Laboratory Procedure Manual

Analyte: **Triglycrides**
Matrix: **Serum**

Method: **Hitachi 912**

as performed by: *Lipid Laboratory Johns Hopkins*

University School of Medicine

Lipoprotein Analytical Laboratory

600 North Wolfe Street
Blalock 1379
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410-614-1030

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March 2008

Important Information for Users
The Johns Hopkins Lipid Laboratory 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 (and SI units)
TRIGLY_D	LBXTR	Triglycerides(mg/dL)
	LBDTRSI	Triglycerides(mmol/L

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Triglycerides are measured enzymatically in serum using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol. Glycerol is then oxidized using glycerol oxidase, and H_2O_2, one of the reaction products, is measured as described above for cholesterol. Absorbance is measured at 500 nm.

High levels of serum triglycerides help mark conditions that are associated with increased risk for CHD and peripheral atherosclerosis. High triglycerides are associated with increased risk for CAD in patients with other risk factors, such as low HDL-cholesterol, some patient groups with elevated apolipoprotein B concentrations, and patients with forms of LDL that may be particularly atherogenic. Very high triglycerides can result in pancreatitis and should be promptly evaluated and treated. Triglycerides are also measured because the value is used to calculate low density lipoprotein (LDL)-cholesterol concentrations (see below). In NHANES 2005-2008, triglycerides are only measured in specimens from fasting participants, i.e., those sampled in Session 1.

2. SAFETY PRECAUTIONS

A. Daily Safety Precautions.

All personnel working in the laboratory must wear gloves and laboratory coats. Laboratory coats are to be kept snapped. Lab coats must meet OSHA compliance CPL2-2.44D. Splash and spray resistant fabric that is also antistatic is required. Gloves are removed when leaving the immediate work area or when entering offices within the immediate work area. All used gloves, vials, pipettes and other items that come in contact with specimens are disposed of in a Biohazard box lined with a red plastic bag. Work benches are cleaned at the end of each day with a solution of sodium hypochlorite (bleach: water, 10:100, v/v) and then covered with plastic-backed white paper.

