



REFERENCES

1. Levin, J., "The history of the development of the limulus amoebocyte lysate test", Bacterial endotoxin: Structure, Biomedical significant, and detection with the limulus amoebocyte lysate test, pp. 3-28, Alan R. Liss, 1985.
2. Levin, J. and F. B. Bang, "Clottable protein in limulus: its localization and kinetics of its coagulation by endotoxin", Thromb. Diath. Hemorrhag., 19, 186-194, 1968.
3. Jorgensen, J. H.; R. F. Smith, "Preparation, sensitivity and specificity of limulus lysate for endotoxin assay", Appl. Microbiol., 26(1), 43-48, 1973.
4. Piotrowicz, B. I. and A. C. McCartney, "Chromogenic limulus amoebocyte lysate assay for endotoxin: comparison of three commercial products", Med. Lab. Sci., 44, 89-91, 1987.
5. Klein, K. B.; et al, "Detection of endotoxin", Lancet, 2, 50, 1974.
6. Usawattanakul, W.; et al, "Tachypleus lysate test for endotoxin in patients with gram negative bacterial infections", Southeast Asian J. Trop. Med. Pub. Hlth., 10(1), 13-17, 1979.
7. Reinhold, R. B. and J. Fine, "A technique for quantitative measurement of endotoxin in human plasma", Proc. Soc. Exp. Biol. Med., 137, 334-340, 1971.

8. Pearson, A. D., et al, "Assay of endotoxin", Lancet, 26 May, 1194-1195, 1973.
9. "Microbial pyrogen", Pyrogens: endotoxin, LAL testing and depyrogenation, edited by F.C. Pearson, pp.11-17, Mercel Dekker, Inc, 1985.
10. Kanoh, S.; Y. Ogsawa; T. Komuro and H. Kawasaki, "Preparation and properties of a reference endotoxin (LPS) for limulus and pyrogen test", Symposium on Toxins, 35, 112-113, 1982.
11. Galanos, C.; et al., "Chemical, physical and biological properties of bacterial lipopolysaccharides", Biomedical Applications of the Horseshoe Crab (Limulidae), edited by E. Cohen, pp. 319, Alan R. Liss, New York, 1979.
12. Tanamoto, K.; et al., "Biological activities of synthetic lipid A analogs: pyrogenicity, lethal toxicity, anticomplement activity, and induction of gelation of limulus amoebocyte lysate", Infect. Immun., 44(2), 421-426, 1984.
13. Mascoli, C. C. and M. E. Weary, "Limulus amebocyte lysate (LAL) test for detecting pyrogens in parenteral injectable products and medical devices: Advantages to manufacturers and regulatory officials", J. Parenter. Drug Assoc., 33, 81 (1979).
14. Pearson, F. C. and M. Weary, "The significance of Limulus

- amebocyte lysate test specificity on the pyrogen evaluation of parenteral drugs", J. Parenter. Drug Assoc., 34, 103, 1980.
15. Rietsche, E. T.; Y. B. Kim; D. W. Watson; C. Galanos; O. Luderitz; and O. Westphal, "Pyrogenicity and immunogenicity of lipid A complexed with bovine serum albumin or human serum albumin", Infect. Immun., 8, 173 (1973).
16. Weary, M., "The Rabbit Pyrogen Test", Pyrogens: Endotoxins, LAL Testing and Depyrogenation, Pearson, F. C., Marcel Dekker, Inc., p.104-117, 1985.
17. "LAL Assay", Pyrogens: Endotoxins, LAL Testing, and Depyrogenation, Pearson, F.C., Marcel Dekker, Inc., p.119-142, 1985.
18. Nandan R. and D. R. Brown, "An improved in vitro pyrogen test: To detect picograms of endotoxin contamination in intravenous fluids using Limulus amoebocyte lysate", J. Lab. Clin. Med., 89, 910, 1977.
19. Obayashi, T.; et al, "A new chromogenic endotoxin-specific assay using recombined limulus coagulation enzymes and its clinical applications", Clinica. Chimica. Acta, 149, 55-65, 1985.
20. Maitra, S. K.; M. C. Schotz; T. T. Yoshikawa and L. B. Guze, "Determination of Lipid A and endotoxin in serum by mass

spectroscopy", Proc. Natl. Acad. Sci. USA., 75, 3993, 1978.

