Laboratory Procedure Manual

Analyte: **Urine Creatinine**

Matrix: **Urine**

Method: **Roche Cobas 8000**

As performed by: **University of Minnesota**
Advanced Research and Diagnostic Laboratory (ARDL)
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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:

File Name	Variable Names	SAS Label
ALB_CR_L	URXUCR	Creatinine, urine (mg/dL)
	URXCRS	Creatinine, urine (umol/L)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Creatinine in serum or plasma is the most commonly used test to assess renal function. It is a breakdown product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body, depending on muscle mass. Creatinine is 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 the MDRD equation (see Note 1).

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 high-performance liquid chromatography methods, and it has the advantage over Jaffe picric acid-based methods that are susceptible to interferences from non-creatinine chromogens.

2. SAFETY PRECAUTIONS

A. Daily Safety Precautions

All personnel working in the laboratory must wear gloves and laboratory coats. Laboratory coats are to be kept snapped. Lab coats must meet OSHA compliance CPL2-2.44D. Splash and spray resistant fabric that is also antistatic is required.

Gloves are removed when leaving the immediate work area or when entering offices within the immediate work area. All used gloves, vials, pipettes and other items that come in contact with specimens are disposed of in a Biohazard box lined with a red plastic bag. Work surfaces are cleaned at least daily and as needed with appropriate germicidal cleaner

B. Blood Handling

The improper handling of blood samples from patients with infectious diseases, e.g. hepatitis or HIV, can lead to infection of staff that draw, handle, analyze, or store such samples. Transmission can occur by ingestion, inhalation, or direct contact, and staff must exercise care when handling blood samples. Always wear liquid impermeable gloves (e.g., nitrile or plastic) when handling biological samples. The use of latex gloves is not allowed due to concerns for personnel having or developing latex sensitivities. Never pipet samples by mouth. Avoid contact with serum. Cover any scratches or cuts on fingers and hands and wear gloves before handling serum. Store all samples in sealed containers. In order to minimize the formation aerosols, do not leave samples open to the atmosphere longer than necessary.

It is about 30 times easier to become infected with hepatitis than with HIV through sample mishandling, and it has been recommended that the usual precautions for handling blood specimens to prevent hepatitis infection serve as a guide to prevent AIDS infection as well. Handle all specimens as if you know them to be infectious. All staff should adhere to the CDC Guidelines for Prevention of HIV Infection in Health Care Workers.

C. Spills

The contaminated area is cleaned with a solution of sodium hypochlorite (bleach: water, 10:100, v/v) and the wipes are disposed of in a red biohazard box.

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

Use urine for the procedure. Urine is collected without added preservatives. It may be a random specimen or a timed specimen, depending upon the desired application of the test. Centrifuge urine specimens prior to analysis to remove particulate matter from the supernatant. Hydrochloric acid (14-47 mmol/L urine) or boric acid (81 mmol/L urine) are acceptable preservatives. Creatinine in *unpreserved urine* is stable for 6 days at 4ºC, and longer at –70ºC. Creatinine in *preserved urine* is stable for 8 days at 4ºC, and longer at –70ºC.

Specimens must be at room temperature prior to assay. Mix specimens well and centrifuge 10 minutes at 1500xg before use.

Bilirubin does not interfere up to an index of 70. Hemolysis does not interfere up to an H index of 1000.

Minimum sample volume: 100 uL (includes dead volume).

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. Roche/Hitachi Cobas 8000 Analyzer. Roche Diagnostics, 9115 Hague Road, P.O. Box 50457, Indianapolis, IN, USA 46250-0446.

B. Purified water supply. The Cobas 8000 requires a continuous supply of purified water. The system used by the Cobas 8000 is the MedWater Systems MW 90. When the system is

started the inlet valve opens and water enters the system. It then passes through the sediment filter and the carbon filter, which absorb organic chemicals like chlorides, insecticides, pesticides, and herbicides from the water and remove particles larger than 5 microns. The reverse osmosis modules remove about 98% of the salt content out of the water, as well as nearly all of the bacteria, viruses, heavy metal complexes, and organic molecules with a molecular weight higher than 300 Daltons. The cleaned water is called Permeate. The water system should be checked daily for the conductivity/resistivity. The carbon cartridge and water canister should be replaced approximately every 3 months. Service can be contacted by calling 801-529-6705. The system serial number is MW901126.

C. Cobas 8000 system reagents.

ISE module reagents

1) ISE Internal Standard Gen.2. Roche product # 04880455 (2L bottle). No preparation required. Solution of NaCl, sodium acetate, KCl, triethanolamine, and HEPES buffer. Store at room temperature. The on-board stability is 6 weeks after opening. Two bottles are kept on-board the analyzer, one for each ISE unit. This solution is automatically drawn by the Cobas 8000 to clean the ISE dilution vessels and for 1-point calibration before and after measuring the diluted samples.

2) ISE Reference Electrolyte. Roche product #10820652 (500 mL bottle). No preparation required. Solution of 1 mol/L potassium chloride. Store at room temperature. The solution is stable on-board the analyzer until the expiration date stated on the bottle. Two bottles are kept on-board the analyzer, one for each ISE unit. This solution is automatically drawn by the Cobas 8000 to use as reference electrolyte against ISE Internal Standard and against the diluted samples.

3) ISE Diluent Gen.2. Roche product #04880480 (2L bottle). No preparation required. Solution of HEPES buffer and triethanolamine. Store at room temperature. The solution is stable on-board the analyzer for 6 weeks after opening. Two bottles are kept on-board the analyzer, one for each ISE unit. This solution is automatically drawn by the Cobas 8000 to dilute samples at a fixed ratio.

4) ISE Cleaning Solution/Elecys SysClean. Roche Product #11298500 (100 mL bottle). This is a sodium hydroxide and sodium hypochlorite solution. Store at 2-8˚C. The solution is stable up to the stated expiration date when stored at 2-8 °C. This solution is not kept on the instrument; it is used in maintenance functions to clean the ISE flow path, ECL flow path and the pre-wash area.

