### Principle of the Test

**Step 1**: Reagent 1 (R1), an acidic buffer containing an iron-binding dye and ferric chloride is added to the serum sample. The low pH of R1 releases iron from transferrin. The iron then forms a colored complex with the dye. The colored complex at the end of this first step represents both the serum iron and excess iron already present in R1.

**Step 2**: Reagent 2 (R2), a neutral buffer is then added, shifting the pH and resulting in a large increase in affinity of transferrin for iron. The serum transferrin rapidly binds the iron by abstracting it from the dye-iron complex. The observed decrease in absorbance of the colored dye-iron complex is directly proportional to the total iron-binding capacity of the serum sample.

### Reagents

- **Reagent 1 (R1)** contains: 166 µmol/L chromazurol B, 735 µmol/L cetrimide, 16 µmol/L ferric chloride, acetate buffer, stabilizers, and preservatives.
- **Reagent 2 (R2)** contains: 338 mmol/L sodium bicarbonate, buffer, stabilizers, and preservatives.

### Reagent Preparation

The Direct TIBC Reagents, R1 and R2 are ready to use as supplied.

### Reagent Storage and Stability

The reagent is stable until the expiration date shown on the label when stored at 2-8°C.

### Precautions

The Direct TIBC Kit is for in-vitro diagnostic use. Normal precautions for handling laboratory reagents should be taken.

1. Do not ingest reagent, do not pipette by mouth.
2. Prevent contact with skin and eyes.
3. Do not mix reagents of different lot numbers.
4. All specimens and controls being tested should be considered potentially infectious. Universal Precautions, as they apply to your facility, should be used for handling and disposal of materials during and after testing.

### Specimen Storage and Collection

1. Serum is the specimen of choice. DO NOT USE PLASMA.
2. Samples should be separated from the red cells and analyzed promptly. However, the serum may be stored at 2-8°C, or at -20°C for up to one month. Serum can be stored at room temperature (22-28°C) for two weeks.

### Intended Use

For use in the determination of total iron-binding capacity in serum on automated chemistry analyzers. For in vitro diagnostic use only.

### Introduction

Total iron-binding capacity (TIBC) is the measure of the ability of serum proteins, principally transferrin, to bind iron. It is the maximum concentration of iron that the serum proteins can bind. Together with the total serum iron concentration, the TIBC is used in the diagnosis and treatment of iron deficiency anemia, other disorders of iron metabolism, and chronic inflammatory disorders. As an index of nutritional status, Serum TIBC is increased in iron deficiency, and decreased in anemia that is due to chronic disease.

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Run-to-Run (N = 25)

<table>
<thead>
<tr>
<th></th>
<th>Level 1</th>
<th>Level 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (µg/dL)</td>
<td>208</td>
<td>362</td>
</tr>
<tr>
<td>S.D. (µg/dL)</td>
<td>5.2</td>
<td>5.7</td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>2.5</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Expected Values

250 – 450 µg/dL

Since these ranges vary with different populations, it is recommended that each laboratory establish its own expected range.

Limitations

1. Using normal sera (average TIBC: approx. 350 µg/dL), the following substances were tested for possible interferences by addition and demonstrated less than 5% bias at least the limits given:
   - Bilirubin up to 25.6 mg/dL
   - Hemoglobin up to 500 mg/dL
   - Triglycerides up to 1690 mg/dL
   - Copper up to 3 mg/dL
   - Zinc up to 250 µg/dL
   - Nickel up to 500 µg/dL
   - Cuprimine up to 250 µg/dL
   - Imferon (as iron) up to 1430 µg/dL
   - Ascorbate greater than 20 mg/dL of ascorbic acid causes significantly decreased TIBC results.
   - Desferal demonstrated less than 5% bias up to 11.5 µg/mL and less than 10% bias up to at least 23 µg/mL

2. Serum is the preferred sample, Do Not Use Plasma.

References

5. U.S. Patent Number 6,627,448.