

Laboratory Procedure Manual

<i>Analyte:</i>	Creatinine
<i>Matrix:</i>	Urine – Home Collection
<i>Method:</i>	Enzymatic Roche Cobas 6000 Analyzer
<u><i>Revised:</i></u>	
<i>As performed by:</i>	University of Minnesota Minneapolis, Minnesota
<i>Contact:</i>	Dr. John Eckfeldt, MD

Important Information for Users

The University of Minnesota periodically refines 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
HUKM_H_R	URHCRM	Creatinine, Urine AM (mg/dL)
	URHCRE	Creatinine, Urine PM (mg/dL)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Creatinine is produced by creatine and creatinine 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).

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 chromophore in the presence of peroxidase to produce a color 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.

2. SAFETY PRECAUTIONS

- a. Follow the Laboratory Safety and General Laboratory Practice regulations from the College of American Pathologists (CAP), the Clinical Laboratory Improvement Act (CLIA), and Occupational Safety and Health Administration (OSHA). Observe Universal Blood and Body Substance Technique (UBBST) and the Centers for Disease Control (MMWR 36;2S;1987) precautions for prevention of HIV transmission in the health care setting.
- b. Wear laboratory coats and disposable gloves when handling urine specimens. Cover the work surface with disposable, absorbent toweling. Place urine tubes, pipette tips, gloves, toweling, etc., and closed residual urine specimens into plastic bag and secure tightly. Urine can be discarded into the regular waste stream (sewer or trash). Clean the work surfaces with 0.5% bleach.
- c. Recommend to laboratory personnel performing the assay that they receive the HBV vaccine. Maintain records of vaccination or signed declination forms in the laboratory.
- d. Label all reagents indicating the preparation date, expiration date, formula, lot number if applicable, hazards of the reagent, antidote of contact with hazard, and the initials of the technician.
- e. Note the location of the Material Safety Data Sheets for the specified chemicals.
- f. Participate in Annual Safety Training and Laboratory-Specific Safety Tour noting the location of the chemical spill kit (a solid absorbent material), the location of fire extinguishers, alarms, and eye washes. Dial 911 for emergency.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

COMPUTERIZATION

Data is managed using Microsoft Excel software on a PC and the Laboratory Information Systems (L.I.S). The test is analyzed on a Roche/Hitachi Cobas 6000 Chemistry Analyzer. Results from the analyzer printout are manually entered into Excel spreadsheet and double-checked. Reflex testing is set up in the Cobas to order a repeat of any very low result, to verify abnormal values. Statistical evaluation of the runs is accomplished with Microsoft Excel software on a PC.

INFORMATION SECURITY SYSTEM

Computer firewall and password protection are established at startup. Files are automatically backed up nightly to prevent loss due to alteration or destruction. System passwords are used to limit computer access to authorized users. Access codes are never posted and must be memorized and are altered with changes in personnel. University Information Technology policies regarding privacy and security and e-mail are followed. The laboratory reports are verified against hardcopy before results are reported electronically. The laboratory personnel have received HIPPA training. The medical director has approved the content and format of computer-generated reports.

DATA SYSTEM MANAGEMENT

The integrity of the specimen data is established by routine verification of the transcribed information against the identification on the specimen tube and the hard copy that accompanies all specimens. Urine test results are verified and are returned by hard copy or electronic transmission as per study protocols. For the NHANES, results are returned via internet by File Transfer Protocol (ftp) onto formatted worksheets to Westat. The hard copies are organized in notebooks. Worksheets and results are archived indefinitely and located in the laboratory. All test values are linked to analytic batch and date of analysis, quality control, reagent lot numbers and expiration dates, and testing personnel.

