Quantitative determination of Ferritin in Human Serum. Latex Immuno Turbidimetric assay

**REF** TB1130  R1: 1x40 mL +  R2: 1x10 mL +  R3: 1x1 mL (calibrator)

**METHOD AND PRINCIPLE**
The latex particles coated with human anti-Ferritin antibodies are agglutinated when they react with samples containing Ferritin, producing agglutination with increased turbidity of the solution. The photometric measurement of the absorbance produced is directly proportional to the Ferritin present in the sample. The use of calibrators with a known titre allows the quantitative determination of the unknown samples.

**CLINICAL SIGNIFICANCE**
Ferritin is an universal intracellular protein that stores iron and releases it in a controlled fashion. Ferritin is found in most tissues as a cytosolic protein, but small amounts are secreted into the serum where it functions as an iron carrier. Plasma Ferritin is also an indirect marker of the total amount of iron stored in the body, hence serum ferritin is used as a diagnostic test for iron-deficiency anaemia. Ferritin is a globular protein complex consisting of 24 protein subunits and it is the primary intracellular iron-storage protein, keeping iron in a soluble and non-toxic form.

Serum Ferritin levels are measured in medical laboratories as part of the iron studies workup for iron-deficiency anaemia. The Ferritin levels measured usually have a direct correlation with the total amount of iron stored in the body. However, Ferritin levels may be artificially high in some cases of anaemia of chronic disease where Ferritin is elevated in its capacity as an inflammatory acute phase protein and not as a marker for iron overload. If the Ferritin level is low, there is a risk for lack of iron, which could lead to anaemia. Low Ferritin levels may also indicate hypothyroidism, vitamin C deficiency or celiac disease. Low serum Ferritin levels are seen in some patients with restless legs syndrome, not necessarily related to anaemia, but perhaps due to low iron stores short of anaemia.

If Ferritin is high, there is iron in excess or else there is an acute inflammatory reaction in which Ferritin is mobilized without iron excess. For example, Ferritin may be high in infection without signaling body iron overload. Ferritin is also used as a marker for iron overload disorders, such as hemochromatosis or hemosiderosis. Adult-onset Still's disease, some porphyrias, and hemophagocytic lymphohistiocytosis/macrophage activation syndrome are diseases in which the Ferritin level may be abnormally raised.

**REAGENT COMPOSITION**

**R 1 (Buffer):**
Buffer TRIS/Glycine pH 8.5 20 mmol/L

**R 2 (Antibody):**
Latex particles coated with polyclonal anti-human Ferritin antibodies. pH 8.2.

**R 3 (calibrator):**
Human Ferritin Value on label

The Ferritin concentration is traceable to the 3rd International Reference Material for Ferritin, 94/572 from WHO (NIBSC).

**REAGENT PREPARATION AND STABILITY**
Liquid and ready to use reagents, stable until the expiry date shown, if stored as indicated on the label and avoid contamination, evaporation and prolonged exposure to direct light. Do not freeze the reagents.

Discard the reagent if signs of deterioration appear, such as the presence of particles and turbidity or failure to recover the values of certified control sera. After opening the bottles, it is advisable to withdraw the necessary volume, to immediately close the bottles and store them in the fridge in order to avoid contamination, degradation from direct light and evaporation.

**SPECIMEN**
Fresh serum free of hemolysis.
Avoid hemolysate or lipemic samples. Separate the serum from the clot quickly. Perform a single defrost.
Stability: 7 days at 2-8 °C, 3 months at -20 °C

**PROCEDURE**
Wavelength: 650 nm+/− 20 nm
Temperature: +37°C
Zero adjust: distilled water

**CALIBRATION**
The results depend on the accuracy of the calibration, on the correct setting of the test on the instrument, on the correct volumetric ratio of reagent/sample and on the correct analysis temperature.

**Dilute Ferritin Calibrator in NaCl 9 g/L (physiological solution) as follows:**

<table>
<thead>
<tr>
<th>Dilution</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAL (μL)</td>
<td>-</td>
<td>10</td>
<td>25</td>
<td>50</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td>NaCl 9 g/L (μL)</td>
<td>100</td>
<td>90</td>
<td>75</td>
<td>50</td>
<td>25</td>
<td>-</td>
</tr>
<tr>
<td>Factor</td>
<td>0.0</td>
<td>0.1</td>
<td>0.25</td>
<td>0.5</td>
<td>0.75</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Multiply the concentration of Ferritin Calibrator by the corresponding factor to obtain the Ferritin concentration of each dilution.

To avoid manual calibrator dilutions, it is possible to use

**FERRITIN CALIBRATION SET - REF: TUC1080 (5 x 1 mL)**
a calibration set consisting of bottles of pre-diluted calibrators, with the exact value indicated on the label.

**CALCULATION**
Calculate for each sample and for each calibrator the ∆ Abs (Abs2 - Abs1). Construct a calibration curve (∆ Abs against concentrations of each calibrator) and report the values of the ∆ Abs (Abs2 - Abs1) of the samples to obtain their concentrations. For automatic calculations (provided by automatic analyzers), the recommended data processing method is the SPLINE Curve but other methods can be used (Point-to-Point, Logit-Log 4P, etc.).

