

Laboratory Procedure Manual

Analyte: **Sodium**

Matrix: **Serum**

Method: **Roche Cobas 6000 (c501 module)**

Method No.:

Revised:

As performed by: **University of Minnesota
Advanced Research and Diagnostic Laboratory (ARDL)
1200 Washington Ave S, Suite 175
Minneapolis, MN 55415**

Contact: **Anthony Killeen, MD, PhD, Laboratory Director
Jennifer Peters, MT, ASCP, Laboratory Manager**

Important Information for Users

The Advanced Research and Diagnostic Laboratory (ARDL) periodically refine these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for testing the items listed in the following table:

Data File Name	Variable Name	SAS Label
BIOPRO_J	LBXSNASI	Sodium (mmol/L)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Electrolytes are involved in most major metabolic functions in the body. Sodium, potassium and chloride are amongst the most important physiological ions and the most often assayed electrolytes. They are supplied primarily through the diet, absorbed in the gastro-intestinal tract, and excreted via the kidneys.

Sodium (Na) is the major extracellular cation and functions to maintain fluid distribution and osmotic pressure. Some causes of decreased levels of sodium include prolonged vomiting or diarrhea, diminished reabsorption in the kidney and excessive fluid retention. Common causes of increased sodium include excessive fluid loss, high salt intake and increased kidney reabsorption.

This method utilizes an indirect (specimen is diluted by the instrument prior to analysis) ion-selective electrode (ISE) method for determination of the serum electrolyte concentrations.

An Ion-Selective Electrode (ISE) makes use of the unique properties of certain membrane materials to develop an electrical potential (electromotive force, EMF) for the measurements of ions in solution. The electrode has a selective membrane in contact with both the test solution and an internal filling solution. The internal filling solution contains the test ion at a fixed concentration. Because of the particular nature of the membrane, the test ions will closely associate with the membrane on each side. The membrane EMF is determined by the difference in concentration of the test ion in the test solution and the internal filling solution.

The complete measurement system for a particular ion includes the ISE, a reference electrode and electronic circuits to measure and process the EMF to give the test ion concentration. The sodium and potassium electrodes are based on neutral carriers and the chloride electrode is based on an ion exchanger.

Cobas 6000 Application Code: 989(Na)

2. SAFETY PRECAUTIONS

Caution: This product is of human and animal origin. Handle as though capable of transmitting infectious disease. Wear appropriate PPE when handling equipment, reagents, and samples.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

ARDL utilizes a highly specialized Laboratory Information System (LIS) (STARLIMS, Abbott Informatics Corporation; Hollywood, FL, 33021-6755) for all lab functions. Major instrument platforms are interfaced directly to the LIS, allowing data to be electronically transferred directly to the main database. The system provides an extensive quality assurance package and data management tools. Numerous networked computer workstations are used in the laboratory for data management and transmission, and also include software for word and spreadsheet creation and manipulation, statistical analysis, report presentation, and electronic communication. All workstations are user password protected with job specific security access levels and have idle time out functionality. All systems are redundantly backed up on a real time basis.

4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

a. **Specimen Type and Requirements:** Serum, lithium heparin plasma, and urine are acceptable specimens. The NHANES Biochem study uses refrigerated serum. This test is analyzed from NHANES Vial 018.

b. **Specimen Volume:** Optimum/Minimum volume: 100 μ L in a sample cup or 2 mL microtube (9.7 μ L serum or plasma; remainder for dead volume).

c. **Acceptable Specimens/Unacceptable Specimens:** Serum or lithium heparin plasma is acceptable. Other anticoagulants are not acceptable.

d. **Specimen Stability and Storage:** Separated serum or plasma should be removed from the cells within one hour of collection. Serum or plasma is stable for 14 days at 2-8°C, and indefinitely at -20°C or colder. Specimens must be at room temperature prior to assay.

e. **Interferences or limitations:** Hemolysis does not interfere with the assay up to an H index of 1000 (approximately 1000 mg/dL hemoglobin). Intralipid does not interfere in

the tested concentration range up to 2000 mg/dL (L index of 2000). However, there is poor correlation between triglycerides concentration and L index. Gross lipemia can result in pseudohyponatremia as a result of fluid displacement. Excessively lipemic specimens should be ultracentrifuged, and specimens with particulate matter should be cleared by centrifugation. Bilirubin does not interfere up to an I index of 60.

f. **Specimen Handling and Transport:** Mix specimens well, allow clot to fully form (if serum), and centrifuge 10 minutes at 2000 x g before use. Aliquot a minimum of 0.1 mL. Store sample in refrigerator immediately until shipment. Ship at refrigerated temperature.