21. Kimura, H., "Measurement of endotoxin. II. Comparison of reactivities measured by radioimmunoassay and with the *Limulus test*", Acta Med. Okayama, 30, 257, 1976.
22. Stevens P.; S. Alam; L. S. Young; and K. Chesebro, "Sensitive measurement of endotoxin by radio-rocket immuno electrophoresis using [¹²⁵I]*Staphylococcus aureus* protein A", J. Immunol. Methods, 43, 199, 1981.
23. Fink, P. C. and C. Galanos, "Determination of anti-lipid A by enzyme immunoassay", Immunobiology, 158, 380, 1981.
24. U. S. Pharmacopeial Convention Inc., "Bacterial endotoxin test". U. S. Pharmacopeia, XXI edition, Mack Printing Co., Easton, Pennsylvania, pp. 1165-1167, 1985.
25. Sheagren, J. N. and S. M. Wolff, "Demonstration of endogenous pyrogen in afebrile rabbits", Nature, 210, 539, 1966.
26. Sinchaiphanid, J., "Biology of Horseshoe crabs" (in Thai), Thai. Fish. Gaz., 38(2), 85-92, 1985.
27. "Class merostomata", Invertebrate Zoology, vol. II, A. Kaestner, Interscience Publishing, pp. 69-82, 1968.
28. Tomasulo, P. A.; et al, "Biological activities of tritiated endotoxins: correlation of the limulus lysate assay with rabbit pyrogen and complement-activation assays for

- endotoxin", J. Lab. Clin. Med., 89(2), 308-315, 1977.
29. "Endotoxin Standards", Pyrogens: Endotoxins, LAL Testing, and Depyrogenation, Pearson, F. C., Marcel Dekker Inc., p. 89-101, 1985.
30. Dabbah, R.; et al., "Pyrogenicity of *E. coli* 055:B5 endotoxin by the USP rabbit test: HIMA collaborative study. J. Parenter. Drug Assoc., 34, 212, 1980.
31. Tsuji, K.; K. A. Steindler, and S. J. Harrison, "Limulus amoebocyte lysate assay for detection and quantitation of endotoxin in a small-volume parenteral product", Appl. Environ. Microbiol., 40, 533, 1980.
32. Keene, W. R.; H. R. Siberian and M. Landy, "Observation on the pyrogenic response and its applications to the bioassay of endotoxin", J. Clin. Invest., 40, 295, 1961.
33. Greisman, S. E. and R. B. Hornick, "Comparative pyrogenic reactivity of rabbit and man to bacterial endotoxin", Proc. Soc. Exp. Biol. Med., 131, 1154, 1969.
34. Young, N. S.; J. Levin and R. Prendergast, "An invertebrate coagulation system activated by endotoxin: Evidence for enzymatic mediation", J. Clin. Invest., 51, 1790, 1972.
35. Tai, J. Y. and T. Y. Liu, "Studies on *Limulus* lysate. Isolation of pro-clotting enzyme", J. Biol. Chem., 252, 2178, 1977.

36. Solum, N. O., "Some characteristics of the clottable protein of *Limulus polyphemus* blood cells", Thromb. Diath. Haemorrh., 23, 169, 1970.
37. Nakamura, S.; S. Iwanaga, T. Harada, and M. Niwa, "A clottable protein (coagulation) of the horseshoe crab hemocytes structural change of its polypeptide chain during gel formation", J. Biochem., 80, 649, 1976.
38. Mosesson, M. W.; L. Wolfenstein-Todel; J. Levin, and O. Bertrand, "Characterization of amebocyte coagulation from the horseshoe crab (*Limulus polyphemus*)", Thromb. Res., 14, 765, 1979.
39. Tai, J. Y.; R. C. Seid; R. D. Huhn and T. Y. Liu, "Studies on *Limulus* amebocyte lysate. II. Purification of the coagulogen and the mechanism of clotting", J. Biol. Chem., 252, 4773, 1977.
40. Nakamura, S.; T. Morita and S. Iwanaga, "Lipopolysaccharide sensitive serine protease zymogen (factor C) found in limulus hemocytes: isolation and characterization", J. Biochem., 154, 511-521, 1986.
41. Ohki, M.; T. Nakamura; T. Morita and S. Iwanaga, "A new endotoxin sensitive factor associated with hemolymph coagulation system of horseshoe crab (Limulidae)", FEBS Lett., 120, 217, 1980.

