Laboratory Procedure Manual

Analyte: Urinary Creatinine

Matrix: Urine

Method: Enzymatic by Roche/Hitachi Modular P Chemistry Analyzer

Method No.: 

Revised: January 2011

as performed by: University of Minnesota

Contact: Dr. Michael Steffes

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:

<table>
<thead>
<tr>
<th>File Name</th>
<th>Variable Name</th>
<th>SAS Label (and SI units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALB_CR_G</td>
<td>URXUCR</td>
<td>Urinary Creatinine (mg/dL)</td>
</tr>
<tr>
<td></td>
<td>URXUCRSI</td>
<td>Urinary Creatinine (umol/L)</td>
</tr>
</tbody>
</table>
1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

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

Creatinine is produced by creatine and creatine phosphate as a result of muscle metabolic processes. Creatinine is the waste product derived from muscle creatinine and is released into the blood at a relatively constant rate. It is then excreted by glomerular filtration during normal renal function. The amount of creatinine per unit of muscle mass is constant; therefore, increased blood creatinine is the best indicator of impaired kidney 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). The ratio of urine albumin to urine creatinine is used to predict nephropathy risk in diabetic patients. (For the NHANES IV survey, urine creatinine is used as a reference analyte against which are measured other urine analytes, such as pesticides and heavy metals, and urine albumin/creatinine ratio).

2. SAFETY PRECAUTIONS

A. Follow all procedures and policies in the FUMC Laboratory Safety Manual, including the Universal Blood and Body Substance Technique (UBBST). Consider all specimens received for analysis potentially positive for infectious agents.

B. Wear gloves, lab coat, and safety glasses while handling all specimens. Dispose analyzed specimens and contaminated supplies in autoclave/biohazard bags; seal and autoclave. Wipe all work surfaces with disinfectant solution.

C. Recommend to laboratory personnel performing the assay that they receive the HBV vaccine. Maintain records of vaccination or signed declination forms.

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 (MSDS) for sodium hypochlorite, and ethanol: room UH3-555 CC1 paperwork area.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

A. Microsoft Excel software on a PC and our Laboratory Information Systems (L.I.S.) are used to manage the data. The test is analyzed on a Roche/Hitachi Modular P (ModP) Chemistry Analyzer.

B. Results are manually entered into spreadsheet and double-checked.

C. Reflex testing is set up in the ModP to order a repeat of any very low result, to verify abnormal values.

D. Statistical evaluation of the runs is accomplished with Microsoft Excel software on a PC.

E. The run numbers, and date specimens were received are entered into the Excel file. The Excel spreadsheet results file data are copied to the shipment Excel file and sent using Internet FTP transfer of files or e-mailed to Westat within 21 days of sample receipt.

F. The Excel files containing all raw data and results are backed up daily using a CD writer or External drive for storage. Files stored on the L.I.S. network are automatically backed up nightly to tape.
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Documentation for data system maintenance is contained in printed copies of data records, as well as in "system log" files on the local hard drives used for the archival of data.

G. The integrity of specimen identification is maintained through a two-step verification process. A collection list and specimens in numbered analyzer cups that corresponds to NHANES IV specimen identification is obtained from the Microalbumin Laboratory. This corresponding specimen number is read via barcode in the ModP. The ModP printout results are entered manually onto the collection list. A technologist reviews result data prior to result verification and release.

H. NHANES IV results are available to the Microalbumin Laboratory within 36 hours via hard copy. The electronic transmission of results to NHANES IV is the responsibility of the Microalbumin Laboratory. The data is stored on the computer drive, organized in notebooks, and located in the Microalbumin Laboratory.

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

A. Specimen Collection Procedure
   1) Timed or random urine collections are obtained from study subjects as per study protocols. Urine is collected without added preservatives.
   2) No special instructions such as fasting or special diets are requested.
   3) The optimum specimen volume is 1 mL, and the minimum acceptable volume is 100 uL (includes dead volume). Urine is stable for four days at 4°C, and longer at -20°C.
   4) For the NHANES IV survey 250 uL or urine in 0.5 mL capped analyzer sample cups is received from the Microalbumin Laboratory for creatinine analysis. A collection list accompanies all specimens.

B. Specimen Storage Procedure
   1) Store aliquots in analyzer sample cups at 2-8°C until analysis.
   2) Complete analyses within 36 hours of receipt.
   3) Discard specimens after analyses.

