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CHAPTER 18

CENTRAL LABORATORY PROCEDURES

18.1 Sample Handling in the Laboratory

18.1.1 Receipt of Samples by Laboratory

1. When the samples arrive in the laboratory, they will be logged into the laboratory by batch according to the log sheets. The laboratory will record on the log sheet the date the samples were received and will record the condition of the samples when received (O.K., thawed, etc.) using the appropriate sample condition code (Appendix A).
2. The permanent storage vials will be transferred to a -70°C freezer for permanent storage. If the remaining aliquots are not to be analyzed immediately, they will also be stored at -70°C until they are analyzed. At this time the laboratory will make a note of any missing aliquots in the "Comments" column of the log sheet.

18.1.2 Preparation of Samples for Analyses

The laboratory technician will remove the samples from the freezer, place them upright and allow them to thaw at room temperature. The technician will place the still-sealed vials on a blood wheel or similar mixing device and rotate for 30 min at room temperature to insure complete sample mixing. The technician will then unseal the samples and remove aliquots for the appropriate tests.

18.2 Lipid and Lipoprotein Analysis

18.2.1 Preparation of HDL Fraction

18.2.1.1 Reagents

1. Manganese Chloride Solution, 1.0 mol/L

Manganese chloride is stored in a tightly closed container or in a desiccator to minimize water uptake. The solution is prepared by adding 19.79 g manganese chloride, $MnCl_2.4\ H_2O$, to a 100 mL volumetric flask, dissolving and diluting to volume with distilled water. Solution is stored in the refrigerator and prepared fresh monthly.

2. Heparin, 5000 USP Units/ml (1.3 mg/ml)

A solution of 5000 USP units/ml (approximately 1.3 mg/ml) is prepared by diluting stock heparin with 0.15 mol/L sodium chloride solution. For example, 1.0 ml of a stock solution of 40,000 units/ml is diluted with 7 ml of 0.15 mol/L sodium chloride solution. The weight concentration is approximately 35 mg/ml. Solution is stored in the refrigerator and prepared fresh weekly. (Note: Heparin exerts its maximal effect at a concentration of about 1.1 mg/ml, and no further precipitation is observed up to about 7 mg/ml.

18.2.1.2 Separation Procedure

1. Specimens should be organized in batches of no more than 40 specimens. One vial of the current HDL control pool (AQ) is thawed and mixed on a rotator as described above. The control pool is prepared and analyzed in the same manner as the samples. One batch of specimens is precipitated at a time. Specimens should be allowed to equilibrate to room temperature before pipetting.

2. Two (2.0) ml of each specimen is transferred to an appropriately labeled tube. (Note: A smaller volume of specimens may be used, if necessary. The volumes of precipitating reagents are decreased proportionately.)

3. Eighty (80) ul heparin solution (5000 units/ml) are added to each tube and immediately mixed thoroughly, using a vortex-type mixer. An intermittent mixing action should be used to prevent the formation of separate reagent-sample layers when vortexing. Although foaming should be avoided, mixing is continued for at least 5 sec.

4. To each tube 100 ul of 1.0 mol/L manganese chloride solution is added and immediately mixed as above. A precipitate forms immediately.

5. The tubes are allowed to stand for 30 min in an ice bath, or 10 min at room temperature.

6. The precipitate is sedimented by centrifuging at 1500 x g for 30 min at 4°C.

7. Tubes are removed from the centrifuge and the supernatant immediately examined for turbidity. Turbidity in the supernatant indicates incomplete sedimentation of the insoluble VLDL and LDL complex. This generally occurs in specimens with elevated triglyceride values but may also be observed in specimens with normal triglyceride levels. This occurs when the density of the insoluble lipoprotein aggregate approximates that of the sample, precluding sedimentation by low speed centrifugation. Cholesterol measurement in a turbid supernatant solution will grossly overestimate HDL cholesterol. If the heparin-$MnCl_2$ supernatant is turbid, the original plasma is diluted two fold with 0.15 M NaCl and the heparin-$MnCl_2$ precipitation repeated. Do not dilute the sample more than two-fold because excessive dilution will make it difficult to measure cholesterol in the heparin-$MnCL_2$ supernatant accurately.

