TRAINING UPDATE

Lab Location:	SGMC	**Date Distributed:**	5/26/2021
Department:	Core Lab	**Due Date:**	6/26/2021

DESCRIPTION OF PROCEDURES

Name of procedure:

SOP #	Title
SGMC.C3056	Human Chorionic Gonadotropin, Total hCG by Atellica IM Analyzer
SGMC.C3047	Intact Parathyroid Hormone (PTH) by Atellica IM Analyzer
SGMC.C3052	Prostate-Specific Antigen (PSA) by Atellica IM Analyzer
SGMC.C3035	Free Thyroxine (FT4) by Atellica IM Analyzer
SGMC.C3038	Thyroid Stimulating Hormone (TSH3-UL) by Atellica IM Analyzer
SGMC.C3045	Total Iron Binding Capacity (TIBC) by Atellica CH Analyzer
SGMC.C3033	Ferritin (Fer) by Atellica IM Analyzer
SGMC.C3034	Folate (Fol) by Atellica IM Analyzer
SGMC.C3044	Iron (Iron-2) by Atellica CH Analyzer
SGMC.C3037	Vitamin B12 (VB12) by Atellica IM Analyzer

Description of change(s):

These are the new assay SOPs for the Atellica Solution analyzers. Core technical staff must review and be familiar with -

- Specimen requirements
- Reagent, calibrator & QC stability and storage
- Ranges and dilutions

These SOPs were implemented on May 19, 2021

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

Technical SOP

Title	**Human Chorionic Gonadotropin, Total hCG by Atellica IM Analyzer**
Prepared by	Ashkan Chini Date: 5/3/2021
Owner	Robert SanLuis Date: 5/3/2021

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

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Test Code
Human Chorionic Gonadotropin, Quantitative	Atellica IM Analyzer	HCGQ

Synonyms/Abbreviations
Pregnancy test, Quant; Quant hCG; Total HCG, THCG

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The Atellica IM ThCG assay is a 2-site sandwich immunoassay using direct chemiluminescent technology, which uses constant amounts of 2 antibodies. The first antibody, in the Lite Reagent, is a goat polyclonal anti‑hCG antibody that has been affinity purified and labeled with acridinium ester. The second antibody, in the Solid Phase, is a purified mouse monoclonal anti‑hCG antibody, which is covalently coupled to paramagnetic particles. These 2 antibodies are specific for different epitopes that are present on both the free ß‑subunit and the ß‑subunit of intact hCG.
A direct relationship exists between the amount of hCG present in the patient sample and the amount of relative light units (RLUs)detected by the system

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	Serum
-Other Acceptable	Plasma (Lithium Heparin)

Criteria	
Collection Container	Serum: Red top tube, Serum separator tube (SST) Plasma: Mint green top tube (PST)
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: 48 hours
	Frozen: Not established
Timing Considerations	N/A
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. Before placing on system, ensure samples are free of: • Bubbles or foam • Fibrin or other particulate matter

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
Total hCG (ThCG)	Siemens, Atellica IM, Cat. No. 10995690
ThCG DIL	Siemens, Atellica IM, Cat. No. 10995691

4.2 Reagent Preparation and Storage

Reagent	**Total hCG (ThCG)**
Storage	• Store at 2-8°C • Store in an upright position. • Protect from heat and light.
Stability	Reagents are stable onboard the system for 21 days.
Preparation	Reagent is liquid and ready to use. Before loading the reagent onto the system, mix it by hand and visually inspect the bottom of the reagent pack to ensure that all particles are re-suspended.

Reagent	**ThCG DIL**
Storage	Store at 2-8°C in an upright position.
Stability	Stable onboard the system for 28 days.
Preparation	Liquid and ready to use.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	**Supplier and Catalog Number**
Calibrator B (CAL B)	Siemens Atellica IM, Cat. No. 10995503

5.2 Calibrator Preparation and Storage

Calibrator	Calibrator B (CAL B)
Preparation	1. Add 5.0 mL of reagent grade water into Low and High Calibrator vials using a calibrated pipette. Replace cap. 2. Let the vials stand for 15–20 minutes at room temperature to allow the lyophilized material to dissolve. 3. Gently mix and invert the vials to ensure homogeneity of the material.
Storage/Stability	• Store at 2-8°C in an upright position. • **Unopened:** stable until expiration date stamped on the box. • **Reconstituted:** remains stable for 4 hours at room temperature and 28 days refrigerated.

5.3 Calibration Parameter

Criteria	**Special Notations**
Reference Material	Calibrator B (CAL B)
Assay Range	See Package Insert for specific assay ranges.

Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in mIU/mL.
Frequency	• When changing lot numbers of primary reagent packs. • At the end of the lot calibration interval (34 days), for a specified lot of calibrated reagent on the system. • At the end of pack calibration interval (28 days), for calibrated reagent packs on the system. • When indicated by quality control results. • After major maintenance or service. At the end of the onboard stability interval, replace the reagent pack on the system with a new reagent pack. Recalibration is not required, unless the lot calibration interval is exceeded.
Calibration Scheme	See Package Insert for specific calibration scheme.
Procedure	To load a new lot of reagent on IM Module: Note: Calibrate the new lot of reagent as soon as it is loaded on the instrument. If the reagent sits un-calibrated for a short period of time (24 hours) then it will not be eligible for a "Lot Calibration" and another new reagent will need to be loaded onboard. 1. From the home page of the IM Module Screen (small screen attached on the IM Module) make sure the analyzer status is in Standby. 2. On the IM Module Screen select Reagent Loader. Make sure the Reagent Drawer status is unlocked. Open the reagent drawer, load the reagent and then close it. Once the reagent is scanned, the IM Module Screen will populate message "Missing TDef for lot" next to the reagent. The Reagent Drawer status remains unlocked. 3. Both Reagent Master Curve and Calibrator Package Insert need to be scanned using the Atellica Solution's main monitor. To differentiate between the two: • Reagent Master Curve has MC TDEF printed right below the assay name. • Calibrator Package Insert has CAL printed right above the assay name. 4. To scan the Reagent Master Curve, go to Set up – Test Definition – IM Test Definition. Scan the barcode. 5. To scan the Calibrator Package Insert, go to Calibration – Calibrator Definition. Scan the barcode. 6. Re-open the Reagent Drawer and close it. This time its status should change to locked, meaning the reagent is going to be loaded onboard ready for calibration.

Procedure	Refer to the Atellica Solution Operating, QC, Calibration and Maintenance procedure for specific instructions.

5.4 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
InteliQ Immunoassay Plus Controls, Levels 1, 2 & 3	Bio-Rad Laboratories Cat. No. 12009948, 12009949, 12009950

6.2 Control Preparation and Storage

Control	InteliQ Immunoassay Plus Controls
Preparation	Allow to thaw at room temperature (18-25C) for approximately 60 minutes or until completely thawed. Once thawed, gently invert the tube several times to ensure homogeneity.
Storage/Stability	**Frozen**: until the expiration date if unopened at -20 to -70C **Thawed and Unopened:** 30 days at 2-8C **Thawed and Onboard:** 14 days at 2-8C Note: Stability for PSA and Folate is shorter.

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 Siemens Atellica QC Schedule and the Siemens Atellica 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

Siemens Atellica IM Analyzer

7.2 Equipment

- Refrigerator capable of sustaining 2–8°C.
- Freezer capable of sustaining range not to exceed -20 to -70°C.
- Centrifuge

7.3 Supplies

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

8. PROCEDURE

Atellica IM Total hCG (ThCG) is required to perform this test.

Total hCG is performed on the Atellica IM Analyzer after the method is calibrated 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	Instrument Set-up Protocol
1.	Perform any required instrument maintenance.
2.	Ensure that the instrument has sufficient primary and ancillary reagents.
3.	Check status of cuvettes and tips. Check waste levels. Fill or empty as appropriate.
4.	Check calibration status and re-calibrate as needed.

8.2	Specimen Testing
1.	Centrifuge the specimens.

8.2	Specimen Testing
2.	Load the sample in the Atellica rack and place the rack into the Sample Handler to initiate testing. **NOTE: If not equipped with an in-line decapper unit, samples must be de-capped prior to loading on the Atellica system
3.	Refer to the general operating procedure for detailed steps.
4.	Follow protocol in section 10.6 "Repeat Criteria and Resulting" for samples with results above the analytical measurement range (AMR). Investigate any flagged results and repeat as 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.

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 Total hCG in mIU/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

mIU/mL

10.4 Clinically Reportable Range (CRR)

3 – 1,000,000 mIU/mL

10.5 Review Patient Data

Each result is reviewed for error messages. 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 below or within the AMR or CRR may be reported without repeat. Values that exceed the upper ranges must be repeated.

IF the result is …	THEN…
< 3 mIU/mL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 3 mIU/mL
≥ 1,000 mIU/mL	**On Board Automated Dilution:** Results ≥ 1000 mIU/mL will automatically have repeat testing performed into the instrument using dilution factor of 1,000. No multiplication is necessary.
> 1,000,000 mIU/mL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 1,000,000 mIU/mL - REP" Bring to the attention of Tech in Charge (TIC) or Group Lead to check for integrity issues prior to release of results.

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

11. EXPECTED VALUES

11.1 Reference Ranges

Age	Non- Pregnant Female	Male
All	1 - 3 mIU/mL	0 - 2 mIU/mL

11.2 Critical Values

None established

11.3 Standard Required Messages

Each result has the following interpretation automatically added to the report by the LIS:

HCG levels with Gestational Age

Gestational Age	hCG mIU/mL (IU/L)
0.2-1 week	5-50
1-2 weeks	50-500
2-3 weeks	100-5,000
3-4 weeks	500-10,000
4-5 weeks	1,000-50,000
5-6 weeks	10,000-100,000
6-8 weeks	15,000-200,000
2-3 months	10,000-100,000

12. CLINICAL SIGNIFICANCE

Human chorionic gonadotropin (hCG) is a heterodimeric (α plus s) sialoglycoprotein hormone produced by the placenta soon after a fertilized ovum implants into the uterine wall. Presence of hCG in serum shortly after conception, followed by a rapid rise in concentration, makes it an excellent marker to confirm and monitor a pregnancy. Physiologically, hCG appears to maintain the corpus luteum and support the endometrium. Serum and plasma hCG concentrations peak during the first trimester, then decrease and plateau during the remainder of pregnancy, circulating as the intact heterodimer in the blood of healthy women who have an uncomplicated pregnancy.

13. PROCEDURE NOTES

- **FDA Status:** FDA Approved/modified
- **Validated Test Modifications:** Plasma sample types validated

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.

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

3 – 1,000 mIU/mL

14.2 Precision

Material	Mean mIU/mL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Serum 1	2.4	0.2	8.8
Serum 2	12.6	0.4	2.8
Serum 3	787	14	1.8
Control 1	6.8	0.3	4.5
Control 2	23.4	0.6	2.6
Control 3	202.1	3.6	1.8

14.3 Interfering Substances

HIL Interference:
Interfering substances at the levels indicated in the table below were tested in accordance with CLSI Document EP07-A2.

Substance tested	Substance Concentration	mIU/mL	Bias %
Hemoglobin	1000 mg/dL	5.6	8.9
Bilirubin (conjugated)	40 mg/dL	6.1	-0.3
Bilirubin (unconjugated)	40 mg/dL	6.0	0.3
Lipemia Intralipid®	3000 mg/dL	6.1	2.0

14.4 Clinical Sensitivity/Specificity/Predictive Values

Detection Capability
The assay is designed to have a limit of blank (LoB) ≤ 2.0 mIU/mL, a limit of detection (LoD) ≤ 4.0 mIU/mL, and a limit of quantitation (LoQ) ≤ 6.0 mIU/mL.
The LoB of the Atellica IM ThCG assay is 1.5 mIU/mL. The LoD for the Atellica IM ThCG assay is 1.7 mIU/mL. The LoQ of the Atellica IM ThCG assay is 2.6 mIU/mL.

Patient samples with high hCG levels can cause a paradoxical decrease in RLUs (high-dose hook effect). In this assay, hCG levels as high as 1,000,000 mIU/mL will assay greater than 1,000 mIU/mL.

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.

16. RELATED DOCUMENTS

1. Atellica Solution Operating, QC, Calibration and Maintenance procedure
2. Laboratory Quality Control Program
3. QC Schedule for Siemens Atellica Solution
4. Laboratory Safety Manual
5. Safety Data Sheets (SDS)
6. Atellica Solution Limits Chart
7. Quest Diagnostics Records Management Procedure
8. Atellica Solution System Error Messages Chart
9. Centrifuge Use, Maintenance and Function Checks (Lab policy)
10. Specimen Acceptability Requirements (Lab policy)
11. Repeat Testing Requirement (Lab policy)
12. Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls
13. Current package insert of Total hCG Reagent

17. REFERENCES

1. Package Insert, Total hCG Reagent, Siemens Healthcare Diagnostics Inc., 02/2020.
2. Package Insert, Calibrator B (CAL B), Siemens Healthcare Diagnostics Inc., 08/2019.
3. Package Insert, InteliQ Immunoassay Plus Controls, Bio-Rad Laboratories, 12/2020

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

None

Technical SOP

Title	**Intact Parathyroid Hormone (PTH) by Atellica IM Analyzer**
Prepared by	Ashkan Chini Date: 4/30/2021
Owner	Robert SanLuis Date: 4/30/2021

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

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Test Code
Intact Parathyroid Hormone	Atellica IM Analyzer	IOIPTH, ITPTH

Synonyms/Abbreviations
iPTH, Intraoperative IPTH

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The Atellica IM PTH assay is a 2-site sandwich immunoassay using direct chemiluminescent technology, which uses constant amounts of 2 anti-human PTH antibodies. The first antibody, in the Lite Reagent, is a mouse monoclonal anti-human PTH (N‑terminal) antibody labeled with acridinium ester. The second antibody is a biotinylated mouse monoclonal anti-human PTH (C‑terminal) antibody that is bound to streptavidin-coated paramagnetic latex particles in the Solid Phase. A direct relationship exists between the amount of intact PTH present in the patient sample and the amount of relative light units detected by the system.