B. Blood Handling.

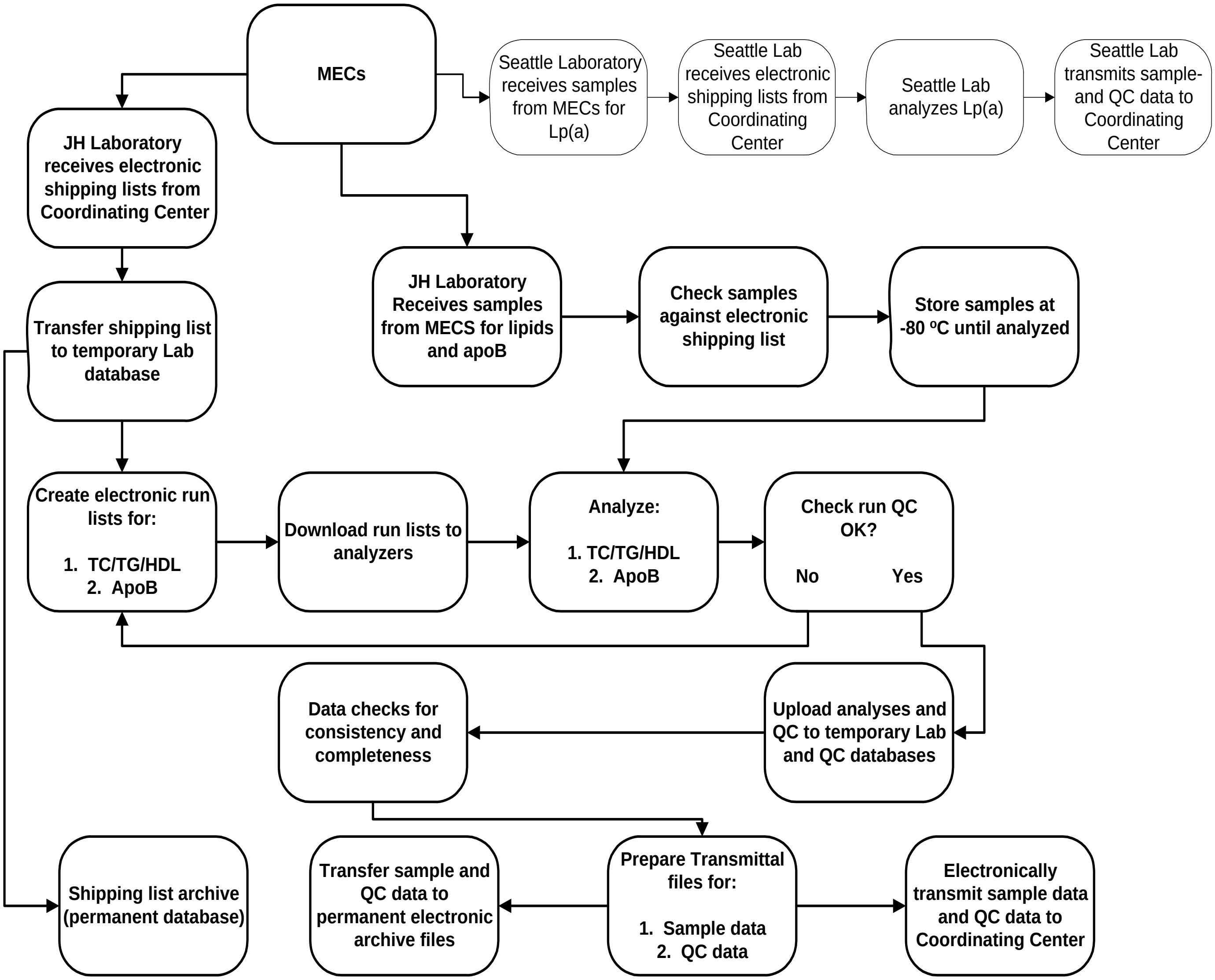
The improper handling of blood samples from patients with infectious diseases, e.g., hepatitis or HIV, can lead to infection of staff that draw, handle, analyze or store such samples. Transmission can occur by ingestion, inhalation or direct contact, and staff must exercise care when handling blood samples. <u>Always wear liquid impermeable gloves (e.g., nitrile or plastic) when handling biological samples.</u> The use of latex gloves is not allowed due to concerns for personnel having or developing latex sensitivities. <u>Never pipet samples by mouth</u>. Avoid contact with serum. Cover any scratches or cuts on fingers and hands and wear gloves before handling serum. Store all samples in sealed containers. In order to minimize the formation of aerosols, do not leave samples open to the atmosphere longer than necessary.

It is about <u>30 times easier</u> to become infected with hepatitis than with HIV through sample mishandling, and it has been recommended that the usual precautions for handling blood specimens to prevent hepatitis infection serve as a guide to prevent AIDS infection as well. Handle all specimens as if you know them to be infectious. All staff should adhere to the CDC Guidelines for Prevention of HIV Infection in Health Care Workers.

C. Spills.

The contaminated area is cleaned with a solution of sodium hypochlorite (bleach: water, 10:100, v/v) and the wipes are disposed of in a red biohazard box.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT



The NHANES Lab number is 13, and we will receive vessel(s) 21(serum).Samples will be sent to the following address via FedEx overnight shipping:

Lipoprotein Analytical Lab/JHU
Attn: Donna Virgil
600 North Wolfe Street
Blalock 1379
Baltimore, MD 21287
410-614-1030

Containers of samples will be sent from the collection locations on scheduled shipping days.

On the day the samples are shipped, our lab will receive data files in Excel format (efiles) from the database coordinating center email account. The efiles will be sent to:

Donna Virgil
dvirgil1@jhem.jhmi.edu, or dvirgil1@jhmi.edu
Ella Levy
elevy2@jhem.jhmi.edu
Cindy Wiley
cwiley2@jhmi.edu

The files will follow the file naming convention NH05_######.xls. The "NH5_" will distinguish NHANES 2005 containers from NHANES 1999 container files. The efile contains 19 pre-formatted columns.

