References

1. Bimol, C.B., and Charles, G.R. Tumor markers. In C.G. Bimal, and G.Luma (eds.), Tumor markers and tumor associated antigens, pp.1-9. United States of America : Mcgraw-hill book company, 1987.
2. Tatarinov, Y.S. Finding of embryo-specific alpha-globulin in blood serum in a patient with primary hepatic cancer. Vop. Med. Khim. 19(1964):90-91.
3. Vacher, J., and Tilghman, S.M. Dominant negative regulation of the mouse alpha-fetoprotein gene in adult liver Science 250(1991) : 1732-1735.
4. Seppala, M., and Ruoslahti, E. Radioimmunoassay of maternal serum alpha-fetoprotein during pregnancy and delivery. Am. J. Obstet. Gynecol. 112(1972) : 208-212.
5. Ishiguro, T., Sugitachi, I., Sakaguchi, H., and Itani, S. Serum alpha-fetoprotein subtractions in patients with primary hepatoma or hepatic metastasis of gastric cancer. Cancer 55(1985) : 156-159.
6. Kohler, G., and Milstein, C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature (London) 256(1975) : 495-497.

7. Eisenbarth, G.S. Application of monoclonal antibody techniques to biochemical research. Anal. Biochem. 111(1981) : 1-16.

8. Addison, G.M., and Hales, C.N. Two site assay of human growth hormone. Hormone metab. Res. 3(1971) : 59-64.

9. Tormey, D.C., et al. Circulating tumor marker. In M.D. David, and P. Rose (eds.), Endocrinology of cancer, pp. 125-129. Florida : CRC Press, 1979.

10. In S. Sell. (ed.), Immunology, Immunopathology and Immunity, pp. 748-752. New York : Elsevire Science Publishing Cmpany, 1987.

11. Bergstrand, C.G. and Czar, B. Paper electrophoretic study of human fetal serum proteins with demonstration of a new protein fraction. Scand. J. Clin. Lab. Invest. 9(1957) : 277-286.

12. Abelev, G.I., Perova, S.D., Khvamkova, N.I., Postnihova, Z.A., and Irlin, I.S. Production of embryonal alpha-globulin by the transplantable mouse hepatomas. Transplantation 1(1963) : 174-180.

13. Tatarinov, Y.S. Detection of embryo-specific alpha-globulin in the blood sera of patients with primary liver tumors. Vopr. Med. Khim. 10(1964) : 90-91.

14. McIntire, K.R., Waldmann, T.A., Moertel, C.G., and Go, V.L.W. Serum AFP in patients with neoplasms of

the gastrointestinal tract. Cancer Res. 35(1975) : 991-996.

15. Ruoslahti, E., and Seppala, M. Studies of cacino-fetal proteins : Physical and chemical properties of human alpha-fetoprotein. Int. J. cancer. 7(1971) : 218-228.

16. Young, J.L., and Webb, B.A. Two methods for the separation of human alpha-fetoprotein and albumin. Anal. Biochem. 88(1978) : 619-623.

17. Sittenfeld, A., and Moreno, E. A sensitive blotting system for detection of alpha-fetoprotein variants with monoclonal and polyclonal antibodies. J. Immunol. Methods 106(1988) : 19-26.

18. Pucci, P., Sicilians, R., Malorni, A., Marino, G., Tecce, M.F., and Ceccarini, C. Human alpha-fetoprotein Primary Structure : A mass spectrometric study. Biochemistry 30(1991) : 5061-5066.

19. Gibbs, E.M., Zielinski, R., Boyd, C., and Dugaiczyk, A. Structure, Polymorphism, and Novel Repeated DNA Elements Revealed by a Complete Sequence of the Human α-Fetoprotein Gene. Biochemistry 26(1987) : 1332- 1343.

20. Lemire, J.M., and Fausto, N. Multiple alpha-fetoprotein RNAs in adult rat liver : Cell type-specific expression and differential regulation. Cancer Res. 51(1991) : 4656-4664.

