REFERENCES

1. Venereal Disease Division, "Report on Statistics of Sexually Transmitted Diseases," V.D. Division, Department of Communicable Disease Control, Ministry of Public Health, Bangkok, 1986.
2. Stamm, W. E., B. Cole, C. Fennell, P. Bonin, A. S. Armstrong, J. E. Herrmann, and K. K. Holmes, "Antigen Detection for the Diagnosis of Gonorrhea, J. Clin. Microbiol., 19 (3), 399-403, 1984.
3. Goh, B. T., K. B. Varia, P. F. Ayliffe, and F. K. S. Lim, "Diagnosis of Gonorrhea by Gram-stained Smears and Cultures in Men and Women: Roloe of the Urethral Smear, "Sex. Transm. Dis., 12(3), 135-139, 1985.
4. Schink, J. C., and L. G. Keith, "Ploblems in the Culture Diagnosis of Gonorrhea," J. Reprod. Med., 30 (suppl.3), 244-249, 1985.
5. Spence, M. R., D. S. Guzick, and L. R. Katta, "The Isolation of Neisseria gonorrhoeae: A Comparison of three Culture Transport Systems, "Sex.Transm. Dis., 10, 138-140, 1983.
6. Deacon, W. E., W. L. Peacock, E. M. Freeman, A. D. Purris, and W. L. Bunch, "Fluorescent Antibody Tests for Detection of the Gonococcus in Women, "Public Health Reports, 75, 125-129, 1960.
7. Finch, C. A., "Assessment of an Enzyme Immunoassay for

Diagnosing Gonorrhea, "J.Reprod.Med., 30 (Suppl. 3), 250-257, 1985.

8. Judson, F. N., "A Clinic-based System for Monitoring the quality of Techniques for the Diagnosis of Gonorrhea, Sex. Transm. Dis., 5,141-145, 1978.

9. Monif, G. R. G., P. A. Jordan, J. L. Thompson, and R. Shaara, "Pragmatic Factors Influencing the Detection of Neisseria gonorrhoeae, "Obstet. Gynecol., 59, 649-652, 1982.

10. Windall, J. J., M. M. Hall and J. A. Washington, "Inhibitory Effects of Vancomycin on Neisseria gonorrhoeae in Thayer-Martin Medium, "J. Infect. Dis., 142, 775, 1980.

11. Taylor, E., and I. Phillips, "Assessment of a Selective Medium for the Isolation of Neisseria gonorrhoeae," Br. J. Vener. Dis., 55, 183-185, 1979.

12. Griffin, C. W. III, M. A. Mehaffey, and E. C. Cook, "Five years of Experience with a National External Quality control Program for the Culture and Identification of Neisseria gnorrhoeae, "J. Clin. Microbiol., 18, 1150-1159, 1983.

13. Freundlich, L. F., S. L. Rosenthal, and F. P. Hochberg, "Comparison of Methods for the Immunological Identification of Neisseria gonorrhoeae in Clinical Specimens Using Commercially Obtained Reagents, "Am. J. Clin. Pathol., 77, 456 - 458, 1982.

14. Arko, R. J., K. G. Finley-Price, and K. H. Wong, "Identification of Problem Neisseria gonorrhoeae Cultures by Standard and Experimental Tests, "J. Clin. Microbiol., 15, 435-438, 1982.

15. Burns, M., P. A. Rossi, B. W. Cox, T. Edwards, M. Kramer, and S. J. Kraus, "A Preliminary Evaluàtion of the Gonozyme Test, "Sex. Transm. Dis., 10 (4), 180-183, 1983.

16. Carlson, B. L., M. S. Haley, N. A. Tisei, and W. M. McCormack, "Evaluation of four Methods for Isolation of Neisseria gonorrhoeae, " J. Clin. Microbiol., 12, 301-303, 1980.

17. Brorson, J. E., J. Holmberg, B. Nygrem, "Vancomycin-Sensitive Strains of Neisseria gonorrhoeae. A Problem for the Diagnostic Laboratory, "Br. J. Vener. Dis., 49, 452-453, 1973.

18. Faur, Y. C, M. H. Weisburd, and M. E. Wilson, "The Selectivity of Vancomycin and Lincomycin in NYC Medium for the Recovery of N. gonorrhoeae from Clinical Specimens, "Health Lab. Sci., 15, 22-27, 1978.

19. Granato, P. A., and M. Roefaro, "Comparative Evaluation of Enzyme Immunoassay and Culture for the Laboratory Diagnosis of Gonorrhea, "Am. J.Clin. Pathol., 83 (5), 613-618, 1985.

20. Hainer, B. L., P. Danylchuk, J. Cooper, and C. W. Weart., "Limulus Lysate Assay in Detection of Gonorrhea

in Women from a Low Incidence Population, "Am. J. Clin. Gynecol., 144, 67-71, 1982.

21. Spagna, V. A., R. B. Prior and R. L. Perkins, "Rapid Presumptive Diagnosis of Gonococcal Urethritis in Men by the Limulus Lysate Test, "Br. J. Vener. Dis., 55, 179-182, 1979.

22. Prior, R. B., V. A. Spagna, "Comparative Evaluation of tube and Microdilution Limulus Lysate Techniques for Rapid Presumptive Diagnosis of Gonococcal Urethritis in Men," J. Clin. Microbiol., 11, 340-342, 1980.

23. Chapel, T.A.,M. Adcock, B.Smith, T.Barnes, and M. Goodman, "Evaluation of the Limulus Lysate Assay for Presumptive Diagnosis of Gonorrhea in Men at a Clinic for Sexually Transmitted Diseases, "Sex. Transm. Dis., 8, 175-178, 1981.

24. Young, H., S. K. Sarafian, and A. McMillan, "Reactivity of the Limulus Lysate with Uterine cervical Secretion," Br.J. Vener. Dis., 57, 200-203, 1981.

25. Spagna, V. A., R. B. Prior, and R. L. Perkins, "Rapid Presumptive Diagnosis of Gonococcal Cervicitis by the Limulus Lysate Assay, "Am. J. Obstet. Gynecol., 137, 595-599, 1980.

26. Takeguchi, N. M., H. H. westall, D. K. Smith, H. C. McDonald, K. A. Livsey, C. C. Detar, and T. A. chapel, "Enzymatic Detection of Neisseria gonorrhoeae, "Br. J. Vener. Dis., 56, 304-307, 1980.

27. Zubrzycki, L., and S. S. Weinberger, "Laboratory Diagnosis of Gonorrhea by a Simple Transformation Test with a Temperature sensitive Mutant of Neisseria gonorrhoeae, "Sex. Transm. Dis., 7, 183-187, 1980.

28. Janik, A., E. Juni, and G. A. Heym, "Genetic Transformation as a Tool for Detection of Neisseria gonorrhoeae," J.Clin. Microbiol., 4, 71-81, 1976.