Laboratory data handling.
The efile received from the database contractor email attachment is imported into the stand alone NHANES dedicated study computer. From this excel file an electronic run file is created for determining total cholesterol, triglyceride and HDL cholesterol analyses on the Hitachi 912 platform. All samples have total cholesterol and HDL cholesterol assayed. Only fasted specimens have triglycerides assayed.

The new host computer for the Hitachi 912 uses the Hitachi 912 computer interface cabled to it to collect data from the analyzer. The operating program purchased to act as the host computer interface, Cache Intersystems, will drop the data from the Hitachi 912 into an excel file on the host computer. The excel file will be linked to the efile received and the data copied to the appropriate specimen field. The efile is visually reviewed by Donna Virgil and if corrections are necessary or a change in the default comment code of 0 is necessary she makes them at this time. The specimen data transferred to the shipping file is reviewed again by both Donna Virgil and Cynthia Wiley prior to submitting results to the database coordinating center.

Submitting Results
Beginning with column I in Excel, the technician inserts results copied from the Excel csv file created by the host computer. Not all columns will apply to every result, and those columns that do not apply should be left blank. The laboratory returns the completed results by sending the Excel attachment to the database coordinating center email account within the defined 21 day limit.

Result Comment Codes
Numerical comment codes are used to indicate valid results, turbidity, insufficient quantity for analysis, results less than the limit of detection, etc. The comment code is listed next to the results column for each assay value submitted

Updating and Deleting Results
If any results already submitted need to be updated or deleted a change reason numerical code is used to resubmit values to the database coordinating center. No data will be changed or deleted without a change reason.

We do not need to version files each time we resend efiles to make updates or corrections. If the lab needs to correct large amounts of data that encompass many containers we must contact the systems analyst at the database coordinating center. We can then transmit the data in one large, single file.

Late Results
We will receive late result email notifications from the database contractor for results that are past due. If our records do not agree with the late results email, we must contact the database contractor to define the discrepancy. If the specimen does not have a result and we must submit a comment code (for example: vial broken) that most closely explains the reason for the null result, the specimen can still be marked as received.

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

A. Specimen handling

1. Collect blood into a red top Vacutainer® blood collection tube.

2. Allow the blood to stand for 45 min at room temperature to allow complete clotting and clot retraction. A shorter period may result in incomplete clotting and secondary clots may form later. During the clotting period leave the collection tube sealed.

3. Centrifuge the samples at 1,500 x g for 30 min at 4ºC. It is preferable to use a refrigerated centrifuge for this purpose, but an unrefrigerated centrifuge can be used if necessary. In either case, the samples should be placed into an ice bath immediately after centrifuging and maintained at 2-4° C thereafter.

4. Samples should be kept frozen at -20ºC, in a non-self defrosting freezer until shipped to the laboratory. If a shipment must be delayed longer than 4 weeks, the specimens should be kept at -80^{o}C. In the event a shipment may have been thawed and refrozen prior to shipment, this should be noted on the transmittal form.

5. Samples are shipped by overnight carrier, such as Federal Express. Samples are not shipped on Friday or the day before a holiday, since the laboratory is closed on weekends or holidays. NCHS provided lists of shipment dates that take account of the weekend and holiday schedule. However, in the event it becomes necessary for the laboratory to receive a shipment on a weekend or holiday, NCHS will inform the laboratory of this, and the laboratory makes arrangements to receive the shipment.

6. Samples are stored at -80ºC until thawed for analysis. Samples are thawed for 45 minutes on a rotating serum mixer and allowed to come to room temperature. An aliquot is first taken for TC, TG and HDLC analysis on the Hitachi 912. All samples verified to be on the shipment log and of sufficient volume are run. Insufficient volume is the only criteria for rejection for samples received according to study protocol. If a shipment was delayed and the samples are received thawed the database contractor is notified and analysis is delayed until a replacement shipment is received in the laboratory.

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

Hitachi 912

B. Other Materials

Serum mixer, transfer pipettes, sample cups, quality control normal and high serum from Solomon Park, SL2 and SL3 series.

C. Reagent Preparation

None, reagent provided as working reagent solution.

