Brown Clinic Northridge
511 14th Ave. NE
Watertown, SD

Brown Clinic
506 First Ave. SE
Watertown, SD

BROWN CLINIC
LABORATORY PROCEDURE MANUAL

PROCEDURE: Iron

PURPOSE:
The IRON method used on the Dimension EXL200 integrated Chemistry system is an *in vitro* diagnostic test intended for the quantitative determination of iron in **serum**. Iron measurements are used in the diagnosis and treatment of diseases such as iron deficiency anemia and other disorders of iron metabolism.

PRINCIPLE:
The iron method is an adaptation of direct iron assays developed by Smith et al.[1] using the chromophore Ferene®. Earlier work by Higgins,[2] Artiss et al.[3, 4] and Hennessy et al.[5] demonstrated the high sensitivity of Ferene® and its utility in iron assays. Potential copper interference is minimized by the addition of thiourea.

Ferene® is a registered trademark of Diagnostic Chemicals, LTD., Charlottetown, P.E.I., Canada C1A4H5.

Under acidic conditions, iron (Fe^{3+}) bound to the protein transferrin is released. In the presence of the reducing agent ascorbic acid the resulting product, Fe^{3+} is reduced to Fe^{2+}. Fe^{2+} forms a blue complex with 5,5'(3-(2-pyridyl)-1,2,4-triazine-5,6-diyl)-bis-2-furansulfonic acid disodium salt (Ferene®). The absorbance of the complex, measured using a bichromatic (600, 700 nm) endpoint technique, is directly proportional to the concentration of iron in the serum.

$$Fe^{+++} - \text{Transferrin} \longrightarrow Fe^{+++} + \text{Transferrin}$$

$$2\ Fe^{+++} + \text{Ascorbic Acid} \longrightarrow 2\ Fe^{++} + \text{Dehydroascorbic Acid} + 2\ H^{+}$$

$$Fe^{++} + 3\ \text{Ferene®} \longrightarrow Fe^{++} - \text{Ferene®}_3\ \text{complex (absorbs at 600 nm)}$$

INSTRUMENT, REAGENTS & SUPPLIES:

Wells[a]	Form	Ingredient	Concentration[b]
1–4	Liquid	Citric Acid monohydrate	150mM
		Thiourea Detergent	180mM
5-6	Liquid	Ferene®	6.0 mM
		Ascorbic Acid	240 mM

	Thiourea	240 mM

a. Wells are numbered consecutively from the wide end of the cartridge.
b. Nominal value per test at manufacture.
c. Wells 5 and 6 contain stabilizer

Risk and Safety:

H351

P201, P202, P308+P313, P501

Warning!

Suspected of causing cancer.

Obtain special instructions before use. Do not handle all safety precautions have been read and understood. Use personal protective equipment as required. If exposed or concerned: Get medical advice/attention. Dispose of contents and container in accordance with local, regional, and national regulations.

Contains: Thiorea

Safety data sheets(MSDS/SDS) available on www.siemens.com/healthcare

Contains thiourea. Possible risk of irreversible effects. Wear suitable protective clothing.

Used cuvettes contain human body fluids; handle with appropriate care to avoid skin contact and ingestion.[6]

For *in vitro* diagnostic use

Reagent Preparation: All reagents are liquid and ready to use.

REAGENT 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 1–4 have been entered by the instrument, they are stable for 72 hours. Once wells 5-6 have been entered by the instrument, they are stable for 14 days.

SPECIMEN REQUIREMENTS:

Serum can be collected by normal procedures.[2] Follow the instructions provided with your specimen collection device for use and processing.

Serum specimens should be separated from cells within 2 hours after venipuncture.[2]

Specimens should be free of particulate matter. To prevent the appearance of fibrin in serum samples, complete clot formation should take place before centrifugation. Clotting time may be increased due to thrombolytic or anticoagulant therapy.

