REFERENCES

1. Schachter, J."Chlamydial Infections" N. Eng. J. Med. 298(1978): 428-435, 490-495, 540-549.

2. Taylor-Robinson, D. and Thomas, B.J. "The Role of Chlamydia trachomatis in Genital Tract and Associated Diseases" J. Clin. Pathol. 33(1980):205-233.

3. Page, L.A. Order II. Chlamydiales Storz and Page 1971, 334 in Bergey's Manual of Determinative Bacteriology, (Buchanan, R.E. and Gibbons, N.E. eds.) 8th ed., pp. 914-918, The Williams & Wilkins Company, Baltimore, 1974.

4. Storz, J. and Page, L.A. "Taxonomy of the Chlamydiae: Reasons for Classifying Organisms of the Genus Chlamydia, Family Chlamydiaceae, in a Separate Order, Chlamydiales ord nov" Int. J. Syst. Bacteriol. 21(1971):332-334.

5. Sweet, R.L., Schachter, J. and Landers, D.V. "Chlamydial Infections in Obstetrics and Gynecology" Clin. Obstet. Gynecol. 26 (1983):143-164.

6. Stamm, W.E. and Holmes, K.K. Chlamydia trachomatis Infections of the Adult in Sexually Transmitted Diseases, (Holmes, K.K., Mardh, P.A., Sparling, P.F. and Wiesner, P.J. ed.) pp. 258-270, McGraw-Hill Book Company, U.S.A.,1984.

7. Schachter, J., Grossman, M., Holt, J., Sweet, R., Goodner, E. and Mills, J. "Prospective Study of Chlamydial Infection in Neonates" Lancet. 2(1979):377-379.

8. Hammerschlag, M.R., Anderka, M., Semine, D.Z., McComb, D. and

McCormack, W.M. "Prospective Study of Maternal and Infantile Infection with Chlamydia trachomatis" Pediatrics. 64(1979):142-148.

9. Frommell, G.T., Rothenberg, R., Wang, S.P. and McIntosh, K. Chlamydial Infection of Mothers and Their Infants" J. Pediat. 95(1979):28-32.

10. Mardh, P.A., Helin, I., Bobeck, S., Lavin, J and Nilsson, T. "Coloization of Pregnant and Puerperal Women and Neonates with Chlamydia trachomatis" Br. J. Vener. Dis. 56(1980): 96-100.

11. Chandler, J.W., Alexander, E.R., Pheiffer, T.A., Wang, S.P., Holmes, K.K.and English, M. "Ophthalmia Neonatorum Associated with Maternal Chlamydial Infections" Trans. Am. Acad. Ophtholmol. Otolaryngol. 83(1977):302-308.

12. Heggie, A.D., Lumicoa, C.G., Stuart, L.A. and Gyues,M.T. "Chlamydia trachomatis Infection in Mothers and Infants. A Prospective Study" Am. J. Dis. Child. 135(1981):507-511.

13. Wiesmeier, E., Lovetl, M.A. and Forsythe, A.B. "Chlamydia trachomatis Isolation in a Symptomatic University Student Population " Obstet. Gynecol. 63(1984):81-83.

14. Goh, B.T., Morgan-Capner, P. and Lim, K.S. "Chlamydial Screening of Pregnant Women in a Sexually Transmitted Diseases Clinic" Br. J. Vener. Dis. 58(1982):327-329.

15. Paavonen, J. and Vesterinen, E. "Chlamydia trachomatis in Cervicitis and Urethritis in Women" Scand. J. Infect. Dis. Suppl.32 (1982):45-54.

16. Shafer, M.A., Beck, A., Blain, B., Dole, P., Irwin, C.E., Sweet, R. and Schachter, J. "Chlamydia trachomatis: Important Relationships to Race, Contraceptive, Lower Gennital Tract Infection and Papanicolaou Smear" J. Pediatr. 104(1984): 141-146.

17. Wang, S.P. and Grayston, J.T. "Immunological Relationship Between Genital TRIC, Lymphogranuloma Venereum, and Related Organisms in a New Mitrotiter Indirect Immunofluorescence Test" Am. J. Ophthalmol. 70(1970):367-374.

18. Wang, S.P. and Grayston, J.T. "Human Serology in Chlamydia trachomatis Infection with Microimmunofluorescence" J. Infect. Dis. 130(1974):388-397.

19. Wang, S.P., Grayston, J.T., Alexander, E.R. and Holmes, K.K. "Simplified Microimmunofluorescence Test with Trachoma-Lymphogranuloma Venereum (Chlamydia trachomatis) Antigens for Use as a Screening Test for Antibody" J. Clin. Microbiol. 1(1975):250-255.

20. Treharne,J.D., Darougar, S.and Jones, B.R. "Modification of the Microimmunofluorescence Test to Provide a Routine Serodiagnostic Test for Chlamydial Infection" J. Clin. Pathol. 30(1977):510-517.

21. Treharne, J.D., Darougar, S., Simmons, P.D. and Thin, R.N. "Rapid Diagnosis of Chlamydial Infection of the Cervix " Br. J. Vener. Dis. 54(1978):403-408.

22. Saiku, P. "Chlamydial Serology" Scand. J. Infect. Dis. Suppl.32 (1982):34-37.

23. Terho, P. "Treatment of Chlamydia-Positive and Chlamydia-Negative

Nonspecific and Postgonococcal Urethritis" Ann. Clin. Res. 10(1978):299-302.