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

SPECIMEN COLLECTION PROCEDURE

- a. Timed or random urine collections are obtained from study participants as per study protocols. Urine is collected in appropriate urine sample cups or 24-hour jugs. The laboratories determine urine quantity either by volume or by weight.
- b. Coordination of protocols is performed at the NHANES Mobile Examination Center (MEC): Random urine collections are collected at the MEC where 4 mL of urine is dispensed into vials and then shipped on dry ice by overnight carrier to the laboratory. For home urine collections (HUC) the participants are given a kit containing sample cups, ice packs, and Styrofoam mailer and are then directed to ship via overnight U.S Priority Mail to the laboratory. For the 24-Hour sample, participants are given a jug to bring home for collection, and are directed to return the jug to the MEC where the urine is dispensed into vials followed by shipping on dry ice by overnight carrier to the laboratory.
- c. There are no special instructions such as fasting or special diets requested of the participants. Protocols are designed to ensure consistent specimen collection procedures for variables such as exercise, time of day, water loading, and posture.
- d. The optimum specimen tube is a 3- to 5-mL screw-top cryogenic vial. Tubes are selected per study protocols and are labeled with the participant's ID and barcode.
- e. The optimum sample volume is 3 mL. The minimum acceptable volume is 1 mL.

SPECIMEN STORAGE PROCEDURE

- a. Urine specimens arrive frozen (on dry ice) or fresh (on ice packs) as per study protocols. Specimens arriving on dry ice are refrigerated to thaw upon arrival to the laboratory. Specimens can remain refrigerated up to 3 weeks until the completion of analysis.

- b. Storage of frozen specimens is located in Dr. Chavers' laboratory in room 13-126 of the Health Sciences Moos Tower at the University of Minnesota.
- c. Analyzed specimens are returned to frozen storage at -80 °C. According to study protocols, specimens are retained for a designated period, then either discarded or returned as described by protocol.
- d. Specimen stability at -20 °C for at least 1 year has been documented.

SPECIMEN HANDLING PROCEDURE

- a. Handle all urine specimens as if they are capable of transmitting any infectious agent.
- b. Record notation of unusual appearance such as blood, precipitate, or color.
- c. Return specimens to specified storage as soon as possible to avoid prolonged time at room temperature.
- d. For the Cobas analyzer, specimens may be direct-sampled in the original NHANES storage vial.
- e. Care must be taken to ensure that the vials are very thoroughly mixed prior to sampling. Mix by inverting the specimen tubes ten times. Mixing can cause bubble formation (which interferes with the Cobas 6000 sample detection system). Bubbles must be removed 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.
- f. For test procedures that require an acidified specimen (pH <3) the procedure is the following: to 3 mL of urine add 100uL of 6 mol/L HCl.

CRITERIA FOR SPECIMEN REJECTION

Corrupted specimen integrity; cracked or leaking tube, unreadable or missing label.

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. EQUIPMENT AND INSTRUMENTATION

1-Purified water supply. Millipore Elix Gulfstream Clinical System 35c. Millipore SAS 67120 Molsheim, France.

This water system provides a continuous supply of purified water to the Cobas 6000 analyzer. The water meets CLSI Clinical Laboratory Reagent Water (CLRW) standards. Water purification is achieved by reverse osmosis, electrodeionization, bactericidal 254nm UV lamp, and 0.22µm filtration.

2-Roche/Hitachi Cobas 6000 Analyzer. Roche Diagnostics, 9115 Hague Road, P.O. Box 50457, Indianapolis, IN, USA 46250-0446.

This analyzer is a fully automated, random-access, software-controlled system intended for quantitative and qualitative *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 determinations on the c501 module and electrochemiluminescence signal in the immunoassay analysis module, e601. The c501 module is used for this test procedure. Samples for ISE determination and photometric measurement can be directed by the user or an interfaced order. Each ISE specimen is pre-diluted by the instrument in one of 160 reaction cells. The diluted specimen is then measured in the ISE measuring system. 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 ISE and photometric reactions, the cell rinse unit washes the reaction cell, and the cell is re-used. All analyses occur at 37°C.

