TRAINING UPDATE

Lab Location:	SGMC	**Date Distributed:**	7/12/2017
Department:	Core Lab	**Due Date:**	8/9/2017
		Implementation:	**8/9/2017**

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Iron by Dimension Vista® System SGAH.C906 v1

Total Iron Binding Capacity (TIBC) by Dimension Vista® System SGAH.C907 v2

Description of change(s):

These changes apply to BOTH SOPs

(note QC change is already in effect)

Section	Reason
3.2	Remove specimen onboard stability
4,5,6	Remove individual section labeling instructions and add general one
6.1, 6.2	Update QC material and storage
7.2	Change freezer upper limit to -50C
10.5	Move patient review from section 6
15	Update to new standard wording, add reagent hazard warning
17	Update QC product and PI dates

This change is ONLY for TIBC

Section	Reason
3.2	change refrigerated & frozen stability to match PI

These revised SOPs will be implemented on August 9, 2017

Document your compliance with this training update by taking the quiz in the MTS system.

Technical SOP

Title	**Iron by Dimension Vista® System**	
Prepared by	Ashkan Chini	Date: 4/15/2015
Owner	Robert SanLuis	Date: 4/15/2015

Laboratory Approval	**Local Effective Date:**	
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		

Review		
Print Name	**Signature**	**Date**

Quest Diagnostics
Site: Shady Grove Medical Center
Title: **Iron by Dimension Vista® System**

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Iron	Dimension Vista® System	FE

Synonyms/Abbreviations
FE

Department
Chemistry

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2. ANALYTICAL PRINCIPLE

Under acidic conditions, iron (Fe3+) bound to the protein transferrin is released. In the presence of the reducing agent ascorbic acid, (Fe3+) is reduced to (Fe2+). (Fe2+) 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 sample.

Fe^{3+} – Transferrin ----------> Fe^{3+} + Transferrin

2 Fe^{3+} + Ascorbic Acid ----------> 2 Fe^{2+} + Dehydroascorbic Acid + 2 H^{+}

Fe^{2+} + 3 Ferene® ----------> Fe^{2+} – Ferene®$_3$ complex (absorbs at 600 nm)

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	Normal procedures for collecting and storing serum and plasma may be used for samples to be analyzed by this method.
Special Collection Procedures	N/A
Other	N/A

3.2 Specimen Type & Handling

Criteria	
Type -Preferred	Plasma (Lithium Heparin)
-Other Acceptable	Serum
Collection Container	Plasma: Mint green top tube (PST) Serum: Red top tube, Serum separator tube (SST)
Volume - Optimum	1.0 mL
- Minimum	0.5 mL
Transport Container and Temperature	Collection container or Plastic vial at room temperature
Stability & Storage Requirements	Room Temperature: 8 hours*
	Refrigerated: 7 days
	Frozen: 6 months

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Criteria	
Timing Considerations	Serum should be physically separated from cells as soon as possible with a maximum limit of two hours from the time of collection.
Unacceptable Specimens & Actions to Take	Specimens that are unlabeled, improperly labeled, or those that do not meet the stated criteria are unacceptable. Request a recollection and credit the test with the appropriate LIS English text code for "test not performed" message. Examples: Quantity not sufficient-QNS; Wrong collection-UNAC. Document the request for recollection in the LIS.
Compromising Physical Characteristics	Hemolyzed samples may give elevated Iron results and should not be used with the Iron method. Reject sample and request a recollection. Credit the test with the appropriate LIS English text code explanation of HMT (Specimen markedly hemolyzed)
Other Considerations	Allow Red Top or SST to clot completely prior to centrifugation.

*This information does not match Siemens Package Insert. It is based on the validation which is performed internally in this laboratory. Validation information is available for review.

NOTE: Labeling requirements for all reagents, calibrators and controls include: (1) Open date, (2) Substance name, (3) Lot number, (4) Date of preparation, (5) Expiration date, (6) Initials of tech, and (7) Any special storage instructions. Check all for visible signs of degradation. When placed onboard the analyzer, the instrument captures the date / time loaded and calculates and tracks the opened expiration.

4. REAGENTS

The package insert for a new lot of kits must be reviewed for any changes before the kit is used. A current Package Insert is included as a Related Document.

4.1 Reagent Summary

Reagents	Supplier & Catalog Number
IRON	Siemens, Flex® reagent cartridge, Cat. No. K3085

4.2 Reagent Preparation and Storage

Reagent	IRON
Container	Reagent cartridge
Storage	Store at 2-8°C
Stability	• Stable until expiration date stamped on the cartridges. • Sealed wells on the instrument are stable for 30 days. • Open well stability – o 3 days for wells 1 - 8

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	o 14 days for wells 9 - 12
Preparation	All reagents are liquid and ready to use.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
IRON CAL	Siemens Dimension Vista®, Cat. No. KC240

5.2 Calibrator Preparation and Storage

Calibrator	IRON CAL
Preparation	No preparation is required.
Storage/Stability	• Store at 2 - 8°C • **Unopened:** stable until expiration date stamped on the ampule. • **Opened:** once the ampule is opened, it should be used immediately and any portion not used should be discarded.