8. One (1.0) ml of the clear supernate is carefully transferred to a 1.5 ml microfuge tube. Add 100 ul of one (1) M $NaHCO_3$. The solution is allowed to stand for 10 min at room temperature. The

precipitate is sedimented at 10,000 x g for 10 min and an aliquot removed for HDL-cholesterol measurements (see below).

18.2.2 Calculation of HDL- and LDL-Cholesterol

18.2.2.1 HDL-Cholesterol

The observed cholesterol concentration in each heparin-manganese supernatant is multiplied by the factor 1.18 to correct for the dilution that occurs when the heparin-manganese chloride and $NaHCO_3$ solutions are added. The result is rounded to the nearest integer. The result is then multiplied by the appropriate dilution factor if the serum sample was diluted before precipitation.

18.2.2.2 LDL-Cholesterol

LDL-cholesterol in children is calculated from the equation [LDL-chol] = [total chol]-[HDL-chol]-[TG]/6.5 (the denominator 6.3 is used for adults) where all concentrations are given in mg/dl.

18.2.3 Cholesterol Analysis

Cholesterol in serum or the heparin-$MnCL_2$ supernatant is measured using an enzymatic cholesterol method (Cholesterol CHOD-PAP method, Boehringer-Mannheim Diagnostics, Cat. No. 816302). The analyses are performed on the Hitachi 704 Clinical Chemistry Analyzer.

A. Principle

Approximately two-thirds of the cholesterol in serum is present as cholesterol ester. When the sample is mixed with the cholesterol reagent, the following reactions occur:

a. cholesterol esters $\xrightarrow[\text{esterase}]{\text{cholesterol}}$ cholesterol + fatty acid

b. $\text{cholesterol} + O_2 \xrightarrow[\text{oxidase}]{\text{cholesterol}} \text{cholestenone} + H_2O_2$

c. $H_2O_2 + \text{phenol} + \text{4-aminophenazone} \xrightarrow{\text{peroxidase}}$

4-(p-benzoquinone monoimino)-phenazone + $4H_2O$

The depth of color is proportional to the cholesterol concentration and is measured at 505 nm.

B. Procedure

1. The ID numbers for the samples to be analyzed for total cholesterol (and triglyceride) are keyed into the Hitachi 704 analyzer. Similarly, samples to be analyzed for HDL-cholesterol are also entered into the instrument.
2. A 100 ul aliquot of each sample is placed in the sample cups on the instrument in the order in which they are to be analyzed. Place the quality control samples and calibrators into their assigned positions on the instrument and begin the analysis. The results are printed on the Hitachi printout and are also sent to a laboratory computer file located on a dedicated personal computer.
3. Inspect the results for the quality control samples (see Quality Control section below). If the run is within control limits, the results are accepted and the data are down loaded to the Laboratory Report Form. If the quality control results exceed control limits, the source of the difficulty is identified and corrected, and the entire analytical run is repeated.

18.2.4 Triglyceride Analysis

A. Principle

Triglycerides are hydrolyzed to produce glycerol and fatty acid. The amount of glycerol produced is proportional to the triglyceride concentration. The reactions are as follows:

$$\text{a. Triglyceride} + 3H_2O \xrightarrow{\text{lipase}} \text{glycerol} + \text{fatty acids}$$

$$\text{b. glycerol} + \text{ATP} \xrightarrow{\text{glycerokinase}} \text{glycerol-3-PO}_4 + \text{ADP}$$

$$\text{c. glycerol-3-PO}_4 + O_2 \xrightarrow[\text{oxidase}]{\text{glycerol phosphate}} \text{dihydroxyacetone phosphate} + H_2O_2$$

$$\text{d. } H_2O_2 + \text{+ 4-aminophenazone + 4-chlorophenol} \xrightarrow{\text{peroxidase}} \text{4-(p-benzoquinone monoimino)-phenazone} + 2H_2O + HCl.$$

The depth of color is proportional to triglyceride concentration and is measured at 505 nm.

B. Procedure

1. Triglycerides are analyzed enzymatically simultaneously with cholesterol on the Hitachi 704 analyzer using reagents from Boehringer Mannheim Diagnostics (Triglycerides/GPO, Cat. No. 816370). Triglyceride blanks are measured in the CDC control materials using the same reagent, but without lipase. It is necessary to run blanks in CDC quality control materials because some of these pool acquire significantly high blanks (50-80 mg/dl) during the preparation of the pools.