5) EcoTergent/Hitergent/Eco-D. Roche product # 6544410 (12 bottles/box). No preparation required. Solution of ethanolamine, hexahydro-1,3,5-tris (Betahydroxyethyl) triazine and nonidet P-40. Store at room temperature, protected from light. Roche product #08063168 (Bottle set for Cobas 8000 ISE). Pour the contents of 3 bottles of 59 mL EcoTergent into one of the empty bottles of the Bottle Set to approximately 180 mL. The on-board stability is 28 days after placement on the analyzer.

502 Module Reagents

6) Roche Product #03263991190, CREP2 reagent kit (250 tests):
   • 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.
   • R3 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.

7) Roche Calibrator for Automated Systems (C.F.A.S) Catalog #10759350360

8) Pooled urine control, 2 levels (UD1 and UD2). The control is prepared from pooled human urine. Stable at -70°C for up to four years, at 2-8°C for four days, and at 15-25°C for two hours.

9) Cell Wash Solution I/NaOH-D. Roche product #4880285 (1800 mL bottle). No preparation required. Solution of sodium hydroxide (1N) and detergent. Store at room temperature. The on-board bottle stability is 10 weeks after opening. This solution is automatically drawn by the Cobas 8000 while cleaning reaction cuvettes during analysis.

10) Cell Wash Solution II/Acid Wash. Roche product # 4880307 (2L bottle). No preparation required. Solution of citric acid monohydrate, buffer, and detergent. Store at room temperature. The on-board stability is 12 weeks after opening. This solution is automatically drawn by the Cobas 8000 while cleaning reaction cuvettes during analysis.
11) Reaction cell cuvette segments. Roche product #04854241 (24 segments/box). Eight segments complete the entire rotor). Perform cell wash and cell blank functions after installation. Change cuvettes monthly.

12) ECOTergent/Eco-D. Roche product # 6544410 (59 mL bottle). No preparation required. Solution of ethanolamine, hexahydro-1,3,5-tris (Betahydroxyethyl) triazine and nonidet P-40. Store at room temperature. The on-board stability is 12 days after opening. EcoTergent is an on-board reagent automatically drawn by the Cobas 8000 during the daily incubator bath exchange; it reduces surface tension in the reaction bath water.

13) Sample Cleaner 1/Multiclean. Roche product #05352991 (20 mL bottle). No preparation required. Solution of 1 mol/L NaOH. Store at room temperature. The on-board stability is 2 weeks after opening. This solution is used to wash sample probes and reaction cells.

14) Sample Cleaner 2. Roche product #05968828 (20 mL bottle). No preparation required. Solution of buffer and detergent. Store at room temperature. The on-board stability is 2 weeks after opening. This solution is used to wash the sample probes.

15) NAOHD cassette (Detergent 1). Roche product #04489241 (66 mL cassette). No preparation required. Solution of 1 mol/L NaOH. The on-board stability is 12 weeks

after placement on the analyzer. The solution in this cassette is used for reagent probe wash, reaction cell wash, and the maintenance action wash reaction parts.

16) SMS cassette (Detergent 2). Roche product #04489225 (50 mL cassette). No preparation required. Solution of 200 mmol/L HCl. The on-board stability is 12 weeks after placement on the analyzer. This cassette is used for reagent probe wash and reaction cell wash.

801 Module Reagents

17) ProCell II M. Roche product # 06908799 (2 L bottle). No preparation required. Solution of Tripropylamine (TPA) and Oxaban A. Store at room temperature. The on-board stability is 21 days after opening. This is a buffer solution that is used for conditioning the electrodes, transporting the assay reaction mixture, washing the streptavidin-coated microbeads and in signal generation. ProCell II M is automatically drawn by the Cobas 8000 during analysis.

18) CleanCell M. Roche product #04880293 (2 L bottle). No preparation required. Solution of potassium hydroxide and polidocanol. Store at room temperature. The on-board stability is 21 days after opening. The solution is automatically drawn by the Cobas 8000 to clean the measuring channel after each measurement and conditioning the electrodes.

19) PreClean II M. Roche product #06908853 (2 L bottle). No preparation required. Solution of phosphate buffer, NaCl, and detergent. Store at room temperature. The on-board stability is 4 weeks after opening. This is a phosphate buffer that is used to wash and resuspend microbeads during the pre-wash step. It removes substances which potentially interfere with the detection of signals. PreClean II M is automatically drawn by the Cobas 8000 during analysis.

General Use

20) Sample cups (micro). Roche product #05085713.

21) Sample cups (standard). Roche product #10394246.

22) Printer paper, 8.5 x 11 inch. Various sources including Bose Multipurpose Paper.

23) Printer cartridge. HP Laserjet CF226A, black. Hewlett-Packard Company.

24) Reagents and calibrators. See specific assay procedures.

25) Quality control materials. Two levels of controls are assayed each day that a specific test is performed. Check current QC records for lot in use and acceptable values.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

There are a variety of calibration models used on the Cobas 8000. There are factored methods, blank calibrations, two-point calibrations and multi-point calibrations. The type of calibration is dictated by the Roche application parameters for each method. The calibrator material may be a Roche product, an in-house preparation, or a product from another company. A new set point

value is typically assigned whenever a calibrator lot number changes. If the calibrator is a Roche product, the updated set point value must be downloaded via the COBAS Link. This is a direct, web-based link from the Cobas 8000 to the Roche database of lot-specific calibrator and control values. If the set point change is for a non-Roche product, then the update must be performed manually in the data manager, then downloaded to the Cobas 8000 control unit. Frequency of calibration is dictated by an automatic, time-dependent re-calibration built into the application parameters for each test, and by observing the quality control data. Not all methods have an automatic time-out calibration feature. Details for each assay may be found in specific procedures and in the application parameters on the Cobas 8000. Acceptable accuracy and precision limits are defined in each chemistry parameter file. A two-point calibration (H2O + calibrator) must be performed when there is a reagent lot number change for this assay. The Cobas 8000 will not allow testing to proceed until a successful calibration has been completed.