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	IRON CAL
Assay Range	5 – 1000 µg/dL
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in µg/dL
Frequency	• Every new reagent cartridge lot. • Every 90 days for any one lot • When major maintenance is performed on the analyzer. • When control data indicates a significant shift in assay.
Calibration Scheme	2 levels, n = 5

5.4 Calibration Procedure

Auto Calibration:

1. Place the required calibrator vials in a carrier. Make sure the barcode labels are entirely visible through the slots.
2. Place the carrier in the loading area.
3. Position the carrier with the labels facing away from the user.
4. Press the **Load** button.

5. Automatic calibration requires that calibrators be on the instrument. As the time for processing approaches, the instrument reviews onboard inventory for the appropriate calibrators.

Manual Calibration:

1. Verify that calibrators and reagents are in inventory on the instrument.
2. Press **System > Method Summary > Calibration**.
3. Select a method from the sidebar menu. Press the **Order Calibration** button on the screen.
4. Verify that the information on the screen is correct. Verify that the calibrator lot is correct using the drop-down menu.
   a. When calibrating using Vials press **OK**.
   b. When calibrating using Cups, check the Use Cups box. This displays the rack and cup position fields. For additional cups use the positions in ascending order. Be sure to use the number of calibration levels and cups as specified in the method IFU. Scan the rack barcode and place calibrator cups in an adapter in position 1 on a rack. Press **OK** and load the rack on the instrument.
5. The status field in the calibration screen changes sequentially to Awaiting Scheduling, Preparing Calibrators and Processing.

5.5 Tolerance Limits

IF……	THEN……
If result fall within assay-specific specification, and QC values are within acceptable limits,	proceed with analysis
If result falls outside assay-specific specification, or QC values are out of Acceptable limits,	troubleshoot the assay and/or instrument and repeat calibration

6. QUALITY CONTROL

6.1 Controls Used

Controls	Supplier and Catalog Number
Liquid Assayed Multiqual® Levels 1 and 3	Bio-Rad Laboratories Cat. No. 337 and 339

6.2 Control Preparation and Storage

Control	Liquid Assayed Multiqual® Levels 1 and 3
Preparation	Allow the frozen control to stand at room temperature (18-25°C) for 30 minutes or until completely thawed. Swirl the contents gently to ensure homogeneity. (Do not use a mechanical mixer) Use immediately. After each use, promptly replace the stopper and return to 2-8°C storage.

Storage/Stability	Frozen controls are stable until the expiration date at -20 to -50°C. Thawed and Unopened: When this product is stored at 2-8°C and the stopper is not punctured, it will be stable for 30 days for iron. This product can be used for 7 days when stored on-board the Siemens Dimension Vista at 2- 8°C. Thawed and Opened: Once the product stopper is punctured, all analytes will be stable for 5 days when stored at 2- 8°C. Store away from light.

6.3 Frequency

Analyze all levels of QC material after every calibration and each day of testing (notated on the QC frequency sheets posted on the instruments).

Refer to the Dimension Vista® QC Schedule in the Laboratory policy Quality Control Program and in the Dimension Vista® Quick Reference Guide.

6.4 Tolerance Limits and Criteria for Acceptable QC

Step	Action
1	Acceptable ranges for QC are programmed into the instrument's Quality Control software system and Unity Real Time, and may be posted near the instrument for use during computer downtime.
2	**Run Rejection Criteria** • Anytime the established parameters are exceeded (if one QC result exceeds 2 SD), the run is considered out of control (failed) and patient results must not be reported. • The technologist must follow the procedure in the Laboratory QC Program to resolve the problem.
3	**Corrective Action**: • All rejected runs must be effectively addressed through corrective action. Steps taken in response to QC failures must be documented. Patient samples in failed analytical runs must be reanalyzed according to the Laboratory QC Program. Supervisors may override rejection of partial or complete runs only with detailed documentation and criteria for overrides that are approved by the Medical Director. Consult corrective action guidelines in Laboratory QC Program. Follow corrective action guidelines in the Laboratory QC Program. • Corrective action documentation must follow the Laboratory Quality Control Program.

Step	Action
4	**Review of QC** • QC must be reviewed weekly by the Group Lead or designee and monthly by the Supervisor/Manager or designee. • If the SD and/or CV are greater than established ranges, investigate the cause for the imprecision and document implementation of corrective actions.

6.5 Documentation

- QC tolerance limits are programmed into the instrument and Unity Real Time; it calculates cumulative mean, SD and CV and stores all information for easy retrieval.
- Quality control records are reviewed daily at the bench, weekly by the Group Lead or designee, and monthly by the Supervisor/Manager or designee.
- Refer to complete policies and procedures for QC documentation and for record retention requirements in the Laboratory QC Program.

