REFERENCE

1. กวี ภู่ไพบูลย์, Medical Mycology, ภาควิชาจุลชีววิทยา, คณะแพทยศาสตร์, จุฬาลงกรณ์มหาวิทยาลัย, กรุงเทพมหานคร, 2524

2. Sauballe, M.A., "Clinical Significance and Laboratory Identification of Yeast," Laboratory Medicine, 15(2), 98-103, 1984.

3. Rippon, J.W., Medical Mycology; The Pathogenic Fungi and Pathogenic Actinomycetes, pp. 484-531, W.B. Saunders Company, Philadelphia, 2nd ed., 1982.

4. Ahearn, D.G., "Medically Important Yeasts," Ann. Rev. Microbiol., 32, 59-68, 1978.

5. Furman, R.M. and D.G. Ahearn, "Candida ciferrii and Candida chiropterorum Isolated from Clinical Specimen," J. Clin. Microbiol., 18(5), 1252-1255, 1983.

6. Doland, C.T., "Letter to the Editor: Why should Medical Laboratories Identify Yeasts?." Am. J. Clin. Path., 55, 632-634, 1971.

7. Qadri, S.M.H., D.J. Flournog, S.G.M. Qadri, and E.G. Ramieres, "Rapid Identification of Yeasts by Semi-Automated and Conventional Methods," Med. Microbiol. Immunol., 175, 307-316, 1986.

8. Schlitzen, R.L., and D.G. Ahearn, "Characterization of Atypical Candida tropicalis and Other Uncommon Clinical Yeast Isoaltes," J. Clin. Microbiol., 15(3), 511-516, 1982.

9. Guinet, R., J. Chanas, A. Goullier, g. Bonnefoy, and P. Ambroise-Thomas, "Fatal Septicemia Due to Amphotericin B Resistant Candida lusitaniae, J. Clin. Microbiol., 18(2), 443-444, 1983.

10. Baker, J.G., I.F. Salkin, D.H. Pincus, and R.F.D. Amato, "Diagnostic Character of an Atypical Candida," J. Clin. Microbiol., 13(1), 199-203, 1981.

11. Schlitzer, R.L., and D.G. Ahearn, : Characterization of Atypcal Candida tropicalis and Other Uncommon Clinical Yeast Isolates," J. Clin. Microbiol., 15(3), 511-516, 1982.

12. Randhawa, H.S., and Mahendra Pal, "Occurence and Significance of Cryptococcus neoformans in the Respiratory Tract of Patients with Bronchopulmonary Disorders," J. Clin. Microbiol., 5(1), 5-8, 1977.

13. Bowman, P.I., and D.B. Ahear, "Evaluation of the Uni-Yeast-Tek Kit for the Identification of Medically Important Yeasts," J. Clin. Microbiol., 2(4), 354-358, 1975.

14. Oblack, d.L., J.C. Rhodes, and W.J. Martin "Clinical Evaluation of the Auto Miicrobic System Yeast Biochemical Card for Rapid Identification of Medically Important Yeasts," J. Clin. Microbiol., 13(2), 351-355, 1981.

15. Zwadyk, P.JR., R.A. Tarton, and A. Proctor, "Evaluation of the API 20 C for Identification of Yeasts," Am. J. Clin. Patho., 67, 269-271, 1977.

16. Isenberg, H.D., T.L. Gavan, A. Sonnenwirth, W.I. Taylor, "Clinical Laboratory Evaluation of Automated Microbial Detection/Identi-fication System in Analysis of Clinical Urine Specimens," J. Clin. Microbiol., 10, 226-230, 1979.

17. Martin, M.V., and J.D. Schneidau, "A Simple and Reliable Assimilation Test for the Identification of Candida species," Am. J. Clin. Pathol., 55, 875-879, 1970.

18. Gangopadhyay, P.K., H. Thadepall, I. Roy, A. Ansari, "Identification of Species of Candida, Cryptococcus and Torulopis by Gas-Liquid Chromatography," J. Inf. Dis., 140(6), 952-958, 1979.

19. Taguchi, M., M. Tsukij, and T. Tsuchiya, "Rapid Identification of Yeasts by Serological Methods a Combined Serological and Biological Method," Sabouraudia, 17, 185-191, 1979.

