

Intended Use

The POINTE Ferritin assay is an *in vitro* diagnostic reagent for the quantitative determination of ferritin (an iron-storing protein) in human serum and plasma by immunoturbidimetric assay on the Roche / Hitachi 917 and other analyzers. Measurements of ferritin aid in the diagnosis of diseases affecting iron overload and iron deficiency anemia. FOR *IN VITRO* DIAGNOSTIC USE.

Introduction and Summary

Ferritin is an iron-containing protein with a molecular weight of approximately 450,000 daltons. It is found mainly in the human liver and spleen, where its function is to eliminate and store iron, and is also found in small amounts in human serum. Ferritin is decreased in iron deficiency anemia and increased in iron overload. Ferritin levels correlate with and are useful in evaluation of total body storage iron.

The POINTE Ferritin is a latex-enhanced immunoturbidimetric assay, developed to accurately and reproducibly measure ferritin levels in serum and plasma samples.

Principle of Test

When an antigen-antibody reaction occurs between ferritin in a sample and an anti-ferritin antibody, which has been sensitized to latex particles, agglutination occurs. This agglutination is detected as an absorbance change, with the magnitude of the change being proportional to the quantity of ferritin in the sample. The actual concentration is then determined by interpolation from a calibration curve prepared from calibrators of known ferritin concentration.

Kit Composition

Reagents (Liquid Stable)

R1: Buffer Reagent Glycine buffer solution (170 mM)	1x 18 mL
R2: Latex Suspension 0.07 % w/v suspension of latex particles sensitized with rabbit anti-human ferritin antibody	1 x 9 mL

Warnings and Precautions

FOR *IN VITRO* DIAGNOSTIC USE. Rx only.

Not to be used internally in humans or animals. Normal precautions exercised in handling laboratory reagents should be followed.

Do not mix or use reagents from one test kit with those from a different lot number.

Do not use reagents past their expiration date stated on each reagent container label.

Do not pipette by mouth. Avoid ingestion and contact with skin.

Reagents in this kit contain sodium azide as a preservative. Sodium azide may form explosive compounds in metal drain lines. When disposing of reagents through plumbing fixtures, flush with copious amounts of water. For further information, refer to "Decontamination of Laboratory Sink Drains to Remove Azide Salts," in the Manual Guide-Safety Management No. CDC-22 issued by the Centers for Disease Control, Atlanta, Georgia.

Reagent Preparation

Reagents are ready to use and do not require reconstitution.

Storage and Handling

All reagents should be stored refrigerated (2-8°C) and protected from light. Return all reagents to 2-8°C promptly after use. Unopened reagents can be used for 18 months from the date of manufacture as indicated on the expiration date on the package and bottle labels.

Reagent Stability

Discard reagents if they become contaminated. Evidence of precipitation in reagent 2 (R -2) solution is cause to discard. Opened reagents can be used for 1 month if stored at 2-8°C.

Specimen Collection and Handling

Serum

Blood should be collected from a patient and the serum separated as soon as possible. Soon after the blood is drawn, it should be allowed to clot, centrifuged, and the serum separated from the clot tube. For highly turbid samples, it is recommended that the sample be spun and the lipid layer be removed. It is recommended that the specimen collection be carried out in accordance with the NCCLS document M29-A2. After sampling, the specimen should be immediately stored at 2-8°C and assayed as soon as possible. If the assay cannot be performed within 24 hours, the sample should be tightly capped and frozen at -20 °C. Avoid repeated freeze-thaw cycles. Samples should be thawed at room temperature in a water bath or air. Undiluted samples should be used for this assay.

Plasma

Whole blood is collected in sodium EDTA or sodium heparin anticoagulant. After collection, immediately centrifuge. In the U.S., follow NCCLS guideline H3-A2. If the assay cannot be performed within 24 hours, then the sample should be tightly capped and frozen at -20°C. Avoid repeated freeze-thaw cycles. Samples should be thawed at room temperature in a water bath or air. Undiluted samples should be used for this assay.

Procedure

Materials Supplied

Reagent 1 (R-1) Buffer Reagent	1 x 18 mL
Reagent 2 (R-2) Latex Suspension	1 x 9 mL

Materials Required But Not Supplied

- Calibrators: POINTE Ferritin Calibrator (F7505-CAL)
(Containing known levels of Ferritin).
- Saline (0.9 % w/v NaCl)
- Roche / Hitachi 917 or other chemistry analyzer

Calibration

A five-point calibration curve should be made using the POINTE Ferritin Calibrator and saline (0 ng/mL). In our laboratory, the calibration curve was stable for at least 4 weeks. It is recommended that the user determine calibration frequency on their analyzer as the calibration curve stability may change due to analyzer condition and use. Calibration is recommended whenever a new lot of reagent is used or when quality control material is outside the specified range.