6.6 Quality Assurance Program

- Each new lot number of reagent or new shipment of the same lot of reagent must be tested with external control materials and previously analyzed samples. Performance of the new lot must be equivalent to the previous lot; utilize published TEA for acceptability criteria.
- Training must be successfully completed and documented prior to performing this test. This procedure must be incorporated into the departmental competency assessment program.
- The laboratory participates in CAP proficiency testing. All proficiency testing materials must be treated in the same manner as patient samples.
- Monthly QC must be presented to the Medical Director or designee for review and signature.
- Monthly QC mean and SD are sent to Bio-Rad Laboratories for peer group comparison.
- Consult the Laboratory QC Program for complete details.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

Dimension Vista® System

7.2 Equipment

- Refrigerator capable of sustaining 2–8°C.

- Freezer capable of sustaining range not to exceed -20 to -50°C.
- Centrifuge

7.3 Supplies

- Aliquot Plates
- System Fluids
- Assorted calibrated pipettes (MLA or equivalent) and disposable tips

8. PROCEDURE

IRON Flex® reagent cartridge Cat. No. K3085 is required to perform this test.

IRON is performed on the Dimension Vista® System after the method is calibrated (see Reference Material in Calibration section) and Quality Controls are acceptable.

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

8.1	Sample Processing
1.	A sample rack holding tubes or cups is placed on the rack input lane.
2.	The sample shuttle moves the rack to the barcode reader which identifies the rack and samples to the system.
3.	The rack moves into the sample server and to the rack positioner.
4.	At the same time, aliquot plates move from the aliquot loader into position.
5.	The aliquot probe aspirates the sample from the tubes or cups and dispenses it into the wells of the aliquot plates.
6.	After each aspirate-dispense action, the probe is thoroughly rinsed inside and out to prevent sample carryover.
7.	When sample aspiration is completed, the sample server moves the rack back to the sample shuttle, where it is placed on the output lane and can be removed by the operator.

8.2	Specimen Testing
1.	For QC placement and frequency, refer to the Dimension Vista® QC Schedule in the Laboratory QC Program.
2.	Follow the instructions, outlined in the Dimension Vista® Operator's Manual
3.	The instrument reporting system contains error messages to warn the user of specific malfunctions. Results followed by such error messages should be held for follow-up. Refer to the Dimension Vista® system manual "Error messages" section for troubleshooting.
4.	Follow protocol in Section 10.5 "Repeat criteria and resulting" for samples with results above or below the Analytical Measurement Range (AMR).

8.2	Specimen Testing
	Investigate any failed delta result and repeat, if necessary.
5.	Append the appropriate English text code qualifier messages to any samples requiring a comment regarding sample quality and/or any other pertinent factors.

Test Conditions	
Sample Volume:	20 µL
Reagent 1 Volume:	100 µL
Reagent 2 Volume:	35 µL
Reaction Time:	5.6 minutes
Test Temperature:	37° C
Wavelength:	600 & 700 nm
Type of measurement:	Bichromatic endpoint

NOTE: In the event that the test system becomes inoperable, notify supervision or designee for further direction. Patient specimens must be stored in a manner that maintains the integrity of the specimen.

9. CALCULATIONS

The instrument automatically calculates the concentration of IRON in µg/dL.

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]

All calculations are performed by the instrument.

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

None required

10.2 Rounding

No rounding is necessary. Instrument reports results as a whole number.

10.3 Units of Measure

µg/dL

10.4 Clinically Reportable Range (CRR)

5 – 3,000 µg/dL

10.5 Review Patient Data

Each result is reviewed for error messages. Refer to the Dimension Vista system manual "Error messages" section for troubleshooting. Resolve any problems noted before issuing patient reports.

10.6 Repeat Criteria and Resulting

All repeats must replicate the original result within the total allowable error (TEa) of the assay. Refer to TEa listing for specific information.

Values that fall within the AMR or CRR may be reported without repeat. Values that fall outside these ranges must be repeated.

IF the result is …	THEN…
< 5 µg/dL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 5 µg/dL
≥ 1,000 µg/dL	**On Board Automated Dilution:** Results ≥ 1,000 µg/dL will automatically have repeat testing performed into the instrument using dilution factor of 2. No multiplication is necessary.
> 2,000 µg/dL	**Manual Dilution**: Using the primary tube, make the smallest dilution possible to bring the raw data within the AMR. Maximum allowable dilution: x 3 **DILUENT**: Water Enter dilution factor as a whole number. Re-assay. Readout is corrected for dilution.
> 3,000 µg/dL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 3,000 µg/dL-REP" Bring to the attention of your supervisor prior to releasing result.

Message	Code
Verified by repeat analysis	Append –REP to the result.

11. EXPECTED VALUES

11.1 Reference Ranges

Age	Female	Male
Adult (>18 years):	50 – 170 µg/dL	65 – 175 µg/dL
Pediatric:		
10 – 18 years	20 - 145	20 - 100
2 – 9 years	20 - 145	20 - 105
0 days – 23 months	20 - 140	20 - 105

11.2 Critical Values

None established

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

Iron measurements are used in the diagnosis and treatment of disorders of iron metabolism including iron deficiency anemia and iron overload conditions such as hemosiderosis, hemochromatosis, and sideroblastic anemia. They may be useful in evaluating iron intoxication in infants and children after accidental ingestion of vitamins containing iron.

