CHAPTER 23

LABORATORY QUALITY CONTROL IN DIETARY TRIALS

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Abbreviations

CDC	Centers for Disease Control and Prevention (US Public Health Service, Atlanta, GA)
CV	Coefficient of variation
CV_a	Coefficient of analytical variation
CV_p	Coefficient of physiological variation
$CV_{spec, tot}$	Coefficient of total variation in serial specimens (quantity of biological material obtained on a given occasion; divisible in aliquots or samples that may be analyzed or stored)
EDTA	Ethylenediaminetetraacetic acid
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
NCEP	National Cholesterol Education Program (National Heart, Lung, and Blood Institute, Bethesda, MD)
SD	Standard deviation
TE	Total error
$\bar{x}$	Mean of an individual analytical run
$\bar{\bar{x}}$	Overall mean of the individual run means
$\bar{x}_s$	Mean of serial measurements

The design and implementation of well-controlled diet studies are perhaps among the most demanding scientific endeavors. The logistics and cost of such studies as well as the validity of their conclusions depend in large part on the reliability of the primary measurements. Measurements of diet can include determining the nutrient composition of foods or diet composites and assessing the amount of food consumed by individual participants. Such measurements require a host of analytical methods and can be difficult to make accurately and precisely.

It is also necessary to determine the effects of dietary manipulations on concentrations of components of interest in biological specimens. This process requires measuring these components before and after dietary treatment and determining the magnitude and significance of the changes. Food composition, dietary intake, and measurements of blood and other biological specimens require the collaboration of investigators with different kinds of expertise and are made with a variety of analytical techniques. The one common requirement of the measurements, however, is the assessment of bias and imprecision, and how they affect the study data.

Quality control is fundamental to the conduct of dietary studies. In general, the more reproducible the measurements, the smaller the changes that can be observed in response to treatment, and the more reliably they can be estimated. This, in turn, influences the number of participants that must be enrolled and the number of measurements required per participant. In addition, it is often desirable to compare data collected in different studies. This comparison is facilitated by basing the measurements on accepted reference methods and specifying laboratory bias when the data are reported. For these reasons, it is important that measurement issues be confronted in detail as the study is being planned.

Quality control issues commonly are considered primarily in terms of minimizing laboratory error. This is certainly a major goal of any quality control system, but much broader issues are involved. Laboratory error per se is only one contributor to the error of the measurements; for most measurements, it is not even the major contributor. Issues pertinent to the chemical analysis of diets are discussed in Chapter 22. This chapter uses the example of the major sources of variation associated with lipid and lipoprotein measurements in blood to illustrate how the principles of quality control apply regardless of what is being measured or where the measurements are made.

SOURCES OF VARIATION

The factors that contribute to changes in the measured values of biological parameters such as blood lipids and lipoproteins can be broadly separated into three major categories: (1) laboratory error; (2) normal physiological variation; and (3) response to treatment. Of these categories, the first two determine the reliability with which the third (response to treatment) can be measured.

Laboratory Error

Laboratory error refers to a reported value that is wrong; for example, when the reported cholesterol concentration of a particular specimen does not reflect its true concentration at the time the specimen was drawn. The error can result from measurement error per se but can also result from improper sample preparation, identification, storage, or transport to the laboratory. Such factors can produce inaccurate results but may be beyond the control of the laboratory. The lack of control is not surprising because in many cases the individuals charged with drawing blood specimens, preparing and storing serum or plasma, and transporting or shipping the specimens to the laboratory may not have formal laboratory training and may not be aware of the various factors that can produce an inaccurate result.

This unawareness is of some concern, because the consequences of improper sample handling can be difficult to detect. It is prudent in the initial phase of a study to conduct a training session in which the individuals charged with blood drawing and sample handling are instructed in the proper techniques of patient preparation and specimen collection, and are made aware of how to document and preserve the integrity of the sample until it is received by the laboratory. Instruction should be followed by a small pilot study in which these individuals are asked to obtain, prepare, document, and ship specimens to the laboratory. The performance of these personnel and the condition of the specimens when they arrive in the laboratory are then assessed, and any necessary corrections or alterations of the protocol are made before the main study begins. Such a pilot study also allows the laboratory staff to practice any special handling or documentation procedures that may be specific to the study. Although the pilot study requires some commitment of time and resources, it can prevent unnecessary delays later in the study and help protect the validity of the measurements. The pilot study should be included in the planning phase of the study.

