Laboratory Procedure Manual

Analyte: **Creatinine**

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	LBXSCR	Creatinine (mg/dL)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Creatinine is a breakdown product of creatine phosphate as a result of muscle metabolic processes. It is then excreted by glomerular filtration during normal renal function. Creatinine may be measured in both serum and urine. Creatinine measurement is useful in the diagnosis and treatment of renal diseases, in monitoring renal dialysis, and as a calculation basis for other urinary analytes (e.g. total protein, microalbumin). Another application of serum creatinine measurement has been its use in the estimation of glomerular filtration rate by a variety of calculation models.

In this enzymatic method creatinine is converted to creatine under the activity of creatininase. Creatine is then acted upon by creatinase to form sarcosine and urea. Sarcosine oxidase converts sarcosine to glycine and hydrogen peroxide, and the hydrogen peroxide reacts with a chromophore in the presence of peroxidase to produce a colored product that is measured at 546 nm (secondary wavelength = 700 nm). This is an endpoint reaction that agrees well with recognized HPLC methods, and it has the advantage over Jaffe picric acid-based methods that are susceptible to interferences from non-creatinine chromogens.

Cobas 6000 Application Code: 452

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, K_2-EDTA 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 µL in a sample cup or 2 mL microtube (5 µL serum or plasma for test; remainder for dead volume).

c. **Acceptable Specimens/Unacceptable Specimens:** Serum, lithium heparin plasma, K_2-EDTA plasma and urine are acceptable specimens. 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 7 days at 15-25°C, seven days at 2-8ºC, three months at -15 to -25°C, and longer at –70ºC.

e. **Interferences or limitations:** Icteric index <15: no interference. Hemolytic index < 800: no interference. Lipemic index < 2000: no interference. Ascorbic acid: < 1.70 mmol/L or < 300 mg/L does not interfere. Drugs: No interference was found at therapeutic concentrations using common drug panels. Exceptions: Rifampicin, Levodopa and Calcium dobesilate (e.g. Dexium) cause artificially low creatinine results. N-ethylglycine at therapeutic concentrations and DL-proline at concentrations ≥1 mmol/L (≥115 mg/L) give falsely high results. No significant interference up to a creatine level of 4 mmol/L (524 mg/L). Hemolyzed samples from neonates, infants or adults with HbF values ≥600 mg/dL interfere with the test. 2-Phenyl-1,3-indandion (Phenindion) at therapeutic concentrations interferes with the assay. In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

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 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

Roche Cat. No. 03263991190, CREP2 reagent kit (250 tests):

b. Reagent Preparation (*Reagents are ready to use; no preparation required)

- R1 reagent. TAPS buffer (N-Tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid): 30 mmol/L, pH 8.1; creatinase (microorganisms): ≥ 332 µkat/L; sarcosine oxidase (microorganisms): ≥132 µkat/L; ascorbate oxidase (microorganisms): ≥33 µkat/L; catalase (microorganisms): ≥1.67 µkat/L; HTIB: 1.2 g/L; detergents; preservative.

- R2 reagent. TAPS buffer: 50 mmol/L, pH 8.0; creatininase (microorganisms): ≥ 498 µkat/L; peroxidase (horseradish): ≥16.6 µkat/L; 4-aminophenazone: 0.5 g/L; potassium hexacyanoferrate (II): 60 mg/L; detergent; preservative.

Storage and stability. Keep reagents refrigerated until use. The reagents are stable for 8 weeks refrigerated 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 Precipath U Plus Control (catalog #12149443160). 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

Roche Calibrator for Automated Systems (C.F.A.S.), catalog #10759350190. The calibrator is stable until the expiration date on the bottle when stored at 4ºC. The lyophilized calibrator is prepared with 3.0 mL of deionized water. Pipette the water into the bottle, and then dissolve by gentle swirling within 30 minutes. Avoid formation of foam while mixing. The prepared calibrator is stable for eight hours at room temperature, two days at 4ºC, and one month at –20ºC (frozen once). Traceability: This method has been standardized against ID/MS.

Calibration frequency: The Cobas 6000 will automatically perform a blank calibration (H2O) when a reagent lot has been on board the analyzer for four weeks. A two-point calibration (H2O + C.F.A.S.) must be performed when there is a reagent lot number change. The Cobas 6000 will not allow testing to proceed until a successful calibration has been completed. Monitor control values to determine stability of the current calibration.

