POINTE SCIENTIFIC, INC.
5449 Research Drive • Canton MI 48188 • USA

Direct TIBC Reagent Set

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.

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.

Materials Required but not Provided
General laboratory equipment.
Direct TIBC Calibrator Set

Calibration
The Direct TIBC Calibrator Set is required for calibration; refer to the Calibrator set package insert for directions. Follow the instrument manufacturer's guidelines for calibration performance and frequency, using quality control samples with each run to verify satisfactory calibration. [Results expressed in µg/dL may be converted to µmol/L by multiplying by 0.179]

Automated Procedure for Hitachi Analyzers

Wavelength:	660 nm
Temperature:	37°C
Mode:	Endpoint
Reaction time step 1:	5 min
Reaction time step 2:	10 min
Sample volume:	18 µL
Reagent 1 (R1) volume:	225 µL
Reagent 2 (R2) volume:	68 µL

The assay can be performed in a variety of automated chemistry analyzers. Details available on request.
All performance data included here were obtained using a Roche Hitachi 911® analyzer.

Calculation of Results
The instrument automatically calculates the results.

Quality Control
Reliability of test results should be monitored by including control sera, with known TIBC concentrations, in each assay run. These controls should be carried through the process and treated in the same manner as the patient's serum samples. The recovery of control values within the established acceptable range should be the criteria used in the evaluation of assay performance.

Linearity
The Direct TIBC method demonstrated linearity between 77 and 694 µg/dL TIBC.

Accuracy
A total of 65 sera having TIBC concentrations ranging from 150-550 µg/dL were assayed with the Direct TIBC assay and a commercially available magnetic separation based TIBC method. Regression analysis of the results yielded y = 1.02 (x) + 11.3, where y = the Direct TIBC method on the Hitachi 911 and x = DTIBC on Cobas Fara, and a correlation coefficient (r) of 0.991.

Precision
Two levels of TIBC were tested, using Multiqual® quality control material. Within-run and run-to-run precision (seven days) studies yielded the following:

Within-Run Precision (N = 25)

	Level 1	Level 2
Mean (µg/dL)	216	361
S.D. (µg/dL)	6.0	3.9
C.V. (%)	2.8	1.1

Run-to-Run (N = 25)

	Level 1	Level 2
Mean (µg/dL)	208	362
S.D. (µg/dL)	5.2	5.7
C.V. (%)	2.5	1.6

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

1. Tietz NW (ed). Textbook of Clinical Chemistry, 3rd ed. Philadelphia, PA: WB Saunders; 1701-1703; 1999.
2. NCCLS. Determination of Serum Iron and Total Iron Binding Capacity; Proposed Standard, NCCLS Document H17-P. Wayne, PA: NCCLS, Vol. 10, No. 4; 1990.
3. Starr RT. Use of an Alumina Column in Estimating Total Iron-Binding Capacity. Clin. Chem. 26: 156-158, 1980.
4. Gambino R., et al. The Relation Between Chemically Measured Total Iron-Binding Capacity Concentrations and Immunologically Measured Transferrin Concentrations in Human Serum. Clin. Chem. 43: 2408-2412, 1997.
5. U.S. Patent Number 6,627,448.

Manufactured for Pointe Scientific, Inc.
5449 Research Drive Canton, MI 48188

"European Authorized Representative"
(O.E.A.R.C.) Av. De Tervueren 34 bte
44 B-1040 Brussels, Belgium

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