C. Specimen Handling Procedure
   1) Handle all urine specimens as if they are capable of transmitting any infectious agent.
   2) Record notation of unusual appearance such as blood, precipitate, or color prior to analysis.

D. Criteria for Specimen Rejection
   1) Corrupted specimen container integrity; cracked or leaking tube, unreadable or missing label.
   2) Note: Angiogram/IVP dyes do not interfere with analysis.
   3) Note: Acidified urine specimens are acceptable.

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. Instrumentation: Roche Modular P Chemistry Analyzer. Roche Diagnostics, 9115 Hague Road, Indianapolis, IN 46250

B. Materials:
   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. No stability time window after opening. This solution is automatically drawn by the Mod P while cleaning reaction cuvettes during analysis.
   2) Cell Wash Solution I/NaOH-D. Roche product #1551540 (1800 mL bottle). No preparation required. Solution of sodium hydroxide (1N). Store at room temperature. Stable until
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expiration date on bottle. After opening a bottle it is stable for 14 days on the instrument. This solution is automatically drawn by the Mod P while cleaning reaction cuvettes during analysis.

3) Reaction cell cuvette segments. Roche product #714-0650 (Four sets of eight segments. Eight segments complete the entire rotor). Soak cuvettes overnight in a solution of 2% Hitergent before installing on the instrument. Perform cell wash and cell blank functions after installation. Change cuvettes quarterly.

4) Hitergent. Roche product #409149 (1L bottle). 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. Hitergent is an on-board reagent automatically drawn by the Mod P during the daily incubator bath exchange. Hitergent is transferred, as needed, from the 1L bottle to the 66 mL bottle located in position 2D3.

5) Sample cups (micro). Roche product #11406680001.

6) Sample cups (standard). Roche product #729177.

C. Reagent Preparation: Roche product #1775685, CREA plus reagent kit:
1) R1 reagent (6 x 64 mL). TAPS buffer, pH 8.1, HTIB (chromophore), creatininase, sarcosine oxidase, ascorbate oxidase. See insert for concentrations. No preparation required. There are approximately 350 tests per bottle.

2) R2 reagent (6 x 35 mL). TAPS buffer, pH 8.0, creatininase, peroxidase, 4-aminophenazone, potassium hexacyanoferrate. See insert for concentrations. No preparation required. There are approximately 380 tests per bottle.

3) Storage and stability. Keep reagents stored in refrigerator until use. R1 is stable for 28 days refrigerated on the analyzer. R2 is stable for 28 days refrigerated on the analyzer.

4) Though the number of tests per bottle is slightly different, always change the reagents as a pair. When loading the reagents onto the Mod P, make sure R1 is placed in the R1 rotor, and R2 is placed in the R2 rotor. Remove any bubbles in the reagents prior to loading. Place the reagents in like-numbered locations in the two rotors. This makes it easier to track the chronology of the reagents on the instrument.

5) Milli-Q water. Milli-Q is the trade name of the water system purchase from the Millipore Corporation. Milli-Q is deionized water treated with activated carbon and deionization cartridges and filtered to remove microorganisms larger than 0.22 micrometers. This meets CAP class I water requirements.

D. Calibrators: Roche Calibrator for Automated Systems (C.F.A.S.), catalog #759350.
1) The calibrator is stable until the expiration date on the bottle when stored at 4ºC. The lyophilized calibrator is prepared with 3.0 mL of Milli-Q water. Volumetrically add the water, and then dissolve by gentle swirling within 30 minutes. Avoid formation of foam while mixing. The prepared calibrator is stable for eight hours at room temperature, two days at 4ºC, and one month at –20ºC (frozen once).

2) The C.F.A.S. calibrator is traceable to reference material SRM 909b (Isotope Dilution Mass Spectroscopy–IDMS). This is a reference material provided by the National Institute of Standards and Technology. This traceability means that this creatinine method yields results that are routinely lower (5-10%) than those creatinine methods using a “traditional” calibrator.

E. Controls:
1) Four levels of commercially prepared control material are used for quality control. The approximate creatinine concentrations of these four levels are 1, 5, 20, and 100 mg/dL.