Blood collection tubes containing EDTA, a strong chelator of metal ions, sodium citrate, or a combination of potassium oxalate and sodium fluoride should not be used.[9]

Serum iron concentrations exhibit diurnal variation, with the highest values being obtained in the morning. Because of this diurnal variation, serum iron values may vary by up to 30% during the course of a day.[10] Iron values may remain elevated for several weeks after administration of therapeutic iron-containing compounds such as iron dextran.[10]

Hemolyzed samples may give elevated IRON results and should not be used with the IRON method

Specimen:

patient preparation: no patient preparation required

specimen type: serum

Stability:

Room Temperature: 4 days
Refrigerated at 2-8°C: up to 7 days
Frozen at -20°C or colder: up to 2 months

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 required for testing:
IRON Flex™ reagent cartridge, Cat. No. DF85
Iron Calibrator, Cat. NO DC85
Quality control Materials
Purified Water diluent (CAT. No. 710615901) or Reagent Grade Water

Test Steps

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

The sample container (if not a primary tube) must contain sufficient quantity to accommodate the sample volume plus the dead volume; precise container filling is not required.

An alternate sample size of 25 µL can be programmed; refer to the Operator's Guide for the use of an alternate sample size.

Test Conditions

- Sample Size: 40 µL, (25 µL)g
- Reagent 1 Volume: 200 µL
- Reagent 2 Volume: 70 µL
- Test Temperature: 37°C
- Wavelength: 600 and 700 nm
- Type of Measurement: bichromatic endpoint

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 iron method:

Assay Range:	5 – 1000 µg/dL[0.9 – 179 µmol/L]
Reference Material:	Primary standards or secondary calibrators such as IRON Calibrator (Cat. No. DC85).
Suggested Calibration Levels:	0, 500, 1075 µg/dL [0, 89.5, 192.4 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:	Standard sample size = 50 µL C0 –1.50 C1 3.46 Alternate sample size = 25 µL C0 –4.97 C1 3.49

Note: Level 1 calibrator is not included in this carton, Purified Water Diluent (Cat. No. 710615901) or reagent grade water should be used as the Level 1 calibrator.

Backup Process:
Refer to Brown Clinic Backup Policy

INTERPRETATION:
The instrument automatically calculates and prints the concentration of iron in µg/dL [µmol/L] using the calculation scheme illustrated in your Dimension® system manual.

Iron determinations can be used in conjunction with Dimension total iron binding capacity (IBCT) results to calculate percent transferrin saturation (ISAT) and unbound iron binding capacity (UIBC).

Calculated results: Transferrin Saturation (%): ISAT = 100[IRON/IBCT]

Unbound iron binding capacity: UIBC = [IBCT-IRON]

Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

EXPECTED VALUES:
Males & Females: 50-175 µg/dL

Each laboratory should establish its own reference interval for IRON as performed on the Dimension® system.

REPORTING:

report format: ug/dl

LIMITATIONS:
Readings in excess of 1000 µg/dL [179 µmol/L] should be repeated after diluting the sample with Purified Water or equivalent (see Reference Manual) to produce a sample concentration within the assay range. The resulting readout will then be multiplied by the dilution factor entered into the system to give the concentration of the undiluted sample.

Samples with results less than 5 µg/dL [0.9 µmol/L] should be reported as "less than 5 µg/dL [0.9 µmol.L]

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.

Use of this assay is not recommended for patients undergoing treatment with dexoferoxamine (e.g. Desferal) or other iron-chelating compounds.

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: IRN Flex™ reagent cartridge insert sheet PN 717085.001 Issue Date 1/16/2017

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: __10/23/2017___
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
10-14	Lori Murray	removed plasma sample type
6/11/15	Heather Hall	Updated information to package insert dated 7/25/13
8/26/15	Amy Harms	Updated information to package insert dated 1/30/15 Risk and Safety
9/17/15	Sam Legg	Updated expected range for both male and female to be the same value per validation.
10/18/2017	Heather Hall	Modified backup process, updated information to insert dated 1/16/2017