Tumor Res. **18**, 29-39 (1983)

Latex Photoimmunoassay of Serum Ferritin

Yoshiro GOTO, Yoshiro NIITSU, Chikara ADACHI,
Makoto FUKUSHIMA, Fumio TAKAHASHI, Yutaka KOHGO,
Nobuko HOKARI and Ichiro URUSHIZAKI
Department of Internal Medicine (Section 4),
Sapporo Medical College,
Sapporo 060, Japan

SUMMARY

A basic analysis including recovery, reproducibility and dilution tests and clinical analysis of the latex photoimmunoassay, LPIA, produced acceptable results. One sample could be processed in a few minutes, which is a much shorter period of time than is required by the RIA or EIA, anzyme immunoassay. Sensitivity was a few nanograms per milliliter. The correlation coefficient with the IRMA was 0.973. Specificity was high and without the influence of various interfering factors in serum.

The LPIA, therefore, seems to be a simple but reliable tool for estimating iron storage conditions and a tumor marker for rapidly screening of malignant diseases.

Key words: Serodiagnosis, Serum ferritin, Latex photoimmunoassay, Turbidimetry

INTRODUCTION

Ferritin, known as an iron storage protein, is abundantly present in liver, spleen, bone marrow and the reticuloendothelial system (2, 7). Recent radioimmunoassay (RIA) advances have made it possible to detect a very small amount of ferritin in serum (1, 11), and have widened the clinical application of serum ferritin in the evaluation of storage iron or in monitoring therapy with iron administered to patients with iron deficiency anemia and under hemodialysis (13), However, several disadvantages, such as regulatory restrictions on the use of radioactive materials and a longer assay incubation period have arisen. Therefore, a new method with the same grade of sensitivity and specificity as the RIA that would also be simple and more rapid has been expected.

The latex photoimmunoassay, LPIA, based on near infrared turbidimetry to observe the immunological agglutination reaction between antigen and latex carrier

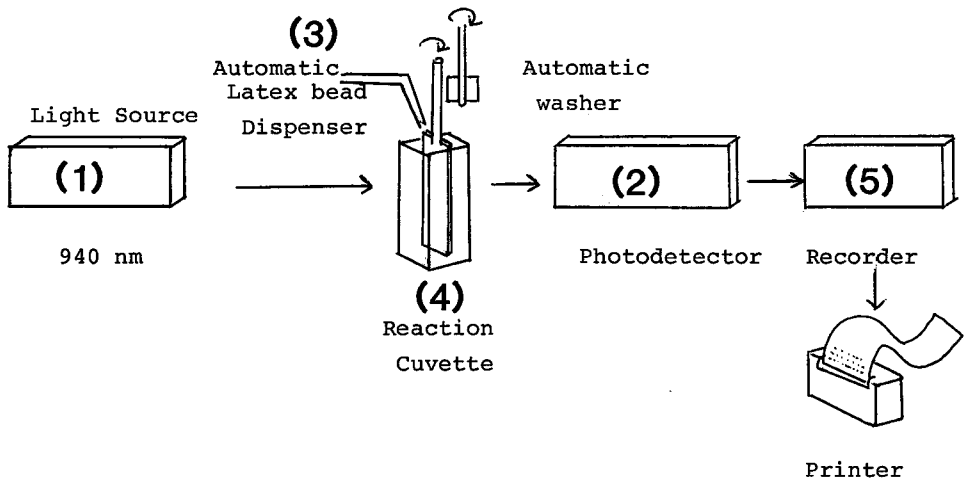

Fig. 1 Schematic Model of Automated Turbidimeter for Latex Photoimmunoassay

particles coupled with antibody in biological fluids is the new quantitative method. This method has been already accepted in evaluating AFP (5), CRP (4), FDP (7), and AT III (7) in serum. An automated model has been manufactured by Mitsubishi Chemical Industry, Ltd. for measurement of serum ferritin concentration. Basic and clinical analyses are evaluated in this report.

I. Principle of LPIA

LPIA is based on measurement of the latex agglutination reaction by the turbidimetric method as described by Sudo, *et al.* (8). Turbidity change near the infrared region correlates well with the amount of antigen or antibody to be examined and the reproducibility of the measurement is acceptable.

