Brown Clinic Northridge
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BROWN CLINIC
LABORATORY PROCEDURE MANUAL

PROCEDURE: Total Iron Binding Capacity

PURPOSE:

The IBCT method used on the Dimension EXL200 Integrated chemistry system is an *in vitro* diagnostic test intended to quantitatively measure Total Iron Binding Capacity in **serum**.

PRINCIPLE:

Total iron binding capacity (IBCT) is a measure of the serum transferrin iron binding capacity. Measurements of serum iron and total iron binding capacity are widely used in the diagnosis and treatment of iron deficiency anemia and chronic inflammatory disorders.[1] This fully automated method adds saturating iron to the sample to saturate the transferrin iron-binding sites. The excess unbound iron is photometrically determined (instead of being physically removed by adsorption), in a manner similar to those described by Yamanishi et. al.[2] Subsequent addition of acid causes the release of bound iron from transferrin, which is then analyzed using the chromogen Ferene®. Earlier work by Higgins,[3] Artiss, et. al.[4] and Hennessy et. al.[5] demonstrated the high sensitivity of Ferene® and its utility in iron assays. A surfactant is used to prevent protein precipitation. Reaction times are optimized to minimize interferences, and thiourea is present to complex copper.

Ferene® is a registered trademark of Diagnostic Chemicals, LTD., Charlottetown, P.E.I., Canada C1A4H5, for the compound 3-(2-pyridyl)-5,6-bis-2-(5-furl sulfonic acid)-1,2.4-triazine disodium salt.

The serum sample is automatically mixed with a ferric iron solution, which saturates all available iron-binding sites of transferrin. Under non-acidic conditions (pH 8.6), only unbound, excess saturating iron is available to be reduced to ferrous iron by ascorbic acid and to form a blue complex with Ferene®. Subsequent addition of acid (final pH of 4.5) releases the iron bound to transferrin; this additional iron is reduced to ferrous iron by ascorbic acid and forms an increased amount of blue complex with Ferene®. The increase in absorbance upon shifting from pH 8.6 to pH 4.5, measured using a bichromatic (600,700 nm) endpoint technique, is proportional to the concentration of transferrin-bound iron, and thus to the iron binding capacity (total) of the serum or plasma sample.

$$\text{Transferrin} + Fe^{+++} \xrightarrow{\text{pH 8.6}} Fe^{+++} - \text{Transferrin} + Fe^{+++}$$

Fe^{+++} + Ferene® + Ascorbic Acid ⟶ Dehydroascorbic acid + 2 H^+ + Fe^{++} – Ferene® complex (absorbs at 600 nm)

Fe^{+++} – Transferrin $\xrightarrow{\text{pH 4.5}}$ Transferrin + Fe^{+++}

2Fe^{+++} + Ferene® + Ascorbic Acid ⟶ Dehydroascorbic acid + 2 H^+ + Fe^{++} – Ferene® complex (absorbs at 600 nm)

INSTRUMENT, REAGENTS & SUPPLIES:

Wells[a]	Form	Ingredient	Concentration[b]
1,2,3	Tablet	Ascorbic acid	19 mM
4	Liquid	Ferene®	0.56 mM
5,6	Liquid	Ferric chloride	0.02 mM
		Citric acid	0.2 mM
7	Liquid	Acetate buffer	500 mM
		Thiourea	33 mM
8	Liquid	Tris Buffer	200 mM

a. Wells are numbered consecutively from the wide end of the cartridge.

b. Nominal value in final reaction mixture.

Precautions:

Harmful. Contains thiourea.

Contains mixture of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1)

Limited evidence of a carcinogenic effect. Irritating to eyes and skin. May cause sensitization by skin contact. Avoid contact with skin. Wear suitable gloves.

Used cuvettes contain human body fluids. Handle with appropriate care to avoid skin contact and ingestion.

For *in vitro* diagnostic use

Safety Data Sheets (SDS/MSDS) can be found at www.siemens.com/healthcare

Reagent Preparation: Mixing and diluting are automatically performed by the Dimension® system.

STORAGE & STABILITY:

Store at 2 - 8°C.

Expiration: Refer to carton for expiration date of individual unopened reagent cartridges. Sealed cartridge wells on the instrument are stable for 30 days. Once wells 1 through 3 have been entered by the instrument, they are stable for 6 days. Once wells 4 through 8 have been entered by the instrument, they are stable for 30 days.