20. Salkin, I.F., H.K. Schadow, L.A. Bankaitis, M.R. McGinnis, and M.E. Kemna, "Evaluation of Abbott Quantum 2 Yeast Identification System," J. Cli. Microbiol., 22(3), 442-444, 1985.

21. Kiihn, T.M., F.F. Edwards, D. Tom, G. Lieberman, E.M. Bernard, and D. Amstrong, "Evaluation of the Quantum 2 Yeast Identification System," J. Clin. Microbiol., 22(2), 216-219, 1985.

22. Hasyn, J.J., and H.R. Buckley, "Evaluation of the Auto-Microbic System for Identification of Yeast," J. Clin. Microbiol., (16(5), 901-905, 1982.

23. Ngui Yen, J.H., and J.A. Smith, "Use of Autobact 1 for Rapid Assimilation Testing of Candida and Torulopsis species," J. Clin. Microbiol., 7, 118-121, 1978.

24. Shinoda, T., L. Kaufman, and A.A. Padhye, "Comparative Evaluation of the Iatron Serological Candida Check Kit and The API 20 C Kit for Identification of Medically Important Candida species," J. Clin. Microbiol., 13, 513-518, 1981.

25. Land, G.A., B.A. Harrison, K.L. Hulme, B.H. Cooper, and J.C. Byrd., "Evaluation of the New API 20 C Strip for Yeast Identification Against a Conventional Method," J. Clin. Microbiol., 10(3), 357-364, 1979.

26. Cooper, H.B., J.B. Johnson, and E.S. Thaxton, "Clinical Evaluation of the Uni-Yeast-Tek System for Rapid Presumption Identification of Medically Important Yeasts," J. Clin. Microbiol., 7(4), 349-355.

27. Buesching, W.J., K. Kuren, and G.D. Roberts, "Evaluation of the Modified API 20 C System for Identificaiton of Clinically Important Yeasts," J. Clin. Microbiol., 9(5), 565-569, 1979.

28. Beneke, E.S., and A.L., Rogers, Medical Mycology Manual with Human Mycoses Monograph, pp. 20-48 and 117-140, Burgess Publishing Company, Minneapolis, Minnesota, 4th. ed., 1980.

29. Frazier, W.C. and D.C. Westhoff, Food Microbiology., pp. 37-45, Tata McGraw-Hill Publishing Co. Ltd., New Delhi, 3rd. ed., 1981.

30. Chengappa, M.M., R.L. Maddur, S.c. Grur, D.H. Pincus, and L.L. Geist, "Isolation and Identificaiton of Yeast-Like Organisms from Clinical Veterinary Sources," J. Clin. Microbiol., 19(3), 427-428. 1984.

31. Negroni, P.M.B. Negroni De Bonvehi, "Man as a host of Rhodotorula" Rev. Med. Vert. Mycol., 20(3), 166, 1985.

32. Barth, J., and M.F. Barthe, "The Ecology of Torulopsis in Man", Sabouraudia, 11, 192-195, 1973.

33. Martin, M.V., and g.R. Wilkinson, "The Oral Yeast Flora of 10 Year-Old Schoolchildren," Sabouraudia, 21, 129-135, 1983.

34. Volz, P.A., D.E. Jerger, A.J. Wurzburger, and J.L. Hister, : A Preliminary Survey of Yeasts Isolated from Marine Habitats at Abaco Island, The Bahamas., Mycopathologia et Mycologia Applicata, 54(93), 313-316, 1974.

35. Howard, D.H. and L.F. Howerd, Fungi Pathogenic for Humans and Animals, pp. 25-39, part B, Marcel Dekker, Inc. 1983.

36. Littman M.L., "Seminar on Mycotic Infection : Cryptococcosis (Torulopsis) Current Concepts and Therapy," Am. J. Med., 27, 976-998, 1956.

37. Bunnag C., B. Dhorraninta, and A. Plangpatanapanichya., A Comparative Study of the Incidence of In door and Out door Mold Spores in Bangkok, Thailand," Annals of Allergy, 48(6), 333-339, 1982.