2. The instrument analyzes triglycerides from the same sample cups used for cholesterol, and sends the results to a hardcopy printout and to a computer file located on a dedicated PC.
3. Inspect the results for the quality control samples (see Quality Control Section below). If the run is within control limits, the results are accepted and the data are downloaded to the Laboratory Report Form. If the quality control results exceed control limits, the results are not accepted and the entire run is repeated after the problem has been corrected.

18.2.5 Priority of Analysis

In the event insufficient sample is obtained for all analyses, the order of priority will be

1. Total cholesterol and triglyceride
2. HDL-cholesterol
3. Apolipoproteins (only on specified samples)

18.3 Apolipoprotein Analyses

ApoAI and B

Apolipoproteins AI and B are measured simultaneously by rate immunonephelometry using a Behring BN100 immunonephelometer. This is a computer controlled automated system. The instrument is calibrated weekly using a sixpoint standard curve. The reagents (wash solution, reaction Puffer, antisera to apoAI and apoB, and calibration serum) are supplied by the manufacturer and are ready to use as received.

A manufacturer-supplied control serum is analyzed at the beginning of each analytical run to ensure that the system operates within specifications. (Note that the manufacturer-supplied pool is not used for day-to-day quality control because a single lot cannot be reliably

procured in sufficient quantity to be useful over a six to 12 month period).

Sample and control pool ID numbers are entered into a controlling computer, after which a sample map showing the location of each sample is printed. This map is used as a guide to place the samples and controls into the proper sample cups.

The samples, controls and reagents are loaded onto the instrument and the run is initiated. Results are automatically printed onto a run sheet which records various test parameters, sample ID numbers, and apoAI and apoB results and flags any analyses that may be unreliable (i.e., off the standard curve, turbid, etc.), as well as quality control values that may be out of limits. The results are then transferred to the DISC log sheets, reviewed for completeness and consistency, and entered into the DISC database for transmission to the Coordinating Center.

Quality control is maintained using several levels of control pools analyzed in quadruplicate throughout the run. Both laboratory prepared and commercial control pools are used. These are procured or prepared in sufficient quantity for use over periods of about a year.

18.4 Quality Control

18.4.1 Internal Quality Control

18.4.1.1 General

The central laboratory has monitored its performance by analyzing bench control materials supplied by the Clinical Chemistry Standardization Section (CCSS) of the CDC. As of December, 1994, because of economic constraints at CDC these pools are no longer supplied by CDC. The pools are now purchased from Solomon Park Laboratories under an arrangement with CDC whereby the pools are prepared by Solomon Park, and the reference values are determined and assigned by CDC as previously.

The cholesterol and triglyceride values are assigned to these pools by CDC using reference methods. The standard curves for cholesterol analyses are based on the use of alcohol/detergent solveilized primary standard cholesterol, a serum calibrator supplied by the reagent manufacturer. Triglyceride standard curves are based upon the use of calibration sera provided by the manufacturer. The estimates of analytical error obtained from the analysis of bench control materials are assumed to represent the error obtained from the analysis of participant samples, and the control samples are therefore subjected to the same analytical manipulation as participant samples.

Precision of lipid and lipoprotein analyses is determined from duplicate analysis of control materials in each run (see Section S.2). The precision of the apoprotein analyses is determined from quadruplicate analyses of the control pools (see Section S.3). For lipid analysis two control pools, one with normal and one with elevated lipid concentration, are used to monitor the analysis of total plasma cholesterol and triglyceride. A third pool with a total cholesterol concentration in the HDL-cholesterol concentration range and a fourth pool with normal concentrations of total and HDL cholesterol are analyzed to monitor HDL cholesterol measurements. The control limits for each pool are calculated from the average and standard deviations of the daily means, and daily ranges of the analyses of the control pools. Temporary control limits for each pool are calculated from the first 20 runs. Permanent control limits are determined after 50 runs and then used until the particular pool is exhausted. Continuity from control pool to control pool is maintained through at least 20 overlapping runs in which the replacement pool is analyzed in parallel with the current pool. 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 analyses are based on the current pool, that is, the analyses must be "in control" before the data are accepted and used to establish control limits for the replacement pool. Two types of control charts are prepared for each level of each analyte. The mean, or $\bar{x}$ chart, monitors the deviation of individual run means ($\bar{x}$) from the overall mean, $\bar{\bar{x}}$. Any shift, drift, or between-day variability is assessed from the X chart. The range, or R chart, monitors within-run variability.

