Laboratory Procedure Manual

Analyte: **Creatinine**

Matrix: **Refrigerated Serum**

Method: **Beckman Synchron LX20**

Method No.:

Revised:

as performed by: *Collaborative Laboratory Services, L.L.C*

Contact: *Dr. David Witte*

Important Information for Users

Collaborative Laboratory Services 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: :

Lab Number	Analyte	SAS Label
BIOPRO_E	LBXSCR	Creatinine (mg/dL)
	LBDSCRSI	Creatinine (umol/L)

There was a change in instruments in 2008. In 2007 the Beckman Synchron LX20 was used and in 2008 the Beckman Coulter UniCel® DxC800 was used. The methods used in 2007 are described in a separate document.

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The LX20 modular chemistry side uses the Jaffe rate method (kinetic alkaline picrate) to determine the concentration of creatinine in serum, plasma, or urine. A precise volume of sample is introduced into a reaction cup containing an alkaline picrate solution. Absorbance readings are taken at both 520 nm and 560 nm. Creatinine from the sample combines with the reagent to produce a red color complex. The observed rate measurement at 25.6 seconds after sample introduction has been shown to be a direct measure of the concentration of the creatinine in the sample.

Creatinine measurements are useful in the diagnosis and treatment of renal diseases.

2. SAFETY PRECAUTIONS

Consider all plasma or serum specimens as potentially positive for infectious agents including HIV and the hepatitis B virus. We recommend the hepatitis B vaccination series for all analysts working with whole blood and/or plasma. Observe universal precautions; wear protective gloves, laboratory coats. Place disposable plastic, glass, and paper (pipette tips, gloves, etc.) that contact plasma and any residual sample material in a biohazard bag and keep these bags in appropriate containers until disposal by maceration chlorination. Wipe down all work surfaces with Sani-Cloth HB, Germicidal Disposable Wipe when work is finished.

Handle acids and bases with extreme care; they are caustic and toxic. Handle organic solvents only in a well-ventilated area or, as required, under a chemical fume hood.

Reagents and solvents used in this study include those listed in Section 6. Material safety data sheets (MSDSs) for these chemicals are readily accessible as hard copies in the lab.

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 tests are analyzed on a Beckman Synchron LX20. When all ordered tests are completed for each sample, the results are printed out by Beckman Synchron LX20 instrument.

   The LX20 is interfaced to the Laboratory Information Systems (L.I.S.) with a bi-directional interface. After results have printed at the LX20 printer, the results will go to the L.I.S. Host Interface Workstation to be collated and then certified by qualified analyst.

B. Statistical evaluations of the runs are accomplished with Microsoft Excel software on a PC. An ad hoc report of the completed runs data is saved to a floppy disk in a comma delimited format (CSV) text file. The file is opened and copied to an Excel spreadsheet for evaluation. The Excel spreadsheet results file data are copied to the shipment file and saved as a CSV file and e-mailed to Westat within 21 days of sample receipt.

C. The Excel files containing all raw data and results are backed up once a week using a CD writer or Zip drive for storage. Files stored on the L.I.S. network are automatically backed up nightly to tape.

D. 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.

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

A. Interferences:

   (1) No interference from bilirubin or lipemia.

   (2) No interference from hemolysis.

B. Separated serum or plasma should not remain at +15 to +30°C longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2 to +8°C. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at –15 to –20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.

C. Fasting is not required.

D. A minimum of 0.6 ml serum is needed for the Multi-Analyte Panel.

E. Sample volume for individual test is 40 µl added to 1.27 ml of buffer reagent, and 40 µl added to 3.23 ml of reference reagent. Sample volume prior to June 10, 2002 was 62 µl.

F. Sample is run singly as part of Multi-analyte Biochemistry Panel.

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: Beckman Synchron LX20

B. Materials

(1) Beckman Synchron CX Micro Sample Tube (Part #448774)

(2) S/P Plastic Transfer Pipet (Cat. #P5214-10)

(3) S/P Brand Accutube Flange Caps (Cat. #T1226-37)

C. Reagent Preparation: Synchron LX Creatinine Reagent Kit (Part #472525).

(1) Prepare Creatinine Reagent by pouring Picric Acid Solution into Alkaline Buffer Solution. Replace cap and mix at least 10 times by gentle inversion.