II. Automated Turbidimeter for LPIA

The automated model consists of 5 parts; 1) a light source, infrared 940 nm, 2) a photodetector for transmittance, 3) an automatic latex bead dispenser and washer, 4) a stirring device for latex beads and samples in a reaction cuvette and 5) a recorder. (Fig. 1)

The latex agglutination reaction is observed as a change in transmittance in a cuvette, integrated every second and recorded at 8 sec intervals for 180 sec. The kinetic values of each sample are recalculated with consideration for the temperature of the reaction fluid, and ferritin concentration is determined in reference to standard values. The sample number, reaction velocity, ferritin concentration and

temperature are printed out automatically.

III. Content of Kit

One kit consists of antiferritin antibody coated latex bead solution (1.5 m*l*), standard ferritin solution (1,000 ng/m*l*, 1 m*l*), stabilizing solution (13 m*l*), buffer (20 m*l*) and diluent (6 m*l*).

IV. Operational Procedure

1) Three serial dilutions of standard ferritin solution (1,000 ng/m*l*) with given diluent; i.e., 333, 111, 37 and 12.3 ng/m*l*, are prepared. Serum samples may be diluted ten times prior to the assay.
2) 200 μl of stabilizing solution is dispensed into each cuvette and then 50 μl of serum sample is added. Cuvettes are set in a cuvette holder.
3) Selecting a program filed for measuring ferritin, the cuvette holder moves to the assaying position, into which 50 μl of antibody coated latex bead solution is dispensed and then stirred. Transmittance of the agglutination reaction is measured automatically.
4) Computed results are displayed on chart paper.

V. Serum Samples and their Storage

Serum specimens were taken from 40 normal subjects (19 males and 21 females). Benign cases consisted of 4 patients with iron deficiency anemia, 4 with acute hepatitis, 10 with chronic hepatitis, 5 with liver cirrhosis and 6 with cerebral infarction. Malignant cases consisted of 6 patients with lung cancer, 9 with hepatoma, 8 with pancreas cancer, 19 with stomach cancer including 5 with metastasis to the liver, and 2 with malignant lymphoma.

All sera were quickly separated and kept frozen at −20℃ until assayed.

VI. Two Site Immunoradiometric Assay (IRMA)

The ferritin concentrations of identical specimens were measured by comparing the results of a two-site immunoradiometric assay, employing the SPAC® Ferritin Kit (Daiichi Radioisotope Laboratories, Tokyo), with those obtained by the LPIA.

RESULTS

Basic Analysis

A standard curve for the ferritin assay is shown in Fig 2., indicating the relationship between ferritin concentration and reaction velocity on a log-log plot and transmittance change in a time course study. It demonstrates that reaction

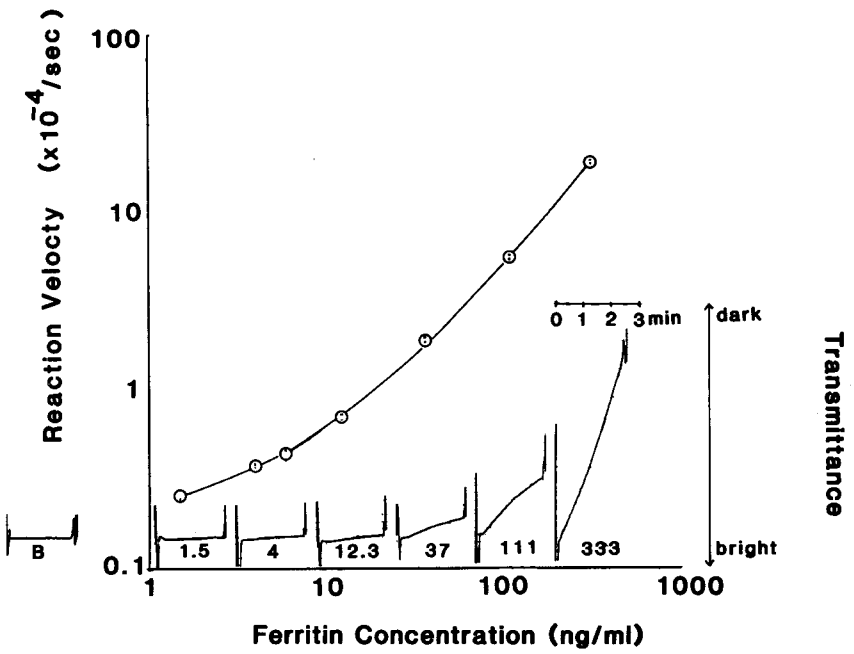

Fig. 2 Standard Curve for Ferritin Assay and Transmittance Change in a Time Course Study

velocity and turbidity change are depenent upon the ferritin concentration added.