13. PROCEDURE NOTES

- **FDA Status:** FDA Approved/modified
- Validated Test Modifications: Specimen stabilities have been modified from the package insert based on in-house stability studies performed at Shady Grove Medical Center.

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 Vista Operator's Guide.

The expected maximum observed standard deviations for repeatability using n = 5 replicates at the following IRON concentrations are:

IRON Concentration	Acceptable S.D. Maximum
55 µg/dL	5 µg/dL
220 µg/dL	8 µg/dL

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

5 – 1000 µg/dL

14.2 Precision

Material	Mean µg/dL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Serum Pool			
Level 1	106.2	1.4 (1.4)	1.5 (1.4)
Level 2	38.4	1.1 (3.0)	1.4 (3.7)
Level 3	715.7	5.3 (0.7)	8.7 (1.2)
Multiqual Control			
Level 3	228.4	1.9 (0.8)	2.0 (0.9)

14.3 Interfering Substances

At iron concentration of 36 µg/dL: Iron Dextran at 60 µg/mL increases IRON results by 175%
At iron concentration of 131 µg/dL: Iron Dextran at 60 µg/mL increases IRON results by 53%
At iron concentration of 35 µg/dL: Hemoglobin at 500 mg/dL increases IRON results by 82% and Hemoglobin at 1000 mg/dL increases IRON results by 141%
At iron concentration of 130 µg/dL: Hemoglobin at 500 mg/dL increases IRON results by 24% and Hemoglobin at 1000 mg/dL increases IRON results by 43%
At iron concentration of 42 µg/dL: Triglycerides at 1109 mg/dL increases IRON results by 71%
Interference may vary depending upon the lipid composition and subsequent degree of turbidity in the sample. Lipemic samples may be flagged with an "Abnormal Reaction" error message.

HIL Interference:
The IRON method was evaluated for interference according to CLSI/NCCLS EP7-A2. Bias, defined as the difference between the control sample (does not contain interferent) and the test sample (contains interferent), is shown in the table below. Bias exceeding 10% is considered "interference".

Substance tested	Substance Concentration	IRON µg/dL	Bias %
Hemoglobin (hemolysate)	25 mg/dL	35	<10
	50 mg/dL		15
	200 mg/dL		42
	500 mg/dL		82
	1000 mg/dL		141
Hemoglobin (hemolysate)	50 mg/dL	130	<10
	200 mg/dL		14
	500 mg/dL		24
	1000 mg/dL		43
Bilirubin (unconjugated)	20 mg/dL	35	<10
Bilirubin (unconjugated)	40 mg/dL	40	<10
	60 mg/dL		<10
Bilirubin (unconjugated)	60 mg/dL	130	<10
Lipemia Intralipid®	3000 mg/dL	35	<10
	3000 mg/dL	130	<10

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14.4 Clinical Sensitivity/Specificity/Predictive Values

Not available

15. SAFETY

Refer to your local and corporate safety manuals and Safety Data Sheet (SDS) for detailed information on safety practices and procedures and a complete description of hazards

IRON Flex® reagent cartridge is suspected of causing cancer (contains Thiourea). Use personal protective equipment as required. IF exposed or concerned: Get medical advice/attention.

16. RELATED DOCUMENTS

1. Dimension Vista® Clinical Chemistry System Operator's Manual
2. Dimension Vista® Calibration/Verification Procedure
3. Dimension Vista® Cal Accept Guidelines
4. Dimension Vista® Calibration summary
5. Dimension Vista® Sample Processing, Startup and Maintenance procedure
6. Laboratory Quality Control Program
7. QC Schedule for Siemens Dimension Vista®
8. Laboratory Safety Manual
9. Safety Data Sheets (SDS)
10. Dimension Vista® Limits Chart (AG.F200)
11. Quest Diagnostics Records Management Procedure
12. Dimension Vista® System Error Messages Chart
13. Centrifuge Use, Maintenance and Function Checks (Lab policy)
14. Hemolysis, Icteria and Lipemia Interference (Lab policy)
15. Repeat Testing Requirement (Lab policy)
16. Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls
17. Current package insert IRON Flex® Reagent Cartridge K3085

17. REFERENCES

1. Ghoshal, Amit K. and Soldin, Steven J., Evaluation of the Dade Behring Dimension® RxL: Integrated chemistry system-pediatric reference ranges. Clinica Chimica Acta 2003; 331:144.
2. Package Insert, IRON Flex® Reagent Cartridge K3085, Siemens Healthcare Diagnostics Inc., 05/13/2015.