(2) Unopened Creatinine Reagent Kit is stable until expiration date when stored at room temperature.

(3) The combined Creatinine Reagent is stable on the instrument for 30 days from the date of preparation. Do not freeze or refrigerate.

(4) Avoid contact of reagent with skin, eyes, or clothing. In case of spill, flush with large amounts of water.

D. Standards Preparation: None required.

(1) Beckman Synchron LX Aqua Cal 1 and 2 (Part #471288 and 471291).

E. Control Material

(1) Beckman Triad Custom Unassayed Chemistry Control Serum (Part #465405)

- In use through August 23, 2002.

(2) Bio-Rad Liquid Unassayed Multiqual (Cat. #697, 699).

- In use from August 24, 2002
- Thaw new bottle weekly. Mix very well, using rocker prior to use.
- Thawed control is stable 7 days. Mix well prior to each use.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

A. Calibrators: Synchron LX Aqua Cal 1 and 2 (Part #471288 and 471291). Refer to LX Operation Procedure for storage and stability information.

B. Calibration frequency: 72 hours.

C. Calibration required after loading new reagent and after certain parts replacement and maintenance procedures.

D. Refer to Operation Procedures or instrument specific Quick Reference Guides for programming a calibration.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

A. Preliminaries

(1) Enter test in L.I.S. as a part of a panel according to procedure listed in this document (See Attachment A).

B. Sample Preparation

(1) Procedure for labeling CX sample tubes and transferring serum (See Attachment B).

C. Operation

(1) Refer to Operation Procedures for programming controls/patients and loading sectors/racks in the Beckman LX20 Chemistry Information Manual, 2001 (See Attachment C for specific procedure for NHANES samples).

D. Recording of Data

(1) Operator will review results and collate and certify in the L.I.S.

(2) Operator will place printouts in box labeled for NHANES samples.

(3) Project supervisor will do an ad hoc report onto a floppy disk in a comma delimited text file from the L.I.S.

(4) Comma delimited file is opened in Excel on a PC and copied into another Excel file to further evaluate the data.

(5) A printout of the Excel spreadsheet for each container ID results is made and comments noted.

(6) Project supervisor reviews the results. If problems noted with patient results or QC, Project Supervisor investigates and discusses issues if necessary with Laboratory Director. Repeat samples if necessary.

(7) Daily log sheets are completed and any problems or issues noted.

(8) Repeat values are used when match the original results within 3 CVs.

E. Replacement and Periodic Maintenance of Key Components

(See Attachment AB for LX20 Maintenance Schedule).

F. Calculations

Synchron LX Systems perform all calculations internally to produce the final reported result. The system will calculate the final result for sample dilutions made by the operator when the dilution factor is entered into the system during sample programming.

9. REPORTABLE RANGE OF RESULTS

A. Analytical Range:

(1) 0.1–25 mg/dL

(2) Samples out of analytical range high should be diluted with saline or deionized water and reanalyzed. Enter dilution factor at sample information screen or multiply printout by dilution factor to obtain the final result.

(3) Limits of detection (LOD) are established by Beckman Coulter and linearity data verifies the reportable range. Detection of results below the reportable range is not relevant and formal limit of detection study is unnecessary.

(4) Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for creatinine determination is 0.1 mg/dL.

(5) 0 is not a reportable value.

10. QUALITY CONTROL (QC) PROCEDURES

A. Blind QC Specimens are included in the samples received from NHANES.

B. Beckman Triad Custom Unassayed Chemistry Controls Levels 2 and 3 are assayed in early A.M. and if a new reagent pack is loaded, controls are assayed again. One level is assayed in middle of the day and both control levels are assayed after running NHANES sample.

C. BioRad Liquid Unassayed Multiqual Controls Levels 1 and 3 are substituted for Beckman Triad controls as of August 24, 2002 for CDC-NHANES runs to allow long term control use. Multiqual controls are analyzed at beginning and end of runs with CDC-NHANES samples.