Precision Studies

1) Within-Assay and Between-Assay Variation

Within-assay or intra-assay precision was evaluated by assaying three different serum ferritin concentrations 10 times each in a single run. The mean± SD (standard deviation) and CV (coefficient of variation) are shown in Table 1. CVs were 8.8, 3.7 and 1.8% with a mean of 4.8±3.8%. Sera with a lower ferritin concentration demonstrated higher CV values, but they did not exceed 10%.

Between-assay or inter-assay precision was evaluated by assaying three different ferritin concentrations in 10 consecutive assays. CVs were 6.4, 8.8, and 8.6% with a mean of 7.9±1.3% (Table 2).

The precision is therefore satisfactory measurement of ferritin.

2) Dilution Analysis

Serial dilution of three different ferritin concentrations was performed (Fig. 3). Lineality was observed in concentrations from 6.5 to 258 ng/m*l*.

3) Analytical Recovery of Added Ferritin

This was examined by enriching three different concentrations of sera, 39.6,

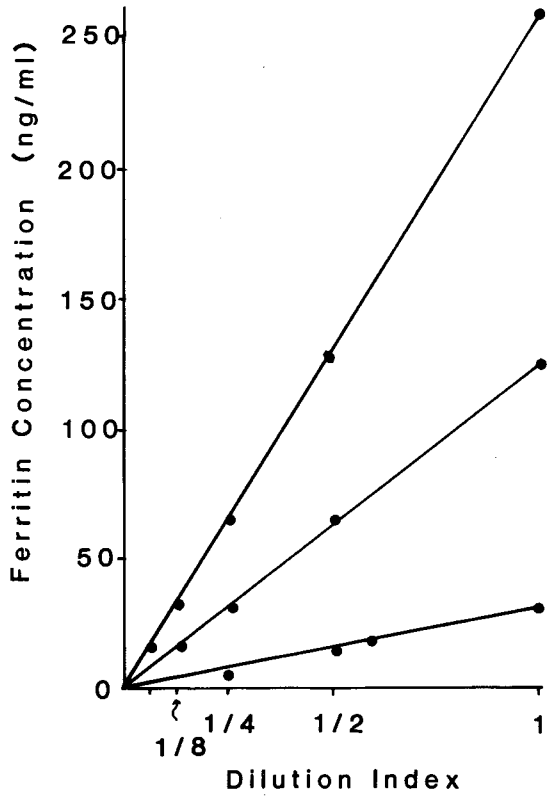

Fig. 3 Serial Dilution Analysis

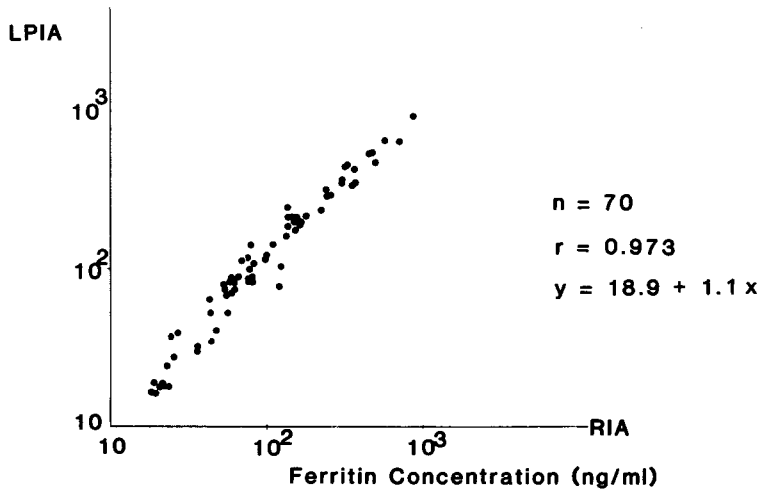

Fig. 4 Correlation between RIA (x) and LPIA (y)