38. Ruiz, A., J.B. Neilson, and G.S. Bulmer, "A One Year Study on the Viability of Cryptococcus neoformans in Nature," Mycopathologia, 77, 177-222, 1982.

39. John De Louvois, A. Mulhall, and R. Hurley, "Biochemical identification of Clinically Important Yeasts." J. Clin. Pathol. 32, 715-718, 1978.

40. McGinis, M.R., Laboratory Hand Book of Medical Mycology, pp. 337-410, Academic Press, New York, 1980.

41. Kreer-van Rij, N.J.W., The Yeast : A Taxonomic Study, pp. 1-103 and 557-1066, Elsevier Science Plublishers B.V., Amsterdum, 3rd., 1984.

42. Odds, F.C., and A.B. Abbott, "A Simple System for Presumtive Identification of Candida albicans and Differentiation of Strains within the Species," Sabouraudia, 18, 301-317, 1980.

43. Iwata, K., "Toxin Produced by Candida albicans." Contrib. Microbiol. Immunol., 4, 77-85, 1977.

44. Cutler, J.E., L. Friedman, and K.C. Milner, "Biological and Chemical Characterization of Toxic Substance from Candida albicans." Infect. Immun. 96, 1535-1539, 1972.

45. Kobayashi, T., I. Nakashima, and N. Kato, "Endotoxic Substance of Cryptococcus neoformans." Mycopathol. Mycol. Appl., 54, 391-404, 1974.

46. Muller, H.E., and K.K. Sethi, "Proteolytic Activity of Cryptococcus neoformans Against Human Plasma Proteins." Med. Microbiol. Immunol., 158, 129-134, 1972.

47. Kozel, T.R., and R.P. Mastroianni, "Inhibition of Phagocytosis by Cryptococcal Polysaccharide : Dissociation of the Attachment and Ingestion Phases of Phagocytosis." Infect. Immun., 14, 62-67, 1976.

48. Hatch, T.F., "Distribution and Deposition of Inhaled Particles in respiratory Tract." Bacteriol. Rev., 25, 237-240, 1961.

49. Madeira-Lopes A., and Uden, N. Van. "The Temperature Profile of Cr. neoformans." Sabouraudia 20(4), 331-334, 1982.

50. Lodder, J., The Yeast : A Taxonomic Study, pp. 1-107 and 863-1352, North-Holland Publishing Company Amsterdum. 2nd. ed., 1970.

51. Campbel, M.C., J.L. Stewart, and H.W. Larsh, The Medical Mycology Handbook, John Wiely and sons, New York, 1980.

52. Phillip, A., A. Tsui, and J.e$\frac{1}{3}$E. O'Brien, "Evaluation of API Microtube GT System for Detection of Germ Tube Production by Clinically Significant Yeasts," J. Clin. Microbiol. 18(5) 1280-1281, 1983.

53. Berardinelli, S., and D.J. Opheim. "New Germ Tube Induction medium for the Identification of Candida albicans., J. Clin. Microboil., 22(5), 861-862. 1985.

54. Mattia, E., G. Carruba, L. Angiolella, and A. Cassone, "Induction of Germ Tube Formation by N-Acetyl-D-Glucosamine in Candida albicans : Uptake of Inducer and Germinative response," J. Clin. Microbiol, 152(2), 555-562, 1982.

55. Sukroongreung, S., "A Glutinous Rice Culture Medium for Demonstration of Clamydospores of Candida albicans," Mycopathologia et Mycologia applicata, 43(3-4), 320-335, 1971.

56. Beheshit, F., a.G. Smith, and G.W. Krause, "Germ Tube and Chlamydospore Formation by Candida albicans," J. Clin. Microbiol., 2, 345-348, 1975.

57. Gow, N.A.R., and G.W. Gooday, "A Model for the Germ Tube Formation and Mycelial Growth from of Candida albicans," Sabouraudia, 22, 137-147, 1984.

58. Bakerspigel, A., and S. Burke, "A Possible Function of the Chlamydospores of Candida albicans," Mycopathologia et Mycologia applicata., 54(91), 147-152, 1974.

59. Frienrich, E., and H. Bohme, "The Growth of Yeast on Media Containging Cycloheximide," Mykosen 17(8), 191-198, 1974.

60. Barnett, J.A., R.W. Payne, D. Yarrow, Yeast Characteristics and Identification, Cambridge University Press.,1st ed., 1983.