B. MATERIALS AND REAGENTS

Purified water supply materials:

1. Progard TL Pretreatment Pack
2. Reverse Osmosis Permeate Divert Solenoid Valve
3. strainer
4. 254 nm UV Lamp
5. Q-Gard TL Polisher Pack
6. PrePak L1 Pretreatment Pak
7. Automatic Sanitization Module

Reagents for testing creatinine:

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

1. R1 reagent. TAPS buffer (N-Tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid): 30 mmol/L, pH 8.1; creatinase (microorganisms): $\geq 332 \mu\text{kat/L}$; sarcosine oxidase (microorganisms): $\geq 132 \mu\text{kat/L}$; ascorbate oxidase (microorganisms): $\geq 33 \mu\text{kat/L}$; catalase (microorganisms): $\geq 1.67 \mu\text{kat/L}$; HTIB: 1.2 g/L; detergents; preservative.
2. R2 reagent. TAPS buffer 50 mmol/L, pH 8.0; creatininase (microorganisms): $\geq 498 \mu\text{kat/L}$; peroxidase (horseradish): $\geq 16.6 \mu\text{kat/L}$; 4-aminophenazone 0.5 g/L; potassium hexacyanoferrate (II): 60 mg/L; detergent; preservative.

**Storage and stability. Keep reagents stored at room temperature until use. The reagents are stable for 8 weeks refrigerated on the analyzer.*

Cobas 6000 analyzer materials and reagents:

1. Cell Wash Solution II/Acid Wash. Roche product #4880307 (2L bottle). No preparation required. Solution of formic acid, citric acid and nikkol BT-9. Store at room temperature. Stable until expiration date on bottle, the on-board stability is 12 weeks after opening. This solution is automatically drawn by the Cobas 6000 while cleaning reaction cuvettes during analysis.
2. Cell Wash Solution I/NaOH-D. Roche product #4880285 (1800 mL bottle). No preparation required. Solution of sodium hydroxide (1N). Store at room temperature. Stable until expiration date on bottle, the on-board bottle stability is 10 weeks after opening. This solution is automatically drawn by the Cobas 6000 while cleaning reaction cuvettes during analysis.
3. 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.

4. 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. Stable until expiration date on bottle, the on-board stability is 12 days after opening. Hitergent is an on-board reagent automatically drawn by the Cobas 6000 during the daily incubator bath exchange.
5. ProCellM. Roche Product # 04880340 (2 L bottle). No preparation required. Solution of Tripropylamine (TPA) and Oxaban A. Store at room temperature. Stable until expiration date on bottle, the on-board stability is 5 days. This is a buffer solution that is used for conditioning the electrodes, transporting the assay reaction mixture, washing the streptavidin-coated microbeads and signal generation. ProcellM is automatically drawn by the Cobas 6000 during analysis.
6. CleanCellM. Roche Product #04880293 (2L bottle). No preparation required. Solution of Potassium Hydroxide and Polidocanol. Store at room temperature. Stable until expiration date on bottle, the on-board stability is 5 days. The solution is automatically drawn by the Cobas 6000 to clean the measuring channel after each measurement and conditioning the electrodes.
7. PreCleanM. Roche Product #03004899 (600 mL bottle). No preparation required. Solution of Polidocanol and OxabanA. Store at room temperature. Stable until expiration date on bottle, the on-board stability is 4 weeks. This is a phosphate buffer that is used to wash and resuspend microbeads during the pre-wash step. PreCleanM is automatically drawn by the Cobas 6000 during analysis.
8. ProbeWashM. Roche Product #3005712 (70 mL bottle). No preparation required. Solution of Polidocanol and Potassium Hydroxide. Store at room temperature. Stable until expiration date on bottle, the on-board stability is 4 weeks. The solution is used to clean the reagent probe during special wash steps and at the end of the run.
9. 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.
10. Sample cups (micro). Roche product #05085713.
11. Sample cups (standard). Roche product #10394246.
12. Printer paper, 8.5 x 11 inch. Various sources including Bose Multipurpose Paper.
13. Printer cartridge. HP Laserjet CE505A, black. Hewlett-Packard Company.
14. Reagents and calibrators. See specific assay procedures.
15. 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.

C. CALIBRATORS (STANDARDS)

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 4°C. The lyophilized calibrator is prepared with 3.0mL 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 4°C, and one month at -20°C (frozen once).

D. CONTROL MATERIALS

Normal pooled urine control is selected for two distinct levels of concentration. Prepare aliquots of the pools and store frozen at -80 °C. This is 'QC Level-1' and 'QC Level-2'. It is 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.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

Traceability: This method has been standardized against SRM 967 (IDMS).

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

Monitor control values: to determine the stability of the current calibration.

