Iron

Purpose

This procedure provides instructions for performing the IRON test procedure. The IRON method is an *in vitro* diagnostic test for the quantitative measurement of iron in human serum and plasma on the Dimension Vista® System.

Policy Statements

This procedure applies to all personnel responsible for operating the Siemens Dimension Vista ® at Children's Hospitals and Clinics of Minnesota.

Principle

Under acidic conditions, iron (Fe^{3+}) bound to the protein transferrin is released. In the presence of the reducing agent ascorbic acid, (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 transferrin-bound iron in the serum.

The IRON method is an adaptation of direct iron assays developed by Smith et al. using the chromophore Ferene®. Earlier work by Higgins, Artiss et al. and Hennessy et al. 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

Clinical Significance

Iron is distributed in the body in such compartments as hemoglobin, tissue, myoglobin, and a labile pool, with the largest amount of iron being found in the hemoglobin of red blood cells or their precursors in bone marrow. Approximately 2.5 mg of physiologic iron is found in plasma, as compared to the approximate 2.5 g of iron contained in hemoglobin. Disorders of iron metabolism include iron deficiency anemia and iron overload conditions such as hemosiderosis, hemochromatosis, and sideroblastic anemia.

Increased Serum Iron: Excessive parenteral administration of iron either as iron salts or in the form of hemoglobin in transfused red blood cells: The administration of iron in a manner which bypasses the regulatory mechanism in the bowel wall may result in abnormally high and often harmful body iron content. The usual circumstance is the patient with a congenital hemolytic anemia who requires repeated blood transfusions over a period of many years. The overuse of intramuscular or intravenous injections of iron in the treatment of iron deficient anemia may also be an occasional cause of this problem.

Acute Iron Toxicity: The accidental ingestion of very large quantities of iron by infants or young children may result in death.

Decreased Serum Iron: A decrease in serum iron is much more common than is an increase. Low serum iron may be due either to an absolute, total body deficiency of iron or to a reduction in the quantity of transferrin. The assay for serum iron is of importance

Iron Deficiency: The lack of iron in the diet is an unusual cause of iron deficiency except in very young infants who have been maintained on a milk-only diet for many months.

Analyzer

PRIMARY METHOD: Siemens Dimension Vista® 500 System
SECONDARY (BACKUP) METHOD: Siemens Dimension Vista® 500 on opposite campus

Sunquest Test Code

FE Iron in µg/dL

Specimen

Serum preferred sample. Sodium heparin and lithium heparin plasma are also acceptable. Refer to Specimen Collection procedures.

Minimum volume: 200 µL preferred, 100 µL minimum, 20 µL actual test volume

Patient Preparation:
Patient should be fasting. Blood levels should be determined on morning collection. Serum iron levels are 30% higher in the morning.

Iron values may remain elevated for several weeks after administration of therapeutic iron-containing compounds such as iron dextran.

Hemolyzed samples may give falsely elevated IRON results. See Result Reporting

Stability:
2 – 8 °C. / 7 days, **-20 °C** for up to 6 months

Rejection criteria:
- Unlabeled tube
- Other than serum or heparinized plasma
- Blood collection tubes containing EDTA, a strong chelator of metal ions, sodium citrate, or a combination of potassium oxalate and sodium fluoride

Preparation:
1. Whole blood specimens should be centrifuged following complete clot formation, according to Specimen Processing procedures prior to analysis. See Processing Procedure Manual.
2. Serum or plasma should be physically separated from cells as soon as possible with a maximum limit of two hours from the time of collection.
3. Specimens should be free of particulate matter.
4. Transfer serum or plasma to a properly labeled Siemens SSC nested on a bar-coded pilot tube. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time.