Quality Control

A quality control program is recommended for all clinical testing laboratories. It is recommended that controls, both normal and abnormal, be run with each batch of samples to monitor the procedure. Each laboratory should establish its own control range by assaying the control a sufficient number of times to generate a valid mean and acceptable range.

Calculations

Ferritin levels are determined using the prepared calibration curve.

Assay Procedure

Note: Allow all reagents and specimens to warm to room temperature. Mix all reagents gently before using.

An example of automated application:

Sample	7 µL
↓	
● ← R1 (Buffer Reagent)	140 µL
↓	
● ← R2 (Latex Suspension)	70 µL
↓	
37 °C, 5 min.	
↓	
Endpoint or Rate, 570 nm (main) / 800 nm (sub)	

Automated Method (Example)

Chemistry Parameters for Automatic Analyzer

INSTRUMENT	Roche / Hitachi 917
TEMPERATURE	37°C
TEST	(FER)
ASSAY CODE	(2 POINT END)(10) (18)(29)(0)(0)
WAVELENGTH	(800) (570)
SAMPLE VOLUME	(7.0) (0.0) (0)
REAGENT VOL (R1)	(140) (0)
REAGENT VOL (R2)	(0) (0)
REAGENT VOL (R3)	(70) (0)
REAGENT VOL (R4)	(0) (0)
ABS. LIMIT (SLOPE)	(32000) (INCREASE)
PROZONE LIMIT	(-32000) (0) (LOWER)
CALIB. TYPE	(SPLINE)
POINT	(5)
SPAN POINT	(5)
SD LIMIT	(999.9)
DUPLICATE LIMIT	(32000)
SENSITIVITY LIMIT	(0)
S1ABS RANGE	(-32000) (32000)
INSTRUMENT FACTOR	a=(1.0) b=(0.0)
UNIT	(ng/mL)
STD.(1) Conc.-POS.	(*1) - (1)
STD.(2) Conc.-POS.	(*2) - (2)
STD.(3) Conc.-POS.	(*3) - (3)
STD.(4) Conc.-POS.	(*4) - (4)
STD.(5) Conc.-POS.	(*5) - (5)
STD.(6) Conc.-POS.	() - ()

Use isotonic saline as STD (1)

*2-5: Input concentration of calibrators

Parameters for other automated analyzers are available.

Limitations

The measurable range for this ferritin test kit is between 2 and 1000 ng/mL. If the ferritin concentration of a sample is greater than the highest calibrator, dilute the sample with isotonic saline and repeat assay. Multiply the result by the dilution factor to compensate for the dilution. Performance of this assay has only been evaluated on adult specimens. Because there is no reference range for adolescent patients, this assay should only be used for adults. Intra-fat concentrations greater than 3% have been shown to interfere with the assay, however, intra-lipid concentrations up to 5% do not interfere with the assay.

Performance

Recovery

When a serum sample with a known ferritin value is assayed, the results obtained should be within ±8 %.

	Assigned Value	Measured	% Recovery
Sample 1	103	102.1	99.1 %
Sample 2	254	249.9	98.4 %
Sample 3	509	493.6	97.0 %
Sample 4	16	15.6	99.4 %

Precision

Within Run

Acceptance Criteria: When a sample is repeatedly assayed 10 times, the absorbance C.V. is less than 7 %.

Serum control samples were assayed 21 times on the same day, and the following results were generated.

Control #	Within-Run		
	1	2	3
N	21	21	21
Mean	14.9	100.0	431.1
Low	13.8	98.7	427.6
High	15.9	101.2	435.5
SD	0.600	0.647	2.203
CV%	4.03	0.65	0.51

Concentration in ng/mL

Between run: Serum control samples were assayed over a course of 20 different days, and the following results were generated.