61. Zimmer, B.L., and G.D. Roberts, "Rapid Selective Urease Test for Presumptive Identification of Cryptococcus neoformans," J. Clin. Microbiol., 10(3), 380-381, 1970.

62. Roberts, G.D., C.D. Horstmeier, G.A. Land, and J.H. Foxworth, "Rapid Urea Broth Test for Yeasts," J. Clin. Microbiol., 7, 584-588, 1978.

63. Hopkins, J.M., and G.A. Land, "Rapid Method for Determining Nitrate Utilization by Yeast," J. Clin. Microbiol., 5(4), 497-500, 1979.

64. Odds, F.C., and A.B. Abbott, "Modification and Extension of Tests for Differentiation of Candida Species and Strain," Sabouraudia, 21, 79-81, 1983.

65. Paliwal, D.K., and H.S. Randhawa, "Evaluation of a Simplifed Guizzotia abyssinica Seed Medium for Differentiation of Cryptococcus neoformans," J. Clin. Microbiol., 7(4), 346-348, 1978.

66. Hopfer, R.L., and D. Groschel, "Six-Hour Pigmentation Test for the Identification of Cryptococcus neoformans," J. Clin. Microbiol., 2(2), 96-98, 1975.

67. Hopfer, R.L., and F. Blank, "Caffeic Acid-Containing Medium for Identification of Cryptococcus neoformans," J. Clin. Microbiol., 2(2), 115-120, 1979.

68. Show, C.E., and L. Kapica, "Production of Diagnostic Pigment by Phenoloxidase Activity of Cryptococcus neoformans," J. Clin. Microbiol., 24(5), 824-830, 1972.

69. Korth, H. and G. Pulverer, "Pigment Formation for Differentiating Cryptococcus neoformans from Candida albicans," J. Clin. Micribiol., 21(3), 541-542, 1979.

70. Strachan, A.A., R.J. YU, and F. Blank, "Pigment Production of Cryptococcos neoformans Grown with Extracts of Guizotia abyssinica," App. Microbiol., 22(3), 478-479, 1971.

71. Young, R.C., J.E. Bennet, G.W. Geelhoed, A.S. Levine., "Fungemia with Compromised Host Resistance : A Study of 70 Cases," Ann. Int. Med., 80, 605-612, 1974.

72. Wang, H.S., R.T. Zeimis, and G.D. Roberts, "Evaluation of a Caffeic Acid-Ferric Citrate Test for Rapid Identification of Cryptococcus neoformans," J. Clin. Microbiol., 6(5), 445-449, 1977.

73. Kaufmann, C.S., and W.G. Merz, "Two Rapid Pigmentation Test for Identification of Cryptococcus neoformans," J. Clin. Microbiol. 15(2), 339-341, 1982.

74. Paliwal, D.K., and H.S. Randhawa, "A Rapid Pigmentation Test for Identification of Cryptococcus neoformans," Antonic van Leeuwenhoek, 44, 243-246, 1978.

75. Wickerham, L.J., and K.A. Burton, "Carbon Assimilation Tests for the Classification of Yeasts," J. Bact., 56, 363-371, 1948.

76. Adams, E.D. JR., and B.H. Cooper, "Evaluation of a Modified Wickerman Medium for Identifying Medically Important Yeasts," Am. J. Med. Technol., 40(9), 377-388, 1974.

77. Michelsen, P.A., L.R. McCarthy, and M.A. Propst, "Future Modifications of the Auxanographic Method for Identification of Yeasts," J. Clin. Microbiol., 5(3), 297-301, 1977.

78. Lane, G.A., E.C. Vinton, G.B. Adcock, and J.M. Hopkins, "Imporved Auxanographic Method For Yeast Assimilations Comparison with Other Approaches," J. Clin. Microbiol., 2(1), 206-217, 1975.

79. Shafiee A. and T. Rahmain, "Almospheric mold spores in Teharan", Iran. Ann. Allerg., 40, 138, 1978.

80. George, S., and G.H.G. Davis, "Rapid Identification of Enterobacteriaceae by Using Noncommercial Micro-test in Conjunction with API20E Profile Data," J. Clin. Microbiol., 10(4), 399-403, 1979.