D. Preparation of Quality Control Materials

Aliquot thawed, mixed, and transferred to two sample cups for duplicate analysis per run.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

The Hitachi analyzer is calibrated at the beginning of the week and as necessary thereafter. A one point calibration procedure is used for total cholesterol. A frozen serum calibration pool (Solomon Park Laboratories, Kirkland, WA) is used to calibrate total cholesterol.

A. Calibration

1. <u>Full Calibration</u>:
Select Calibration menu button.
Select Status
Choose type of calibration (Start up* or Repeat)
Select test(s) to be calibrated
Choose the appropriate level of calibration by selecting edit (blank, 2point*, or Full)
Select Calibrate now if performing a repeat or timeout calibration, select start up in start conditions for a start up calibration.

2. Reagent/calibrator changes

a. The primary means of following any manufacturer initiated changes in reagent or calibrator formulations is the laboratory quality control system. Such reagent or calibrator changes are generally minor, if visible at all. However, they have the potential for introducing minor abrupt shifts in the laboratory mean, which are normally detected through the QC system. When the laboratory is informed of an impending change, the Laboratory Operations Coordinator will take steps to procure the new reagent or calibrator in sufficient time to perform analyses in parallel with the old and new reagent or calibrator. Such parallel analyses will be conducted over a period of 3-4 weeks in 10 runs, each of which is performed on a different day. Each parallel run will be performed on the same day, and will include the appropriate QC pools and at least 20 specimens, for a total of 200 specimens over the 10 runs. This is accomplished by setting up additional instrument channels with the new reagent or calibrator and analyzing the sample simultaneously in both the normal and new channels.

At the end of the 10 runs, descriptive statistics (mean (SD), %CV) will be determined for each analyte in each QC pool, and a paired t-test will be used to assess the significance of the differences between like analytes in each pool. Descriptive statistics (mean (SD), paired t-tests, and linear regression analyses relating the two arms of the parallel analyses will also be conducted for the affected analytes in the 200 split specimens analyzed with the old and new reagents or calibrators. These data will serve to characterize the effect of new reagent or calibrator formulations on NHANES 2005-2006 analyses.

Note that the laboratory has no control over reagent or calibrator formulations, and will have to use the new reagent if it replaces the current formulation. The data collected above, however, will be useful during the data analysis phase when the desirability of adjusting NHANES 2005-2006 data to account for such systematic bias changes can be considered. Based on past experience, such systematic biases are expected to be minimal and would probably be considered acceptable without adjustment. Nonetheless, the data will be available for such adjustment should this not be the case.

B. Verification

All reagent and calibrator lots are validated with 5-10 samples run with both the old calibrator reagent run values vs. the newly calibrated channel or new reagent lot. All values must be within 5% of the older lot analysis. If the values are greater than 5% the lot is rejected for use.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

A. Sample preparation

Samples are thawed and mixed. Sample is then transferred to sample cup and placed in the sample wheel for analysis.

B. Instrument setup

Triglycerides	Temperature: 37°C
Test	[TRIG]
Assay Code	[1POINT]:[50]-[0]
Sample Volume	[3] [1]
R1 Volume	[250][100][NO]
R2 Volume	[0][100][NO]
Wavelength	[700][505]

Calib. Method	[LINEAR][0] [0]
Std. (1) Conc.-Pos.	[0]-[1]
Std. (2) Conc.-Pos.	[*]-[2]
Std. (3) Conc.-Pos.	[0]-[0]
Std. (4) Conc.-Pos.	[0]-[0]
Std. (5) Conc.-Pos.	[0]-[0]
Std. (6) Conc.-Pos.	[0]-[0]
Unit	[MG/DL]
SD Limit	[0.1]
Duplicate Limit	[200]
Sensitivity Limit	[1100]
ABS. Limit (INC/DEC)	[0][INC.]
Prozone Limit	[0][LOWER]
Expected Value	[0]-[200]
TECH Limit	[0]-[1000]
Instrument Factor	[1.00]

[*] Enter the lot specific calibrator value

C. Operation of Assay Procedure

Daily Check/ Power Up

1. Check levels of detergents
2. Ensure external water supply is on
3. Empty waste container.
4. Check printer paper supply
5. Power on or wake up Hitachi 912
6. Log on to the system
7. Check reagents screen for inventory

Start Up procedures

1. Clean and adjust sample and reagent probes.
2. Clean stir paddles.
3. Perform Incubation Bath exchange.
4. Perform Photometer Check
5. Place 500 µL of System Cleaning Solution in W1 and 500 µL 4N NaOH in W2 on the sample tray.
6. Schedule calibration if necessary
7. Press <Print> to print an appropriate calibrator load list
8. Load calibrators and controls.
9. Verify start conditions
10. Press Start.