42. Rojas - Corona, R. R.; R. Skarnes; S. Tamakura; J. Fire, "The limulus coagulation test for endotoxin. A comparison with other assay methods", Proc. Soc. Exp. Biol. Med., 132, 599-601, 1969.
43. Pearson, F. C., "The Limulus amebocyte lysate endotoxin assay: Current status", Am. J. Med. Technol., 45, 704, 1979.
44. Frauch, P., "Slide test as a micromethod of a modified *Limulus* endotoxin test", J. Pharm. Sci., 63, 808, 1974.
- 45 Morita, T.; T. Harada; S. Nakamura; S. Iwanaga and M. Niwa, "Horseshoe crab (*Tachypleus tridentatus*) clotting enzyme: A new sensitive assay method for bacterial endotoxin", Jpn. J. Med. Sci. Biol., 31, 178, 1978.
46. Hussaini, S. N. and H. T. Hassanali, "Limulus amoebocyte lysate assay of endotoxin :a method for visual detection of the positive gel reaction", J. Med. Microbiol., 24, 89-90, 1987.
47. "LAL assay: application and equivalence", Pyrogens: endotoxin, LAL testing and depyrogenation, Person, F.C., Mercel Dekker Inc., p.3-8, 1985.
48. Food and Drug Administration, National Center for Drugs and Biologics, "Draft guideline for validation of the limulus amoebocyte test as an end-product endotoxin test for human and animal parenteral drugs, biological products, and

- medical devices", Pharmacopeial Forum, May-June, 3012-3020, 1983.
49. Rastogi, S. C.; H. D. Hochstein, and E. B. Seligmann, Jr., "Statistical determination of endotoxin content in influenza virus vaccine by limulus amoebocyte lysate test", J. Clin. Microbiol., 6(2), 144-148, 1977.
50. Paulssen, J.; E. B. Bjornson, and D. Fystro, "Ultrafiltration as a means to eliminate inhibition of the limulus amoebocyte lysate (LAL) assay", Acta Path. Microbiol. Immunol. Scand. Sect. B, 93, 73-75, 1985.
51. Jorgensen, J. H. and R. F. Smith, "Rapid detection of contaminated intravenous fluids using the limulus *in vitro* endotoxin assay", Appl. Microbiol., 26(4), 521-524, 1973.
52. Mascoli, C. C. and M. E. Weary, "Limulus amoebocyte lysate (LAL) test for detecting pyrogens in parenteral injectable products and medical devices: advantage to manufacturers and regulatory officials", J. Parenter. Drug Assoc., 33(2), 81-95, 1979.
53. Sullivan, Jr., J. D.; et al., "Comparison of the limulus amoebocyte lysate test with plate counts and chemical analyses for assessment of the quality of lean fish", Appl. Environ. Microbiol., 45(2), 720-722, 1983.
54. Paulssen, J. and P. Michaelsen, "The limulus amoebocyte lysate

- (LAL) assay for the detection of endotoxin in fat emulsions for total parenteral nutrition (TPN)", Acta Path. Microbiol. Immunol. Scand. Sect. B, 92, 177-179, 1984.
55. Hosotsubo, K. K.; et al., "Elimination of false positive limulus amoebocyte lysate tests in patients with hyperlipidemia", Crit. Care Med., 13(12), 1061-1063, 1985.
56. Munford, R. S.; C. L. Hall and L. Grimm, "Detection of free endotoxin in cerebrospinal fluid by limulus lysate test", Infec. Immun., 45(2), 531-533, 1984.
57. Elden, J. and R. H. Yolken, "C-reactive protein and limulus amoebocyte lysate assay in diagnosis of bacterial meningitis", J. Pediatrics., 108(3), 423-426, 1986.
58. Jorgensen, J. H. and G. A. Alexander, "Rapid detection of significant bacteriuria by use of an automated limulus amoebocyte lysate assay", J. Clin. Microbiol., 16(3), 587-589, 1982.
59. Nachum, R.; J. J. Arce and R. N. Berzofsky, "Gram negative bacteriuria of pregnancy: rapid detection by a chromogenic limulus amoebocyte lysate assay", Obstetrics & Gynecology, 68(2), 215-219, 1986.
60. Spagna, V. A. and R. B. Prior, "Limulus lysate assay for gonorrhea", A.F.P., 27(3), 227-231, 1983.