2) Quality control material for concentrations 1 and 5 mg/dL are serum control: Normal donor serum pool control and Roche Precipath U plus control, respectively. Follow the instructions in the package insert. Thaw one vial, mix, and store the material at 2–8ºC for 3 days. Prepare Precipath U with 3ml diluent.

3) Quality control material for 20 and 100 mg/dL are urine control: Pooled urine control (centrifuged and filtered), straight and 1:5 dilution prepared in saline. Undiluted vials are stored at -70 ºC. A fresh vial is thawed daily.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

A. Calibration frequency:
1) The Mod P will automatically perform a two-point calibration when there is a reagent lot number change or a reagent bottle set change. It will also perform a two-point calibration every seven days thereafter.

2) The Mod P will not allow testing to proceed until a successful calibration has been completed. Monitor control values to determine stability of the current calibration.

3) After calibration and controls have been measured and evaluated, the test specimens may be loaded onto the Mod P. An abbreviated description of the measurement procedure follows. A more thorough description may be found in the Mod P general operations protocol.

**Caution:** This product is of human and animal origin. Handle as though capable of transmitting infectious disease.

8. **PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS**

**A. Instrument Setup:**

1) Log into the Mod P using assigned username and password.

2) Reagents. All reagents used on the Mod P are stored in a refrigerated reagent compartment. Enzymatic creatinine is a two-reagent system. Reagent 1 must be placed in the outer (R1) rotor; reagent 2 must be placed in the inner (R2) rotor. All reagents have a unique barcoded identifier. Before starting the analysis sequence check the reagent status on the Mod P to confirm there is adequate reagent to complete the anticipated test volume for the day. Discard any bottles that have gone empty. Check the volume of the two wash reagents.

3) Maintenance. Complete the scheduled daily maintenance as described in the Mod P general operations protocol.

4) Order calibration, if indicated (see Mod P general operations protocol).

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

**B. Sample Preparation:**

1) If specimens have been frozen, allow them to thaw completely, and then mix well. Centrifuge urines for 10 minutes at 1,500 x g to remove particulate matter.

**C. Recording of Data:**

1) To order non-barcoded tests on the Mod P:
   a. <Workplace>
   b. <Test Selection>
   c. Enter specimen ID in the Sample ID field, then <Enter>
   d. Select test CR-EZ by touching the screen or clicking on it with the mouse.
   e. <Barcode Read Error>
   f. Enter the rack number and rack position in the Rack No.-Pos. fields.
   g. <Add>
   h. <OK>
   i. <Save>

2) Note the order of positions 1-5 in the sample rack: position 1 is on the right and position 5 is on the left. Place the specimen in the rack so that ¼ to 1 inch of the vial is above the sample rack. This allows the Mod P to detect the presence of the vial in the rack. Orient the vial in the rack so that any barcodes are turned inward, and therefore unreadable. If the testing vials are to be re-capped, arrange the caps so they can be matched up following analysis.

3) To order barcoded tests on the Mod P: Follow instructions as in step 3 above, except that the barcode now must face outward so the Mod P can read it. The barcode must be oriented vertically. No test ordering is required on the instrument. In this case test ordering has occurred in Misys, and a label has been generated for that purpose, or the specimen has a non-Misys barcode label and a user-defined default battery has been installed on the Mod P.

4) After the specimens are in place, put the racks onto the loading platform. The racks will only load in one orientation, as the center track is offset. Do not prepare more than three racks at a time, as evaporation could occur while the instrument goes through the sampling process.

5) Close the cover on the loading platform.

6) On the Mod P computer terminal, press or click <Start>, then <Start> again.
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7) Only calibration and control data automatically print out. Patient data hard copies must be requested in <Workplace>, <Data Review>. Highlight the desired records, then <Print>, and <Print> again.
8) Non-barcoded records must be manually entered into Misys, a designated spreadsheet, or website.
9) Barcoded records are accepted using the OEM program in Misys. The method code for the Mod P is UR9.

D. Operation:

1) Refer to ModP general operations protocol for programming controls/patients.

E. Recording of Data:

1) Operator will review and verify results in the L.I.S.
2) The L.I.S. reorders tests to verify any critical results. These results are stored in the L.I.S. along with the original results. Original values are used when repeat results match the original within 3 CV’s.
3) Project supervisor will export data from the L.I.S. into an Excel file. The data is copied in into another Excel file for further evaluation.
4) An Excel spreadsheet printout of the results for each container ID is made and comments noted.
5) Project supervisor reviews the results. If problems noted with results or QC, Project Supervisor investigates and discusses issues if necessary with Laboratory Director. Repeat samples if necessary.
6) Daily log sheets are completed and any problems or issues noted.

