# Laboratory Procedure Manual

*Analyte:* **Alanine Amino Transferase (ALT)**

*Matrix:* **Refrigerated Serum**

*Method:* **Beckman Synchron LX20**

*Method No.:*

*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_E | LBXSATSI | Alanine Aminotransferase (U/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.

SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The LX20 uses an enzymatic rate method to measure ALT activity in serum or plasma. In the reaction, ALT catalyzes the reversible transamination of L-alanine and $\alpha$-ketoglutarate to pyruvate and L-glutamate. The pyruvate is then reduced to lactate in the presence of lactate dehydrogenase (LDH) with the concurrent oxidation of NADH to NAD. The system monitors the rate of change in absorbance at 340 nm over a fixed-time interval. The rate of change in absorbance is directly proportional to the ALT activity in the sample.

ALT measurements are used in the diagnosis and treatment of liver and heart disease.

1. 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.

2. 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 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 evaluations of the runs are accomplished with Microsoft Excel software on a PC.

D. 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 sent using Internet FTP transfer of file 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 Hard 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.

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

A. Interferences:

   >20.0 mg/dL bilirubin, >3+ lipemia. Samples with >3+ lipemia should be treated with Lipoclear clarifying agent prior to analysis (see Lipoclear procedure).

Do not use hemolyzed specimens.

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 23 µl added to 250 µl of reagent.

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

## 4. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure

## 5. 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: Beckman Synchron System ALT Reagent (Part #442620, 200 tests/cartridge or Part #476826, 400 tests/cartridge).

1. 200 test cartridge: Prior to use transfer the entire contents of reagent compartment C to reagent compartment A using a disposable transfer pipette. Gently invert cartridge several times to mix.
2. 400 test cartridge: Dispense entire contents of reagent “A” into compartment “A”. Mix gently.
3. Unopened reagent is stable until expiration date when stored at 2–8°C.
4. Premixed reagent is stable for 30 days when installed on the instrument or stored at 2–8°C, unless the expiration date is exceeded.
5. Do not freeze.
6. Contains sodium azide. Avoid skin contact with reagent. Use water to wash skin.

D. Standards Preparation: None required.

E. Control Material

1. 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.

6. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

A. Calibrators: None required.

B. Calibration: Calibration is based on physical principles of dilution ratio extinction coefficient and time. Known samples verify calibration.

7. 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 and verify results in the L.I.S.
2. Operator will place printouts in box labeled for NHANES samples.
3. 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.
4. Project supervisor will export data from the L.I.S. into an Excel file. The data is copied into another Excel file for further evaluation.
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.

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.

8. REPORTABLE RANGE OF RESULTS

A. Analytical Range:

1. 5–400 IU/L; Up to 2600 IU/L using ORDAC on the LX20.
2. Samples which are out of ORDAC (Overrange Detection and Correction) range high should be reanalyzed after doing a manual dilution of the sample. The dilution factor must be entered into the sample information. If the dilution factor is not entered into the system, the printout must be multiplied by the dilution factor to obtain the final answer.

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. Samples with high enzyme activity may consume all of the NADH Substrate before the first absorbance measurement is taken after sample addition. These samples can either report very low enzyme activity or suppress the result at “OIR LO”.
5. Samples with results suppressed “OIR LO” should be diluted 1:20 with saline and reanalyzed.
6. Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for the ALT determination is 5 IU/L.
7. 0 is not a reportable value.

## 9. 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:

E. Controls must be within ±2 S.D.

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

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

Remedial actions for out of control conditions include 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.

## 11. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

A. Hemoglobin causes falsely elevated ALT results. Do not do test if sample is hemolyzed.

B. Bilirubin has no significant interference.

C. Lipemia has no significant interference.

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

12. REFERENCE RANGES (NORMAL VALUES)

ALT

| **Serum or Plasma Age Group** | **Reference Range IU/L** | |
|---|---|---|
| | Male | Female |
| 0–6 Y | 11–39 | 10–32 |
| 6–10 Y | 12–34 | 11–28 |
| 10–20 Y | 8–36 | 8–29 |
| >20 Y | 11–47 | 7–30 |

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.

13. CRITICAL CALL RESULTS (PANIC VALUES)

There are no critical call back values for ALT. For this study results greater than 90 IU/L will be faxed to NHANES.

14. 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.

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

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

16. 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.

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 or dial up modem transfer options are available.