Traceability: This method has been standardized against ID/MS.

Verification: Calibration verification is an ongoing process conducted through the measurement of the LN6 linearity survey from CAP (College of American Pathologists). Linearity is assessed semi-annually.

Calibrator information. Roche Calibrator for Automated Systems (C.F.A.S.), catalog #10759350 360. The calibrator is stable until the expiration date on the bottle when stored at 2-8ºC. The lyophilized calibrator is prepared with 3.0 mL of deionized water. Volumetrically 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 15-25°C, two days at 2-8ºC, and one month at - 15 to -25ºC (frozen once).

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

The Roche/Hitachi Cobas 8000 analyzer series is a fully automated, high throughput laboratory system for clinical chemistry and immunology tests. The system is designed for a large array of quantitative and qualitative in vitro tests. It consists of the following components: data manager, control unit, core components, ISE module, clinical chemistry module (c502), and immunoassay module (e801). The ISE system is used in the quantitation of sodium (Na+), potassium (K+), and chloride (Cl-). In addition to the standard ISE module, this instrument also uses an ISE support box. The photometric system (c502) can measure colorimetric or immunoturbidimetric reactions utilizing end point or kinetic (rate) absorbance measurements. The immunoassay module (e801) uses electrochemiluminescence to perform up to 300 immunoassay tests per hour. Test ordering and execution on the Cobas 8000 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 8000 can utilize both systems simultaneously.

A. Theory of Operation

Samples for ISE determination, photometric measurement, and immunoassay determination can be directed by the user or an interfaced order. The measuring area of the ISE module has two ISE units. Each ISE specimen is pre-diluted by the instrument in one of two dilution vessels. The diluted specimen is then measured in the ISE measuring system. Each ISE unit has a temperature-controlled compartment with 3 ionselective electrodes and 1 reference electrode. The voltage between each of the ion-selective electrodes and the

reference electrode is measured and used to determine the concentration of Na+, K+, and Cl- ions in the diluted samples. Samples for photometric analysis are measured by the photometer. The photometer measures either endpoint or rate reactions that have occurred in the reaction cell with absorbance changes measured using discrete wavelength settings. Following completion of photometric reactions, the cell rinse unit washes the reaction cell, and the cell is re-used. All analyses occur at 37ºC. Samples for immunoassay determination require a new disposable tip to be used for every pipetting sequence in order to prevent carryover. Samples are pipetted into one of 94 sample cups in an incubator disk that is maintained at 37ºC. Two individually calibrated electrochemiluminescence (ECL) detection cells are the central components performing determination of samples. The measuring cells magnetically capture microbeads with the attached immune complex that is to be determined. All other serum constituents and unbound reagents in the measuring cells are washed away. A photomultiplier measures the light signal that is emitted by the ruthenium marker on the immune complex under the effect of an electrical field. Two test principles are used: competitive assay principle (very small analytes) and sandwich assay principle (larger analytes). Following analysis, sample cups are disposed into a solid waste container on the instrument.

B. Specimens

Follow all usual precautions for obtaining a specimen by venipuncture. To determine the correct specimen type, refer to the specific assay procedure. Follow ARDL safety procedure Exposure Control Plan for blood and body fluid precautions.

The approximate dead volume for the Cobas 8000 microsample cups is 50 uL. It is 100 uL for the standard cups.

Specimens may also be direct-sampled in the original storage vial. These vials are typically prepared by field center laboratories after collection of specimens at their site. Because they are frequently very small with small volumes of specimen, care must be taken that these vials are very thoroughly mixed prior to sampling. Since mixing or vortexing can cause bubble formation (which interferes with the Cobas 8000 sample detection system), care must be taken to remove these bubbles before analysis begins. This can be done by poking the bubbles with a wooden stick, or by a short (1 minute) centrifugation at 1,500 x g. However, if lipids are being measured centrifugation is not an option because the triglycerides could layer out into the surface of the specimen, resulting in an artifactual increase in their concentration. Storage vials come in a variety of shapes and sizes, and the clip design of the Cobas 8000 sample racks accommodates most of them.

C. Instrument Overview

A general description of the instrument and its components is found in Section 1 of the Cobas 8000 analyzer series Operator’s Manual, Version 5.3.

The c502 module of the Cobas 8000 utilizes a spectrophotometric measuring system. The instrument utilizes optically pure, reusable plastic reaction cells that are changed on a monthly basis. The reaction cells are automatically washed by the instrument after completion of each test cycle. Sample and reagents are added to the reaction cells at specific timed intervals, varying by the program parameters defined for each test. Most methods utilize two reagents (within the same cassette), but a few use one. All Roche reagent bottles have a uniquely barcoded ID label on them so they are recognized when

loaded onto the instrument. NonRoche assays use a generic Closed Development Channel Cassette, with each assay assigned to a specifically numbered cassette corresponding to its on-board application. The Cobas 8000 measures the reagent volume in the bottle as it is withdrawn, so the instrument provides a real time update on the number of tests available in each of the bottles.

The measuring system in the e801 module is based upon the principle of electrochemiluminescence (ECL). Single-use cuvettes and sample tips are used instead of reusable ones. Like the c502 module, reagent vessels of the e801 are uniquely barcoded and the test count is updated in real time. In general, each reagent vessel consists of three reagents: streptavidin-coated microparticles, biotinylated antigen or sandwich antibody, and ruthenium-labelled antibody. Reagents may be loaded while the instrument is in Operation mode.