D. Acceptable Answer:

(1) Controls must be within ±2 S.D.

(2) Refer to Quality Control Flow Chart for action decisions guidelines (See Attachment I).

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

Remedial action for out of control conditions includes examination of the pipetting and detection equipment and examination of reagent materials. 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 METHOD; INTERFERING SUBSTANCES AND CONDITIONS

A. Hemolysis has no significant interference.

B. <20 mg/L bilirubin has no significant interference.

C. At 30 mg/dL of bilirubin, the creatinine value is decreased by <0.4 mg/dL.

D. Lipemia has no significant interference.

E. Acetoacetic acid, cefaclor, cefoxitin, cephalothin, D-glucose, gluthathione, and pyruvic acid demonstrate positive interference.

F. L-Dopa methyl ester demonstrates negative interference.

G. Fluorescein interference suppressed results.

H. Sulfasalazine and sulfobromophthalein have no significant interference.

I. Refer to References for other interferences caused by drugs, disease, and preanalytical variables.

13. REFERENCE RANGES (NORMAL VALUES)

Creatinine

Serum or Plasma Age Group	mg/dL		
	Male		**Female**
0–1 month		0.3–0.8	
1 mo. – 1 Y		0.3–0.6	
1–15 Y		0.3–1.0	
>15 Y	0.7–1.3		0.6–1.1

Adult reference range values were established from wellness participants with an age mix similar to our patients. These data were analyzed using non-parametric techniques described by Reed (Clin Chem. 1971;17:275) and Herrara (J Lab Clin Med. 1958;52:34–42) which are summarized in recent editions of Tietz' textbook. Descriptions appear in Clin Chem. 1988;34:1447 and Clin Lab Med. 1993;13:481.

Pediatric Reference Range Guidelines for Synchron Systems- Multicenter study using data from Montreal, Quebec; Miami, FL; and Denver, CO. Beckman 1995.

14. CRITICAL CALL RESULTS (PANIC VALUES)

Values greater than 2.0 mg/dL are called for CLS patients. For this study results greater than 2.4 mg/dL will be faxed to NHANES.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens arrive refrigerated. Specimens are kept refrigerated until ready to transfer to CX multi sample tubes. Capped CX sample tubes are kept refrigerated until ready to put on instrument.

Specimen vials are returned to container and refrigerated after transfer of aliquot and double checking of pour off tubes. Specimen vial container is placed in –70°C Freezer after testing is complete. CX sample tubes are refrigerated, then frozen after analysis.

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

Samples will remain in refrigerator until instrument is back in operation.

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

The collaborating agency with access to patient identifiers or the responsible medical officer is notified by FAX by the Project Supervisor of any critical values. Copies of faxes sent concerning abnormal results are kept in a folder by the supervisor for the duration of the study.

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 a comma delimited file, either through electronic mail or other electronic means.

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

Internet FTP transfers of files or dial-up modem transfer options are available.

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

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 CX-Micro Sample 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.

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.

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.