18.4.1.2 Cholesterol and Triglyceride Controls

Two quality control pools (Level 2, Level 3) are used to monitor the analysis of total cholesterol and triglyceride. One pool has normal and the other has elevated concentrations of total cholesterol and triglycerides. Two aliquots from one vial of each pool are analyzed in each run.

18.4.1.3 HDL-Cholesterol Controls

The measurement of HDL-cholesterol is controlled with two different control pools. One pool with a low total cholesterol concentration (Level 1) is used to monitor the cholesterol analyses, per se, in the concentration range of HDL-cholesterol. The other pool (Level 4) has a total cholesterol concentration in the range of 150-170 mg/dl, triglyceride below 100 mg/dl and an HDL-cholesterol of 50-70 mg/dl. As described in Section S.2, one aliquot of the Level 4 pool is prepared with each batch of participant samples. Thus, several aliquots of Level 4 may be analyzed in a single run whereas only two aliquots of the Level 1 pool are analyzed in each run.

The Level 1 pool provides control only for the cholesterol measurement, and the Level 4 pool provides control for the entire HDL cholesterol procedure, including the precipitation. Should an HDL run

be "out of control", the results form the analysis of the two control pools may help to find the source of the difficulty. For example, if the results of the analysis on the Level 1 pool are in control, it is assumed that the analytical procedure for cholesterol measurement was performed properly. If both Level 1 and Level 4 are in control, the results of the HDL cholesterol analyses are accepted and reported. If Level 1 results are out of control, corrective measures are directed at the cholesterol measurement procedure itself, and the heparin-Mn^{+2} supernatants are reanalyzed. On the other hand, if only the Level 4 results are out of control, it is assumed that the precipitation step was at fault, and new heparin-Mn^{+2} supernatants are prepared for analysis.

18.4.1.4 Introduction of Replacement Control Pools: Overlapping Analyses

Before a control pool is depleted, a replacement pool is introduced and analyzed in a minimum of 20 runs concurrently with the current pool. The mean, standard deviation, and range for the replacement pool are established. During this overlapping period, quality control is maintained with the current pool.

Limits for the new 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 20-run limits are established, control is transferred to the replacement pool and the original pool is no longer analyzed. Permanent control limits are established for each pool after 50 runs. These procedures are used for all pools. Since more than one aliquot of the Level 4 pool might be analyzed in a single run,

however, only the first aliquot of this pool is used to establish the 20 or 50 run limits.

18.4.1.5 Quality Control of ApoA-I and LDL-B Analyses

Quality Control of ApoAI and ApoB measurements is presently monitored using several levels of control sera Omega, provided by Technicon Instruments, Tarrytown, NY; Pool 13 laboratory prepared. Note that the Omega pool is a lyophilized material that is no longer available. We have sufficient supplies of this pool on hand for another several years use and will continue to use it for the DISC follow-up visits. This is necessary because it is the only pool currently used that was used in the DISC I study and should allow direct comparison with the earlier analyses. Pool 13 is a frozen pool that is stored at -70°C until needed. We are also evaluating the use of Solomon Park Levels 2 and 3 pools (used for cholesterol and triglycerides) as possible replacements for currently used pools. The Level 2 and Level 3 pools are frozen pools stored at -70°C and should be suitable for apoAI and apoB measurements. When we have sufficient data to calculate 50 run limits for these pools, we will be able to determine their suitability. The control pools for apoAI and apoB are used primarily to monitor the precision and stability of the measurements. There is presently no generally available standardization program for apoAI or apoB. In 1993, however, The World Health Organization (WHO) - International Federation of Clinical Chemistry (IFCC) First International Reference Materials for Apolipoproteins AI and B became available for use by manufacturers in assigning apoAI and apoB values to their calibration materials. These values are supplied with the calibration pools and should allow the apoAI and apoB valves measured in the DISC study to be expressed in terms of WHO-IFCC cased values.