SPECIMEN REQUIREMENTS:
Normal procedures for collecting serum may be used for samples to be analyzed by this method
Follow the instructions provided with your specimen collection device for use and processing.[7]
For serum, complete clot formation should take place before centrifugation.[8]
Serum should be removed from cells within two hours.[9]
Heparinized plasma samples may show falsely elevated IBCT results, therefore do not use plasma samples.

Specimen:

Patient preparation: no special preparation required
Specimen type: **serum**
Stability:
Room temperature: 4 days
Refrigerated at 2-8°C: up to 7 days
Frozen at -20°C: for up to 2 months
The purpose of specimen storage information is to provide guidance to users; however, users may validate their own procedures for storing patient samples.

CONTROLS:
At least once daily run solutions at two levels of a quality control material with known concentrations.

For further details, refer to your Dimension® system manual. The result obtained should fall within limits defined by the day-to-day variability of the system as measured in the user's laboratory. If the results fall outside the laboratory's acceptable limits, follow the procedure in the quality control policy.

PROCEDURE:
Materials Needed: IBCT Flex® reagent cartridge, Cat. No. DF84
IBCT Calibrator, Cat. No. DC84

Test Steps

Sampling, reagent delivery, mixing, separation, processing and printing of results are automatically performed by the Dimension® system. For details of this processing, refer to your Dimension® Operator's manual.

Test Conditions

• Sample size:	25 µL
• Reagent 1 Volume:	36 µL
• Reagent 2 Volume:	25 µL
• Reagent 3 Volume:	25 µL
• Reagent 4 Volume:	75 µL
• Test Temperature:	37°C ±1.0°C
• Wavelength:	600 and 700 nm
• Type of measurement:	Bichromatic, endpoint
• Units	µg/dL [µmol/L]

Calibration

The general calibration procedure is described in your Dimension® system manual (also see Appendix B). The following information should be considered when calibrating the IBCT method:

• Assay Range	36 – 1000 µg/dL [6.4 – 179.1 µmol/L]
• Reference Material	IBCT Calibrator (Cat. No. DC84)
• Suggested Calibration Levels	0, 550, 1100 µg/dL [0, 98, 197 µmol/L]
• Calibration Scheme	Three levels in triplicate
• Calibration Frequency:	Every new reagent cartridge lot Every 90 days for any one lot For each new lot of Flex reagent cartridges After major maintenance or service, if indicated by quality control results As indicated in laboratory quality control procedures When required by government regulations
• Assigned Coefficients:	C_0 = -50.00 C_1 = 7.0

Back-up Testing :

Refer to Brown Clinic Backup Policy

INTERPRETATION:

The instrument automatically calculates and prints the concentration of Total Iron Binding Capacity (IBCT) in µg/dL [µmol/L] using the calculation scheme illustrated in your Dimension® system manual

EXPECTED VALUES:

250-450 µg/Dl

REPORTING:

report format: ug/dl

LIMITATIONS:

Results: >1000 µg/dL [179.1 µmol/L]

Manual dilution: Make appropriate dilution with Purified Water to obtain results within assay range. Enter dilution factor. Reassay. Resulting readout is corrected for dilution.

Autodilution: Refer to your Dimension® system manual. Recommended Auto Dilute volume is 12 µL.

The instrument reporting system contains error messages to warn the operator of specific malfunctions. Any report slip containing such error messages should be held for follow-up. Refer to your Dimension® system manual.

Analytical Specificity

For Known Interfering Substances section refer to package insert.

For Known Non-Interfering Substance refer to package insert.

For Additional Technical Information refer to package insert.

Reference: Flex® reagent cartridge insert sheet PN 717084.001 Issue date 1-30-2015

Origination Date: 9-10-07
Date of Implementation: 11-10-10
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Written By: ____Lori Murray MT(ASCP)_____ Date: _8-8-12

Approved By: _____Aaron Shives, MD_____ Date: ___10/21/2017_____
Laboratory Director

REVIEW - REVISION SUMMARY DOCUMENTATION

Date	By	Revision Summary
07/10	Lori Murray	New format
8-8-12	Lori Murray	Changed instrumentation to EXL200 and specimen Requirements
6/16/15	Heather Hall	Updated specimen requirements and AMR
10/18/17	Heather Hall	Updated information to insert dated 1/30/2015, modified backup process