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure.

6. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS

a. Reagents and Supplies

- ISE Diluent (ready for use), Roche Cat. No. 04522630190, 5x300 mL. HEPES buffer: 10 mmol/L; Triethanolamine: 7 mmol/L; Preservative. Store at 15-25°C. On board stability 14 days.
- ISE Internal Standard (ready for use), Roche Cat. No. 04522320190, 5x600 mL. HEPES buffer: 10 mmol/L; Triethanolamine: 7 mmol/L; Sodium chloride: 3.06 mmol/L; Sodium acetate: 1.45 mmol/L; Potassium chloride: 0.16 mmol/L; Preservative. Store at 15-25°C. On board stability 14 days.
- ISE Reference Electrolyte (ready for use), Roche Cat. No. 11360981216, 5x300 mL. Potassium chloride: 1 mol/L. Store at 15-25°C. On board stability 28 days.
- Electrodes, Roche Cat. No. 03246353001 (Chloride) and Roche Cat. No. 03149501001 (Reference). Store electrodes at 7-40 °C. See label for expiration date. Store at 15-25°C. On board stability: chloride (2 months), reference (6 months).

- ISE Cleaning Solution, Roche Cat. No. 11298500316, 5x100 mL. Sodium hydroxide solution (12%) with sodium hypochloride solution < 2 % active Cl. Store at 2-8°C. If always closed immediately after usage and stored at 2-8°C the ISE Cleaning Solution can be used up to the expiration date. For daily maintenance use only fresh cleaning solution.
- b. Reagent Preparation (*Reagents are ready to use; no preparation required)
- To achieve the stated on-board stability, an Internal Standard insert (chimney) must be used. Place a new insert (chimney) in each Internal Standard bottle. Slowly push the chimney downwards into the bottle. Do not cover the hole of the chimney as it is being pushed down. Ensure the chimney is inserted as far as possible. Place the bottle, with chimney, into its slot on the analyzer.
- c. Equipment/Instrumentation-
- Roche Cobas 6000 Chemistry Analyzer (Roche Diagnostics Corporation, Indianapolis, IN 46250)
 - The Millipore Elix Gulfstream Clinical 35 System is designed to meet CLSI Clinical Laboratory Reagent Water (CLRW) standards. Water purification is achieved by reverse osmosis, electrodeionization, bactericidal 254 nm UV lamp and 0.22 µm filtration.
- d. Specimens are run in singleton
- e. Quality Control
- Normal pooled serum control (CQ). Stable at -80°C for up to 4 years, at refrigerated temperature for up to 1 day and at room temperature for up to 4 hours.
 - Roche Precinorm U Plus Control (Roche Cat. No. #12149435160), 10x3 mL. Roche Diagnostics 9115 Hague Road Indianapolis, IN 46250-0457). Stable until expiration date on package when unopened and stored at 2-8°C. To prepare, open bottle 1 and pipette in exactly 3.0 mL of diluent (bottle 2). Dissolve by gentle swirling for 30 minutes. Prepared control is stable for 12 hours at room temperature, 5 days at 2-8°C, and one month at -20°C (when frozen once).

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

Calibrators S1, S2 and S3

S1: ISE Standard Low, Roche Cat. No. 11183974216, 10x3 mL.

120 mmol/L Na⁺, 3 mmol/L K⁺, 80 mmol/L Cl⁻

S2: ISE Standard High, Roche Cat. No. 11183982216, 10x3 mL

160 mmol/L Na⁺, 7 mmol/L K⁺, 120 mmol/L Cl⁻

S3: ISE Standard High (compensated), Roche Cat. No. 11183982216, 10x3 mL

160 mmol/L Na⁺, 7 mmol/L K⁺, 120 mmol/L Cl⁻

Storage and stability

Store S1, S2 and S3 at 15-25°C.

See label for expiration date.

On-board stability Calibrators S1, S2 and S3: to be used for one calibration only.

Full calibration for Na⁺, K⁺ and Cl⁻ requires the following 3 calibrator solutions:
ISE Standard Low, ISE Standard High, and ISE Standard High (compensated).

The slope of the calibration curve is calculated from Standards 1 and 2. ISE Standard High (compensated) is designed to reduce matrix effects; it only affects the intercept, not the slope. An internal standard is also measured during calibration and between samples to compensate for any system deviations.

Refer to the Operator's Manual of the analyzer for detailed calibration instructions.

