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.C3029	Hemoglobin A1c (A1c-E) by Atellica CH Analyzer
SGMC.C3049	Bilirubin, Direct (DBil-2) by Atellica CH Analyzer
SGMC.C3050	Bilirubin, Total (TBil-2) by Atellica CH Analyzer
SGMC.C3001	Albumin (Alb) by Atellica CH Analyzer
SGMC.C3032	Prealbumin (PreAlb) by Atellica CH Analyzer
SGMC.C3051	Protein, Urine and Cerebrospinal Fluid (UCFP) by Atellica CH Analyzer
SGMC.C3028	Total Protein (TP) by Atellica CH 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 May19, 2021

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

Technical SOP

Title	**Hemoglobin A1c (A1c-E) by Atellica CH 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
Hemoglobin A1c	Atellica CH Analyzer	A1C

Synonyms/Abbreviations
Enzymatic Hemoglobin A1c, Glycohgb, HbA1c, A1C

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The Atellica CH A1c_E assay consists of two separate measurements: glycated hemoglobin (A1c_E) and total hemoglobin (tHb_E). The two measurements are used to determine the %HbA1c (NGSP units) or the hemoglobin A1c_E/tHb_E ratio in mmol/mol (IFCC units). The individual concentration values of A1c_E and tHb_E generated by this assay are used only for calculating the %HbA1c or A1c_E/tHb_E ratio, and must not be used individually for diagnostic purposes.

The anticoagulated whole blood specimen is lysed on the system using the Atellica CH A1c_E pretreatment solution to obtain hemolysate for the Atellica CH A1c_E assay.

The Atellica CH A1c_E assay is an enzymatic method that specifically measures N-terminal fructosyl dipeptides on the beta-chain of HbA1c. In the pretreatment step, the erythrocytes are lysed and the hemoglobin is oxidized to methemoglobin by reaction with sodium nitrite. In the first step of the reaction (the Atellica CH A1c_E reagent 1 (R1) + sample), the N-terminal fructosyl dipeptide fragment is cleaved from the hemoglobin beta chain with a protease. Concurrently, methemoglobin is converted into stable azide-methemoglobin in the presence of sodium azide and the total hemoglobin concentration is determined by measuring the absorbance at 478/694 nm. In the second step of the reaction, fructosyl peptide oxidase (FPOX) is added to react with the fructosyl dipeptide to generate hydrogen peroxide. The hydrogen peroxide reacts with the chromogen in the presence of peroxidase to develop a color that is measured at 658/805 nm. The Atellica CH A1c_E assay incorporates a turbidity normalization mechanism (cHb_E) that is measured at 805 nm to effectively remove any sample turbidity which could impact the tHb_E measurement.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A

Component	Special Notations
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**	K_2-EDTA or K_3-EDTA Whole Blood None
Collection Container	Lavender Top Tube
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: 48 hours
	Refrigerated: 7 days
	Frozen: 21 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	Thoroughly mix all samples immediately prior to testing. Avoid the formation of bubbles or foam. Ensure samples are free of fibrin or particulate matter.
Other Considerations	None

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
Enzymatic Hemoglobin (A1c-E) – Contains • Reagent Pack 1 (P1), 2 each • Reagent Pack 2 (P2), 2 each • A1c-E PRE (Vial 1), 2 each	Siemens, Atellica CH, Cat. No. 11097536

4.2 Reagent Preparation and Storage

Reagent	**Enzymatic Hemoglobin (A1c-E)**
Storage	• Store at 2-8°C • Protect from heat and light sources.
Stability	Reagents are stable onboard the system for 63 days.
Preparation	Reagent is liquid and ready to use.
Instructions	Reagents Pack 1 and 2 are loaded onboard like other reagents. A1c-E PRE is loaded in a different place. To load A1c-E PRE onboard the CH Module cover needs to open: • From the CH Module Screen select **Home – Module State** • Press **Pause** and wait until the system status changes to **Stopped** • Select **Unlock Front Cover** • Once the front cover is unlocked open the cover and load A1c-E PRE onboard • Close the front cover and reset the instrument.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
Enzymatic Hemoglobin A1c-E Calibrator (A1c-E CAL), 3 levels	Siemens Atellica CH, Cat. No. 11099338

5.2 Calibrator Preparation and Storage

Calibrator	Enzymatic Hemoglobin A1c-E Calibrator (A1c-E CAL)
Preparation	**Note:** A1c-E CAL Level 1 is light-sensitive. When handling the reagent, protect it from light and ensure that the CH Analyzer top cover is closed when running the A1c-E assay. A1c-E CAL Level 1 is liquid and ready to use. Prepare Levels 2 and 3 using the following steps:

	1. Add 1.0 mL of reagent grade water into each vial using a calibrated pipette. 2. Let the vials stand for 30 minutes at room temperature to allow the lyophilized material to dissolve. 3. Gently swirl and invert the vials to ensure homogeneity of the material.
Storage/Stability	• Store at 2-8°C • Protect from heat and light sources • **Unopened:** stable until expiration date stamped on the box. • **Level 1:** 214 days when recapped immediately after use. • **Levels 2 & 3:** stable for 11 days after reconstitution.