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3. Package Insert, IRON CAL, Siemens Healthcare Diagnostics Inc., 05/2016.
4. Package Insert, Liquid Assayed Multiqual® Chemistry Controls, Bio-Rad Laboratories, 09/2015.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
0	6/19/17	3.2	Remove specimen onboard stability	L Barrett	R SanLuis
0	6/19/17	4,5,6	Remove individual section labeling instructions and add general one	L Barrett	R SanLuis
0	6/19/17	6.1, 6.2	Update QC material and storage	L Barrett	R SanLuis
0	6/19/17	7.2	Change freezer upper limit to -50C	L Barrett	R SanLuis
0	6/19/17	10.5	Move patient review from section 6	L Barrett	R SanLuis
0	6/19/17	15	Update to new standard wording, add reagent hazard statement	L Barrett	R SanLuis
0	6/19/17	17	Update QC product and PI dates	L Barrett	R SanLuis

19. ADDENDA

None

Technical SOP

Title	**Total Iron Binding Capacity (TIBC) by Dimension Vista® System**
Prepared by	Ashkan Chini Date: 4/15/2015
Owner	Robert SanLuis Date: 4/15/2015

Laboratory Approval	**Local Effective Date:**	
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		

Review		
Print Name	**Signature**	**Date**

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TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Total Iron Binding Capacity	Dimension Vista® System	TIBCP

Synonyms/Abbreviations
TIBC, IBCT

Department
Chemistry

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2. ANALYTICAL PRINCIPLE

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. Subsequent addition of acid causes the release of bound iron from transferrin, which is then analyzed using the chromogen Ferene. Earlier work by Higgins, Artiss and Hennessy 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.

The serum or plasma 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.

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.

pH 8.6

$Transferrin + Fe^{+++}$ ----------> Fe^{+++} – Transferrin + Fe^{+++}

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

pH 4.5

Fe^{+++} – Transferrin ----------> Transferrin + Fe^{+++}

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

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	Normal procedures for collecting and storing serum and plasma may be used for samples to be analyzed by this method.
Special Collection Procedures	N/A
Other	N/A

3.2 Specimen Type & Handling

Criteria	
Type -Preferred **-Other Acceptable**	Plasma (Lithium Heparin) Serum
Collection Container	Plasma: Mint green top tube (PST) Serum: Red top tube, Serum separator tube (SST)
Volume - Optimum **- Minimum**	1.0 mL 0.5 mL
Transport Container and Temperature	Collection container or Plastic vial at room temperature
Stability & Storage Requirements	Room Temperature: 8 hours*
	Refrigerated: ~~24 hours*~~ 7 days
	Frozen: ~~7 days*~~ 6 months
Timing Considerations	Serum should be physically separated from cells as soon as possible with a maximum limit of two hours from the time of collection.
Unacceptable Specimens & Actions to Take	Specimens that are unlabeled, improperly labeled, or those that do not meet the stated criteria are unacceptable. Request a recollection and credit the test with the appropriate LIS English text code for "test not performed" message. Examples: Quantity not sufficient-QNS; Wrong collection-UNAC. Document the request for recollection in the LIS.
Compromising Physical Characteristics	Gross hemolysis. Reject sample and request a recollection. Credit the test with the appropriate LIS English text code explanation of HMT (Specimen markedly hemolyzed)
Other Considerations	Allow Red Top or SST to clot completely prior to centrifugation.

*This information does not match Siemens Package Insert. It is based on the validation which is performed internally in this laboratory. Validation information is available for review.

NOTE: Labeling requirements for all reagents, calibrators and controls include: (1) Open date, (2) Substance name, (3) Lot number, (4) Date of preparation, (5) Expiration date, (6) Initials of tech, and (7) Any special storage instructions. Check all for visible signs of degradation. When placed onboard the analyzer, the instrument captures the date / time loaded and calculates and tracks the opened expiration.

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Quest Diagnostics
Site: Shady Grove Medical Center
Title: **Total Iron Binding Capacity (TIBC) by Dimension Vista® System**

Form revised 2/02/2007

4. REAGENTS

The package insert for a new lot of kits must be reviewed for any changes before the kit is used. A current Package Insert is included as a Related Document.

4.1 Reagent Summary

Reagents	Supplier & Catalog Number
TIBC	Siemens, Flex® reagent cartridge, Cat. No. K3084

4.2 Reagent Preparation and Storage

Reagent	**TIBC**
Container	Reagent cartridge
Storage	Store at 2-8°C
Stability	• Stable until expiration date stamped on the cartridges. • Sealed wells on the instrument are stable for 30 days. • Open well stability – 7 days for wells 1 - 12
Preparation	Hydration, mixing and diluting are automatically performed by the instrument.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
TIBC CAL	Siemens Dimension Vista®, Cat. No. KC230

5.2 Calibrator Preparation and Storage

Calibrator	TIBC CAL
Preparation	No preparation is required.
Storage/Stability	• Store at 2 - 8°C • **Unopened calibrator** is stable until expiration date stamped on the vial. • **Opened Calibrator:** once the stopper of the vial is punctured, assigned values are stable for 7 days when stored on board the Dimension Vista System.