Table 1 *Within-Assay Variation*

Sample	Mean (ng/m*l*)	S. D.	C. V.
No. 1 (n=10)	19.4	1.7	8.8
No. 2 (n=10)	56.4	2.1	3.7
No. 3 (n=10)	319.1	5.8	1.8

Table 2 *Between-Assay Variation*

Sample	mean (mg/m*l*)	S. D.	C. V.
No. 1 (n=10)	207.3	13.3	6.4
No. 2 (n=10)	106.9	9.4	8.8
No. 3 (n=10)	32.1	2.7	8.6

Table 3 *Analytical Recovery of Added Ferritin*

Serum 1

added ng/m*l*	measured ng/m*l*	expectation ng/m*l*	reoovery %
0	39.6		
3.5	43.1	43.1	100
10.6	48.1	50.2	95.8
31.7	74.2	71.3	104.1
95.1	148.2	134.7	110
mean			102.5±6.1

Serum 2

measured ng/m*l*	expectation ng/m*l*	reoovery %
111.8		
116.9	115.3	101.4
123.3	122.4	100.7
142.3	143.5	99.2
228.5	206.9	110.4
		102.9±5.1

Serum 3

added ng/m*l*	measured ng/m*l*	expectation ng/m*l*	reoovery %
0	188.5		
3.5	191.7	192	99.8
10.6	218.7	199.1	109.8
31.7	229.3	220.2	104.1
95.1	298.6	283.6	105.3
mean			104.8±4.1

111.8 and 188.5 ng/m*l* with standard ferritin to produce increments in ferritin concentration. The results ranged from 95.8% to 109.8% (103.4±4.8) (Table 3).

Table 4 *Effect of Sera in Various Pathological Conditions on Measurement of Serum Ferritin*

Serum Sample	Added (ng/m*l*)	Measured (ng/m*l*)	Expected (ng/m*l*)	Recovery (%)
RA (+)	0	257.0		
	6.2	260.6	263.2	99.0
	18.5	277.7	275.5	100.8
	55.5	313.8	312.5	100.4
	0	176.0		
	18.5	188.7	194.5	97.0
	55.0	225.9	231.5	97.6
	0	30.0		
	18.5	49.6	48.5	102.3
	166.5	205.3	196.5	104.5
	0	21.4		
	18.5	44.7	39.9	112.0
	166.5	213.2	187.9	113.5
			Mean±S.D.	103.0±6.0
Total Bil.				
4.1 mg/dl	0	179.1		
	6.2	180.2	185.3	97.2
	18.5	190.3	197.6	96.3
	55.5	244.7	234.6	104.3
1.4 mg/dl	0	169.4		
	18.5	201.6	187.9	107.3
	55.5	207.1	224.9	92.1
1.4 mg/dl	0	245.7		
	6.2	258.7	251.9	102.7
	18.5	283.7	264.2	107.4
	55.5	322.2	301.2	107.0
			Mean±S.D.	101.8±5.9

Serum Sample	Added (ng/m*l*)	Measured (ng/m*l*)	Expected (ng/m*l*)	Recovery (%)
Chylemia	0	171.7		
	18.5	183.7	190.2	96.6
	55.5	216.1	227.2	95.1
	166.5	317.1	338.2	93.8
	0	16.0		
	18.5	34.4	34.5	99.7
	55.5	71.8	71.5	100.4
	166.5	206.3	182.5	113.0
	0	38.4		
	18.5	57.4	56.9	100.9
	55.5	81.2	93.9	86.5
	166.5	202.1	204.9	98.6
			Mean±S.D.	98.3±7.1
Hemoglobin				
13.4 g/d*l*	0	185.2		
	18.5	201.1	203.7	98.7
	55.5	244.2	240.7	101.5
11.8 g/d*l*	0	31.3		
	18.5	46.9	49.8	94.2
	55.5	78.6	86.8	90.6
	166.5	195.3	197.8	98.7
2.1 g/d*l*	0	146.3		
	18.5	156.1	164.8	94.7
	55.5	191.3	201.8	94.8
	166.5	282.8	312.8	90.4
			Mean±S.D.	95.5±4.0
IgA				
2,370 mg/d*l*	0	114.2		
	18.5	125.1	132.7	94.3
	55.5	172.8	169.7	101.8
	166.5	299.5	280.7	106.7
IgG				
3,619 mg/d*l*	0	128.8		
	18.5	153.7	147.6	104.1
	55.5	191.8	184.3	104.1
	166.5	298.3	295.3	101.0
			Mean±S.D.	102.0±4.3