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	Enzymatic Hemoglobin A1c-E Calibrator (A1c-E CAL)
Assay Range	See Package Insert for specific assay ranges.
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in % HbA1c
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 (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	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
Liquichek Diabetes Control, Levels 1, 2 & 3	Bio-Rad Laboratories Cat. No. 171, 172, 173

6.2 Control Preparation and Storage

Control	Liquichek Diabetes Control, Levels 1, 2 & 3
Preparation	Allow the control to reach room temperature (18-25°C) until completely thawed and swirl gently to ensure homogeneity. Use immediately. After each use, promptly replace the stopper and return to 2-8°C storage.
Storage/Stability	**Frozen:** stable until the expiration date at -10 to -70ºC. **Thawed:** all analytes will be stable for 14 days at 2-8°C.

6.3 Frequency

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

Refer to the 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.

Step	Action
	• 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 Enzymatic Hemoglobin A1c (A1c-E) is required to perform this test.

A1c-E 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.	Thoroughly mix the specimens immediately before testing.
2.	Load A1C samples in the Atellica STAT rack **ONLY** 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 Enzymatic Hemoglobin A1c.

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

% HbA1c

10.4 Clinically Reportable Range (CRR)

3.8 – 14.0 % HbA1c

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…
< 3.8 % HbA1c	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 3.8 % HbA1c
> 14.0 % HbA1c	Report as: "> 14.0 % HbA1c -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

< 5.7 %

11.2 Critical Values

None established

11.3 Standard Required Messages

The following comment is automatically added to the report by the LIS:
"Reference range and Suggested Diagnosis:

HbA1c (%)
Diabetic ≥6.5
Prediabetes 5.7 – 6.4
Normal <5.7

The frequency of HbA1c testing should depend on the clinical situation, the treatment regimen, and the clinician's judgment. The American Diabetes Association recommends a reasonable HbA1c goal for many nonpregnant adults is <7%. Less stringent HbA1c goals may be appropriate for some patients with diabetes and other risk factors, such as severe hypoglycemia or extensive comorbid conditions.

American Diabetes Association, Diagnosis and Classification of Diabetes Mellitus, Diabetes Care 2017; 40 (Supplement 1): S11-S24."

12. CLINICAL SIGNIFICANCE

HbA1c refers to the product of a non-enzymatic reaction between glucose and hemoglobin A1. The human erythrocyte is freely permeable to glucose, which can non-enzymatically combine with hemoglobin to form HbA1c. This non-enzymatic reaction between the alpha-amino group of the N-terminal valine of the hemoglobin beta-chain and glucose takes place to form an unstable aldimine or Schiff base intermediate (labile fraction). This reaction is slow and reversible and occurs at a rate that is proportional to the glucose concentration in the blood. The aldimine intermediate subsequently undergoes a non-reversible Amadori rearrangement to form the stable ketoamine 1 – glucofrutovaline product. Since the reaction is driven by the concentration of reactants, the degree of glycosylation (resported as HbA1c relative to the total hemoglobin) is proportional to the average concentration of blood glucose over the circulating life span of hemoglobin in the red cell (approximately 120 days).

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)

3.8 – 14.0 % HbA1c

14.2 Precision

Material	Mean % HbA1c	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Control 1	4.62	0.03	0.6
Control 2	8.94	0.03	0.3
5.0 % HbA1c	5.28	0.02	0.5
6.5 % HbA1c	6.49	0.03	0.4
8.0 % HbA1c	7.89	0.03	0.3
12.0 % HbA1c	11.79	0.03	0.3

14.3 Interfering Substances

- The A1c_E assay has significant interference with fetal hemoglobin (HbF) and samples may produce a negative bias (lower than actual results).
- This assay should not be used to diagnosis diabetes during pregnancy. Hemoglobin A1c reflects the average blood glucose levels over the preceding 8-12 weeks and therefore may be falsely low during pregnancy or any other condition associated with recent onset of hyperglycemia and/or decreased RBC survival.
- This assay should not be used to diagnose or monitor diabetes in patients with the following conditions: hemoglobinopathies except as demonstrated to produce acceptable performance (sickle cell trait), abnormal RBC turnover (such as anemias with hemolysis and iron deficiency), malignancies, and severe chronic hepatic and renal disease.
- Do not use sodium fluoride / potassium oxalate collection tubes as they may interfere with results.

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	Analyte Concentration 6.5 % HbA1c	Analyte Concentration 8.0 % HbA1c
Bilirubin (conjugated)	10 mg/dL	NSI*	NSI*
Bilirubin (unconjugated)	10 mg/dL	NSI*	NSI*
Lipemia Intralipid®	1000 mg/dL	NSI*	NSI*

*NSI = No significant interference. A percentage effect > 5% is considered a significant interference.

14.4 Clinical Sensitivity/Specificity/Predictive Values

Detection Capability
The assay is designed to have a limit of blank (LoB) < 3.8 %HbA1c. The assay is designed to have a limit of detection (LoD) ≤ 3.8 %HbA1c.

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 A1c-E reagent and Calibrator level 1 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.
Contains: 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H-isothiazol- 3-one (R1 and A1c_E PRE); Maleic acid (A1c_E PRE)

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 Enzymatic Hemoglobin A1c (A1c-E) Reagent

17. REFERENCES

1. Package Insert, A1c-E Reagent, Siemens Healthcare Diagnostics Inc., 09/2019.
2. Package Insert, A1c-E CAL, Siemens Healthcare Diagnostics Inc., 01/2020.
3. Package Insert, Liquichek Diabetes Controls, Bio-Rad Laboratories, 10/2019.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

None

Technical SOP

Title	**Bilirubin, Direct (DBil-2) by Atellica CH 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
Bilirubin, Direct	Atellica CH Analyzer	DBIL, DBILN

Synonyms/Abbreviations
Bilirubin Direct and Total are included in Batteries/Packages: COMP/LIVP Bilirubin Neonatal is included in Batteries/Packages: NBIL

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The bilirubin is oxidized by vanadate at about pH 3 to produce biliverdin. In the presence of detergent and vanadate, conjugated (direct) bilirubin is oxidized. This oxidation reaction causes a decrease in the optical density of the yellow color, which is specific to bilirubin. The decrease in optical density at 451/545 nm is proportional to the direct bilirubin concentration in the sample. The concentration is measured as an endpoint reaction.