21. Yoshima, H., Mizuochi, T., Ishii, M., and Kobata, A. Structure of the asparagine-linked sugar chains alpha-fetoprotein purified from human ascites fluid. Cancer Res. 40(1980) : 4276-4281.

22. Yamashita, K., Hitoi, A., Tsuchida, Y., Nishi, S., and Kobata, A. Sugar chain alpha-fetoprotein produced in human yolk sac tumor. Cancer Res. 43(1983) : 4691-4695.

23. Gitlin, D., Perricelli, A., and Gitlin, G.M. Synthesis of alpha-fetoprotein by liver, yolk sac, and gastrointestinal tract of the human conceptus. Cancer Res. 32(1972) : 979-982.

24. Gitlin, D. Normal biology of alpha-fetoprotein. Ann. NY. Acad. Sci. 259(1975) : 7-16.

25. Gitlin, D., and Perricelli, A. Synthesis of serum albumin, prealbumin, α-fetoprotein, α_1-antitrysin and transferrin by the human yolk sac. Nature 228(1970) : 995-997.

26. Ruoslahti, E., Hirai, H. (chairman), and Group members. Alpha-fetoprotein. Scand. J. Immunol. 8, Suppl 8(1978) : 3-26.

27. Seppala, M. Immunologic detection of alpha-fetoprotein as a marker of fetal pathology. Clin. Obstet. Gynecol. 20(1977) : 737-757.

28. Masseyeff, R., Gilli, J., Krets, B., Calluand, A., and Bonet, C. Evolution of α-fetoprotein serum levels throughout life in human and rats, and during pregnancy in the rat. Ann. NY. Acad. Sci.

259(1975) : 17-28.

29. Ruoslahti, E., and Seppata, M. α-fetoprotein in normal human serum. Nature 235(1972) : 161-162.

30. Xu, Y., Halstall, H.B., and Heineman, W.R. Heterogeneous enzyme immunoassay of alpha-fetoprotein in maternal serum by flow-injection amperometric detection of 4-aminophenol. Clin. Chem. 36(1990) : 1941-1944.

31. Seppala, M. and Ruoslahti, E. Alpha-fetoprotein in amniotic fluid : An index of gastational age. Am. J. Obstet. Gynecol. 114(1972) : 595-598.

32. Seppala, M. Fetal pathophysiology of human AFP. Ann. NY. Acad. Sci. 259(1975) : 59-73.

33. Gitlin, D. Normal biology of AFP. Ann. NY. Acad. Sci. 259(1975) : 7-16.

34. Nomura, M., Imai, M. Kumakura, T., Tachibana, K., Aoyagi, S., Usuda, S., Nakamura, T., and Mayumi, M. Three-site sandwich radioimmunoassay with monoclonal antibodies for a sensitive determination of human alpha-fetoprotein. J. Immunol. Methods 58(1983) : 293-300.

35. Chan, D.W., Kelsten, M., Rock, R., and Bruzek, D. Evaluation of a monoclonal immunoenzymometric assay for alpha-fetoprotein. Clin. Chem. 32(1986) : 1318-1322.

36. Ruoslahti, E., and Seppala, M. Studies of carcino fetal protein : Development of a

radioimmunoassay for α-fetoprotein in serum of healthy adults. Int. J. Cancer. 8(1971) : 374-383.

37. Trichopoulos, D., Gerety, R.J., Sparros, L., Tabor, E., Xirouchaki, E., Munoz, N., and Linsell, C.A. Hepatitis B and primary hepato-cellular carcinoma in a European population. Lancet 2(1978) : 1217-1219.

38. Purves, L.R., Bershon, I., and Geddes, E.W. Serum alpha-fetoprotein and primary cancer of the liver in man. Cancer 25(1970) : 1261-1270.

39. Kiji, T., Munehisa, T., Yamaguchi, K., Kusumoto, Y., and Nakamura, S. Epidemiological studies of alpha-fetoprotein and hepatitis B antigen in Tomie town, Nangasaki, Japan. Ann. NY. Acad. Sci. 259(1975) : 239-247.