29. Jaffe, H. W., S. J. Kraus, T. A. Edwards, S. S. Weinberger, and L. Zubrzycki, "Diagnosis of Gonorrhea Using a Genetic Transformation Test on Mailed Clinical Specimens, "J. Infect. Dis., 146 (2), 275-279, 1982.

30. Buchanan, T. M., J. Swanson, K. K. Holmes, S. J. Kraus, and E. C. Gotschlich, "Quantitative Determination of Antibody to Gonococcal Pili. Changes in Antibody Level with Gonococcal Infection, "J. Clin. Invest., 52, 2896-2909, 1973.

31. Luoma, M. K., W. R. Cross, and J. A. Rudbach, "Radioimmunoassay for Quantifying Antibody to N. gonorrhoeae in Human Sera, "Br. J.Vener. Dis., 51, 387-391, 1975.

32. Oates, S. A., W. A. Falkler, J. M. Joseph, and L. E. Warfel, "Asymptomatic Females: Detection of Antibody Activity to Gonococcal Pili Antigen by Radioimmunoassay, "J.Clin. Microbiol., 5, 26-30, 1977.

33. Gaafar, H. A., "Evaluation of the Fluorescent Gonococcal Test : Heated, "J. Clin. Microbiol., 4, 423-428, 1976.

34. Gaafar, H.A., and D.C. D'Arcangelis, "Fluorescent Antibody Test for the Serological Diagnosis of Gonorrhoea, J. Clin. Microbiol., 3, 438 - 442, 1976.

35. O' Reilly, R. J., B. G. Welch, and D. S. Kellogg, "An Indirect Fluorescent Antibody Technique for Study of Uncomplicated Gonorrhea. II. Selection and Characterization of the Strain of Neisseria gonorrhoeae Used as Antigen," J. Infect. Dis., 127, 77-83, 1973.

36. Welch, B. G., and R. J. O' Reilly, "An Indirect Fluorescent-Antibody Technique for Study of Uncomplicated Gonorrhea. I. Methodology, "J. Infect. Dis., 127, 69-76, 1973.

37. Wilkinson, A. E., "Indirect Fluorescence Test for the Detection of Anti-gonococcal Antibodies, "Br. J. Vener.Dis., 51, 28-30, 1975.

38. Swanson, J., and O. Barrera, "Gonococcal Pilus Subunit Size Heterogeneity Correlates with Transitions in Colony Piliation Phenotype, Not with Changes in Colony Opacity,". J. Exp. Med., 158, 1459-1472, 1983.

39. Reimann, K., and I.Lind, "An Indirect Haemagglutination Test for Demonstration of Gonococcal Antibodies

Using Gonococcal Pili as Antigen. I. Methodology and Preliminary Results, "Acta. Path. Microbiol. Scand. (C), 85, 115-122, 1977.

40. Reimann, K., I. Lind, and K. E. Andersen, "An Indirect Haemagglutination Test for Demonstration of Gonococcal Antibodies Using Gonococcal Pili as Antigen, II. Serological Investigation of Patients Attending a Dermato-Venereological Outpatients Clinic in Copenhagen, "Acta. Path. Microbiol. Scand. (C), 88, 155-162, 1980.

41. Young, H.,C. Henrichsen, and A. McMillan, "The Diagnostic Value of a Gonococcal Complement Fixation Test." Med. Lab. Sci., 37, 165-170, 1980.

42. Glynn, A. A., and C. Ison, "Serological Diagnosis of Gonorrhea by an Enzyme-Linked Immunosorbent Assay, "Br.J.Vener.Dis., 54, 97-102, 1978.

43. Broudeur, B. R., F. E. Ashton, and B. B. Diena, "Enzyme Linked Immunosorbent Assay with Polyvalent Gonococcal Antigen, "J. Med.Microbiol., 15, 1-9, 1982.

44. Oranje, A., K. Reimann, R. V. W. vanEijk, H. J. A. Schouten, A. De Roc, G. J. Tideman, E. Stolz, and M. F. Michel, "Gonococcal Serology. A comparison of three Different tests, "Br.J.Vener. dis., 59, 47-52, 1983.

45. Thornley, M. J., D. V. Wilson, R. D. De Harmaeche, J. K. cates, and R. R. A. coombs, "Detection of Gonococcal Antigens in Urine by Radioimmunoassay, "J.Med.Microbiol., 12, 161-175, 1979.

46. Deacon, W. E., W. L. Peacock, E. M. Freeman, A. Harris, and W. L. Bunch, "Fluorescent Antibody Tests for Detection of the Gonococcus in Women, "Public Health Reports, 75, 125-129, 1960.

47. Danielsson, D., "The Demoustration of N. gonorrhoeae with the Aid of Fluorescent Antibodies, "Acta, Dermato. Venereol., 43 (5), 451-464, 1983.

48. White, L. A., and D. S. Kellagg, "Neisseria gonorrhoeae Identification in Direct Smears by a Fluorescent Antibody-Counterstain Method, "Appl. Microbiol., 13 (2), 171-174, 1965.

49. Lind, I., "Identification of Neisseria gonorrhoeae by Means of Fluorescent Antibody Technique, "Acta. Path. Microbioscand., 70, 613-629, 1967.

50. Sarafian, S. K., and H. Young, "Detection of Gonococcal Antigens by an Indirect Sandwich Enzyme-Linked Immunosorbe Assay, "J. Med. Microbiol., 15, 541-550, 1982.

51. Aardoom, H. A., D. De Hoop, Co. A. Iserief, M. F. Michel, and E. Stolz, "Detection of Neisseria gonorrhoeae Antigen by a Solid-Phase Enzyme Immunoassay, "Br.J.Vener.Dis., 58,359-362, 1982.

52. Papasian, C. J., W. R. Bartholomew, and D. Amsteredam, "Validity of an Enzyme Immunoassay for Detection of Neisseria gonorrhoeae Antigens, "J. Clin. Microbiol., 19 (3), 347-350, 1984.

53. Nasello, M. A., D. R. Callihan, M. A.Menegus, and R. T.

steigbigel, "A Solid-Phase Enzyme Immunoassay (Gonozyme) Test for Direct Detection of Neisseria gonorrhoeae Antigen in Urogenital Specimens from Patients at a Sexually Transmitted Disease Clinic, "Sex. Transm. Dis., 12 (4), 198-202, 1985.

54. Lind, I., L. Berthelsen, O. Birkov, T. Karlsmark, F. U. Verdich, and K. Weismann, "The Diagnosis of Gonorrhoea by Means of the Gonozyme Test," Europ. J. Sex. Transm. Dis., 3, 37-42,1985.

55. Kellogg, D. S., W. L. Peacock, W. E. Deacon, L. Brown, and C. I. Pirkle, "Neisseria gonorrhoeae. I. Vinelence Genetically Linked to clonal variation, "J.Bacteriol., 85, 1274-1279, 1963.

56. Kellogg, D. S., I. R. Cohen, L. C. Norins, A. L. Schroeter, and G. Reising, "Neisseria gonorrhoeae. II. Colonial Variation and Pathogenicity During 35 Months in Vitro, "J. Bacteriol., 96, 596-605, 1968.