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	Bilirubin is extremely photosensitive. Care should be taken to protect sample from both daylight and fluorescent light to avoid photodegradation.
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: To be determined
	Refrigerated: 5 days
	Frozen: 3 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

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
Direct Bilirubin 2 (DBil-2)	Siemens, Atellica CH, Cat. No. 11097532

4.2 Reagent Preparation and Storage

Reagent	**Direct Bilirubin 2 (DBil-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 8 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 mg/dL
Frequency	• When changing lot numbers of primary reagent packs. • At the end of the lot calibration interval (60 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
Liquichek Pediatric Control, Level 2	Bio-Rad Laboratories Cat. No. 355

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**: 11 days at 2-8C for DBIL **Thawed and Opened**: 7 days at 2-8C for DBIL Note: stability varies by assay

Control	**Liquichek Pediatric Control, Level 2**
Preparation	Allow to stand at room temperature (18-25C) until completely thawed. 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**: 3 months at 2-8C **Thawed and Opened**: 14 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.
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 Direct Bilirubin 2 (DBil-2) is required to perform this test.

Direct Bilirubin 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.

8.1	Instrument Set-up Protocol
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 Direct Bilirubin in mg/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 one decimal point.

10.3 Units of Measure

mg/dL

10.4 Clinically Reportable Range (CRR)

0.1 – 22.5 mg/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…
< 0.1 mg/dL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 0.1 mg/dL
≥ 15.0 mg/dL	**On Board Automated Dilution:** Results ≥ 15.0 mg/dL will automatically have repeat testing performed into the instrument using dilution factor of 1.5. No multiplication is necessary.
> 22.5 mg/dL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 22.5 mg/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

Direct Bilirubin, all ages ≤ 0.3 mg/dL

11.2 Critical Values

None established

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

Measurements of bilirubin are used in the diagnosis and treatment of liver, hemolytic hematological and metabolic disorders, including hepatitis and gall bladder disease. There are at least four distinct bilirubin species that make up the total bilirubin in serum. The direct reacting species are mono-and diconjugated bilirubin (β- and γ-bilirubin) and the delta fraction (δ-bilirubin), which is tightly bound to albumin. Unconjugated bilirubin (α-bilirubin) is water-insoluble and reacts only after addition of an accelerator such as caffeine.

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)

0.1 – 15.0 mg/dL

14.2 Precision

Material	Mean mg/dL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
QC	0.4	0.00	0.0
Plasma	3.5	0.04	0.05
Serum	11.9	0.02	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	mg/dL	Bias %
Hemoglobin	1000 mg/dL	1.0	-10
Lipemia (Triglycerides Co.)	750 mg/dL	1.0	0

14.4 Clinical Sensitivity/Specificity/Predictive Values

Not available

15. SAFETY

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

Reagent 1 contains hydroxylammonium chloride. May produce an allergic reaction.

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 Direct Bilirubin 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, Direct Bilirubin 2 Reagent, Siemens Healthcare Diagnostics Inc., 08/2020.
3. Package Insert, Chemistry Calibrator (CHEM CAL), Siemens Healthcare Diagnostics Inc., 05/2020.
4. Package Insert, InteliQ Assayed Multiqual Controls, Bio-Rad Laboratories, 07/2020
5. Package Insert, Liquichek Pediatric Controls, Bio-Rad Laboratories, 03/2020

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
1	5/12/21	11.1	Changed to match to manufacture range	L Barrett	R SanLuis

19. ADDENDA

None

Technical SOP

Title	**Bilirubin, Total (TBil-2) by Atellica CH 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
Bilirubin, Total	Atellica CH Analyzer	TBIL, TBILN
Bilirubin, Cord		CBIL

Synonyms/Abbreviations
Bilirubin Total is included in Batteries/Packages: COMP, LIVP Bilirubin Neonatal is included in Batteries/Packages: NBIL

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The bilirubin is oxidized by vanadate at about pH 2.9 to produce biliverdin. In the presence of the detergent and the vanadate, both conjugated (direct) and unconjugated bilirubin are oxidized. This oxidation reaction causes the decrease in the optical density of the yellow color, which is specific to bilirubin. The decrease in optical density at 451/545 nm is proportional to the total bilirubin concentration in the sample. The concentration is measured as an endpoint reaction.