40. Heyward, W.L., Lanier, A.P., McMahon, B.J., Fitzgerald, M.A., Kilkenny, S., and Paprocki, T.R. Early detection of primary hepatocellular carcinoma. J.A.M.A. 254(December 1985) : 3052-3054.

41. McIntire, K.R., Vogel, C.L., Princler, G.L., and Patel, I.R. Serum α-fetoprotein as a biochemical marker for hepatocellular carcinoma. Cancer Res. 32(1972) : 1941-1946.

42. Lehmann, F.G. Early detection of hepatoma : Prospective study in liver cirrhosis using passive hemagglutination and the radioimmunoassay. Ann. NY. Acad Sci.

259(1975) : 196-210.

43. Nakakuma, K., Tashiro, S., Uemura, K., and Takayama, K. Alpha-fetoprotein and human chorionic gonadotropin in embryonal carcinoma of the ovary : An 8-year survival case. Cancer 52(1983) : 1470-1472.

44. Yoshimoto, T., Higashino, K., Hada, T., Tamura, S., Nakanishi, K., Mitsunobu, M., Uematsu, K., Matsuoka, T., and Taketa, K. A primary lung carcinoma producing alpha-fetoprotein, carcinoembryonic antigen and human chorionic gonadotropin. Cancer 60(1987) : 2744-2750.

45. Alpert, m.E., Uriel, J., and de Nechaud, B. Alpha-fetoglobulin in the diagnosis of human hepatoma. N. Engl. J. Med. 278(1968) : 984-986.

46. Shijo, H., Okazaki, M., Koganemaru, f., Higashi, M., Sakaguchi, S., and Okumura, M. Influence of hepatitis B virus infection and age on mode of growth of hepatocellular carcinoma. Cancer 67(1991) : 2626-2632.

47. Bloomer, J.R., Waldmann, T.A., McIntire, K.R., and Klatskin, G. Alpha-fetoprotein in non-specific hepatic disorders. J.A.M.A. 233(1975) : 38-41.

48. Endo, Y., Kania, E., Lino, S., and Oda, T. Clinical significance of alpha-fetoprotein with special reference to primary cancer of the liver. Gann

Monog. Cancer Res. 14(1973) : 67-78.

49. Sakamoto, S., Yachi, A., Anzai, T., and Wada, T. AFP producing cells in hepatitis and in liver cirrhosis. Ann. NY. Acad. Sci. 259(1975) : 253-258.

50. Obata, H., Hayashi, N., and Motoike, Y. A prospective study on the development of hepatocellular carcinoma from liver cirrhosis with persistent hepatitis B virus infection. Int. J. Cancer 25(1980) : 741-747.

51. Heyward, W.L., Lanier, A.P., Bender, T.R., McMahon, B.J., Kilkenny, S., Paprocki, T.R., Kline, K.T., Silimperi, D.R., and Maynard, J.E. Early detection of primary hepatocellular carcinoma by screening for alpha-fetoprotein in high-risk families. Lancet 2(1983) : 1161-1162.

52. Brock, D.J.H., and Sutcliffe, R.G. Alpha-fetoprotein in the antenatal diagnosis of anencephaly and spina bifida. Lancet 2(1972) : 197-199.

53. Macri, J.N., Buchanan, P.D., and Gold, M.P. Low α-fetoprotein and trisomy. Lancet 2(1986) : 405.

54. Brambati, B., Simoni, G., Bonacchi, I., and Piceni, L. Fetal chromosomal aneuploidies and maternal serum alpha-fetoprotein levels in first trimester. Lancet 2(1986) : 165-166.

55. Report of U.K. Collaborative Study on Alpha-fetoprotein in Relation to Neural-tube Defects : Maternal

serum alpha-fetoprotein measurement in antenatal screening for anencephaly and spina bifida in early pregnancy. Lancet 1(1977) : 1323-1332.

56. Jones, S.R., Evans, S.E., Mackenzie, W.E., and Hodgkins, P. First trimester alpha-fetoprotein levels and fetal chromosomal aneuploidies. Lancet 1(1988) : 826-827.