D. Recording of Data.

Data is transferred to a 3.5 floppy disk as a dbf file and imported into a dedicated stand alone PC which imports the data into the raw data report. At this point a visual review of the data is done prior to exporting the data to another dbf format file which is then opened in excel. The excel file will be edited to include rundate, runnumber and technician number and a csv file is created. From the comma delimited file the data is copied and pasted into the original excel spreadsheet that is received electronically prior to sample shipment. Once all analyses are compiled into the shipment file it is transmitted to Database coordinating center via email attachment. Each datafile is acknowledged with a processing status reply email letting the lab know if the data imported into the dbase correctly. If notification is made that the file needs editing then edits are made and the file resent and renamed with a higher version number.

E. Calculations

The Hitachi 912 computer uses absorbance signals to calculate analyte concentrations. Specific details are available in the Hitachi Operator's Manual.

9. REPORTABLE RANGE OF RESULTS

LINEARITY: 4 - 3000 mg/dL

When the result exceeds 1000 mg/dL dilute the specimen with 0.9% saline and re-assay.

Report patient results less than 4 mg/dL as < 4 mg/dL.

Report patient results greater that 3000 mg/dL as > 3000 mg/dL.

10. QUALITY CONTROL (QC) PROCEDURES

The Central Laboratory monitors its performance by analyzing quality control sera for which the values have been assigned by the Centers for Disease Control (CDC) Lipid Standardization Laboratory using CDC reference methods. The estimates of analytical error obtained from the analysis of quality control materials are assumed to represent the error of the measurements in survey samples. The control pools are therefore subjected to the same analytical manipulations as the survey samples.

The precision of lipid and lipoprotein analyses is determined from replicate analyses of the control sera in each run. Two control pools, one with normal and one with elevated lipid concentration, are used to monitor the analysis of total plasma cholesterol and triglyceride. Similarly, two levels of control sera are used for HDL-cholesterol, one at the level of about 28 mg/dL and the other at about 50 mg/dL.

A. Control limits

The control limits for each pool are calculated from the overall mean and standard deviation of the run means, and ranges for the pool. Temporary control limits for each pool are calculated from the first 20 run days. Permanent control limits are determined after 50 run days and remain in effect until the pool is exhausted. Continuity between the current and replacement pool is maintained from at least 20 overlapping runs in which both pools are analyzed in parallel. It is from this period of overlap that the 20 run temporary limits are established for the replacement pool. During this period the acceptability of the measurements is based on the current pool. Furthermore, the analyses must be "in control" before the data are accepted for use to establish control limits for the replacement pool. Two types of control charts are prepared for each level of each analyte. The mean chart monitors the deviation of individual run means X from the overall laboratory mean, X. Any shift, drift, or among day variability is assessed from the mean chart. The range, or R chart, monitors within-run variability.

B. Quality Control Pools

Two quality control pools are used to monitor the analysis of total cholesterol and triglyceride. In each case, one pool has normal, and the other elevated concentrations of the respective analytes. An aliquot from each pool is analyzed two times in each run.

C. Introduction of Replacement Control Pools

Before a control pool is depleted, a replacement pool is purchased from Solomon Park Laboratories, Kirkland, WA. These pools have CDC-assigned reference values. Each is analyzed on a minimum of 20 run days (temporary limits) concurrently with the current pool. The mean, standard deviation, and range for the replacement pool are established. During this overlap period, quality control is maintained with the current pool.