24. Halberstaedter, L. and von Provazek, S. "Zur Atiologie des Trachoms" Dtsch. Med. Wochenschr. 33(1907):1285-1287.

25. Lindner, K. "Gonoblennorrhoe, Einschlussblennorrhoe und Trachoma" Albrech. Von. Graefes. Arch. Opthalmol. 78(1911):380.

26. Bedson, S.P., Western, G.T. and Simpson, S.L. "Observations on the Atiology of Psittacosis" Lancet. 1(1930):235-236.

27. Hellerstrom, S. and Wassen, E. Meningo- Encephalitische Veranderungen bei Affen nach Intracerebraler Impfung mit Lymphogranuloma ingunale in Proc. Verh., 8 er., pp. 1147-1151, Internat. Kongr. Dermatol. n Syph., 1930.

28. T'ang, F.F., Chang, H.L., Huang, Y.T. and Wang, K.C. "Studies on the Etiology of Trachoma with Special Reference to Isolation of the Virus in Chick Embryo" Chinese. Med. J. 75(1957): 429-447.

29. JOnes, B.R., Collier, L.H. and Smith, C.H. "Isolation of Virus from Inclusion Blennorrhoeae" Lancet. 1(1959):902-905.

30. Jones, B.R. "Ocular Syndromes of TRIC Virus Infection and their Possible Genital Significance" Br. J. Vener. Dis. 40(1964): 3-15.

31. Schachter, J. Biology of Chlamydia trachomatis in Sexually Transmitted Diseases, (Holmes, K.K., Mardh, P.A., Sparling, P.E. and Wiesner, P.J. ed.) pp.243-257, McGraw-Hill Book Company, U.S.A.,1984.

32. Gordon, F.B. and Quan, A.L. "Isolation of the Trachoma Agent in Cell Culture" Proc. Soc. Exp. Biol. Med. 118(1965):354-359.

33. Matsumoto, A. and Manire, G.P. " Electron Microscopic Observations on the Fine Structure of Cell Walls of Chlamydia psittaci" J. Bacteriol. 104(1970):1332-1337.

34. Schachter, J. and Caldwell, H.D. "Chlamydiae" Ann. Rev. Microbiol. 34(1980):285-309.

35. Bergan, T. "Biology of Chlamydiae" Scand. J. Infect. Dis. Suppl.32 (1982):11-15.

36. Ward, M.E. "Chlamydial Classification, Development and Structure" Br. Med. Bull. 39(1983):109-115.

37. Meyer, K.F. "The Host Spectrum of Psittacosis- Lymphogranuloma venereum (PL) Agents" Am. J. Ophthalmol. 63(1967):1225-1246.

38. Mount, D.T., Bigazzi, P.E. and Barron, A.L. "Infection of Genital Tract and Transmission of Ocular Infection to Newborns by the Agent of Guinea Pig Inclusion Conjunctivitis" Infect. Immun. 6(1972):921-926.

39. Lamont, H.C., Semine, D.Z., Leveille, C. and Nichols, R.L. "Immunity to Vaginal Reinfection in Female Guinea Pig Infected Sexually with Chlamydia of Guinea Pig Inclusion Conjunctivitis" Infect. Immun. 19(1978):807-813.

40. Moulder, J.W. "Intracellular Parasitism: Life in an extreme Environment" J. Infect. Dis. 130(1974):300-306.

41. Manire, G.P. The Chlamydiae in Microbiology (Braude, A.I., Devis, C.E. and Fierrer, J. ed.) pp. 516-522, W.B. Saunders Company, Japan, 1983.

42. Byrne, G.I. "Requirements of Ingestion of Chlamydia psittaci by Mouse Fubroblasts (L Cells)" Infect. Immun. 14(1976):645-651.

43. Friis, R.R. "Interaction of L Cells and Chlamydia psittaci: Entry of the Parasite and Host Responses to Its Development" J. Bacteriol. 110(1972):706-721.

44. Levy, N.J. "Wheat Germ Agglutinin Blockage of Chlamydial Attachment Sites: Antagonism by N-Acetyl-D-Glucosamine" Infect. Immun. 25(1979):946-953.

45. Moulder, J.W. "The Relation of the Psittacosis Group (Chlamydiae) to Bacteria and Viruses" Ann. Rev. Microbiol. 20(1966): 107-130.

46. Richmond, S.J. and Stirling, P."Localization of Chlamydial Group Antigen in McCoy Cell Monolayers Infected with Chlamydia trachomatis or Chlamydia psittaci" Infect. Immun. 34(1981): 561-570.

47. Hatch, T.P. "Competition Between Chlamydia psittaci and L Cells for Host Isoleucine Pools: a Limiting Factor in Chlamydial Multiplication" Infect. Immun. 12(1975):211-220.

48. Horoschak, K.D. and Moulder, J.W. "Division of Single Host Cells after Infection with Chlamydiae" Infect. Immun. 19(1978): 281-286.

49. Kondo, L., Hanna, L. and Keshishyan, H. "Reduction in Chlamydial Infectivity by Lysozyme (36974)" Proc. Soc. Exp. Biol. Med. 142(1973):131-132.