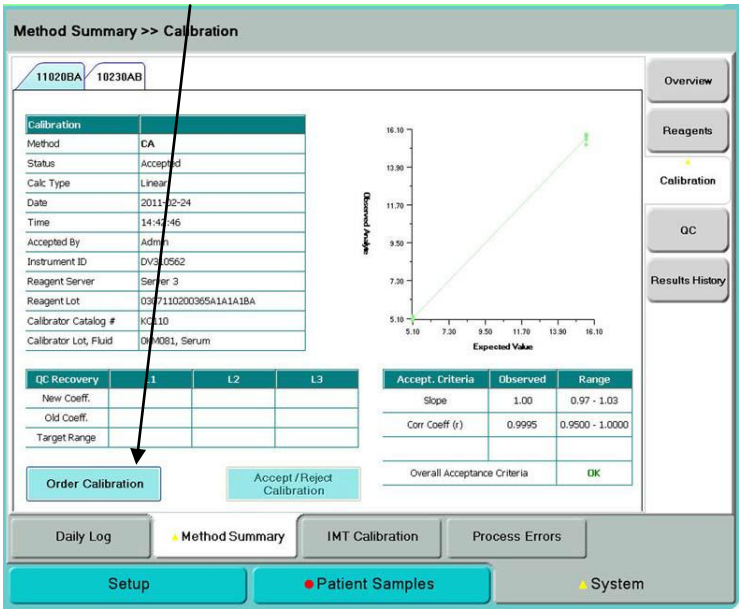
Reagents

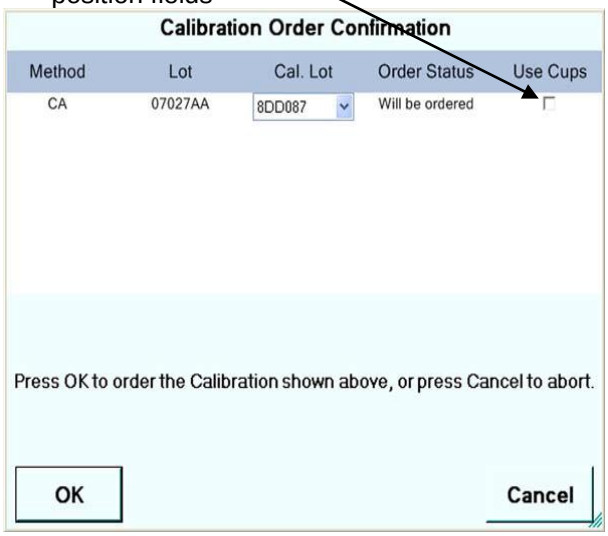
Product Description	*Product Code*	*Stability*
IRON Flex® Reagent Cartridge (Vista) All reagents are liquid and ready to use	K3085	**Store at:** 2 - 8 °C. **Unopened:** Refer to carton for expiration date of individual unopened reagent cartridges. **On-board:** Sealed wells on the instrument are stable for 30 days. **Open well stability:** 3 days for wells 1 – 8, 14 days for wells 9–12
IRON CAL – National Institutes of Standards and Technology SRM 934	KC240	**Store at:** 2 - 8 °C. **Unopened:** Refer to carton for expiration date. **On-board:** Do not place ampoules directly on board the Dimension Vista® System; do not place the calibrator in empty Vista vials. **Opened:** Opened ampoules must be used immediately, and any unused portion discarded in the Acid waste container.

Risk and Safety

- Harmful. Contains Thiourea. Limited evidence of a carcinogenic effect. Wear suitable protective clothing and gloves.
- Safety data sheets (MSDS/SDS) available on www.siemens.com/diagnostics
- Used cuvettes contain human body fluids; handle with appropriate care to avoid skin contact or ingestion.
- Follow Children's Laboratory Safety guidelines when handling patient samples and reagents.

Calibration

Analytical Measuring Range	5 – 1000 mcg/dL
Calibration Material:	IRON CAL, Cat. No. KC240. **Note:** Level 1 calibrator is System Water. It is not necessary to load Level 1.
Calibration Levels:	Level 1 (System water): 0.0 µg/dL Level 2 (Calibrator A): 1075 µg/dL
Calibration Scheme:	2 levels, n=5
Calibration Frequency:	• Every 90 days for any one lot • 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
Calibration Instructions:	Calibrate IRON using sample cups on the Vista • Verify that reagents are in inventory on the instrument. • Press **System > Method Summary > Calibration** • Select IRON method from the sidebar menu. Press the **Order Calibration** button on the screen.  (continued on next page)

Calibration Instructions(cont)	• When the Calibration Order Confirmation box appears, verify that the information on the screen is correct. Verify that the calibrator lot is correct using the drop-down menu. • Check the "Use Cups" box. This displays the rack and cup position fields  • Scan the rack barcode. Open the ampoule of Calibrator A and place in a sample cup in a teal or brown adapter in position 1 on the rack. Press **OK** and load the rack on the instrument. • QC must be manually ordered after calibration is complete. • Refer to the Vista Operator's Guide to Review Calibration

Analytical Measuring Range (AMR)

- Cal Verification and AMR verification are performed at least once every six (6) months.
- Touch Advanced → Calibrations → Calibrations by Lot, select method IRON and "Order a Linearity Study"
- See iGuide "Calibration by Lot" for more information.

