

In vitro diagnostic reagents for the quantitative determination of human 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 anaemia.

Principle

The Ferritin test is based upon the reactions between Ferritin in the sample and latex-covalently bound antibodies against human Ferritin. Ferritin values are determined turbidimetrically using fixed-time measurement with sample blank correction. The relationship between absorbance and concentration permits a multipoint calibration with a measuring range between 0 and 500 ng/mL. The measuring temperature is 37°C. The assay can be performed on different instruments allowing turbidimetric measurements at 500 to 600 nm.

Reagents

Each Ferritin kit contains:

A.- Buffer - 25 mL of phosphate buffer, pH: 6.7, containing protein stabilizers and 0,09 % sodium azide as preservative.

B.- Latex reagent - 7,5 mL of a suspension of latex microparticles covalently bound anti-ferritin antibodies suspended in a neutral aqueous solution, with 0,09 % sodium azide as preservative.

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 but not provided

Automatic analyzer.
Saline solution.
Set of calibration.
Set of controls.

Storage and Stability

The ferritin reagents should be stored tightly capped at +2...+8°C when not in use. **Do not freeze.** Reagents in the original vials are stable to the expiration date on the vial label when capped and stored at +2...+8°C. Immediately following the completion of an assay run, the reagent vials should be capped until next use in order to maximize curve stability. Once opened the reagent can be used within 1 month if stored tightly closed at +2...+8°C after use.

The ferritin buffer reagent should be clear and colourless. Any turbidity may be sign of deterioration and reagent should be discarded.

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 discarded.

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...+8°C) 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

The reagents are ready to use as supplied. Reagents should be gently shaken before each use.

Follow the instructions of the operator's manual to load the cartridge, technique programation, calibration, sample measurement and control.

Calibration. Quality control

Standardization: use CRM® diagnostic systems Ferritin Calibrators. The method was standardized against OMS 80/578 international standard.

A multi-point calibration curve must be prepared using the **Ferritin calibration set** and newly opened reagents. This curve is stored in memory by the analyzer and recalled for later use. Calibration curves are stable for up to 14 days, after which a new curve must be generated. Additionally, recalibration must be performed whenever reagent lots are changed or quality control so indicates.

For quality control use CRM® diagnostic systems Ferritin Controls or other 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.

Calculation

The turbidimetric analysers automatically calculate the Ferritin concentration of each sample.

Reference Intervals

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 µg/L, followed by a fall of down to 1 µg/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.

Assay range and Sensitivity

The assay range is established from 0 to 500 ng/mL.

Calculating the mean plus 2SD of twenty replicates of zero standard resulted in a lower limit of detection of 5,2 ng/mL.

Specificity

The assay is specific for Ferritin determination. Interference from bilirubin, hemolysis or rheumatoid factors has not been observed.

Other substances can interfere. For a comprehensive review of interfering substances, refer to the publication by Young.

Assay Precision

Intra-assay coefficients of variation (CV) for three samples (ferritin values ranging from 30 to 300 ng/mL) were between 1,5 and 7,5 %; daily calibrated inter-assay CVs were between 3,2 and 8,5 %.

Assay Linearity

Linearity was evaluated using serial dilutions, prepared with saline solution, of two different samples, which contained values of Ferritin in the range of analysis. Linear regression values of Ferritin ng/mL vs concentration yielded correlation coefficients, r>0,999, for all samples. Within the assay's measuring range, the deviations of measurement from theoretical values did not exceed the 10 % level. In addition, the system did not show prozone phenomenon at least up to 5500 ng/mL.

Method comparison

46 samples (conc. approx. 5 to 500 ng/mL) were correlated with a nephelometric commercial procedure. When comparing the results by lineal regression the results was: $y = 1,01x + 0,34$ and $r=0,995$.

Analytical characteristics have been obtained in a single experiment in a Cobas-Mira plus analyser. 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 expressed in the present document should be interpreted as a guide example.

Literature

Wick M, Pinnggera W, Lehmann P. Ferritin in iron metabolism. Diagnosis of anemias. 2nd ed. Springer-Verlag, Wien 1994.
Miles LEM, et al. Measurement of serum ferritin by a 2-site immunoradiometric assay. Anal Biochem 1974; 61:209-224
Milmann N, Sondergaard M, Sorensen CM. Iron stores in female blood donors evaluated by serum ferritin. Blut 1985;51:337-345.
Young DS. Effects of Drugs on Clinical Laboratory Test. 5th Edition, AACC Press, 2000.
Sonderdruck aus DG Klinische Chemie Mitteilungen 1995; 26: 207 – 224.

