TRAINING UPDATE

Lab Location:	SGMC	**Date Distributed:**	7/5/2017
Department:	Core Lab	**Due Date:**	7/25/2017
		Implementation:	**7/25/2017**

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Ferritin by Dimension Vista® System SGAH.C908 v1

Folate by Dimension Vista® System SGAH.C909 v1

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 limits to match specific products
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 Ferritin

Section	Reason
10.4, 14.1	Change lower limit from 0.5 to 1 to match practice (reporting whole number)

These revised SOPs will be implemented on July 25, 2017

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

Technical SOP

Title	**Ferritin 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: **Ferritin by Dimension Vista® System**

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Ferritin	Dimension Vista® System	FERIT

Synonyms/Abbreviations
None

Department
Chemistry

Form revised 2/02/2007

2. ANALYTICAL PRINCIPLE

The LOCI FERR method is a homogeneous, sandwich chemiluminescent immunoassay based on LOCI technology. The LOCI reagents include two synthetic bead reagents and a biotinylated anti-ferritin monoclonal antibody fragment. The first bead reagent (Sensibeads) is coated with streptavidin and contains a photosensitizer dye. The second bead reagent (Chemibeads) is coated with a second anti-ferritin monoclonal antibody and contains chemiluminescent dye. Sample is incubated with biotinylated antibody and Chemibeads to form a particle/ferritin/biotinylated antibody sandwich.
Sensibeads are added and bind to the biotin to form bead-pair immunocomplexes. Illumination of the complex at 680 nm generates singlet oxygen from Sensibeads which diffuses into the Chemibeads, triggering a chemiluminescent reaction. The resulting signal is measured at 612 nm and is a direct function of the ferritin concentration in the sample.

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: 7 days
	Frozen: 6 months
Timing Considerations	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.

Criteria	
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.

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
Ferritin	Siemens, Flex® reagent cartridge, Cat. No. K6440

4.2 Reagent Preparation and Storage

Reagent	Ferritin
Container	Reagent Cartridge
Storage	Store at 2-8°C
Stability	• Reagent is stable until expiration date stamped on the reagent cartridges. • Sealed wells on the instrument are stable for 30 days. • Once wells 1 - 12 have been entered by the instrument, they are stable for 7 days.
Preparation	All reagents are liquid and ready to use.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
LOCI 4 CAL	Siemens Dimension Vista®, Cat. No. KC640A

5.2 Calibrator Preparation and Storage

Calibrator	LOCI 4 CAL
Preparation	LOCI 4 CAL is frozen. Before use, thaw at room temperature for at least 30 minutes. Mix the contents of the vial by inverting gently ten (10) times.
Storage/Stability	• Store at -25 to -15°C • **Unopened calibrator** is stable until expiration date stamped on the box. • **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	**LOCI 4 CAL**
Assay Range	0.5 – 2000 ng/mL (per manufacture)
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in ng/mL
Frequency	• Every new reagent cartridge lot. • Every 30 days for any one lot • When major maintenance is performed on the analyzer. • When control data indicates a significant shift in assay.
Calibration Scheme	5 levels, n = 3

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
Liquichek Immunoassay Plus Control Levels 1, 2 and 3	Bio-Rad Laboratories Cat. No. 267, 268 and 269

6.2 Control Preparation and Storage

Control	Immunoassay Plus Control Levels 1, 2 and 3
Preparation	Allow the vials to stand at room temperature (18-25°C) until it is completely thawed. Gently swirl the vial several times to ensure homogeneity and immediately load on instrument. After each use, promptly replace the stopper and return to 2-8°C storage.

Storage/Stability	Once the control is thawed, all analytes will be stable for 4 days at 2-8°C. Unthawed controls are stable until the expiration date at -20 to -50°C.

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.
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 to not exceed -15 to -25°C for calibrator.
- Freezer capable of sustaining range not to exceed -20 to -50°C for QC product
- Centrifuge

7.3 Supplies

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

8. PROCEDURE

Ferritin Flex® reagent cartridge Cat. No. K6440 is required to perform this test.

Ferritin 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). 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:	2 µL
Chemibeads Reagent Volume:	20 µL
Biotinylated Antibody Reagent Volume:	20 µL
Sensibeads Reagent Volume:	100 µL
Test Temperature:	37 °C
Reaction Time	10 min
Wavelengths	680 & 612 nm
Type of measurement:	Chemiluminescence

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 Ferritin in ng/mL.