Quality Control

Biorad Multiqual (Human) Levels 1 & 3

Frequency: Two levels each day of use

Stability: Stable until the date on vial when stored at -20 to -50 °C protect from light. Thawed and unopened, 7 days at 2 -8 °C. 7 days on board the Vista, and 5 days opened and stored at 2 – 8 °C

Preparation: Allow the control to stand at 18 – 25 °C for 30 minutes until completely thawed. Gently mix the vials until homogeneous to dissolve any precipitate.

Sunquest Control names: Level 1 = C-MQ1, Level 2 = C-MQ3

Acceptable ranges:

- Ranges are current in Sunquest and the instrument. Refer to the Quality Control Procedure for QC exception codes.
- If a control value is outside the confidence interval, the determination must be repeated. If the repeated determination confirms the deviation, a new reference curve should be established.
- Do not release patient results until the cause of deviation has been identified and corrected
- When a new lot of control is received, validate the manufacturer's insert range by running the new lot in parallel with the current lot, and confirming that the results obtained are within the stated range

Calculations

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

Interferences

Hemolysis, Icterus & Lipemia (HIL) Index Values:

H	I	L
3	-	-

Iron Dextran at a concentration of 60 mcg/mL causes significantly elevated IRON results.
Moderate and gross hemolysis cause falsely elevated IRON results.
Lipemia causes falsely elevated IRON results, and/or may cause an "Abnormal Reaction" error message. Ultrafuge before analysis.
Patients treated with metal-binding drugs (e.g. deferoxamine) for acute iron toxicity may have depressed iron values, as chelated iron may not react effectively in this assay.

Refer to the Siemens IFU for additional information on non-interfering substances.

Reference Range

Age	Iron
0 – 2 months	100-250 µg/dL
2 months – 2 years	40-100 µg/dL
> 2 years	50-120 µg/dL

Critical Values

None defined

Limitations

Linear range of detection: 5 – 1000 µg/dL

The instrument reporting system contains flags and comments to provide the user with information regarding instrument processing errors, instrument status information and potential errors in iron results. Refer to your Dimension Vista® Operator's Guide for the meaning of report flags and comments. Any report containing flags and/or comments should be addressed and not reported.

Dilutions

Maximum Dilution:	1:2
Surplus Rack:	Samples with results >1000 µg/dL reflex to a 1:2 dilution.
Limited Rack:	Samples with results >1000 µg/dL should be repeated as an Add-On Test with a 1:2 dilution.
Manual Dilution:	Do not perform manual dilutions.

Result Reporting

- Results between 5 – 1000 µg/dL without error messages are released
- Results below 5 µg/dL are reported as < 5 µg/dL.
- Results with "diluted" appended are reportable.
- Results above 1000 µg/dL without error messages are reported following a maximum dilution of 1:2
- Results with "above assay range" appended following a maximum dilution of 1:2 are reported as >2000 µg/dL
- Append appropriate HIL comments to hemolyzed samples. Refer to CH5.101 HIL on Dimension Vista

Specimen Storage

Promptly stopper tested specimen and store upright in specimen rack. Every 8 hours remove specimens to refrigerator/freezer storage. Samples are retained 7 days in specimen storage freezer.

References

1. Siemens Dimension Vista ® IRON Flex® reagent cartridge Instructions for Use, Siemens Healthcare Diagnostics, PN 717085.001 Issue Date 2013-08-20 D PN 781085.001 – US
2. Siemens Dimension Vista ® IRON Calibrator Instructions for Use, Siemens Healthcare Diagnostics, July 11, 2014
3. Jacobs & DeMott Laboratory Test Handbook, Lexi-Comp, Inc, Hudson, OH, 5th Edition, 2001
4. Biorad Multiqual Chemistry Control Product Insert, Bio-Rad Laboratories, Irvine, CA

Historical Record

Version	Written/Revised by:	Effective Date:	Summary of Revisions
1.	D. Riedel	Nov. 2000	Initial Version
2.	Patti Yelich	02/2001	
3.	L. Lichty	07/2003	
4.	L. Lichty	10/09/2006	
5.	L. Lichty	01/18/2011	New format, new reagent, revised calibration, renumbered from CH 3.29
6.	L. Lichty	3/19/13	Clarify maximum dilution reporting
7.	L. Lichty	12/17/2013	Siemens Healthcare IRON CLSI Procedure - Dimension Vista - Rev D, Sep 3, 2013
8.	L. Lichty	August 4, 2014	Replaces Iron on Dimension RxL
9.	L. Lichty	09/15/2016	Urgent Device correction for deferoxamine interferent