81. Paliwal, D.K., H.S. Randhawa, and T. Kowashik, "A Modified Method for Testing Inositol Assimilation by Cryptococcus spp.," Can. J. Microbiol., 25, 1188-1190, 1979.

82. Chaskes, S., and R.L. Tyndall, "Pigment Production by Cryptococcus neoformans and Other Cryptococcus spp. from Aminophenols and Diaminobenzenes," J. Clin. Microbiol., 7(2), 146-152, 1978.

83. Warwood, N.M. and D.J. Blazevic, "Comparison of Cream of Rice Agar and Horse Serum for Differentiating Germ Tubes of Candida albicans from Filaments of Candida tropicalis," J. Clin. Microbiol., 5(4), 501-502, 1977.

84. Bohm, K.H. "Study on Temperature resistance of Cr. neoformans-A Contribution to Epidemiology." Rev. Med. Vet. Mycol., 16(1), 50, 1981.

85. Joshi, K.R., J.B. Gavin and D.A. Bremmer, "The Formation of Germ Tubes by Candida albicans in Various Peptone Media," Sabouraudia, 11, 259-262, 1973.

86. Syverson, R.E., "Variable Assimilation of Compounds by Candida albicans." J. Clin. Micriobiol., 13(1), 163-166, 1981.

87. Arthur, L.B., The Antimicrobic Susceptibility Test : Principle and Practices, Henry Kimpton Published, London. pp. 7, 1976.

88. Sydney M.F., and W.J. Mortin, Diagnostic Microbiology, The C.V. Mosby Company. pp. 671, 6th ed, 1982.

89. Chindamporn A., P. Prapuntanwatana and K. Pupaibul. "Identification of Cryptococcus neoformans by Phenoloxidase Strip Test", J. Med. Tech. Assoc. Thailand, 10(4), 139-143.

APPENDIX

APPENDIX

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 ml.
Distilled water	1000 ml.

Preparation

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 °C, add bromcresol purple solution and adjust pH to 7.2+0.5 by 0.1 N NaOH.

2. Dispense 15 ml. in test tube and cotton plug.

3. Sterilize by autoclaveing at 121 °C for 15 min.

4. Cool to room temperature before keep in cold room (0-4 °C)

2. Carbohydrate discs

Formula

Carbohydrate	10.0 gm.
(except for raffinose in 20.0 gm.)	
Distilled water	100.0 ml.

Preparation

1. Dissolve carbohydrate powder in distilled water and steriled by filtration (0.45 micron,millipore membrane filter).

2. 50 microliters of 10% carbohydrate solution was dropped on 8 mm. steriled paper disc (Tokyo,Japan) by aseptic technique.

3. Allow the carbohydrate disc to dry at room temperature before keep in cold room (0-4 °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

1. Suspend all ingredients in distilled water.
2. Adjust pH to 7.2 by 0.1 N NaOH.
3. Dispense 9 ml. aliquotes in test tube with Durham tube.
4. Sterilize by autoclaving at 121°C for 15 min.

4. Bromcresol purple (stock solution)

Bromcresol purple powder	1.6 gm.
95% Ethyl alcohol	100.0 ml.

5. Stock carbohydrate solution

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

6. Christensen's urea agar

Formula

Urea agar base	29 gm.
Distiled water	1000 ml.
Agar powder	17 gm.

Preparation

1. Dissolve 29 gm. of urea agar base in 100 ml. Distilled water and sterileby filtration.

2. Melt agar powder in 900 ml. distilled water, sterile by autoclaving and cool to 50°C.

3. Mix urea agar base and melted steriled agar thoroughly.

4. Aseptically dispense in steriled test tube and cool as slants.

7. Nitrate assimilation test medium

Formular

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

Preparation

1. Mix all ingredients in distilled water and heat to boiling until the medium is completely dissolved.

2. Dispense 15.0 ml. aliquote in to 25-125 mm. test tube and steriled by autoclaving at 121°C for 15 min.

3. Cool to room temperature and keep in cold room (0-4°C).

8. Peptone disc

Formula

Peptone	1.0 gm.
Distilled water	100.0 ml.

