TRAINING UPDATE

Lab Location:	SGMC	**Date Distributed:**	10/19/2022
Department:	Core lab	**Due Date:**	11/2/2022

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

SOP Title:Vitamin B12 (VB12) by Atellica IM Analyzer

Description of change(s):

Due to supply chain issues, we have changed our procedure to eliminate the auto dilution.

CRR has changed to 45 – 2,000

Results that exceed the CRR will be reported as >2,000 pg/mL

Review procedure and take the quiz.

This SOP review was assigned October 19th, 2022

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

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.

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

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 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 – 2,000 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	Report as > 2,000 pg/mL 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
1	10/19/22	4.2	Removed Diluent	D Collier	R SanLuis
1	10/19/22	10.4	Updated CRR	D Collier	R SanLuis
1	10/19/22	10.6	Revised reporting results	D Collier	R SanLuis

19. ADDENDA

None