Tests can be ordered manually or they can be executed by use of the bi-directional interface connected to the StarLims host computer. Similarly, reporting can be achieved through the interface, by manually keying the results into StarLims from the instrument's hard copy printout, by data entry into a spreadsheet or a website, or by manipulation of the instrument's downloaded data file. Which avenue is chosen for these functions is dictated by the parameters of specific studies

D. Instrument Setup

1) Log into the Cobas 8000 using assigned username and password.

2) Reagents. All reagents used on the Cobas 8000 are stored in a refrigerated reagent compartment. All reagents have a unique barcoded identifier. Before starting the analysis sequence check the reagent status on the Cobas 8000 to confirm there is adequate reagent to complete the anticipated test volume for the day. Discard any bottles that have gone empty.

3) Maintenance. Complete the scheduled daily maintenance as described in the Cobas 8000 maintenance protocol.

4) Order calibration, if indicated (see Cobas 8000 operations protocol).

5) Order controls. If a calibration was requested, the controls should not be ordered until the calibration has completed. If the controls are ordered and executed before the calibration is complete, the controls will be measured on the previously stored calibration line.

E. Loading Calibrators and Controls

On the c502 module, each calibrator and control has a defined location on either the black calibrator sample racks or white control racks. A complete posting of all of these assigned locations is available on the Cobas 8000 computer. The assigned locations are also marked on the calibrator and quality control racks themselves. A standard sample cup is acceptable to hold calibrator or quality control material, but a micro cup is not. Fill the sample cup with enough volume to complete the full calibration sequence (this will vary by method; some multipoint calibrations will sample the standard several times in order to make serial dilutions, while in a two-point calibration, the standard is only sampled in duplicate) or

control testing. Most calibrations require water as the “zero” standard. Make sure it is loaded. The sample cups are loaded into the calibrator and control sample racks. If using 2 mL Sarstedt vials for a control, the Cobas 8000 will not be able to detect the vial if it is seated too low in the rack. The bottom of the 2 mL vial should be seated to approximately the bottom of the metal prongs in the rack. Take care to ensure that the vial is seated far enough down so it is not jarred from the rack during transport on the Cobas 8000. Alternatively, a 2 mL Sartstedt vial can also be placed in a 13 x 75 mm plastic support tube.

On the e801 module, CalSet and ControlSet vials from Roche are used, and each vial has a barcode provided in the calibrator or control kit. Check that the lot number of the barcoded calibrator or control matches what is active in the Cobas 8000 control unit, otherwise the system will not recognize it until it is downloaded onto the system. Most e801 calibrators have two barcodes on each label; be sure that the 8000 barcode is the one that faces out, or the calibrator will not be run. The 8000 barcode is smaller with a yellow stripe at the top and bottom of the label. Additionally, after the Cobas 8000 has read the same calibrator barcode 4 times, it will not accept it again. Roche recommends that each aliquot of calibrator be used only once on the analyzer.

See specific protocols for the assigned calibrators and controls to be used for each test.

F. Requesting a Calibration:

1) At the home screen, click or touch screen on tab. Click or touch screen on <Status>.

2) Click or touch screen on <Status.> A list of all the Cobas 8000 tests appears. If more than one bottle set of reagents is on-board, a separate listing will appear for each set. Calibrations that are recommended to be performed have the Cause column highlighted in yellow.

3) Click or touch screen on the tests to be calibrated.

4) In the “Method” box on the right side of the screen, select the appropriate type of calibration to be performed on the selected test. The correct type of calibration for each method can be found in the specific test protocols located in the Cobas 8000 Applications folder. Most c502 methods utilize a two-point calibration, while all e801 methods utilize a full calibration. Generally, if a screen button is white, that means it is active/available. Blue indicates completion, gray indicates inactive/unavailable. Make sure that the Method box is white before clicking on Save below.

5) If a calibration has timed out, or if there has been a reagent lot change, this information appears in the Cause column and the calibration is already recommended and highlighted in blue. In these cases, touch or click on and the calibration is ordered.
    a. A note on lot v. reagent pack calibrations: Calibrating a reagent cassette that has been loaded on the instrument for less than 24 hours generates a lot calibration and is valid for all reagent cassettes belonging to the same reagent lot. However, calibrating a reagent cassette that has been loaded on the instrument for more than 24 hours generates a reagent pack calibration, which is only valid for the particular reagent cassette.

6) Click or touch screen on <Save>

7) Failed calibrations will generate an error message by the Cobas 8000. On the c502 module, the two most common flags found in a failed calibration are SENS and DUP. On the e801 module, the most common flags found in a failed calibrator are Monotony of Curve, Deviation of Duplicates and Factor.

G. Loading Reagents:

In general there are two types of reagents: Primary reagents are test-specific and supplied in reagent cassettes. System reagents are used across all tests and supplied in larger quantities.

On the e801 module, there are also consumables. Consumables are tips for pipetting and cups for incubating. These are provided together in packs that are loaded periodically onto the instrument, when the Cobas generates an alarm that they are running low.

Review available reagents by <Reagents>, <Status>. Sort the reagents by clicking on Available Tests. This lists the reagents in ascending order of the number of tests performable with the reagents currently on the Cobas 8000. You can sort by the ISE, c502, or e801 module by selecting it from the Module: dropdown just above test name. When a test is highlighted on this list, the reagent bottle locations, number of tests remaining, and stability by bottle are shown in the window on the right side of the screen.