Limits for the replacement pool are calculated and evaluated, and control charts are prepared as described in the following sections. Care is taken to assure that data used in the calculations are only from runs that are "in control" i.e. that meet established quality control criteria. As soon as acceptable temporary limits are reestablished, control is transferred to the replacement pool, and the original pool is retired. Permanent control limits are established after 50 run days.

D. Calculation of Control Limits

The Lipid Laboratory uses statistical control charts to evaluate performance and make quality control decisions. Control limits are calculated from the means, standard deviations and ranges as described in this section. It is important that the data used to calculate control limits be collected during a stable analytical period when they are representative of overall laboratory performance.

The daily mean, X, for a control pool is calculated for each run by averaging the replicate values for the pool:
X = sum of control values/number of replicates= x/n

For NHANES 2005-2006, n = 2.
The overall mean for the pool, $\underline{X}$, is calculated by summing the individual run means and dividing by the number of runs, N:
$\underline{X}$ = sum of run means/number of runs = X/N
The overall mean is rounded to the nearest whole number.
N = 20 run days for temporary limits
N = 50 run days for permanent limits

The standard deviation of the run means, S_X, is also calculated for the control pool. The basic equation for calculating standard deviation is as follows:
$S_X = \quad (x - x)^2 /(N - 1)$

The range, R, for each run is the difference between the highest and the lowest value obtained for the pool in that run:
$R = X_{high} - X_{low}$

The average range, R, for a series of runs is calculated by dividing the sum of the ranges for the series by the number of runs:
$\underline{R} = R/N$
N = 20 for temporary limits
N = 50 for permanent limits.

The control limits (99%) for the X chart are calculated as follows:
Upper control limit = $\underline{X} + 3S_X$
Lower control limit = $\underline{X} - 3S_X$

Control limits are rounded to the nearest whole number.

The warning (95%) limits for the X chart are calculated as follows:
Upper warning limit = $\underline{X} + 2S_X$
Lower warning limit = $\underline{X} - 2S_X$

Warning limits are rounded to nearest whole number.
The limits on X are evaluated as described below.
The limits used for the R chart are calculated in a similar fashion.
Range control limit = $\underline{R} + 3 S_r$
Range warning limit = $\underline{R} + 2 S_r$
Where S_r is the standard deviation of $\underline{R}$.

The lower limit for the range chart is zero since there is no negative range.

E. Evaluation of Control Limits

Before the control chart can be used for quality control, it is reviewed to determine that the data have been collected during a stable analytical period. The chart is examined for outliers, for periods of questionable or unstable performance, and for evidence of excessive bias. An outlier will distort the control limits if incorporated into the final calculations. An outlier is considered to be any value of X which falls outside the control limits ($\underline{X} \pm 3S_X$) or any value of R which exceeds the control limit for R. These values are eliminated as are values from any questionable period of performance. The values of $\underline{X}$, S_X, and the control limits are recalculated and the charts are evaluated again.

When values from at least 20 acceptable runs are used for the final calculations, the control charts are constructed according to the criteria listed below. If there are not 20 acceptable runs after eliminating unacceptable data, continue analyzing the pool until at least 20 acceptable runs have been completed.

The criteria used in the Lipid Laboratory were those that served as guidelines for the Lipid Research Clinics Program and are designed to minimize both bias and variability. As used in this manual, the bias of the cholesterol is calculated as the algebraic difference between the $\underline{X}$ and the CDC reference value (RV) for the pool.

F. Construction of Control Charts

A separate control chart is constructed for each analyte in each control pool. Construct each chart so that plots for X and R are arranged one above the other on the same sheet of graph paper. Draw the X line across the entire sheet; draw the warning- and control limits parallel to the X line. At the top of the chart, indicate the CDC reference value. Draw the R line and R limits on the R plot.

Plot the run mean and range values. The chart should be kept current; the values should be plotted after each run. Make liberal use of annotations indicating events that might affect the analyses (personnel changes, reagent problems, changes in instrument components, etc.).

G. Use and Interpretation of Control Charts

Values for X which exceed the $3S_X$ limit or values of R that exceed the range control limit indicate the run is 'out-of-control'. The run must be repeated. Statistically, one in 100 runs can be expected to be 'out-of-control during normal stable operation. A value exceeding the warning limit, but not the control limit, is interpreted as an indication of possible trouble, but does not necessarily require action. Statistically, about one in 20 values will exceed the warning limits.