## 17. 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.

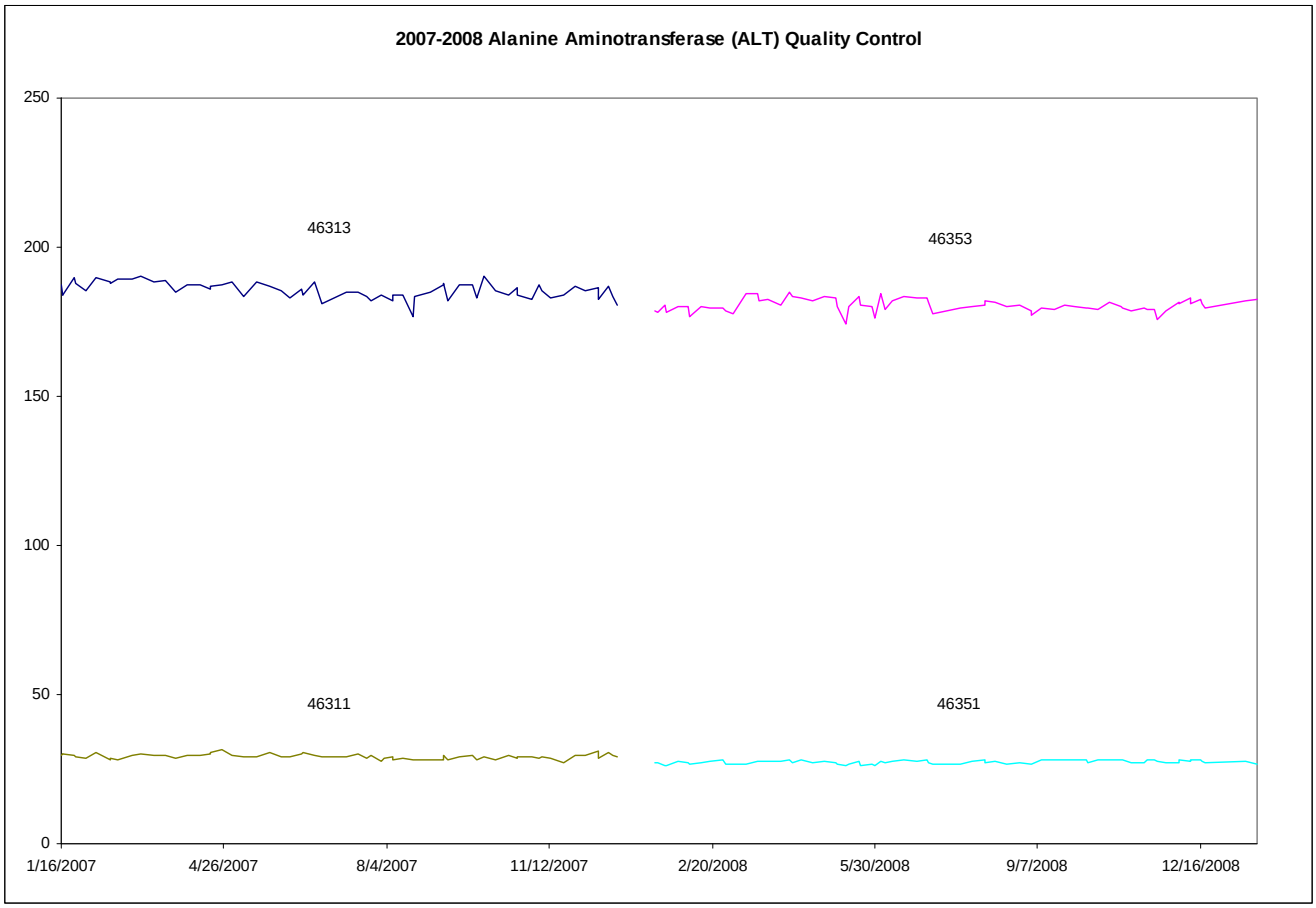
Microsoft Excel spreadsheets are used to keep records and track specimens with the data taken from the Laboratory Information System. Logs are kept including information of when samples arrive, are processed and tested, when frozen after testing, and when returned to NHANES for long term storage.

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 Alanine Aminotransferase (ALT) by Lot**

| Lot | N | Start Date | End Date | Mean | Standard Deviation | Coefficient of Variation |
|---|---|---|---|---|---|---|
| 46311 | 67 | 1/16/2007 | 12/24/2007 | 29.097 | 0.858 | 2.9 |
| 46313 | 67 | 1/16/2007 | 12/24/2007 | 185.679 | 2.735 | 1.5 |
| 46351 | 72 | 1/16/2008 | 1/20/2009 | 27.236 | 0.633 | 2.3 |
| 46353 | 72 | 1/16/2008 | 1/20/2009 | 180.424 | 2.207 | 1.2 |



**REFERENCES**

1. Beckman Synchron LX Systems Chemistry Information Manual, 2001.
2. Tietz, N.W. Textbook of Clinical Chemistry, W.B. Saunders, Philadelphia, PA (1986).
3. Tietz, N.W., "Specimen Collection and Processing; Sources of Biological Variation," Textbook of Clinical Chemistry, 2nd Edition, W.B. Saunders, Philadelphia, PA (1994).
4. National Committee for Clinical Laboratory Standards, Procedures for the Handling and Processing of Blood Specimens, Approved Guideline, NCCLS publication H18-A, Villanova, PA (1990).
5. Tietz, N.W., ed., Clinical Guide to Laboratory Tests, 3rd Edition, W.B. Saunders, Philadelphia, PA (1995).
6. National Committee for Clinical Laboratory Standards, How to Define, Determine, and Utilize Reference Intervals in the Clinical Laboratory, Approved Guideline, NCCLS publication C28-A, Villanova, PA (1995).
7. Tietz, N.W., ed., Fundamentals of Clinical Chemistry, 3rd Edition, W.B. Saunders, Philadelphia, PA (1987).
8. Henry, J.B., ed., Clinical Diagnosis and Management by Laboratory Methods, 18th Edition, W.B. Saunders, Philadelphia, PA (1991).
9. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 4th Edition, AACC Press, Washington, D.C. (1995).
10. Friedman, R.B. and D.S. Young, Effects of Disease on Clinical Laboratory Tests, 3rd Edition, AACC Press, Washington, D.C. (1997).
11. Young, D.S., Effects of Preanalytical Variables on Clinical Laboratory Tests, 2nd Edition, AACC Press, Washington, D.C. (1997).
12. National Committee for Clinical Laboratory Standards, Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline, NCCLS publication EP9-A, Villanova, PA (1995).
13. National Committee for Clinical Laboratory Standards, Precision Performance of Clinical Chemistry Devices, Tentative Guideline, 2nd Edition, NCCLS publication EP5-T2, Villanova, PA (1992).