Manual calibration should be performed if:

- A reagent lot change has not occurred in the past 6 month
- After major service or repairs
- As needed for troubleshooting

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.

Acceptable accuracy and precision: For each chemistry test, the limits are defined in the application parameter file on the Cobas analyzer.

Validation of the analytical measurement range (amr): is performed every 6 months and after major maintenance or service.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

Further instructions are available from the following:
Cobas 6000 Application Code: 452 (used for creatinine)
Cobas 6000 General Operating Procedure: COBAS 690.1
Cobas 6000 Analyzer Series Operator's Manual Version 5.

OPERATING INSTRUCTIONS: purified water system

The water is filtered from the reservoir through the inlet solenoid valve to the Progard TL Pretreatment Pack. It then passes through the Reverse Osmosis Permeate Divert Solenoid Valve, a strainer, and a 254 nm UV Lamp. The water also passes through the Q-Gard TL Polisher Pack and PrePak L1 Pretreatment Pak. The water system should be checked daily to indicate the distribution is ≥ 10 mg. Every three months the RL membrane must be cleaned, and every four months the Progard TL Pretreatment Pack, Q-Gard TL Polisher Pack, and PrePak L1 Pretreatment Pak require replacement. The Automatic Sanitization Module requires replacement after two years. Request service as needed.

OPERATING INSTRUCTIONS: Cobas 6000 analyzer

Module c501 overview

The c501 module is utilized for this test procedure. This module is for workloads using a combination of photometric and ion-selective electrode determinations. The instrument utilizes reusable optically pure plastic reaction cells that are changed on a monthly basis. The reaction cells are automatically washed by the instrument after completion of the 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, 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. Non-Roche assays use a generic Multi Cassette, and the operator assigns the appropriate test to these cassettes as they are loaded. The Cobas 6000 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.

Ordering Tests

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.

Test Entry for creatinine:

STARLIMS test code: CR, UCR

Manual entry.

- The Cobas prints results to two decimal places (as x.xx) in mg/dL.
- The Cobas prints low results as <1.10 mg/dL for urine.
- Check results for error flags and take appropriate corrective action.
- Investigate alert values and delta checks.

Specimen Set-up:

Prepare urine specimens for testing as described in Sample Handling.

Instrument Set-Up:

Perform all scheduled instrument maintenance before beginning testing. The Cobas 6000 has scheduled daily, weekly, bi-monthly, monthly, quarterly and as-needed maintenance. These tasks are listed in the check-off chart at the instrument, and described in detail in Section C of the Operator's Manual, Version5.

The Cobas 6000 is programmed to re-boot every weekday morning at 0500, and Monday at 0400. It does not automatically reboot on weekends. If the Cobas 6000 needs to be turned on prior to its pre-programmed start time, follow the screen prompts to activate the system. In this case a Daily Pipe must be manually ordered.

Calibration Set-up:

There are a variety of calibration models used on the Cobas 6000. 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 6000 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.

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. All methods do not 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 6000. Acceptable accuracy and precision limits are defined in each chemistry parameter file.

Loading Calibrators and Controls:

For the c501 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 6000 computer. The assigned locations are also marked on the calibrator and quality control racks. Either a standard sample cup or micro sample cup is acceptable to hold the calibrators and quality control material. Fill the sample cup with enough volume to complete the full calibration sequence (this will vary by method; some multi-point 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 6000 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 make sure the vial is seated far enough down so it is not jarred from the rack during transport on the Cobas 6000. Alternatively, a 2 mL Sartstedt vial can also be placed in a 13 x 75 mm plastic support tube.

Requesting a Calibration: (Operator’s Manual Section B, Book 1) 0

1. At the home screen, click or touch screen on <Calibration> tab.
2. Click or touch screen on <Status>. A list of all the Cobas 6000 tests appears. If more than one bottle set of reagents is on-board, a separate listing will appear for each set.
3. Click or touch screen on the tests to be calibrated.
4. In the “Method” box on the right side of the screen, select (click) 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 6000 Applications folder. Most c501 methods utilize a two-point calibration, while all e601 methods utilize a full calibration. Generally, if a screen button is white, that means it is active/available. Yellow 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. In these cases just highlight the test, skip the Method box, and touch or click on <Save>.
6. Click or touch screen on <Save>.
7. Failed calibrations will generate an error message by the Cobas 6000.