**CONVERSION FACTOR:** 1 μg/mL = 1 ng/mL

**QUALITY CONTROL**
Normal and abnormal control sera of known concentration should be analysed routinely with each group of unknown samples.
Ferritin Turbidimetric

MTD Diagnostics Quality Control Material are:
- Plasma Protein Control. Level 1 REF: TUC1040 (3x1 mL)
- Plasma Protein Control. Level 2 REF: TUC1050(3 x1 mL)

The range of the values of the controls must be evaluated as a guideline, since it can be determined by the application of the method or by the user's manual skills or by other factors. The values obtained must be used for the evaluation of the Precision of the method (Repeatability). For the evaluation of the Accuracy of the method (Reproducibility) it is necessary to adhere to a program of External Quality Assessment (EQA) managed by certified bodies.

EXPECTED VALUES

<table>
<thead>
<tr>
<th>Category</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children</td>
<td>7 – 140 µg/L</td>
</tr>
<tr>
<td>Women</td>
<td>20 – 250 µg/L</td>
</tr>
<tr>
<td>Men</td>
<td>20 – 200 µg/L</td>
</tr>
</tbody>
</table>

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes the results should always be assayed in conjunction with the patient's medical history, clinical examinations and other findings.

PERFORMANCE

PRECISION:
- Low Level: Serum Samples (n) = 20 : Mean 65 – SD 2.31 – CV% 3.56
- High Level: Serum Samples (n) = 20 : Mean 178 – SD 3.32 – CV% 1.87

ACCURACY (CORRELATION):
A comparison between MTD Diagnostics method (y) and a commercially available test (x) gave following results:

\[ y = 1.002 x - 0.71 \quad r = 0.999 \]

LINEARITY: 10 – 300 µg/L
SENSITIVITY: 10 µg/L
PROZONE LIMIT: No prozone effect up to concentration of 4000 µg/L.

SPECIFICITY / INTERFERENCES
Bilirubin up to 20 mg/dL, Haemoglobin up to 1000 mg/dL, Reumathoid Factor up to 600 IU/mL do not interfere. Lipemia interferes. Other substances may interfere.

NOTES
1. This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meets the performance characteristics of the method. It is recommended to validate periodically the instrument.
2. The linearity limit depends on the sample/reagent ratio, as well as the analyzer used. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.
3. Do not dilute the calibrator in plastic tubes because Ferritin tends to bind to the plastic.
4. Heterophilic antibodies can interfere by reacting with the reagent antibody. This occurs especially in patients who are routinely exposed to animals or biological products of animal origin.

PRECAUTIONS
R1 and R2 contain TRIS BUFFER 100 mmol/L – pH 7.5 - CAS 1185-53-1

H315: Causes skin irritation
H319: Causes serious eye damage
H335: May cause respiratory irritation

The antibody present in the preparation are of animal origin and are not capable of transmitting infectious diseases to humans. However, since there are no methods to ensure the total absence of such infectious agents or of other microbes, this product must be handled as if it were risky and potentially capable of transmitting infectious diseases of any kind, in accordance with Good Laboratory Practice standards.

The human serum in the preparation (R3 calibrator) was tested by CE and FDA approved methods and found to be negative for the presence of hepatitis B virus surface antigen (HBsAg), human immunodeficiency virus (HIV) 1&2 and hepatitis C virus (HCV). However, because no test method can provide complete assurance that infectious agents or of other microbes are absent, this product should be handled as a potentially biohazardous material in accordance with Universal/Standard Precautions.

The products do not contain other dangerous substances or mixtures, according to the EC Regulation n ° 1272/2008 or their concentrations are such as not to be considered persistent, bioaccumulative or toxic (PBT). The product is classified and labeled in accordance with EC directives or respective national laws. Sodium azide, less than 0.1%.

However, in compliance with the normal prudential rules that everyone has to keep managing any chemical or laboratory reagent, in case of contact of reagents with the operator, you must apply the following first aid:

S26 (P305 – P351 – P338): In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S28 (P302–P352): After contact with skin, wash immediately with plenty of water.
S36/37/39 (P280): Wear suitable protective clothing, gloves and eye/face protection.

S46 (P301 – P310): If swallowed, seek medical advice immediately and show container or label. If victim is conscious and alert, wash out mouth with water.
S56 (P273): Dispose of this material and its container at hazardous or special waste collection point.
S63 (P304 – P340): In case of accident by inhalation: remove casualty to fresh air and keep at rest. If breathing is difficult, give oxygen and get medical aid.

All samples, calibrators and controls should be treated as potentially infectious material capable of transmitting HIV and hepatitis.

FOR MORE INFORMATION, REQUEST SAFETY DATA SHEET FOR REAGENT (MSDS) AT THE MANUFACTURER.

SIMBOLOGY

<table>
<thead>
<tr>
<th>CE Mark (EC Directive 98/79)</th>
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<tbody>
<tr>
<td>In Vitro Diagnostic</td>
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<td>Consult instructions for use</td>
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<tr>
<td>Contains sufficient for &lt;0 test</td>
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</tr>
<tr>
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BIBLIOGRAPHY
- Worwood M. Blood Reviews. 4:259-269 (1990)