Preparation

1. Dissolve peptone in distilled water and sterile by autoclaving at 121 °C for 15 minutes.

2. Aseptically disspense 50 microliters of peptone solution on sterile disc (Tokyo,Japan).

3. Allow disc to dry at room temperature and keep in cold room.

9. Potassium nitrate disc

Formula

Potassium nitrate	1 gm.
Distilled water	100 ml.

Preparation : The same as peptone disc.

10. Sabouraud broth (Difco)

Formula

Neopeptone	10 gm.
Bacto-dextrose	20 gm.
Distilled water	1000 ml.

Preparation

1. Suspend 30 gm. of the powder Sabouraud broth in 1000 ml. distilled water.

2. Mix thoroughly until medium is completely dissolved.

3. Dispense 5 ml. aliquotes in screw-capped tubes.

4. Sterilize by autoclaving at 121° C for 15 min. and keep in cold room.

11. Sabouraud broth pH 1.5

Formula

Neopeptone	10 gm.
Bacto-Dextrose	20 gm.
Distilled water	1000 ml.

Preparation

1. Suspend 30 gm. of the powder Sabouraud broth in 1000 ml. distilled water.

2. Mixed throughly until the medium is completely dissolved.

3. Adjust pH to 1.5 by 1 N HCl

4. Sterilize by filtration (0.45 micron, Milipore membrane filter).

5. Aseptically dispense 5 ml. aliquots in steriled test tubes.

6. Keep in cold room (0-4 °C) and warm to room temperature before use.

12. Carbohydrate assimilation medium for rapid method

Formula

Yeast nitrogen base(Difco)	0.335 gm.
Distilled water	400 ml.
Bromcresol purple solution	10.0 ml.
(1 gm./lit)	

Preparation

1. Suspend 0.335 gm. yeast nitrogen base in 400 ml. of distilled water.

2. Add 10 ml. of bromcresol purple solution and adjust pH to 7.10 $\pm$ 0.05

3. Mix thoroughly until medium is completely dissolved.

4. Sterilize by autoclaving at 121 °C for 15 minutes.

5. Cool to room temperature and keep in cold room.

13. Carbohydrate solution

10 % of 15 difference kind of carbohydrate ageous solution except for raffinose (20%)were sterilized by filtration (0.45 micron, Millipore membrane filter)

Combination of carbohydrate solution with yeast nitrogen base

1. Add 50 ml Sterilized carbohydrate solution to 450 ml. sterilized yeast nitrogen base. Final concentration is 1% sugar.

2. A septically dispense 5 ml. aloquotes in steriled test tube.

3. Keep in cold room (0-4 °C) , warm to room temperature before use.

14. Urea impregnated cotton-tip [modified from Zimmer and Robert (61)]

Formula

Urea agar base	14.5 gm.
Distilled water	100.0 ml.

Preparation

1. Dissolve urea agar base in distilled water, adjust pH to 5.5 and sterilized by filtration.

2. The steriled 6-inch cotton swab was soaked in urea agar base solution.

3. Put the soaked cotton swab in steriled test tube, cotton pluge and allow it to dry at room temperature for 3 days. The lyophilization was needed by the method of Zimmer and Robert (61)

4. Keep dried cotton swab in steriled aluminium foil and store at room temperature.

15. Nitrate-Zephirans treated swabs. [modified from Hopkins and Land (63)]

Formula

KNO_3	2.00 gm.
$Na_2H_2PO_4H_2O$	11.70 gm.
Na_2HPO_4	1.20 gm.
Zephiran chloride (benzalkonium chloride) (17% solution)	1.20 gm
Distilled water	200.00 ml.

Preparation

1. Weigh and measuare all ingredient carefully.

2. Add 1.20 ml. Zephiran chloride to 200 ml. distilled water.

3. Add dry chemicals and mix to dissolve completely.

Preparation of swab

1. Place 0.1 ml of reagent in each of desired number of straight-side clean test tube.

2. Place a 6-inch cotton swab in each tube so that fluid will be absorbed.

3. Allow swabs to dry in test tubes overnight at room temperature. The lyophilization was needed by method of Hopkins and Land (63)

4. Remove swabs from tube , Place in aluminium foil.

5. Sterilize by autoclaving at 121 °C for 15 min.

6. Keep in cold room (0-4 °C) and warm to room temperature before using.

16. Glutineous rice agar for chlamydoconidia production

Formula

Glutineous rice powder	5.0 gm.
Distilled water	1000.0 ml.

