Total Iron - Ferrozine

**Intended Use**

Spectrum Diagnostics iron is intended for the in-vitro quantitative, diagnostic determination of total iron in human serum.

**Background**

The majority of iron in the body (∼3 – 3.5 g) is found in the haemoglobin of the red blood cells or their precursors in the bone marrow. Plasma contains very small fraction of iron (∼ 2.5 mg). Iron is transported from one organ to another as a complex formed of ferric ions and a protein called apotransferrin, this iron-protein complex is called transferrin. The major iron-storage compound in the body is ferritin; it occurs in almost all body cells but particularly in hepatocytes. Serum iron is measured by the quantity of iron bound to transferrin, while TIBC is a direct measurement to transferrin. Elevated serum iron levels have been found in cases of haemochromatosis, hepatitis, hepatic necrosis and hemolytic anemia. Decreased levels have been associated with iron deficiency anemia, chronic blood loss, chronic disorders and insufficient dietary iron. TIBC varies in disorders of iron metabolism so, TIBC is elevated in iron deficiency anemia. The measurements of both serum iron and TIBC is fundamental in evaluation and differential diagnosis of various types of anemia, liver disease and chronic illness.

**Method**

Guanidine / Ferrozine method.

**Assay Principle**

Iron

Ferric ions are released from transferrin by guanidine hydrochloride and reduced to ferrous state by hydroxylamine. Ferrous ions react with ferrozine forming a coloured complex. To prevent copper interference, cupric ions are bound to thiourea.

\[
\text{Transferin-Fe(III)} \xrightarrow{\text{Guanidine-HCl}} \text{Apoptransferrin +Fe(III)} \xrightarrow{\text{Hydroxylamine}} \text{Fe(II)}
\]

Fe(II) + Ferrozine \rightarrow Colored complex

The color intensity is directly proportional to the iron concentration and is determined by monitoring the increase in absorbance at 546 nm.

**Reagents**

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Standard Iron (ST)</th>
<th>200 μg/dL</th>
<th>35.8 μmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 1 (buffer pH 4.5)</td>
<td>Acetate buffer</td>
<td>0.4 mol/L</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Guanidine hydrochloride</td>
<td>1.5 mol/L</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hydroxylamine hydrochloride</td>
<td>0.6 mol/L</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Thiourea</td>
<td>100 mmol/L</td>
<td></td>
</tr>
<tr>
<td>Reagent 1B (buffer pH 4.5)</td>
<td>Acetate buffer</td>
<td>0.4 mol/L</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Guanidine hydrochloride</td>
<td>1.5 mol/L</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Thiourea</td>
<td>100 mmol/L</td>
<td></td>
</tr>
</tbody>
</table>

**Reagent 2:** Ferrozine 60 mmol/L

**Reagent Preparation**

Prepare the working solution by adding 5 ml of chromogen (R2) to one bottle of buffer (R1). Or prepare the working solution according to the number of tests required by mixing 9 volumes of R1 and 1 volume of R2, e.g. 900 μL R1+100 μLR2.

**Precautions and Warnings**

Do not ingest or inhale. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries, seek medical advice immediately.

**Reagent Storage and Stability**

All reagents are stable until expiration date stated on label when stored refrigerated at 2 – 8 °C. Working solution is stable for 6 months at 2 – 8 °C.

**Specimen Collection and Preservation**

The recommended specimen is serum or heparinized plasma. Plasma specimens collected with EDTA, oxalate, or citrate as anticoagulants are unsatisfactory since they bind iron, preventing its reaction with the chromogen. Morning specimen is preferable to avoid low result due to diurnal variation. The biological half life of iron in blood is few hours.

**Stability:** 7 days at 15 –25 °C ; 3 weeks at 2 – 8 °C; 1 year at -20 °C.

**Procedure A-( Iron )**

<table>
<thead>
<tr>
<th>Reagent Blank</th>
<th>Standard</th>
<th>Sample Blank</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working Solution</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>-----</td>
</tr>
<tr>
<td>Dist.water</td>
<td>200μl</td>
<td>-----</td>
<td>200μl</td>
</tr>
<tr>
<td>Sample</td>
<td>-----</td>
<td>-----</td>
<td>200μl</td>
</tr>
<tr>
<td>Reagent 1B</td>
<td>-----</td>
<td>-----</td>
<td>1.0 ml</td>
</tr>
</tbody>
</table>

Mix, and incubate for 5 to 10 minutes at 20 – 25 °C. Read the absorbance of the standard and sample against reagent blank, and the absorbance of sample blank against distilled water within 30 minutes at 546 nm.

**Calculation**

Iron conc. (μg/dL) = \[
\frac{A_{\text{Sample}} - A_{\text{Sample blank}}}{A_{\text{Standard}}} \times 200
\]

**Quality Control**

Normal & abnormal commercial control serum of known concentrations should be analyzed with each run.
Performance Characteristics

Precise
Within run (Repeatability)

<table>
<thead>
<tr>
<th>Total Iron</th>
<th>Level 1</th>
<th>Level 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean (µg/dL)</td>
<td>159</td>
<td>344</td>
</tr>
<tr>
<td>SD</td>
<td>2.1</td>
<td>1.9</td>
</tr>
<tr>
<td>CV%</td>
<td>2.3</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Run to run (Reproducibility)

<table>
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<tr>
<th>Total Iron</th>
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</thead>
<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean (µg/dL)</td>
<td>162</td>
<td>351</td>
</tr>
<tr>
<td>SD</td>
<td>2.9</td>
<td>2.6</td>
</tr>
<tr>
<td>CV%</td>
<td>2.9</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Methods Comparison
A comparison between Spectrum Diagnostics Iron reagent and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.983 was obtained.

Sensitivity
When run as recommended, the sensitivity of this assay is 5 µg/dL for serum iron.

Linearity
The reaction is linear up to iron concentration of 500 µg/dL. Specimens showing higher concentration should be diluted 1+1 using physiological saline and repeat the assay (result × 2).

Interfering Substances
Serum, plasma

Haemolysis
No interference up to haemoglobin level of 5 g/L (0.3 mmol/L) in determining serum iron and up to 1 g/L.

Icterus
No significant interference up to a bilirubin level of 30 mg/dL.

Lipemia
Lipemic specimens are not recommended since they may cause negative bias. Lipemic specimens can be diluted before assay and the dilution factor should be considered during calculation.

Anticoagulants
Citrate, EDTA, and oxalate should be avoided.

Others
Pathological albumin levels more than 7 g/dL.

Expected values
Iron
Neonates : 36 – 184 µg/dL (6.4 - 33 µmol/L)
< 7 months : 37 – 145 µg/dL (7.7 - 33 µmol/L)
Adults Women : 37 – 145 µg/dL (6.6 - 26 µmol/L)
Men : 59 – 158 µg/dL (10.6 - 28.4 µmol/L)

Spectrum Diagnostics does not interpret the results of a clinical laboratory procedure; interpretation of the results is considered the responsibility of qualified medical personnel. All indications of clinical significance are supported by literature reference.

Analytical Range
5 – 500 µg/dL (0.9 – 89.5 µmol/L).

Waste Disposal
This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.
S56: dispose of this material and its container at hazardous or special waste collection point.
S67: use appropriate container to avoid environmental contamination.
S61: avoid release in environment. refer to special instructions/safety data sheets.

References

ORDERING INFORMATION

<table>
<thead>
<tr>
<th>CATALOG NO.</th>
<th>QUANTITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>272 001</td>
<td>100 Test</td>
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EC REP

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