57. Seller, M.J., Creasy, M.R., and Alberman, E.D. Alpha-fetoprotein levels in amniotic fluids from spontaneous abortions. Br. Med. J. 2(1974) : 524-525.

58. Cuckle, H.S., Wald, N.J., and Lindenbaum, R.H. Maternal serum alpha-fetoprotein measurement : a screening test for Down syndrome. Lancet 1(1984) : 926-929.

59. Fuhrman, W., Wendt, P., and Weitzel, H.K. Maternal serum AFP as screening test for Down syndrome. Lancet 2(1984) : 413.

60. Cuckle, H.S., and Wald, N.J. Amniotic fluid alpha-fetoprotein levels in Down syndrome. Lancet 2(1986) : 290-291.

61. Abelev, G.I., Perova, S., Khramkova, N.I., Postnikova, Z.A., and Irlin, I. Production of embryonal alpha-globulin by the transplantable mouse hepatoma. Transplantation 1(1963) : 174-180.

62. Lin, T., Chu, S., Chen, M., and Chen, C. Serum alpha-fetoglobulin and primary cancer of the liver in

Taiwan. Cancer 30(1972) : 435-443.

63. Smith, J.B., and Todd, D. Foetoglobulin and primary liver cancer. Lancet 2(1968) : 833.

64. Sasaki, T., Tsukada, Y, and Hirai, H. A new procedure for coupling antibody to paper discs for radioimmunoassay : Application to the determination of alpha-fetoprotein. J.Immunol. Methods 64(1983) : 25-29.

65. Ruoslahti, E., Uotila, M., and Engvall, E. Radioimmunoassay of alpha-fetoprotein with polyclonal and monoclonal antibodies. Methods Enzymol. 84(1982) : 3-19.

66. Belanger, L., Sylvestre, C., and Dufour, D. Enzyme-linked immunoassay for alpha-fetoprotein by competitive and sandwich procedures. Clin. Chem. Acta. 48(1973) : 15-18.

67. Porstmann, B., Aorameas, S., Ternynck, T., Porstmann, T., Micheel, B., and Guesdon, J.L. An antibody chimera technique applied to enzyme immunoassay for human alpha-1-fetoprotein with monoclonal and polyclonal antibodies. J. Immunol. Methods 66(1984) : 179-185.

68. Brock, D.J.H., Barron, L., and Van Heyningen, V. Enzyme-linked immunospecific assays for human alpha-fetoprotein using monoclonal antibodies. Clin. Chem. Acta. 122(1982) : 353-358.

69. Belanger, L. and Masseyeff, r. Enzyme immunoassay of human α_1-fetoprotein. Methods. Enzymol.

84(1982) : 19-31.

70. Kelsten, M.L., Chan, D.W., Bruzek, D.J., and Rock, R.C. Monitoring hepatocellular carcinoma by using a monoclonal immunoenzymometric assay for alpha-fetoprotein. Clin. Chem. 34(1988) : 76-81.

71. Miles, L.E.M., and Hales, C.N. Labelled antibodies and immunological assay systems. Nature 219(1968) : 186-189.

72. Hunter, W.M., Bennie, J.G., Brock, D.J.H., and Van Heyningen, V. Monoclonal antibodies for use in an immunoradiometric assay for α-fetoprotein. J. Immunol. Methods 50(1982) : 133-144.

73. Catty, D. Properties of antibodies and antigens. In D. Catty (ed.), Antibodies Volume I : a practical approach, pp. 7-18. Oxford : IRL Press, 1988.

74. Catty, D. Production and quality control of polyclonal antibodies. In D. Catty (ed.), Antibodies Volume I : a practical approach, pp. 19-52. Oxford : IRL Press, 1988.

75. Fazekas de St. Groth, S., and Scheidegger, D. Production of monoclonal antibodies : Strategy and tactics. J. Immunol. Methods 35(1980) : 1-21.

76. Kearney, J.F., Radbrach, A., Liesegang, B., and Rajienaky, K. A new mouse myeloma cell line that has lost immunoglobulin expression but permits the construction of antibody-secreting

hybrid cell lines. J. Immunol. 123(1979) : 1548-1550.