57. Sparling, P. F., and A. R. Yobs, "Colonial Morphology of Neisseria gonorrhoeae Isolated from Males and Females, " J. Bacteriol., 93 (1), 513 1967.

58. Elmros, T., P. Horstedt, and B. Winblad, "Scanning Electron Microscopic Study of Virulent and Avirulent Colonies of Neisseria gonorrhoeae, "Infect. Immun., 12 (3), 630-637, 1975.

59. Jephcott, A. E., A. Reyn, and A. Birch-Anderson, "Neisseria gonorrhoeae III. Demonstration of

Presumed Appendages to cells from Different Colony Types, "Acta. Path. Microbiol Scand. (B), 79, 437-439, 1971.

60. Swanson, J., S. J. Kraus, and E. C. Gotschlich, "Studies on Gonococcus Infection. I. Pili and Zones of Adhèsion: Their Relation to Gonococcal Growth Patterns," J. Exp. Med., 134, 886-906, 1971.

61. Kraus, S. J., and L. H. Glassman, "Scanning Electron Microscope Study of Neisseria gonorrhoeae," Appl. Microbiol., 27, (3), 584-592, 1974.

62. Swanson, J., "Studies on Gonococcus Infection. IV. Pili: Their Role in Attachment of Gonococci to Tissue Culture Cellls, "J.Exp.Med., 137, 571-589, 1973.

63. James-Holmquest, A. N., J. Swamson, T. M. Buchanan, R. D. Wende, and R. P. Williams, "Differeential Attachment by Piliated and Nonpiliated Neisseria gonorrhoeae to Human Sperm, "Infect.Immun., 9, 897-902, 1974.

64. James, A. N., J. M. Knox, and R. P. William, "Attachment of Gonococci to Sperm : Influence of Physical and Chemical Factors, "Br. J. Vener. Dis. , 52, 128-135, 1976.

65. Buchanan, T. M., and W. A. Pearce, "Pili as a Mediator of the Attachment of Gonococci to Human Erythrocytes, "Infect. Immun., 13, 1483-1489, 1976.

66. Punsalang, A. P., and W. D. Sawyer, "Role of Pili in

the Virulence of Neisseria gonorrhoeae, "Infect. Immune., 8, 255-263, 1973.

67. Thongthai, C., and W. D. Sawyer, "Studies on the Virulence of Neisseria gonorrhoeae : I. Relation of Colonial Morphology and Resistance to Phagocytosis by Ploymorphonuclear Leukocytes, "Infect. Immun., 7, 373-379, 1973.

68. Ofek, I., E. H. Beachey, and A. Bisno, "Resistance of Neisseria gonorrhoeae to Phagocytosis:Relationship to Colonial Morphology and Surface Pili," J. Infect. Dis., 129, 310-316, 1973.

69. Hendley, J. O., K. R. Powell, J. R. Jordan, R. D. Rodewald, and W. A. Volk, "Capsules of Neisseria gonorrhoeae, "Immunobiology of Neisseria gonorrhoeae (Brooks, G. F., E. C. Gotschlich, K. K. Holmes, W. D. Sawyer, and F. E. Young,eds.) pp. 116-120, American Scciety for Microbiology, Washington, 1978.

70. Hendley, J. O., K. R. Powell, R. D. Rodewald, H. H. Holzgrefe, and R. Lyles, "Demonstration of a Capsule on Neisseria gonorrhoeae, "N.Engl.J.Med., 296, 608-611, 1977.

71. James, J. F., and J. Swanson, "The Capsule of the Gonococcus, "J. Exp. Med., 145, 1082-1086, 1977.

72. Richardson, W. P., and J. C. Sadoff, "Production of a Capsule by Neisseria gonorrhoeae, "Infect. Immun., 15, 663-664, 1977.

73. Demarco de Hormaeche, R., M. J. Thornley, and A. M. Glauert, "Demonstration by Light and Electron Microscopy of Capsules on Gonococci Recently Grown in Vivo, "J. Gen. Microbiol., 106,81-91,1978.

74. Donoghue, H.D., and J. E. Tyler, "Antagonisms Amongst Streptococci Isolated from the Human Oral Cavity, "Arch. Oral. Biol., 20, 381-387, 1975.

75. Morse, S. A., and B. H. Hebeler, "Effect of pH on the Growth and Glucose Metabolism of Neisseria gonorrhoeae, "Infect.Immun., 21, 87-95, 1978.

76. Bundle, D. R., H. J. Jennings, and C. P. Kenny, "Studies on the Group-specific Polysaccharide of Neisseria meningitidis Serogroup X and an Improved Procedure for its Isolation, "J. Biol.Chem., 249, 4797-4801, 1974.

77. Hebeler, B. H., and F. E. Young, "Autolysis of Neisseria gonorrhoeae," J. Bacteriol., 122, 385-392, 1975.

78. Wolf-Watz, H., T. Elmros, S. Normak, and G. D. Bloom, "Cell Envelope of Neisseria gonorrhoeae : Outer Membrane and Peptidoglycan Composition of Penicillin - Sensitive and - Resistant Strains," Infect. Immun., 11, 1332-1341, 1975.

79. Hebeler, B. H., and F. E. Young, "Chemical composition and Turnover of Peptidoglycan in Neisseria gonorrhoeae," J. Bacteriol., 126, 1180-1185, 1976.

80. Morse, S. A., and L. Bartenstein, "Factors Affecting Autolysis of Neisseria gonorrhoeae," Proc. Soc.

Exp. Biol. Med., 145,1418-1421, 1974.

81. Wegerner, W. S., B. H.Hebeler, and S. A. Morse, "The cell Envelope of Neisseria gonorrhoeae : Relationship between Autolysis in Buffer and the Hydrolysis of Peptidoglycan," Infect. Immun., 18, 210-219, 1977.

82. Elmros, T. G., Sandstrom, and L. Burman, "Autolysis of Neisseria gonorrhoeae. Relation between Mechanical Stability and Viability," Br. J. Vener. Dis., 52,246-249, 1976.

83. Morse, S. A., "The Biology of the Gonococcus, "CRC Crit. Rev. Microbiol., 7, 93-189, 1979.

84. McGee, Z. A., A. P. Johnson, and D. Tayor-Robinson, "Pathogenic Mechanisms of Neisseria gonorrhoeae : Observations on Damage to Human Fallopian tubes in Organ culture by Gonococci of Colony Type 1 or Type 4," J. Infect. Dis., 143-422, 1981.

85. Perry, M. B., V. Daoust, B. B. Diena, F. E. Ashton, and R. Wallace, "The lipopolysaccharides of Neisseria gonorrhoeae Colony Types 1 and 4," Can. J. Biochem., 55,623-629, 1975.

86. Wiseman, G. M., and J. D. Caid, "Composition of the Lipopolysaccharide of Neisseria gonorrhoeae," Infect. Immun., 16, 550-556, 1977.