Bias and Imprecision

Errors can also occur after sample collection. Under the best of circumstances there is always some degree of uncertainty associated with a laboratory measurement. *Bias* refers to the proximity of the measured value in a particular specimen to the true concentration in that specimen and depends in large part on proper test calibration. The assessment of bias requires the use of appropriate serum control pools that contain known concentrations of the components of interest and can be analyzed along with the participant specimens.

Imprecision refers to the reproducibility of several measurements in the same specimen. When a component is assayed several times in a specimen, the individual measurements will usually differ somewhat because of variations in the delivery of specimen or reagent volumes, lot-to-lot variations in reagent preparations, instrument function, or other factors. The influence of imprecision can be reduced by making replicate measurements of each specimen and averaging the values. In practice, this is rarely done for either research or routine clinical purposes because of the time and expense involved.

Consider the example of total serum cholesterol. A specimen is generally analyzed once, and the result is assumed to be correct within certain limits that are defined by the bias and imprecision of the analytical procedure. Part of the function of the laboratory quality control system is to define these limits. It is not possible to say with absolute certainty that any particular value is correct. Instead, staff try to minimize the probability that the result is outside the acceptable error limit. The overall reliability of the laboratory results is generally stated in terms of the bias and imprecision of the measurements. Such statements of reliability do not refer to measurements in particular samples but rather to the average proximity of the measurements in specimens during the course of the study to their true values.

For cholesterol, bias and imprecision are monitored through the use of at least two serum control pools with known cholesterol concentrations, one in the 180 mg/dL to 200 mg/dL range and the other in the 240 mg/dL to 280 mg/dL range. Aliquots of each pool are included in each analytical run and each is analyzed at least in duplicate. The mean value for each pool is calculated and the values are used to indicate bias in that run. When considered along with the quality control results from a series of analytical runs, the daily means are also used to estimate run-to-run variation as well as the average bias of the measurements made in specimens in those runs. The difference between the highest and lowest value in a single run, referred to as the *range*, is used as a measure of the reproducibility of the measurements in that run.

Quality control results are displayed visually, as illustrated in Figure 23–1. In the example shown in the figure, the serum control pool is assumed to have a true cholesterol concentration of 200 mg/dL. Each point indicates the mean value of an individual run ($\bar{x}$). The chart also indicates the mean of the individual run means, ($\bar{\bar{x}}$), or the overall mean. In this example this overall mean is 202 mg/dL. The overall mean is usually referred to as the *laboratory mean*. The laboratory bias, in this case +1%, can be calculated from the following equation:

$$\% \text{ bias} = [(\bar{\bar{x}} - \text{true value}) \div \text{true value}] \times 100$$