77. Tsung, Y.K., Milunsky, A., and Alpert, E. Derivation and characterization of a monoclonal hybridroma antibody specific for human alpha-fetoprotein. J. Immunol. Methods 39(1980) : 363-368.

78. Shahangian, S., Fritsche, H.A.Jr., and Hughes, J.I. A double[monoclonal immunoenzymometric assay for α_1-fetoprotein modified for increased analytical precision. Clin. Chem. 33(1987) : 583-586.

79. Littlefield, J.W. Selection of hybrids from matings of fibroblast in vitro and their presumed recombinants. Science 145(1964) : 709-711.

Appendix I

Reagents and Preparations

1. Reagent for cell culture

1.1. RPMI 1640 medium

RPMI 1640	10.4 g
HEPES	5.96 g
Sodium bicarbonate	2.02 g
Distilled water	1 L

The medium was sterilized by milipore membrane fitration of pore size 0.45 micron.

1.2. Complete RPMI medium

RPMI 1640	10.4 g
HEPES	5.96 g
D-glucose	3.6 g
Sodium pyruvate	1.1 g
Sodium bicarbonate	2.02 g
Distilled water	1 L
L-glutamine	0.29 g

The solution of penicillin G, steptomycin and kanamycin were added to the final concentrations of 10,000 units, 100 mg and 100 mg/L, respectively. The medium was sterilized by passing through a millipore membrane filter. The complete medium was prepared by supplementing this medium with 100 ml heat inactivated fetal calf serum.

2. Reagent for mouse monoclonal antibody isotyping kit

2.1. Phosphate buffer saline (PBS) containing 0.05% Tween 20 and 1% BSA

Sodium chloride	8	g
Potassium chloride	0.2	g
di-Sodium hydrogenphosphate	1.15	g
Potassium dihydrogen phosphate	0.2	g
Tween 20	0.5	ml
Bovine serum albumin	10	ml
Distilled water	1	L

3. Reagent for purification

3.1. 100% Ammonium sulfate

Ammonium sulfate	100	g
Distilled water	100	ml

3.2. 0.5 M Phosphate buffer pH 7.4

di-Sodium hydrogenphosphate	57.1	g
Sodium dihydrogen phosphate monohydrate	13.5	g
Distilled water	1	L

adjust pH with 5 N NaOH

3.3. 0.1 M Citric acid

citric acid anhydrous	19.21	g
Distilled water	1	L

4. Reagent for Polyacrylamide gel electrophoresis

4.1. 30% acrylamide-0.8% bis-acrylamide solution

acrylamide	30	g
N,N'-Methylene-bis	0.8	g
Distilled water	100	ml

4.2. 3 M Tris-HCl pH 8.8

Tris-base	181.65	g
Distilled water	400	ml
adjust pH to 8.8 by adding concentrated HCl	45	ml

4.3. 0.5 M Tris-HCl pH 6.8

Tris-base	30.27	g
Distilled water	400	ml
adjust pH to 6.8 by adding concentrated HCl	20	ml

4.4. Sample buffer in SDS PAGE

40% sucrose solution	10	ml
1% SDS solution	10	ml
mercaptoethanol	5	ml
0.1% bromphenol blue	10	ml

4.5. Preparation of gel

4.5.1. 10% seperation gel mixture

30% acrylamide solution	2.3	ml
3 M Tris-HCl pH 8.8	1.75	ml
1% SDS solution	0.7	ml
1.5% ammonium persulfate	0.35	ml
TEMED	10	ul
Distilled water	1.9	ml

4.5.2. 3.75% stacking gel

30% acrylamide solution	0.5	ml
1% SDS solution	0.4	ml
0.5 M Tris-HCl pH 6.8	1	ml
1.5% ammonium persulfate	0.2	ml
TEMED	5	ul
Distilled water	1.9	ml

4.6. Running buffer

Tris	6.06	g
glycine	28.8	g
SDS	2	g
Distilled water	2	L

4.7. Coomassie blue staining solution

Coomassie blue	0.5	g
methanol	115	ml
glacial acetic acid	20	ml
Distilled water	115	ml

