



DIRECT - TIBC

(LIQUID)

3x30, 3x18 ml

RE - ORDER IT1160

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 µ mol/L chromazurol B, 735 µ mol/L cetrimide, 16 µ mol/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 STABILITY AND STORAGE

The reagent is stable until expiration date shown on 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.

Do not ingest reagent, do not pipette by mouth.

Prevent contact with skin and eyes.

Do not mix reagents of different lot numbers.

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

Serum is the specimen of choice. DO NOT USE PLASMA.

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.

MATERIAL 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 µ g/dL may be converted to µ mol/L by multiplying by 0.179]

| | |
|------------------------|----------|
| Wavelength: | 660 nm |
| Temperature: | 37°C |
| Mode: | Endpoint |
| Reaction time step1: | 5 min |
| Reaction time step2: | 10 min |
| Sample volume: | 18 µ L |
| Reagent 1 (R1) volume: | 225 µ L |
| Reagent 2 (R2) volume: | 68 µ L |

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 µ g/dL TIBC.

ACCURACY

A total of 65 sera having TIBC concentrations ranging from 150-550 µ g/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 Multiqua® 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 (µ g/dL) | 216 | 361 |
| S.D. (µ g/dL) | 6.0 | 3.9 |
| C.V. (%) | 2.8 | 1.1 |

Run-to-Run Precision (N=25)

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

EXPECTED VALUES

250-450 µ g/dL

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

AUTOMATED PROCEDURE FOR HITACHI ANALYZERS

LIMITATIONS

Using normal sera (average TIBC: approx. 350 μ g/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 25.6mg/dL

Hemoglobin up to 500 mg/dL

Triglycerides up to 1690 mg/dL

Copper up to 3 mg/dL

Zinc up to 250 μ g/dL

Nickel up to 500 μ g/dL

Cuprimine up to 250 μ g/dL

Imferon (as iron) up to 1430 μ g/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 μ g/mL and less than 10% bias up to at least 23 μ g/dL.

Serum is the preferred sample, Do Not Use Plasma.

REFERENCES

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NCCLS. Determination of Serum Iron and Total Iron Binding Capacity; Proposed Standard, NCCLS Document H17-P. Wayne, PA: NCCLS, Vol. 10, No. 4; 1990.

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