61. Prior, R. B. and V. A. Spagna, "Rapid evaluation of gonococcal and nongonococcal urethritis in men with limulus amoebocyte lysate and a chromogenic substrate", J. Clin. Microbiol., 17(3), 485-488, 1983.
62. Spagna, V. A.; R. B. Prior; and G. A. Sawaya, "Sensitivity, Specificity, and Predictive values of the limulus lysate assay for detection or exclusion of gonococcal cervicitis", J. Clin. Microbiol., 16(1), 77-81, 1982.
63. Harris, R. I.; et al., "An improved chromogenic substrate endotoxin assay for clinical use", J. Clin. Pathol., 36, 1145-1149, 1983.
64. Levin, J.; P. A. Tomasulo and R. S. Oser, "Detection of endotoxin in human blood and demonstration of an inhibitor", J. Lab. Clin. Med., 75(6), 903-911, 1970.
65. Harthug, S.; B. Bjorvatn; B. Osterud, "Quantitative of endotoxin in blood from patients with meningococcal disease using limulus lysate test in combination with chromogenic substrate", Infection 11, 4, 192-195, 1983.
66. Obayashi, T.; et al., "New limulus amoebocyte lysate test for endotoxaemia", Lancet, Jan 30, 289, 1982.
67. Case, M. J.; S. S. Ryther and T. J. Novitsky, "Detection of endotoxin in antibiotic solutions with limulus amoebocyte

- lysate", Antimicrob. Agents Chemother., 23(5), 649-652, 1983.
68. Piotrowicz, B. I.; I. Watt; S. Edlin and A. C. McCartney, "A micromethod for endotoxin assay in human plasma using limulus amoebocyte lysate and a chromogenic substrate", Eur. J. Clin. Microbiol., 4(1), 52-54, 1985.
69. Levin, J.; et al., "Detection of endotoxin in the blood of patients with sepsis due to gram negative bacteria", New England J. Med., 283(24), 1313-1316, 1970.
70. Obayashi, T.; et al., "Endotoxin-inactivating activity in normal and pathological human blood samples", Infec. Immun., 53(2), 294-297, 1986.
71. Ross, V. C. and C. W. Twohy, "Endotoxin and medical devices", Bacterial Endotoxins: Structure, Biomedical significant, and Detection with the limulus amoebocyte lysate test., pp. 267-280, Alan R. Liss, New York, 1985.
72. Nowotny, A.; U. H. Behing; H. L. Chang, "Relation of structure to function in bacterial endotoxins. VIII Biological activities in a polysaccharide-rich fraction", J. Immunol., 115(1), 199-203, 1975.
73. Niwa, M.; M. Hiramatsu; O. Waguri, "The gelation reaction between horseshoe crab amoebocytes and endotoxins studied by the quantitative protein method", Jpn. J. Med. Sci. Biol., 27,

108-111, 1974.

74. Lowry, H. O.; et al., "Protein measurement with folin phenol reagent", J. Biol. Chem., 193, 265-275, 1951.
75. Sullivan, J. D., Jr. and S. W. Watson, "Factors affecting the sensitivity of limulus lysate", Appl. Microbiol., 28, 1023-1026, 1974.
76. Tsuji, K. and K. A. Steindler, "Use of magnesium to increase sensitivity of limulus amoebocyte lysate for detection of endotoxin", Appl. Environ. Microbiol., 45(4), 1342-1350, 1983.
77. Devleeschouwer, M. J.; M. F. Cornil; J. Dony, "Studies on the sensitivity and specificity of the limulus amoebocyte lysate test and pyrogen assays", Appl. Environ. Microbiol., 50(6), 1509-1511, 1985.
78. Wildfeuer, A.; B. Heymer; K. H. Schleifer and O. Haferkamp, "Investigations on the specificity of the Limulus test for the detection of endotoxin", Appl. Microbiol., 28, 867-871, 1974.
79. Lechman, L.; P. Deluca, "Kinetic principles and stability testing", The theory and practice of industrial pharmacy, Lachman, Lieberman, Kanig, second edition, Lea & Febiger, U.S.A., 1976.
80. "E-toxate for detection and semi-quantitative endotoxin", Sigma

technical bulletin no. 201, Sigma Chemical Co., U.S.A.,
Revised 1983.