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

ng/mL

10.4 Clinically Reportable Range (CRR)

~~0.5~~ 1 – 40,000 ng/mL

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…
< 1 ng/mL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 1 ng/mL
≥ 2,000 ng/mL	**On Board Automated Dilution:** Results ≥ 2,000 ng/mL will automatically have repeat testing performed into the instrument using dilution factor of 20. No multiplication is necessary.
≥ 40,000 ng/mL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 40,000 ng/mL-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

8 – 388 ng/mL

11.2 Critical Values

None established

11.3 Priority 3 Limit(s)

None established

12. CLINICAL SIGNIFICANCE

Measurements of ferritin aid in the diagnosis of diseases affecting iron metabolism, such as hemochromatosis (iron overload) and iron deficiency anemia. Circulating ferritin levels accurately reflect iron stores in the body and are useful when either iron deficiency or iron overload is suspected. The protein originates in the reticuloendothelial cells of the liver and spleen and in the erythroblasts of bone marrow.
Iron deficiency anemia (IDA) is common among menstruating and reproductively active females, children, older adults, and vegetarians. A low ferritin level is an early indicator of IDA; occurring before serum iron is decreased and morphological abnormalities appear in red blood cells. Normal ferritin levels cannot be used to exclude IDA if a hepatic, malignant or inflammatory condition exists in the patient (anemia of chronic disease, ACD). Patients

with ACD may show normal or slightly increased ferritin levels due to an increase in ferritin, caused by the acute phase response associated with chronic inflammation, which overrides the decrease in ferritin associated with IDA.

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 Ferritin concentrations are:

Ferritin Concentration	Acceptable S.D. Maximum
10 ng/mL	0.6 ng/mL
450 ng/mL	27 ng/mL

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

1 – 2000 ng/mL

Note: manufacture's low limit for AMR is 0.5 but SGMC reports values as whole numbers.

14.2 Precision

Material	Mean ng/mL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Lyphochek Anemia Control			
Serum Pool 1	8.8	0.16	0.31
Serum Pool 2	436.4	5.58	14.33
Serum Pool 3	1696.3	27.18	57.44

14.3 Interfering Substances

HIL Interference:
The Ferritin 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	Ferritin ng/mL	Bias %
Hemoglobin (hemolysate)	500 mg/dL	20 , 502.7	<10
Bilirubin (unconjugated)	20 mg/dL	20 , 502.7	<10
Bilirubin (conjugated)	60 mg/dL	20 , 502.7	<10
Lipemia Intralipid®	3000 mg/dL	20 , 502.7	<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.

FERR Flex® Reagent Cartridge and LOCI 4 CAL may cause an allergic skin reaction. Contains 5-chloro-2-methyl-3(2h)-isothiazolone mixture with 2-methyl-3(2h)-isothiazolone. IF ON SKIN: Wash with plenty of soap and water. If skin irritation or rash occurs: 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 Functions 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 FERR Flex® Reagent Cartridge K6440

17. REFERENCES

1. Package Insert, FERR Flex® Reagent Cartridge K6440, Siemens Healthcare Diagnostics Inc., 3/25/2015.
2. Package Insert, LOCI 4 CAL, Siemens Healthcare Diagnostics Inc., 03/2015.
3. Package Insert, Liquichek Immunoassay Plus Control, Bio-Rad Laboratories, revised 12/2015.
4. Ferritin by Siemens Centaur SOP from Quest Diagnostics Nichols Institute in Chantilly, VA. Document CHA QDIC713 v2.2

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
0	6/2/17	3.2	Remove specimen onboard stability	L Barrett	R SanLuis
0	6/2/17	4,5,6	Remove individual section labeling instructions and add general one	L Barrett	R SanLuis
0	6/2/17	6.1, 6.2	Update QC material and storage	L Barrett	R SanLuis
0	6/2/17	10.4, 14.1	Change lower limit from 0.5 to 1 to match practice (reporting whole number)	L Barrett	R SanLuis
0	6/2/17	7.2	Specify freezer ranges for products	L Barrett	R SanLuis
0	6/2/17	10.5	Move patient review from section 6	L Barrett	R SanLuis
0	6/2/17	15	Update to new standard wording, add reagent hazard warning	L Barrett	R SanLuis
0	6/2/17	17	Update QC product and PI dates	L Barrett	R SanLuis