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	Bilirubin is extremely photosensitive. Care should be taken to protect sample from both daylight and fluorescent light to avoid photodegradation.
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)

Criteria	
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: To be determined
	Refrigerated: 5 days
	Frozen: 3 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

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 Bilirubin-2 (TBil-2)	Siemens, Atellica CH, Cat. No. 11097531

4.2 Reagent Preparation and Storage

Reagent	**Total Bilirubin-2 (TBil-2)**
Storage	Store at 2-30°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 8 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 mg/dL
Frequency	• When changing lot numbers of primary reagent packs. • At the end of the lot calibration interval (60 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
Liquichek Pediatric Control, Level 2	Bio-Rad Laboratories Cat. No. 355

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 TBIL **Thawed and Opened**: 9 days at 2-8C for TBIL Note: stability varies by assay

Control	**Liquichek Pediatric Control, Level 2**
Preparation	Allow to stand at room temperature (18-25C) until completely thawed. 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**: 3 months at 2-8C **Thawed and Opened**: 14 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 in 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 Bilirubin-2 (TBil-2) is required to perform this test.

Total Bilirubin 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.

8.1	Instrument Set-up Protocol
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 Total Bilirubin-2 in mg/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 one decimal point.

10.3 Units of Measure

mg/dL

10.4 Clinically Reportable Range (CRR)

0.2 – 70.0 mg/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…
< 0.2 mg/dL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 0.2 mg/dL
≥ 35.0 mg/dL	**On Board Automated Dilution:** Results ≥ 35.0 mg/dL will automatically have repeat testing performed into the instrument using dilution factor of 2. No multiplication is necessary.
> 70.0 mg/dL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 70.0 mg/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	Male/Female
Adult (> 17 years):	< 1.0 mg/dL
Pediatric:	
1 month – 17 years	< 0.8
3 days – 30 days	< 10.3
1 – 2 days	< 7.2
0 – 24 hours	< 5.1

Cord Blood Bilirubin < 2.0 mg/dL

11.2 Critical Values

Total Bilirubin, all ages > 17.9 mg/dL

Cord Blood Bilirubin > 17.9 mg/dL

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

Measurements of bilirubin are used in the diagnosis and treatment of liver, hemolytic hematological and metabolic disorders, including hepatitis and gall bladder disease. There are at least four distinct bilirubin species that make up the total bilirubin in serum. The direct reacting species are mono-and diconjugated bilirubin (β- and γ-bilirubin) and the delta fraction (δ-bilirubin), which is tightly bound to albumin. Unconjugated bilirubin (α-bilirubin) is water-insoluble and reacts only after addition of an accelerator such as caffeine.

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)

0.2 – 35.0 mg/dL (lower value adjusted to one decimal)

14.2 Precision

Material	Mean mg/dL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Serum Pool	0.8	0.02	0.05
Plasma Pool	1.5	0.02	0.05
QC	7.1	0.03	0.07

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.

Interfering Substances	Substance Concentration	mg/dL	Bias %
Hemoglobin A	500 mg/dL	1.1	9
Hemoglobin F	1000 mg/dL	1.1	-9
Lipemia (Triglycerides C.)	750 mg/dL	1.0	10

14.4 Clinical Sensitivity/Specificity/Predictive Values

Not available

15. SAFETY

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

Atellica CH Tbil_2 reagent is harmful to aquatic life. Avoid release to the environment.
Contains: Cetrimonium bromide

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 Bilirubin-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, Total Bilirubin-2 Reagent, Siemens Healthcare Diagnostics Inc., 08/2020.
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
5. Package Insert, Liquichek Pediatric Controls, Bio-Rad Laboratories, 03/2020

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

None

Technical SOP

Title	**Albumin (Alb) by Atellica CH 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
Albumin	Atellica CH Analyzer	ALB

Synonyms/Abbreviations
ALB, included in Batteries/Packages: COMP, LIVP, RENP

Department
Chemistry

2. ANALYTICAL PRINCIPLE

Serum or plasma albumin quantitatively binds to BCG to form an albumin-BCG complex that is measured as an endpoint reaction at 596/694 nm.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

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

3.2 Specimen Type & Handling

Criteria		
Type -Preferred **-Other Acceptable**	Plasma (Lithium Heparin) Serum	
Collection Container	Plasma: Mint green top tube (PST) Serum: Red top tube, Serum separator tube (SST)	
Volume - Optimum **- Minimum**	1.0 mL 0.5 mL	
Transport Container and Temperature	Collection container or Plastic vial at room temperature	
Stability & Storage Requirements	Room Temperature:	To be determined
	Refrigerated:	3 days
	Frozen:	60 days

Criteria	
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
Albumin (Alb)	Siemens, Atellica CH, Cat. No. 11097590

4.2 Reagent Preparation and Storage

Reagent	**Albumin (Alb)**
Storage	Store at 15-25°C
Stability	Onboard per well: 60 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 g/dL
Frequency	• When changing lot numbers of primary reagent packs. • At the end of the lot calibration interval (97 days), for a specified lot of calibrated reagent on the system. • At the end of pack calibration interval (60 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 Albumin **Thawed and Opened**: 14 days at 2-8C for Albumin 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 Albumin (Alb) is required to perform this test.

Albumin 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.

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 Albumin in g/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 one decimal point.