Quality control pools are analyzed in quadruplicate and control limits are calculated as described below.

18.4.1.6 Calculation of Control Limits

The Central Laboratory uses statistical control charts to evaluate performance and make quality control decisions. Control limits for these charts are calculated from the means, standard deviations and ranges as described in the following 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, $\bar{x}$, for a control pool is calculated for each run by averaging the two values for the pool:

$$\bar{x} = \frac{\text{sum of control values}}{\text{number of control values}} = \frac{\Sigma x}{2}$$

The overall mean for the pool, $\bar{\bar{x}}$, is calculated by summing the individual run means and dividing by the number of runs, n:

$$\bar{\bar{x}} = \frac{\text{sum of individual run means}}{\text{number of runs}} = \frac{\Sigma \bar{x}}{n}$$

For temporary limits n = 20; for permanent limits n = 50.

The standard deviation of the run means, $S_{\bar{x}}$, is also calculated for the control pool. The basic equation for calculating standard deviation is as follows:

$$S_{\bar{x}} = \sqrt{\frac{\Sigma(\bar{x}-\bar{\bar{x}})^2}{n-1}}$$

The range, R, for each run is the difference between the highest and the lowest value obtained for the pool in that run:

$$R = |x_1 - x_2| \ .$$

Since only duplicates are used, the range is the difference between duplicates.

The average range, $\bar{R}$, for a series of runs is calculated by dividing the sum of the ranges for the series by the number of runs:

$$\bar{R} = \frac{\Sigma R}{n}$$

Again, n=20 for temporary limits and 50 for permanent limits.

The control limits for the daily means are calculated as follows:

$$\text{Upper control limit} = \bar{\bar{x}} + 3S_{\bar{x}}$$

$$\text{Lower control limit} = \bar{\bar{x}} - 3S_{\bar{x}}$$

Round to nearest whole number.

The warning (95% probability) limits for the daily means for control pools are calculated as follows:

$$\text{Upper warning limit} = \bar{\bar{x}} + 2S_{\bar{x}}$$

$$\text{Lower warning limit} = \bar{\bar{x}} - 2S_{\bar{x}}$$

Round to nearest whole number.

Control and warning limits for the daily ranges are calculated by multiplying R for the pool by the following probability factors which are appropriate for duplicate analyses:

$$\text{Range control limit} = 3.27\ \bar{R}$$

$$\text{Range warning limit} = 2.46\ \bar{R}$$

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

18.4.1.7 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. Look 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 $\bar{x}$ which falls outside the control limits ($\bar{\bar{x}} \pm 3S_{\bar{x}}$ or any value of R which exceeds the upper control limit for R. These values are eliminated as are values from any questionable period of performance. Recalculate $\bar{\bar{x}}$, $S_{\bar{x}}$, and the limits and evaluate again.

When values from at least 20 acceptable runs are used for the final calculations, the control charts are used according to the criteria listed in the table 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 Central 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 or triglyceride in the pool is the difference between that measurement and the CDC reference value (RV) for the pool. The bias is calculated as the difference between $\bar{x}$ and RV.

18.4.1.8 Construction of Control Charts

Construct control charts so that the $\bar{x}$ chart and R chart for each pool are one above the other on the same piece of graph paper (Fig 1). Draw the $\bar{x}$ line across the entire sheet and the limit lines for the $\bar{x}$ chart parallel to the $\bar{\bar{x}}$ line. Draw and label lines to represent the CDC RV. Draw the limit lines and a line to represent $\bar{R}$ on the R chart.

Enter the daily mean and range values on the appropriate chart. The chart should be kept current to be useful. Make liberal use of notes about events which might affect the quality of analyses (personnel changes, reagent problems, changes in instrument components, etc.).

18.4.1.9 Use and Interpretation of Control Charts

Values for $\bar{x}$ which exceed the $\bar{\bar{x}} + 3S_{\bar{x}}$ limit or values of R that exceed the $3.27\bar{R}$ limit indicate the run is out of control. The run must be repeated. A value exceeding the warning limits but not the control limit is interpreted as an indication of possible trouble which should be of concern, but does not necessarily require action. Statistically, about one in 20 values will exceed the warning limits.