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

Criteria	
Transport Container and Temperature	Collection container or Plastic vial at room temperature
Stability & Storage Requirements	Room Temperature: 9 hours
	Refrigerated: 72 hours
	Frozen: Not recommended
Timing Considerations	N/A
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. Before placing on system, ensure samples are free of: • Bubbles or foam • Fibrin or other particulate matter

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
Intact Parathyroid Hormone (PTH)	Siemens, Atellica IM, Cat. No. 10995621

4.2 Reagent Preparation and Storage

Reagent	Intact Parathyroid Hormone (PTH)
Storage	• Store at 2-8°C • Store in an upright position. • Protect from heat and light.

Stability	Reagents are stable onboard the system for 28 days.
Preparation	Reagent is liquid and ready to use. Before loading primary reagent packs onto the system, mix them by hand and visually inspect the bottom of the reagent pack to ensure that all particles are re-suspended.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
Intact Parathyroid Hormone (PTH) Calibrator	Siemens, Atellica IM, Cat. No. 10995621

5.2 Calibrator Preparation and Storage

Calibrator	Intact Parathyroid Hormone (PTH) Calibrator
Preparation	1. Add 1.0 mL of reagent grade water into each vial using a calibrated pipette. Replace cap. 2. Let the vials stand for 30 minutes at room temperature to allow the lyophilized material to dissolve. 3. Gently mix and invert the vials to ensure homogeneity of the material.
Storage/Stability	• Store at 2-8°C in an upright position. • **Unopened:** stable until expiration date stamped on the box. • **Reconstituted:** remains stable for 8 hours at room temperature and 60 days frozen (thaw one time ONLY).

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	Intact Parathyroid Hormone (PTH) Calibrator
Assay Range	See Package Insert for specific assay ranges.
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in pg/mL
Frequency	• When changing lot numbers of primary reagent packs. • At the end of the lot calibration interval (91 days), for a specified lot of calibrated reagent on the system. • At the end of pack calibration interval (14 days), for calibrated reagent packs on the system. • When indicated by quality control results. • After major maintenance or service.

	At the end of the onboard stability interval, replace the reagent pack on the system with a new reagent pack. Recalibration is not required, unless the lot calibration interval is exceeded.
Calibration Scheme	See Package Insert for specific calibration scheme.
Procedure	To load a new lot of reagent on IM Module: Note: Calibrate the new lot of reagent as soon as it is loaded on the instrument. If the reagent sits un-calibrated for a short period of time (24 hours) then it will not be eligible for a "Lot Calibration" and another new reagent will need to be loaded onboard. 1. From the home page of the IM Module Screen (small screen attached on the IM Module) make sure the analyzer status is in Standby. 2. On the IM Module Screen select Reagent Loader. Make sure the Reagent Drawer status is unlocked. Open the reagent drawer, load the reagent and then close it. Once the reagent is scanned, the IM Module Screen will populate message "Missing TDef for lot" next to the reagent. The Reagent Drawer status remains unlocked. 3. Both Reagent Master Curve and Calibrator Package Insert need to be scanned using the Atellica Solution's main monitor. To differentiate between the two: • Reagent Master Curve has MC TDEF printed right below the assay name. • Calibrator Package Insert has CAL printed right above the assay name. 4. To scan the Reagent Master Curve, go to Set up – Test Definition – IM Test Definition. Scan the barcode. 5. To scan the Calibrator Package Insert, go to Calibration – Calibrator Definition. Scan the barcode. 6. Re-open the Reagent Drawer and close it. This time its status should change to locked, meaning the reagent is going to be loaded onboard ready for calibration.
Procedure	Refer to the Atellica Solution Operating, QC, Calibration and Maintenance procedure for specific instructions.

5.4 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
Lyphocheck Specialty Immunoassay Control, Levels 1, 2 and 3	Bio-Rad Laboratories Cat. No. 27124, 27125, 27126

6.2 Control Preparation and Storage

Control	Lyphocheck Specialty Immunoassay Control, Levels 1, 2 and 3
Preparation	Reconstitute each vial with 2 mL of reagent grade water. Replace the stopper and allow this product to stand for approximately 15 minutes swirling occasionally. Before sampling, gently swirl the vials several times to ensure homogeneity.
Storage/Stability	**Unopened**: until expiration date when stored at 2-8C. **Reconstituted & stored tightly capped at 2-8C**: 4 days for PTH **Reconstituted and stored in tightly capped aliquot vials at -20 to -70C**: stable for 30 days. Use the content of each aliquot vial only once and discard the remainder.

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 Siemens Atellica QC Schedule and the Siemens Atellica 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.

Step	Action
	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

Siemens Atellica IM Analyzer

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

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

8. PROCEDURE

Atellica IM Intact Parathyroid Hormone (PTH) is required to perform this test.

PTH is performed on the Atellica IM Analyzer after the method is calibrated 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	Instrument Set-up Protocol
1.	Perform any required instrument maintenance.
2.	Ensure that the instrument has sufficient primary and ancillary reagents.
3.	Check status of cuvettes and tips. Check waste levels. Fill or empty as appropriate.
4.	Check calibration status and re-calibrate as needed.

8.2	Specimen Testing
1.	Centrifuge the specimens.
2.	Load the sample in the Atellica rack and place the rack into the Sample Handler to initiate testing. **NOTE: If not equipped with an in-line decapper unit, samples must be de-capped prior to loading on the Atellica system
3.	Refer to the general operating procedure for detailed steps.
4.	Follow protocol in section 10.6 “Repeat Criteria and Resulting” for samples with results above the analytical measurement range (AMR). Investigate any flagged results and repeat as 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.

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 PTH in pg/mL.

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

None required

10.2 Rounding

No rounding is necessary. Instrument reports results up to one decimal point.

10.3 Units of Measure

pg/mL

10.4 Clinically Reportable Range (CRR)

6.3 – 2000.0 pg/mL

10.5 Review Patient Data

Each result is reviewed for error messages. 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 below or within the AMR or CRR may be reported without repeat. Values that exceed the upper ranges must be repeated.

IF the result is …	THEN…
< 6.3 pg/mL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 6.3 pg/mL
> 2000.0 pg/mL	Report as: "> 2000.0 pg/mL"

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

11. EXPECTED VALUES

11.1 Reference Ranges

18.4 – 80.1 pg/mL

11.2 Critical Values

None established

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

This assay is intended to be used to aid in the differential diagnosis of hyperparathyroidism, hypoparathyroidism, or hypercalcemia of malignancy. Parathyroid hormone (PTH), produced by the parathyroid gland, is the major circulating factor regulating extracellular calcium concentration. Abnormally low-ionized calcium concentrations trigger the secretion of PTH. The PTH molecules bind to type 1 parathyroid hormone receptors in target tissues and initiate a sequence of reactions resulting in increased extracellular calcium concentrations. PTH stimulates osteoclastic bone resorption resulting in the release of calcium from bone. PTH stimulates transcellular calcium reabsorption from the renal tubules and stimulates the kidney to produce 1,25-dihydroxyvitamin D which acts on the intestines to increase calcium reabsorption. In most clinical conditions, rising levels of extracellular calcium suppresses PTH secretion through a negative feedback mechanism. PTH increases the rate of bone metabolism. Depending on the age of the patient, the bones involved, and the concentrations of the hormone in circulation over time, the effect on the bone can be either catabolic or anabolic. Consistently high concentrations of PTH generally have a catabolic effect and intermittent, slightly elevated concentrations have an anabolic effect.

13. PROCEDURE NOTES

- **FDA Status:** FDA Approved/cleared
- **Validated Test Modifications:** None

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.

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

6.3 – 2000.0 pg/mL

14.2 Precision

Material	Mean pg/mL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Serum A	3.8	0.53	N/A
Serum B	20.7	0.7	3.4
Control 1	42.0	1.91	4.6
Control 2	240.7	3.99	1.7
Control 3	868.7	12.24	1.4

14.3 Interfering Substances

HIL Interference:
Interfering substances at the levels indicated in the table below were tested in accordance with CLSI Document EP07-A2.

Substance tested	Substance Concentration	pg/mL	Bias %
Hemoglobin	500 mg/dL	16.8	-3
Bilirubin (conjugated)	60 mg/dL	15.8	-3
Bilirubin (unconjugated)	60 mg/dL	16.2	-1
Lipemia Intralipid®	3275 mg/dL	12.8	-4

14.4 Clinical Sensitivity/Specificity/Predictive Values

Detection Capability
The assay is designed to have a limit of blank (LoB) < 6.0 pg/mL, a limit of detection (LoD) ≤ 6.0 pg/mL, and a limit of quantitation (LoQ) ≤ 6.0 pg/mL. The LoB of the Atellica IM PTH assay is 1.4 pg/mL. The LoD for the Atellica IM PTH assay is 1.5 pg/mL. The LoQ of the Atellica IM PTH assay is 2.3 pg/mL.

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.
Product is toxic to aquatic life. Avoid release into the environment. May produce an allergic reaction. **Contains:** 2-methyl-2H-isothiazol-3-one.

16. RELATED DOCUMENTS

1. Atellica Solution Operating, QC, Calibration and Maintenance procedure
2. Laboratory Quality Control Program
3. QC Schedule for Siemens Atellica Solution
4. Laboratory Safety Manual
5. Safety Data Sheets (SDS)
6. Atellica Solution Limits Chart
7. Quest Diagnostics Records Management Procedure
8. Atellica Solution System Error Messages Chart
9. Centrifuge Use, Maintenance and Function Checks (Lab policy)

10. Specimen Acceptability Requirements (Lab policy)
11. Repeat Testing Requirement (Lab policy)
12. Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls
13. Current package insert of Intact Parathyroid Hormone (PTH) Reagent

17. REFERENCES

1. Package Insert, PTH Reagent, Siemens Healthcare Diagnostics Inc., 11/2020.
2. Package Insert, Lyphocheck Specialty Immunoassay Control, Bio-Rad Laboratories, 04/2019

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

None

Technical SOP

Title	**Prostate-Specific Antigen (PSA) by Atellica IM Analyzer**
Prepared by	Ashkan Chini Date: 4/25/2021
Owner	Robert SanLuis Date: 4/25/2021

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

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Test Code
PSA Total	Atellica IM Analyzer	PSAT

Synonyms/Abbreviations
PSA, Prostatic Antigen, Prostate-Specific Antigen, TPSA

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The Atellica IM PSA assay is a 2‑site sandwich immunoassay using direct chemiluminescent technology, which uses constant amounts of 2 antibodies. The first antibody, in the Lite Reagent, is a goat polyclonal anti‑PSA antibody labeled with acridinium ester. The second antibody, in the Solid Phase, is a mouse monoclonal anti‑PSA antibody, which is covalently coupled to paramagnetic particles. A direct relationship exists between the amount of PSA present in the sample and the amount of relative light units detected by the system.

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 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	Serum
Collection Container	Serum: Red top tube, Serum separator tube (SST)
Volume - Optimum **- Minimum**	1.0 mL 0.5 mL

Criteria	
Transport Container and Temperature	Collection container or Plastic vial at room temperature
Stability & Storage Requirements	Room Temperature: 8 hours
	Refrigerated: 48 hours
	Frozen: Not specified by manufacturer
Timing Considerations	N/A
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. Before placing on system, ensure samples are free of: • Bubbles or foam • Fibrin or other particulate matter

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
Prostate-Specific Antigen (PSA)	Siemens, Atellica IM, Cat. No. 10995662
Multi-Diluent 2	Siemens, Atellica IM, Cat. No. 10995644

4.2 Reagent Preparation and Storage

Reagent	Prostate-Specific Antigen (PSA)
Storage	• Store at 2-8°C • Store in an upright position. • Protect from heat and light.

Stability	Reagents are stable onboard the system for 28 days.
Preparation	Reagent is liquid and ready to use. Before loading primary reagent packs onto the system, mix them by hand and visually inspect the bottom of the reagent pack to ensure that all particles are re-suspended.

Reagent	**Multi-Diluent 2**
Storage	Store at 2-8°C in an upright position.
Stability	It is stable onboard the system for 28 days.
Preparation	Liquid and ready to use.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
Calibrator Q (CAL Q)	Siemens Atellica IM, Cat. No. 10995517

5.2 Calibrator Preparation and Storage

Calibrator	Calibrator Q (CAL Q)
Preparation	1. Add 2.0 mL of reagent grade water into each vial using a calibrated pipette. Replace cap. 2. Let the vials stand for 15–20 minutes at room temperature to allow the lyophilized material to dissolve. 3. Gently mix and invert the vials to ensure homogeneity of the material.
Storage/Stability	• Store at 2-8°C in an upright position. • **Unopened:** stable until expiration date stamped on the box. • **Reconstituted:** stable for 8 hours at room temperature and 21 days refrigerated.

5.3 Calibration Parameter

Criteria	**Special Notations**
Reference Material	Calibrator Q (CAL Q)
Assay Range	See Package Insert for specific assay ranges.
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in ng/mL
Frequency	• When changing lot numbers of primary reagent packs. • At the end of the lot calibration interval (29 days), for a specified lot of calibrated reagent on the system.