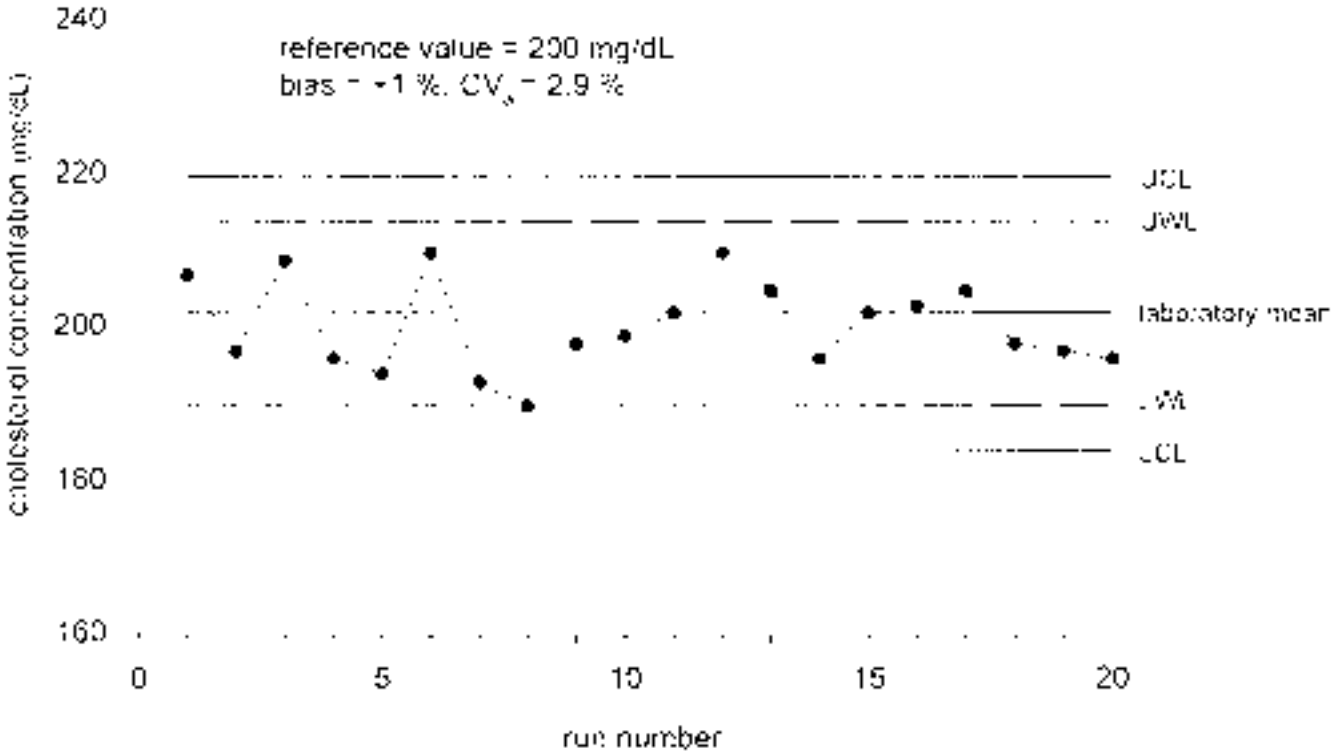

FIGURE 23-1. Sample quality control chart. UCL, LCL, upper and lower control limits, calculated as $\bar{\bar{x}} \pm 2.58$ SD; UWL, LWL, upper and lower warning limits, calculated as $\bar{\bar{x}} \pm 1.96$ SD. In practice, the multipliers are usually rounded to the next whole number. CV_a, coefficient of analytical variation.

The standard deviation (SD) of the run means is 5.9 mg/dL. The imprecision of the measurements is expressed in terms of the coefficient of variation (CV), defined as

$$\%CV = (SD \div \bar{\bar{x}}) \times 100$$

In this case, the CV is 2.9%. The dotted lines on the chart mark the 95% limits, calculated as

$$95\% \text{ limits} = \bar{\bar{x}} \pm 1.96(SD)$$

The heavy solid lines correspond to the 99% limits, which were calculated from the equation

$$99\% \text{ limits} = \bar{\bar{x}} \pm 2.58(SD)$$

In practice, the multipliers are usually rounded to the next whole numbers, 1.96 to 2 and 2.58 to 3.

The laboratory mean and control limits are calculated when sufficient data have been accumulated for the pool, generally after 20 to 50 runs. The true value (also called the reference value) for the pool is assigned using a recognized reference method if available. In the case of total cholesterol, the basis for assigning true values is the reference method for cholesterol (1) used by the Clinical Chemistry Standardization Section, Centers for Disease Control and Prevention (CDC), Atlanta, Ga. Bias estimates determined from the control pools are used to describe the average accuracy of measurements for the specimens analyzed in those particular analytical runs. When investigators report study data, it is common to summarize the bias estimates for the entire data set from the quality control measurements made during the course of the entire study. The CV expresses the SD of the individual run means as a percent of the laboratory mean. This is a convenient way of expressing the imprecision without having to specify the concentration of the particular serum pool used. For cholesterol, CV values are fairly similar over the concentration range of interest. It should be noted, however, that %CV defines the range in which approximately two-thirds of the measurements can be expected to fall, ie, %CV reflects $\bar{\bar{x}} \pm 1$ SD.