The principle reagent containers for the c502 modules are reagent packs that contain up to three reagent vials. Reagent packs are stored in a refrigerated reagent compartment on the c502 which holds up to 60 reagent cassettes. Most test methods use two reagents, though some use only one. Generally, R1 is a buffered reagent that establishes the optimum pH and reaction conditions for the test, and R2 has the enzymes and/or chromogenic components that complete the reaction. If a test volume greater than the capacity of one reagent container is anticipated, additional packs may be placed on the instrument. If an automatic calibration is not required on pack change, or if the new pack was calibrated when it was placed on the instrument, then the Cobas 8000 will automatically begin pipetting from the new pack once the previous one is empty. Roche-provided reagent packs have a two-dimensional barcoded ID on one side. This barcode contains lot number, test code, expiration date and available test count information. When loading reagent packs into the cassette loading area on the c502, the barcode must face to the right. As a reminder, there is a diagram illustrating this on the loading stage. Unloading and Reloading Cobas c packs: The system counts down each cassette’s initial number of available tests each time it pipets out of the cassette. If a pack is “unloaded” and later reloaded, the system recognizes the cassette and begins counting down at the point when it was unloaded, assuming the cassette’s reagent volume remains unchanged. However, if a reagent cassette is “dumped”, that cassette cannot be returned to the instrument.

The principle reagent container for the Cobas e801 module is the Cobas e pack. This pack consists of three separate capped reagent containers. Each pack is equipped with a RFID label that contains reagent, lot number, control, calibration, expiration and stability information. These packs can be loaded directly from the refrigerator onto the Cobas 8000 without any delay. The Cobas e packs are loaded into one of the 48 positions on the e801 reagent disk, which is stored at a lower level inside the analyzer. To load reagents, press the reagent loader button, located to the right of the reagent loading ports on the e801 module. The reagent loader button calls the reagent loader to come up to receive reagents, and also sends it down once reagent packs are placed onboard. The LED of the button

indicates the position of the reagent loader. To load a reagent cassette, it must be pushed through one of the five loading ports into the reagent loader. Then press the reagent loader button again to send the cassette down into storage. If the reagent loader button is pressed quickly, the loader will descend and come back up. This is useful if more than five reagent cassettes need to be loaded. If the reagent loader button is held down for more than five seconds, the loader will descend and not return.

H. Loading Specimens:

Given that ARDL receives specimens from so many different sources, in many different containers, and provides results via different mechanisms (report from host, manual entry to spreadsheet, manual entry to website, processing of the instrument data download), there are multiple ways to load specimens and order testing on the analyzer. Prior to placing specimens on the instrument, it is mandatory that all specimens be thoroughly mixed. Most specimens analyzed in ARDL have been frozen, so this step is critical. Mixing sometimes causes surface bubbles to form, and these must be remedied before sampling. Poking the bubbles with a wooden applicator stick is recommended. Urine specimens must be centrifuged following mixing. All specimens analyzed on the e801 module must be at room temperature before they are placed on the instrument.

Interfaced test ordering

1) It is not necessary to “connect” the Cobas 8000 to the host. This connection is always in place, with a Data Innovations middleware system serving as a buffer between the Cobas 8000 and StarLims.
2) The desired tests are ordered in the host computer system, StarLims.

3) A container ID (CID) is generated by the system, and a label is produced with the barcoded CID on it.

4) This label is affixed to a vial that will be used for direct sampling on the Cobas 8000.

5) After arrival of the specimen at the analyzer, and following mixing and/or centrifugation, the labeled vial is placed into a proper rack.

6) When loading the vials onto the sample racks, the caps are removed and stored in a sequential system to allow re-capping of the vials with the same cap following analysis. A board with numbered holding positions in the processing area facilitates this process.

7) The barcode must face out through the groove in the rack, and the vial must be positioned so it is high enough in the rack to be seen by the location detector, but not so high that it might be ejected from the rack while being transported through the instrument.

8) Close the cover on the loading platform.

9) On the Cobas 8000 computer terminal, press or click <Start>, then <Start> again.

10) Patient data hard copies must be requested in <Workplace>, <Data Review>. Highlight the desired records, then <Print>, and then <Print Preview>. Wait until the icon on the Preview button flashes once and then returns before you press <History>; then the

selection will be available as a choice to print out on the left-hand side. Result lists will be listed as <Result List>; press <OK> to print out the selection.

I. Instrument shutdown

After bringing the instrument to Standby mode, and successfully transferring the data to the mass storage and S: drive locations (see separate procedure), the Cobas 8000 is ready for activation of the Sleep Pipe.
First, load the designated green rack as follows, using standard sample cups, half-filled:
Pos 1: Sample Cleaner 1
Pos 2: SysClean
Pos 3: Leftover serum-based control material, or Roche Activator (Roche Product #04663632).
Place the rack on the sample loading tray. Then request the Sleep Pipe: 1. <Utility> 2. <Maintenance> 3. <Pipe Functions> 4. <Sleep Pipe> 5. <Execute> The instrument samples the green rack elements, and completes the Sleep Pipe functions in approximately 45 minutes. It then enters sleep mode until re-starting at the pre-programmed time the following morning.

After coming to Stand-by status the data from each day's run is downloaded from the Cobas 8000 computer to a USB, then to the network folder.

Consult the procedure describing this process for details.

Print all Cobas 8000 test results, and file in chronological order with the other daily printouts.

An automatic timer has been set so that the Cobas 8000 turns on each weekday morning at 0430, automatically performing an air purge, photometer check, and incubator bath exchange during the process. The automatic timer has been set so that the Cobas 8000 remains off during weekends.

Return all leftover controls and calibrators to the refrigerator at the end of the day.

J. Recording of Data

Any printouts must be requested on the Cobas 8000 by highlighting or selecting the desired information to be printed, then hitting the <Print> button, then <Preview>. Wait until the icon on the Preview button flashes once and then returns before you press <History>; then the selection will be available as a choice to print out on the left-hand side. Result lists will be listed as <Result List>; press <OK> to print out the selection.

