production

Formula

Glutinous	rice powder	5.0	gm
Distilled	water	1000.0	ml
Agar		10.0	gm
Tween		2.5	gm

- 12.1 Dissolve glutinous rice powder in 100 ml distilled water and heat to boiling for 10 minutes.
- 12.2 Allow to cool and precipitate at room temperature for 2 hours and filtrate through cotton and gauze. The supernatant is collected about 500 ml solution.
- 12.3 Add agar to the solution and heat to boiling. Frequently agitate until it is completely dissolved.

- 12.4 Allow solution to cool to 50 $^{\circ}$ C, adjust pH to 7.1 7.2 with 0.1 N NaOH and add 2.5 ml of Tween 80.
- $12.5\,$ Autoclave at 121 $^{\rm o}{\rm C}$ for 15 minutes and pour on plate.

13. Sabouraud Dextrose Agar (Difco)

Formula

Neopeptone	10	gm
Bacto-Dextrose	40	gm
Bacto-peptone	17	gm
Distilled water	1000	m: 1

- 13.1 Suspend 65 gm of powdered Sabouraud Dextrose Agar in 1000 ml of distilled water and mix thoroughly.
- 13.2 Heat to boiling with frequent agitation until the medium is completely dissolved.
- 13.3 Dispense 5 ml in test tube and cotton pluge.
- 13.4 Sterilize by autoclaving at 121 $^{\circ}$ C for 15 minutes.
- 13.5 Cool to room temperature in a slant position and keep in cold room.

14. Sabouraud Dextrose Agar with antibiotics

Formula

Neopeptone	10 gm
Bacto-Dextrose	40 gm
Bacto-Peptone	17 gm
Distilled water	1000 ml
Sodium benzyl penicillin	1.5 mg/ml
(Pagemex, Dumex)	
Streptomycin sulfate	5 mg/ml
(Streptomycin, Dumex)	

- 14.1 Suspend 52 gm of powdered Sabouraud Dextrose Agar in 800 ml of distilled water and mix thoroughly.
- 14.2 Heat to boiling with frequent agitation until the medium is completely dissolved.
- 14.3 Sterilize by autoclaving at 121 $^{\circ}$ C for 15 minutes.
- 14.4 Cool to 40 45 °C and mix with 1,000,000 units of sodium benzyl penicillin and 2 gm of Streptomycin sulfate, agitate until the antibiotics are completely dissolved.
 - 14.5 Dispense 20 ml in sterile petri dish.
- 14.6 Cool to room temperature and keep in cold room.

15. McFarland nephelometer standards (68, 69)

	tube number							
	0.5	1	2	3	4	5	6	7
Barium chloride (ml) Sulfuric acid (ml)	0.05 9.95							
Approx. cell density (x10 ⁸ cells/ml)	1	3	6	9	12	15	18	21

APPENDIX II

Table 18 Frequency of detection of Candida in 3 groups study

	GROUP 1	GROUP 2	GROUP 3	
AP	21.05	59.26	95.00	
PP	21.05	51.85	87.50	
RB	25.00	51.85	80.00	
LB	22.37	40.74	85.00	
RC	25.00	51.85	67.50	
LC	25.00	40.74	67.50	
AT	31.58	62.69	97.50	
PT	52.63	66.67	97.50	
LA	19.74	33.33	55.00	
FL	14.47	25.93	55.00	
D	not done	59.26	95.00	

Table 19 Mean density by site in 3 groups study

 	GROUP 1	GROUP 2	GROUP 3
AP	0.39	0.85	2.40
PP	0.32	0.93	1.88
RB	0.49	0.85	1.48
LB	0.53	0.74	1.58
RC	0.36	0.78	1.08
LC	0.32	0.63	1.00
AT	0.58	1.19	2.70
PT	0.96	1.37	2.85
LA	0.38	0.48	0.80
FL	0.29	0.41	0.85
D	not done	1.78	3.55



BIOGRAPHY

Miss Patchara Pipattanagovit was born on January 1, 1956, in Bangkok, Thailand. She graduated with the Bachelor of Science degree from the Faculty of Dentistry, Chulalongkorn University, in 1979 and the Doctor of Dental Surgery degree from the Faculty of Dentistry, Chulalongkorn University, in 1981.

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