81. Schmitz, A. J. and T. E. Munson, "Development of endotoxin limit for USP articles, USP articles in-process, and potential articles", Pharmacopeial Forum, Sept.-Oct., 2947-2971, 1987.
82. "Draft guideline for LAL end-product testing of biologicals, parenteral drugs, and medical devices", Pyrogens: endotoxin, LAL testing and depyrogenation, Person, F.C., Marcel Dekker Inc., p.3-8, 1985.
83. "Detection of endotoxin", Manual instruction of LAL test, Wako Pure Chemical Ltd., Japan, pp. 1-8.
84. "Limulus Amoebocyte Lysate", Manual Instruction, LAL test, Marine Biologicals, Inc., New Jersey, U.S.A., 1985.
85. Steindler, K.A.; et al, "Potentiating effect of calcium gluconate on the limulus amoebocyte lysate (LAL) gelation-end-point assay for endotoxin", J. Parenter. Sci. Technol., 35(5), 242-247, 1981.
86. Weary, M; et al, "Relative potencies of four reference endotoxin standards as measured by the limulus amoebocyte lysate and USP rabbit pyrogen tests", Appl. Environ. Microbiol., 40, 1148-1151, 1980.

87. Ronneberger, H. J., "Comparison of the pyrogen tests in rabbit and with limulus lysate", Dev. Biol. Stand., 34, 27-32, 1977.
88. Van Noordwijk, F. and Y. DeYong, "Comparison of the limulus test for endotoxin with the rabbit pyrogens of the European Pharmacopeia", J. Biol. Stand., 4, 131-139, 1976.
89. "Ultrastructure and function", The Life of Yeast, Phaff, H. J., Second edition, Harvard University Press, England, 29-68, 1978.
90. Obayashi, T., "Limulus coagulation factor G as a detector of fungal polysaccharide", Abstracts of X congress of the International Society for Human and Animal Mycology, Barceiona, 5(1), 21, 1988.

APPENDIX



Glassware

1. 250 mL Measuring cylinders
2. 0.1, 1, 2, 5, and 10 mL pipettes
3. 50, 125, 250, and 500 mL beakers
4. 100 mL conical flasks
5. 10x75 mm. tubes
6. 16x150 mm. screw-cap tubes
7. 100x15 mm. petri dishes
8. 50 mL vials

Plasticware

1. 500 mL polypropylene centrifuge bottles
2. 50 mL polycarbonate centrifuge tubes
3. 50 mL polypropylene centrifuge tubes
4. 1 mL polycarbonate centrifuge tubes
5. 1 mL disposable syringes
6. 50 uL micropipette tips
7. Magnetic bars

Reagents

Colox-lypon F mixture

1. Colox	100 mL
2. Lypon F	100 mL

Mix the two solution together and store at room temperature for further use in washing of glassware and plasticware.

Water for Injection

It was obtained from Queen Soavabha Memorial Institute, Thai Red Cross Society, Bangkok, Thailand. It was prepared according to U.S.P. specification.

10x 5mM Tris-HCl buffer

1. Trizma (Sigma)	6.06 g.
2. water for injection to	1,000 mL

2.5 N HCl was used to adjust pH to 7.4 and the buffer was tested with commercial LAL test before diluted to 5 mM Tris buffer using water for injection.

5 mM Tris-HCl buffer

1. 50 mM Tris-HCl buffer	100 mL
2. water for injection to	1,000 mL

1 M Magnesium Chloride solution

1. MgCl ₂ . 6H ₂ O (Fluka)	20.331 g.
2. water for injection	to 100 mL

The solution was prepared by dissolving magnesium chloride completely using magnetic stirrer. The solution was tested with commercial LAL test prior to use.

0.125% EDTA 3% NaCl in 5 mM Tris buffer

1. EDTA.2H₂O (E. Merck) 1.25 g.
2. NaCl (E. Merck) 30 g.
3. 5 mM Tris-HCl buffer to 1,000 mL

Dissolved EDTA and NaCl in Tris buffer using magnetic stirrer. The solution was tested with commercial LAL.

0.05 M EDTA 3% NaCl in 5 mM Tris buffer

1. EDTA.2H₂O (E. Merck) 18.61 g.
2. NaCl (E. Merck) 30 g.
3. 5 mM Tris-HCl buffer to 1,000 mL

Dissolved EDTA and NaCl in Tris buffer using magnetic stirrer. The solution was tested with commercial LAL.

