

Laboratory Procedure Manual

Analyte: **Total Calcium**

Matrix: **Refrigerated Serum**

Method: **Beckman UniCel[®] DxC800 Synchron**

Revised:

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

Contact:

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:

| File Name | Variable Name | SAS Label |
|------------------|----------------------|------------------------|
| BIOPRO_F | LBXSCA | Total Calcium (mg/dL) |
| | LBDSCASI | Total Calcium (mmol/L) |

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The DxC800 system uses indirect (or diluted) I.S.E. (ion selective electrode) methodology to measure calcium concentration in serum, plasma, or urine. A calcium ion selective electrode measures un-bound free calcium ions in solution. The system determines calcium concentration by measuring calcium ion activity in solution. When the sample buffer mixture contacts the electrode, calcium ions complex with the ionophore at the electrode surface. Changes in potential develop at the electrode surface as the reaction occurs. These changes in potential are referenced to a sodium reference electrode. The reference signal is used in calculating the analyte concentrations based on the Nernst equation.

Calcium measurements are used in the diagnosis and treatment of parathyroid disease, bone diseases, chronic renal disease and tetany. Urinary calcium measurement is used in the differential diagnosis of hypercaluria.

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 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 Coulter UniCel[®] DxC800 Synchron Clinical System. The DxC800 is interfaced to the Laboratory Information Systems (L.I.S.) with a bi-directional interface. After tests are completed, the results will go to the L.I.S. Host Computer Interface to be verified by qualified analyst.
- b. Reflex testing is set up in the L.I.S. to order a repeat of any critical result, to verify abnormal values.
- c. Statistical evaluation of the runs is accomplished with Microsoft Excel software on a PC.
- d. A result file is generated in the L.I.S. database. The file is opened and copied to an Excel spreadsheet for evaluation. 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.
- e. The Excel files containing all raw data and results are backed up once a week using a CD writer or External drive for storage. Files stored on the L.I.S. network are automatically backed up nightly to tape.
- f. 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.
 - 3) Samples with >3+ lipemia should be treated with Lipoclear Clarifying agent prior to analysis (see Lipoclear procedure).
- 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 40 µl added to 1.27 mL buffer reagent and 40 µL added to 3.23 mL reference 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 Coulter UniCel® Dx C800 Synchron Clinical System
- b. Materials
 - 1) Beckman Micro 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:
 - 1) No preparation required.
 - 2) ISE Electrolyte Buffer (*Part #A28945*)
 - 3) ISE Electrolyte Reference (*Part #A28937*)
 - 4) Unopened reagents are stable until expiration date when stored at room temperature.
 - 5) Once opened and installed on the instrument, the reagents are stable for 30 days unless expiration date is exceeded.
 - 6) If electrolyte reagents are frozen in transit, warm to room temperature and mix thoroughly by inverting at least 20 times.
- d. Standards Preparation: No preparation required.
 - 1) Beckman Synchron Aqua Calibrators 1 and 2 (*Part #471288 and 471291*).
- e. Control Material
 - 1) Beckman Triad Custom Unassayed Chemistry Control Serum (*Part #465405*).
After August 19, 2009 Beckman Triad was discontinued. This control was used as regular control for this analyte, but not reported for this project.
 - 2) Bio-Rad Liquid Unassayed Multiqual (*Cat. #697, 699*).
 - Thaw bottle of control and mix very well.
 - Thawed control is stable 7 days. Mix well prior to each use.After August 19, 2009 Bio-Rad Multiqual used in our lab as the regular control for this analyte.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

- a. Calibrators: Beckman Synchron Aqua Calibrator 1 and 2 (*Part #471288 and 471291*).
 - 1) Refer to UniCel DxC 600/800 System *Instructions For Use (IFU)* manual for storage and stability information.
- b. Calibration:
 - 1) Calibration frequency: 24 hours.
 - 2) Required after loading new reagent
 - 3) Required after certain parts replacement and maintenance procedures.
 - 4) Refer to UniCel DxC 600/800 System *Instructions For Use (IFU)* manual or *DxC800 and DxC600 Operating Procedure* 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 Micro tube (CX tube) and transferring serum (See *Attachment B*).
- c. Operation
 - 1) Refer to Operation Procedures for programming controls/patients and loading sectors/racks in the Beckman Coulter Synchron UniCel DxC 600/800 System *Instructions For Use (IFU)* manual or *DxC800 and DxC600 Operating Procedure*. (See *Attachment C* for specific procedure for NHANES samples).
- d. 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.
 - 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. Replacement and Periodic Maintenance of Key Components
(See *Attachment D* for DxC800 Maintenance Schedule).
- f. Calculations
Synchron 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) 2.0-20.0 mg/dL.
 - 2) Samples with concentrations exceeding the high end of the analytical range should be diluted with deionized water and reanalyzed. Enter dilution factor at sample information screen or multiply printout by dilution factor to obtain final results.
 - 3) Samples out of instrument range low should be reported at "<2.0".
 - 4) 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.
 - 5) Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for calcium determination is 2.0 mg/dL.
 - 6) 0 is not a reportable value.

10. QUALITY CONTROL (QC) PROCEDURES

- a. Blind QC Specimens are included in the samples received from NHANES.
- b. Controls 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 samples.
- c. BioRad Liquid Unassayed Multiquel Controls Levels 1 and 3 are assayed for CDC-NHANES runs to allow long term control use. Multiquel controls are analyzed at beginning and end of runs with CDC-NHANES samples. Before August 19, 2009 Beckman Triad Custom Unassayed Chemistry Controls Levels 2 and 3 were analyzed in early AM and at times when CDC-NHANES were not being analyzed. As of August 19, 2009 we have discontinued use of Beckman Triad controls.
- d. Acceptable Answer:
 - 1) Controls must be within ± 2 S.D.
 - 2) Refer to Quality Control Flow Chart for action decisions guidelines (*See Attachment F*).

