CHAPTER V

Refrence

1. มะเร็งแห่งชาติ , สถาบัน , มะเร็งปอดกับภาวะแวดล้อมและบุหรี่, กรุงเทพมหานคร, กรมการแพทย์, กระทรวงสาธารณะสุข, ไม่ระบุปี (จุลสาร).

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APPENDIX I

Prepared Solution

1.1 Agar coated slide

agar	0.5	g.
distilled water to	1000	ml.

Melt the agar in boiling water, stand until the temperature was 60-70 °C , then 3 ml of the melting agar was poured on microscopic slide, stand until the agar was hard,incubate at 37°C until the agar was dried on the slide.

1.2 1% Amido black

Amido black	1	g.
H_2O	45	ml.
Methanal	45	ml.
glacial acetic acid	10	ml.

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1.3 barbitone buffer (0.05 m. pH 8.2)

Sodium barbital	10	g.
1 N. HCl	15.8	ml.
distilled water to	1000	ml.

1.4 coating buffer

Sodium carbonate	1.59	g.
Sodium bicarbonate	2.94	g.
Distilled water to	1000	ml.

The pH was adjusted to 9.6

1.5 chromogen diluent solution

di-sodium hydrogen phosphate	22.5	g.
citric acid	5.6	g.
distilled water to	1000	ml.

The pH was adjusted to 6.0

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1.6 chromogen solution

O-phenylene diamine	10	mg.
chromogen diluent solution	30	ml.

30% H_2O_2 was added immediately before use.

1.7 conjugate diluent solution

0.9% saline (NSS)	3	parts.
chicken serum	1	part.

12 ml. of the solution was suitable for 50 Microliter of conjugate and plus with 0.1 ml. of sodium citrate. (1 mol/l. pH 7.4)

1.8 Phosphate buffer saline (0.15 M, pH 7.2)

Sodium chloride	8.0	g.
Potassium chloride	0.2	g.
di-potassium hydrogen phosphate	1.15	g.
Potassium di-hydrogen phosphate	0.2	g.
distilled water to	1000	ml.

The pH was adjusted to 7.2

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1.9 serum diluent

Sodium chloride	9	g.
Trisodium citrate	5.88	g.
Distilled water to	1000	ml.

1.10 sodium citrate solution (1 mol/l. pH 7.4)

Sodium citrate	2.14	g.
Distilled water to	10	ml.

The pH was adjusted to 7.4

1.11 Veronal buffer (0.05 M. pH 8.2)

Barbital	3.44	g.
Sodium barbital	7.57	g.
Distilled water to	1000	ml.

The pH was adjusted to 8.2

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1.12 <u>washing buffer</u>

di-potassium hydrogen phosphate	1.22	g.
potassium dihydrogen phosphate	0.408	g.
Sodium chloride	8.77	g.
Distilled water to	1000	ml.

The pH was adjusted to 7.4 with 4 mol/l sodium hydroxide and 0.1 ml tween 20 was added.

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APPENDIX II

Competitive Radioimmuno Assay (RIA, Gammadab kit)

The assay procedure of competitive RIA technique includes the preparation of a standard curve from which the unknown ferritin content in the sample is determined, each steps of the procedure was followed:

1. Allow all reagents to reach ambient temperature and mix before using.

2. Label the polypropylene or glass test tubes in duplicate according to the following scheme. Total counts (tracer) and ferritin blank may be required for certain data reduction programs.

3. Add to the appropreate tubes in duplicates:

   a. 25 microlitres of ferritin blank, 0 ng/ml.
   b. 25 microlitres of each ferritin standard, 5, 20, 50, 200 and 500 ng/ml.
   c. 25 microlitres of each ferritin control, levels I and II (10 and 100 ng/ml.).
   d. 25 microlitres of each patient samples.

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4. Add 100 microlitres of (^{125} I) Ferritin tracer to all tubes.

5. Add 100 microlitres of Rabbit Anti-Ferritin Serum to all tubes excepts total counts. Mix reagent by vortexing each tube.

6. Incubate tubes for 30 minutes at 37°C ± 2°C in a constant temperature water bath.

7. Add 500 microlitres of Precipitating Antirerum Reagent to all tubes (except total counts). Gently mix each tube on a vertex mixer set at a low speed.

8. Incubates tubes for five minutes at 37°C ± 2°C.

9. Centrifuge all tubes (except total counts) at 2-12°C for 15 minutes at a mimimum relative centrifugal force (RCF) of 1000 xg.

10. Carefully decant each tube (except total counts) into the waste beaker. Tap the rim of each tube vigorously onto the absorbent paper to remove any residual supernatant.