5.3 Calibration Parameter

Criteria	**Special Notations**
Reference Material	TIBC CAL
Assay Range	8 – 1000 µg/dL
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in µg/dL
Frequency	• Every new reagent cartridge lot. • Every 90 days for any one lot • When major maintenance is performed on the analyzer. • When control data indicates a significant shift in assay.
Calibration Scheme	2 levels, n = 5

5.4 Calibration Procedure

Auto Calibration:

1. Place the required calibrator vials in a carrier. Make sure the barcode labels are entirely visible through the slots.
2. Place the carrier in the loading area.
3. Position the carrier with the labels facing away from the user.
4. Press the **Load** button.
5. Automatic calibration requires that calibrators be on the instrument. As the time for processing approaches, the instrument reviews onboard inventory for the appropriate calibrators.

Manual Calibration:

1. Verify that calibrators and reagents are in inventory on the instrument.
2. Press **System > Method Summary > Calibration**.
3. Select a method from the sidebar menu. Press the **Order Calibration** button on the screen.
4. Verify that the information on the screen is correct. Verify that the calibrator lot is correct using the drop-down menu.
   a. When calibrating using Vials press **OK**.
   b. When calibrating using Cups, check the Use Cups box. This displays the rack and cup position fields. For additional cups use the positions in ascending order. Be sure to use the number of calibration levels and cups as specified in the method IFU. Scan the rack barcode and place calibrator cups in an adapter in position 1 on a rack. Press **OK** and load the rack on the instrument.
5. The status field in the calibration screen changes sequentially to Awaiting Scheduling, Preparing Calibrators and Processing.

5.5 Tolerance Limits

IF……	**THEN……**
If result fall within assay-specific specification, and QC values are within acceptable limits,	proceed with analysis

Quest Diagnostics
Site: Shady Grove Medical Center
Title: **Total Iron Binding Capacity (TIBC) by Dimension Vista® System**

Form revised 2/02/2007

If result falls outside assay-specific specification, or QC values are out of Acceptable limits,	troubleshoot the assay and/or instrument and repeat calibration

6. QUALITY CONTROL

6.1 Controls Used

Controls	Supplier and Catalog Number
Liquid Assayed Multiqual® Levels 1 and 3	Bio-Rad Laboratories Cat. No. 337 and 339

6.2 Control Preparation and Storage

Control	Liquid Assayed Multiqual® Levels 1 and 3
Preparation	Allow the frozen control to stand at room temperature (18-25°C) for 30 minutes or until completely thawed. Swirl the contents gently to ensure homogeneity. (Do not use a mechanical mixer) Use immediately. After each use, promptly replace the stopper and return to 2-8°C storage.
Storage/Stability	Frozen controls are stable until the expiration date at -20 to -50°C. Thawed and Unopened: When this product is stored at 2-8°C and the stopper is not punctured, it will be stable for 30 days for iron. This product can be used for 7 days when stored on-board the Siemens Dimension Vista at 2- 8°C. Thawed and Opened: Once the product stopper is punctured, all analytes will be stable for 5 days when stored at 2- 8°C. Store away from light.

6.3 Frequency

Analyze all levels of QC material after every calibration and each day of testing (notated on the QC frequency sheets posted on the instruments).

Refer to the Dimension Vista® QC Schedule in the Laboratory policy Quality Control Program and in the Dimension Vista® Quick Reference Guide.

6.4 Tolerance Limits and Criteria for Acceptable QC

Step	Action
1	Acceptable ranges for QC are programmed into the instrument's Quality Control software system and Unity Real Time, and may be posted near the instrument for use during computer downtime.

Step	Action
2	**Run Rejection Criteria** • Anytime the established parameters are exceeded (if one QC result exceeds 2 SD), the run is considered out of control (failed) and patient results must not be reported. • The technologist must follow the procedure in the Laboratory QC Program to resolve the problem.
3	**Corrective Action**: • All rejected runs must be effectively addressed through corrective action. Steps taken in response to QC failures must be documented. Patient samples in failed analytical runs must be reanalyzed according to the Laboratory QC Program. Supervisors may override rejection of partial or complete runs only with detailed documentation and criteria for overrides that are approved by the Medical Director. Consult corrective action guidelines in Laboratory QC Program. Follow corrective action guidelines in the Laboratory QC Program. • Corrective action documentation must follow the Laboratory Quality Control Program.
4	**Review of QC** • QC must be reviewed weekly by the Group Lead or designee and monthly by the Supervisor/Manager or designee. • If the SD and/or CV are greater than established ranges, investigate the cause for the imprecision and document implementation of corrective actions.

6.5 Documentation

- QC tolerance limits are programmed into the instrument and Unity Real Time; it calculates cumulative mean, SD and CV and stores all information for easy retrieval.
- Quality control records are reviewed daily at the bench, weekly by the Group Lead or designee, and monthly by the Supervisor/Manager or designee.
- Refer to complete policies and procedures for QC documentation and for record retention requirements in the Laboratory QC Program.

6.6 Quality Assurance Program

- Each new lot number of reagent or new shipment of the same lot of reagent must be tested with external control materials and previously analyzed samples. Performance of the new lot must be equivalent to the previous lot; utilize published TEA for acceptability criteria.

