Blood Urea Nitrogen (BUN) in Refrigerated Serum – NHANES 2001-2002 Collaborative Laboratory Services, L.L.C.)

0. Public Release Data Set Information

This document details the Lab Protocol for NHANES 2001-2002 data.

A list of the released analytes follows:

Lab	Analytes	SAS Label	Description
l40_b	LBXSBU	Blood urea nitrogen (mg/dL)	Blood Urea Nitrogen
l40_b	LBDSBUSI	Blood urea nitrogen (mmol/L)	Blood Urea Nitrogen

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The LX20 modular chemistry (BUNm) is used to quantitatively determine the concentration of blood urea nitrogen in serum or plasma by means of the enzymatic conductivity rate method. A precise volume of sample is injected into the urease reagent in a reaction cup containing an electrode that responds to changes in solution conductivity. Electronic circuits determine the rate of increase in conductivity, which is directly proportional to the concentration of urea in the sample.

Urea nitrogen measurements are used in the diagnosis and treatment of certain renal and metabolic diseases in conjunction with creatinine measurements.

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 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. A statistical evaluation of the runs is accomplished with Microsoft Excel software on a PC. An Adhoc 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 comma delimited file (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 bilirubin or lipemia.
   2) No interference from hemolysis.

b. Separated serum or plasma should not remain at +15°C to +30°C longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2°C 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 10 µl added to 765 µl 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 Pipet (Cat. #P5214-10)
   3) S/P Brand Accutube Flange Caps (Cat. #T1226-37)

c. Reagent Preparation: LX BUN Reagent Kit (Part #472482).
   1) Reagent preparation required. Pour bottle of wetting agent into diluent. Mix 10 times by gentle inversion. Pour bottle of urease reagent into diluent and mix 10 times by gentle inversion. Allow prepared BUN reagent to equilibrate at room temperature 12-24 hours before loading onto the instrument.
   2) Unopened BUN Reagent Kit components are stable until expiration date when stored at 2-8°C. (The diluent may be stored at room temperature).
   3) Once mixed and loaded on the instrument, the Bun reagent is stable for 15 days, unless the expiration date is exceeded.
   4) Frozen reagent should be discarded.

d. Standards Preparation: No preparation required.
   1) Beckman Synchron LX Aqua Cal 1, 2 and 3 (Part #471288, 471291, 471294).

e. Control Material
   1) Beckman Triad Custom Unassayed Chemistry Control Serum (Part #465405).
      - υ In use through August 23, 2002.
   2) Bio-Rad Liquid Unassayed Multiqual (Cat. #697, 699).
      - υ In use from August 24, 2002
      - υ Thaw new bottle weekly. Mix very well, using rocker prior to use.
      - υ Thawed control is stable 7 days. Mix well prior to each use.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

a. Calibrators: Beckman Synchron LX Aqua Cal 1, 2, and 3.
b. Calibration frequency: 72 hours
   1) Calibration required after loading new bottle of reagent and after certain parts replacement and maintenance procedures.
   2) Refer to Operation Procedures or instrument specific Quick Reference Guide 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 Procedure 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 Adhoc 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 CSV's.
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) 1-150 mg/dL
   2) Samples which are out of analytical range high should be diluted with saline and reanalyzed.
   3) When test is repeated on a dilution, the dilution factor must be entered at the instrument Sample Information Screen.
   4) If the dilution factor is not entered into the instrument, the print out value must be multiplied by the dilution factor to obtain the final result.
   5) 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.
   6 Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for the BUN determination is 1 mg/dL.
   7) 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. Hemoglobin has no significant interference.
b. Bilirubin has no significant interference.
c. Lipemia has no significant interference.
d. L-Dopa and methylbenzethonium chloride demonstrate negative interference.
e. Refer to References for other interferences caused by drugs, disease and preanalytical variables.

13. REFERENCE RANGES (NORMAL VALUES)

BUN

Serum or Plasma Age Group	Reference Range mg/dL
0-5 Y	5-18
5-15 Y	7-18
>15 Y	6-23

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 Clinics in Laboratory Medicine June 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 60 mg/dL are called for CLS patients. For this study results greater than 49 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.