	• At the end of pack calibration interval (28 days), for calibrated reagent packs on the system. • When indicated by quality control results. • After major maintenance or service. At the end of the onboard stability interval, replace the reagent pack on the system with a new reagent pack. Recalibration is not required, unless the lot calibration interval is exceeded.
Calibration Scheme	See Package Insert for specific calibration scheme.
Procedure	To load a new lot of reagent on IM Module: Note: Calibrate the new lot of reagent as soon as it is loaded on the instrument. If the reagent sits un-calibrated for a short period of time (24 hours) then it will not be eligible for a "Lot Calibration" and another new reagent will need to be loaded onboard. 1. From the home page of the IM Module Screen (small screen attached on the IM Module) make sure the analyzer status is in Standby. 2. On the IM Module Screen select Reagent Loader. Make sure the Reagent Drawer status is unlocked. Open the reagent drawer, load the reagent and then close it. Once the reagent is scanned, the IM Module Screen will populate message "Missing TDef for lot" next to the reagent. The Reagent Drawer status remains unlocked. 3. Both Reagent Master Curve and Calibrator Package Insert need to be scanned using the Atellica Solution's main monitor. To differentiate between the two: • Reagent Master Curve has MC TDEF printed right below the assay name. • Calibrator Package Insert has CAL printed right above the assay name. 4. To scan the Reagent Master Curve, go to Set up – Test Definition – IM Test Definition. Scan the barcode. 5. To scan the Calibrator Package Insert, go to Calibration – Calibrator Definition. Scan the barcode. 6. Re-open the Reagent Drawer and close it. This time its status should change to locked, meaning the reagent is going to be loaded onboard ready for calibration.
Procedure	Refer to the Atellica Solution Operating, QC, Calibration and Maintenance procedure for specific instructions.

5.4 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
InteliQ Immunoassay Plus Controls, Levels 1, 2 & 3	Bio-Rad Laboratories Cat. No. 12009948, 12009949, 12009950

6.2 Control Preparation and Storage

Control	InteliQ Immunoassay Plus Controls
Preparation	Allow to thaw at room temperature (18-25C) for approximately 60 minutes or until completely thawed. Once thawed, gently invert the tube several times to ensure homogeneity.
Storage/Stability	**Frozen**: until the expiration date if unopened at -20 to -70C **Thawed and Unopened:** 14 days at 2-8C **Thawed and Onboard:** 14 days at 2-8C Note: Stability for Folate is shorter.

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 Siemens Atellica QC Schedule and the Siemens Atellica 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

Siemens Atellica IM Analyzer

7.2 Equipment

- Refrigerator capable of sustaining 2–8°C.
- Freezer capable of sustaining range not to exceed -20 to -70°C.
- Centrifuge

7.3 Supplies

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

8. PROCEDURE

Atellica IM Prostate-Specific Antigen (PSA) is required to perform this test.

PSA is performed on the Atellica IM Analyzer after the method is calibrated 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	Instrument Set-up Protocol
1.	Perform any required instrument maintenance.
2.	Ensure that the instrument has sufficient primary and ancillary reagents.
3.	Check status of cuvettes and tips. Check waste levels. Fill or empty as appropriate.
4.	Check calibration status and re-calibrate as needed.

8.2	Specimen Testing
1.	Centrifuge the specimens.

8.2	Specimen Testing
2.	Load the sample in the Atellica rack and place the rack into the Sample Handler to initiate testing. **NOTE: If not equipped with an in-line decapper unit, samples must be de-capped prior to loading on the Atellica system
3.	Refer to the general operating procedure for detailed steps.
4.	Follow protocol in section 10.6 "Repeat Criteria and Resulting" for samples with results above the analytical measurement range (AMR). Investigate any flagged results and repeat as 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.

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 Prostate-Specific Antigen 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 up to one decimal point.

10.3 Units of Measure

ng/mL

10.4 Clinically Reportable Range (CRR)

0.0 – 10,000.0 ng/mL

10.5 Review Patient Data

Each result is reviewed for error messages. 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 below or within the AMR or CRR may be reported without repeat. Values that exceed the upper ranges must be repeated.

IF the result is …	THEN…
0.0 ng/mL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: 0.0 ng/mL
≥ 100.0 ng/mL	**On Board Automated Dilution:** Results ≥ 100.0 ng/mL will automatically have repeat testing performed into the instrument using dilution factor of 100. No multiplication is necessary.
> 10,000.0 ng/mL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 10,000.0 ng/mL -REP" Bring to the attention of Tech in Charge (TIC) or Group Lead to check for integrity issues prior to release of results.

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

11. EXPECTED VALUES

11.1 Reference Ranges

< 4.0 ng/mL

11.2 Critical Values

None established

11.3 Standard Required Messages

The following comment is automatically added to the report by the LIS:

"This test was performed using the Chemiluminescence method. Values obtained from different assay methods cannot be used interchangeably. PSA levels, regardless of value, should not be interpreted as absolute evidence of the presence or absence of disease."

12. CLINICAL SIGNIFICANCE

Prostate cancer is the most common type of cancer found in men in the United States and the second leading cause of male cancer mortality. Prior to the use of PSA for early detection of prostate cancer, the traditional method of digital rectal examination (DRE) detected considerably fewer tumors. The most sensitive method for early detection of prostate cancer uses both DRE and PSA. The American Cancer Society and American Urological Association (AUA) recommend that early detection of prostate cancer should be offered to asymptomatic men 50 years of age or older with an estimated life expectancy of more than

10 years. An abnormal DRE and/or an elevated PSA may suggest the presence of prostate cancer; however, a prostate biopsy is required for final diagnosis. PSA testing is also accepted as an adjunctive test in the management of prostate cancer. Serum levels of PSA are most useful when sequential values are obtained and monitored over time. After complete removal of the prostate gland (radical prostatectomy), PSA levels should decline to a very low or non-detectable level. A rise of the serum PSA level in prostatectomy patients indicates residual prostate tissue; recurrence or metastasis of the disease. Serum PSA levels during radiation treatment should decline and remain at baseline while the patient is in remission.

13. PROCEDURE NOTES

- **FDA Status:** FDA Approved/cleared
- **Validated Test Modifications:** None

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.

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

0.0 – 100.0 ng/mL (lower value adjusted to one decimal place)

14.2 Precision

Material	Mean ng/mL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Serum A	0.07	0.0	N/A
Serum B	0.44	0.01	2.2
Serum C	3.66	0.07	2.0
Serum D	6.48	0.10	1.5
Serum E	13.53	0.22	1.6
Serum F	32.49	0.76	2.3
Serum G	79.63	2.13	2.55

14.3 Interfering Substances

HIL Interference:
Interfering substances at the levels indicated in the table below were tested in accordance with CLSI Document EP07-A2.

Serum specimens that are …	Demonstrate ≤ 5% change in results up to …
Hemolyzed	500 mg/dL of hemoglobin
Icteric	40 mg/dL of bilirubin
Lipemic	1000 mg/dL of triglycerides

14.4 Clinical Sensitivity/Specificity/Predictive Values

Detection Capability
The assay is designed to have a limit of blank (LoB) ≤ 0.01 ng/mL, a limit of detection (LoD) ≤ 0.03 ng/mL, and a limit of quantitation (LoQ) ≤ 0.10 ng/mL. The LoB of the Atellica IM PSA assay is 0.01 ng/mL. The LoD for the Atellica IM PSA assay is 0.02 ng/mL. The LoQ of the Atellica IM PSA assay is 0.04 ng/mL.

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.

16. RELATED DOCUMENTS

1. Atellica Solution Operating, QC, Calibration and Maintenance procedure
2. Laboratory Quality Control Program
3. QC Schedule for Siemens Atellica Solution
4. Laboratory Safety Manual
5. Safety Data Sheets (SDS)
6. Atellica Solution Limits Chart
7. Quest Diagnostics Records Management Procedure
8. Atellica Solution System Error Messages Chart
9. Centrifuge Use, Maintenance and Function Checks (Lab policy)
10. Specimen Acceptability Requirements (Lab policy)
11. Repeat Testing Requirement (Lab policy)
12. Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls
13. Current package insert of Prostate-Specific Antigen Reagent

17. REFERENCES

1. Package Insert, PSA Reagent, Siemens Healthcare Diagnostics Inc., 09/2019
2. Package Insert, CAL Q, Siemens Healthcare Diagnostics Inc., 08/2019
3. Package Insert, InteliQ Immunoassay Plus Controls, Bio-Rad Laboratories, 12/2020

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

None

Technical SOP

Title	**Free Thyroxine (FT4) by Atellica IM Analyzer**
Prepared by	Ashkan Chini Date: 4/21/2021
Owner	Robert SanLuis Date: 4/21/2021

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

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Test Code
Free Thyroxine	Atellica IM Analyzer	FT4

Synonyms/Abbreviations
Free T4

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The Atellica IM FT4 assay is a competitive immunoassay using direct chemiluminescent technology. FT4 in the patient sample competes with acridinium-ester-labeled T4 in the Lite Reagent for a limited amount of biotinylated rabbit polyclonal anti-T4 antibody. Biotin-labeled anti‑T4 is bound to avidin that is covalently coupled to paramagnetic particles in the Solid Phase. An inverse relationship exists between the amount of FT4 present in the patient sample and the amount of relative light units detected by the system.

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

Criteria	
Transport Container and Temperature	Collection container or Plastic vial at room temperature
Stability & Storage Requirements	Room Temperature: 8 hours
	Refrigerated: 48 hours
	Frozen: Not established
Timing Considerations	N/A
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. Before placing on system, ensure samples are free of: • Bubbles or foam • Fibrin or other particulate matter

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
Free Thyroxine (FT4)	Siemens, Atellica IM, Cat. No. 10995589

4.2 Reagent Preparation and Storage

Reagent	Free Thyroxine (FT4)
Storage	• Store at 2-8°C • Store in an upright position. • Protect from heat and light.
Stability	Reagents are stable onboard the system for 21 days.

Preparation	Reagent is liquid and ready to use. Before loading primary reagent packs onto the system, mix them by hand and visually inspect the bottom of the reagent pack to ensure that all particles are re-suspended.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
Calibrator A (CAL A)	Siemens Atellica IM, Cat. No. 10995500

5.2 Calibrator Preparation and Storage

Calibrator	Calibrator A (CAL A)
Preparation	1. Add 5.0 mL of reagent grade water into each vial using a calibrated pipette. Replace cap. 2. Let the vials stand for 15–20 minutes at room temperature to allow the lyophilized material to dissolve. 3. Gently mix and invert the vials to ensure homogeneity of the material.
Storage/Stability	• Store at 2-8°C in an upright position. • **Unopened:** stable until expiration date stamped on the box. • **Reconstituted:** o 4 hours at room temperature o 28 days at 2-8°C

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	Calibrator A (CAL A)
Assay Range	See Package Insert for specific assay ranges.
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in ng/dL
Frequency	• When changing lot numbers of primary reagent packs. • At the end of the lot calibration interval (21 days), for a specified lot of calibrated reagent on the system. • At the end of pack calibration interval (7 days), for calibrated reagent packs on the system. • When indicated by quality control results. • After major maintenance or service.

	At the end of the onboard stability interval, replace the reagent pack on the system with a new reagent pack. Recalibration is not required, unless the lot calibration interval is exceeded.
Calibration Scheme	See Package Insert for specific calibration scheme.
Procedure	To load a new lot of reagent on IM Module: Note: Calibrate the new lot of reagent as soon as it is loaded on the instrument. If the reagent sits un-calibrated for a short period of time (24 hours) then it will not be eligible for a "Lot Calibration" and another new reagent will need to be loaded onboard. 1. From the home page of the IM Module Screen (small screen attached on the IM Module) make sure the analyzer status is in Standby. 2. On the IM Module Screen select Reagent Loader. Make sure the Reagent Drawer status is unlocked. Open the reagent drawer, load the reagent and then close it. Once the reagent is scanned, the IM Module Screen will populate message "Missing TDef for lot" next to the reagent. The Reagent Drawer status remains unlocked. 3. Both Reagent Master Curve and Calibrator Package Insert need to be scanned using the Atellica Solution's main monitor. To differentiate between the two: • Reagent Master Curve has MC TDEF printed right below the assay name. • Calibrator Package Insert has CAL printed right above the assay name. 4. To scan the Reagent Master Curve, go to Set up – Test Definition – IM Test Definition. Scan the barcode. 5. To scan the Calibrator Package Insert, go to Calibration – Calibrator Definition. Scan the barcode. 6. Re-open the Reagent Drawer and close it. This time its status should change to locked, meaning the reagent is going to be loaded onboard ready for calibration.
Procedure	Refer to the Atellica Solution Operating, QC, Calibration and Maintenance procedure for specific instructions.

5.4 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
InteliQ Immunoassay Plus Controls, Levels 1, 2 & 3	Bio-Rad Laboratories Cat. No. 12009948, 12009949, 12009950

6.2 Control Preparation and Storage

Control	InteliQ Immunoassay Plus Controls
Preparation	Allow to thaw at room temperature (18-25C) for approximately 60 minutes or until completely thawed. Once thawed, gently invert the tube several times to ensure homogeneity.
Storage/Stability	**Frozen**: until the expiration date if unopened at -20 to -70C **Thawed and Unopened:** 30 days at 2-8C **Thawed and Onboard:** 14 days at 2-8C Note: Stability for PSA and Folate is shorter.

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 Siemens Atellica QC Schedule and the Siemens Atellica 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

Step	Action
	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

Siemens Atellica IM Analyzer

7.2 Equipment

- Refrigerator capable of sustaining 2–8°C.
- Freezer capable of sustaining range not to exceed -20 to -70°C.
- Centrifuge

7.3 Supplies

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

8. PROCEDURE

Atellica IM Free Thyroxine (FT4) is required to perform this test.

Free Thyroxine is performed on the Atellica IM Analyzer after the method is calibrated 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	Instrument Set-up Protocol
1.	Perform any required instrument maintenance.
2.	Ensure that the instrument has sufficient primary and ancillary reagents.
3.	Check status of cuvettes and tips. Check waste levels. Fill or empty as appropriate.
4.	Check calibration status and re-calibrate as needed.