- Training must be successfully completed and documented prior to performing this test. This procedure must be incorporated into the departmental competency assessment program.
- The laboratory participates in CAP proficiency testing. All proficiency testing materials must be treated in the same manner as patient samples.
- Monthly QC must be presented to the Medical Director or designee for review and signature.
- Monthly QC mean and SD are sent to Bio-Rad Laboratories for peer group comparison.
- Consult the Laboratory QC Program for complete details.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

Dimension Vista® System

7.2 Equipment

- Refrigerator capable of sustaining 2–8°C.
- Freezer capable of sustaining range not to exceed -20 to -50°C.
- Centrifuge

7.3 Supplies

- Aliquot Plates
- System Fluids
- Assorted calibrated pipettes (MLA or equivalent) and disposable tips

8. PROCEDURE

TIBC Flex® reagent cartridge Cat. No. K3084 is required to perform this test.

TIBC is performed on the Dimension Vista® System after the method is calibrated (see Reference Material in Calibration section) and Quality Controls are acceptable.

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

8.1	Sample Processing
1.	A sample rack holding tubes or cups is placed on the rack input lane.
2.	The sample shuttle moves the rack to the barcode reader which identifies the rack and samples to the system.
3.	The rack moves into the sample server and to the rack positioner.

8.1	Sample Processing
4.	At the same time, aliquot plates move from the aliquot loader into position.
5.	The aliquot probe aspirates the sample from the tubes or cups and dispenses it into the wells of the aliquot plates.
6.	After each aspirate-dispense action, the probe is thoroughly rinsed inside and out to prevent sample carryover.
7.	When sample aspiration is completed, the sample server moves the rack back to the sample shuttle, where it is placed on the output lane and can be removed by the operator.

8.2	Specimen Testing
1.	For QC placement and frequency, refer to the Dimension Vista® QC Schedule in the Laboratory QC Program.
2.	Follow the instructions, outlined in the Dimension Vista® Operator's Manual
3.	The instrument reporting system contains error messages to warn the user of specific malfunctions. Results followed by such error messages should be held for follow-up. Refer to the Dimension Vista® system manual "Error messages" section for troubleshooting.
4.	Follow protocol in Section 10.5 "Repeat criteria and resulting" for samples with results above or below the Analytical Measurement Range (AMR). Investigate any failed delta result and repeat, if necessary.
5.	Append the appropriate English text code qualifier messages to any samples requiring a comment regarding sample quality and/or any other pertinent factors.

Test Conditions	
Sample Volume:	10.7 µL
Reagent 1 Volume:	15.4 µL
Reagent 2 Volume:	10.7 µL
Reagent 3 Volume:	10.7 µL
Reagent 4 Volume:	32.1 µL
Reaction Time:	3.8 minutes
Test Temperature:	37°C
Wavelength:	600 & 700 nm
Type of measurement:	Bichromatic endpoint

NOTE: In the event that the test system becomes inoperable, notify supervision or designee for further direction. Patient specimens must be stored in a manner that maintains the integrity of the specimen.

9. CALCULATIONS

The instrument automatically calculates and prints the concentration of TIBC in µg/dL.

LIS Calculated Result: Transferrin Saturation (%) = 100[IRON/IBCT]

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

None required

10.2 Rounding

No rounding is necessary. Instrument reports results as a whole number.

10.3 Units of Measure

µg/dL

10.4 Clinically Reportable Range (CRR)

8 – 3,000 µg/dL

10.5 Review Patient Data

Each result is reviewed for error messages. Refer to the Dimension Vista system manual "Error messages" section for troubleshooting. Resolve any problems noted before issuing patient reports.

10.6 Repeat Criteria and Resulting

All repeats must replicate the original result within the total allowable error (TEa) of the assay. Refer to TEa listing for specific information.

Values that fall within the AMR or CRR may be reported without repeat. Values that fall outside these ranges must be repeated.

IF the result is …	THEN…
< 8 µg/dL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 8 µg/dL
≥ 1000 µg/dL	**On Board Automated Dilution:** Results ≥ 1000 µg/dL will automatically have repeat testing performed into the instrument using dilution factor of 2. No multiplication is necessary.

IF the result is …	THEN…
> 2,000 µg/dL	**Manual Dilution**: Using the primary tube, make the smallest dilution possible to bring the raw data within the AMR. Maximum allowable dilution: x 3 **DILUENT**: Reagent Grade Water Enter dilution factor as a whole number. Re-assay. Readout is corrected for dilution.
> 3,000 µg/dL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 3,000 µg/dL-REP" Bring to the attention of your supervisor prior to releasing result.

Message	Code
Verified by repeat analysis	Append –REP to the result.

11. EXPECTED VALUES

11.1 Reference Ranges

Age	TIBC Female	TIBC Male
Adult (>18 years):	250 – 450 µg/dL	250 – 450 µg/dL
Pediatric:		
15 – 18 years	285 - 410	270 - 415
11 – 14 years	250 - 420	265 - 410
4 – 10 years	260 - 385	185 - 415
13 months – 3 years	160 - 415	215 - 420
3 – 12 months	250 - 455	150 - 380
0 – 90 days	165 - 275	155 - 330

Transferrin Saturation 20 – 50%

11.2 Critical Values

None established

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

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.