10.3 Units of Measure

g/dL

10.4 Clinically Reportable Range (CRR)

1.0 – 12.0 g/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…
< 1.0 g/dL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 1.0 g/dL
≥ 6.0 g/dL	**On Board Automated Dilution:** Results ≥ 6.0 g/dL will automatically have repeat testing performed into the instrument using dilution factor of 2. No multiplication is necessary.
> 12.0 g/dL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 12.0 g/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 (>19 years):	3.4 – 5.0 g/dL	3.4 – 5.0 g/dL
Pediatric:		
10 – 19 years	3.8 - 5.6	3.8 - 5.6
7 – 9 years	3.8 - 5.6	3.8 - 5.6
4 – 6 years	3.6 - 5.2	3.6 - 5.2
13 months – 3 years	3.5 - 4.7	3.5 - 4.2
6 – 12 months	2.3 - 4.7	2.2 - 4.7
91 – 180 days	2.3 - 4.4	2.2 - 4.9
31 – 90 days	2.0 - 4.2	2.1 - 4.8
8 – 30 days	1.9 - 4.4	2.1 - 4.5
0 – 7 days	1.9 - 4.0	2.4 - 3.9

11.2 Critical Values

None established

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

Albumin is the protein of the highest concentration in plasma. Albumin is formed exclusively in the liver and serves as a transport and binding protein for calcium, fatty acids, bilirubin, hormones, vitamins, trace elements and drugs. It is also of prime importance in maintaining the colloidal osmotic pressure in both the vascular and extravascular spaces. Decreased serum albumin concentration can result from liver disease. It can also result from kidney disease, which allows albumin to escape into the urine. Decreased serum albumin may also be explained by malnutrition or a low protein diet.

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)

1.0 – 6.0 g/dL

14.2 Precision

Material	Mean g/dL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Human serum, low	2.1	0.04	0.05
Control 1	3.4	0.04	0.07
Control 2	5.1	0.05	0.08

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	g/dL	Bias %
Hemoglobin (hemolysate)	400 mg/dL	3.4	10
Bilirubin (unconjugated)	30 mg/dL	3.5	-2
Bilirubin (conjugated)	30 mg/dL	3.3	-2
Lipemia Intralipid®	1000 mg/dL	3.4	7

14.4 Clinical Sensitivity/Specificity/Predictive Values

Not available

15. SAFETY

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

Atellica CH Alb Reagent causes serious eye damage. May produce an allergic reaction. Contains succinic acid and 2-methyl-2H-isothiazol-3-one hydrochloride. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Immediately get medical advice/attention.

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 Albumin 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, Albumin 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	**Prealbumin (PreAlb) 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
Prealbumin	Atellica CH Analyzer	PRALB

Synonyms/Abbreviations
Thyroxine – binding prealbumin, TBPA, Transthyretin

Department
Chemistry

2. ANALYTICAL PRINCIPLE

A sample is incubated with assay buffer. The antibody reagent, which is specific for human prealbumin, is then added. The resulting formation of antibody-antigen complex results in an increase in turbidity. The absorbance of the resulting turbid solution at 340 nm is proportional to the concentration of prealbumin in the sample.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

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

3.2 Specimen Type & Handling

Criteria	
Type -Preferred	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

Criteria		
Stability & Storage Requirements	Room Temperature:	8 hours
	Refrigerated:	2 days
	Frozen:	Time not specified in insert
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
Prealbumin (PreAlb)	Siemens, Atellica CH, Cat. No. 11097617

4.2 Reagent Preparation and Storage

Reagent	**Prealbumin (PreAlb)**
Storage	Store at 2-8°C
Stability	Reagents are stable onboard the system for 30 days.
Preparation	Reagent is liquid and ready to use.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
Liquid Specific Protein Calibrators (LSP CAL)	Siemens Atellica CH, Cat. No. 11099434

5.2 Calibrator Preparation and Storage

Calibrator	Liquid Specific Protein Calibrators (LSP CAL)
Preparation	Calibrators are liquid and ready to use.
Storage/Stability	• Store at 2-8°C • **Unopened:** stable until expiration date stamped on the box. • **Opened:** stable for 28 days when recapped immediately after use.

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	Liquid Specific Protein Calibrators (LSP CAL)
Assay Range	See Package Insert for specific assay ranges.
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in mg/dL
Frequency	• When changing lot numbers of primary reagent packs. • At the end of the lot calibration interval (60 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.

5.5 Tolerance Limits

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

IF……	THEN……
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 Immunology Control, Levels 1, 2 & 3	Bio-Rad Laboratories Cat. No. 12009941, 12009942, 12009943

6.2 Control Preparation and Storage

Control	InteliQ Immunology Control Levels 1, 2 & 3
Preparation	Allow to thaw at room temperature (18-25C) for approximately 45 minutes or until completely thawed. Once thawed, gently invert the tube several times to ensure homogeneity.
Storage/Stability	**Frozen**: Until expiration date when unopened at -20 to -70C. **Thawed**: • Unopened for 45 days at 2-8C • Opened & off board for 10 days at 2-8C • Opened & onboard for 30 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.

Step	Action
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 Prealbumin (PreAlb) is required to perform this test.

Prealbumin 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
3.	Refer to the general operating procedure for detailed steps.

8.2	Specimen Testing
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 Prealbumin in mg/dL

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

mg/dL

10.4 Clinically Reportable Range (CRR)

5 – 140 mg/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…
< 5 mg/dL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 5 mg/dL

IF the result is …	THEN…
≥ 70 mg/dL	**On Board Automated Dilution:** Results ≥ 70 mg/dL will automatically have repeat testing performed into the instrument using dilution factor of 2. No multiplication is necessary.
> 140 mg/dL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 140 mg/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

20 – 40 mg/dL

11.2 Critical Values

None established

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

Prealbumin is synthesized in the liver and acts as a binding protein for thyroxine and retinol-binding protein. The serum concentration reflects the synthesis capacity of the liver and is markedly diminished in malnutrition and other conditions. Due to the short half-life of approximately two days, prealbumin may be suitable for monitoring the nutritional status and efficacy of parenteral nutrition.

