Laboratory Procedure Manual

Analyte: **Uric Acid**

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 NHANES 2003–2004 data.

A tabular list of the released analytes follows:

Lab Number	Analyte	SAS Label
l40_c	LBXSUA	Uric acid, serum (mg/dL)
	LBDSUASI	Uric acid, serum (µmol/L)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The LX20 uses a timed endpoint method to measure the concentration of uric acid in serum, plasma, or urine. Uric acid is oxidized by uricase to produce allatoin and hydrogen peroxide. The hydrogen peroxide reacts with 4-aminoantipyrine (4-AAP) and 3.5-dichloro-2-hydroxybenzene sulfonate (DCHBS) in a reaction catalyzed by peroxidase to produce a colored product. The system monitors the change in absorbance at 520 nm at a fixed time interval. The change in absorbance is directly proportional to the concentration of uric acid in the sample.

Uric acid measurements are used in the diagnosis and treatment of numerous renal and metabolic disorders.

2. SAFETY PRECAUTIONS

Consider all plasma or serum specimens 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 test is 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 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. A statistical evaluation of the runs is 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 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 <2+ hemolysis, <2+ lipemia, or <30 mg/dL bilirubin.

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°C 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 12 µl added to 300 µl of reagent.

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 Pipette (Cat. #P5214-10)

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

C. Reagent Preparation: Beckman Synchron Systems Uric Acid Reagent (Part #442785).

(1) No preparation required.

(2) When stored unopened at 2–8°C, the reagent is stable until the expiration printed on the label.

(3) When first opened or installed on the instrument, the reagent is stable for 30 days unless the expiration date is exceeded.

(4) Do not freeze.

(5) Avoid skin contact with reagent. Use water to wash reagent from skin.

D. Standards Preparation: No preparation required.

(1) Beckman Synchron Multi Calibrator (Part #450140).

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: Beckman Synchron MULTI Calibrator (Part #450140).

(1) One level. Concentration will vary from lot to lot.

(2) Refer to Operation Procedure for storage and stability information.

B. Calibration: Required with a new lot of reagent.

C. Calibration frequency: 14 days.

D. Within-lot calibration frequency: 90 days.

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.

B. Sample Preparation

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

C. Operation

(1) Refer to Operation Procedures for programming controls/patients and loading sectors/racks in the Beckman LX20 Chemistry Information Manual, 2001.

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 CSV text file from the L.I.S.

(4) CSV 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 CSVs.

E. Replacement and Periodic Maintenance of Key Components

(See manual 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) 0.5–12 mg/dL.

(2) Samples with concentrations exceeding the high end of the analytical range should be diluted with saline 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 the uric acid determination is 0.5 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.

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 >2+ demonstrates positive interference.

B. Bilirubin <30 mg/dL has no significant interference.

C. On samples with >2+ lipemia, pretreat sample with Lipoclear clarifying agent and reanalyze according to Lipoclear procedure.

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

13. REFERENCE RANGES (NORMAL VALUES)

Uric Acid

Serum or Plasma Age Group	**Reference Range mg/dL**		
	Male		Female
0–10 Y		1.9–5.4	
10–18 Y		3.5–7.3	
>18 Y	3.6–8.4		2.9–7.5

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)

There are no critical call back values for uric acid. For this study we will fax results if uric acid is >12.9 mg/dL.

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 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.

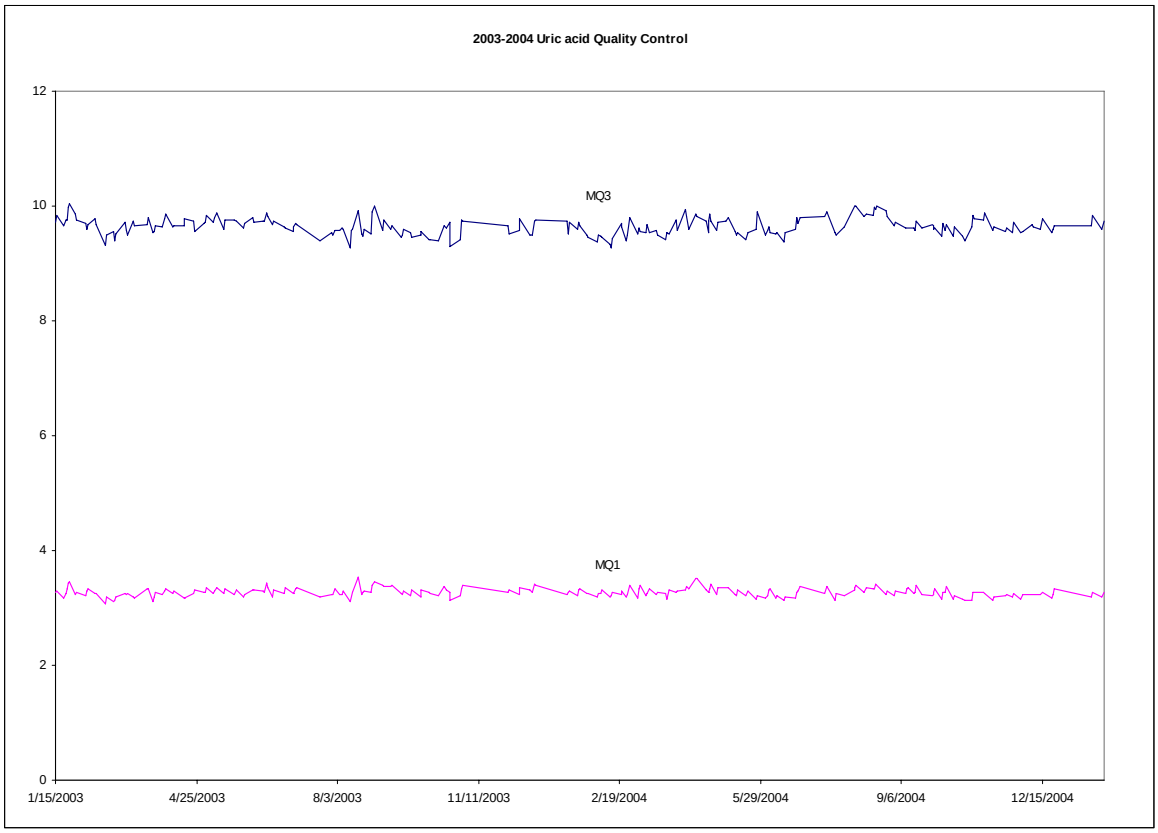
Microsoft Excel spreadsheets are used to keep records and track specimens with the data taken from the Laboratory Information System. Logs are kept including information of when samples arrive, are processed and tested, when frozen after testing, and when returned to NHANES for long term storage.

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. SUMMARY STATISTICS AND QC GRAPHS

Uric Acid

Summary Statistics for Uric Acid by Lot						
Lot	**N**	**Start Date**	**End Date**	**Mean**	**Standard Deviation**	**Coefficient of Variation**
MQ1	237	1/15/2003	1/28/2005	3.27	0.08	2.3
MQ3	237	1/15/2003	1/28/2005	9.64	0.15	1.6



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