Manual calibration should be performed if:

- A reagent lot change has not occurred in the past 6 months
- After major service or repairs

- As needed for troubleshooting

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

a. **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 end 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.

b. **Professional Judgement:** Check results for error flags and take appropriate corrective action. Investigate alert values and delta checks.

c. **Result Entry**

STARLIMS test code: CR, UCR

Manual entry

- Serum or plasma results are reported to two decimal places (as x.xx) in mg/dL.
- Report low results as <0.10 mg/dL for serum, and <1 mg/dL for urine.
- Check results for error flags and take appropriate corrective action.
- Investigate alert 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.

Results are reported to two decimal places (as x.xx) in mg/dL. Report low results as <0.10 mg/dL.

a. Reportable Range of Test Results: Reportable Range 0.10-122.04 mg/dL

   Intra-assay %CV (10 within-day replicates at a concentration of 1.00 mg/dL) 1.0%

   Intra-assay %CV (10 within-day replicates at a concentration of 3.53 mg/dL) 0.9%

   Inter-assay %CV (between day replicates at a concentration of 0.83 mg/dL) 3.0%

   Inter-assay %CV (between day replicates at a concentration of 3.85 mg/dL) 1.9%

   Dilutions: The confirmed analytical measurement range of the assay is 0.10-30.51 mg/dL (serum), and 1-516 mg/dL (urine). Serum specimens exceeding the high limit are automatically diluted (1:4) by the instrument, and results from samples diluted using the rerun function are automatically multiplied by a factor of 4. Urine specimens exceeding the high limit are automatically diluted (1:2.5) by the instrument and results from samples diluted using the rerun function are automatically multiplied by a factor of 2.5. If a manual dilution is required, dilute the specimen in normal saline, and multiply the result by the dilution factor. For example, to perform a 1:5 dilution, pipette 50 µL of the patient sample into 200 µL of normal saline. Mix thoroughly, perform the assay, and multiply the result by a factor of 5. The maximum allowable manual dilution is 1:5.

b. Reference Range: Serum, adult male 0.67-1.17 mg/dL adult female 0.51-0.95 mg/dL

c. Critical Results: None

d. Analytical Measurement Range: 0.10-30.51 mg/dL

Reportable Range: 0.10-122.04 mg/dL

Limit of Detection (standard 1 + 3 SD): 0.10 mg/dL

10. QUALITY CONTROL (QC) PROCEDURE

•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 Precipath U Plus Control (catalog #12149443160). 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).

•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. Creatinine 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 reagent 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.

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. Limit of Detection (standard 1 + 3 SD): 0.10 mg/dL

b. Analytical Measurement Range: 0.10-30.51 mg/dL

c. Interferences or limitations: Icteric index <15: no interference. Hemolytic index < 800: no interference. Lipemic index < 2000: no interference. Ascorbic acid: < 1.70 mmol/L or < 300 mg/L does not interfere. Drugs: No interference was found at therapeutic concentrations using common drug panels. Exceptions: Rifampicin, Levodopa and Calcium dobesilate (e.g. Dexium) cause artificially low creatinine results. N-ethylglycine at therapeutic concentrations and DL-proline at concentrations ≥1 mmol/L (≥115 mg/L) give falsely high results. No significant interference up to a creatine level of 4 mmol/L (524 mg/L). Hemolyzed samples from neonates, infants or adults with HbF values ≥600 mg/dL interfere with the test. 2-Phenyl-1,3-indandion (Phenindion) at therapeutic concentrations interferes with the assay. In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

13. REFERENCE RANGES (NORMAL VALUES)

Serum, adult male 0.67-1.17 mg/dL adult female 0.51-0.95 mg/dL

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 will 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 rpts	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								
		date	# days		return to	# days	1 year discard	NHANES
21-day due date	analyzed date	result sent	results return	thaw date	freezer date	at frig temp	or return date	Quarterly 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:

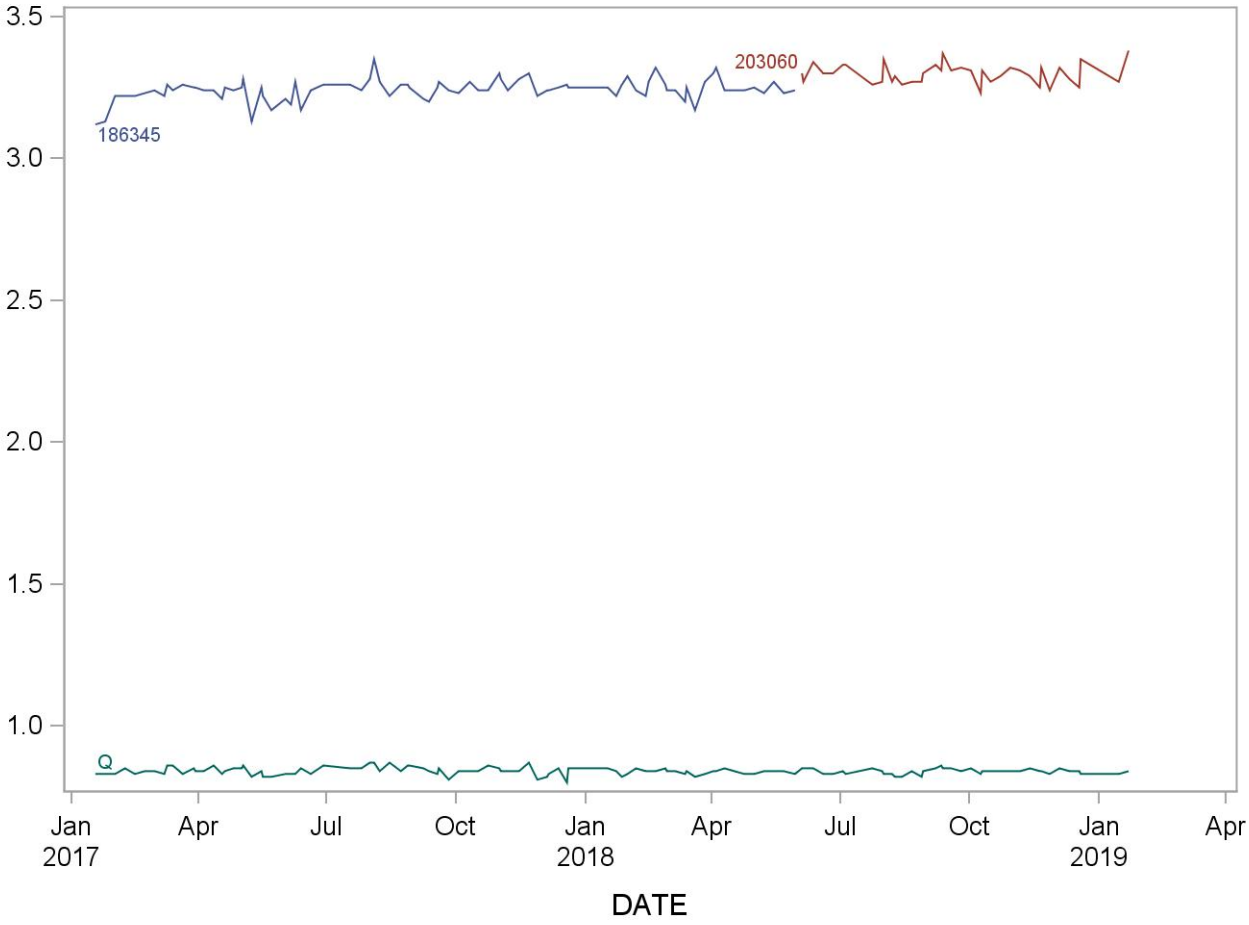
Prior to analysis, the specimens are stored in the designated 2-8°C refrigerator. On the day of analysis, the specimens are selected and thawed by the technician operating the COBAS. After analysis and the QC repeats have been run, the specimens are refrozen. After 1 year, the specimen vials that have 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 Creatinine, refrigerated serum (mg/dL)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
186345	84	18JAN17	30MAY18	3.242	0.038	1.2
Q	122	18JAN17	22JAN19	0.840	0.013	1.5
203060	38	04JUN18	22JAN19	3.298	0.035	1.1



REFERENCES

1. Cobas 6000 Analyzer Operator's Manual. Roche Diagnostics Inc., 9115 Hague Road Indianapolis, IN 46250-0457. Version 5.0.

2. Roche Calibrator for Automated Systems (C.F.A.S.) package insert. Version 2011-08, V6. Roche Diagnostics Inc., 9115 Hague Road Indianapolis, IN 46250-0457.

3. Roche CREP2 reagent package insert. Version 2016-02, V12.0. Roche Diagnostics Inc., 9115 Hague Road Indianapolis, IN 46250-0457.

4. Roche Precipath U Plus package insert, Version 2011-04, V4. Roche Diagnostics Inc., 9115 Hague Road Indianapolis, IN 46250-0457.