US Traceability: This method has been standardized against primary calibrators prepared gravimetrically from purified salts.

Calibration frequency:

Perform a full calibration

- every 24 hours
- after ISE cleaning and maintenance

- after changing the reagent bottles
- after replacing any electrode
- as required following quality control procedures

If calibration fails perform the following corrective action steps in sequence:

- Check reagent and calibrator for appropriate lot numbers, expiration dates, preparation and storage conditions.
- Repeat calibration with new calibrator.
- Repeat calibration with new reagent and new calibrator. If successful calibration is not achieved, discontinue testing and notify the supervisor.

8. OPERATING PROCEDURE INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

- Instrument Operation:** The Roche/Hitachi Cobas 6000 analyzer series is a fully automated, random-access, software controlled system for immunoassay and photometric analyses intended for qualitative and quantitative in vitro determinations using a wide variety of tests. The Cobas 6000 analyzer series is optimized for workloads using a combination of photometric and ion-selective electrode (ISE) determinations (c501 module), and electrochemiluminescence (ECL) signal in the immunoassay analysis module (e601 module). The ISE system is used in the quantitation of sodium, potassium and chloride. The photometric system can measure colorimetric or immunoturbidimetric reactions utilizing end point or kinetic (rate) absorbance measurements. Test ordering and execution on the Cobas 6000 and data entry in the STARLIMS host computer system may be done manually or these tasks may be executed via a barcode-based bi-directional interface. The Cobas 6000 can utilize both of these two systems simultaneously.
- Professional Judgement:** Check results for error flags and take appropriate corrective action. Investigate alert values and delta checks.
- Result Entry**

STARLIMS test code: NA

Manual Results

- Sodium results are reported in whole numbers, as in x mmol/L.
- Check results for error flags and take appropriate corrective action.
- Investigate critical values and delta checks.

9. REPORTABLE RANGE OF RESULTS

Out of Range results: Certain tests have pre-programmed limits that trigger an automatic re-analysis by the COBAS. These limits may be low-end values or high-end values (but within technical range). If the duplicate value is in agreement with the initial value, then the initial value is reported. Sodium values <133 mmol/L or >149 mmol/L are automatically repeated by the instrument.

Results are reported in whole numbers. Check results for error flags and take appropriate corrective action. Investigate critical values and delta checks.

a. Reportable Range of Test Results: Reportable Range 80-180 mmol/L

Intra-assay %CV (10 within-day replicates at a concentration of 118.9 mmol/L) 0.3%

Intra-assay %CV (10 within-day replicates at a concentration of 147.1 mmol/L) 0.2%

Inter-assay %CV (between day replicates at a concentration of 139.1 mmol/L) 1.0%

Inter-assay %CV (between day replicates at a concentration of 120.4 mmol/L) 0.8%

Dilutions: Specimens exceeding the linear limits are not diluted by the Cobas 6000.

Specimens reading above or below the linear range are essentially incompatible with life, and would most likely indicate an analytical problem or a contaminated or incorrect specimen (i.e. urine specimen labeled as a serum specimen).

b. Reference Range: Serum, adult 133-145 mmol/L

c. Critical Results: None

d. Analytical Measurement Range: 80-180 mmol/L

Reportable Range: 80-180 mmol/L

Limit of Detection (standard $1 + 3 \text{ SD}$): Specimens exceeding the linear limits are not repeated or diluted by the Cobas 6000. If a dilution is required, dilute the specimen in CLRW water, and multiply the result by the corresponding dilution factor.

Maximum dilution is 1:2. Specimens reading above or below the linear range are essentially incompatible with life, and would most likely indicate an analytical problem or a contaminated or incorrect specimen (i.e. urine specimen labeled as a serum specimen).

10. QUALITY CONTROL (QC) PROCEDURE

Quality Control

- Normal pooled serum control (CQ). Stable at -80°C for up to 4 years, at refrigerated temperature for up to 1 day and at room temperature for up to 4 hours.
- Roche Precinorm U Plus Control (Roche Cat. No. #12149435160, 10x3 mL.). Roche Diagnostics 9115 Hague Road Indianapolis, IN 46250-0457). Stable until expiration date on package when unopened and stored at $2-8^{\circ}\text{C}$. To prepare, open bottle 1 and pipette in exactly 3.0 mL of diluent (bottle 2). Dissolve by gentle swirling for 30 minutes. Prepared control is stable for 12 hours at room temperature, 5 days at $2-8^{\circ}\text{C}$, and one month at -20°C (when frozen once).
- Both levels of quality control are analyzed at the start of the day and results are verified for acceptability prior to testing specimens. Quality control is also analyzed at the end of the shift, with change in reagent, after major maintenance, or as needed for troubleshooting.
- The analytical measurement range (AMR) must be validated every 6 months or after major maintenance or service procedures. The laboratory enrolls in the College of American Pathologist (CAP) linearity program. Sodium is included in the LN2 kit that is shipped twice per year. Follow kit instructions for preparation. Analyze samples in duplicate. Results are due within two to four weeks of receipt of kit. Results are submitted online to the CAP website by the lead or supervisor. The linearity report is available online at the CAP website shortly after the due