For the c501 module, the two most common flags found in a failed calibration are SENS and DUP.

- a. SENS (sensitivity error) occurs when the difference in absorbance between the zero standard and measuring standard does not fall within a method-specific, defined range. Typically, the absorbance difference is too small, and this usually indicates a deteriorated reagent. Replace the reagent cassette, and repeat the calibration.
- b. All calibrators are assayed in duplicate. DUP (duplication error) occurs when the pair of measurements at the zero or measuring point does not agree satisfactorily with each other. In this case simply repeat the calibration. If the error occurs again, consider sources of imprecision (sample probe, syringe leakage, bubbles in reagent, etc.).

Loading Reagents: (Operator's Manual for Module c501 Section A-60)

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 6000. You can sort by the c501 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 tests remaining and stability by bottle, are shown in the window on the right side of the screen.

The principle reagent containers for the c501 modules are reagent packs that contain up to three reagent vials. Reagent packs are stored in a refrigerated reagent compartment on the c501 that stores 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 bottles may be placed on the instrument. If an automatic calibration is not required on bottle change, or if the new bottle set was calibrated when it was placed on the instrument, then the Cobas 6000 will automatically begin pipetting from the new bottle once the previous bottle is empty.

Roche-provided reagent packs have a two-dimension, 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 c501, 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.

Loading Specimens: (Operator's Manual Section B-52)

The Cobas is able to test specimens from many different sources, in many different containers, and provide 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 to order testing on the analyzer. The following instructions attempt to cover these scenarios, but there may be nuances to certain studies and their associated testing schemes that the user must be aware of.

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.

Manual test ordering:

1. If the original vial is not compatible with the Cobas sampling system, transfer the specimen from its original labeled container to a hand-labeled sample cup.
2. Place the cup into the next available sample position in a gray, 5-place sample rack.
3. Enter the desired demographics for the specimen:
 - a. <Workplace>
 - b. <Test Selection>
 - c. In <Type> field select the specimen type (Ser/Pl or Urine) from the dropdown menu.
 - d. Enter the specimen ID number in the Sample ID field. <Enter>
 - e. Click or touch screen on the desired tests.
 - f. To assign a testing position for the vial, click <Barcode Read Error>.
 - g. Enter Rack No. and Position in the corresponding fields. Note that position 1 on each rack is on the right end of the rack. The gray racks are defined for serum testing; the yellow racks are defined for urine testing.
 - h. <Add>.
 - i. <OK>.
 - j. <Save>.
4. Continue this process until 10 specimens (two racks) have been ordered. Additional specimen loading can occur while the instrument is running.
5. If there is adequate reagent onboard and the necessary calibrations and controls have been successfully completed, load the sample racks onto the instrument. If the access light is green on the left side of the loading area, lift the lid of the sample loading compartment, remove the tray, and place the racks onto the tray. Since the tray (and the slot in the racks) is offset, there is only one way to load them onto the instrument.

6. After the racks are loaded, return the tray to the loading stations, close the lid and click <Start>.
7. The Start Conditions window appears. Click the big <Start> to begin the run.
8. The other options on the Start screen have uses described in the Operator's Manual. The primary field of interest is the Masking field. If any tests are to be turned off, click on Masking, then highlight the tests. Click on T-Mask to turn the test off, or click on P-Mask to turn the test off for patient testing but to allow calibration and quality control.
9. The sample rack arm will move from a vertical position to horizontal, and sweep the sample racks into the barcode scanning station. From there, the racks enter the holding carousel prior to sampling on the c501 or e601.
10. After this process has begun, additional tests may be ordered by returning to the Test Selection screen. Tests may be added anytime, but the Cobas 6000 will not allow the Start button to be activated if it is flashing. This delay occurs after the last sample rack has entered the sampling chamber. It usually lasts ~ 1 minute, unless there is a backup in the holding carousel.