Control #	Between Run		
	1	2	3
N	20	20	20
Mean	16.5	105.2	428.7
Low	15.4	102.7	421.0
High	18.1	107.6	432.6
SD	0.733	1.522	2.510
CV%	4.45	1.45	0.59

Concentration in ng/mL

Total Precision:

Control 1	3.44%
Control 2	1.54%
Control 3	0.83%

Accuracy / Correlation

A comparison of the POINTE Ferritin and another company's Ferritin on the Hitachi 917 was performed with the following results. Statistics were calculated by linear regression. Samples used in the correlation study were serum samples from Asian patients.

$$y = 0.89x - 9.4037$$

$$r = 0.997$$

$$n = 64$$

$$x = \text{Company A's Ferritin}$$

$$y = \text{Pointe Ferritin}$$

$$\text{Slope 95 \% CI} = \pm 0.0177 (0.8723 \text{ to } 0.9077)$$

$$\text{Y-Intercept 95 \% CI} = \pm 6.443 (-15.8467 \text{ to } -2.9608)$$

Linearity

Linearity was tested at the low and high range. The POINTE Ferritin has a linear range of 2 - 1,000 ng/mL.

Lower Limit of Detection

The analytical sensitivity is 2 ng/mL. This means that when saline and serum containing 2 ng/mL of ferritin are tested 10 times, + 2 SD of the respective results do not overlap each other.

Prozone

No hook effect seen up to 30,000 ng/mL ferritin.

Matrix Comparison

Serum vs. Sodium EDTA Plasma

$$y = 0.9895x + 0.2193$$

$$r = 0.997$$

$$n = 20$$

$$x = \text{Serum}$$

$$y = \text{EDTA-2Na Plasma}$$

$$\text{Slope 95 \% CI} = \pm 0.0422$$

$$\text{Y-Intercept 95 \% CI} = \pm 3.2018$$

Serum vs. Sodium Heparin Plasma

$$y = 1.01612x - 0.5524$$

$$r = 0.998$$

$$n = 20$$

$$x = \text{Serum}$$

$$y = \text{Sodium Heparin Plasma}$$

$$\text{Slope 95 \% CI} = \pm 0.0303$$

$$\text{Y-Intercept 95 \% CI} = \pm 2.4465$$

Interference

Bilirubin C	No interference up to 62 mg/dL
Bilirubin F	No interference up to 62 mg/dL
Hemoglobin	No interference up to 1,040 mg/dL
Rheumatoid Factor	No interference up to 520 IU/mL
Lipid	No interference up to 3 % Intra-fat (which contains 10% soybean oil)
	No interference up to 5 % Intra-lipid (which contains 10 % soybean oil, different manufacturer)

Expected Values

226 normal male and 205 normal female serum samples were assayed on a Hitachi 917 analyzer to yield the following expected reference range:

Male:	7-253 ng/mL
Female:	2-110 ng/mL

It is recommended that each laboratory establish its own expected range.

References

1. Cook, J.D., Lipschitz, D.A., Miles, L.E. & Finch, C.A.: Serum ferritin as a measure of iron stores in normal subjects. *Am. J. Clin. Nutr.* 27:681-687, 1974.
2. Addison, G.M., Beamish, M.R., Hales, C.N., Hodgkins, M., Jacobs, A. & Llewellyn, P.: An immunoradiometric assay for ferritin in the serum of normal subjects and patients with iron deficiency and iron overload. *J. Clin. Path.* 25:326-329, 1972.
3. Walters, G.O., Miller, F.M., Worwood, M.: Serum ferritin concentration and iron stores in normal subjects. *J. Clin. Pathol.* 26:770-772, 1973.
4. Marcus, D.M. & Zinberg, N.: Isolation of ferritin from human mammary and pancreatic carcinomas by means of antibody immunoabsorbents. *Arch. Biochem. Biophys.* 162:493-501, 1974.

Symbol Key

Use by (YYYY-MM-DD)	Lot and batch code
Catalog number	Manufacturer
In vitro diagnostic medical device	Temperature limitation
Consult instructions for use	Rx Only: Prescription Use Only
CE mark	Authorized representative in the European Community

F7505

Manufactured for HORIBA
Instruments Inc.- Pointe
Brand 5449 Research Drive
Canton, MI 48188



Manufactured by Kamiya Biomedical Company 12779 Gateway Drive, Seattle, WA 98168 USA	
European Authorized Representative: Advena Ltd. Tower Business Centre, 2 nd Floor Tower Street, Swatar, BKR 4013 Malta	

Certified to Perform Reagents

The Pointe reagents are certified to be manufactured according to specified parameters. Any Pointe reagent product not meeting specifications through its listed expiration date will be remedied immediately without charge.

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