4) Correlation with IRMA (Fig. 4)

Correlation with IRMA, a sandwich method (x), was evaluated by the SPAC® ferritin kit. Comparison with the LPIA (y) resulted in a correlation coefficient of 0.973 and a regression equation of $y=18.9+1.1x$.

5) Interference of Serum Factors (Table 4)

Recovery tests were performed in order to determine the interfering effects of the various serum compoments stated below on measurement of serum ferritin by LPIA. Three different ferritin concentrations of sera, 18.5, 55.0 and 16.5 ng/m*l*

were added to 4 RA (rheumatoid arthritis) positive sera, 3 sera of hyperbilirubinemia, 3 of chylemia, 3 of hyperhemoglobinemia and 2 of hypergammaglobulinemia. Results were 103.0±6.0, 101.8±5.9, 98.3±7.1, 95.5±4.0, 102±4.3-%, respectively. None of these factors demonstrated remarkable interference in the determination of serum ferritin concentration by the LPIA.

Clinical Application

The serum ferritin concentrations of the 19 normal males in the study ranged from 24.3 to 166.1 ng/m*l* with a mean value of 98.5±46.5 ng/m*l*, while those of the 21 females ranged from 6.4 to 144.4 ng/m*l* with 42.8±66.2 ng/m*l*. The mean value in female subjects was approximately one half that in male subjects. (Fig. 5).

Eighty patient samples were examined by the LPIA. As shown in Fig. 5., the means and % positivity (the cut-off level was 192 ng/m*l*; mean+2SD) were 12.2±6.5 ng/m*l* in patients with iron deficiency anemia, 171.6±140.2 in chronic hepatitis, 101.6±89 in liver cirrhosis and 164.2±83.7 ng/m*l* in cerebral infarction. All these values in patients with benign diseases stayed within normal range except for those with acute hepatitis, 236.5±104.5 ng/m*l*, which was 2.4 times higher

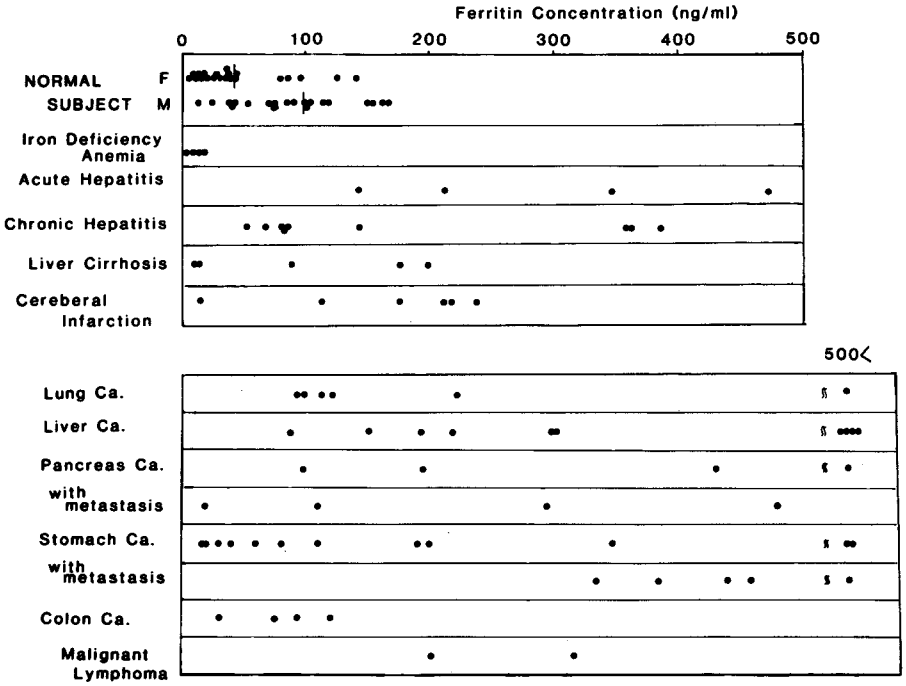

Fig. 5 Serum Ferritin Concentration of Normal Subjects and Patients with Various Diseases Determined by Latex Photoimmunoassay

than that of normal subjects.