Results:

Control and calibration results will automatically print out on the remote printer connected to the Cobas 6000. Patient result printouts must be requested on the Cobas 6000: <Workplace>, <Data Review>, highlight desired records, <Print>, <Print>.

Detailed STARLIMS instructions may be found in specific STARLIMS protocols, but the general process for automated entry is thus:

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 <Release 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.

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 6000 is ready for activation of the Sleep Pipe. First, load the designated green rack as follows, using standard sample cups, half-filled:

Pos 1: MultiClean

Pos 2: Sys Clean

Pos 3: Leftover serum-based control material

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.

The instrument should be placed in Sleep mode when not in use for long periods (e.g. overnight). This prolongs the lamp life and other essential components. When in sleep mode the reagent compartment remains refrigerated, but most other functions are turned off.

CALCULATIONS AND INTERPRETATIONS OF RESULTS

Dilutions

The confirmed analytical measurement range of the assay is **1.10-516 mg/dL** (urine). 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 μ L of the patient sample into 200 μ L 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.

Assay Performance

Analytical Measurement Range	1.10-516 mg/dL urine
Reportable Range	5-1290 mg/dL urine
Limit of Detection (standard 1 + 3SD)	1.10 mg/dL urine
Intra-assay %CV (10 within-day replicates at a urine concentration of 87.mg/dL)	0.4% urine
Inter-assay %CV (18 between-day replicates at a urine concentration of 86 mg/dL)	2.0% urine
Inter-assay %CV (20 between-day replicates at a urine concentration of 17 mg/dL)	3.2 % urine

9. REPORTABLE RANGE OF RESULTS

5-1290 mg/dL, urine creatinine.

For the NHANES, the reportable range is ≥ 5 mg/dL. Results are reported with zero decimal places (whole numbers) in mg/dL. The lower limit is 5. There is no upper limit, as a specimen can be diluted to obtain a result.

10. QUALITY CONTROL (QC) PROCEDURES

Two levels of the normal pooled urine control. They are analyzed at the start of the day and the 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.

Westgard Rules are followed. Determine that the 2-2's and/or 1-3's rules are not violated.

External Quality Assessment (EQA) is by CAP linearity testing (LN2) and CAP proficiency testing.

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 sequence 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 service as needed.

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

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

Remedial action for out of control conditions:

- [1] Includes examination of the pipetting and detection equipment and examination of reagent materials.
- [2] The QC parameters are compared to the patient means to look for confirmatory or disconfirmatory evidence.
- [3] When the 2-2's and/or 1-3's rules are violated.
- [4] Re-test all samples from analysis runs which have quality control results that are not within the established tolerance limits.
- [5] Results are not released from rejected runs.
- [6] Should the testing system become inoperable, the course of action to take is to, discontinue testing and notify the supervisor.
- [7] Notify NHANES if the problem causes reporting delays

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

- Icteric index <15: no interference.
- Hemolytic index < 800: not 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 $\geq 1\text{mmol/L}$ ($\geq 115\text{ mg/L}$) 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 $\geq 600\text{ mg/dL}$ 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)

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")

Not applicable to this procedure.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Store specimens at 2-8 °C until analysis. Specimens are at room temperature during analysis. Complete testing within 36 hours of receipt in the laboratory.

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

If the analytical system fails, the specimens should be refrigerated at 2-8 °C until the analytical system is restored. If long-term interruption is anticipated, specimens are refrozen at -80 °C.

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

Test results are reported to the collaborating agency at a frequency and by a method determined by the study coordinator. Generally, data from these analyses are compiled and sent to the responsible person at the collaborating agency as an Excel file, either through Internet FTP transfer of files or electronic mail.

Critical call reporting is not applicable to this procedure.