Table 3 .Precision and Accuracy of TG Control Pools

Pool	Mean	95% limits	99% limits	95% limits (range)	99% limits (range)	Runs
SL2 I132	86	2SD = 5.6	3SD = 8.4	80.4-90.6	77.6-94.4	436
SL3 I180	203	2 SD = 8.12	3SD = 12.18	195-211.1	190.8-215.18	428

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA Do not release patient data when control errors occur.

A. In cases where a single control pool falls outside specified ranges, but calibration is acceptable and the other control pool is acceptable, a decision may be made to repeat 10% of the samples from the technically out of Control run, and if these values are confirmed as "in control" , the run may be accepted. This decision is made by either the Lab Director or the Laboratory/Study Coordinator.

B. When runs are consistently out of control, the calibrators, reagents and other material are checked to make sure they are not out of date. The Hitachi 912 troubleshooting guide is consulted and calibration is repeated.

Replacement control pools are analyzed to obtain temporary limits (20 run days). Final limits are calculated after 50 run days. A new QC graph is prepared each time a pool lot changes and is recreated when limits are created, temporary or permanent.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

Icterus: No significant negative interference from conjugated bilirubin above an I index of 10 and from unconjugated bilirubin above an I index of 27.

Hemolysis:No significant interference from hemoglobin up to an H index of 500.
Lipemia:No significant interference from lipemia up to an L index of 1000. (NA)(Intralipid).

Dilute extremely lipemic samples 1+ 4 with 0.9% NaCl and multiply the result by 5.

13. REFERENCE RANGES (NORMAL VALUES)

< 150 mg/dL	Normal
150-199 mg/dl	Borderline high
200-499 mg/dl	High
>500 mg/dl	Very high

Source: National Cholesterol Education Program, Adult Treatment Panel III;
JAMA, May 16, 2001- Vol 285; 2486-2497.

14. CRITICAL CALL RESULTS ("PANIC VALUES")

Occasionally, a specimen may have a value considered to be a “critical action value (CAV)”. A critical action value is a value that can reflect a life-threatening problem if not attended to promptly. Extremely high triglyceride levels (i.e., levels > 2,000 mg/dL) can be the result of pancreatitis, a potentially life threatening condition. For this reason, the triglyceride panic value for NHANES 2005-2006 is set at 1,000 mg/dL. Values of 1,000 mg/dL or higher are communicated to NCHS by telephone as soon as the values become reportable.

15 SPECIMEN STORAGE AND HANDLING DURING TESTING

Samples are received frozen and stored at -80^{O}C until testing is performed.

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

Samples are held at -80ºC in the freezer in 1379. If a problem occurs and this freezer begins to warm. samples are transferred to the research freezers located in 1358. A service call is placed to repair the freezer in 1379. A loaner freezer is requested for each service repair that removes the freezer from 1379 for any period greater than 1 day.

No alternate test site has been identified. As far as downtime for equipment repairs, the 21 day turnaround time as established in the contract, has always been sufficient enough to allow the repair to occur prior to the deadline for sample analysis. If the repair could not be accomplished in the time frame allowed we will discuss the three options available to us with the Project Officer. One option is to wait until the repair is made if the proposed repair date is agreeable to the Project Officer. T he second option is to perform the analyses in the

Clinical Chemistry Laboratory of the Johns Hopkins Hospital. While the chemistries are identical, they are performed in the Clinical Chemistry laboratory on a larger platform, namely the Hitachi Modular. While this laboratory is CLIA certified it is not a participant in the CDC LSP program. Split sample comparisons are run between the two laboratories on patient samples so relative bias is known. The third option is would be to use the NWRL since it is a CDC referenced laboratory. If necessary the JHU and NWRL laboratories can establish the bias between the two laboratories.

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

For Triglycerides a value of 1000 or more, maybe be considered life threatening depending on the underlying cause, and must be reported to the medical officer. At the current time that medical officer is Kathryn Porter at 301-458-4441.