8.2	Specimen Testing
1.	Centrifuge the specimens.
2.	Load the sample in the Atellica rack and place the rack into the Sample Handler to initiate testing. **NOTE: If not equipped with an in-line decapper unit, samples must be de-capped prior to loading on the Atellica system
3.	Refer to the general operating procedure for detailed steps.
4.	Follow protocol in section 10.6 “Repeat Criteria and Resulting” for samples with results above the analytical measurement range (AMR). Investigate any flagged results and repeat as 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.

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 Free Thyroxine in ng/dL.

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

None required

10.2 Rounding

No rounding is necessary. Instrument reports results up to two decimal points.

10.3 Units of Measure

ng/dL

10.4 Clinically Reportable Range (CRR)

0.10 – 12.00 ng/dL

10.5 Review Patient Data

Each result is reviewed for error messages. 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 below or within the AMR or CRR may be reported without repeat. Values that exceed the upper ranges must be repeated.

IF the result is …	THEN…
< 0.10 ng/dL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 0.10 ng/dL
> 12.00 ng/dL	Repeat the assay. If replicates agree within the TEa, report as: "> 12.00 ng/dL -REP".

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

11. EXPECTED VALUES

11.1 Reference Ranges

Free T4	Male	Female
Adult (>18 years):	0.76 – 1.46 ng/dL	0.76 – 1.46 ng/dL
Pediatric:		
16 - 18 years	0.97 – 1.25	0.93 – 1.25
11 - 15 years	0.97 – 1.25	0.89 – 1.20
6 - 10 years	0.93 – 1.32	0.93 – 1.25
1 - 5 years	0.97 – 1.25	1.01 – 1.32
1 - 11 months	0.89 – 1.48	0.93 – 1.40
4 - 30 days	0.78 – 1.52	0.81 – 1.44
0 - 3 days	0.97 – 1.87	0.93 – 1.44

11.2 Critical Values

None established

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

Thyroxine is synthesized in the thyroid gland and once in the circulation, almost all thyroxine is protein bound. It is only the few unbound or “free thyroxine” that is capable of binding to cellular receptors resulting in a physiologic response. FT4 concentrations more closely parallel thyroid dysfunction in patients with either hypo- or hyperthyroidism than do the serum levels of total thyroxine.

13. PROCEDURE NOTES

- **FDA Status:** FDA Approved/cleared
- **Validated Test Modifications:** None

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.

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

0.10 – 12.00 ng/dL

14.2 Precision

Material	Mean ng/dL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Serum A	0.4	0.02	4.7
Plasma B	0.9	0.03	3
Plasma C	5.1	0.11	2.1
Serum D	10.7	0.27	2.5
Control 1	0.7	0.01	1.6
Control 2	2.0	0.02	1.2
Control 3	4.4	0.07	1.6

14.3 Interfering Substances

- Phenytoin may interfere due to competition for TBG binding sites.
- FT4 values may be decreased in patients with non-thyroidal conditions and those taking carbamazepine.
- Thyroid autoantibodies in human serum may interfere and cause falsely elevated FT4 results.

HIL Interference:
Interfering substances at the levels indicated in the table below were tested in accordance with CLSI Document EP07-A2.

Serum specimens that are or that contain….	Have an insignificant effect on the assay up to …
Hemolyzed	300 ng/dL of hemoglobin
Lipemic	1000 mg/dL of triglycerides
Icteric	20 mg/dL of bilirubin
Biotin	3500 ng/mL of biotin

14.4 Clinical Sensitivity/Specificity/Predictive Values

Detection Capability
The assay is designed to have an analytical sensitivity ≤ 0.1 ng/dL, a limit of blank (LoB) ≤ 0.1 ng/dL, and a limit of detection (LoD) ≤ 0.3 ng/dL. The analytical sensitivity for the Atellica IM FT4 assay is 0.1 ng/dL. The LoB of the Atellica IM FT4 assay is 0.1 ng/dL. The LoD for the Atellica IM FT4 assay is 0.2 ng/dL, and an LoB of 0.1 ng/dL.

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.

16. RELATED DOCUMENTS

1. Atellica Solution Operating, QC, Calibration and Maintenance procedure
2. Laboratory Quality Control Program
3. QC Schedule for Siemens Atellica Solution
4. Laboratory Safety Manual
5. Safety Data Sheets (SDS)
6. Atellica Solution Limits Chart
7. Quest Diagnostics Records Management Procedure
8. Atellica Solution System Error Messages Chart
9. Centrifuge Use, Maintenance and Function Checks (Lab policy)
10. Specimen Acceptability Requirements (Lab policy)
11. Repeat Testing Requirement (Lab policy)
12. Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls
13. Current package insert of Free Thyroxine Reagent

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, FT4 Reagent, Siemens Healthcare Diagnostics Inc., 11/2020
3. Package Insert, CAL A, Siemens Healthcare Diagnostics Inc., 08/2019
4. Package Insert, InteliQ Immunoassay Plus Controls, Bio-Rad Laboratories, 12/2020

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

None

Technical SOP

Title	**Thyroid Stimulating Hormone (TSH3-UL) by Atellica IM Analyzer**
Prepared by	Ashkan Chini Date: 4/21/2021
Owner	Robert SanLuis Date: 4/21/2021

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

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Test Code
Thyroid Stimulating Hormone	Atellica IM Analyzer	TSH

Synonyms/Abbreviations
TSH

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The Atellica IM TSH3‑UL assay is a third-generation assay that employs anti-FITC monoclonal antibody covalently bound to paramagnetic particles, an FITC-labeled anti-TSH capture mouse monoclonal antibody, and a tracer consisting of a proprietary acridinium ester and an anti‑TSH mouse monoclonal antibody conjugated to bovine serum albumin (BSA) for chemiluminescent detection. A direct relationship exists between the amount of TSH present in the patient sample and the amount of relative light units detected by the system.

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

Criteria	
Transport Container and Temperature	Collection container or Plastic vial at room temperature
Stability & Storage Requirements	Room Temperature: 24 hours
	Refrigerated: 2 days
	Frozen: 14 days
Timing Considerations	N/A
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. Before placing on system, ensure samples are free of: • Bubbles or foam • Fibrin or other particulate matter

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
Thyroid Stimulating Hormone 3-Ultra (TSH3-UL)	Siemens, Atellica IM, Cat. No. 10995703

4.2 Reagent Preparation and Storage

Reagent	Thyroid Stimulating Hormone 3-Ultra (TSH3-UL)
Storage	• Store at 2-8°C • Store in an upright position. • Protect from heat and light.

Stability	Stable onboard for 63 days.
Preparation	Reagent is liquid and ready to use. Before loading primary reagent packs onto the system, mix them by hand and visually inspect the bottom of the reagent pack to ensure that all particles are re-suspended.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
Thyroid Stimulating Hormone 3-Ultra Calibrator	Siemens Atellica IM, Cat. No. 10995703

5.2 Calibrator Preparation and Storage

Calibrator	Thyroid Stimulating Hormone 3-Ultra Calibrator
Preparation	1. Add 2.0 mL of reagent grade water into each vial using a calibrated pipette. Replace cap. 2. Let the vials stand for 15–20 minutes at room temperature to allow the lyophilized material to dissolve. 3. Gently mix and invert the vials to ensure homogeneity of the material.
Storage/Stability	• Store at 2-8°C in an upright position. • **Unopened:** stable until expiration date stamped on the box. • **Reconstituted:** 4 hours at room temperature and 28 days refrigerated.

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	Thyroid Stimulating Hormone 3-Ultra Calibrator
Assay Range	See Package Insert for specific assay ranges.
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in µIU/mL
Frequency	• When changing lot numbers of primary reagent packs. • At the end of the lot calibration interval (49 days), for a specified lot of calibrated reagent on the system. • At the end of pack calibration interval (63 days), for calibrated reagent packs on the system. • When indicated by quality control results. • After major maintenance or service.

	At the end of the onboard stability interval, replace the reagent pack on the system with a new reagent pack. Recalibration is not required, unless the lot calibration interval is exceeded.
Calibration Scheme	See Package Insert for specific calibration scheme.
Procedure	Calibrators provided in an assay kit must only be used with reagents from that kit lot. Do not use calibrators from one kit with reagent from a different kit lot. To load a new lot of reagent on IM Module: Note: Calibrate the new lot of reagent as soon as it is loaded on the instrument. If the reagent sits un-calibrated for a short period of time (24 hours) then it will not be eligible for a "Lot Calibration" and another new reagent will need to be loaded onboard. 1. From the home page of the **IM Module Screen** (small screen attached on the IM Module) make sure the analyzer status is in **Standby**. 2. On the IM Module Screen select **Reagent Loader**. Make sure the **Reagent Drawer** status is **unlocked**. Open the reagent drawer, load the reagent and then close it. Once the reagent is scanned, the IM Module Screen will populate message "**Missing TDef for lot**" next to the reagent. The Reagent Drawer status remains unlocked. 3. Both Reagent Master Curve and Calibrator Package Insert need to be scanned using the **Atellica Solution's main monitor**. To differentiate between the two: • Reagent Master Curve has **MC TDEF** printed right below the assay name. • Calibrator Package Insert has **CAL** printed right above the assay name. 4. To scan the Reagent Master Curve, go to **Set up** – **Test Definition** – **IM Test Definition**. Scan the barcode. 5. To scan the Calibrator Package Insert, go to **Calibration** – **Calibrator Definition**. Scan the barcode. 6. Re-open the **Reagent Drawer** and close it. This time its status should change to **locked**, meaning the reagent is going to be loaded onboard ready for calibration.
Procedure	Refer to the Atellica Solution Operating, QC, Calibration and Maintenance procedure for specific instructions.

5.4 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
InteliQ Immunoassay Plus Controls, Levels 1, 2 & 3	Bio-Rad Laboratories Cat. No. 12009948, 12009949, 12009950

6.2 Control Preparation and Storage

Control	InteliQ Immunoassay Plus Controls
Preparation	Allow to thaw at room temperature (18-25C) for approximately 60 minutes or until completely thawed. Once thawed, gently invert the tube several times to ensure homogeneity.
Storage/Stability	**Frozen**: until the expiration date if unopened at -20 to -70C **Thawed and Unopened:** 30 days at 2-8C **Thawed and Onboard:** 14 days at 2-8C Note: Stability for PSA and Folate is shorter.

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 Siemens Atellica QC Schedule and the Siemens Atellica 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

Siemens Atellica IM Analyzer

7.2 Equipment

- Refrigerator capable of sustaining 2–8°C.
- Freezer capable of sustaining range not to exceed -20 to -70°C.
- Centrifuge

7.3 Supplies

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

8. PROCEDURE

Atellica IM TSH3-UL is required to perform this test.

TSH3-UL is performed on the Atellica IM Analyzer after the method is calibrated 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	Instrument Set-up Protocol
1.	Perform any required instrument maintenance.
2.	Ensure that the instrument has sufficient primary and ancillary reagents.
3.	Check status of cuvettes and tips. Check waste levels. Fill or empty as appropriate.
4.	Check calibration status and re-calibrate as needed.

8.2	Specimen Testing
1.	Centrifuge the specimens.

8.2	Specimen Testing
2.	Load the sample in the Atellica rack and place the rack into the Sample Handler to initiate testing. **NOTE: If not equipped with an in-line decapper unit, samples must be de-capped prior to loading on the Atellica system
3.	Refer to the general operating procedure for detailed steps.
4.	Follow protocol in section 10.6 "Repeat Criteria and Resulting" for samples with results above the analytical measurement range (AMR). Investigate any flagged results and repeat as 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.

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 TSH3-UL in µIU/mL.

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

None required

10.2 Rounding

No rounding is necessary. Instrument reports results up to two decimal points.

10.3 Units of Measure

µIU/mL

10.4 Clinically Reportable Range (CRR)

0.01 – 150.00 µIU/mL

10.5 Review Patient Data

Each result is reviewed for error messages. 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 below or within the AMR or CRR may be reported without repeat. Values that exceed the upper ranges must be repeated.

IF the result is …	THEN…
< 0.01 µIU/mL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 0.01 µIU/mL
> 150.00 µIU/mL	Report as: "> 150.00 µIU/mL"

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

11. EXPECTED VALUES

11.1 Reference Ranges

Age	Female	Male
Adult (>18 years):	0.36 - 3.74 µIU/mL	0.36 - 3.74 µIU/mL
Pediatric:		
6 – 18 years	0.51 – 4.91	0.52 – 5.08
1 month – 5 years	0.59 – 6.78	0.67 – 5.97
0 – 30 days	0.80 – 10.83	0.64 – 12.75

11.2 Critical Values

None established

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

Thyroid stimulating hormone (TSH) is a glycoprotein secreted by the anterior lobe of the pituitary gland. TSH stimulates the normal thyroid gland to synthesize and secrete thyroxine (T4) and triiodothyronine (T3). Although less sensitive measurements of TSH (or free T4) can be used to diagnose severe, clinically apparent hypo- or hyperthyroidism, only a highly sensitive TSH assay has sufficient clinical sensitivity to detect the minor degrees of thyroxine excess or deficiency associated with early, subclinical phases of hypo- or hyperthyroidism.

13. PROCEDURE NOTES

- **FDA Status:** FDA Approved/cleared
- **Validated Test Modifications:** None

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.

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

0.01 – 150.00 µIU/mL
Note: lower limit rounded to 2 decimals places to match practice

14.2 Precision

Material	Mean µIU/mL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Serum A	0.019	0.001	N/A
Serum B	0.157	0.003	2.1
Serum C	0.972	0.012	1.2
Serum D	8.995	0.134	2.5
Serum E	54.319	1.376	2.5
Serum F	118.735	2.477	2.1

14.3 Interfering Substances

Do not use samples that contain fluorescein. Levels > 0.24 µg/mL may decrease results in this assay.