In malignant diseases, on the other hand, the means and % positivity were 459.0±825 (2/6, 33%) in patients with lung cancer; 186.5±205.8 (4/13, 31%) in stomach cancer; 80.4±38.1 (0/4, 0%) in colon cancer; 420.1±380.3 (7/9, 78%) in liver cancer; 374.9±214.5 (3/5, 60%) in pancreas cancer, and 709.0±769.1 (4/4, 100%) in malignant lymphoma. Patients with gastrointestinal dieseases, except for those with liver metastasis, revealed ferritin values within normal range and the positivity was less than 33%. However, those with hepatoma, pancreas cancer and malignant lymphoma showed high values of ferritin and over 60% positivity.

Fig. 6 represents a 39-year-old female with iron deficiency anemia during a course of treatment with intravenous iron therapy, 40 mg per day. 12.1 ng/m*l* was the initial value of serum ferritin, which increased to 60.2 ng/m*l* after 5 consecutive injections of iron preparation, prior to any increase in hemoglobin or reticulocytes. The serum ferritin level of 108.9 ng/m*l* represents a restoration of storage iron after three week treatment, as well as of hemoglobin and RBC.

DISCUSSION

Determination of serum ferritin concentration has been made possible by

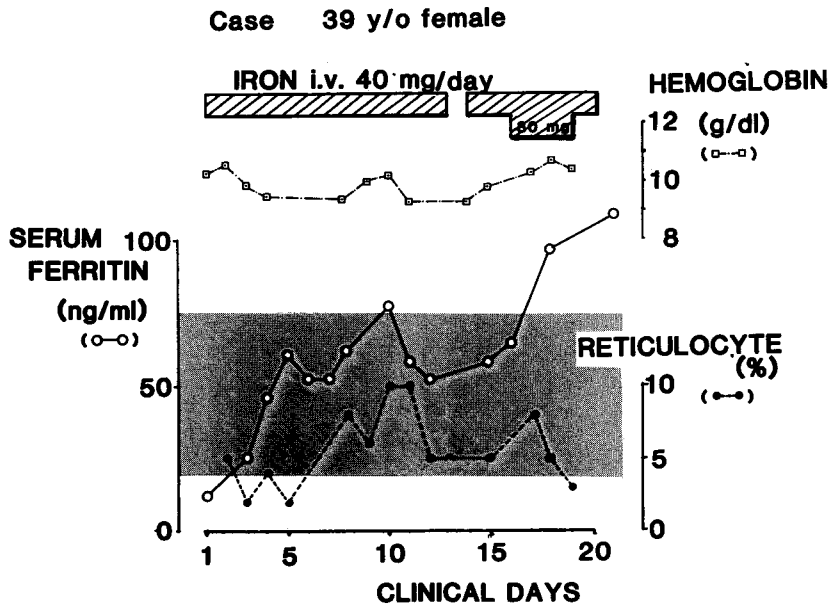

Fig. 6 A Clinical Course of a Patient with Iron Deficiency Anemia Treated with Intravenous Iron Therapy

development of the radioimmunoassay, initiated by Addison *et al* (1), the competitive radioimmunoassay (double antibody method) by Niitsu (13) and Marcus (8) and the immunoradiometric assay by Niitsu (12), Kohgo (6), Miles (9) and Halliday (3).

The LPIA, latex photoimmunoassay, based on a photometric assay using infrared turbidimetry, excludes radiation hazard, and the assay procedure is quite simple and rapid using the computarized model. It takes only a few minutes to determine ferritin concentration for one sample.

Basic analysis revealed satisfactory results in the measurable range of 12.3 to 333 ng/m*l*. Measurement of lower ferritin concentrations is sensitive enough to distinguish a clinical state of iron deficiency.

The " high dose " hook effect (10), one of the demerits of the IRMA, was not observed in the LPIA, since rapid and great change of transmittance in highly concentrated specimens resulted in automatic display of sample dilution requirements.

