FERRITIN

In vitro diagnostic reagents for the quantitative determination of Ferritin in serum by means of particle-enhanced turbidimetric immunoassay.

Diagnostic Relevance

Ferritin is a macromolecule with a molecular weight of at least 440 kD and is formed of apoferritin and an iron core of about 2500 Fe+3 ions

It has been found a direct correlation between the plasma ferritin concentration and the quantity of available iron stored in the body so that its determination is used for diagnosis and monitoring of iron deficiency and iron overload. Additional parameters (transferrin, transferrin saturation, and hematological investigations) could be required for the diagnosis of disturbances of distribution.

In a comparison of the various parameters available for the determination of the body's iron stores, plasma ferritin was the most efficient parameter, demonstrating a sensitivity of 80 %, and a specificity of 96 %.

The serum concentrations of ferritin are found to be elevated in patients with infections, inflammation or in hepatic or chronic renal diseases. The determination of ferritin is particularly useful in the diagnosis of iron therapy, for the determination of iron reserves in high-risk groups, and in the differential diagnosis of anemia.

Principle

This Ferritin test is based upon the reactions between Ferritin in the sample and latex-covalently bound rabbit antihuman Ferritin antibodies. Ferritin values are determined photometrically.

Reagents

Each Ferritin kit contains:

A.- Buffer – of phosphate buffer, pH: 6.7, containing protein stabilizers and 0,09 % sodium azide as preservative.

B.- Latex reagent – of a suspension of latex microparticules covalently bound anti- ferritin antibodies suspended in a neutral aqueous solution, with 0,09 % sodium azide as preservative

C.- Calibrator – Human - based reference fluid. Preservative: sodium azide, 0.09%. All raw materials of human origin used in the manufacture of this product showed no reactivity when tested for HBsAg, anti-HIV-1/2 and HCV with commercially available test methods. However, this product should be handled as though capable of transmitting infectious diseases

Reagent Preparation

Working Reagent is prepared with **75µl of Latex Reagent and 225 µl of Buffer Reagent.** Prepare a fresh WR based on its workload. Shake gently the reagents before pipetting.

Calibration Curve and Controls

Analytical Range up to 500 ng/mL.

Use BIOMED Ferritin Calibrator

For quality control use BIOMED Control or another suitable control material. The control intervals and limits must be adapted to the individual laboratory requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits. Control must be assayed and evaluated as for patient samples.

Storage and Stability

Reagents in the original vial is stable to the expiration date on the vial label when capped and stored at +2 - +8ºC. Immediately following the completion of an assay run, the reagent vial should be capped until next use in order to maximize curve stability. Do not freeze reagents.

The Ferritin latex reagent should have a white, turbid appearance free of granular particulate. Visible agglutination or precipitation may be a sign of deterioration, and the reagent should be discarted.

The Ferritin buffer reagent should be clear and colourless. Any turbidity may be sign of deterioration and reagent should be discarted.

WR is stable for up to 5 days at 4ºC. It is recommended that each Laboratory prepares a fresh Working Reagent based on its workload.

Precautions

For in vitro diagnostic use only. Do not pipette by mouth. Reagents containing sodium azide must be handled with precaution. Sodium azide can form explosive azides with lead and copper plumbing. Since absence of infectious agents cannot be proven, all specimens and reagents obtained from human blood should always be handled with precaution using established good laboratory practices. Disposal of all waste material should be in accordance with local guidelines. As with other diagnostic tests, results should be interpreted considering all other test results and the clinical situation of the patient.

Materials required

Spectrophotometric analyser. Controls.

Specimens

Specimens should be collected by venipuncture following good laboratory practices. Suitable assay specimens are human serum samples, as fresh as possible (stored up to 7 days at +2...+8ºC) or deep-frozen. Any additional clotting or precipitation, which occurs due to the freeze/thaw cycle, should be removed by centrifugation prior to assay.

Very lipemic specimens, or turbid frozen specimens after thawing, must be clarified before the assay by high-speed centrifugation (15 min at approx. 15.000 rpm).

Procedure

Wavelength	600 nm
Temperature	37ºC
Cuvette	1cm light path

Measurement against distilled water blank.
Bring the reagents at 37ºC and pipette:

	Calibrator	Sample
Calibrator	30 µ	---
Sample	---	30 µ
Distilled Water	---	---
Work. Reagent	300 µ	300 µ

Mix and measure absorbance immediately (A1) incubate 5 min (37ºC), after incubation read absorbance (A2).

Calculation

Plot the calibration curve and the sample concentration is obtained by interpolation the sample absorbance (A2-A1) in the calibration curve.

Example for Multi standard curve				
Cal. NO.	Cal. conc.	Cal.(High)	Saline	Total volume
Cal.1	15 ng/ml	5 µl	195 µl	200 µl
Cal.2	30 ng/ml	10 µl	190 µl	200 µl
Cal.3	150 ng/ml	50 µl	150 µl	200 µl
Cal.4	300 ng/ml	100 µl	100 µl	200 µl
Cal.5	600 ng/ml	200 µl	---	200 µl

If is an one point calibration:

$$\frac{(A2\text{-}A1)\,Sample}{(A2\text{-}A1)\,Calibrator} \times \text{Calibrator conc}$$

Reference Values

The determination of reference ranges for ferritin concentrations of clinically healthy individuals is very difficult. Ferritin concentrations are age- and sex- dependent and exhibit a wide range of distribution.

Children: Cord blood contains 100 a 250 ng/mL. In the first two months of life there is a rise of up to 600 g/L, followed by a fall of down to 1 g/L (Hb-neosynthesis).

Children and adolescents 15 - 120 ng/mL. (6 weeks to 18 years of age).

Men 30 - 300 ng/mL

Women (Pre-menopausal) 10 - 160 ng/mL

Women (Post-menopausal) 30 - 300 ng/mL

These data are to be interpreted as a guide. Each laboratory should establish its own reference intervals.

Specific Performance Characteristics

As is well known, the analytical characteristics of a clinical chemistry reagent depend on both the reagents and the instrument used. Multicenter studies indicate important differences in analytical characteristics among similar instruments. Therefore, the data must be calculated by each instrument.

Literature

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