50. Garett, A.J., Harrison, M.J. and Manire, G.P. "A Search for the Bacterial Mucopeptide Component, Muramic Acid in Chlamydia" J. Gen. Microbiol. 80(1974):315-318.

51. Thompson, S.E. and Washinton, A.E. "Epidemiology of Sexually Transmitted Chlamydia trachomatis Infections" Epidemiol.

Rev. 5(1983) : 96-123.

52. Scola, L.J., Paroski, J.S., Burzynski, L and Faden, H.S. "Chlamydia trachomatis Infection in Intants Delivered by Cesarean Section" Clin. Pediatr. 23(1984):118-120.

53. Harrison, H.R. and Alexander, E.R. Chlamydia trachomatis Infections of the Infant in Sexually Transmitted Diseases (Holmes, K.K., Mardh, P.A., Sparling, P.F. and Wiessner, P.J. ed.) pp.270-280, McGraw-Hill Book Company, U.S.A., 1984.

54. Schachter, J., Lum, L., Gooding, C.A. and Ostler, B. "Pneumonitis Following Inclusion Blennorrhoeae" J. Pediatr. 87(1975): 779-780.

55. Beem, M.O., Saxon, E.M. and Tipple, M. "Treatment of Chlamydial Pneumonia in Infancy" Pediatrics : 63(1979): 198-203.

56. Harrison, H.R., Alexander, E.R. Chiang, W.T., Giddens, W.E. Jr., Boyce, J.T., Benjamin, D. and Gale, J.L. "Experimental Nasopharyngitis and Pneumonia Caused by Chlamydia trachomatis in Infants Baboons. Histopathologic Comparison with a Case in a Human Infant" J. Infect. Dis. 139(1979): 141-146.

57. Ridway, G.L., Owen, J.M. and Oriel, J.D. "A Method for Testing the Antibiotic Susceptibility of Chlamydia trachomatis in a Cell Culture System" J. Antimicrob. Chemother. 2(1976): 71-76.

58. Blackman, H.J., Yoneda, C., Dawson, C.R. and Schachter, J. "Antibiotic Susceptibility of Chlamydia trachomatis" Antimicrob. Agents. Chemother. 12(1977): 673-677.

59. Ridway, G.L. and Oriel, J.D. "Activity of Antimicrobials Against Chlamydia trachomatis In Vitro" J. Antimicrob. Chemother. 5(1979): 483-484.

60. Ridway, G.L., Owen, J.M. and Oriel, J.D." The Antimicrobial Susceptibility of Chlamydia trachomatis in Cell Culture" Br. J. Vener. Dis. 54(1978): 103-106.

61. Kuo, C.C., Wang, S.P. and Grayston, J.T. "Antimicrobial Activity of Several Antibiotics and a Sulfonamide Against Chlamydia trachomatis Organisms in Cell Culture" Antimicrob. Agents. Chemother. 12(1977): 80-83.

62. Smith, T.F. and Washton, H.E. "In Vitro Susceptibility of 30 Strains of Chlamydia trachomatis to Rosamicin" Antimicrob. Agents. Chemother. 14(1978): 493-494.

63. Mourad, A., Sweet, R.L., Sugg, N. and Schachter, J. "Relative Resistance to Erythromycin in Chlamydia trachomatis" Antimicrob. Agents. Chemother. 18(1980): 696-698.

64. Bowie, W.R. "In Vitro Activity of Rosaramicin Against Chlamydia trachomatis" Antimicrob. Agents. Chemother. 18(1980): 978-979.

65. Johannisson, G., Sernryd, A. and Lycke, E. "Susceptibility of Chlamydia trachomatis to antibiotic In Vitro and In Vivo" Sexual. Trans. Dis. 6(1979): 50-57.

66. Bowie, W.R., Alexander, E.R., Floyd, J.F., Holmes, J., Miller, Y. and Holmes, K.K. "Differential Response of Chlamydial and Ureplasma-Associated Urethritis to Sulfafurazole (Sulfisoxazole) and Aminocyclitols" Lancet 2(1976): 1276-1278.

67. Coufalik, E.D., Taylor-Robinson, D. and Csonka, G.W. "Treatment of Nongonococcal Urethritis with Rifampicin as a Means of Defining the Role of Ureaplasma urealyticum"Br. J. Vener. Dis. 55(1979): 36-43.

68. Harrison, H.R., Alexander, E.R. Weinstein, L., Lewis, M., Nash, M. and Sim, D.A. "Cervical Chlamydia trachomatis and Mycoplasmal Infections in Pregnancy. Epidemiology and Outcomes" JAMA 250(1983): 1721-1727.

69. Taylor-Robinson, D. and McCormack, W.M. "The Genital Mycoplasma" N. Eng. J. Med. 302(1980): 1003-1010.

70. Oriel, J.D., Reeve, P., Thomas, B.J. and Nicol, C.S. "Infection with Chlamydia Group A in Men with Urethritis Due to Neisseria gonorrhoeae" J. Infect. Dis. 131(1975): 376-382.

71. Juvakoski, T., Allgulander, C. and Lassus, A. "Rosaramicin and Tetracycline Treatment in Chlamydia trachomatis-Positive and Negative Nongonococcal Urethritis" Sex. Trans. Dis. 8(1981): 12-15.