Greater precision in within-assay and between-assay and good correlation with values by the IRMA demonstrated adequate reproducibility of this assay method. Specificity was proven by the results of recovery tests in which no serum components interfered with the assay system.

A computer-controlled automated photometer made performace of the assay easy and shortened the attending time. Any problems, such as insufficient dispensing of latex solution or buffer or incorrect movement of the cuvette holder are indicated visually on the chart paper and by an alarm.

Clinical evaluation of 40 normal subjects showed a difference in sex, the mean ferritin concentration in females being one half of that in males. This result coincides with our own previous findings (13). Sera taken from patients with benign diseases revealed values within normal range, except for those patients with acute hepatitis, which is considered to be a leakage of tissue ferritin by acute cell damage as reported by others (6, 14, 15). Detection of lower ferritin concentrations in patients with iron deficiency anemia provides a useful tool in diagnosing such patients and in estimating the status of storage iron.

Higher values of serum ferritin concentration were observed in patients malignant diseases. This is also consistent with previous reports by the authors (12, 13). Elevation of serum ferritin was not evident among patients with cancers of the gastrointestinal tract unless metastasis was not overt. Therefore measurement of serum ferritin concentration should be helpful not only in screening those with a suspicion of malignancy, but also in monitoring pathological conditions including metastasis.

References

1. ADDISON, G. M., BEAMISH, M. R., HALES, C. N., HODGKIN, M., JACOBS, A. and LLEWELLIN, P.: J. Clin. Pathol. **25,** 326-329 (1972).
2. GRANICK, S.: Clinical Rev. **38,** 379-403 (1946).
3. HALLIDAY, J. W., GERA, K. L. and POWELL, L. W.: Clin. Chim. Acta **58,** 207-214 (1975).
4. IIDA, H., TAKEMURA, M., NIWA, H., KAWASE, M., ITO, M., NAKAMURA, S. and KAWADA, M.: Rinsho Kensa Kiki Shiyaku **6,** 705-710 (1983).
5. KAWAI, T., MUKOJIMA, T., YAMAGISHI, Y., TOMITA, K. and YUDA, M.: Rinsho Kensa **25,** 923-927 (1981).
6. KOHGO, Y., NIITSU, Y., WATANABE, N., OHTSUKA, S., KOSEKI, J., SHIBATA, K. and URUSHIZAKI, I.: Jap. J. Gastroenterol. **73,** 1553-1566 (1976).
7. KOHGO, Y. and NIITSU, Y.: Acta Haematol. Jpn. **45,** 1221-1228 (1982).
8. MARCUS, D. M. and ZINBERG, N.: J. Natl. Cancer Inst. **55,** 791-795 (1975).
9. MILES, L. E. M., LIPSCHITZ, D. A., BIEBER, C. P. and COOK, J. D.: Anal. Biochem. **61,** 209-224 (1974).
10. MILES, L. E. M.: In: Handbook of Radioimmunoassay, p. 132, Abraham, G. ed., Dekker, N. Y. (1977).
11. NIITSU, Y., KOHGO, Y., YOKOTA, M., and URUSHIZAKI, I.: Ann. N. Y. Acad. Sci. **259,** 450-452 (1975).
12. NIITSU, Y., KOHGO, Y., OHTSUKA, S., WATANABE, N., KOSEKI, J., SHIBATA, K., ISHITANI, K., NAGAI, T., GOCHO, Y. and URUSHIZAKI, I.: Onco-Developmental Gene Expression Papars presented in International Research Group for Carcinoembroyonic Protein, San Diego, U. S. A. (1976).
13. NIITSU, Y. and URUSHIZAKI, I.: Acta Haematol. Jpn. **41,** 1301-1308 (1978).
14. PRIETO, J., BARRY, M. and SHERLOCK, S.: Gastroenterology **68,** 525-533 (1975).
15. REISSMAN, K. R. and DIETRICH, M. R.: J. Clin. Invest. **35,** 588-595 (1956).
16. SUDO, T., TSUTSUI, S., ITO, M., MORITA, S. and SAWAI, M.: Kitasato Arch. Exp. Med. **53,** 55-68 (1980).
17. SUDO, T.: Personal Communication.