0.025 M EDTA 3% NaCl in 5 mM Tris buffer

1. EDTA.2H₂O (E. Merck) 9.31 g.
2. NaCl (E. Merck) 30 g.
3. 5 mM Tris-HCl buffer to 1,000 mL

Dissolved EDTA and NaCl in Tris buffer using magnetic stirrer. The solution was tested with commercial LAL.

0.25 M EDTA 3% NaCl solution

1. EDTA.2H₂O (E. Merck) 93.06 g.



2. 3% NaCl solution to 1,000 mL

Dissolved EDTA in 3% sodium chloride solution with the help of magnetic stirrer and tested with commercial LAL.

0.025 M EDTA 3% NaCl solution

1. 0.25 M EDTA 3% NaCl solution 100 mL

2. 3% NaCl solution to 1,000 mL

Mixed the solution by shaking thoroughly for at least 5 minutes.

3% Sodium Chloride solution

1. Sodium Chloride (E. Merck) 21 g.

2. Normal Saline Solution to 1,000 mL

The solution was shaked continuously until NaCl was completely dissolved. The solution was kept at room temperature. The solution was tested with commercial LAL before use.

3% sodium chloride in 5 mM Tris buffer

1. NaCl (E. Merck) 30 g.

2. 5 mM Tris buffer to 1,000 mL

The solution was shaked continuously until NaCl was completely dissolved. The solution was kept at room temperature. The solution was tested with commercial LAL before use.

2.5 N HCl solution

1. 25% HCl (E. Merck) 36.3 mL

2. water for injection to 100 mL

Shaked the mixture well at least 5 minutes and tested with commercial LAL.

0.1 N HCl solution

1. 25% HCl (E. Merck) 1.5 mL

2. water for injection to 100 mL

Shaked the mixture well at least 5 minutes and tested with commercial LAL.

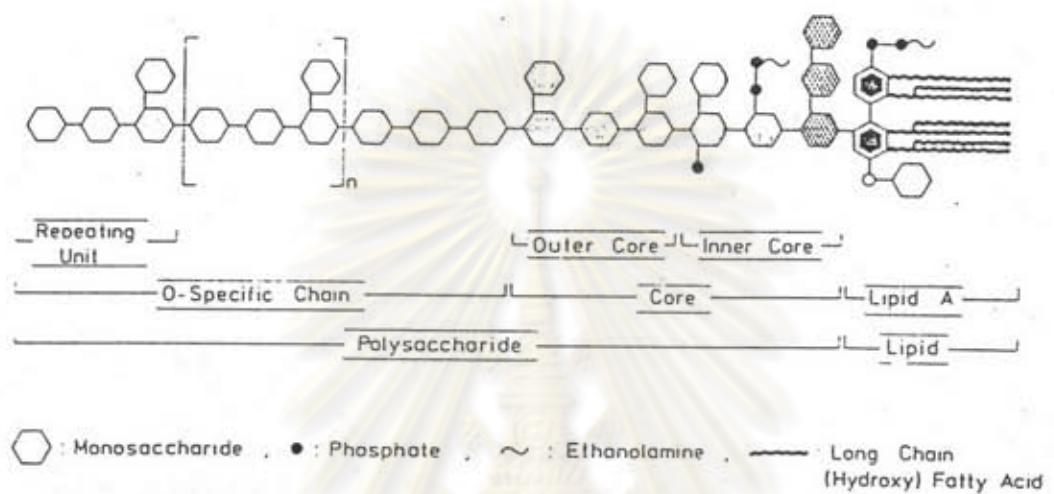
0.1 N NaOH solution

1. NaOH, pellets (E. Merck) 0.40 g.

2. water for injection to 100 mL

Dissolved NaOH completely by gently shaking with water for injection and tested with commercial LAL.

Schematic diagram of LPS.



Amoebocyte under microscope (400x)



គ្រូនយោវិទ្យាព្យាគរ
ជុំដាក់សងក្រោះមហាវិទ្យាប្រជាធិបតេយ្យ



BIOGRAPHY

Mr. Por Punyaratabandhu was born on June 30, 1955 in Prachiburi, Thailand. He graduated with a Bachelor of Pharmacy from the Department of Pharmaceutical Science at Saugor University, Saugor (M.P.), India in 1980. At present, he holds a post of Pharmacist-in-Charge of Vaccine Section at Queen Soavabha Memorial Institute, Thai Red Cross Society, Bangkok, Thailand.

คุณนายวิทูกรัชพอยวิช
อุปนายก湿润膜化验室
ศูนย์การอนามัยไทยฯ