19. ADDENDA

None

Technical SOP

Title	**Folate 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**

Form revised 2/02/2007

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

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Folate	Dimension Vista® System	FOLAC

Synonyms/Abbreviations
Folic Acid; included in battery FOLB12

Department
Chemistry

Form revised 2/02/2007

2. ANALYTICAL PRINCIPLE

The LOCI Folate method is a homogeneous, competitive chemiluminescent immunoassay based on LOCI technology. LOCI reagents include two synthetic bead reagents and labeled folate binding protein (FBP). The first bead reagent (Chemibeads) is coated with a folic acid derivative and contains a chemiluminescent dye. The second bead reagent (Sensibeads) is coated with streptavidin and contains photosensitive dye. Before the immunological portion of the reaction is initiated, the patient sample is pretreated with Sodium Hydroxide (NaOH) and Dithioerythritol (DTE) to release serum folate from endogenous folate binding protein (FBP) and to maintain 5-methyltetrahydrofolate in its reduced form. After the sample pretreatment, chemibeads and labeled folate binding reagent are added sequentially to the reaction vessel. Folate from the patient sample competes with the folate-chemibead for a limited amount of labeled FBP. Sensibeads are then added and bind to the biotinylated portion of the labeled FBP to form bead pair immunocomplexes. Illumination of the complex by light at 680 nm generates singlet oxygen from the Sensibeads which diffuses to the Chemibeads triggering a chemiluminescent reaction. The resulting signal is measured at 612 nm and is an inverse function of the concentration of folate in the sample.

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	Protect samples from light.
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*
	Frozen: 7 days*
Timing Considerations	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.
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	Avoid using hemolyzed samples. 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
Folate	Siemens, Flex® reagent cartridge, Cat. No. K6444

4.2 Reagent Preparation and Storage

Reagent	Folate
Container	Reagent Cartridge
Storage	Store at 2-8° C
Stability	• Reagent is stable until expiration date stamped on the reagent cartridges. • Sealed wells on the instrument are stable for 30 days. • Once wells 3 - 12 have been entered by the instrument, they are stable for 3 days.

Preparation	Hydrating, diluting and mixing are automatically performed by the instrument.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
LOCI 4 CAL	Siemens Dimension Vista®, Cat. No. KC640A

5.2 Calibrator Preparation and Storage

Calibrator	LOCI 4 CAL
Preparation	LOCI 4 CAL is frozen. Before use, thaw at room temperature for at least 30 minutes. Mix the contents of the vial by inverting gently ten times.
Storage/Stability	• Store at -25 to -15° C • **Unopened calibrator** is stable until expiration date stamped on the box. • **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	**LOCI 4 CAL**
Assay Range	0.5 – 20.0 ng/mL
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in ng/mL
Frequency	• Every new reagent cartridge lot. • Every 30 days for any one lot • When major maintenance is performed on the analyzer. • When control data indicates a significant shift in assay.
Calibration Scheme	5 Levels, n = 3

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
Liquichek Immunoassay Plus Control Levels 1, 2 and 3	Bio-Rad Laboratories Cat. No. 267, 268 and 269

6.2 Control Preparation and Storage

Control	Immunoassay Plus Control Levels 1, 2 and 3
Preparation	Allow the vials to stand at room temperature (18-25°C) until it is completely thawed. Gently swirl the vial several times to ensure homogeneity and immediately load on instrument. After each use, promptly replace the stopper and return to 2-8°C storage.

Storage/Stability	Once the control is thawed, Folate will be stable for 4 days at 2-8° C. Unthawed controls are stable until the expiration date at -20 to -50° C.

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.
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 to not exceed -15 to -25°C for calibrator.
- Freezer capable of sustaining range not to exceed -20 to -50°C for QC product
- Centrifuge

7.3 Supplies

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

8. PROCEDURE

Folate Flex® reagent cartridge Cat. No. K6444 is required to perform this test.

Folic Acid 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). 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 µL
DTE Reagent Volume:	10 µL
Extract Reagent Volume:	10 µL
FBE/bio-Mab Reagent Volume:	30 µL
Chemibead Reagent Volume:	20 µL

Sensibead Reagent Volume:	30 µL
Reaction Time:	21 minutes
Test Temperature:	37° C
Wavelength:	Illumination: 680 nm Emission: 612 nm
Type of measurement:	Chemiluminescence

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 Folate in ng/mL.

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

None required

10.2 Rounding

Instrument reports results with one decimal point.