HIL Interference:
Interfering substances at the levels indicated in the table below were tested in accordance with CLSI Document EP07-A2.

Substance tested	Substance Concentration	µIU/mL	Bias %
Hemoglobin	500 mg/dL	0.839	-2
Bilirubin (conjugated)	40 mg/dL	0.873	-4
Bilirubin (unconjugated)	40 mg/dL	0.875	-2
Lipemia Intralipid®	1000 mg/dL	0.839	-0.2

14.4 Clinical Sensitivity/Specificity/Predictive Values

Detection Capability
The assay is designed to have a limit of blank (LoB) ≤ 0.004 µIU/mL, a limit of detection (LoD) ≤ 0.008 µIU/mL, and a limit of quantitation (LoQ) ≤ 0.008 µIU/mL. The LoB of the Atellica IM TSH3‑UL assay is 0.003 µIU/mL. The LoD for the Atellica IM TSH3‑UL assay is 0.007 µIU/mL. The LoQ of the TSH3‑UL assay is 0.007 µIU/mL.

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.

16. RELATED DOCUMENTS

1. Atellica Solution Operating, QC, Calibration and Maintenance procedure
2. Laboratory Quality Control Program
3. QC Schedule for Siemens Atellica Solution
4. Laboratory Safety Manual
5. Safety Data Sheets (SDS)
6. Atellica Solution Limits Chart
7. Quest Diagnostics Records Management Procedure
8. Atellica Solution System Error Messages Chart
9. Centrifuge Use, Maintenance and Function Checks (Lab policy)
10. Specimen Acceptability Requirements (Lab policy)
11. Repeat Testing Requirement (Lab policy)
12. Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls
13. Current package insert of TSH3-UL Reagent

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, TSH3-UL Reagent, Siemens Healthcare Diagnostics Inc., 06/2019.
3. Package Insert, InteliQ Immunoassay Plus Controls, Bio-Rad Laboratories, 12/2020

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

None

Technical SOP

Title	**Total Iron Binding Capacity (TIBC) by Atellica CH Analyzer**
Prepared by	Ashkan Chini Date: 4/28/2021
Owner	Robert SanLuis Date: 4/28/2021

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

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Test Code
Total Iron Binding Capacity	Atellica CH Analyzer	TIBCP

Synonyms/Abbreviations
TIBC, IBCT

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The Atellica CH Total Iron Binding Capacity (TIBC) assay uses two reagents in a sequential process that is monitored spectrophotometrically.

Step 1

1. The system adds R1, an acidic buffer containing an iron-binding dye (Chromazurol B) and ferric chloride, to the serum sample.
2. The low pH of R1 releases iron from transferrin.
3. The iron forms a colored complex with the dye at the end of this first step. The colored complex represents both the serum iron and excess iron already present in R1.

Step 2

1. The system then adds R2, a neutral buffer.
2. The pH shifts, resulting in a large increase in affinity of transferrin for iron.
3. The serum transferrin rapidly binds the iron by abstracting it from the dye-iron complex.
4. The observed decrease in absorbance of the colored dye-iron complex is directly proportional to the total iron-binding capacity of the serum 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: 4 days
	Refrigerated: 7 days
	Frozen: 2 months
Timing Considerations	N/A
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. Before placing on system, ensure samples are free of: • Bubbles or foam • Fibrin or other particulate matter

* plasma samples have been validated

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
Total Iron Binding Capacity (TIBC)	Siemens, Atellica CH, Cat. No. 11097525

4.2 Reagent Preparation and Storage

Reagent	**Total Iron Binding Capacity (TIBC)**
Storage	Store at 2-8°C
Stability	Onboard per well: 7 days
Preparation	Reagent is liquid and ready to use.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	**Supplier and Catalog Number**
Special Chemistry Calibrator (SPCL CHEM CAL)	Siemens Atellica CH, Cat. No. 11099438

5.2 Calibrator Preparation and Storage

Calibrator	Special Chemistry Calibrator (SPCL CHEM CAL)
Preparation	1. Open each vial carefully. 2. Add 5.0 mL of reagent grade water into each vial using a calibrated pipette. Replace rubber stopper. 3. Let the vials stand for 30 minutes at room temperature to allow the lyophilized material to dissolve. 4. Prior to use, to ensure homogeneity and to avoid foam formation, mix the contents by gently inverting the vials.
Storage/Stability	• Store at 2-8°C • **Unopened:** until expiration date stamped on the box • **Reconstituted:** stable for 7 days

5.3 Calibration Parameter

Criteria	**Special Notations**
Reference Material	Special Chemistry Calibrator (SPCL CHEM CAL)
Assay Range	See Package Insert for specific assay ranges.
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in µg/dL
Frequency	• When changing lot numbers of primary reagent packs. • At the end of the lot calibration interval (180 days), for a specified lot of calibrated reagent on the system. • At the end of pack calibration interval (7 days), for calibrated reagent packs on the system. • When indicated by quality control results. • After major maintenance or service.

	At the end of the onboard stability interval, replace the reagent pack on the system with a new reagent pack. Recalibration is not required, unless the lot calibration interval is exceeded.
Calibration Scheme	See Package Insert for specific calibration scheme.
Procedure	Refer to the Atellica Solution Operating, QC, Calibration and Maintenance procedure for specific instructions.

5.4 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
InteliQ Assayed Multiqual Control Levels 1 & 3	Bio-Rad Laboratories Cat. No. 12008256, 12008258

6.2 Control Preparation and Storage

Control	InteliQ Assayed Multiqual Control Levels 1 & 3
Preparation	Allow to stand at room temperature (18-25C) until completely thawed but not more than one (1) hour. Once thawed, gently invert several times to ensure homogeneity.
Storage/Stability	**Frozen**: until the expiration date if unopened at -20 to -70C **Thawed and Unopened**: 30 days at 2-8C for TIBC **Thawed and Opened**: 14 days at 2-8C for TIBC Note: stability varies by assay

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 Siemens Atellica QC Schedule and the Siemens Atellica 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

Siemens Atellica CH Analyzer

7.2 Equipment

- Refrigerator capable of sustaining 2–8°C.
- Freezer capable of sustaining range not to exceed -20 to -70°C.
- Centrifuge

7.3 Supplies

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

8. PROCEDURE

Atellica CH Total Iron Binding Capacity (TIBC) is required to perform this test.

TIBC is performed on the Atellica CH Analyzer after the method is calibrated 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	Instrument Set-up Protocol
1.	Perform any required instrument maintenance.
2.	Ensure that the instrument has sufficient primary and ancillary reagents.
3.	Check status of cuvettes and tips. Check waste levels. Fill or empty as appropriate.

8.1	Instrument Set-up Protocol
4.	Check calibration status and re-calibrate as needed.

8.2	Specimen Testing
1.	Centrifuge the specimens.
2.	Load the sample in the Atellica rack and place the rack into the Sample Handler to initiate testing. **NOTE: If not equipped with an in-line decapper unit, samples must be de-capped prior to loading on the Atellica system
3.	Refer to the general operating procedure for detailed steps.
4.	Follow protocol in section 10.6 “Repeat Criteria and Resulting” for samples with results above the analytical measurement range (AMR). Investigate any flagged results and repeat as 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.

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 TIBC in µg/dL.

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

Calculated Results:

Transferrin Saturation (%): ISAT = (IRON/TIBC)*100 (Calculated in the LIS)

Unbound Iron Binding Capacity: UIBC = [TIBC – IRON] (Not reported out)

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)

40 – 670 µg/dL

10.5 Review Patient Data

Each result is reviewed for error messages. 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 below or within the AMR or CRR may be reported without repeat. Values that exceed the upper ranges must be repeated.

IF the result is …	THEN…
< 40 µg/dL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 40 µg/dL
≥ 670 µg/dL	Report as: "> 670 µg/dL-REP" Bring to the attention of Tech in Charge (TIC) or Group Lead to check for integrity issues prior to release of results.

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 Modified
- **Validated Test Modifications:** Plasma sample types have been validated

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.

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

40 – 670 µg/dL

14.2 Precision

Material	Mean µg/dL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Serum QC	222	1.72	1.3
Serum	370	1.54	0.7
Serum	536	2.05	0.6

14.3 Interfering Substances

HIL Interference:
Interfering substances at the levels indicated in the table below were tested in accordance with CLSI Document EP07-A2.

Substance tested	Substance Concentration	µg/dL	Bias %
Hemoglobin	1000 mg/dL	413	-1
Bilirubin (unconjugated)	25 mg/dL	428	-1
Bilirubin (conjugated)	25 mg/dL	428	-3
Lipemia Intralipid®	375 mg/dL	430	10
Lipemia (Triglycerides)	1000 mg/dL	412	7

14.4 Clinical Sensitivity/Specificity/Predictive Values

Detection Capability
The assay is designed to have a limit of blank (LoB) < limit of detection (LoD) and LoD ≤ 40 µg/dL. The LoD corresponds to the lowest concentration of total iron binding capacity that can be detected with a probability of 95%. The LoD for the Atellica CH TIBC assay is 9 µg/dL, and was determined using 120 determinations, with 60 blank and 60 low level replicates, and a LoB of 6 µg/dL.

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.

Atellica TBIC reagent may cause an allergic skin reaction. Wear protective gloves/protective clothing/eye protection/face protection. Contaminated work clothing should not be allowed out of the workplace. IF ON SKIN: Wash with plenty of soap and water. If skin irritation or rash occurs: Get medical advice/attention. Wash contaminated clothing before reuse. Dispose of contents and container in accordance with all local, regional, and national regulations. **Contains:** reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H-isothiazol-3-one (3:1) (R1 and R2)

16. RELATED DOCUMENTS

1. Atellica Solution Operating, QC, Calibration and Maintenance procedure
2. Laboratory Quality Control Program
3. QC Schedule for Siemens Atellica Solution
4. Laboratory Safety Manual
5. Safety Data Sheets (SDS)
6. Atellica Solution Limits Chart
7. Quest Diagnostics Records Management Procedure
8. Atellica Solution System Error Messages Chart
9. Centrifuge Use, Maintenance and Function Checks (Lab policy)
10. Specimen Acceptability Requirements (Lab policy)
11. Repeat Testing Requirement (Lab policy)
12. Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls
13. Current package insert of Total Iron Binding Capacity Reagent

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 Reagent, Siemens Healthcare Diagnostics Inc., 11/2019.
3. Package Insert, Special Chemistry Calibrator (SPCL CHEM CAL), Siemens Healthcare Diagnostics Inc., 10/2019.
4. Package Insert, InteliQ Assayed Multiqual Controls, Bio-Rad Laboratories, 07/2020

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

None

Technical SOP

Title	**Ferritin (Fer) by Atellica IM Analyzer**
Prepared by	Ashkan Chini Date: 4/21/2021
Owner	Robert SanLuis Date: 4/21/2021

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

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Test Code
Ferritin	Atellica IM Analyzer	FERIT

Synonyms/Abbreviations
None

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The Atellica IM Fer assay is a 2-site sandwich immunoassay using direct chemiluminescent technology, which uses constant amounts of 2 anti-ferritin antibodies. The first antibody, in the Lite Reagent, is a goat polyclonal anti-ferritin antibody labeled with acridinium ester. The second antibody, in the Solid Phase, is a mouse monoclonal anti-ferritin antibody, which is covalently coupled to paramagnetic particles.

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

Criteria	
Transport Container and Temperature	Collection container or Plastic vial at room temperature
Stability & Storage Requirements	Room Temperature: 8 hours
	Refrigerated: 48 hours
	Frozen: Not established
Timing Considerations	N/A
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. Before placing on system, ensure samples are free of: • Bubbles or foam • Fibrin or other particulate matter

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 (Fer)	Siemens, Atellica IM, Cat. No. 10995569
Multi-Diluent 1	Siemens, Atellica IM, Cat. No. 10995637

4.2 Reagent Preparation and Storage

Reagent	Ferritin (Fer)
Storage	• Store at 2-8°C • Store in an upright position. • Protect from heat and light sources.

Stability	Reagents are stable onboard the system for 28 days.
Preparation	Reagent is liquid and ready to use. Before loading primary reagent packs onto the system, mix them by hand and visually inspect the bottom of the reagent pack to ensure that all particles are re-suspended.

Reagent	**Multi-Diluent 1**
Storage	Store at 2-8°C in an upright position.
Stability	Remains stable onboard the system for 28 days.
Preparation	It is liquid and ready to use.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
Calibrator C (CAL C)	Siemens Atellica IM, Cat. No. 10995506

5.2 Calibrator Preparation and Storage

Calibrator	Calibrator C (CAL C)
Preparation	1. Add 5.0 mL of reagent grade water into each vial using a calibrated pipette. Replace cap. 2. Let the vials stand for 15–20 minutes at room temperature to allow the lyophilized material to dissolve. 3. Gently mix and invert the vials to ensure homogeneity of the material.
Storage/Stability	• Store at 2-8°C in an upright position. • **Unopened:** stable until expiration date stamped on the box. • **Reconstituted:** o 4 hours at room temperature o 28 days at 2-8°C

5.3 Calibration Parameter

Criteria	**Special Notations**
Reference Material	Calibrator C (CAL C)
Assay Range	See Package Insert for specific assay ranges.
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in ng/mL
Frequency	• When changing lot numbers of primary reagent packs. • At the end of the lot calibration interval (50 days), for a specified lot of calibrated reagent on the system.

