Total Iron and Total Iron Binding Capacity Reagent Set

REF: 270 001 (50 test)  REF: 270 002 (100 test)

Reagent 1  1 x 45 ml  Reagent 1  1 x 90 ml
Reagent 1B 1 x 50 ml  Reagent 1  1 x 100 ml
Reagent 2  1 x 6 ml  Reagent 2  1 x 11 ml
Reagent 3  1 x 50 ml  Reagent 3  1 x 50 ml
Reagent 4  1 x 2.5 g  Reagent 4  1 x 2.5 g

Intended Use
Spectrum Diagnostics iron and total iron binding capacity (TIBC) reagent set is intended for the in-vitro quantitative, diagnostic determination of total iron and total iron binding capacity 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 of ferroic 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 hemochromatosis, hepatitis, hepatic necrosis and hemolytic anemia. Decreased levels have been associated with iron deficiency anemia, chronic blood loss, chronic disorders and insufficient dietary iron. The 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
Guandine / Ferrozine method.

Assay Principle
Iron
Ferric ions are released from transferrin by guandine 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.

\[
\text{Transferrin-Fe(III)} \rightarrow \text{Guandine-HCl} \rightarrow \text{Apotransferrin +Fe(III)}
\]

\[
\text{Fe(III)} \rightarrow \text{Hydroxylamine} \rightarrow \text{Fe(II)}
\]

\[
\text{Fe(II) + Ferrozine} \rightarrow \text{Colored complex}
\]

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

Total Iron Binding Capacity
The transferrin in the specimen is saturated with iron by exposure to excess ferric ions; then unbound iron is removed by addition of light magnesium carbonate and centrifugation. The iron bound to protein in the supernatant is measured by the Principle applied to total iron described above.

Reagents
Standard Iron (ST)  200 μg/DL  35.8 μmol/L
Reagent 1 (buffer pH 4.5)
Acetate buffer  0.4 mol / L
Guandine hydrochloride  1.5 mol / L
Hydroxylamine hydrochloride  0.6 mol / L
Thiourea  100 mmol/L

Symbols in Product Labelling
- Authorized Representative
- For in-vitro diagnostic use
- Temperature Limitation
- Use by/Expiration Date

Reagent 2: Ferrozine  60 mmol/L
Reagent 3: Ferriic chloride  120 μmol/L
Reagent 4: Magnesium carbonate powder.

Reagent Preparation
REF: 270 001 Prepare the working solution by adding 5 ml of chromogen (R2) to one bottle of buffer (R1).
REF: 270 002 Prepare the working solution by adding 10 ml of chromogen (R2) to one bottle of buffer (R1).

Procedures:
- To 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 μR2.

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></th>
<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>
<td>1.0 ml</td>
</tr>
<tr>
<td>Dist. water Standard</td>
<td>200μl</td>
<td>200μl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample</td>
<td>200μl</td>
<td>200μl</td>
<td></td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Reagent 1</td>
<td></td>
<td>1.0 ml</td>
<td></td>
<td></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**

**Precision**

Within run (Repeatability)

<table>
<thead>
<tr>
<th></th>
<th>Total Iron</th>
<th>TIBC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Level 1</td>
<td>Level 2</td>
</tr>
<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>
<thead>
<tr>
<th></th>
<th>Total Iron</th>
<th>TIBC</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Level 1</td>
<td>Level 2</td>
</tr>
<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>

**Anticoagulants**

Citrated, EDTA, and oxalate should be avoided.

**Others**

Pathological albumin levels more than 7 g/dL decrease the TIBC levels.

**Expected values**

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Iron</td>
<td>Neutones</td>
<td>&lt; 7 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adults Women</td>
</tr>
<tr>
<td></td>
<td>Men</td>
<td></td>
</tr>
<tr>
<td></td>
<td>36 – 184 μg/dL</td>
<td>37 – 145 μg/dL</td>
</tr>
<tr>
<td></td>
<td>(6.4 – 33 μmol/L)</td>
<td>(7.7 – 33 μmol/L)</td>
</tr>
<tr>
<td>TIBC</td>
<td>59 – 159 μg/dL</td>
<td>37 – 145 μg/dL</td>
</tr>
<tr>
<td></td>
<td>(10.6 – 28 μmol/L)</td>
<td>(6.6 – 26 μmol/L)</td>
</tr>
</tbody>
</table>

**Methods Comparison**

A comparison between Spectrum Diagnostics Iron and TIBC reagents 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 and 10 μg/dL for TIBC.

**Linearity**

The reaction is linear up to iron concentration of 500 μg/dL, and TIBC up to concentration of 1000 μg/dL. Specimens showing higher concentration should be diluted 1+1 using physiological saline and repeat the assay (result x 2).

**Procedure B- (TIBC)**

1. Pipette into a labeled tube 1 ml Reagent 3 and 0.5 ml test specimen.
2. Mix thoroughly and let stand for 5–30 minutes at 15 – 25 °C.
3. Add to each tube one spoonful of reagent 4, mix thoroughly and leave for 30 minutes at 15 - 25 °C and mix several times during the incubation time.
4. Centrifuge at 3000 r.p.m for 10 minutes.
5. Carefully remove the supernatant into a clean tube and determine the serum iron as described above in procedure A within one hour.

**Calculation**

Total iron binding capacity = Iron in supernatant × 3 (dilution).

**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 for TIBC.

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.