87. Stead, A., J. S. Main, M. E. Ward, and P. J. Watt, "Studies on Lipopolysaccharides Isolated from Strains of Neisseria gonorrhoeae," J. Gen.

Microbiol., 88,123-131, 1975.

88. Sadoff, J. C., W. D. Zollinger, and H. Sidberry, "Cell Structures of Neisseeria gonorrhoeae, "Immunobiology of Neisseria gonorrhoeae (Brooks, G. F., E. C. Gotschlich, K. K. Holmes, W. D. Sawyer, and F. E. Young, eds.), pp. 33-100, American Society for Microbiology, Washington, 1978.

89. Glynn, A. A., M. E. Ward, "Nature and Heterogeneity of the Antigens of N. gonorrhoeae Involved in the serum Bactericidal Reaction," Infect. Immun., 2, 162-168, 1970.

90. Gregg, C. R., M. A. Melly, C. G. Hellerqvist, J. G. Coniglio, and Z. A. Mcgee, "Toxic Activity of Purified Lipopolysaccharide of Neisseria gonorrhoeae for Human Fallopian Tube Mucosa," J. Infect. Dis., 143, 432-439, 1981.

91. Johnston, K. H., K. K. Holmes, and E. C. Gotschlich, "The Serological Classification of Neisseria gonorrhoeae. I. Isolation of the Outer Membrane Complex Responsible for Serotypic Specificity," J.Exp. Med., 143, 741-758, 1976.

92. Blake, M. S., and E. C. Gotschlich, "Gonococcal Membrane proteins : Speculation on their Role in Pathogenesis," Prog. Allergy, 33, 298-313, 1983.

93. Swanson, J., "Studies on Gonococcus Infection. XVIII. 125-labeled Peptide Mapping of the Major

Protein of the Gonococcal Cell Wall Outer Membrane," Infect. Immun., 23, 799-810, 1979.

94. Blake, M. S., E. C. Gotschlich, and J. Swanson," The Effect of Proteolytic Enzymes on the Outer Membrane Proteins of Neiseria gonorrhoeae," Infect. Immun., 33, 212-222, 1981.

95. Hidebrandt, J. F., L. W. Mayer, S. P. Wang, and T.M. Buchanan, "Neisseria gonorrhoeae Acquire a New Prinipal Outer-Membrane Protein when Transformed to Resistance to Serum Bactericidal Activity," Infect. Immun., 20, 267-272, 1978.

96. Heckels, J. E., "The Surface Properties of Neisseria gonorrhoeae : Isolation of the Major Components of the Outer Membrane, " J. Gen. Microbiol., 99, 333-341, 1977.

97. Zak, K., J-L. Diaz, D. Jackson, and J. E. Heckels, "Antigenic Variation During Infection with Neisseria gonorrhoeae : Detection of Antibodies to Surface poteins in Sera of Patients with Gonorrhea," J. Infect. Dis., 149, 166-174, 1984.

98. Swanson, J., "Studies on Gonococcus Infection. XIV. Cell Wall Protein Differences among Color/ Opacity Colony Variants of Neisseria gonorrhoeae," Infect. Immun., 21,292-302, 1978.

99. James, J. F. and J. swanson, "Color / Opacity colonial variants of Neisseria gonorrhoeae and Their Relationship to the Menstrual Cycle,"

Immunobiology of Neisseria gonorrhoeae (Brooks, G. F., E. C. Gotschlich, K. K. Holmes, W. D. Sawyer, and F. E., Young, eds.), pp. 338-343, American Society for Microbiology, Washington, 1978.

100. Lambden, P. R., J. E. Heckels, L. T. James, and P. J. Watt, "Variations in Surface Protein Composition Associated with Virulence Properties in Opacity Types of Neisseria gonorrhoeae," J.Gen.Microbiol., 114, 305-312, 1979.

101. Britigan, B. E., M. S. Cohen, and P. F. Sparling, "Gonococcal Infection : a Model of Molecular Pathogenesis," N.Engl. J. Med., 312 (26), 1683-1694, 1985.

102. Stern, A., M.Brown, .Nickel, and T.F. Meyer, "Opacity Genes in Neisseria gonorrhoeae : Contrl of Phase and Antigenic Variation," Cell, 47, 61-71, 1986.

103. McDade, R. L., and K. H. Johnston, "Characterization of Serologically Dominant Outer Membrane Poteins of Neisseria gonorrhoeae," J. Bacteriol., 141, 1183-1191, 1980.

104. Jones, R. B., P. A. Jemison, W. J. Newhall, and R. A. Haak, "Resolution of Basic Gonococcal Outer Membrane Proteins by Nonequilibrium pH Gradient Electrophoresis, "Infect. Immun., 30, 773-780, 1980.

105. McGee, Z. A., R. R. Dourmashkin, J. G. Gross, J. B. clark, and D. Taylor-Robinson, "Relationship of Pili to Colonial Morphology Among Pathogenic and Nonpathogenic Species of Neisseria," Infect. Immun., 15, 594-600, 1977.

106. Weistreich, G. A., and R. F. Baker, "The Presence of Fimbriae (Pili) in Three Species of Neisseria," J. Gen. Microbiol., 65, 167-173, 1971.

107. Buchanan, T. M., "Surface Antigens : Pili, "The Gonococcus (Roberts, R. B., ed.), pp. 225-272, John Wiley & Sons, New York, 1977.

108. Mardh, P-A., and L.Westrom, "Adherence of Bacteria to Vaginal Epithelial cells, "Infect. Immun., 13, 661-666, 1976.

109. Ward, M. E., P. J. Watt, and J. N. Robertson, "The Human Falloian Tube : A Laboratory Model for Gonococcal Infection, " J. Infect. Dis., 129, 650-659, 1974.

110. Pearce, W. A., and T. M. Buchanan, "Attachment Role of Gonococcal Pili : Optimum Conditions and Quantitation of Adherence of Isolated pili to Human cells in Vitro, " J. Clin. Invest., 61, 931-943, 1978.

111. Draper, D. L., E.A. Donegan, J. F. James, R. L. Sweet, and G. F. Brooks, "Scanning Electron Microscopy of Attachment of Neisseria gonorrhoeae Colony Phenotypes to Surfaces of Human Genital

Epithelia," Am. J. Obstet. Gynecol., 138, 818-826, 1980.

112. Salit, I. E., M. Blake, and E. C. Gotschlich, "Intra-Strain Heterogeneity of Gonococcal Pili is Related to Opacity Colony Variance," J. Exp. Med., 151, 716-725, 1980.

113. Schoolnik, G. K., J. Y. Tai, and E. C. Gotschlich, "A Pilus Peptide Vaccine for the Prevention of Gonorrhea," Prog. Allergy, 33, 314-331, 1983.

114. Robertson, J. N., P. Vincent, and M.E. Ward, "The Preparation and Proerties of Gonococcal pili," J. Gen. Microbiol., 102, 169-177, 1977.