4.8. Destaining solution

methanol	200	ml
glacial acetic acid	70	ml
Distilled water	730	ml

4.9. The silver stain plus kit

4.9.1. Fixative enhancer solution

methanol	100	ml
acetic acid	20	ml
fixative enhancer concentrate	10	ml
deionized water	70	ml

4.9.2. Staining and developing

deionized water	35	ml
silver complex solution	5	ml
reduction moderator solution	5	ml
image development reagent	5	ml
development accelerator reagent	50	ml

5. Reagents for coupling of antiserum

5.1. 0.5 M bicarbonate buffer pH 8

Sodium bicarbonate	4.2	g
Distilled water	100	ml

5.2. 0.1 M acetic acid

glacial acetic acid	5.7	ml
Distilled water	500	ml

5.3. 0.1 M Sodium acetate

Sodium acetate	4.1	g
Distilled water	500	ml

5.4. 0.1 M Acetate buffer pH 4

0.1 M acetic acid	500	ml
0.1 M Sodium acetate	500	ml

5.5. 0.05 M barbitone buffer pH 8

barbitone	9.21	g
Distilled water	1000	ml

adjust pH to 8.0 with 5 N NaOH

6. Reagents for lowry method

6.1. Solution A : 2% sodium carbonate in 0.1 M NaOH

Sodium carbonate	2 g
0.1 M Sodium hydroxide	100 ml

6.2. Solution B : copper sulfate solution

copper sulfate	0.5 g
1% Sodium Potassium tartrate	100 ml

6.3. Solution C : freshly prepared

Solution A	50 ml
Solution B	1 ml

6.4. Solution D : Folin's Ciocalteau Phenol reagent

Folin's Ciocaltean phenol	1 ml
Distilled water	1 ml

7. Reagent for purification of human AFP

7.1. 0.1 M Tris buffer pH 8

Tris-base	6.055 g
Distilled water	400 ml
adjust pH to 8.0 by adding concentrated HCl	21 ml

7.2. 8 M Urea in 0.02 M PB

Urea	48 g
0.02 M PB	100 ml

7.3. 6 M Sodium thiocyanate

Sodium thiocyanate	486.4 g
Distilled water	1 L

Appendix II

1. Preparation of label standard AFP with I^{125}

The standard of AFP was labelled with I^{125} using N-Bromosuccinimide method. Five microliters of NaI^{125} (0.5 mCi) was added into the eppendrof tube containing 4.8 ug of standard AFP in 10 ul of 0.5 M phosphate buffer. After mixing, 5 ul of N-Bromosuccinimide 200 ug/ml was added and the reaction tube was mixed for 20 sec. Then the reaction was stopped with 100 ul of 0.05 M phosphate buffer pH 7.4. The mixture was injected into HPLC using 7.8 mm (ID) x 30 cm (L)Protein Pak 125 column, at a flow rate of 1 ml/min with 0.05 M phosphate buffer (mobile phase) in isocratic system. The 0.5 ml fractions of label standard AFP were collected and pooled in 0.05 M Phosphate buffer.

2. Protocol of Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) and silver staining

The preparation of seperating gel (10% gel) and stacking gel (3.75% gel) was described in Appendix I,4.5. The gel slab was performed in slab gel casting apparatus. The seperating gel was poured and overlaid by distilled water and allowed to polymerized about 30 minutes. The water was removed and a stacking gel was overlaid on the seperating gel. The comb was inserted on the top and gel was allowed to polymerized about20-30 minutes. Before use,

the comb was carefully removed. The gel was inserted into the chamber of electrophoresis apparatus which readily contained electrode buffer (Appendix I,4.6). The samples were diluted with sample buffer and heated at 100° C for 2 min. THe sample was carefully added into the well. Electrophoresis was carried out at 100 volt, 20 mA constant current until stacking dye reached the bottom of the gel. The gel was stained with the silver stain plus kit (Appendix I,4.9) as follows, the gel was placed in a fixative enhancer solution (Appendix I,4.9.1) for 30 minutes, washed with 200 ml distilled water 2 times, and then placed in the staining and developing solution (Appendix I,4.9.2) for 20 minutes. After staining,the gel was fixed in 5% acetic acid for stoping the reaction. Finally, the gel was dried on Whatman filter paper by slab gel dryer.