72. Paavonen, J., Kousa, M., Saikku, P., Vartiainen, E., Kanerva, L. and Lassus, A. "Treatment of Nongonococcal Urethritis with Trimethoprim-Sulphadiazine and with Placebo. A Double Blind Partner-Controlled Study" Br. J. Vener. Dis. 56 (1980):101-104.

73. Darne, J.F., Ridgway, G.L. and Oriel, J.D. "Rosaramicin and Tetracycline in the Treatment of Nongonococcal Urethritis. A Comparision of Clinical and Microbiological Results" Br. J. Vener. Dis. 58(1982):117-120.

74. Bowie, W.R., Alexander, E.R. and Holmes, K.K. "Eradication of Chlamydia trahcomatis from the Urethras of Men with Nongonococcal Urethritis by Treatment wiyh Amoxicillin" Sex. Trans. Dis. 8(1981):79-81.

75. Bowie, W.R., Manzon, L.M., Borrie-Hume, C.I., Fawcett, A. and Jones, H.D. "Efficacy of Treatment Regimens for Lower Urogenital Chlamydia trachomatis Infection in Women" Am. J. Obstet. Gynecol. 142(1982):125-129.

76. Waugh, M.A. and Nayyar, K.C. "Triple Tetracycline (Deteclo) in the Treatment of Chlamydial Infection of the Female Genital Tract" Br.J. Vener. Dis. 53(1977):96-97.

77. Ripa, K.T., Svensson, L., Mardh, P.A. and Westrom, L. "Chlamydia trachomatis Cervicitis in Gynecologic Patients" Obstet. Gynecol. 52(1978):698-702.

78. Heessen, F.W.A. and Muytjens, H.L. "In Vitro Activities of Ciprofloxacin, Norfloxacin, Pipemidic Acid, Cinoxacin and Nalidixic Acid Against Chlamydia trachomatis" Antimicrob. Agents. Chemother. 25(1984):123-124.

79. Muytjens, H.L., van der Ros-van der Repe, J. and van Veldhuizen, G. "Comparative Activities of Ciprofloxacin (Bay 09867), Norfloxacin, Pipemidic Acid and Nalidixic Acid" Antimicrob. Agents. Chemother. 24(1983):302-304.

80. Khan, M.Y., Siddiqui, Y. and Gruninger, R.P. "Comparptive In Vitro Activity of MK-0366 and Other Selected Oral Antimicrobial Agents Against Neisseria gonorrhoeae" Antimicrob. Agents. Chemother. 20(1981):265-266.

81. Wise, R., Andrews, J.M. and Edwards, L.J. " In Vitro Activity of Bay 09867, A New Quinoline Derivative, Compared with Those of Other Antimicrobial Agents" Antimicrob. Agents. Chemother. 23(1983):559-564.

82. Bailey, J.M.G., Heppleston, C. and Richmond, S.J. "Comparison of the In VItro Activities of Ofloxacin and Tetracycline Against Chlamydia trachomatis as Assessed by Indirect Immunofluorescence" Antimicrob. Agents. Chemother. 26 (1984):13-16.

83. Osada, Y. and Ogawa, H. "Antimycoplasmal Activity of Ofloxacin (DL-82800)" Antimicrob. Agents. Chemother. 23(1983): 509-511.

84. Dobson, R.A., O' Conner, J.R., Poulin, S.A., Kundsin, R.B., Smith, T.F. and Came, P.E. "In Vitro Antimicrobial Activity of Rosoxacin Against Neisseria gonorrhoeae, Chlamydia trachomatis and Ureaplasma urealyticum" Antimicrob. Agents. Chemother. 18(1980):738-740.

85. Schachter, J. Chlamydiae (Psittacosis-Lymphogranuloma Venereum-Trachoma Group), in Manual of Clinical Microbiology, 3rd ed., (Lenette, E.H., Balows, A., Hausler, W.J. and Truant, J.P. ed.) pp.357-364, American Society for Microbiology, Washington, D.C. 1980.

86. Evans, R.T. and Woodland, R.M. "Detection of Chlamydiae by Isolation and Direct Examination" Br. Med. Bul. 39 (1983):181-186.

87. Mardh, P.A. Bacteria, Chlamydiae and Mycoplasma in Sexually Transmitted Disease (Holmes, K.K., Mardh, P.A., Sparling, P.F. and Wiesner, P.J. ed.) pp. 829-856, McGraw-Hill Book Company, U.S.A., 1984.

88. Mardh, P.A., Westrom, L., Collen, S. and Wolner-Hanssen, P. "Sampling, Specimen Handling, and Isolation Techniques in the Diagnosis of Chlamydial and Other Genital Infections" Sex. Trans. Dis. 8(1981):280-285.

89. Dunlop, E.M.C., Vanghan-Jackson, J.D. and Darougar, S. "Chlamydial Infection: Improved Methods for Collection of Material for Culture from the Urogenital Tract and Rectum" Br. J. Vener. Dis. 48(1972):421-424.

90. Mardh, P.A. and Zeeberg, B. "The Toxic Effect of Sampling Swabs and Transportation Tubes on the Formation of Intracytoplasmic Inclusion of Chlamydia trachomatis in Tissue Cell Cultures" Br. J. Vener. Dis. 57(1981):268-272.

91. Kallings, I. and Mardh, P.A. "Sampling and Specimen Handling in the Diagnosis of Genital Chlamydia trachomatis Infections" Scand. J. Infect. Dis. Suppl.32(1982):21-24.

