Pointe Direct TIBC Reagent Set

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Intended Use
For use in the determination of total iron-binding capacity in serum on automated chemistry analyzers. For *in vitro* diagnostic use only. **Rx 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.
3. If the sample cannot be analyzed promptly or is being transported to reference laboratory, the serum must be separated from the cells immediately after collection.
4. Once separated from the cells 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, catalog number I7517-CAL.

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]

Procedure for Automated Analyzers

Wavelength:	660 nm
Temperature:	37°C
Mode:	Endpoint
Reaction time step 1:	5 min
Reaction time step 2:	7.5 min
Sample: Reagent 1: Reagent 2 Ratio	4:50 (R1) : 15 (R2)
Eg: Sample volume:	16 µL
Reagent 1 (R1) volume:	200 µL
Reagent 2 (R2) volume:	60 µL

The assay can be performed on a variety of automated chemistry analyzers. Details available on request.
All performance data included here were obtained using a COBAS Fara II® analyzer.

Calculation of Results
The instrument automatically calculates the results.

Quality Control
To ensure adequate quality control, normal and abnormal controls with assayed values should be run as unknown samples:
- At least once per day or as established by the laboratory.
- When a new bottle reagent is used.
- After preventative maintenance is performed or a critical component is replaced.
- With every calibration.

Control results falling above the upper limit or below the lower limit of the established range indicates the assay may be out of control.

The following corrective actions are recommended in such situations:
- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the controls.
- If results are still out of control, recalibrate with fresh calibrator, then repeat the controls.
- If results are still out of control perform a calibration with fresh reagent, then repeat the controls.
- If results are still out of range, contact Technical Services or your local distributor.

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Linearity
The Direct TIBC method demonstrated linearity between 70 and 700 µg/dL TIBC. Samples above 700 µg/dL should be diluted with 0.9% w/v laboratory saline

Accuracy
A total of 125 serum samples having TIBC concentrations ranging from 95 – 554 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 = 1.05 (x) - 5.4, where y = the Direct TIBC method and x = magnetic method, and a correlation coefficient (r) of 0.987.

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

Within-Run Precision (N=25)		
	Level 1	Level 2
Mean (µg/dL)	250	446
S.D (µg/dL)	9.0	8.2
c.v. (%)	3.6	1.8

Run to Run Precision (N=25)		
	Level 1	Level 2
Mean (µg/dL)	247	451
S.D (µg/dL)	9.5	10.4
c.v. (%)	3.8	2.3

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), several sub- stances were tested for possible interference. The following DID NOT INTERFERE as demonstrated by less than 5% bias to the limits shown:

Bilirubin	up to at least	32 mg/dL
Copper	up to at least	3 mg/dL
Zinc	up to at least	250 µg/dL
Nickel	up to at least	500 µg/dL
Chromium	up to at least	5 µg/dL
Cuprimine	up to at least	250 µg/dL Iron
Dextran (Imferon)	up to at least	1430 µg/dL
Hemoglobin	up to at least	500 mg/dL
Triglycerides	up to at least	1300 mg/dL

2. Ascorbate demonstrated less than 5% bias up to 10 mg/dL and less than 10% bias up to 20 mg/dL. Greater than 20 mg/dL of ascorbic acid causes significantly decreased TIBC results.
3. Desferal demonstrated less than 5% bias up to 11.5 µg/mL and less than 10% positive bias up to at least 23 µg/mL. Greater than 250
4. µg/mL Desferal causes significantly increased TIBC results.
5. Greater than 460 µg/dL of iron (ferrous sulfate) causes significantly decreased TIBC results.
6. 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. 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.


Exclamation Mark

Signal Word: Warning

Hazard Statements
Causes skin irritation
Causes serious eye irritation
Precautionary Statements - Prevention
Wash face, hands and any exposed skin thoroughly after handling Wear protective gloves/protective clothing/eye protection/face protection Wear eye/face protection
Precautionary Statements - Response
Specific treatment (see supplemental first aid instructions on this label)
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
If eye irritation persists: Get medical advice/attention
IF ON SKIN: Wash with plenty of soap and water
If skin irritation occurs: Get medical advice/attention
Take off contaminated clothing and wash before reuse
Precautionary Statements - Storage
None
Precautionary Statements - Disposal
None
Hazards not otherwise classified (HNOC)
Not applicable
Unknown Toxicity
8.4148% of the mixture consists of ingredient(s) of unknown toxicity
Reagent 2:
None

REF I7517


Manufactured for
HORIBA Instruments Incorporated
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IVD

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Certified to Perform Reagents
The Pointe reagents are certified to be manufactured according to specified parameters. Any Pointe reagent product not meeting specifications through its listed expiration date will be remedied immediately without charge.

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Use by (YYYY-MM-DD) LOT Lot and batch code REF Catalog number Manufacturer IVD *In vitro* diagnostic medical device Temperature limitation
Consult instructions for use CE CE mark EC REP Authorized representative in the European Community **Rx Only:** Prescription Use Only