	• At the end of pack calibration interval (28 days), for calibrated reagent packs on the system. • When indicated by quality control results. • After major maintenance or service. At the end of the onboard stability interval, replace the reagent pack on the system with a new reagent pack. Recalibration is not required, unless the lot calibration interval is exceeded.
Calibration Scheme	See Package Insert for specific calibration scheme.
Procedure	To load a new lot of reagent on IM Module: Note: Calibrate the new lot of reagent as soon as it is loaded on the instrument. If the reagent sits un-calibrated for a short period of time (24 hours) then it will not be eligible for a "Lot Calibration" and another new reagent will need to be loaded onboard. 1. From the home page of the **IM Module Screen** (small screen attached on the IM Module) make sure the analyzer status is in **Standby**. 2. On the IM Module Screen select **Reagent Loader**. Make sure the **Reagent Drawer** status is **unlocked**. Open the reagent drawer, load the reagent and then close it. Once the reagent is scanned, the IM Module Screen will populate message "**Missing TDef for lot**" next to the reagent. The Reagent Drawer status remains unlocked. 3. Both Reagent Master Curve and Calibrator Package Insert need to be scanned using the **Atellica Solution's main monitor**. To differentiate between the two: • Reagent Master Curve has **MC TDEF** printed right below the assay name. • Calibrator Package Insert has **CAL** printed right above the assay name. 4. To scan the Reagent Master Curve, go to **Set up** – **Test Definition** – **IM Test Definition**. Scan the barcode. 5. To scan the Calibrator Package Insert, go to **Calibration** – **Calibrator Definition**. Scan the barcode. 6. Re-open the **Reagent Drawer** and close it. This time its status should change to **locked**, meaning the reagent is going to be loaded onboard ready for calibration.
Procedure	Refer to the Atellica Solution Operating, QC, Calibration and Maintenance procedure for specific instructions.

5.4 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
InteliQ Immunoassay Plus Controls, Levels 1, 2 & 3	Bio-Rad Laboratories Cat. No. 12009948, 12009949, 12009950

6.2 Control Preparation and Storage

Control	InteliQ Immunoassay Plus Controls
Preparation	Allow to thaw at room temperature (18-25C) for approximately 60 minutes or until completely thawed. Once thawed, gently invert the tube several times to ensure homogeneity.
Storage/Stability	**Frozen**: until the expiration date if unopened at -20 to -70C **Thawed and Unopened:** 30 days at 2-8C **Thawed and Onboard:** 14 days at 2-8C Note: Stability for PSA and Folate is shorter.

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 Siemens Atellica QC Schedule and the Siemens Atellica 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

Siemens Atellica IM Analyzer

7.2 Equipment

- Refrigerator capable of sustaining 2–8°C.
- Freezer capable of sustaining range not to exceed -20 to -70°C.
- Centrifuge

7.3 Supplies

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

8. PROCEDURE

Atellica IM Ferritin (Fer) is required to perform this test.

Ferritin is performed on the Atellica IM Analyzer after the method is calibrated 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	Instrument Set-up Protocol
1.	Perform any required instrument maintenance.
2.	Ensure that the instrument has sufficient primary and ancillary reagents.
3.	Check status of cuvettes and tips. Check waste levels. Fill or empty as appropriate.
4.	Check calibration status and re-calibrate as needed.

8.2	Specimen Testing
1.	Centrifuge the specimens.

8.2	Specimen Testing
2.	Load the sample in the Atellica rack and place the rack into the Sample Handler to initiate testing. **NOTE: If not equipped with an in-line decapper unit, samples must be de-capped prior to loading on the Atellica system
3.	Refer to the general operating procedure for detailed steps.
4.	Follow protocol in section 10.6 "Repeat Criteria and Resulting" for samples with results above the analytical measurement range (AMR). Investigate any flagged results and repeat as 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.

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)

1 – 16,500 ng/mL

10.5 Review Patient Data

Each result is reviewed for error messages. 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 below or within the AMR or CRR may be reported without repeat. Values that exceed the upper 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
≥ 1,650 ng/mL	**On Board Automated Dilution:** Results ≥ 1,650 ng/mL will automatically have repeat testing performed into the instrument using dilution factor of 10. No multiplication is necessary.
> 16,500 ng/mL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 16,500 ng/mL -REP" Bring to the attention of Tech in Charge (TIC) or Group Lead to check for integrity issues prior to release of results.

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 Standard Required Messages

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/cleared
- **Validated Test Modifications:** None

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.

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

1 – 1,650 ng/mL (lower value adjusted to a whole number)

14.2 Precision

Material	Mean ng/mL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Serum A	4.2	0.15	0.31
Serum B	8.2	0.14	0.44
Serum C	41.9	0.57	1.78
Serum D	65.4	0.82	2.89
Serum E	118.3	1.44	4.79
Serum F	487.1	8.00	20.39
Serum G	779.9	20.69	44.48
Serum H	1453.6	49.51	91.4

14.3 Interfering Substances

HIL Interference:
Interfering substances at the levels indicated in the table below were tested in accordance with CLSI Document EP07-A2.

Substance tested	Substance Concentration	ng/mL	Bias %
Hemoglobin	900 mg/dL	22.8	0
Bilirubin (conjugated)	60 mg/dL	22.9	-2
Bilirubin (unconjugated)	60 mg/dL	22.6	1
Lipemia Intralipid®	2000 mg/dL	21.0	7

Serum ferritin values are elevated in the presence of the following conditions and do not reflect actual body iron stores:

- Inflammation
- Significant tissue destruction
- Liver disease
- Malignancies such as acute leukemia and Hodgkin's disease
- Therapy with iron supplements

14.4 Clinical Sensitivity/Specificity/Predictive Values

Detection Capability
The assay is designed to have a limit of blank (LoB) ≤ 0.5 ng/mL, a limit of detection (LoD) ≤ 1.0 ng/mL, and a limit of quantitation (LoQ) ≤ 5.0 ng/mL. The LoB of the Atellica IM Fer assay is 0.3 ng/mL. The LoD corresponds to the lowest concentration of ferritin that can be detected with a probability of 95%. The LoD for the Atellica IM Fer assay is 0.7 ng/mL, and was determined using 536 determinations, with 456 blank and 80 low-level replicates, and an LoB of 0.3 ng/mL. The LoQ corresponds to the lowest amount of Ferritin in a sample at which the within laboratory CV is ≤ 20%. The LoQ of the Atellica IM Fer assay is 0.9 ng/mL.

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.

Calibrator C (CAL C) is toxic in contact with skin and harmful if swallowed. Wear protective gloves / clothing / eye and face protection. IF SWALLOWED or ON SKIN: Call a poison center or physician if you feel unwell. **Contains**: sodium azide

16. RELATED DOCUMENTS

1. Atellica Solution Operating, QC, Calibration and Maintenance procedure
2. Laboratory Quality Control Program
3. QC Schedule for Siemens Atellica Solution
4. Laboratory Safety Manual
5. Safety Data Sheets (SDS)
6. Atellica Solution Limits Chart
7. Quest Diagnostics Records Management Procedure
8. Atellica Solution System Error Messages Chart
9. Centrifuge Use, Maintenance and Function Checks (Lab policy)
10. Specimen Acceptability Requirements (Lab policy)
11. Repeat Testing Requirement (Lab policy)
12. Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls
13. Current package insert of Ferritin Reagent

17. REFERENCES

1. Package Insert, Ferritin Reagent, Siemens Healthcare Diagnostics Inc., 06/2019
2. Package Insert, Calibrator C (CAL C), Siemens Healthcare Diagnostics Inc., 08/2019
3. Package Insert, InteliQ Immunoassay Plus Controls, Bio-Rad Laboratories, 12/2020

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

None

Technical SOP

Title	**Folate (Fol) by Atellica IM Analyzer**	
Prepared by	Ashkan Chini	Date: 4/21/2021
Owner	Robert SanLuis	Date: 4/21/2021

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

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Test Code
Folate	Atellica IM Analyzer	FOLAC

Synonyms/Abbreviations
Folic Acid; included in battery FOLB12

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The Atellica IM Fol assay is a competitive immunoassay using direct chemiluminescent technology. Folate in the patient sample competes with acridinium-ester-labeled folate in the Lite Reagent for a limited amount of biotin-labeled folate binding protein. Biotin-labeled folate binding protein binds to avidin that is covalently coupled to paramagnetic particles in the Solid Phase. In the Atellica IM Fol assay, the sample is pretreated to release the folate from endogenous binding proteins 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 may be used for samples to be analyzed by this method.
Special Collection Procedures	Folates are light-sensitive. Minimize exposure to light during sample handling and storage.
Other	N/A

3.2 Specimen Type & Handling

Criteria	
Type -Preferred	Serum
Collection Container	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: 48 hours

Criteria	
	Frozen: 30 days
Timing Considerations	N/A
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. Before placing on system, ensure samples are free of: • Bubbles or foam • Fibrin or other particulate matter

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 (Fol)	Siemens, Atellica IM, Cat. No. 10995572
Fol DTT/Releasing Agent	Siemens, Atellica IM, Cat. No. 10995576
Fol DIL	Siemens, Atellica IM, Cat. No. 10995574
APW1	Siemens, Atellica IM, Cat. No. 10995458

4.2 Reagent Preparation and Storage

Reagent	Folate (Fol)
Storage	• Store at 2-8°C • Store in an upright position. • Protect from heat and light.
Stability	Reagents remain stable onboard for 14 days.

Preparation	Reagent is liquid and ready to use. Before loading reagent packs onto the system, mix them by hand and visually inspect the bottom of the reagent pack to make sure that all particles are re-suspended.

Reagent	**Fol DTT/Releasing Agent**
Storage	• Store at 2-8°C • Store in an upright position.
Stability	Stable onboard for 108 hours.
Preparation	**Note:** • Careful preparation of DTT/Releasing Agent is required to obtain accurate and consistent results since the absolute amount of DTT delivered to each test can affect results. • DTT/Releasing Agent Ready Pack ancillary reagent packs are lot-number-specific. Do not use packs from one lot of DTT/Releasing Agent with any other lot. 1. Carefully transfer the contents of one vial of Releasing Agent into one vial of DTT. For convenience, the Releasing Agent can be poured or transferred by pipette into the DTT vial. 2. Firmly screw the cap on the DTT vial and invert the vial several times to mix. 3. Pour or pipette the entire contents of the DTT vial into the disposable ancillary reagent pack provided. 4. Place a pack seal on the disposable ancillary reagent pack. Ensure that the seal is centered over the openings of the pack and press firmly on the adhesive portion of the seal.

Reagent	**Fol DIL**
Storage	• Store at 2-8°C • Store in an upright position.
Stability	Stable onboard for 28 days.
Preparation	It is liquid and ready to use.

Reagent	**APW1**
Storage	• Store at 2-8°C • Store in an upright position.
Stability	Stable onboard for 14 days.
Preparation	It is liquid and ready to use.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
Fol CAL	Siemens Atellica IM, Cat. No. 10995572

5.2 Calibrator Preparation and Storage

Calibrator	Fol CAL
Preparation	1. Add 3.0 mL of reagent grade water into the Low and High Calibrator vials using a calibrated pipette. Replace cap. 2. Let the vials stand for 15–20 minutes at room temperature to allow the lyophilized material to dissolve. 3. Gently mix and invert the vials to ensure homogeneity of the material.
Storage/Stability	• Store at 2-8°C in an upright position. • **Unopened:** stable until expiration date stamped on the box. • **Reconstituted:** stable for 8 hours at room temperature, 7 days refrigerated, and 28 days frozen.

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	Fol CAL
Assay Range	See Package Insert for specific assay ranges.
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in ng/mL
Frequency	• When changing lot numbers of primary reagent packs. • At the end of the lot calibration interval (14 days), for a specified lot of calibrated reagent on the system. • At the end of pack calibration interval (7 days), for calibrated reagent packs on the system. • When indicated by quality control results. • After major maintenance or service. At the end of the onboard stability interval, replace the reagent pack on the system with a new reagent pack. Recalibration is not required, unless the lot calibration interval is exceeded.
Calibration Scheme	See Package Insert for specific calibration scheme.
Procedure	To load a new lot of reagent on IM Module: Note: Calibrate the new lot of reagent as soon as it is loaded on the instrument. If the reagent sits un-calibrated for a short period of time (24 hours) then it will not be eligible

	for a "Lot Calibration" and another new reagent will need to be loaded onboard. 1. From the home page of the IM Module Screen (small screen attached on the IM Module) make sure the analyzer status is in Standby. 2. On the IM Module Screen select Reagent Loader. Make sure the Reagent Drawer status is unlocked. Open the reagent drawer, load the reagent and then close it. Once the reagent is scanned, the IM Module Screen will populate message "Missing TDef for lot" next to the reagent. The Reagent Drawer status remains unlocked. 3. Both Reagent Master Curve and Calibrator Package Insert need to be scanned using the Atellica Solution's main monitor. To differentiate between the two: • Reagent Master Curve has MC TDEF printed right below the assay name. • Calibrator Package Insert has CAL printed right above the assay name. 4. To scan the Reagent Master Curve, go to Set up – Test Definition – IM Test Definition. Scan the barcode. 5. To scan the Calibrator Package Insert, go to Calibration – Calibrator Definition. Scan the barcode. 6. Re-open the Reagent Drawer and close it. This time its status should change to locked, meaning the reagent is going to be loaded onboard ready for calibration.
Procedure	Refer to the Atellica Solution Operating, QC, Calibration and Maintenance procedure for specific instructions.

5.4 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
InteliQ Immunoassay Plus Controls, Levels 1, 2 & 3	Bio-Rad Laboratories Cat. No. 12009948, 12009949, 12009950

6.2 Control Preparation and Storage

Control	InteliQ Immunoassay Plus Controls
Preparation	Allow to thaw at room temperature (18-25C) for approximately 60 minutes or until completely thawed. Once thawed, gently invert the tube several times to ensure homogeneity.
Storage/Stability	**Frozen**: until the expiration date if unopened at -20 to -70C **Thawed and Unopened:** 4 days at 2-8C **Thawed and Onboard:** 4 days at 2-8C

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 Siemens Atellica QC Schedule and the Siemens Atellica 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

Siemens Atellica IM Analyzer

7.2 Equipment

- Refrigerator capable of sustaining 2–8°C.
- Freezer capable of sustaining range not to exceed -20 to -70°C.