E. Instrument Shutdown

1) After the patient specimens and final controls have been evaluated and accepted, load the green rack (W999) and run it through the instrument. Place three standard sample cups in positions 1, 2 and 3. Fill cup 1 with 1N sodium hydroxide, fill cup 2 with 4N sodium hydroxide, and fill cup 3 with leftover serum. Place it onto the loading platform and press <Start>, and <Start> again. After 18 minutes, the Mod P comes to Stand-by status. If the green rack is not run, the Mod P will take at least one hour to come to Stand-by status.
2) After coming to Stand-by status the data from each day’s run is downloaded from the Mod P computer to a diskette, then to the network folder. Consult the procedure describing this process for details.
3) Print all Mod P test results, and file in chronological order with the other daily printouts.
4) The Mod P is turned off each day after all work is complete. The steps are as follows: <Utility>, <Maintenance>, <Nightly Pipe>, <Select>, <Execute>. This shutdown process requires approximately five minutes. The instrument and its computer are automatically turned off. The reagent compartment remains refrigerated.
5) An automatic timer has been set so that the Mod P turns on each weekday morning at 0630, automatically performing an air purge, photometer check, and incubator bath exchange during the process. The automatic timer has been set so that the Mod P remains off during weekends.
6) Return all leftover controls and calibrators to the refrigerator at the end of the day.

9. REPORTABLE RANGE OF RESULTS

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

B. Linear range of the method: 0-600 mg/dL (urine). Specimens exceeding the high limit are automatically diluted (1:2) by the instrument, and reported accordingly. If a manual dilution is required, dilute the specimen in normal saline, and multiply the result by the dilution factor.

C. Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence.
D. 0 is not a reportable value. The lower limit of detection for the assay is 1 mg/dL; there is no upper limit of detection. Therefore, the "reportable range of results" is "1 mg/dL and up" or "> 1 mg/dL". The urine creatinine test result is reported in mg/dL to the nearest whole number.

10. QUALITY CONTROL (QC) PROCEDURES

A. Two levels of control are assayed each time the creatinine method is performed. It is acceptable to run each control at the start of the day, and again at the end of the day. The operator may run them more frequently, if desired. One control is prepared from pooled, normal human serum. The other is an elevated, abnormal commercial control. The urine control is prepared from pooled human urine. Consult quality control charts for current ranges and lots in use.

B. In November 2006 this method, calibrated with Roche C.F.A.S. calibrator was evaluated by assaying NIST SRM 967 Level 1 and Level 2. These two standards have values assigned by IDMS measurement at NIST. They are reputed to have better commutability to human serum than SRM 909b (the comparative standard for establishing the C.F.A.S. creatinine set point). Level 1 has an assigned value of 0.75 mg/dL; Level 2 has an assigned value of 3.92 mg/dL. Over two days of testing the average values on the ModP were 0.75 mg/dL and 3.93 mg/dL, respectively. As future lot changes of C.F.A.S. occur, it is recommended that SRM Levels 1 and 2 are evaluated at that time.

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

A. Remedial action for out of control conditions includes examination of the pipetting and detection equipment and examination of reagent materials.

B. the QC parameters are compared to the patient means to look for confirmatory or disconfirmatory evidence. When the 2 2s and/or 1 3s rules are violated, samples are repeated following corrective maintenance or reagent changes.

12. LIMITATIONS OF THE METHOD; INTERFERING SUBSTANCES AND CONDITIONS

A. Urine

1) Icterus: No significant interference up to an approximate conjugated bilirubin concentration of 70 mg/dL.
2) Hemolysis: No significant interference up to an approximate hemoglobin concentration of 1000 mg/dL.
3) Ascorbic acid < 300 mg/L, glucose < 2000 mg/dL and urobilinogen < 40 mg/dL do not interfere.
4) Calcium dobesilate (e.g. Dexam) and α-methyldopa give falsely low recoveries. Of ten additional commonly used drugs tested in vitro, none interfered with the assay.