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)

5 – 70 mg/dL

14.2 Precision

Material	Mean mg/dL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Serum QC	20.8	0.3	1.4
Serum	40.3	0.5	1.2
Serum	60.3	0.5	0.9

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	mg/dL	Bias %
Hemoglobin	1000 mg/dL	28.4	-5
Bilirubin (conjugated)	25 mg/dL	30.0	1
Bilirubin (unconjugated)	25 mg/dL	28.4	-1
Lipemia Intralipid®	250 mg/dL	28.4	-4

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 a LoD ≤ 5 mg/dL. The LoD for the Atellica CH PreAlb assay is 1.8 mg/dL, and was determined using 120 determinations, with 60 blank and 60 low level replicates, and a LoB of 0.0 mg/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 Prealbumin Reagent

17. REFERENCES

1. Package Insert, Prealbumin Reagent, Siemens Healthcare Diagnostics Inc., 06/2020.
2. Package Insert, LSP CAL, Siemens Healthcare Diagnostics Inc., 07/2019.
3. Package Insert, InteliQ immunology Controls, Bio-Rad Laboratories, 08/2020.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

None

Technical SOP

Title	**Protein, Urine and Cerebrospinal Fluid (UCFP) by Atellica CH 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
Urine Total Protein, Random	Atellica CH Analyzer	UTPR
Urine Total Protein, 24 hour		UTP24
CSF Total Protein		CTP

Synonyms/Abbreviations
UTP, Urinary protein, CSFP CTP is included in Batteries/Packages: CPRO UTPR is included in Batteries/Packages: UTP24

Department
Chemistry

2. ANALYTICAL PRINCIPLE

In the reaction sequence, pyrogallol red combines with sodium molybdate to form a red complex with maximum absorbance at 470 nm. The protein in the sample reacts with this complex in acid solution to form a bluish-purple colored complex, which absorbs at 600 nm. The absorbance at 600 nm is directly proportional to the concentration of protein in the sample. The analyte concentration is determined by calculation using a logit curve fit on a previously stored calibration curve.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	Normal procedures for collecting cerebrospinal fluid may be used for samples to be analyzed by this method. **Urine**: A timed 24 hour collection is preferred. See Laboratory Test Directory (electronic) for collection instructions. **Random Urine**: Clean catch specimen. Deliver to laboratory promptly.
Special Collection Procedures	CSF: Cerebrospinal fluid should be collected with care to avoid contamination with plasma protein.

Component	Special Notations
	24 hour Urine: **Inpatient:** See Laboratory Test Directory (electronic) for details. Refrigerate during collection. **Outpatient:** Provide patient with prepared instructions sheet and container. Random Urine: Urine Collection Kit with specimen transferred to Urine Chemistry Collection Tube (yellow top) is preferred.
Other	N/A

3.2 Specimen Type & Handling

Criteria			
Type -Preferred **-Other Acceptable**	Urine: 24 hour specimen CSF: Sterile tube number 1 from lumbar puncture tray. Urine: Random urine or other timed collections. CSF: Tube 3 may be used, if not needed for other testing.		
Collection Container	Timed Urine Collection: 24 hour container, no additives or preservatives. Random Urine: Urine Chemistry Collection Tube (yellow top) or urine collection cup. CSF: Sterile tubes from lumbar puncture tray.		
Volume - Optimum **- Minimum**	24 hr Urine: Total voided in 24 hrs. N/A	Random Urine: 10 mL 5 mL	CSF: 1 mL 0.5 mL
Transport Container and Temperature	Collection container or Plastic vial at room temperature		
Stability & Storage Requirements	Room Temperature:	Urine: 2 hours CSF: test immediately upon receipt	
	Refrigerated:	3 days	
	Frozen:	Urine: 1 year CSF: 6 months	
Timing Considerations	CSF specimens take priority in specimen handling		
Unacceptable Specimens & Actions to Take	**CSF** samples are unlikely to be recollected; therefore, utilize discretion in rejecting a sample of this type. **Consult your supervisor**. Specimens that are unlabeled, improperly labeled, markedly hemolyzed, or those that do not meet the stated criteria are unacceptable. **Urine**: Urine samples in Urine Analysis Preservative Tubes are NOT acceptable. Request a recollection and credit the test with the appropriate code. Examples: Quantity not sufficient-QNS; Wrong collection-UNAC. Consult the English text code list for "test not performed"		

Criteria	
	messages from the LIS. Document the request for recollection in the LIS.
Compromising Physical Characteristics	**CSF**: Blood present in the cerebrospinal fluid invalidates the protein values since it reflects contamination with plasma proteins. **Urine**: Centrifuge urine before analyzing to remove particulates.
Other Considerations	• Measure total 24 hour volume and enter volume into LIS. Prepare, label, and refrigerate an aliquot in a small urine collection cup. Record 24 hour volume on aliquot. • Avoid hemolyzed samples. Hemolysis increase Atellica CH UCFP assay results at 25 mg/dL hemoglobin. • Specimens should not contain blood.