- Centrifuge

7.3 Supplies

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

8. PROCEDURE

Atellica IM Folate (Fol) is required to perform this test.

Folate is performed on the Atellica IM Analyzer after the method is calibrated 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	Instrument Set-up Protocol
1.	Perform any required instrument maintenance.
2.	Ensure that the instrument has sufficient primary and ancillary reagents.
3.	Check status of cuvettes and tips. Check waste levels. Fill or empty as appropriate.
4.	Check calibration status and re-calibrate as needed.

8.2	Specimen Testing
1.	Centrifuge the specimens.
2.	Load the sample in the Atellica rack and place the rack into the Sample Handler to initiate testing. **NOTE: If not equipped with an in-line decapper unit, samples must be de-capped prior to loading on the Atellica system
3.	Refer to the general operating procedure for detailed steps.
4.	Follow protocol in section 10.6 “Repeat Criteria and Resulting” for samples with results above the analytical measurement range (AMR). Investigate any flagged results and repeat as 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.

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

No rounding is necessary. Instrument reports results up to one decimal point.

10.3 Units of Measure

ng/mL

10.4 Clinically Reportable Range (CRR)

0.6 – 48.0 ng/mL

10.5 Review Patient Data

Each result is reviewed for error messages. 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 below or within the AMR or CRR may be reported without repeat. Values that exceed the upper ranges must be repeated.

IF the result is …	THEN…
< 0.6 ng/mL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 0.6 ng/mL
≥ 24.0 ng/mL	**On Board Automated Dilution:** Results ≥ 24.0 ng/mL will automatically have repeat testing performed into the instrument using dilution factor of 2. No multiplication is necessary.
> 48.0 ng/mL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 48.0 ng/mL -REP" Bring to the attention of Tech in Charge (TIC) or Group Lead to check for integrity issues prior to release of 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/cleared
- **Validated Test Modifications:** None

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.

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

0.6 – 24.0 ng/mL
Note: lower limit rounded to one decimal place to match practice

14.2 Precision

Material	Mean ng/mL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Serum A	1.42	0.05	N/A
Serum B	4.13	0.10	2.4
Serum C	6.19	0.18	2.9
Serum D	9.23	0.24	2.6
Serum Control 1	2.82	0.09	3.3
Serum Control 2	5.43	0.14	2.5

14.3 Interfering Substances

Methotrexate and leucovorin interfere with folate measurement because these drugs cross react with folate binding proteins.

HIL Interference:
Interfering substances at the levels indicated in the table below were tested in accordance with CLSI Document EP07-A2.

Substance tested	Substance Concentration	ng/mL	Bias %
Bilirubin (conjugated)	20 mg/dL	3.83	0.5
Bilirubin (unconjugated)	20 mg/dL	3.62	-5.2
Lipemia Intralipid®	2000 mg/dL	5.02	1.8

14.4 Clinical Sensitivity/Specificity/Predictive Values

Detection Capability

The assay is designed to have a limit of blank (LoB) ≤ 0.40 ng/mL, a limit of detection (LoD) ≤ 0.70 ng/mL, and a limit of quantitation (LoQ) ≤ 0.70 ng/mL. The LoB of the Atellica IM Fol assay is 0.19 ng/mL for serum and 0.00 ng/mL for RBC hemolysate. The LoD for the Atellica IM Fol assay is 0.38 ng/mL for serum, and was determined using 540 determinations, with 480 blank and 60 low-level replicates, and an LoB of 0.19 ng/mL. The LoD for Atellica IM Fol assay is 0.21 ng/mL for RBC hemolysate. The LoQ of the Atellica IM Fol assay is 0.56 ng/mL.

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.

Reagents may be corrosive to metals. Causes severe skin burns and eye damage. Wear protective gloves/protective clothing/eye protection/face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. IF SWALLOWED: rinse mouth. Do NOT induce vomiting. IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. Absorb spillage to prevent material damage.
Contains: sodium hydroxide (in Atellica IM Fol DTT/Releasing Agent and in Atellica IM APW1)

16. RELATED DOCUMENTS

1. Atellica Solution Operating, QC, Calibration and Maintenance procedure
2. Laboratory Quality Control Program
3. QC Schedule for Siemens Atellica Solution
4. Laboratory Safety Manual
5. Safety Data Sheets (SDS)
6. Atellica Solution Limits Chart

7. Quest Diagnostics Records Management Procedure
8. Atellica Solution System Error Messages Chart
9. Centrifuge Use, Maintenance and Function Checks (Lab policy)
10. Specimen Acceptability Requirements (Lab policy)
11. Repeat Testing Requirement (Lab policy)
12. Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls
13. Current package insert of Folate Reagent

17. REFERENCES

1. Package Insert, Folate Reagent, Siemens Healthcare Diagnostics Inc., 11/2020.
2. Package Insert, InteliQ Immunoassay Plus Controls, Bio-Rad Laboratories, 12/2020

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

None

Technical SOP

Title	**Iron (Iron-2) by Atellica CH Analyzer**	
Prepared by	Ashkan Chini	Date: 4/28/2021
Owner	Robert SanLuis	Date: 4/28/2021

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

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Test Code
Iron	Atellica CH Analyzer	FE

Synonyms/Abbreviations
FE

Department
Chemistry

2. ANALYTICAL PRINCIPLE

Ferric iron is dissociated from its carrier protein, transferrin, in an acid medium and simultaneously reduced to the ferrous form. The ferrous iron is then complexed with ferrozine, a sensitive iron indicator, to produce a colored chromophore, which absorbs at 571/658 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

Criteria	
Transport Container and Temperature	Collection container or Plastic vial at room temperature
Stability & Storage Requirements	Room Temperature: 4 days
	Refrigerated: 7 days
	Frozen: 60 days
Timing Considerations	N/A
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. Before placing on system, ensure samples are free of: • Bubbles or foam • Fibrin or other particulate matter

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-2	Siemens, Atellica CH, Cat. No. 11097601

4.2 Reagent Preparation and Storage

Reagent	**Iron-2**
Storage	Store at 2-8°C
Stability	Onboard per well: 30 days
Preparation	Reagent is liquid and ready to use.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
Chemistry Calibrator (CHEM CAL)	Siemens Atellica CH, Cat. No. 11099411

5.2 Calibrator Preparation and Storage

Calibrator	Chemistry Calibrator (CHEM CAL)
Preparation	1. Shake to break up lyophilized cake. 2. Open each vial carefully. 3. Using a calibrated pipette, add exactly 3.0 mL of reagent grade water into the vial. Replace the stopper. 4. Manually mix by inverting 10 times every 10 minutes for a period of 30 minutes, or until reconstitution is complete. 5. Prior to use, mix by inversion at least 5 times to ensure homogeneity. 6. Refrigerate any unused material. Prior to reuse, mix contents thoroughly.
Storage/Stability	• Protect from heat and light sources. • Store at 2-8°C • **Unopened:** stable until expiration date stamped on the box. • **Reconstituted:** remains stable for 48 hours

5.3 Calibration Parameter

Criteria	**Special Notations**
Reference Material	Chemistry Calibrator (CHEM CAL)
Assay Range	See Package Insert for specific assay ranges.
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in µg/dL
Frequency	• When changing lot numbers of primary reagent packs. • At the end of the lot calibration interval (180 days), for a specified lot of calibrated reagent on the system. • At the end of pack calibration interval (30 days), for calibrated reagent packs on the system. • When indicated by quality control results. • After major maintenance or service. At the end of the onboard stability interval, replace the reagent pack on the system with a new reagent pack. Recalibration is not required, unless the lot calibration interval is exceeded.

Calibration Scheme	See Package Insert for specific calibration scheme.
Procedure	Refer to the Atellica Solution Operating, QC, Calibration and Maintenance procedure for specific instructions.

5.4 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
InteliQ Assayed Multiqual Control Levels 1 & 3	Bio-Rad Laboratories Cat. No. 12008256, 12008258

6.2 Control Preparation and Storage

Control	InteliQ Assayed Multiqual Control Levels 1 & 3
Preparation	Allow to stand at room temperature (18-25C) until completely thawed but not more than one (1) hour. Once thawed, gently invert several times to ensure homogeneity.
Storage/Stability	**Frozen**: until the expiration date if unopened at -20 to -70C **Thawed and Unopened**: 30 days at 2-8C for Iron **Thawed and Opened**: 14 days at 2-8C for Iron Note: stability varies by assay

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 Siemens Atellica QC Schedule and the Siemens Atellica 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

Siemens Atellica CH Analyzer

7.2 Equipment

- Refrigerator capable of sustaining 2–8°C.
- Freezer capable of sustaining range not to exceed -20 to -70°C.
- Centrifuge

7.3 Supplies

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

8. PROCEDURE

Atellica CH Iron-2 is required to perform this test.

Iron-2 is performed on the Atellica CH Analyzer after the method is calibrated 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	Instrument Set-up Protocol
1.	Perform any required instrument maintenance.
2.	Ensure that the instrument has sufficient primary and ancillary reagents.
3.	Check status of cuvettes and tips. Check waste levels. Fill or empty as appropriate.
4.	Check calibration status and re-calibrate as needed.

8.2	Specimen Testing
1.	Centrifuge the specimens.
2.	Load the sample in the Atellica rack and place the rack into the Sample Handler to initiate testing. **NOTE: If not equipped with an in-line decapper unit, samples must be de-capped prior to loading on the Atellica system

8.2	Specimen Testing
3.	Refer to the general operating procedure for detailed steps.
4.	Follow protocol in section 10.6 "Repeat Criteria and Resulting" for samples with results above the analytical measurement range (AMR). Investigate any flagged results and repeat as 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.

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 total iron binding capacity (TIBC) results to calculate percent transferrin saturation (SAT) and unbound iron binding capacity (UIBC).

Calculated Results:

Transferrin Saturation (%): ISAT = (IRON/TIBC)*100 (Calculated in the LIS)

Unbound Iron Binding Capacity: UIBC = [TIBC – IRON] (Not reported out)

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)

2 – 2000 µg/dL

10.5 Review Patient Data

Each result is reviewed for error messages. 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 below or within the AMR or CRR may be reported without repeat. Values that exceed the upper ranges must be repeated.

IF the result is …	THEN…
< 2 µg/dL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 2 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.
> 2000 µg/dL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 2000 µg/dL -REP" Bring to the attention of Tech in Charge (TIC) or Group Lead to check for integrity issues prior to release of results.

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

Transferrin Saturation 20 – 50%

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/cleared
- **Validated Test Modifications:** None

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.

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

2 – 1000 µg/dL

14.2 Precision

Material	Mean µg/dL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Plasma Pool	62	0.49	0.8
Control 1	63	0.54	0.9
Control 2	157	0.65	0.4
Control 3	253	0.71	0.3
Serum Pool	813	2.11	0.3

14.3 Interfering Substances

HIL Interference:
Interfering substances at the levels indicated in the table below were tested in accordance with CLSI Document EP07-A2.

Substance tested	Substance Concentration	µg/dL	Bias %
Bilirubin (conjugated)	50 mg/dL	51	1
Bilirubin (unconjugated)	50 mg/dL	50	6
Lipemia Intralipid®	500 mg/dL	50	-7

14.4 Clinical Sensitivity/Specificity/Predictive Values

Detection Capability
The assay is designed to have a limit of blank (LoB) ≤ limit of detection (LoD) and LoD ≤ 10 µg/dL. The LoD for the Atellica CH Iron-2 assay is 2 µg/dL, and a LoB of 1 µg/dL.

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.

Atellica Iron-2 reagent is harmful if swallowed. Causes serious eye irritation. Causes skin irritation. Wear protective gloves/protective clothing/eye protection/face protection. Wash hands thoroughly after handling. IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell. IF IN EYES: Rinse cautiously with water for several minutes. **Contains:** Guanidinium chloride (R1 and R2)

16. RELATED DOCUMENTS

1. Atellica Solution Operating, QC, Calibration and Maintenance procedure
2. Laboratory Quality Control Program
3. QC Schedule for Siemens Atellica Solution
4. Laboratory Safety Manual
5. Safety Data Sheets (SDS)
6. Atellica Solution Limits Chart
7. Quest Diagnostics Records Management Procedure
8. Atellica Solution System Error Messages Chart
9. Centrifuge Use, Maintenance and Function Checks (Lab policy)
10. Specimen Acceptability Requirements (Lab policy)
11. Repeat Testing Requirement (Lab policy)
12. Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls
13. Current package insert of Iron-2 Reagent

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-2 Reagent, Siemens Healthcare Diagnostics Inc., 07/2019.
3. Package Insert, Chemistry Calibrator (CHEM CAL), Siemens Healthcare Diagnostics Inc., 04/2020.
4. Package Insert, InteliQ Assayed Multiqual Controls, Bio-Rad Laboratories, 07/2020

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

None

Technical SOP

Title	**Vitamin B12 (VB12) by Atellica IM Analyzer**
Prepared by	Ashkan Chini Date: 4/21/2021
Owner	Robert SanLuis Date: 4/21/2021

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

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Test Code
Vitamin B12	Atellica IM Analyzer	VTB12

Synonyms/Abbreviations
VB12, cobalamin; included in battery FOLB12

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The Atellica IM VB12 assay is a competitive immunoassay using direct chemiluminescent technology in which vitamin B12 from the patient sample competes with vitamin B12 labeled with acridinium ester in the Lite Reagent, for a limited amount of purified intrinsic factor, which is covalently coupled to paramagnetic particles in the Solid Phase. The assay uses Releasing Agent (sodium hydroxide) and DTT to release the vitamin B12 from the endogenous binding proteins in the sample and cobinamide to prevent rebinding after the Solid Phase is added to the sample. An inverse relationship exists between the amount of vitamin B12 present in the sample and the amount of relative light units (RLUs) detected by the system.