date. Confirm reported values are within acceptability limits. Place instrument printouts, worksheets and CAP results in the CAP three ring binder.

- New Lot Verification: Each new electrode lot must be verified for acceptability before being placed into use. Calibration, quality control, and comparison of at least 5 patient samples on the old and new lots must be performed and found to be within acceptable limits before a new lot can be placed into use.

- Quarterly Technical Progress Report:

Progress reports for each quarter of the calendar year are submitted to NHANES. The report includes 1) a summary of the status of all specimens including date of arrival, deviations from protocol, handling, storage, and manipulation of vials and all laboratory procedures used, 2) a description of any major problems such as missing data, out of range values or inconsistent data and what was done to overcome them, internal and external quality control for runs containing NHANES specimens, instrument calibration and repairs, reagent lots and dates of use.

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

- If QC values are outside of specified ranges, do the following, in order, until QC is acceptable:
 1. Repeat the analysis using fresh QC material.
 2. Perform a calibration.
 3. Check for system problems.
 4. Contact Roche Technical Support for assistance and possible service dispatch. Phone: 1-800-428-2336; account number: 55042919

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

- a. Analytical Measurement Range: 80-180 mmol/L
- b. Hemolysis does not interfere with the assay up to an H index of 1000 (approximately 1000 mg/dL hemoglobin). Intralipid does not interfere in the tested

concentration range up to 2000 mg/dL (L index of 2000). However, there is poor correlation between triglycerides concentration and L index. Gross lipemia can result in pseudohyponatremia as a result of fluid displacement. Excessively lipemic specimens should be ultracentrifuged, and specimens with particulate matter should be cleared by centrifugation. Bilirubin does not interfere up to an I index of 60. Drugs: No interference was found at therapeutic concentrations using common drug panels. See package insert for complete listing.

13. REFERENCE RANGES (NORMAL VALUES)

Serum, adult: 133-145 mmol/L

14. CRITICAL CALL RESULTS ("PANIC VALUES")

Not applicable.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens are stored at refrigerated temperature between sample receipt and analysis on the instrument. Specimens must be at room temperature prior to assay. Specimens are returned to refrigerated or frozen temperature post analysis depending on the study specific requirements.

16. ALTERNATE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

Should the testing system become inoperable, discontinue testing and notify the supervisor. While instrument trouble-shooting or repair occurs; keep specimens at refrigerated or frozen temperature depending on study specific requirements.

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

All data is reported electronically via an eFile that is uploaded to the WESTAT secure website within 21 days of receipt of specimens.

18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

Specimen Receipt:

Shipments for NHANES generally arrive on Tuesdays and/or Wednesdays. These shipments are recorded on the Log of Quality Assurance located in a binder labeled NHANES Shipping Log in the receiving area. The specimen barcode numbers in the boxes are checked against the manifests. The receipt date is written on top of the boxes. The frozen samples (vial 11-Iron/UIBC & vial 13-CRP) are placed in the designated -70°C freezer and the refrigerated samples (vial 18-Biochem panel) are placed in the designated 2-8°C refrigerator until analysis. The manifests are filed in a binder labeled NHANES Shipping Manifests located in the receiving area. All labels are removed from the shipping box and the provided airbill is attached for return shipment.