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
Urinary/Cerebrospinal Fluid Protein (UCFP)	Siemens, Atellica CH, Cat. No. 11097543

4.2 Reagent Preparation and Storage

Reagent	Urinary/Cerebrospinal Fluid Protein (UCFP)
Storage	• Store at 2-8°C • Store reagent in an upright position, away from light and heat
Stability	Onboard per well: 30 days
Preparation	Reagent is liquid and ready to use. **Note**: Do not use reagents that are cloudy, discolored, or contain precipitates.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
Urinary/Cerebrospinal Fluid Protein Calibrator (UCFP CAL)	Siemens Atellica CH, Cat. No. 11099339

5.2 Calibrator Preparation and Storage

Calibrator	Urinary/Cerebrospinal Fluid Protein Calibrator (UCFP CAL)
Preparation	Calibrators are liquid and ready to use. Allow to equilibrate to room temperature and mix thoroughly before use.
Storage/Stability	• Store at 2-8°C • Store all calibrators in an upright position. • **Unopened:** stable until expiration date stamped on box • **Opened:** remains stable for 60 days.

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	Urinary/Cerebrospinal Fluid Protein Calibrator (UCFP CAL)
Assay Range	See Package Insert for specific assay ranges.
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in mg/dL
Frequency	• When changing lot numbers of primary reagent packs. • At the end of the lot calibration interval (60 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……	THEN……
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 Urine Chemistry Control Levels 1 & 2	Bio-Rad Laboratories Cat. No. 12009995, 12009996
Liquichek Spinal Fluid Control Levels 1 & 2	Bio-Rad Laboratories Cat. No. 751, 752

6.2 Control Preparation and Storage

Control	**InteliQ Urine Chemistry Control, Levels 1 & 2**
Preparation	Remove cap and place in instrument for testing
Storage/Stability	**Unopened**: until expiration date at 2-8C **Opened & On-board**: days at 2-8C

Control	**Liquichek Spinal Fluid Control, Levels 1 & 2**
Preparation	Allow to reach room temperature before sampling. Gently swirl vial several time to ensure homogeneity.
Storage/Stability	**Unopened**: until expiration date at 2-8C **Opened**: stable for 30 days at 2-8C, store tightly capped.

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 -50°C.
- Centrifuge

7.3 Supplies

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

8. PROCEDURE

Atellica CH Urinary/Cerebrospinal Fluid Protein (UCFP) is required to perform this test.

UCFP 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.

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 urinary/cerebrospinal fluid protein in mg/dL.

For 24 hour urines, the LIS will calculate the total mg of protein/24hrs if the protein result from the aliquot (Urine Total Protein Random) is within the CRR. The formula is:

$$\frac{\text{(urine TP random) x (Total Urine Volume)}}{100} = \text{\# mg/24hrs}$$

If the Urine Total Protein Random value is above or below the CRR, then report the Urine Total Protein 24hr value as UTC (unable to calculate)

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

mg/dL

10.4 Clinically Reportable Range (CRR)

6 – 2500 mg/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…
< 6 mg/dL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 6 mg/dL
≥ 250 mg/dL	**On Board Automated Dilution:** Results ≥ 250 mg/dL will automatically have repeat testing performed into the instrument using dilution factor of 10. No multiplication is necessary.
> 2500 mg/dL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 2500 mg/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

CSF Total Protein:

Age	Female	Male
Adult (>18 years):	15 – 45 mg/dL	15 – 45 mg/dL
Pediatric:		
8 – 18 years	15 - 45	15 - 40
2 – 7 years	15 - 45	15 - 45
7 – 23 months	15 - 48	15 - 50
3 – 6 months	15 - 44	15 - 48
31 days – 2 months	15 - 93	15 - 48
15 – 30 days	15 - 100	15 - 96
0 – 14 days	15 - 153	15 - 100

Urine Total Protein Random:
<12 mg/dL

Urine Protein, 24 hour:
<149 mg/24 hr

11.2 Critical Values

None established

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

Measurement of the protein content in urine is used in diagnosis and treatment of kidney diseases. Measurement of the protein content in cerebrospinal fluid is used in the diagnosis and treatment of central nervous system diseases.

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 – 250 mg/dL

14.2 Precision

Material	Mean mg/dL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Urine Pool 1	23.3	0.57	2.4
Urine Pool 2	149.4	0.96	0.6
CSF Control 1	45.4	0.45	1.0
CSF Control 2	86.9	0.61	0.7
Urine Control 1	27.7	0.58	2.1
Urine Control 2	70.8	0.56	0.8

14.3 Interfering Substances

Samples containing amikacin, gentamycin, kanamycin, and tobramycin should be avoided since these substances falsely increase Atellica CH UCFP assay results.

14.4 Clinical Sensitivity/Specificity/Predictive Values

Detection Capability
The Limit of Blank (LoB) corresponds to the highest measurement result that is likely to be observed for a blank sample. The assay is designed to have an LoB ≤ limit of detection (LoD). The Limit of Detection (LoD) corresponds to the lowest concentration of protein that can be detected with a probability of 95%. The assay is designed to have an LoD ≤ 6.0 mg/dL. The Limit of Quantitation (LoQ) corresponds to the lowest concentration of protein in a sample at which the total allowable error is ≤ 35%. The assay is designed to have an LoQ ≤ 6.0 mg/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 UCFP Reagent may cause damage to organs. Do not breathe vapors. Wear protective gloves/protective clothing/eye protection/face protection. IF exposed or concerned: Call a POISON CENTER or doctor/physician.
Contains: Methanol (Atellica CH UCFP assay P1)