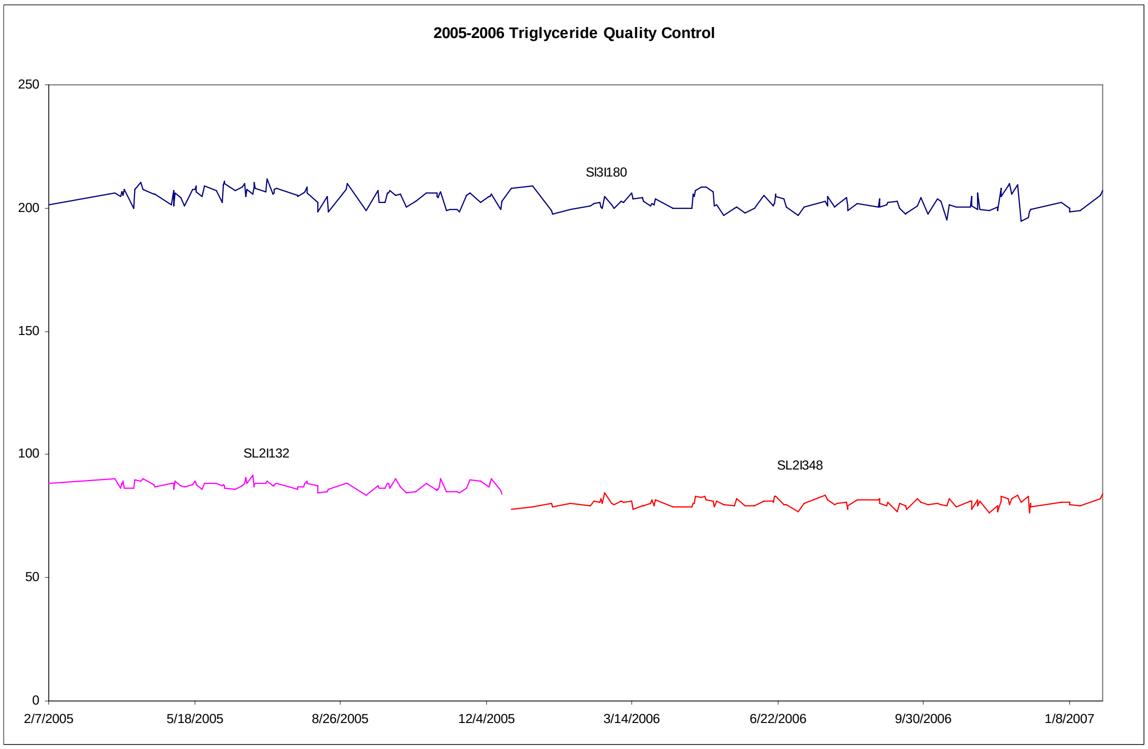
18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

The shipping list that is emailed to the laboratory is used to create runs for the Hitachi Chemistry Analyzer. Shipments are checked against the email shipping lists upon arrival in the laboratory. Problems with vials such as condition, QNS etc are noted upon physical inspection. The number comment codes provided to the laboratory by The database contractor and NCHS for reporting data are used to indicate individual analyte comments. For example, if the individual vial was empty upon inspection then the empty vial code of 18 is entered in the comment field for each analysis requested for that individual specimen ID. The report form for NHANES 2005 is an Excel spreadsheet sent originally as the shipping list email attachment with the data entry columns blank. Data is transferred from the instruments to the spreadsheet and visually checked for transcription errors by the Lab and Study Coordinators prior to email transfer to database coordinating center. The laboratory has 21 days from the receipt of samples in the laboratory to report the specimen data to the database coordinating center. Should the laboratory exceed the 21 day contractual limit, the database contractor notifies the Study Coordinator by email of the individual specimens and the test data owed for each specimen.

19. SUMMARY STATISTICS AND QC GRAPHS

Summary Statistics for Triglyceride by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SL2I132	86	2/7/2005	12/15/2005	87.5	1.6	1.8
Sl3I180	187	2/7/2005	1/31/2007	203.6	3.6	1.8
SL2I348	101	12/21/2005	1/31/2007	80.3	1.7	2.1



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