Direct Serum Total Iron Binding Capacity (TIBC)

REF: 273 001-D  100 Test

Reagent 1  2 x 50 ml
Reagent 2  1 x 16 ml
Calibrator  1 x 1 ml

Intended Use

Spectrum Diagnostics total iron binding capacity (TIBC) reagent is intended for the in-vitro quantitative, diagnostic determination of total iron binding capacity in human serum.

Background

The serum total iron-binding capacity (TIBC) represents the maximum concentration of iron that can be bound by an individual’s serum protein. Determination of TIBC is one of several commonly used assays in assessment of iron status and TIBC is highly correlated with serum transferrin (the primary serum iron transport protein) because > 95% of serum nonhem iron is bound by transferrin. Usually, only 30% of the available serum iron-binding sites are occupied, and changes in ratio of serum iron to TIBC reflect changes in the body iron stores.

Assay Principle

In the first step, the serum sample is added to reagent 1 (R1). R1 contains iron as ferric ion in sufficient quantity to saturate the highest anticipated TIBC in a complex with an excess of chromazol B in acetate buffer at pH 4.8. When the sample is saturated, the unbound iron is released from transferrin because of the low pH. The iron from sample then forms a complex with the remaining excess of chromazol B, increasing the absorbance. In the second step, reagent 2 (R2) which is strongly buffered is added. The affinity of transferrin for iron increases and the transferrin extracts iron from the iron-dye complex, decreasing the absorbance. The decrease in absorbance is directly proportional to TIBC.

Reagents

Reagent 1 (R1)
Acetate Buffer  pH 4.8  0.4 mol/L
Chromazol B  300 μmol/L
Surfactant  0.1 %
Non active ingredients.

Reagent 2 (R2)
MOPs buffer pH 8.0  100 mmol/L

Calibrator (C)
Actual concentration is stated on the vial label

Reagent Preparation, Storage and Stability

Reagents are supplied ready-to-use and stable up to the expiry date label on the bottles when properly stored refrigerated at 2 – 8 °C.

Calibrator:

The calibrator is vacuum sealed; therefore the vial should be reconstituted carefully with exactly 1 ml of distilled water. Close the vial carefully and allow the calibrator to stand for 30 minutes swirling occasionally: Avoid foaming! Do not shake! After reconstitution divide the calibrator into several aliquots. The tightly closed calibrator can be used within 60 days at –25 °C. Avoid repeated freezing and thawing.

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.

SYMBOLOGY IN PRODUCT LABELLING

EC Ref.  Authorised Representative
for in-vitro diagnostic use
TVD  Temperature Limitation
LOT  Batch Code/lot number
REF  Consult instructions for use
CAUTION: Consult instructions for use
Manufactured by

Specimen Collection and Preservation

The recommended specimen is serum. Plasma specimens collected with EDTA, oxalate, or citrate as anticoagulants are unsatisfactory since they bind iron, preventing its reaction with the reagent. 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.

System Parameters

Wavelength  630 nm
Assay type  End point
Direction  Decrease
Temperature  37 °C

Procedure

<table>
<thead>
<tr>
<th>Calibrator Blank</th>
<th>Calibrator</th>
<th>Sample Blank</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 1 500 μl</td>
<td>500 μl</td>
<td>500 μl</td>
<td>500 μl</td>
</tr>
<tr>
<td>Calibrator 40 μl</td>
<td>40 μl</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Sample -----</td>
<td>40 μl</td>
<td>40 μl</td>
<td>40 μl</td>
</tr>
</tbody>
</table>

Mix and incubate for 5 min, at 37 °C, then add R2

<table>
<thead>
<tr>
<th>Reagent 2 150 μl</th>
<th>150 μl</th>
</tr>
</thead>
</table>

Mix and incubate for 7 minutes then read the absorbance of the Calibrator against Calibrator Blank and absorbance of sample against sample Blank.

Calculation

Total iron binding capacity = \( \frac{A_{\text{sample}}}{A_{\text{calibrator}}} \times \text{calibrator Conc.} \)

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>TIBC</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>200</td>
<td>299</td>
</tr>
<tr>
<td>SD</td>
<td>2.12</td>
<td>1.36</td>
</tr>
<tr>
<td>CV%</td>
<td>1.06</td>
<td>0.45</td>
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</table>

Run to run (Reproducibility)

<table>
<thead>
<tr>
<th>TIBC</th>
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<td>n</td>
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<tr>
<td>Mean (μg/dL)</td>
<td>203</td>
<td>303</td>
</tr>
<tr>
<td>SD</td>
<td>2.19</td>
<td>1.42</td>
</tr>
<tr>
<td>CV%</td>
<td>1.12</td>
<td>0.51</td>
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</table>
Methods Comparison
A comparison between Spectrum Diagnostics 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 70 µg/dL.

Linearity
The reaction is linear up to concentration of 700 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 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.

Anticoagulants
Citrate, EDTA, and oxalate should be avoided.

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

Expected values

<table>
<thead>
<tr>
<th>TiBC</th>
<th>1 day</th>
<th>1 week</th>
<th>Infants</th>
<th>3 – 12 months</th>
<th>1 – 10 years</th>
<th>11 – 16 years</th>
<th>Adults Women</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg/dL</td>
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<tr>
<td></td>
<td>(24 – 57</td>
<td>(34 – 58</td>
<td>(27 – 61</td>
<td>(52 – 78</td>
<td>(47 – 89</td>
<td>(49 – 89</td>
<td>(49 – 89</td>
<td>(52 – 77</td>
</tr>
<tr>
<td></td>
<td>µmol/L</td>
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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

70 – 700 µg/dL (12.5 – 125 µmol/L).

Waste Disposal
This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

SS6: dispose of this material and its container at hazardous or special waste collection point.

SS7: use appropriate container to avoid environmental contamination.

SS1: avoid release in environment. Refer to special instructions/safety data sheets.

References

ORDERING INFORMATION

<table>
<thead>
<tr>
<th>CATALOG NO.</th>
<th>QUANTITY</th>
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<tr>
<td>273 001-D</td>
<td>100</td>
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