Examine the chart for evidence of long term drift and excessive variability. Take corrective actions as necessary.

Excessive within-run variability is detected from the R chart when the R values exceeds the upper control limit, in which case the run is declared out of control, and the analyses must be repeated.

Finally, when $\bar{x}$ falls between the control and warning limits on several occasions, the analyst should be alert for possible drifts. Although drift can usually be detected visually, the following guide lines can be used to detect changes. First, it is unlikely that by chance seven successive $\bar{x}$ values will be on one or the other side of $\bar{\bar{x}}$. If this occurs abruptly or progressively, a change in the usual analytical performance should be suspected. Excess variability may also be indicated on the $\bar{x}$ chart in the form of wider-than-usual deviations of $\bar{x}$ from $\bar{\bar{x}}$, even though $\bar{x}$ still falls within limits. Excessive within-run variability should be suspected if seven or more successive R values exceed the $\bar{R}$ line.

18.4.2 Laboratory Standardization

The Central Laboratory participates in the CDC-NHLBI programs for cholesterol, triglyceride and HDL-cholesterol standardization. This standardization program evolved in large part from the needs and level of laboratory performance that was achieved in the Lipid Research Clinic Program. It consisted of three parts. In each part, the laboratory analyzed frozen serum samples covering a wide concentration range of cholesterol, triglyceride and HDL-cholesterol concentrations.

Structure of Lipid Standardization Program
used when Central Laboratory was standardized

Part	Samples*	Number Frozen Samples*	Number Analytical Runs	Minimum-Maximum Time
I	Evaluation	120	4	2-4 weeks
II	Standardization	336	28	7-16 weeks
	Set 1	168	14	4-8 weeks
	Set 2	168	14	4-8 weeks
III	Surveillance**			
	Annually	288	48	12-14 months

* A single cholesterol and triglyceride determination is performed on each sample.

** Each months, surveillance samples are analyzed at the rate of 24 samples in 4 runs.

18.4.2.1 Part I: Evaluation

This was essentially a pilot phase. Its purpose was to identify any analytical problems and to determine if the laboratory's performance was adequate to proceed to the standardization phase.

18.4.2.2 Part II: Standardization

Part II was an intensive long-range evaluation of the laboratory's performance. Its purpose was to document with blind samples that precision and accuracy could be maintained at acceptable levels over a 2- to 4- month period. The 336 frozen samples for Part II were arranged in two sets and shipped on different dates. Each set consisted of 168 samples to be analyzed in 14 runs in 4-8 weeks. Each run was performed on a separate day. Results were reported to CDC for evaluation after each group of 24 samples was analyzed. After the laboratory completed this phase successfully, it was designated as "Standardized" and then entered Phase III.

18.4.2.3 Part III: Surveillance

Surveillance is an ongoing activity in which the Central laboratory receives 36 samples per quarter with unknown values. These are analyzed at the rate of 9 samples each three weeks in duplicate and the results are sent to CDC for evaluation.

REFERENCES

1. Thompson WO, Thompson PK, Dailey ME. J Clin Invest 5: 573-604, 1928.
2. Waterfield RL. J Physiol 72: 110-120, 1931.
3. Fawcett JK, Wynn V. J Clin Path 13: 304-310, 1960.
4. Stoker DJ, Wynn V, Robertson G. Brit Med J 1: 336-338, 1966.
5. Humphrey KR, Gruemer HD, Lott JA. Clin Chem 23: 1343-1345, 1977.
6. Tan MH, Wilmhurst EG, Gleason RE, Soeldner JS. New Engl J Med 289: 416-419, 1973.
7. Dixon M, Paterson CR. Clin Chem 24: 824-826, 1978.
8. Koerselman HB, Lewis B, Pilkington TRE. J Atheroscler Res 1: 85-88, 1961.