Hard copies of patient data should be generated only if reviewing the data for verification in StarLims, or if the results are to be manually entered into a spreadsheet or website. Detailed StarLims instructions may be found in specific StarLims protocols, but the general process for automated entry is as follows:
1. Log in with personal user ID and password.
2. <Start Batch>
3. Select appropriate batch category from drop down menu.
4. <Close Batch>
5. <LifeCycle icon>
6. <Result/Finish Batch>

7. Select appropriate batch category/number from drop down menu.
8. Review data. Accept, correct or comment as necessary.
9. <Finish Batch>

Manual data entry in StarLims is done via the <Order/Result Review> option on the Dashboard. Select <Advanced>, then enter the CID of interest. The entry fields appear in the lower portion of the screen. After data entry, select <Finish Result>, then <Approve Pending>. Data entry into spreadsheets is typically accompanied by an additional tab for a Data Dictionary where details regarding the methodology can be provided. This information is available in the ARDL Data Dictionary folder on the S: drive.

K. Dilutions

The confirmed analytical measurement range of the assay is 1.1-610 mg/dL (urine). Specimens exceeding the high limit are automatically diluted (net 1:2.5) by the instrument, and reported accordingly. The maximum allowable manual dilution is 1:2.5.

L. AMR

The analytical measurement range (AMR) must be validated every 6 months or after major maintenance or service procedures. CAP LN6 product is used to validate the urine creatinine analytical measurement range and shipped automatically twice per year. Follow package insert instructions to prepare the samples. Analyze the samples in duplicate, programming in the same manner as patient samples.

Determine the mean value for each level. Results are submitted to CAP electronically by the lead or supervisor and paper copies of the documentation is saved in the CAP binder. CAP reports acceptability limits and linear range shortly after all results are reported.

M. 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.

9. REPORTABLE RANGE OF RESULTS

Linear range of the method: 1.1-610 mg/dL. Specimens exceeding the high limit are automatically diluted (1:2.5) by the instrument, and reported accordingly. The maximum allowable dilution is 1:2.5.

Analytical Measurement Range: 1.1-610 mg/dL

Clinically Reportable Range: 5-1525 mg/dL

Assay Performance	Ranges
Analytical Measurement Range	1.1-610 mg/dL
Reportable Range	5-1525 mg/dL
Limit of Detection (standard 1 + 3SD)	1.1 mg/dL

Intra-assay %CV (10 within-day replicates at a concentration of 52.08 mg/dL)	0.8%
Inter-assay %CV (47 between-day replicates at a concentration of 88.2 mg/dL)	1.9%

10. QUALITY CONTROL (QC) PROCEDURES

A. 1. Pooled urine control, UD1. The control is prepared from pooled human urine. Stable at -70°C for up to four years, at 2-8°C for four days, and at 15-25°C for two hours.

2. Pooled urine control, UD2. The control is prepared from pooled human urine. Stable at -70°C for up to four years, at 2-8°C for four days, and at 15-25°C for two hours.

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.

B. Evaluation of Control Limits

Before the control chart can be used for quality control, it is reviewed to determine that the data have been collected during a stable analytical period. The chart is examined for outliers, for periods of questionable or unstable performance, and for evidence of excessive bias. An outlier will distort the control limits if incorporated into the final calculations. An outlier is considered to be any value of X which falls outside the control limits (X + 3Sx) or any value of R which exceeds the control limit for R. These values are eliminated as are values from any questionable period of performance. The values of X, Sx, and the control limits are recalculated and the charts are evaluated again.

The criteria used in the Laboratory were those designed to minimize both bias and variability.

C. Construction of Control Charts

A separate control chart is constructed for each analyte in each control pool. Construct each chart so that plots for X are arranged one above the other on the same sheet of graph paper. Draw the X line across the entire sheet; draw the warning- and control limits parallel to the X line.

Plot the run mean and range values. The chart should be kept current; the values should be plotted after each run. Make liberal use of annotations indicating events that might affect the analyses (personnel changes, reagent problems, changes in instrument components, etc.).

D. Use and Interpretation of Control Charts

Values for X which exceed the 3Sx limit indicate the run is ‘out-of-control’. The run must be repeated. Statistically, one in 100 runs can be expected to be ‘out-of- control during normal stable operation. A value exceeding the warning limit, but not the control limit, is interpreted

as an indication of possible trouble, but does not necessarily require action. Statistically, about one in 20 values will exceed the warning limits.

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:

- Repeat the analysis using fresh QC material.
- Perform a calibration.
- Check for system problems.
- Contact Roche Technical Support for assistance and possible service dispatch.

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

- Check reagent and calibrator for appropriate lot numbers, expiration dates, preparation
- processes 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. Contact Roche Technical Support for assistance and possible service dispatch.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

- Icterus index limit: 70
- Hemolysis index limit: 1000
- Ascorbic acid: Ammonium ions may cause erroneously elevated results.
- Drugs: No interference was found at therapeutic concentrations using common drug panels. Exceptions: Rifampicin, Levodopa, Dicynone (Etamsylate) 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 from creatine up to a concentration 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 IgM (Waldenstrom’s macroglobulinemia), may cause unreliable results.

13. REFERENCE RANGES (NORMAL VALUES)

Roche ranges:

- Urine, adult male, first morning void: 40-278 mg/dL
- Urine, adult female, first morning void: 29-226 mg/dL

14. CRITICAL CALL RESULTS ("PANIC VALUES")

None

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Samples are received frozen and stored at -70C until testing is performed. The samples are thawed at 37C and mixed before centrifuging. The samples remain at room temperature until testing is completed. After analysis and the QC repeats have been run, the specimens are refrozen. Upon completion of analysis, specimens are stored for 1 week. NHANES specimens are frozen at -70ºC and discarded or returned after 1 year.

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

Samples are held at -70C in the freezer. Should the testing system become inoperable, discontinue testing and notify the supervisor. If a problem occurs with the freezer, and this freezer begins to warm, samples are transferred to a different unit. A service call is placed to repair the refrigerator or freezer.