10.3 Units of Measure

ng/mL

10.4 Clinically Reportable Range (CRR)

0.5 – 100.0 ng/mL

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…
< 0.5 ng/mL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 0.5 ng/mL
≥ 20.0 ng/mL	**On Board Automated Dilution:** Results ≥ 20.0 ng/mL will automatically have repeat testing performed into the instrument using dilution factor of 5. No multiplication is necessary.
> 100.0 ng/mL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 100.0 ng/mL-REP" Bring to the attention of your supervisor prior to releasing results.

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

11. EXPECTED VALUES

11.1 Reference Ranges

3.1 – 17.5 ng/mL

11.2 Critical Values

None established

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

Macrocytic anemia is the major clinical manifestation of folate deficiency. It is characterized by abnormal maturation of red blood cell precursors in the bone marrow, the presence of megaloblasts and decreased red blood cell survival. Both folate and vitamin B12 deficiency can cause macrocytic anemia. Folate supplementation can mask B12 deficiency because the associated anemia responds to folate alone. Misdiagnosis delays treatment of the deficiency allowing irreversible neurological abnormalities to progress. Appropriate treatment depends on the differential diagnosis of the deficiency.

The main causes of folate deficiency are absence of intestinal microorganisms, poor intestinal absorption (surgical resection, celiac disease), increased demands (pregnancy, liver disease, and malignancies), insufficient dietary uptake (alcoholism), anti-folate drugs (methotrexate) and anticonvulsants (carbamazepine, phenobarbital, phenytoin, valproic acid).

Although serum folate measurement provides an early index of folate status, red blood cell

folate more closely reflects tissue stores and is considered the most reliable indicator of folate status.

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 Folate concentrations are:

FOL Concentration	Acceptable S.D. Maximum
2.5 ng/mL	0.46 ng/mL
10 ng/mL	1.44 ng/mL

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

0.5 – 20.0 ng/mL

14.2 Precision

Material	Mean ng/mL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Bio-Rad Liquichek			
Level 1	1.9	0.11	0.16
Level 2	4.5	0.18	0.22
Level 3	6	0.26	0.35

14.3 Interfering Substances

- Albumin at 6 g/dL decreases folate results by 13% at 2.9 ng/mL
- Chlorpromazine at 0.2 mg/dL increases folate results by 16% at 2.9 ng/mL
- Cimetidine at 2 mg/dL increases folate results by 47% 2.9 ng/mL
- Total Protein at 12 g/dL decreases folate results by 49% at 2.9 mL

HIL Interference:
The Folate 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	Folate	Bias %
Bilirubin (unconjugated)	20 mg/dL	2.9	<10
Bilirubin (conjugated)	20 mg/dL	2.9	<10
Lipemia Intralipid®	3000 mg/dL	2.9	<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.

Folate Flex® Reagent Cartridge and LOCI 4 CAL may cause an allergic skin reaction. Contains 5-chloro-2-methyl-3(2h)-isothiazolone mixture with 2-methyl-3(2h)-isothiazolone. IF ON SKIN: Wash with plenty of soap and water. If skin irritation or rash occurs: 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 Functions 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 Folate Flex® Reagent Cartridge K6444

17. REFERENCES

1. Package Insert, Folate Flex® Reagent Cartridge K6444, Siemens Healthcare Diagnostics Inc., 3/25/2015.
2. Package Insert, LOCI 4 CAL, Siemens Healthcare Diagnostics Inc., 03/2015.
3. Package Insert, Liquichek Immunoassay Plus Controls, Bio-Rad Laboratories, revised 12/2015.
4. Folate, Serum and RBC by Siemens Centaur SOP from Quest Diagnostics Nichols Institute in Chantilly, VA. Document CHA QDIC723 v3.3L

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
0	6/2/17	3.2	Remove specimen onboard stability	L Barrett	R SanLuis
0	6/2/17	4,5,6	Remove individual section labeling instructions and add general one	L Barrett	R SanLuis
0	6/2/17	6.1, 6.2	Update QC material and storage	L Barrett	R SanLuis
0	6/2/17	7.2	Specify freezer ranges for products	L Barrett	R SanLuis
0	6/2/17	10.5	Move patient review from section 6	L Barrett	R SanLuis
0	6/2/17	15	Update to new standard wording, add reagent hazard warning	L Barrett	R SanLuis
0	6/2/17	17	Update QC product and PI dates	L Barrett	R SanLuis

19. ADDENDA

None