115. Lambden, P. R., J. N. Robertson, and P. J. Watt, "The Preparation and Properties of a and B Pili from Variants of Neisseria gonorrhoeae Pg," J. Gen. Microbiol., 124, 109-117, 1981.

116. Lambden, P. R., J. N. Robertson, and P.J. Watt, "Biological Properties of Two Distinct Pilus Types Produced by Isogenic Variants of Neisseria gonorrhaeae Pg," J. Bacteriol, 141(1), 393-396, 1980.

117. Hermodson, M. A., K. C. S. chen, and T. M. Buchanan, "Neisseria Pili Proteins : Amino-Terminal Amino Acid Sequences and Identification of an Unusual Amino Acid," Biochemistry, 17(3), 442-445, 1978.

118. Buchanan, T. M. K. C. S. Chen, R. B. Jones, J. F. Hildebrandt, W. A. Pearce, M.A. Hermodson, J. C.

Newland, and D. L. Luchtel, "Pili and Principal Outer Membrane Protein of Neisseria gonorhoeae : Immunochemical, Structural, and Pathogenic Aspects," Immunobiology of Neisseria gonorrhoeae (Brook, G. F., E. C. Gotschlich, K. K. Holmes, W. D. Sawyer, and F. E. young; eds.), pp.145-154, American Society for Microbiology, Washington, 1978.

119. Schoolnik, G. K., R. Fernandez, J. Y. Tai, J. Rothbard, and E. C. Gotschlich," Gonococcal Pili : Primary Structure and Receptor Binding Domain," J.Exp. Med., 159, 1351-1370, 1984.

120. Schoolnik, G. K., J. Y. Tai, and E. C. Gotschlich, "The Human Erythrocyte Binding Domain of Gonococcal Pili,". Seminars in Infectious Disease (Weinstein, L., B. N. Fields, J. B. Robbins, J. C. Hill, and G. Sadoff, eds.), Vol. IV, pp. 172-180, Thieme-Stratton, New York, 1982.

121. Billyard, M. S., C. Deal, E.Getzoff, P.Hagblom, T.F. Meyer, E. Segal, and J. Tainer, "Gonococcal Plus : Genetics and Structure," Cur. Top. Microbiol. Immunol., 118, 13-28, 1985.

122. Hill, J. H., "Fundamental Problems for Laboratory Research on Neisseria gonorrhoeae and Gonococcal Infection," Am. J. Syph., 32, 165-189, 1948.

123. Jephcott, A. E., and A. Reyn, "Neisseria gonorrhoeae. Colony Variation I," Acta. Path. Microbiol.

Scand. [B], 79, 609-614; 1971.

124. Chan, K., and G.M. Wiseman, "A New Colonial Type of N. gonorrhoeae," Br.J.Vener.Dis., 51, 251-256, 1975.

125. Jacobs, N. F., S.J. Kraus, C. T. Thornsberry, and J. Bullard, "Isolation and Characterization of a Rough Colony Type of Neisseria gonorrhoeae," J. Clin. Microbiol., 5(3), 365-369, 1977.

126. Bergstrom. S., K. Robbins, J. M.Koomey, and J. Swanson, "Piliation control Mechanisms in Neisseria gonorrhoeae," Proc. Natl. Acad. Sci. USA., 83, 3890-3894, 1986.

127. Sparling, P. F., J. G. Cannon, and M.So, "Phase and Antigenic Variation of Pili and Outer Membrane Protein II of Neisseria gonorrhoeae," J. Infect. Dis., 153(2), 196-201, 1986.

128. Meyer, T. F., N. Mlawer, and M. So. "Pilus Expression in Neisseria gonorrhoeae Involves Chromosomal Rearrangement," Cell, 30, 45-52, 1982.

129. Meyer, T. F., E. Billyard, R. Haas, S. Storzbach, and M. So, "Pilus Genes of Neisseria gonorrhoeae: Chromosomal Organization and DNA sequence," Proc. Natl. Acad. Sci. USA., 81, 6110-6114, 1984.

130. Swanson, J., S. Bergstrom, K. Robbins, O. Barrera, D. Corwin, and J. M. Koomey," Gene Conversion Involving the pilin Structural Gene correlates with Pilus$^+$ Pilus$^-$ Changes in Neisseria gonorrhoeae," 47, 267-276, 1986.

131. Segal, E., E. Billyard, M. So, S. Storzbach, and T.F. Meyer, "Role of Chromosomal Rearrangement in N. gonorrhoeae Pilus Phase Variation," Cell, 40, 293-300, 1985.

132. Swanson, J., S. Bergstrom, O. Barrera, K. Robbins, and D. Corwin, "Pilus Gonococcal Variants: Evidence for Multiple Forms of Piliation control," J. Exp. Med., 162, 729-744, 1985.

133. Hagblom, P., E. Segal, E. Billyard, and M. So, "Intragenic Recombination Leads to Pilus Antigenic Variation in Neisseria gonorrhoeae, "Nature, 315(6015), 156-158, 1985.

134. Brinton, C. C., J. Bryan, J. -A. Dillon, N. Guerina, L. J. Jacobson, A. Labik, S. Lee, A. Levine, S. Lim, J. McMichael, S. Polen, K. Rogers, A. C. C. To, and S. C. M. To, "Use of Pili in Gonorrhea Control : Role of Bacterial Pili in Disease, Purification and Properties of Gonococcal Pili, and Progress in the Development of a Gonococcal pilus Vaccine for Gonorrhea, "Immunobiology of Neisseria gonorrhoeae (Brooks, G. F., E. C. Gotschlich, K. K. Holmes, W. D. Sawyer, and F. E. Young, eds.) pp. 155-178, American Society for Microbiology, Washington, 1978.

135. Novotny, P., and W. H. Turner, "Immunological Heterogeneity of Pili of Neisseria gonorrhoeae," J.Gen.

Microbiol., 89, 87-92, 1975.

136. Buchanan, T. M., "Antigenic Heterogeneity of Gonococcal Pili," J. Exp. Med., 141, 1470-1475, 1975.

137. Duckworth, M., D. Jackson, K.Zak, and J.E. Heckels, "Structural Variations in Pili Expressed during Gonococcal Infection," J. Gen. Microbiol., 129, 1593-1596, 1983.

138. Reimann, K., A. P. Oranje, and M. F. Michel, "Demonstration of Antigenic Heterogeneity of Neisseria gonorrhoeae Pili Antigens Using Human Sera in the Test System," Acta. Path. Microbiol. Scand. [C], 90, 47-52, 1982.

139. Cannon, J. G., and F. Sparling, "The Genetics of the Gonococcus," Ann. Rev. Microbiol., 38, 111-133, 1984.

140. Rothbard, J. B., R. Fernandez, and G. K. Schoolnik, "Strain - Specific and Common Epitopes of Gonococcal Pili," J. Exp. Med., 160, 208-221, 1984.

141. Haas, R., and T.F. Meyer, "The Repertoir of Silent Pilus Genes in Neisseria gonorrhoeae : Evidence for Gene Conversion," Cell, 44, 107-115, 1986.