9. Page IH, Moinuddin M. Circulation 25: 651-652, 1962.

10. Laboratory Methods Committee, Lipid Research Clinics Program. Clin Chem 23: 60-63, 1977.

11. Folsom AR, Kuba K, Leupker RV, Jacobs DR, Frantz ID. Clin Chem 29: 505-508, 1983.

12. Peric-Golia L. Lancent 2: 876, 1972.

13. Mann JA, Truswell AS. Am J Clin Nutr 24: 1300, 1971.

14. Denborough MA, Patterson B. Clin Sci 25: 115-122, 1963.

15. Morbidity and Mortality Weekly Report, Volume 34, No. 45, Nov. 15, 1985, Centers for Disease Control, Atlanta.

16. Sniderman AD, Teng B, Jerry M. J Lipid Res 16: 465-469, 1975.

17. Sniderman AD, Shapiro S, Marpole D, Malcolm I, Skinner B, Kwiterovich Jr PO. Proc natl Acad Sci 77: 604-608, 1980.

APPENDIX A

DISC Form 30
Rev. 1 2/10/88
1 Page

DISC SERUM SHIPMENT LOG A.. REPORT FORM (LIPIDS)

Center Name: ______________________ Page No.: __________ File No.: __________

Person who prepared shipment: ______________________

	Month	Day	Year
Date of shipment:	__ __	__ __	__ __
Date of receipt:	__ __	__ __	__ __

	Date of Analysis Month	Day	Year	Technician No.	Run No.
TG:	__ __	__ __	__ __	________	________
TC:	__ __	__ __	__ __	________	________
HDL:	__ __	__ __	__ __	________	________
A1:	__ __	__ __	__ __	________	________
B:	__ __	__ __	__ __	________	________

PARTICIPANT ID AND NAME CODE (AFFIX LABEL)	VISIT NO.	SEX M/F	CONDITION	TOTAL CHOLESTEROL	TRIGLYCERIDES	CHOLESTEROL HDL	CHOLESTEROL LDL	HDL SERUM	APO A-I	APO B	COMMENTS
		_	_	___	___	___	___	_	___	___	
		_	_	___	___	___	___	_	___	___	
		_	_	___	___	___	___	_	___	___	
		_	_	___	___	___	___	_	___	___	
		_	_	___	___	___	___	_	___	___	
		_	_	___	___	___	___	_	___	___	

KEEP A COPY OF THE SHIPMENT LOG FOR YOUR FILES.

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CHAPTER 20

PROCEDURES FOR NON-CLINIC DATA COLLECTION VISITS IN DISC

20.1 HOME VISITS

For those participants who can be visited at their home (or some other convenient location outside of the clinic), clinic personnel should collect as much visit information as possible. The following information (listed in other of priority) should be considered as the minimum data collection for every visit and should be collected unless the participant refuses.

20.1.1 Height

Height can be accurately measured using the DISC portable stadiometers. The standard DISC measurement procedures should be followed and measurements taken by certified observers. Whenever possible, two certified observers should be present to measure height, as specified in the height measurement procedures in Chapter 10. A single observer is acceptable, but should be avoided if at all possible.

20.1.2 Weight

Weight can be measured using the Seca model 770 electronic scale. As with height, the standard DISC measurement procedures should be followed and measurements taken by certified observers.

20.1.3 Blood Draw (when required for visit)

A blood draw can be performed so that serum can be sent for lipid, apolipoprotein, CBC and sex hormone determinations. For the blood draw, the tourniquet should not be left on the arm more than two minutes and the blood should be centrifuged within two hours. If at all possible,

three red-top vacutainers (two 15 ml and one 10 ml tubes for 40 ml of blood to provide serum for lipid and apolipoprotein determines at the Central Lipid Laboratory and sex hormone assays) and one lavender-top vacutainer (for local CBC determinations) should be collected.

Blood collected by non-DISC personnel should be sent to the DISC clinic for checking, packaging, and the addition of study forms. Only DISC clinics are permitted to send blood to the DISC Central Laboratory.

20.1.4 Menses Data (when required for visit)

Menses data collection telephone contacts and mailings can be accomplished following usual procedures. Review of calendars with participants can be performed in-person at home or by telephone interview. Participants' mothers can assist with all aspects of menses data collection. When calendars are reviewed by telephone, it may be helpful to talk to both the girl and her mother.

20.1.5 Medical History and Tobacco Use

The medical history form can be completed to determine the use of medications. This form can be completed during the home visit or over the telephone.