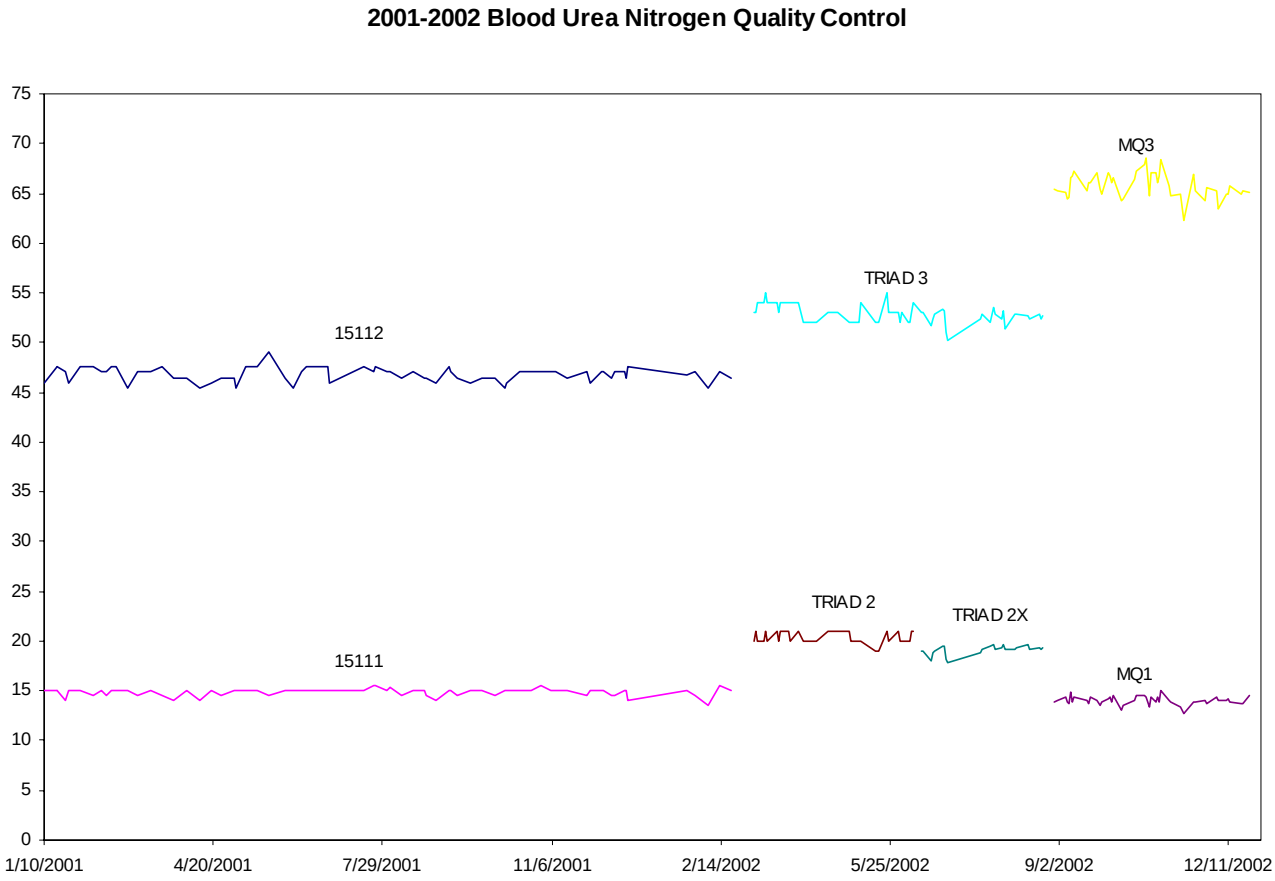
Microsoft Excel spreadsheets are used to keep records and track specimens with the data taken from the Laboratory Information System. Logs are kept including information of when samples arrive, are processed and tested, when frozen after testing, and when returned to NHANES for long term storage.

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. SUMMARY STATISTICS AND QC GRAPHS

Summary Statistics for Blood Urea Nitrogen by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
15111	71	1/10/2001	2/20/2002	14.8	0.38	2.6
15112	71	1/10/2001	2/20/2002	46.8	0.66	1.4
TRIAD 2	33	3/6/2002	6/8/2002	20.4	0.6	3.0
TRIAD 3	57	3/6/2002	8/23/2002	52.8	0.9	1.7
TRIAD 2x	24	6/12/2002	8/23/2002	19.1	0.5	2.6
MQ1	46	8/30/2002	12/23/2002	14.0	0.4	3.0
MQ3	46	8/30/2002	12/23/2002	65.8	1.2	1.9



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