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

Results are downloaded from StarLIMS. See separate procedure “NHANES LIS Workflow” from University of Minnesota/ARDL for additional detail. The results spreadsheet is sent electronically by the ARDL LIS contact person to Westat.

There is no critical result for this procedure.

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

A. Specimen Receipt

Shipments for NHANES generally will arrive on Tuesdays and/or Wednesdays and Fridays. These shipments are recorded on the Log of Quality Assurance located in the ARDL Share Drive. 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 are placed in the designated -70C freezer and the refrigerated samples are placed in the designated 2-8C refrigerator until analysis. The manifests are filed in a binder labeled NHANES Shipping Manifests located in the receiving area. The shipping box is returned back to the study.

B. 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, and Biorepository receipt number. A screen shot example of the Quality Assurance Log is provided below:

QUALITY ASSUR SPECIMEN RECEIPT					# received, analyzed and transmitted			QUALITY ASSURANCE OF SPECIMEN RETURN								1 year	srisai
NHANES shipper I.D.	NHANES container I.D. #	vial #	date rec'd	receive spec. cond.	spec.	2.5% qc rpts	total	21-day due date	analyzed date	date result sent	# days results return	thaw date	return to freezer date	# days at frig temp	discard or return date	Biopharma receipt number
776968931579	439129	018	5/27/2022	ok	8	1	9	6/17/2022	5/27/2022	6/6/2022	10		5/31/2022	4	8/22/2023	C52-23R0015
776983196298	438272	018	5/31/2022	ok	25	1	26	6/21/2022	5/31/2022	6/6/2022	6		5/31/2022	0	8/22/2023	C52-23R0015
776987255984	439131	018	6/3/2022	ok	27	1	28	6/24/2022	6/3/2022	6/6/2022	3		6/7/2022	4	8/22/2023	C52-23R0015
777041297947	440008	018	6/7/2022	ok	47	1	48	6/28/2022	6/7/2022	6/8/2022	1		6/7/2022	0	8/22/2023	C52-23R0015
777042345191	439173	018	6/7/2022	ok	29	1	30	6/28/2022	6/7/2022	6/8/2022	1		6/7/2022	0	8/22/2023	C52-23R0015

C. Specimen Ordering and Preparation for Analysis

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 respective NHANES 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 8000 analyzer reads the bar-coded label for the sample ID and test information.

D. 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.

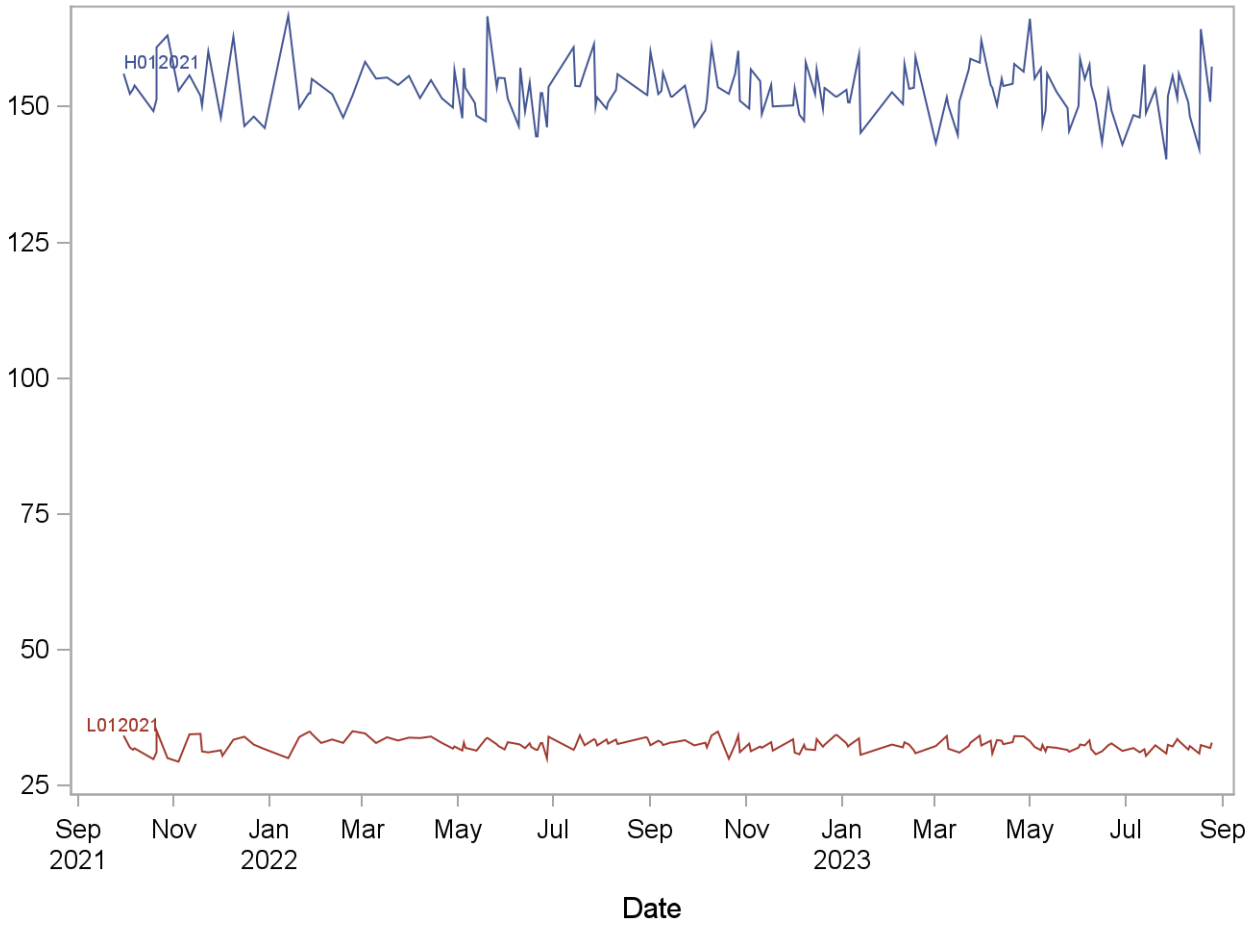
E. Specimen Handling and Return

On the day of analysis, the specimens are selected and thawed by the Cobas 8000 operator. The samples are thawed at 37C and mixed before centrifuging. The samples remain at room temperature until testing is completed. After analysis and the QC repeats have been run, the specimens are refrozen. After 1 year, the specimen vials that are designated for return are shipped to SriSai Biopharmaceuticals in Frederick, MD. These specimens are shipped on dry ice via FedEX