Quality Assurance Log:

A Quality Assurance Specimen Receipt and Specimen Return Log is maintained by laboratory staff. The following parameters are tracked: NHANES shipper I.D., NHANES Container I.D., Vial #, Date Received, Specimen Receipt Conditions, Number of Specimens Received, 2.5% QC Repeats, Total Number of Specimens, 21 Day Due Date, Analysis Date, Date Results Sent, Number of Days For Result Return, Thaw Date (if applicable), Return To Freezer Date, Number of Days at Refrigerated Temperature, 1 Year Discard or Return Date, NHANES Quarterly Report Date

QUALITY ASSUR SPECIMEN RECEIPT					# received, analyzed and transmitted		
NHANES shipper I.D.	NHANES container I.D. #	vial #	date rec'd	receive spec. cond.	spec.	2.5% qc rpt	total
778177102370	368009	018	1/17/2017	ok	47	1	48
778204066788	368058	018	1/24/2017	ok	44	1	45
778299222501	368100	018	1/31/2017	ok	41	1	42
778354403016	368143	018	2/7/2017	ok	25	1	26
778355410426	369009	018	2/7/2017	ok	42	1	43
778408602420	369092	018	2/14/2017	ok	54	1	55
778407805716	368181	018	2/14/2017	ok	22	1	23
778454414575	368221	018	2/21/2017	ok	21	1	22
778463664926	369135	018	2/21/2017	ok	44	1	45
778511320887	369176	018	2/28/2017	ok	29	1	30
778517067017	370009	018	2/28/2017	ok	44	1	45
778564705940	370058	018	3/7/2017	ok	64	1	65

QUALITY ASSURANCE OF SPECIMEN RETURN								1 year	
21-day	analyzed	date	# days	thaw	return to	# days	discard	NHANES	
due date	date	result	results	date	freezer	at frig	or return	Quarterly	
date	sent	return	date	date	temp	date	Report		
2/7/2017	1/18/2017	1/30/2017	13	NA	1/20/2017	2			
2/14/2017	1/25/2017	2/6/2017	13	NA	1/27/2017	2			
2/21/2017	2/1/2017	2/6/2017	6	NA	2/2/2017	1			
2/28/2017	2/8/2017	2/10/2017	3	NA	2/10/2017	2			
2/28/2017	2/8/2017	2/10/2017	3	NA	2/10/2017	2			
3/7/2017	2/15/2017	2/17/2017	3	NA	2/16/2017	1			
3/7/2017	2/15/2017	2/17/2017	3	NA	2/16/2017	1			
3/14/2017	2/22/2017	2/27/2017	6	NA	2/24/2017	2			
3/14/2017	2/22/2017	2/27/2017	6	NA	2/24/2017	2			
3/21/2017	3/1/2017	3/3/2017	3	NA	3/3/2017	2			
3/21/2017	3/1/2017	3/3/2017	3	NA	3/3/2017	2			
3/28/2017	3/8/2017	3/10/2017	3	NA	3/10/2017	2			

Specimen Ordering/Labeling:

Electronic files for all NHANES specimens are sent via email from Westat, Inc to the NHANES contact person shortly before they are to be received. These files include the Sample ID, Analyte Type, Slot No, Sample Collection Date, Sample Comment, Age Grouping, Astro ID, Receipt Date, Analysis Date, Run Number, Tech ID, Analyte Result, Result Comment, Adjusted Result, QC Repeat, LOD, Change Reason, and Change Reason Other. The first seven columns are protected and cannot be altered. The files are saved on the laboratory's common S drive in the NHANES Biochem folder. After analysis, the contact person returns the completed files via their website to Westat, Inc.

The NHANES spreadsheets are used to set up pending batches for batch accession upload in the Laboratory Information system (STARLIMs). New labels are generated out of the Laboratory Information System (STARLIMs). The new bar-coded labels are attached to a carrier tube. The Cobas analyzer reads the bar-coded label for the sample ID and test information.

Specimen Storage:

The temperatures for all freezers and refrigerators are monitored 24 hours a day/ 7 days a week. If the temperature for any unit falls outside the allowable range, action is taken to resolve the problem. If the temperature cannot be corrected, the contents are moved to a different unit.

Specimen Handling/Specimen Return:

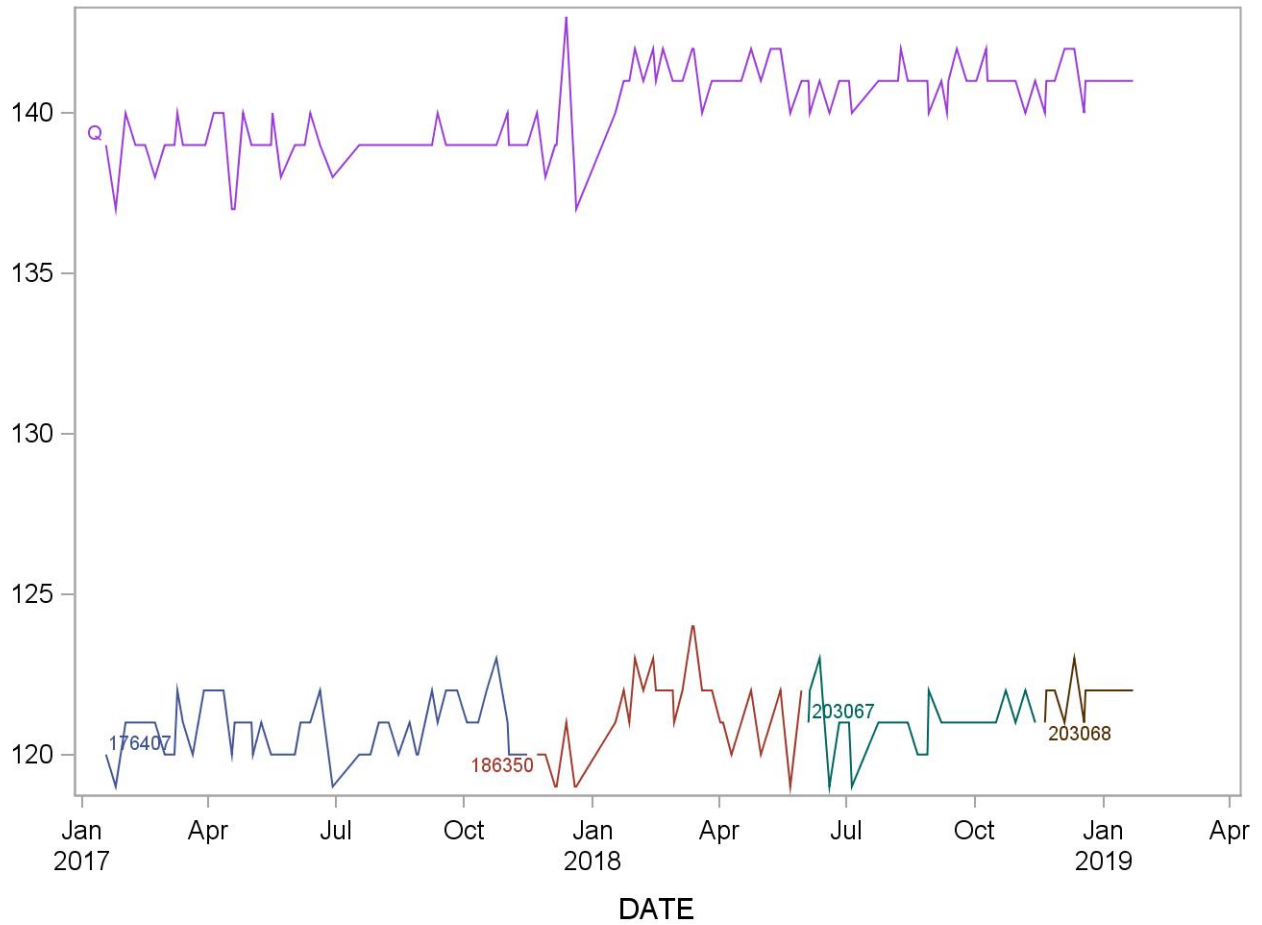
Prior to analysis, the specimens are stored in the designated 2-8°C refrigerator. On the day of analysis, the specimens are selected by the technician operating the COBAS. After analysis and the QC repeats have been run, the specimens are frozen. After 1 year, the specimen vials that have at least 0.2ml of sample remaining will be shipped to SriSai Biopharmaceuticals in Frederick, MD. These specimens will be shipped on dry ice via Federal Express.

19. SUMMARY STATISTICS AND QC GRAPHS

See following page.

Summary Statistics and QC Chart for Sodium (mmol/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
Q	122	18JAN17	22JAN19	140.1	1.3	0.9
176407	52	18JAN17	15NOV17	120.8	0.9	0.7
186350	32	22NOV17	30MAY18	121.2	1.4	1.1
203067	29	04JUN18	13NOV18	121.0	0.8	0.7
203068	9	20NOV18	22JAN19	121.8	0.7	0.5



REFERENCES

1. Cobas 6000 Analyzer Operator's Manual. Roche Diagnostics Inc., 9115 Hague Road Indianapolis, IN 46250-0457. Version 5.0.
2. Roche ISE reagent package insert. Version 2016-04, V13.0. Roche Diagnostics Inc., 9115 Hague Road Indianapolis, IN 46250-0457.
3. Roche Precinorm U Plus package insert, Version 2015-03, V7.0. Roche Diagnostics Inc., 9115 Hague Road Indianapolis, IN 46250-0457.