Agar	10.0 gm.
Tween 80	2.5 gm.

Preparation

1. Dissolve glutineous rice powder in 100 ml. distilled water and heat to boiling for 10 min.

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.

3. Add agar to the solution and heat to boiling with frequently agitate until it completely dissolved.

4. Allow solution to cool to 50°C ,adjust pH to 7.1-7.2 with 0.1 N NaOH and add 2.5 ml of Tween 80.

5. Autoclave at 121°C for 15 minutes and pour plate.

17. Mycobiotic agar for cycloheximide resistance

Formula

Bacto-soytine	10 gm.
Bacto-dextrose	10 gm.
Bacto-agar	15 gm.
Actidione	0.5 gm.
Chloramycetin	0.05 gm.
Distilled water	1000 ml.
Actidione for add	0.5 gm.

Preparation

1. Suspend 36 gm. of powderd mycobiotic agar in 1000 ml. of distilled water and mix throughly.

2. Heat to boiling with frequent agitation until the medium is completely dissolved. Add 0.5 gm. of Actidione which dissolved in 2-3 drops of DMSO and add water to 5 ml.

3. Dispense 5 ml. in test tube and cotton pluge.

4. Sterile by autoclaving at 121°C for 10 minutes.

5. Cool to room temperature in a slant position and keep in cold room.

18. L-DOPA paper strip

Formula

L-DOPA	6 mg.
(L-3,4-Dihydroxyphenylalanine)	
Phosphate buffer pH 6.8	7 ml.
Ferric citrate	1 mg.

Preparation

1. Dissolve L-DOPA in few drop of DMSO and add distilled water to 3 mg/ml. final concentration.

2. Dissolve ferric citrate in 1 ml. distilled water and gently heat until it completely dissolved.

3. Mix L-DOPA solution, ferric citrate solution and 7 ml. of phosphate buffer thoroughtly. And saturated the filter paper.

4. Alow the paper to dry in room temperature and keep in refrigirator and refresh by distilled water.

Phosphate buffer pH 6.8

Solution A : Dissolve KH_2PO_4 9.07 gm. in 100 ml. distilled water

Solution B : Dissolve $Na_2HPO_4 7H_2O$ 17.87 gm. in 1000 ml. distilled water.

Mix solution A and B with equal volume then make the final pH 6.8

19. Sabouraud dextrose agar (Difco)

Formula

Neopeptone	10 gm.
Bacto-Dextrose	40 gm.
Bacto-Peptone	17 gm.
Distilled water	1000 ml.

Preparation

1. Suspend 65 gm. of powdered Sabouraud dextrose agar in 1000 ml. of distilled water and mix thoroughtly.

2. Heat to boiling with frequent agitation until the medium is completely dissolved.

3. Dispense 5 ml. in test tube and cotton pluge.

4. Sterile by autoclaving at 121°C for 15 min.

5. Cool to room temperature in a slant position and keep in cold room.

20. Recycle of microtiter plate

Preparation

1. The completed test plates will be soaked in 20% Chlorox solution for 24 hours to kill all organism.

2. After killing all organism, the plates will be soaked in detergent (lipon-F) for 3 hours ,rinse in tap water for 3 times and finally soaked in distilled water overnight.

3. After the plate dried, resterilized by gas sterilization.

21. McFarland nephelometer standards (77, 78)

	tube number							
	0.5	1	2	3	4	5	6	7
Barium chloride (ml)	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7
Sulfuric acid (ml)	9.95	9.9	9.8	9.7.	9.6	9.5	9.4	9.3
Approximate cell density ($x10^8$ cell/ml)	1	3	6	9	12	15	18	21

BIOGRAPHY

Miss Chantana Waropastrakul was born on July 8, 1961 in Ayuthaya, Thailand, She graduated with the degree of Bachelor of Science from the Faculty of Science, Kasetsart University in 1983.

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