3. Protocol used for the determination of specificity of monoclonal anti AFP

To perform the test, all reagents were added as follows.

100 ul standard protein
50 ul labelled AFP
50 ul monoclonal anti AFP Ior II (1.6ug)
200 ul 0.05 M phosphate buffer
vortex all tubes and incubate 4 c overnight
500 ul goat anti mouse (5 ug)
vortex, incubate room temperature 15 min

centrifuge 3000 rpm,40 min

decant and count

The specificity of monoclonal antibody was shown from the curve plotted between B/T and concentration of protein.

4. Protocol used for the determination of association constant (K)

The association constant of monoclonal antibody was calculated by means of a Scatchard plot. In this plot, K was derived from the slope of the straight line plotted between B/F and total bound. In the actual experiment performed, the concentration of AFP was given in pg. Conversion scales to mass and molar units was noted below. It was L/mole that was used in the calculation of K.

Association constant (K) = $D \times J \times Z \times 10^9$ L/mole

D = slope

J = incubation volume (ml)

Z = molecular weight

$$B/F = \frac{\text{cpm bound - cpm NSB}}{\text{Tc - cpm bound - cpm NSB}}$$

Total bound = unlabelled bound/tube + labelled bound/tube

labelled bound/tube (pg) = $B \times 0.045\ ZY/XS$

unlabelled bound/tube (pg) = $B \times I \times A \times 10^3$

Tc = total count

Y = cpm of Tc

X = efficiency of counter (%)

S = specific activity (Ci/mmole)

B = bound fraction (cpm)

I = volume of sample added (ml)

A = concentration of standard (ng/ml)

5. Preparation of IRMA AFP standards

Sixty-five microliters of standard AFP (sigma) concentration 770 ug/ml was diluted in 1 ml horse serum, previously shown to be free from AFP, to give a stock standard concentration 50 ug/ml.Then the stock standard(50 ug/ml) was diluted to 1:5,1:50 and 1:156 with horse serum to make standard AFP concentration 10,1 ug/ml and 320 ng/ml. Finally the AFP standard concentration 320 ng/ml was diluted by serial two - fold dilutions to give a range of working standard concentration 160,80,40,20 and 10 ng/ml. All of standards were aliqouted and stored at -20° C.

6. Protocol used for the cellulose solid phase AFP IRMA

To perform the test, all reagents were added as follows:

6.1. one step method

20 ul sample/standard

100 ul cellulose antibody

50 ul labelled antibody (100,000 cpm)

200 ul assay buffer

vortex all tubes and rotate at room temperature 3 hours or overnight

1 ml wash buffer

centrifuge 10 min, 3,000 rpm and decant

1 ml wash buffer

centrifuge 10 min, 3,000 rpm and decant

count tubes

6.2. <u>two step method</u>

20 ul sample/standard

50 ul labelled antibody (100,000 cpm)

vortex all tubes and rotate at room temperature 3 hours or 37° C 1,3 hr

100 ul cellulose antibody

200 ul assay buffer

vortex all tubes and rotate at room temperature 2 or 3 hours

1 ml wash buffer

centrifuge 10 min, 3,000 rpm and decant

1 ml wash buffer

centrifuge 10 min, 3,000 rpm and decant

count tubes

The AFP concentration was calculated from a standard curve plotted between B/T and concentration of AFP.

Vitae

Name	Miss Teerakul Arpornsuwan
Date of Birth	May 18, 1962
Place of Birth	Phetchburi, Thailand
Education	Bachelor of Science (Med. Tech.) from Chiang Mai University, Thailand Attended to the Faculty of Graduate Studies, Chulalongkorn University in the academic year 1991.
Office	Medical Scientist at Radioisotope laboratory section, Health Science Research Institute, Department of Medical Sciences, Ministry of Public Health