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

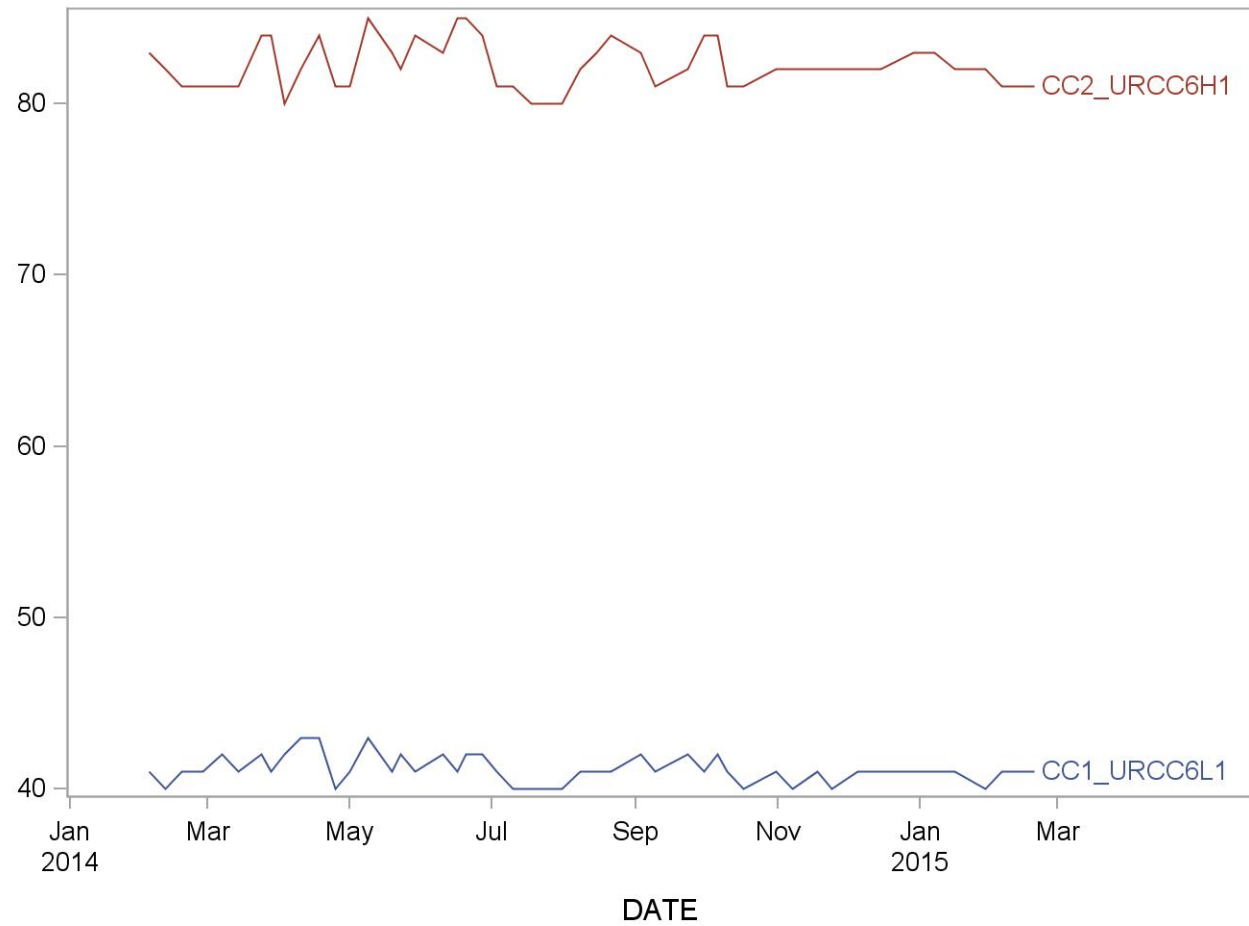
Standard record-keeping means (e.g., electronic, data files, laboratory notebook) are used to track specimens. Records are maintained indefinitely. Specimens are

retained at the laboratory for at least one year. Only numerical identifiers are used. All personal identifiers are kept masked and available only to the project coordinator in order to safeguard confidentiality.