92. Gordon, F.B., Harper, I.A., Quan, A.L., Treharne, J.D., Dwyer, R. St. C. and Garland, J.A. "Detection of Chlamydia (Bedsonia) in Certain Infections of Man: I. Laboratory Procedures: Comparison of Yolk Sac and Cell Culture for Detection and Isolation" J. Infect. Dis. 120(1969):

451-462.

93. Reeve, P., Owen, J. and Oriel, J.D. "Laboratory Procedures for the Isolation of Chlamydia trachomatis from the Human Genital Tract" J. Clin. Pathol. 28(1975):910-914.

94. Ripa, K.T. "Biological Principles of the Culture of Chlamydia trachomatis in Cell Monolayers" Scand. J. Infect. Dis. Suppl. 32(1982):25-29.

95. Gordon, F.B., Dessler, H.R., Quan, A.L. , McQuilkin, W.T. and Thomas, J.I. "Effect of Ionizing Irradiation on Susceptibility of McCoy Cell Culture to Chlamydia trachomatis". Appl. Microbiol. 23(1972):123-129.

96. Gordon, F.B., Magruder, G.B., Quan, A.L. and Arm, H.G. "Cell Cultures for Detection of Trachoma Virus from Experimental Simian Infections" Proc. Soc. Exp. Biol. Med. 112(1963): 236-241.

97. Scherer, W.F., Syverton, J.T. and Gey, G.O. "Studies on the Propagation In Vitro of Poliomyelitis Virus. IV. Viral Multiplication in a Stable Strain of Human Malignant Epithelial Cells (Strain Hela) Derived from an Epidermoid Carcinoma of the Cervix" J. Exp. Med. 97(1953):695-709.

98. Jenkin, H.M. "The Continuous Passage of Agents of Trachoma in Cell Culture. I. Characteristics of TWB and Bour Strains of Trachoma Cultivated in Serial Passage in Hela 229 Cells" J. Infect. Dis. 116(1966):390-399.

99. Blythe, W.A. and Taverne, J. "Cultivation of TRIC Agents: A Comparison Between the Use of BHK-21 and Irradiated McCoy

Cells" J. Hyg. (Cambridge) 72(1974):121-128.

100. Rota, T.R. and Nicols, R.L. "Chlamydia trachomatis in Cell Culture. I. Comparison of the Efficacies of Infection in several Chemically Defined Media, at Various pH and Temperature Values and after Exposure to Diethylamino-Ethyl-Dextran" Appl. Microbiol. 26(1973):560-565.

101. Johnson, F.W.A. and Hobson, D. "Facters Affecting the Sensitivity of Replicating McCoy Cells in the Isolation and Growth of Chlamydia A (TRIC Agents)" J. Hyg. (Cambridge) 76 (1976):441-451.

102. Wentworth, B.B. and Alexander, E.R. "Isolation of Chlamydia trachomatis in Cycloheximide Treated McCoy Cells" J. Clin. Microbiol. 6(1977):328-331.

103. Sompolinsky, D. and Richmond, S. :Growth of Chlamydia trachomatis in McCoy Cells Treated with Cytochalasin B" Appl. Microbiol. 28(1974):912-914.

104. Ripa, K.T. and Mardh, P.A. "Cultivation of Chlamydia trachomatis in Cycloheximide Treated McCoy Cells" J. Clin. Microbiol. 6(1977):328-331.

105. Evans, R.T. and Taylor-Robinson, D. "Comparison of Various McCoy Cell Treatment Procedures Used for Detection of Chlamydia trachomatis" J. Clin. Microbiol. 10(1977):198-201.

106. La Scolea, L.J.Jr. and Keddell, J.E. "Efficacy of Various Cell Culture Procedures for Detection of Chlamydia trachomatis and Applicability to Diagnosis of Pediatric Infections" J. Clin. Microbiol. 13(1981):705-708.

107. Karayiannis, P., Hobson,d. and Lee, N. "Effect of Cycloheximide on the Infective Yield of a Genital Strain of Chlamydia trachomatis in McCoy Cells" Infect. Immun. 33(1981):309-311.

108. Kuo, C.C., Wang, S.P., Wenworth, B.B. and Grayston, J.T. "Primary Isolation of TRIC Organism in Hela 229 CellS Traeted with DEAE-Dextran" J. Infect. Dis. 125(1972):665-668.

109. Darougar, S., Cubitt, S. and Jones, B.R. "Effect of High-Speed Centrifugation on the Sensitivity of Irradiated McCoy Cell Culture for the Isolation of Chlamydia" Br. J. Vener. Dis. 50(1974):308-312.

110. Thomas, B.J., Evans, R.T., Hutchinson, G.R. and Taylor-Robinson, D. "Early Detection of Chlamydia Inclusions Combining the use of Cycloheximide Treated McCoy Cells and Immunofluorescence Staining" J. Clin. Microbiol. 6(1977):285-292.

111. Schachter, J. and Dawson, C.R. Comparative Efficacy of Various Diagnostic Methods for Chlamydial Infection in Nongonococcal Urethritis and Related Infections (Holmes, K.K. and Hobson, D. ed.) pp. 337-341, American Society for Microbiology, Washington, D.C., 1977.