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. Bilirubin has no significant interference.
- c. Lipemia has no significant interference.
- d. Methicillin and Methylbenzethonium have negative interference.
- e. Bromide has a positive interference.
- f. Serum from patients receiving EDTA therapy may yield depressed calcium values.
- g. Refer to References for other interferences caused by drugs, disease and preanalytical variables.

13. REFERENCE RANGES (NORMAL VALUES)

Calcium

| <i>Serum or Plasma Age Group</i> | <i>mg/dL</i> |
|--------------------------------------|--------------|
| 0-10 days | 7.6-10.4 |
| 10 days - 2 Y | 9.0-11.0 |
| 2-12 Y | 8.8-10.8 |
| >12 Y | 8.5-10.5 |

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 Herrera (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 less than 7.0 or greater than 14.0 mg/dL are called for CLS patients. For this study we will early report results if Calcium is less than 7.6 or greater than 11.4 mg/dL.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens arrive refrigerated. Specimens are kept refrigerated until ready to transfer to Micro tubes. Capped Micro 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. Micro tubes are refrigerated, and 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 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.

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.

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

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

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

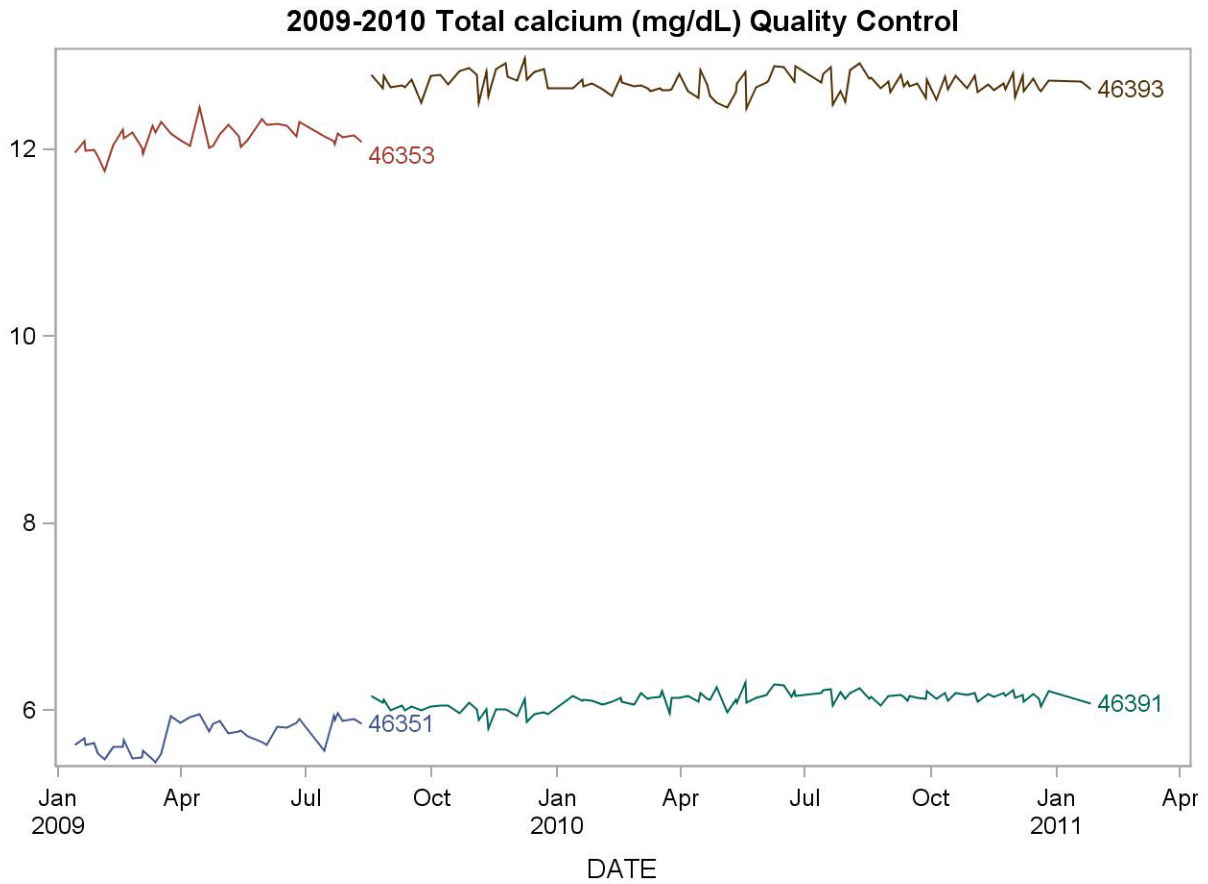
In general, when specimens are received, the specimen ID number, and a name identifying the container ID and slot number is entered into the Laboratory Information System (L.I.S.) database. New barcodes are printed and the specimens stored in a refrigerator. Samples are aliquoted to a Micro tube with 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.

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

| Lot | N | Start Date | End Date | Mean | Standard Deviation | Coefficient of Variation |
|-------|-----|------------|----------|---------|--------------------|--------------------------|
| 46353 | 44 | 13JAN09 | 11AUG09 | 12.1223 | 0.1319 | 1.1 |
| 46351 | 44 | 13JAN09 | 11AUG09 | 5.7351 | 0.1599 | 2.8 |
| 46393 | 106 | 18AUG09 | 26JAN11 | 12.7036 | 0.1124 | 0.9 |
| 46391 | 106 | 18AUG09 | 26JAN11 | 6.1081 | 0.0886 | 1.5 |



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