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 Urinary/Cerebrospinal Fluid Protein 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, Urinary/Cerebrospinal Fluid Protein Reagent, Siemens Healthcare Diagnostics Inc., 01/2020.
3. Package Insert, Urinary/Cerebrospinal Fluid Protein Calibrator (UCFP CAL), Siemens Healthcare Diagnostics Inc., 10/2019.
4. Package Insert, InteliQ Urine Chemistry Controls, Bio-Rad Laboratories, 11/2020
5. Package Insert, Liquichek Spinal Fluid Controls, Bio-Rad Laboratories, 07/2020

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

None

Technical SOP

Title	**Total Protein (TP) 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 Protein, Serum / Plasma	Atellica CH Analyzer	TP
Total Protein, Body Fluid (serous)		FTP

Synonyms/Abbreviations
TP, included in Batteries/Packages: COMP, LIVP

Department
Chemistry

2. ANALYTICAL PRINCIPLE

Protein peptide bonds interact with the cupric ions to form a purple complex that is measured as an endpoint reaction at 545 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, plasma and body fluid 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), Body Fluid (serous) Serum
Collection Container	Plasma: Mint green top tube (PST) Serum: Red top tube, Serum separator tube (SST) Body Fluid (serous): Mint green top tube (PST) preferred, Red top tube
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:	Plasma/Serum: 8 hours Body Fluid: To be determined
	Refrigerated:	Plasma/Serum: 3 days Body Fluid: To be determined
	Frozen:	Plasma/Serum: 180 days Body Fluid: 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. Centrifuge and check fluid for clots before testing. Synovial fluid is sent to reference lab for testing. 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 Protein II (TP)	Siemens, Atellica CH, Cat. No. 11097604

4.2 Reagent Preparation and Storage

Reagent	**Total Protein II (TP)**
Storage	Store at 15-25°C
Stability	Reagents are stable onboard the system for 90 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 g/dL
Frequency	• When changing lot numbers of primary reagent packs. • At the end of the lot calibration interval (181 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 TP **Thawed and Opened**: 14 days at 2-8C for TP 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 Protein II (TP) is required to perform this test.

Total Protein 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.

8.1	Instrument Set-up Protocol
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 Total Protein in g/dL.

Albumin/globulin (A/G) ratio is given whenever the Total Protein and Albumin are ordered at the same time. Since the total protein value is elevated by the inclusion of fibrinogen in plasma specimens, the *Total Protein is corrected for this calculation*. Therefore, the formula is as follows:

(Total Protein in g/dL – 0.3g/dL) – Albumin (g/dL) = the Globulin Proteins g/dL

$$\frac{\text{Albumin (g/dL)}}{\text{Globulin Proteins g/dL}} = \text{A / G ratio}$$

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

g/dL

10.4 Clinically Reportable Range (CRR)

2.0 – 24.0 g/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…
< 2.0 g/dL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 2.0 g/dL
≥ 12.0 g/dL	**On Board Automated Dilution:** Results ≥ 12.0 g/dL will automatically have repeat testing performed into the instrument using dilution factor of 2. No multiplication is necessary.
> 24.0 g/dL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 24.0 g/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

Serum / Plasma:

Age	Female	Male
Adult (>19 years):	6.4 – 8.2 g/dL	6.4 – 8.2 g/dL
Pediatric:		
10 – 19 years	6.4 - 8.6	6.4 - 8.6
7 – 9 years	6.3 - 8.1	6.3 - 8.1
1 – 6 years	6.0 - 7.8	6.0 - 8.0
6 – 11 months	4.6 - 7.8	4.2 - 7.9
61 – 180 days	4.0 - 7.6	4.0 - 7.0
0 – 60 days	3.6 - 7.0	4.0 - 7.6

Body Fluid: Reference ranges have not been established for this sample type

A/G ratio: 1.1 - 2.0

11.2 Critical Values

None established

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

Measurements of total protein are used in the diagnosis and treatment of a variety of diseases involving the liver, kidney or bone marrow as well as metabolic or nutritional disorders.

13. PROCEDURE NOTES

- **FDA Status:** FDA Approved/cleared for plasma and serum
- **FDA Status:** FDA Approved/modified for body fluid
- **Validated Test Modifications:** Testing validated for body (serous) fluid specimens

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)

2.0 – 12.0 g/dL

14.2 Precision

Material	Mean g/dL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Serum QC	4.0	0.03	0.04
Serum	7.7	0.13	0.13
Plasma	9.6	0.05	0.13

14.3 Interfering Substances

A potential interference may be seen in results from patients receiving dextran as volume expanders. This would appear as an overestimation or a positive bias in results.

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	g/dL	Bias %
Hemoglobin	500 mg/dL	6.1	6
Bilirubin (unconjugated)	25 mg/dL	5.9	2
Bilirubin (conjugated)	25 mg/dL	6.1	-1
Lipemia Intralipid®	500 mg/dL	6.1	-2

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 ≤ 2.0 g/dL. The LoD corresponds to the lowest concentration of total protein that can be detected with a probability of 95%. The LoD for the Atellica CH TP assay is 0.7 g/dL, and was determined using 120 determinations, with 60 blank and 60 low level replicates, and a LoB of 0.6 g/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 TP reagent may be corrosive to metals. Causes severe skin burns and eye damage. Harmful to aquatic life with long lasting effects. Wear protective gloves/protective clothing/eye protection/face protection. Avoid release to the environment. IF SWALLOWED: rinse mouth. Do NOT induce vomiting. IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a poison center or physician.
Contains: Sodium hydroxide (R1, R2): Sulfuric acid copper (2+) salt (1:1), hydrate (1:5) (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 Protein II 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.
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18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

None