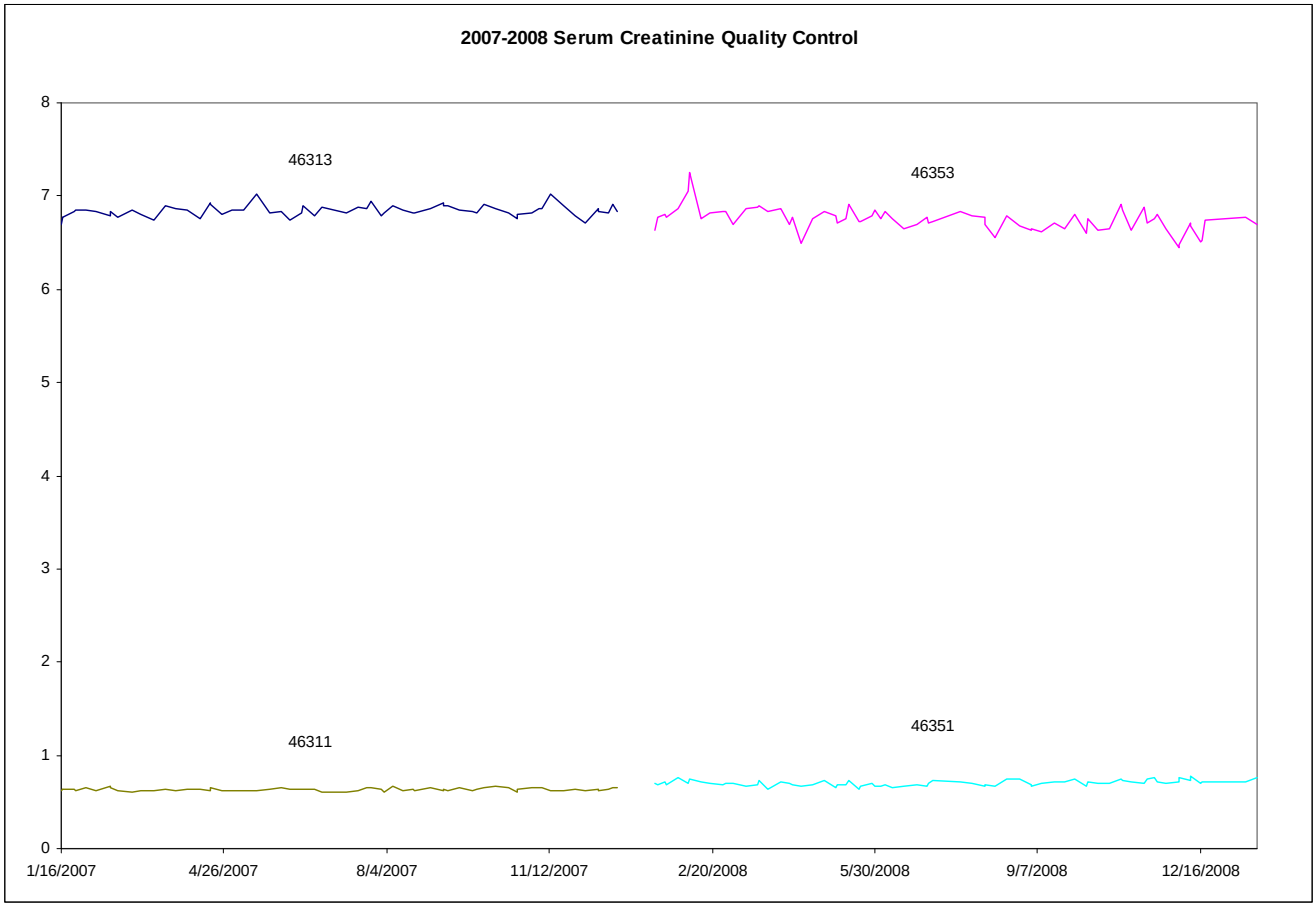
19. SERUM CREATININE IN ESTIMATING GLOMERULAR FILTRATION RATE

Serum creatinine alone is not sufficient to assess glomerular filtration rate (GFR) and tends to underestimate chronic kidney disease. The GFR can be estimated from serum creatinine using several equations including one developed using data from the Modification of Diet in Renal Disease (MDRD) Study (14). Use of equations to estimate GFR are dependent on the calibration of the serum creatinine assay used in developing those equations and serum creatinine assay calibration can vary substantially across laboratories (15). Coresh *et al*. compared serum creatinine performed on frozen samples at the Coulston Laboratory (NHANES 1999–2000) to a serum creatinine “reference” method done at the Cleveland Clinic Laboratories (MDRD study). Coresh suggested adding 0.13 mg/dL to the serum creatinine concentration for NHANES 1999–2000 participants and subtracting the value of 0.23 mg/dL from the serum creatinine concentration for NHANES 1988–1994 participants (16).

20. SUMMARY STATISTICS AND QC GRAPHS

Summary Statistics for Serum Creatinine by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
46311	67	1/16/2007	12/24/2007	0.634	0.016	2.6
46313	67	1/16/2007	12/24/2007	6.845	0.065	0.9
46351	72	1/16/2008	1/20/2009	0.704	0.030	4.3
46353	72	1/16/2008	1/20/2009	6.748	0.126	1.9



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