142. Segal, E., P. Hagblom, H. S. Seifert, and M. So. "Antigenic Variation of Gonococcal Pilus Involves Assembly of Separated Silent Gene Segments," Proc. Natl. Acad. Sci. USA., 83, 2177-2181, 1986.

143. Koransky, J. R., R. W. Scales, and S. J. Kraus, "Bacterial Hemagglutination by Neisseria gonorrhoeae," Infect. Immune., 12 (3), 495-498, 1975.

144. Brinton, C. C., "Non-Flagellar Appendages of Bacteria," Nature, 183, 782-786, 1959.

145. Dugid, J. P., and R. R. Gillies, "Fimbrial and Adhesive Properties in Dysentery Bacilli," J. Pathol. Bacteriol., 74, 397-411, 1957.

146. Salit, I. E., "Hemagglutination by Neisseria meningitidis," Can. J.Microbiol., 27, 586-593, 1981.

147. Waitkins, S. A., "Fimbrial Haemagglutination by Neisseria gonorrhoeae, "Br. J. Vener. Dis., 50, 272-278, 1974.

148. Rothbard, J. B., R.Fernandez, L.Wang, N. N. H. Teng, and G. K. Schoolnik, "Antibodies to Peptides Corresponding to a Conserved Sequence of Gonococcal Pilins Block Bacterial Adhesion," Proc. Natl. Acad. Sci.USA., 82, 915-919, 1985.

149. Virji, M., and J. E. Heckels, "The Role of Common and Type-Specific Pilus Antigenic Domains in Adhesion and Virulence of Gonococci for Human Epithelial Cells, "J.Gen. Microbiol., 130, 1089-1095, 1984.

150. James, J.F., and J. Swanson "Piliation of Gonococci in Vivo," J. Infect. Dis., 137, 94-96, 1978.

151. Grimble, A., and L.R.G. Armitage, "Surface Structures of the Gonococcus," Br. J. Vener. Dis., 50, 354-359, 1974.

152. Jones, R. B., J. C. Newland, D.A. Olsen, and T. M. Buchanan, "Immune-Enhanced Phagocytosis of Neisseria gonorrhoeae by Macrophages : Characterization of the Major Antigens to Which Opsonins Are Detected," J. Gen. Microbiol., 121, 365-372, 1980.

153. Swanson, J., G. King, and B. Zeligs, "Studies on Gonococcus Infection : VIII. ^{125} Iodine labelling of Gonococci and Studies of Their Interactions with Eukaryotic Cells in Vitro," Infect. Immun., 11, 453-459, 1975.

154. Ward, M.E., and P.J. Watt, "Adherence of Neisseria gonorrhoeae to Urethral Mucosal Cells : An Electron Microscope Study of Human Gonorrhea," J. Infect. Dis., 126, 601-605, 1972.

155. Tebbutt, G. M., D. R. Veale, J. G. P. Hutchison, and H. Smith, "The Adherence of Pilate and Nonpilate Strains of Neisseria gonorrhoeae to Human and Guinea-Pig Epithelial Tissues," J. Med. Microbiol., 9. 263-273, 1976.

156. Tramont, E. C., J. Ciak, J. Boslego, D. G. McChesney, C. C. Brinton, and W. Zolliger, "Antigenic Specificity of Antibodies in Vaginal Secretions During Infection with Neisseria

gonorrhoeae," J.Infect. Dis., 142(1), 23-31,1980.

157. Tramont, E. C., J. C. Sadoff, J. W. Boslego, J. Ciak, and D. McChesney, "Gonococcal Pilus Vaccine," J. Clin. Invest., 68, 881-888, 1981.

158. Heckels, J. E., B. Blackett, J. S. Everson, and M. E. Watt, "The Influence of Surface Charge on the Attachment of Neisseria gonorrhoeae to Human Cells," J. Gen. Microbiol., 96, 359-364, 1976.

159. Buchanan, T. M., W. A. Pearce, and K. C. S. Chen, "Attachment of Neisseria gonorrhoeae Pili," Immunobiology of Neisseria gonorrhoeae (Brooks, G. F., E. C. Gotschlich, K. K. Holmes, W. D. Sawyer, and F. E. Young, eds.), pp. 242-249, American Society for Microbiology, Washington, 1978.

160. Haschemeyer, R. H., and R. J. Meyers, "Negative Staining, "Principles and Techniques of Electron Microscopy (Hayat, M. A., ed.), Vol. 2, Van Nostrand Reinhold Company, New York, 972.

161. Lowry, O. H., N. J. Rosenbrough, A. L. Farr, and R. J. Randall, "Protein Measurement with the Folin Phenol Reagent, "J. Biol. Chem., 193, 265-275, 1951.

162. Laemmli, U. K., "Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4," Nature, 227, 680-685, 1970.

163. Johnston, N. A. "Evaluation of the Coagglutination Test

for the Identification of Neisseria gonorrhoeae in Primary Cultures, "Br. J. Vener. Dis., 57, 315-319, 1981.

164. Johnstone, A., and R. Thorpe, Immunochemistry in Practice, pp. 44 - 46, Blackwell Scientific Publications, Boston, 1982.

165. Avrameas, S., "Coupling of Enzymes to Proteins with Glutaraldehyde. Use of the Conjugates for the Detection of Antigens and Antibodies," Immunochemistry, 6, 43-52, 1969.

166. Voller, A., and D. Bidwell, "Enzyme-Linked Immunosorbent Assay," Manual of Clinical Laboratory Immunology (Rose, N. R., H. Friedman, and J. L. Fahey, eds.), pp.99-109, American Society. For Microbiology, Washington, 3 rd.ed., 1986.

167. Eshdat, Y., V. Speth, and K. Jann, "Participation of Pili and Cell Wall Adhesin in the Yeast Agglutination Activity of Escherichia coli," Infect. Immun., 34(3), 980-986, 1981.

168. Dodd, D. C., and B. L. Eisenstein, "Antigemic Quantitation of Type 1 Fimbriae on the Surface of Escherichia coli Cells by an Enzyme-Linked Immunosorbent Inhibition Assay," Infect. Immun., 38, 764-773, 1982.

169. Coulter, A. R., R. D. Harris, and S. K. Sutherland, "Enzyme Immunoassay for Rapid Clinical Identification of Snake Venom," Med. J. Aust., 1, 433-435, 1980.

170. Virji, M., J. S. Everson, and P. R. Lambden, "Effect of Anti-pilus Antisera on Virulence of Variants of Neisseria gonorrhoeae for Cultured Epithelial Cells," J. Gen. Microbiol., 128, 1095-1100, 1982.

APPENDIX

1. Media and Ingredients

1.1. The defined supplement

The defined supplement was a modification of that described by kellogg et al. (55) and was composed of 2 mg co-carboxylase, 1 g L-glutamine, 40 g glucose, and 50 mg ferric nitrate per 100 ml of distilled water. The mixture was sterilized by filtration and stored at 4 °C until use.