112. Hanna, L., Okumoto, M., Thygeson, P., Rose, L. and Dawson, C.R. "TRIC Agents Isolated in the United States. X. Immunofluorescence in the Diagnosis of TRIC Agent Infection in Man" Proc. Soc. Exp. Biol. Med. 119(1965):722-728.

113. Sowa, J., Collier, L.H. and Sowa, S. "A Comparison of the Iodine and Fluorescent Antibody Methods for Staining Trachoma Inclusions in the Conjunctiva" J. Hyg. (Cambridge)

69(1971):693-708.

114. Yoneda, C., Dawson, C.R., Dagtous, T., Hoshiwara, I., Jones, P., Messadi, M. and Schadhter, J. "Cytology as a Guide to the Presence of Chlamydial Inclusions in Giemsa Stained Conjunctival Smears in Severe Endemic Trachoma" Br. J. Ophthalmol. 59(1975):116-124.

115. Thatcher, R.W., Darougar, S. and Jones, B.R. "Conjunctival Impression Cytology" Arch. Ophtholmol. 95(1977):678-681.

116. Treharne, J.D., Forsey, T. and Thomas, B.J. "Chlamydial Serology" Br. Med. Bull. 39(1983):194-200.

117. Bedson, S.P. "Use of Complement Fixation Reaction in Diagnosis of Human Psittacosis" Lancet.2(1935):1277-1280.

118. Tarizzo, M.L., Nabli, B. and Laborne, J. "Studies on Trachoma: 11 Evaluation of Laboratory Diagnostic Methods under Field Conditions" Bull. Wld. Hlth. Org. 38(1968):897-905.

119.Dhir, S.P., Hakomori, S., Kenny, G.E. and Grayston, J.T. "Immunochemical Studies on Chlamydial Group Antigen (Presence of a 2-Keto-3-Deoxycarbohydrate as Immunodominant Group)" J. Immunol. 109(1972):116-122.

120. Schachter, J. Chlamydiae in Manual of Clinical Immunology, 2nd ed. (Rose, N.R. and Friedman, H. ed.) pp. 700-706, American Society for Microbiology, Washington, D.C.,1980.

121. Caldwell, H.D., Kromhout, J. and Schachter, J. "Purification and Partial Characterization of the Major Outer Membrane P Protein of Chlamydia trachomatis" Infect. Immun.31(1981):

1161-1176.

122. Hanna, L., Jawetz, E., Nabli, B., Hoshiwara, I., Ostler, B. and Dawson, C. "Titration and Typing of Serum Antibodies in TRIC Infections by Immunofluorescence" J. Immun. 108 (1972):102-107.

123. Svensson, L., Westrom, L., Ripa, K.T. and Mardh, P.A. "Difference in Some Clinical and Laboratory Parameter in Acute Salpingitis Related to Culture and Serological Findings" Am. J. Obstet. Gynecol. 138(1980):1017-1021.

124. Been, M.O. and Saxon, E.M. "Respiratory-Tract Colonization and a Distinctive Pneumonia Syndrome in Infants Infected with Chlamydia trachomatis" N. Eng. J. Med. 296(1977):306-310.

125. Smith, T.F., Weed, L.A., Segura, J.W., Pettersen, G.R. and Washinton, J.A. "Isolation of Chlamydia from Patients with Urethritis" Mayo. Clin. Proc. 50(1975):105-110.

126. Washington II, J.A. and Sutter, V.L. Dilution Susceptibility Test: Agar and Micro-Broth Dilution Procedures in Manaul of Clinical Microbiology 3rd ed. (Lennette, E.H., Balows, A., Hausler, W.J. and Truant, J.P. ed.) pp. 253-258, American Society for Microbiology, Washington,D.C., 1980.

127. Martin, D.H., Koutsky, L., Eschengbach, D.A., Daling, J.R., Alexander, E.R., Beneditti, J.K. and Holmes, K.K. "Prematurity and Perinatal Mortality in Pregnancies Complicated by Maternal Chlamydia trachomatis Infections" JAMA. 247(1982):1585-1588.

128. Harrison, H.R., Boyce, W.T., Haffner, W.H.T., Crowley, B. Weinstein., L., Lewis, M. and Alexander, E.R. "The

Prevalance of Genital Chlamydia trachomatis and Mycoplasmal Infections during Pregnancy in an American Indian Population " Sex. Trans. Dis. 10(1983): 184-186.

129. Harrison, H. R., Alexander, E.R., Weinstein, L.,Lewis, M., Nash, M. and Sim, D.A. "Cervical Chlamydia trachomatis and Mycoplasmal Infections in Pregnancy" JAMA. 250 (1983): 1721-1727.

130. Jones, R.N.,Barry,A.L. and Thornsberry,C. "In Vitro Evaluation of Three New Macrolide Antimicrobial Agents, RU 28965, and RU 29065, and Comparison with Other Orally Administered Drugs" Antimicrob. Agents. Chemother. 24(1983): 209-215.

131. Barlam, T. and Neu,H.C."In Vitro Comparison of the Activity of the Activity of RU28965, A New Macrolide with that of Erythromycin Against Aerobic and Anaerobic Bacteria" Antimicrob. Agents. Chemother. 25(1984): 529-531.

APPENDIX I

ANTIBIOTICS PREPARATION

Amphotericin B 0.25 mg/ml

1. Dissolve amphotericin B 50 mg in 200 ml sterile double distilled water with aseptic technique.

2. Devide each 4 ml and 0.8 ml of amphotericin B by aseptic technique into sterile test tube.

They were used to inhibit fungi in transport medium, maintenance medium and cell culture medium.