11. Count all tubes, including total counts, for one minutes in a gamma counter with the window suitably adjusted for Iodine-125.

12. calculate results with standard curve (Figure 26)

Note The method followed the (^{125} I) Ferritin Radioimmuno assay kit (Cat No.CA-590) of Clinical assay division of Travenol laboratories, INC. Cambridge, Massachusetts.

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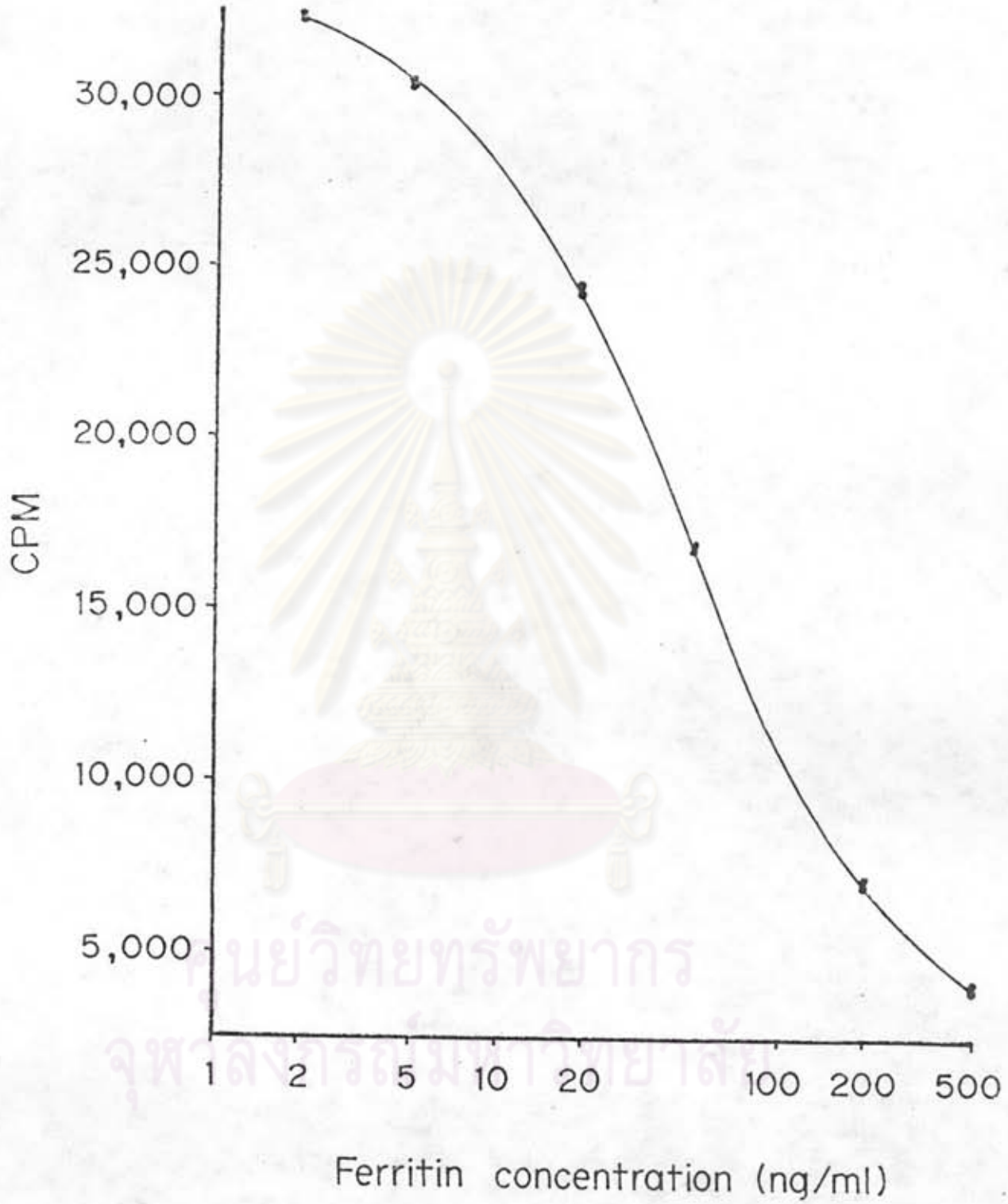

Figure 26(98) Standard curve of the RIA technique

Biography

Mr. Tirasak Pasharawipas was born on December 15, 1960 in Bangkok, Thailand. He graduated with the Bachelor degree of Science (Medical Technology) from the Faculty of Medical Technology, Chiengmai University in 1983.

He had experienced in The Unit of Immunology and Serology, Department of Pathology, Faculty of Medicine, Songkhlanakarin University.

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