13. PROCEDURE NOTES

- **FDA Status:** FDA Approved/modified
- **Validated Test Modifications**: Specimen stabilities have been modified from the package insert based on in-house stability studies performed at Shady Grove Medical Center.

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 Vista Operator's Guide.

The expected maximum observed standard deviations for repeatability using n = 5 replicates at the following TIBC concentrations are:

TIBC Concentration	Acceptable S.D. Maximum
205 µg/dL	20 µg/dL
565 µg/dL	23 µg/dL

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

8 – 1000 µg/dL

14.2 Precision

Material	Mean µg/dL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Multiqual Control			
Level 1	205	4.7 (2.3)	8.2 (4.0)
Level 2	317	5.5 (1.7)	9.7 (3.1)
Level 3	565	5.6 (1.0)	11.4 (2.0)

14.3 Interfering Substances

Measurements of TIBC may be inaccurate if performed within 14 days of IV iron dextran administration.
Ferrous sulfate at 250 µg/dL increased TIBC results by 17%.
Hemoglobin at 200 mg/dL increased TIBC results by 13% at TIBC concentrations of 416 µg/dL.

HIL Interference:
The TIBC method was evaluated for interference according to CLSI/NCCLS EP7-A2. Bias, defined as the difference between the control sample (does not contain interferent) and the test sample (contains interferent), is shown in the table below. Bias exceeding 10% is considered "interference".

Substance tested	Substance Concentration	TIBC µg/dL	Bias %
Hemoglobin (hemolysate)	50 mg/dL	416	<10
Bilirubin (unconjugated)	60 mg/dL	483	<10
Bilirubin (conjugated)	60 mg/dL	483	<10
Lipemia Intralipid®	800 mg/dL	525	<10

14.4 Clinical Sensitivity/Specificity/Predictive Values

Not available

15. SAFETY

Refer to your local and corporate safety manuals and Safety Data Sheet (SDS) for detailed information on safety practices and procedures and a complete description of hazards

TIBC Flex® reagent cartridge causes serious eye and skin irritation; may cause an allergic skin reaction; suspected of causing cancer. Use personal protective equipment as required. IF exposed or concerned: Get medical advice/attention. IF IN EYES: Rinse cautiously with water for several minutes. Contains: Acetic acid; Thiourea; 5-chloro-2-methyl-3(2h)-isothiazolone mixture with 2-methyl-3(2h)-isothiazolone

16. RELATED DOCUMENTS

1. Dimension Vista® Clinical Chemistry System Operator's Manual
2. Dimension Vista® Calibration/Verification Procedure
3. Dimension Vista® Cal Accept Guidelines
4. Dimension Vista® Calibration summary
5. Dimension Vista® Sample Processing, Startup and Maintenance procedure
6. Laboratory Quality Control Program
7. QC Schedule for Siemens Dimension Vista®
8. Laboratory Safety Manual
9. Safety Data Sheets (SDS)
10. Dimension Vista® Limits Chart (AG.F200)
11. Quest Diagnostics Records Management Procedure
12. Dimension Vista® System Error Messages Chart
13. Centrifuge Use, Maintenance and Function Checks (Lab policy)
14. Hemolysis, Icteria and Lipemia Interference (Lab policy)
15. Repeat Testing Requirement (Lab policy)
16. Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls
17. Current package insert TIBC Flex® Reagent Cartridge K3084

17. REFERENCES

1. Ghoshal, Amit K. and Soldin, Steven J., Evaluation of the Dade Behring Dimension® RxL: Integrated chemistry system-pediatric reference ranges. Clinica Chimica Acta 2003; 331:144.
2. Package Insert, TIBC Flex® Reagent Cartridge K3084, Siemens Healthcare Diagnostics Inc., 05/11/2015.
3. Package Insert, TIBC CAL, Siemens Healthcare Diagnostics Inc., 06/30/2012.
4. Package Insert, Liquid Assayed Multiqual® Chemistry Controls, Bio-Rad Laboratories, 09/2015.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
0	4/23/15	9	Correct calculation information	R SanLuis	R SanLuis
0	4/23/15	11.1	Add range for transferrin saturation	R SanLuis	R SanLuis
1	6/19/17	3.2	Remove specimen onboard stability, change refrigerated & frozen stability to match PI	L Barrett	R SanLuis
1	6/19/17	4,5,6	Remove individual section labeling instructions and add general one	L Barrett	R SanLuis
1	6/19/17	6.1, 6.2	Update QC material and storage	L Barrett	R SanLuis
1	6/19/17	7.2	Change freezer upper limit to -50C	L Barrett	R SanLuis
1	6/19/17	10.5	Move patient review from section 6	L Barrett	R SanLuis
1	6/19/17	15	Update to new standard wording, add reagent hazard statement	L Barrett	R SanLuis
1	6/19/17	17	Update QC product and PI dates	L Barrett	R SanLuis

19. ADDENDA

None