1.2. Kelogg medium (GCBS)

The Kellogg medium was prepared by dissolving 36 g of GC agar base (BBL) in 1000 ml of distilled water, heated until dissolve completely and sterilized at 15 lbs for 15 min. Ten ml of defined supplement were added when the medium was warm, and poured into each sterile petri dish. All GCBS media were dried at 36-37 °C to remove surface moisture before use.

2. Reagents for Purification of Gonococcal Pili

2.1. Tris-HCl buffer 0.01M, pH 9.5

1.2114 g of Tris (hydroxymethyl) aminomethane was dissolved in about 800 ml of distilled water. Then adjust the pH to 9.5 with 1 N HCl and made up the volume to 1000 ml with distilled water.

2.2. Tris-HCl buffer 0.01 M, pH 7.0, 0.01 M NaN_3

The buffer was prepared by dissolving 0.3028 g of Tris, 0.1625 g of sodium azide in distilled water, then adjusted to pH 7.0 with 1N HCl and was made up the volume to 250 ml.

2.3. Ammonium sulfate, saturated solution

One kilogram of ammonium sulfate (Merk) was dissolved in 1000 ml distilled water and heated until dissolved completely, filtered, allowed to stand overnight at room temperature and pH adjusted to 7.0 with ammonium hydroxide solution.

2.4. Ammonium sulfate, 20% saturated solution

One hundred ml of saturated ammonium sulfate was diluted with 400 ml distilled water.

3. Reagents for Lowry Method (Protein Determination)

3.1. Reagent A

The grams of sodium carbonate (Na_2CO_3) was dissolved in 0.5 N NaOH solution.

3.2. Reagent B

One gram of copper sulfate, hydrated ($CuSO_4 \cdot 5H_2O$) was dissolved in 100 ml distilled water.

3.3. Reagent C

The two grams of potassium tartrate was dissolved in 100 ml of distilled water.

3.4. Diluted Folin-Ciocalteu phenol reagent

This solution was freshly prepared by diluting 5 ml of Folin Ciocalteu Phenol reagent (Sigma) with 45 ml of distilled water.

3.5. Bovine serum albumin (CSL) standard (0.3 mg/ml)

4. Reagents for Electron Microscopy

4.1. Fixative solution

4 % glutaraldehyde in 0.1 M phosphate buffer pH 7.4 was prepared by adding 2 ml of 25% glutaraldehyde to 6.25 ml of 0.2 M phosphate buffer pH 7.4 and volume adjusted to 12.5 ml with distilled water.

4.2. Phosphotungstate solution 0.5%, pH 7.0

The staining solution was prepared by dissolving 50 mg of phosphotungstic acid in 10 ml of distilled water, pH adjusted to 7.0 with NaOH solution, filtered through Whatman filter paper No.1 and stored in a dark bottle at 4°C.

5. Reagents for SDS-PAGE

5.1. Stock acrylamide

Thirty grams of acrylamide (LKB) and 0.8 g of N, N'-methylene bisacrylamide (Sigma) were dissolved in 100 ml of distilled water, then filtered and stored in the dark at 4°C.

5.2. Tris-HCl buffer 1.5 M, pH 8.8

18.15 g of Tris was dissolved in 50 ml of distilled water, pH adjusted to 8.8 with in HCl and final volume added to 100 ml.

5.3 Tris-HCl buffer 0.5 M, pH 6.8

Three grams of Tris was dissolved in 20 ml distilled water, pH adjusted to 6.8. The final volume was made up to 50 ml with distilled water.

5.4. EDTA 0.2 M

The 2.92 gram of EDTA was dissolved in 50 ml distilled water.

5.5. Sodium Dodecyl Sulfate (SDS), 20%

Ten grams of SDS was dissolved in 50 ml of distilled water.

5.6. N,N,N',N'-tetramethylethylenediamine (TEMED) (Sigma)

5.7. Ammonium persulfate solution, 10% (W/V)

The 0.1 g of ammonium persulfate (Sigma) was dissolved in 1 ml distilled water. This reagent was freshly prepared before use.

5.8. Electrode buffer

The buffer was prepared by dissolving 3 g of Tris, 14.4 g of glycine, and 5 ml of 20% SDS in 1000 ml of distille water, then pH adjusted to 8.3.

5.9. Stock sample buffer (5X)

The stock solution consisted of 2.5 ml of 1.5 M Tris-HCl buffer, pH 6.8, 1 g of SDS, 5 ml of glycerol, 2.5 ml of mercaptoethanol, and 0.5 mg of bromphenol blue. The volume was adjusted to 10 ml with distilled water.

5.10. Staining solution

The staining solution was prepared by dissolving 2.5 g of Coomassie brilliant blue R (Sigma) in 500 ml methanol, 76 ml glacial acetic acide and making up the volume to 1000 ml with distilled water.

5.11. Destaining solution

5.11.1. Destaining I

One litre of destaining solution was prepared as follows : 500 ml methanol, 76 ml glacial acetic acid, and 424 ml of distilled water.

5.11.2. Destaining II

One litre of destaining solution was composed of 50 ml methanol, 75 ml glacial acetic acid, and 875 ml of distilled water.

5.12. SDS molecular weight markers

The standard molecular weight markers purchased from Sigma Chemical Company were composed of egg white lysozyme (14,300), B-lactoglobulin (18,400), Trypsinogen (24,000), Pepsin (34,700), egg albumin (45,000), and bovine plasma albumin (66,000).

6. Reagents for Rabbit Immunization

6.1. Tris-HCl buffer 0.01 M, pH 7.0

0.1211 g of Tris was dissolved in distilled water, pH adjusted to 7.0, sterilized by filtration through a sterile Millipore membrance (0.45 um).

6.2. Freund's complete adjuvant (Difco)

6.3. Freund's incomplete adjuvant (Difco)

7. Reagents for Immunodiffusion

7.1. Agarose 1%

0.2 g of agarose was dissolved in 20 ml normal saline solution, boiled until dissolved completely.

7.2. Goat anti rabbit whole serum (Sigma)

7.3. Goat anti rabbit Immunoglobulin G (Sigma)

8. Reagents for Indirect Hemagglutination Test (IHA)

8.1. Formalin treatment of sheep red blood cells.

8.1.1. Sheep red blood cells (SRBC)

Sheep blood was collected in an equal volume of Alsever's solution and stored for 5-10 days at 4 °C before use.

8.1.2. Normal saline solution (NSS)

Nine grams of NaCl dissolved in 1 litre of distilled water.

8.1.3. Formalin solution 7.5%

The 18.7 ml of 37% formaldehyde solution is diluted with 81.3 ml of NSS.

8.2. Tannic acid treatment

8.2.1. Phosphate buffered salin (PBS) pH 7.2

4.672 g $Na_2HPO_4 2H_2O$, 1.53 g KH_2PO_4, and 6.75 g NaCl disssolved in 1 litre distilled water.