Cycloheximide 0.1 mg/ml

1. Dissolve cycloheximide 0.01 g in 100 ml double distilled water.

2. Sterile by filter through 0.22 micron millipore filter paper.

3. Devide each 4 ml of cycloheximide by aseptic technique into sterile test tube.

Store at -20°C.

It was used to inhibit growth of McCoy cells in maintenance medium.

Gentamicin 0.05 mg/ml

1. Dilute 2 ml of 80 mg gentamicin in 160 ml sterile double distilled water.

2. Devide each 2 ml of gentamicin by aseptic technique into steril test tube.

Store at -20°C.

It was used to inhibit Gram negative bacteria in cell culture medium, maintenance medium and transport medium.

Vancomycin 5 mg/ml

1. Dissolve vancomycin 500 mg in 100 ml sterile double distilled water with aseptic technique.

2. Devide eacg 4 ml of vancomycin by aseptic technique into sterile test tube.

Store at -20°C.

It was used to inhibit Gram positive bacteria in cell culture medium, maintenance medium and transport medium.

APPENDIX II

BUFFER

Phosphate Buffer Saline (PBS), pH 7.2

NaCl	10.0	g
KCl	0.25	g
$NaHPO_4$ $2H_2O$	1.78	g
KH_2PO_4	0.25	g
DDW	1000	ml

Dissolve these reagents, adjust pH to 7.2 and autoclave at 121°C for 15 min. Store at 4°C.

PBS pH 7.2 was used for washing the McCoy cells culture.

Phosphate Buffer Saline (PBS), pH 7.2

NaCl	8.0	g
KCl	0.2	g
Na_2HPO_4	1.15	g
KH_2PO_4	0.196	g
DDW	1000	ml

Final pH 7.2

Store at 4°C

This PBS was used to dilute serum and wash slides when micro-immunofluorescent test for antichlamydial antibody was performed.

Tris Buffer, pH 8

Tris	1.2114	g
EDTA	0.2922	g
NaCl	5.544	g
DDW	1000	ml

Final pH 8.0

Store at 4°C

Tris buffer pH 8.0 was used to mix together with glycerine volume by volume for mounting slide in micro-immunofluorescent test.

APPENDIX III

MEDIA AND REAGENTS

Cell Culture Medium

RPMI 1640	200	ml
Fetal Bovine Serum (heat inactivate)	20	ml
Vancomycin 5 mg/ml	4	ml
Gentamicin 0.5 mg/ml	2	ml
Amphotericin B 0.25 mg/ml	0.8	ml

Final pH 7.4

Store at 4°C

Cell cuture medium was used to grow the McCoy cells. When antibiotic susceptibility was tested, the medium should not have any antibiotics.

Cell Maintenance Medium

RPMI 1640	200	ml
Fetal Bovine Serum (heat inactivated)	10	ml
Glucose (0.11 g/ml)	10	ml
Vancomycin 5 mg/ml	4	ml
Gentamicin 0.5 mg/ml	2	ml
Amphotericin B 0.25 mg/ml	0.8	ml
Cycloheximide 0.1 mg/ml	4	ml

Final pH 7.4

Store at 4°C

This medium was used for culturing of C. trachomatis. When C. trachomatis were propagated, the medium should not have cycloheximide

since it was noted that cycloheximide was toxic to C. trachomatis in McCoy cells (107).

If antibiotic susceptibility was tested, the medium should not have any antibiotics except cycloheximide and antibiotic to be tested.

Glucose 0.11 g/ml

Dissolve glucose 10.76 g in 100 ml RPMI 1640 medium, sterile by filter through a millipore filter paper diameter 0.22 µm. Devide each 5 ml of glucose by aseptic technique into sterile test tube. Store at -20°C.

It was used as a supplement for cell maintenance medium.

RPMI 1640 Medium

RPMI 1640 powder	10,38 g
DDW	1000 ml

Suspend RPMI 1640 powder in double distilled water and sterile by filter through 0.22 µm millipore filter paper. Store at 4°C.

RPMI 1640 was used to prepare cell culture medium and cell maintenance medium.

2 SP Transport Medium

A. Preparation of 0.2 M Sucrose Phosphate Buffer (2 SP) (130)

1. Solution A : 68.46 g of sucrose in DDW

   Solution B : 2.088 g of anhydrous K_2HPO_4 in 60 ml DDW

   Solution C : 1.088 g of anhydrous KH_2PO_4 in 40 ml DDW

2. Combine solution A, B and C; bring to close up 1000 ml with DDW

3. Adjust pH to 7.0

4. Bring the volume to 1000 ml with DDW

5. Autoclave at 115°C 15 min

Store at 4°C

B. Composition of 2 SP Transport Medium

2 SP (from A)	200	ml
Fetal Bovine Serum	20	ml
Vancomycin 5 mg/ml	4	ml
Gentamicin 0.5 mg/ml	4	ml
Amphotericin B 0.25 mg/ml	4	ml

Dispense the 2 SP transport medium into sterile centrifuge plastic tube, approximately 1 ml per tube. Store at -20°C.

This transport medium was used for collection and shipment of chlamydiae to laboratory for culturing.