13. REFERENCE RANGES (NORMAL VALUES)

Although urine creatinine concentrations are very dependent upon skeletal muscle mass, approximate normal ranges (based on 24-hour urine collections) are presented below:

Normal Ranges for Urine Creatinine

<table>
<thead>
<tr>
<th>Age, years</th>
<th>Range per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-3</td>
<td>6-22 mg/kg/d</td>
</tr>
<tr>
<td>4-16</td>
<td>12-30 mg/kg/d</td>
</tr>
<tr>
<td>&gt;16, male</td>
<td>1.0-2.0 g/d</td>
</tr>
<tr>
<td>&gt;16, female</td>
<td>0.8-1.8 g/d</td>
</tr>
</tbody>
</table>

14. CRITICAL CALL RESULTS ("PANIC VALUES")
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No medical intervention is indicated for unusual urine creatinine results on random urine specimens

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Store specimens at 2-8°C until analysis. Specimens reach 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

Samples will remain in refrigerator until instrument is back in operation. If the system is inoperable >36 hours, fresh urine aliquots in analyzer sample cups are requested from the Microalbumin Laboratory. Specimens in the original NHANES IV tubes may be refrozen until analysis is possible.

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

A. Test Result Reporting System

1) The collaborating agency with access to patient identifiers or the responsible medical officer receives an Excel file with all results for a specimen with any critical values. These files with critical values are sent in advance of results that are not abnormal, unless all results are ready to send at the same time. The earliest reporting of results would be the day after arrival of specimens. More frequently two to three days after receiving specimens.

2) Test results that are not abnormal are reported to the collaborating agency at a frequency and by a method determined by the study coordinator. Generally, data from this analysis are compiled with results from other analyses and sent to the responsible person at the collaborating agency as an Excel file, either through Internet FTP transfer of files or electronic mail or other electronic means.

3) All data are reported electronically to Westat within 21 days of receipt of specimens.

4) Internet FTP transfer of files is available and is preferred for data transfer.

B. Protocol for reporting critical calls

Not applicable for this procedure.

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

A. Shipments of frozen specimens are logged in upon receipt by the Microalbumin Laboratory. Aliquots in analyzer sample cups and accompanying collection lists are transferred to the Creatinine Laboratory by the Microalbumin Laboratory personnel. Specimens in the original tubes are re-frozen at -70°C and returned on dry ice to the NHANES IV contract storage facility McKesson Bioservices by the Microalbumin Laboratory. All notebooks disks and files containing raw data, final data, QC information, communications, etc. are saved. These serve as documentation for specimen accountability and tracking.

B. In general, when specimens are received, the specimen ID number, and a name identifying the container ID and slot number is entered into the Laboratory Information System (L.I.S.) database. New barcodes are printed and the specimens stored in a refrigerator. Samples are aliquoted to a Micro tube with the new barcodes. The specimen ID is read off of the tube by a barcode reader. Tracked in the database are the date and time of entry into the L.I.S., date and time analysis completed, and who certified the results.

C. Microsoft Excel spreadsheets are used to keep records and track specimens with the data taken from the Laboratory Information System. Logs are kept including information of when samples arrive, are processed and tested, when frozen after testing, and when returned to NHANES for long term storage.

D. The Project supervisor is responsible for keeping a logbook containing the ID numbers of specimens prepared incorrectly, those with labeling problems, and those with abnormal results, together with information about these discrepancies. It is recommended that records, including related QA/QC data, be maintained for 10 years after completion of the NHANES study.
19. SUMMARY STATISTICS AND QC GRAPHS

See following page.
Summary Statistics for Creatinine, urine (mg/dL)

<table>
<thead>
<tr>
<th>Lot</th>
<th>N</th>
<th>Start Date</th>
<th>End Date</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation</th>
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<tbody>
<tr>
<td>AH1_URCUCRH1</td>
<td>14</td>
<td>20JAN11</td>
<td>18MAR11</td>
<td>97.1</td>
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<td>20JAN11</td>
<td>18MAR11</td>
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<tr>
<td>UB 1:5_URCUCRM1</td>
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<td>24MAR11</td>
<td>24JAN13</td>
<td>16.0</td>
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<td>4.3</td>
</tr>
</tbody>
</table>

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REFERENCES

Roche/Hitachi System Application Sheet for CREA plus, 2006.


Roche/Hitachi Modular Analytics Operator’s manual, version 2.0, October 2006.