19. SUMMARY STATISTICS AND QC GRAPHS
See following pages.

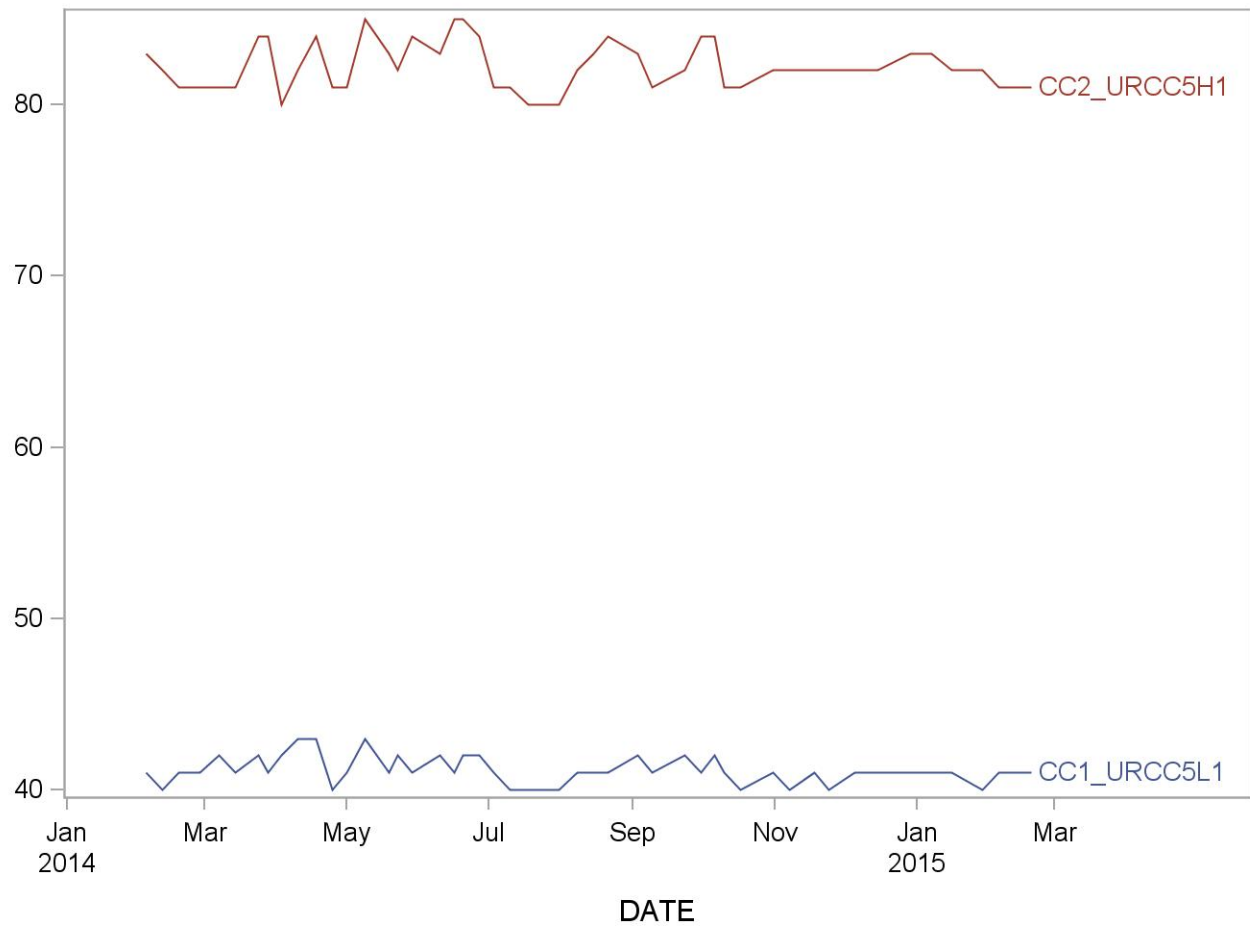
2014 Summary Statistics and QC Chart for Creatinine, Urine AM Collection (mg/dL)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
CC2_URCC6H1	48	04FEB14	19FEB15	82.3	1.4	1.7
CC1_URCC6L1	48	04FEB14	19FEB15	41.1	0.8	1.9



2014 Summary Statistics and QC Chart for Creatinine, Urine PM Collection (mg/dL)

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REFERENCES

1. Cobas 6000 Analyzer Operator's Manual. Roche Diagnostics Inc., 9115 Hague Road Indianapolis, IN 46250-0457. Version 5.0.
2. Roche CREP2 reagent package insert. Version 2012-02, V10. Roche Diagnostics Inc., 9115 Hague Road Indianapolis, IN 46250-0457.
3. 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.