20.1.6 24-Hour Dietary Recalls (when required for visit)

Three 24-hour recalls can be collected through telephone interview or, if possible, with one fact-to-face interview followed by two telephone interviews.

20.1.7 Physical Activity Assessment (when required for visit)

Physical activity assessment can be collected either in person or through telephone interview.

20.1.8 Maturation Assessment

Maturation assessment must be collected by trained and certified clinic personnel. The assessment of maturation may depend on the availability of a private setting in the non-clinic environment.

20.1.9 Complete Anthropometry (when required for visit)

Complete anthropometry (including skinfolds and circumferences) must be collected by trained and certified clinic personnel. These measurements may depend on the availability of a private setting in the non-clinic environment.

20.1.10 Blood Pressure (when required for visit)

Blood pressure must be collected by trained and certified clinic personnel. The measurement of blood pressure may depend on the availability of a quiet setting in the non-clinic environment. The random-zero sphygmomanometer should still be used if it can be transported properly. If transportation is a problem, a standard sphygmomanometer can be used.

20.1.11 Psychosocial Assessments (when required for visit)

Psychosocial assessment can be collected during the visit or through telephone interview.

20.2 DISTANT VISITS

20.2.1 Visits Completed by DISC Personnel

For visits in which DISC staff can travel, the staff person certified for the greatest number of procedures should carry out the visit. This should be conducted in the same manner as home visits (see Section 20.1 above). If necessary, local help can be obtained to carry out the venipuncture and specimen preparation.

20.2.2 Data Collected by Non-DISC Personnel

For those visits too far away from the clinic for clinic personnel to collect the information, attempts should be made to enlist the help of a local research project or medical facility to collect the essential information for DISC. The supplies for the visit (i.e., forms and lab supplies with appropriate ID labels already in place) should be sent to the cooperating medical facility in advance of the visit. The information (listed in order of priority) collected for every visit should include:

20.2.2.1 Height

Height may be measured using the best available local instrument. The accuracy of these instruments will vary greatly, but they should be within a reasonable distance of the true measurement.

20.2.2.2 Weight

Because weight scales in a medical setting are frequently certified by local health authorities, most scales are reasonably accurate and can be use for this purpose in DISC.

20.2.2.3 Blood Drawn (when required for visit)

If it is possible to arrange for a blood draw and shipment of the specimen, a single serum tube (15 ml of blood) for the lipid and apolipoprotein determinations is acceptable, but three tubes (40 ml of blood to provide serum for the lipid and apolipoprotein determinations as well as for the sex hormone assays) are preferable. If it is possible to obtain a local CBC (with the results sent to the DISC clinical center), an additional lavender-top vacutainer should be collected. For the blood draw, the local technician should be instructed in DISC procedures: tourniquet tightened for less than two minutes

and released when blood appears. The participant should be supine. Blood should be centrifuged within two hours and shipped by overnight service to the local DISC center for processing. Serum should be kept cold but not frozen unless a serum vial is available.

20.2.2.4 Menses Data (when required for visit)

Depending on the distance and how long it takes for mail to be received, it may be possible to accomplish menses data telephone contacts and mailings following usual procedures. If the distance is too great or mail is to slow, calendars can be completed by weekly telephone contacts. If the participant lives outside the continental United States and a clinic visit including blood drawing will be completed, menses data collection may be attempted. In this situation, the girl and her mother can be sent a full set of calendars for the weeks around the scheduled blood drawing when menses data would usually be collected and can be asked to mail the completed set of calendars back to the clinic when the girl starts her next menstrual period after the blood draw. Mothers should be encouraged to assist their daughters in completing the calendars. Additionally, if at all feasible, clinic staff should call the participant and her mother at least once during the weeks of menses data collection to make sure they are not having any problems completing the calendars. Review of calendars with participants and their mothers for all distant visits can be completed by telephone interview.

20.2.2.5 Medical History and Tobacco Use

The medical history form can be completed over the phone with the child.

20.2.2.6 24-Hour Recalls (when required for visit)

Three 24-hour recalls can be collected through telephone interview.

20.2.2.7 Physical Activity Assessment (when required for visit)

Physical activity assessment can be collected through telephone interview.