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	Plasma (Lithium Heparin)
-Other Acceptable	Serum

Criteria	
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: 48 hours
	Frozen: Not established
Timing Considerations	N/A
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. Before placing on system, ensure samples are free of: • Bubbles or foam • Fibrin or other particulate matter

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
Vitamin B12 (VB12)	Siemens, Atellica IM, Cat. No. 10995714
VB12 DTT/Releasing Agent	Siemens, Atellica IM, Cat. No. 10995718
T3/T4/VB12 ANC	Siemens, Atellica IM, Cat. No. 10995682
VB12 DIL	Siemens, Atellica IM, Cat. No. 10995716

4.2 Reagent Preparation and Storage

Reagent	**Vitamin B12 (VB12)**
Storage	• Store at 2-8°C • Store in an upright position. • Protect from heat and light.
Stability	Stable onboard for 18 days.
Preparation	Reagent is liquid and ready to use. Before loading primary reagent packs onto the system, mix them by hand and visually inspect the bottom of the reagent pack to ensure that all particles are re-suspended.

Reagent	**VB12 DTT/Releasing Agent**
Storage	Store at 2-8°C in an upright position.
Stability	Stable onboard the system for 108 hours.
Preparation	**Notes:** • DTT/Releasing Agent ReadyPack ancillary reagent packs are lot-number-specific. Do not use packs from one lot of DTT/Releasing Agent with any other lot. • Careful preparation of DTT/Releasing Agent is required to obtain accurate and consistent results since the absolute amount of DTT delivered for each test can affect results. • Prepare immediately before using. 1. Add 300 µL DTT to 12.0 mL Releasing Agent in a test tube using a calibrated pipette. 2. Mix the DTT and Releasing Agent in the test tube. Cover the test tube with para-film and invert the test tube several times to mix. 3. Remove the self-sealing laboratory film and pour the entire contents into the disposable ReadyPack ancillary reagent pack provided. 4. Place a pack seal on the disposable ancillary reagent pack. Ensure that the seal is centered over the opening of the pack, and press firmly on the adhesive portion of the seal.

Reagent	**T3/T4/VB12 ANC**
Storage	Store at 2-8°C in an upright position.
Stability	Stable onboard the system for 14 days.
Preparation	Liquid and ready to use.

Reagent	**VB12 DIL**
Storage	Store at 2-8°C in an upright position.
Stability	Stable onboard the system for 28 days.
Preparation	Liquid and ready to use.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
Calibrator C (CAL C)	Siemens Atellica IM, Cat. No. 10995506

5.2 Calibrator Preparation and Storage

Calibrator	Calibrator C (CAL C)
Preparation	1. Add 5.0 mL of reagent grade water into each vial using a calibrated pipette. Replace cap. 2. Let the vials stand for 15–20 minutes at room temperature to allow the lyophilized material to dissolve. Gently mix and invert the vials to ensure homogeneity of the material.
Storage/Stability	• Store at 2-8°C in an upright position. • **Unopened:** stable until expiration date stamped on the box. • **Reconstituted:** o 4 hours at room temperature o 28 days at 2-8°C

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	Calibrator C (CAL C)
Assay Range	See Package Insert for specific assay ranges.
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in pg/mL
Frequency	• When changing lot numbers of primary reagent packs. • At the end of the lot calibration interval (30 days), for a specified lot of calibrated reagent on the system. • At the end of pack calibration interval (18 days), for calibrated reagent packs on the system. • When indicated by quality control results. • After major maintenance or service. At the end of the onboard stability interval, replace the reagent pack on the system with a new reagent pack. Recalibration is not required, unless the lot calibration interval is exceeded.
Calibration Scheme	See Package Insert for specific calibration scheme.

Procedure	To load a new lot of reagent on IM Module: Note: Calibrate the new lot of reagent as soon as it is loaded on the instrument. If the reagent sits un-calibrated for a short period of time (24 hours) then it will not be eligible for a "Lot Calibration" and another new reagent will need to be loaded onboard. 1. From the home page of the **IM Module Screen** (small screen attached on the IM Module) make sure the analyzer status is in **Standby**. 2. On the IM Module Screen select **Reagent Loader**. Make sure the **Reagent Drawer** status is **unlocked**. Open the reagent drawer, load the reagent and then close it. Once the reagent is scanned, the IM Module Screen will populate message "**Missing TDef for lot**" next to the reagent. The Reagent Drawer status remains unlocked. 3. Both Reagent Master Curve and Calibrator Package Insert need to be scanned using the **Atellica Solution's main monitor**. To differentiate between the two: • Reagent Master Curve has **MC TDEF** printed right below the assay name. • Calibrator Package Insert has **CAL** printed right above the assay name. 4. To scan the Reagent Master Curve, go to **Set up** – **Test Definition** – **IM Test Definition**. Scan the barcode. 5. To scan the Calibrator Package Insert, go to **Calibration** – **Calibrator Definition**. Scan the barcode. 6. Re-open the **Reagent Drawer** and close it. This time its status should change to **locked**, meaning the reagent is going to be loaded onboard ready for calibration.
Procedure	Refer to the Atellica Solution Operating, QC, Calibration and Maintenance procedure for specific instructions.

5.4 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
InteliQ Immunoassay Plus Controls, Levels 1, 2 & 3	Bio-Rad Laboratories Cat. No. 12009948, 12009949, 12009950

6.2 Control Preparation and Storage

Control	InteliQ Immunoassay Plus Controls
Preparation	Allow to thaw at room temperature (18-25C) for approximately 60 minutes or until completely thawed. Once thawed, gently invert the tube several times to ensure homogeneity.
Storage/Stability	**Frozen**: until the expiration date if unopened at -20 to -70C **Thawed and Unopened:** 30 days at 2-8C **Thawed and Onboard:** 14 days at 2-8C Note: Stability for PSA and Folate is shorter.

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 Siemens Atellica QC Schedule and the Siemens Atellica 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

Step	Action
	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

Siemens Atellica IM Analyzer

7.2 Equipment

- Refrigerator capable of sustaining 2–8°C.
- Freezer capable of sustaining range not to exceed -20 to -70°C.
- Centrifuge

7.3 Supplies

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

8. PROCEDURE

Atellica IM Vitamin B12 (VB12) is required to perform this test.

Vitamin B12 is performed on the Atellica IM Analyzer after the method is calibrated 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	Instrument Set-up Protocol
1.	Perform any required instrument maintenance.
2.	Ensure that the instrument has sufficient primary and ancillary reagents.
3.	Check status of cuvettes and tips. Check waste levels. Fill or empty as appropriate.
4.	Check calibration status and re-calibrate as needed.

8.2	Specimen Testing
1.	Centrifuge the specimens.
2.	Load the sample in the Atellica rack and place the rack into the Sample Handler to initiate testing. **NOTE: If not equipped with an in-line decapper unit, samples must be de-capped prior to loading on the Atellica system
3.	Refer to the general operating procedure for detailed steps.
4.	Follow protocol in section 10.6 “Repeat Criteria and Resulting” for samples with results above the analytical measurement range (AMR). Investigate any flagged results and repeat as 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.

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 Vitamin B12 in pg/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

pg/mL

10.4 Clinically Reportable Range (CRR)

45 – 20,000 pg/mL

10.5 Review Patient Data

Each result is reviewed for error messages. 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 below or within the AMR or CRR may be reported without repeat. Values that exceed the upper ranges must be repeated.

IF the result is …	THEN…
< 45 pg/mL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 45 pg/mL
≥ 2000 pg/mL	**On Board Automated Dilution:** Results ≥ 2000 pg/mL will automatically have repeat testing performed into the instrument using dilution factor of 10. No multiplication is necessary.
> 20,000 pg/mL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 20,000 pg/mL -REP" Bring to the attention of Tech in Charge (TIC) or Group Lead to check for integrity issues prior to release of results.

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

11. EXPECTED VALUES

11.1 Reference Ranges

193-986 pg/mL

11.2 Critical Values

None established

11.3 Standard Required Messages

The following comment is automatically added to the report by the LIS if the results fall within 193 – 400 pg/mL:

> Although the reference range for Vitamin B12 is 193 – 986 pg/mL, it has been reported that between 5 to 10% of patients with values between 193 and 400 pg/mL may experience neuropsychiatric and hematologic abnormalities due to occult B12 deficiency; less than 1% of patients with values above 400 will have symptoms.

12. CLINICAL SIGNIFICANCE

Vitamin B12, or cobalamin, is found in variety of foods such as fish, shellfish, meats and dairy products. Intrinsic factor (IF), transcobalamin II (TCII) and haptocorrin (HC) are binding proteins necessary for the assimilation, transport and delivery of B12 to the blood and body tissues. Vitamin B12 is primarily stored in the liver and released on demand. The body uses B12 very efficiently, reabsorbing B12 from the small intestine and returning it to the liver so little is excreted and nutritional deficiency is extremely rare. Vitamin B12 is necessary for DNA synthesis, normal red blood cell maturation and myelin sheath formation and maintenance. It is a coenzyme in the conversion of methylmalonic acid to succinic acid and in the synthesis of methionine. Vitamin B12 deficiency is one of the causes of megaloblastic anemia, a disease in which red blood cells are larger than normal and the ratio of nucleus size to cell cytoplasm in increased. Since folic acid deficiency can also cause megaloblastic anemia, measurement of serum B12 levels is an important part of the differential diagnosis. Vitamin B12 deficiency also causes macrocytic anemias which are characterized by abnormal red blood cell maturation and early release from the bone marrow. Pernicious anemia is a macrocytic anemia. In this disease, an absence of IF prevents normal absorption of B12. In both megaloblastic anemia caused by B12 deficiency and pernicious anemia; treatment with B12 is the therapeutic course. Vitamin B12 deficiency can also lead to abnormal neurologic and psychiatric symptoms such as ataxia, muscle weakness, dementia, psychosis and mood disturbances. Many patients show neurological changes without developing macrocytic anemia Populations at risk for B12 deficiency include strict vegetarians, the elderly and populations with increased B12 requirements associated with pregnancy, thyrotoxicosis, hemolytic anemia, hemorrhage, malignancy and liver or kidney disease. Early diagnosis of B12 deficiency is crucial because of the latent nature of this disorder and the risk of irreversible neurological damage. Recent studies suggest that in addition to serum B12 levels, folic acid, methylmalonic acid and homocysteine should be measured to improve the specificity of the diagnosis. Elevated B12 levels are seen in hematological disorders (chronic myelogenous leukemia, promyelocytic leukemia,

polycythemia vera) and in liver disorders (acute hepatitis, cirrhosis, hepatocellular carcinoma).

13. PROCEDURE NOTES

- **FDA Status:** FDA Approved/cleared
- **Validated Test Modifications:** None

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.

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

45 – 2000 pg/mL

14.2 Precision

Material	Mean pg/mL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Lithium Heparin A	146	3.7	2.5
Serum B	368	7.4	2.0
Serum C	486	11.0	2.3
Control 1	911	14.8	1.6
Control 2	1575	22.0	1.4
Control 3	1778	32.5	1.8

14.3 Interfering Substances

Preservatives such as fluoride and ascorbic acid interfere with this assay.

HIL Interference:
Interfering substances at the levels indicated in the table below were tested in accordance with CLSI Document EP07-A2.

Specimens that are …	Have an insignificant effect on the assay up to …
Hemolyzed	150 mg/dL of hemoglobin
Lipemic	3000 mg/dL of triglycerides
Icteric	20 mg/dL of bilirubin

14.4 Clinical Sensitivity/Specificity/Predictive Values

Detection Capability
The assay is designed to have analytical sensitivity of ≤ 45 pg/mL, limit of blank (LoB) ≤ 45 pg/mL and limit of detection (LoD) ≤ 90 pg/mL. The analytical sensitivity for the Atellica IM VB12 assay is 38 pg/mL. The LoB of the Atellica IM VB12 assay is 38 pg/mL. The LoD for the Atellica IM VB12 assay is 54 pg/mL, and

was determined using 147 determinations, with 75 blank and 72 low-level replicates, and an LoB of 38 pg/mL.

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.

Regent causes serious eye irritation. Causes skin irritation. May be corrosive to metals. Wear protective gloves/protective clothing/eye protection/face protection. Wash hands thoroughly after handling. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Contains: sodium hydroxide (in Atellica IM T3/T4/VB12 Ancillary Reagent and Atellica IM VB12 DTT/Releasing Agent)

16. RELATED DOCUMENTS

1. Atellica Solution Operating, QC, Calibration and Maintenance procedure
2. Atellica Solution Calibration procedure
3. Laboratory Quality Control Program
4. QC Schedule for Siemens Atellica Solution
5. Laboratory Safety Manual
6. Safety Data Sheets (SDS)
7. Atellica Solution Limits Chart
8. Quest Diagnostics Records Management Procedure
9. Atellica Solution System Error Messages Chart
10. Centrifuge Use, Maintenance and Function Checks (Lab policy)
11. Specimen Acceptability Requirements (Lab policy)
12. Repeat Testing Requirement (Lab policy)
13. Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls
14. Current package insert of Vitamin B12 Reagent

17. REFERENCES

1. Package Insert, Vitamin B12 Reagent, Siemens Healthcare Diagnostics Inc., 08/2019.
2. Package Insert, Calibrator C (CAL C), Siemens Healthcare Diagnostics Inc., 08/2019.
3. Package Insert, InteliQ Immunoassay Plus Controls, Bio-Rad Laboratories, 12/2020

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

None