4 SP Medium

1. Solution A : 136.92 g of sucrose in 600 ml DDW

Solution B : 2.268 g of Na_2HPO_4 in 200 ml DDW

2. Combine solution A and solution B

3. Add 2.0 ml of 0.5% phenol red

4. Bring the volume close up 1000 ml, adjust pH to 7.0

5. Bring the volume to 1000 ml with DDW

6. Sterile by autoclaving at 115°C, for 15 min

Store at 4°C.

4 SP medium (0.4 M Sucrose phosphate buffer) was used to collect propagated C. trachomatis by adding equal volume of 4 SP into cell maintenance medium which had propagated C. trachomatis.

1% Trypsin

Trypsin	1	g
DDW	100	ml

Suspend trypsin in double distilled water and sterile by filter through 0.22 µm millipore filter paper. Store at 4°C.

1% trypsin was used for digesting McCoy cells.

APPENDIX IV

STAIN

Jones' Iodine (5% Iodine Solution)

KI	5	g
I_2	5	g
Methanol or absolute ethanol	50	ml
DDW	50	ml

Mix well and filter before use with Whatman filter paper No.1, store at room temperature in a bottle protected from light.

Jones' Iodine was used to stain C. trachomatis in McCoy cells.

Jones' Iodine-Glycerine

Mix Jones' Iodine and glycerine equal volume together. Store at room temperature in a stoppered bottle protected from light.

Jones' Iodine-Glycerine was used to mount the slide and cover-slip stained with Jones' Iodine.

Methanol-Formalin

Formalin or formaldehyde 37%	100	ml
Methanol or Absolute ethanol	900	ml

Mix them together, store at roomtemperature.

Methanol-Formalin was used to fix the McCoy cells to the cover-slip in iodine staining technique for detection of C. trachomatis.

APPENDIX V

CHLAMYDIAL ANTIGEN

Preparation of Chlamydial Antigen from Yolk Sac Grown Organisms for Microimmunofluorescent Test (87)

1. Inject 0.5 ml of a dilution of highly infectious sample of Chlamydia into the yolk sac of 6- to 8-day-old embryonated hen's eggs. Incubate the eggs at 37°C. Examine the eggs daily under a candle light. Eggs that die within 72 hr are rejected.

2. When half of the eggs have died, the yolk sacs of the surviving eggs are harvested.

3. A piece (1 to 2 mm^2) of the yolk sac, close to the umbilicus, is removed, crushed with tweezers, and smeared on a glass slide. The slide is fixed by heat and stained by the Macchiavello or Giemsa method. The slide is read under the oil-immersion lens of the dark-field microscope (x1000). The elementary bodies (EB) are seen as cherry-red particles (yellow-green with Giemsa) on the background of yolk-sac tissue, which stain blue.

4. Yolk sacs from eggs producing numerous EB are selected for antigen production. Egg yolk material should be removed as much as possible. The infected yolk sacs are kept frozen at -80°C until use.

5. Weight the yolk sac from which antigen is to be prepared. A 40% (w/v) suspension of the yolk sac in phosphate buffer saline (PBS) pH 7.2, is prepared.

6. The yolk sac is ground either by a mortar and pestle, by a Tenbroeck tissue grinder, or by shaking with glass beads, and 0.01 M

PBS is added to make a 40% (w/v) yolk-sac suspension.

7. Centrifuge the suspension at 900 g for 15 min at 4°C.

8. Avoid the surface layer of fat and the bottom layer of gross debris, but collect the in-between layer suspension and centrifuge again at 900 g for 15 min.

9. The suspension devoid of fat and gross debris is then transferred to a 40 ml centrifuge tube, and PBS is added to fill the tube.

10. Mix and centrifuge at 20,000g at 4°C for 45 min.

11. Collect the pellet, which consists of relatively pure EBs. PBS is added to make a 40% suspension.

12. Centrifuge the suspension at 900 g for 10 to 15 min to remove any gross debris.

ADDITION

Treatment of Infected Pregnant Women with Talsutin

15 pregnant women whose chlamydial culture positive in the third trimester were further study for treatment. They were treated with talsutin (tetracycline plus amphotericin B) vaginally for 10 days. It was the first study that local tetracycline treatment to chlamydial pregnant women were tested. During delivery or 4 days after the complete treatment, the endocervical specimens were repeated.

Two of which were culture positive. One of treatment failure was delivered by cesarean section, so her infant did not have chlamydial infection. The other stopped using medicine after 3 or 4 days, since she considered herself no symptoms, however, after delivery conjunctivitis was developed and proved by positive chlamydial culture.

Infants Observation

In this study, infants delivered either by infected or non-infected women were observed. Two infants developed inclusion conjunctivitis. One was discussed before that her mother stopped using medicine; the other infant delivered from the women with chla chlamydial culture positive and have no treatment.

None of the babies delivered from chlamydial culture negative women developed inclusion conjunctivitis. Therefore, from this result and Table 9, routine culture for C. trachomatis in the third trimester was important and treatment of chlamydial culture positive women before delivery was necessary.

BIOGRAPHY

Miss Suwanna Boonrumlucktanom was born on August 8, 1956 in Bangkok, Thailand. She graduated with the Bechelor degree of Science, in Microbiology from the Faclty of Science, Chulalongkorn University in 1980.

She had experienced in The Research Center, Ramathibodhi Hospital(1981-1983).