19. SUMMARY STATISTICS AND QC GRAPHS

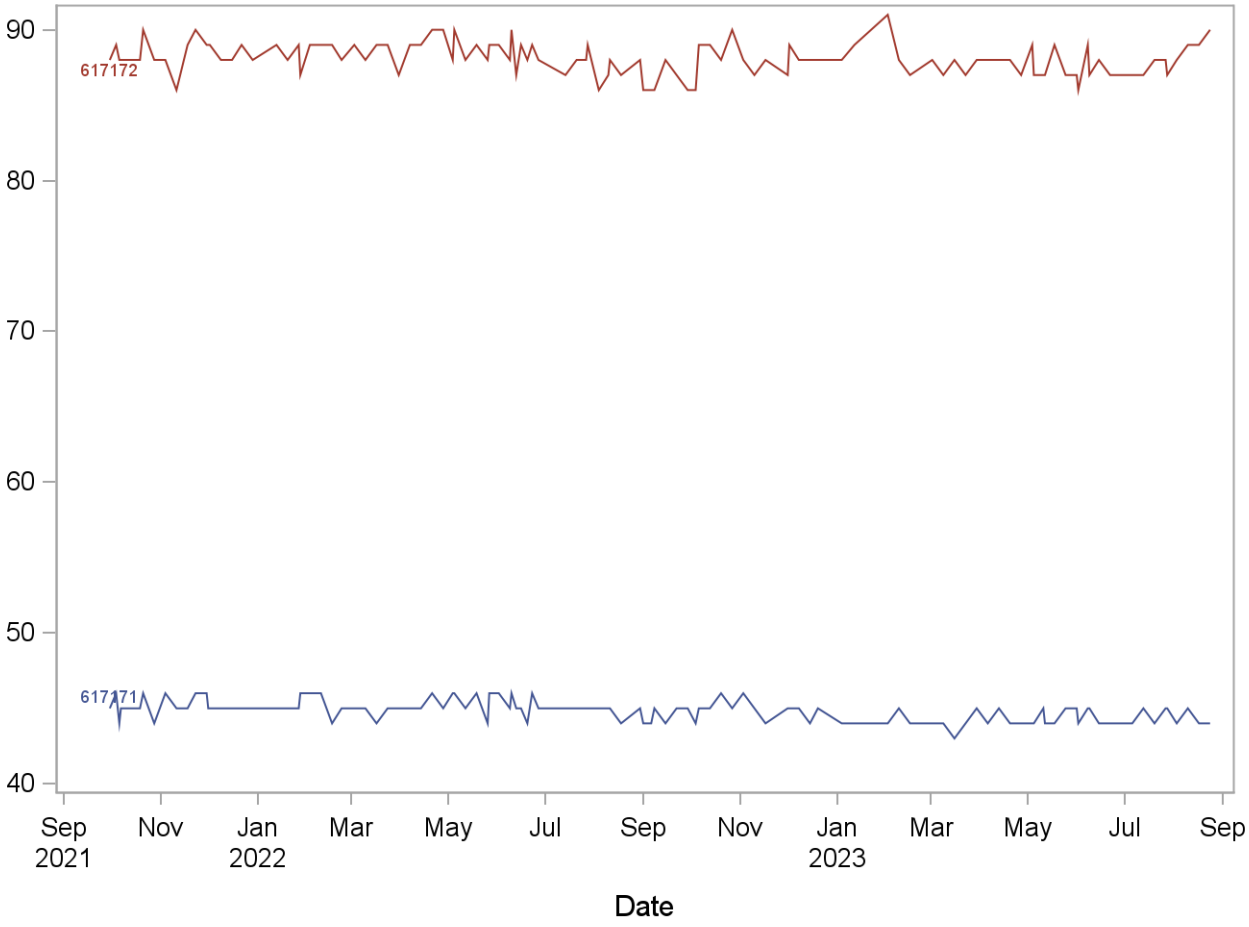
August 2021-August 2023 Summary Statistics and QC Chart
URXUMA (Albumin, urine (ug/mL))

Lot	N	Start Date	End Date	MEAN	Standard Deviation	Coefficient of Variation
H012021	170	30SEP21	25AUG23	152.920	4.706	3.1
L012021	170	30SEP21	25AUG23	32.492	1.163	3.6



August 2021-August 2023 Summary Statistics and QC Chart
URXUCR (Creatinine, urine (mg/dL))

Lot	N	Start Date	End Date	MEAN	Standard Deviation	Coefficient of Variation
617171	113	30SEP21	24AUG23	44.8	0.7	1.6
617172	113	30SEP21	24AUG23	88.1	1.1	1.2



REFERENCES

- Cobas 8000 Analyzer Operator's Manual. Roche Diagnostics Inc., 9115 Hague Road Indianapolis, IN 46250-0457. Version 5.3.

- Roche CREP2 reagent package insert. Version 2022-02, V16.0. Roche Diagnostics Inc., 9115 Hague Road Indianapolis, IN 46250-0457

- Roche Calibrator for Automated Systems (C.F.A.S.) package insert. Version 2020-03, V8.0. Roche Diagnostics Inc., 9115 Hague Road Indianapolis, IN 46250-0457.