Significados de los símbolos indicados en las etiquetas. Explanation of symbols used on labelling. Explication des symboles figurant sur les étiquettes. Spiegolung dei simboli utilizzati sull'etichetta. Significado dos símbolos indicados nas etiquetas. Erläuterung der Symbole auf den Etiketten.

| | LOT | REF | CE | REAG | CAL | Buffer | LYOPH | Conc. | Control H / Control L | | | |
|---|---|---|---|--|---|--|--|--|---|---|--|---|
| Fecha de Caducidad Expirate Date Date de Pénemption Data di Scadenza Data Expiração Verwendbar bis | Temperatura de almáren Storage Temperature Température de Conservation Temperatura de Conservação Lagertemperatur | Número de Lote Lot Number Número de Lot Número di Lotto Número de Lote Chargen-Nr. | Para Diagnóstico In Vitro For In Vitro Diagnostic Usage In Vitro Per Uso Diagnostico In Vitro Utilizar em Diagnostico In Vitro In Vitro Diagnostikum | Número de catálogo Catalog Number Número de catalogue Número di catalogo Número de catálogo Katalognummer | Conformidad Europea European Conformity Conformité aux normes européennes Conformità europea Conformidade com as normas europeias CE-Konformitätskennzeichnung | Fabricado por Manufactured by Fabrique par Fabbriato da Fabricado por Hergestellt | Reactivo Reagent Réactif Reagente Reagent Reagens | Calibrador Calibrator Calibrateur Calibratore Calibrator Kalibrator | Tampón Buffer Tampon Tampone Buffer Puffer | Liofilizado Lyophilised Lyophilisé Liofilo Liofilizado Lyophilisiert | Concentración Concentration Concentration Concentrazione Concentração Konzentration | Control Alto / Control Bajo Control High / Control Low Contrôle élevé / Contrôle Bas Controllo Alto / Controllo Basso Controlo Alto / Controllo Baixo Kontrolle Hoch / Kontrolle Niedrig |

Ferritin / Ferritina Procedure for / Procedimiento para COBAS MIRA PLUS

| | | | | | | | | | |
|--|---|-----------|------------|------------|-------------|-------------|-------------|-------|-------|
| <p>GENERAL</p> <p>Measurement mode: Absorb</p> <p>Reaction mode: R-S-SR1</p> <p>Calibration mode: LOGIT/LOG5</p> <p>Reagent blank: REAG/DIL</p> <p>Cleaner: NO</p> <p>Wavelength: 600 nm</p> <p>Decimal position: 2</p> <p>Units: ng/ml</p> <p>ANALYSIS</p> <p>Post. Dil. Factor: 10.00</p> <p>Conc. factor: NO</p> <p>SAMPLE Vol: 25 µL Cycle: 3</p> <p>DILUENT Name: Saline Vol: 20 µL</p> <p>REAGENT Vol: 200 µL Cycle: 1</p> <p>START REAGENT Vol: 75 µL Cycle: 1</p> <p>DILUENT Name: Saline Vol: 0.0 µL</p> <p>CALCULATION</p> <p>Sample limit: NO</p> <p>Reaction Direction: INCREASE</p> <p>Check: ON</p> | <p>CALCULATION (continued)</p> <p>Antigen excess NO</p> <p>Conversion factor: 1.00000</p> <p>Offset: 0.00000</p> <p>Normal range low: NO</p> <p>Normal range high: NO</p> <p>Number of steps: 1</p> <p>Calc. Step A: KINETIC</p> <p>Reading first: 3</p> <p>Reading last: 12</p> <p>Reaction limit: 3.5000 ΔA</p> <p>Point: T2</p> <p>CALIBRATION</p> <p>Calibr. Interval: ON REQUEST</p> <p>Reagent blank:</p> <p>Reag. Range low: NO High: NO</p> <p>Blank range low: NO High: NO</p> <p>STD POS**:</p> <table style="width: 100%; border: none;"> <tr> <td style="width: 50%;">1: 0.0***</td> <td style="width: 50%;">2: 37.5***</td> </tr> <tr> <td>3: 75.0***</td> <td>4: 150.0***</td> </tr> <tr> <td>5: 300.0***</td> <td>6: 500.0***</td> </tr> <tr> <td>7: NO</td> <td>8: NO</td> </tr> </table> <p>REPLICATE: SINGLE</p> <p>Deviation:</p> <p>Correction std:</p> | 1: 0.0*** | 2: 37.5*** | 3: 75.0*** | 4: 150.0*** | 5: 300.0*** | 6: 500.0*** | 7: NO | 8: NO |
| 1: 0.0*** | 2: 37.5*** | | | | | | | | |
| 3: 75.0*** | 4: 150.0*** | | | | | | | | |
| 5: 300.0*** | 6: 500.0*** | | | | | | | | |
| 7: NO | 8: NO | | | | | | | | |

(**) Select one position on the standard rack
 (***) Standard values on the labels / inserts