8.2.2. Phosphate buffered saline (PBS) pH 6.4

4.673 g Na_2HPO_4 $2H_2O$, 6.63 g KH_2PO_4, and 4.5 g NaCl were dissolved in 1 litre distilled water.

8.2.3. Tannin solution (1:40,000)

The solution was freshly prepared by dissolving 2.5 mg Tannic acid (Merk) in 100 ml of PBS pH 7.2

8.3. Normal rabbit serum, 1% (1%PBS-R)

Normal rabbit serum (Gibco) was inactivated at 56 °C for 30 min and absorbed with an equal volume of packed formalin treated SRBC for 10 min at room temperature. After centrifugation, the absorbed normal rabbit serum was then diluted to 1 percent solution in PBS pH 6.4. This diluted serum was kept at -20°C until use.

9. Reagents for Coagglutination Test

9.1. Tryptic Soy Broth (TSB)

Thirty grams of Tryptic soy broth (BBL) was dissolved in 1000 ml distilled water, then swirled until completely dissolved, sterilized by autoclave at 15 lbs for 15 min.

9.2. Phosphate buffer pH 7.2

0.1925 g of KH_2PO_4 and 0.6244 g of K_2HPO_4 were

dissolved in 1 litre of distilled water.

9.3. Formalin solution, 0.5%

0.25 ml of formaldehyde solution was diluted to 20 ml with phosphate buffer pH 7.2.

9.4. PBS pH 7.2, 0.1% NaN_3

Buffer solution prepared by dissolving 1.53 g KH_2PO_4, 3.72 g Na_2HPO_4, 6.75 g NaCl, and 1 g NaN_3 in 1000 ml distilled water. This solution was sterilized by filtration.

10. Reagents for Isolation of IgG from Rabbit Antiserum

10.1. Solution A

1.36 g KH_2PO_4 was dissolved in 100 ml distilled water

10.2. Solution B

17.4 g K_2HPO_4 was dissolved in 1000 ml distilled water.

10.3. Starting buffer

Phosphate buffer, 0.01 M pH 8.0 was prepared by mixing 10 ml of solution A and 190 ml of solution B, and the solution was diluted to 2000 ml with distilled water.

10.4. Solution C

4.08 g of KH_2PO_4 was dissolved in 100 ml of distilled water.

10.5. Solution D

52.2 g of K_2HPO_4 was dissolved in 1000 ml of distilled water.

10.6. Final buffer

Phosphate buffer, 0.3 M pH 8.0 prepared by diluting 50 ml of solution C with 950 ml of solution D.

10.7. DEAE cellulose (DE-52 Whatman)

11. Reagents for Immunoelectrophoresis (IEP)

11.1. Sodium barbital-HCl 0.05 M, pH 8.2

Barbital buffer was composed of 11.3 g of barbital sodium, 0.1 g of NaN_3 and 16.4 ml of 1N HCl, volume adjusted to 1 litre with distilled water.

11.2. Agarose, 1.5%

0.3 g agarose was dissolved in 20 ml of barbital buffer, boiled until agarose completely dissolved.

12. Reagents for Conjugation of Alkaline Phosphatase to Rabbit IgG

12.1. Alkaline phosphatase enzyme , type VII-S (Sigma),was kept at 4 °C.

12.2. Isophosphate buffer, pH 7.4

3.44 g of NaH_2PO_4 H_2O and 15.2 g of Na_2HPO_4 were dissolved in 1 litre of deionized water.

12.3. $MgCl_2$ solution, 1 M

2.033 g of $MgCl_2$ $6H_2O$ was dissolved in 10 ml deionized water.

12.4. PBS-Mg^{2+}

Addition of 1 ml of 1 M $MgCl_2$ to 100 ml of isophosphate buffer and the volume made upto 1 liter with NSS.

12.5. Glutaraldehyde, 10 %

The solution was prepared by diluting 1 ml of 50% glutaraldehyde with 4 ml of isophosphate buffer.

12.6. Lysine 1 M, pH 7.0

0.731 g of Lysine was dissolved in 5 ml of deionized water, pH adjusted to 7.0 with 1 N NaOH.

12.7. Bovine Serum Albumin, 20 mg/ml

0.2 g of BSA was dissolved in 10 ml of deionized water.

12.8. Tris-HCl buffer 0.05 M, pH 8.0

The buffer was prepared by dissolving 0.6055 g of Tris in 100 ml of deionized water, then pH adjusted to 8.0 with 1 N HCl.

13. Reagents for Enzyme-Linked Immunosorbent Assay (ELISA)

13.1. Coating buffer

Carbonate-bicarbonate buffer, pH 9.6 consisted of 0.159 g of Na_2CO_3, 0.293 g of $NaHCO_3$, and 0.02 g of NaN_3, made up to 100 ml with distilled water, and stored at 4 °C for not more than 2 weeks.

13.2. PBS-T

PBS-Tween pH 7.4 was composed of 8 g NaCl, 0.2 g KH_2PO_4, 2.9 g $Na_2HPO_4\ 12H_2O$, 0.2 g KCl, 0.5 ml Tween 20, and 0.2 g NaN_3 in 1 litre of distilled water and stored at 4°C.

13.3. Sample diluent

2 g of BSA was mixed with 100 ml of PBS-T, and stored at 4°C.

13.4. Triethanolamine 0.25 M,pH 7.6

3.3 ml of triethanolamine was added to distilled water, then adjusted pH to 7.6 Made up the volume to 100 ml

13.5. Mg/Zn solution (100X)

The stock solution prepared by dissolving 10.16 g of $MgCl_2$ $6H_2O$ and 0.017 g of $ZnCl_2$ in 500 ml of distilled water.

13.6. Enzyme diluent

The enzyme diluent for dituting the conjugate contained 4 g of NaCl, 25 ml of 0.25 M Triethanolamine pH 7.6, 5 ml of 100X Mg/Zn , 5 ml of 20 mg/ml BSA ,and 0.5 g of NaN_3, made up to 500 ml with distilled water, and stored at 4 °C.

13.7. Substrate diluent

Diethanolamine buffer, 10 % consisted of 9.7 ml of diethanolamine, 80 ml of distilled water, 20 mg of NaN_3, and 10 mg of $MgCl_2$ $6H_2O$. 1N HCl was added until the pH was 9.8, and distilled water was added to final volume of 100 ml It was stored at 4°C in the dark.

13.8. Substrate

p-nitrophenyl phosphate (5 mg/tablet, Sigma) tablets were stored at -20°C in the dark until use. Immediately before use, one tablet was dissolved in each 5 ml of substrate diluent at room temperature, and must be used the same day.

13.9. Stopping solution

3 M NaOH was prepared by dissolving 12 g of NaOH in 100 ml of distilled water.

BIOGRAPHY

Mr. Suchart Chanama was born on May 23, 1961 in Bangkok, Thailand. He graduated with the degree of Bechelor of Science in Medical Technology, from the Faculty of Medical Technology, Mahidol University in 1984.