Limits of Acceptability

Limits of acceptable laboratory performance for the measurement of total cholesterol have been defined by the National Cholesterol Education Program (NCEP) Laboratory Standardization Panel (LSP) (2). Subsequently, the NCEP Working Group on Lipoprotein Measurement defined limits of acceptbability for triglyceride and HDL- and LDL-cholesterol measurements (3). These guidelines are summarized in Table 23–1. For cholesterol, acceptable *bias* was defined as $\pm$ 3% with respect to CDC reference values; acceptable *precision* was defined as a CV $\leq$ 3% (2). This means that

TABLE 23-1

National Cholesterol Education Program Recommendations for Lipid and Lipoprotein Measurement

Component[1]	% Bias	% CV_a[2]	Total Error[3]
Total Cholesterol	≤ 3%	≤3%	≤ 9%
Triglyceride	≤ 5%	≤5%	≤15%
HDL-cholesterol	≤10%	≤6%[4]	≤22%
LDL-cholesterol	≤ 4%	≤4%	≤12%

[1]From US Department of Health and Human Services (2) and Working Group on Lipoprotein Measurement (3).
[2]CV_a: coefficient of analytical variation.
[3]NCEP recommendations use total error as the primary criterion to determine the limits of acceptable performance. Total error accounts for bias and imprecision at the same time, and in each case, the bias and CV_a shown are examples of values that would satisfy the limits for total error. A greater bias requires a lower CV_a in order to satisfy the goal for total error. Conversely, a larger CV_a would require a smaller bias to meet the requirements for total error. Laboratories that operate within the bias and imprecision limits shown also meet the goal for total error. The values shown for total error assume maximum allowable bias and CV_a, and a 95% confidence limit for CV_a.
[4]$CV_a \geq 42$ mg/dL; at lower HDL concentrations, it is recommended that SD ≤2.5 mg/dL.

average laboratory bias should be within 3% of true values and that two-thirds of the run means should be no more than 3% from the laboratory mean ($\overline{\overline{x}}$). If measurement error is completely random and the laboratory measurements are stable, the individual run means ($\overline{x}$) are expected to fall above and below the laboratory mean ($\overline{\overline{x}}$) with equal frequency (Figure 23–1).

By definition, 1% of the values would be expected to fall outside the 99% limits when the assay procedures are operating properly. The laboratory control limit is generally set at the 99% limit. Although the results are expected to exceed this limit occasionally, such an occurrence would be uncommon, and it is usual practice to consider any analytical run that falls outside this limit to be ''out of control.'' The results from such a run would not be accepted, and the analyses would be repeated. The 95% limits define the warning zone; a single analytical run falling above the 95% limit and below or on the 99% limit would be accepted; however, two or more sequential runs falling in this area would be cause for concern. The second and subsequent runs would be considered ''out of control,'' and the laboratory would initiate troubleshooting procedures.

Total Error

The overall reliability of a laboratory measurement can also be expressed in terms of *total error* (TE), which is a single parameter that accounts for bias and imprecision at the same time. TE is calculated as follows:

$$TE = \% \text{ bias} + (1.96 \times \%CV)$$

For a laboratory operating at the extremes of the NCEP criteria (Table 23–1), the total error for cholesterol would be 8.9%:

$$TE = 3\% + (1.96 \times 3\%)$$

Again, 1.96 can be rounded to 2, producing a total error estimate of 9%.

In actual practice, most well-controlled laboratories that use modern automated methods to measure total cholesterol are capable of accuracy within 1% to 2% of reference values and CV values in the range of 1% to 2%. (There are many other types of measurements that cannot achieve the level of precision that is possible for cholesterol assays.) Figure 23–2 illustrates the analytical variation for a laboratory with a positive bias of 1% and a CV of 1.1%. The bias and CV used for this example were obtained in one of the authors' (PSB) laboratories. In this case, TE would be:

$$TE = 1\% + (.96 \times 1.1\%) = 3.2\%$$

Normal Physiological Variation

When an individual is in a steady state (ie, consuming a regular diet, not losing or gaining weight, pursuing a normal routine of activity), and a component such as cholesterol is measured in different specimens taken on several occasions, the measured values will differ somewhat but cluster around a mean value that can be considered the ''usual value'' for that individual. As shown earlier, part of this variation arises from the process of making the measurements. In addition, however, lipid and lipoprotein concentrations fluctuate throughout the course of normal daily activity, contributing to the temporal fluctuations observed in a particular individual (4). This would occur even in the absence of analytical error. Such normal fluctuations occur for various reasons, including recent food intake, postural changes that occur throughout the day, and small seasonal variations (5–13).

For this reason, it is incorrect to speak of the participant's cholesterol concentration as a fixed value. Rather, it is more accurate to consider the participant's usual (ie, average) cholesterol concentration or, better, his or her range of concentrations. The factors that contribute to normal physiological variation are not completely understood, but several, including postural and postprandial changes, have been examined.

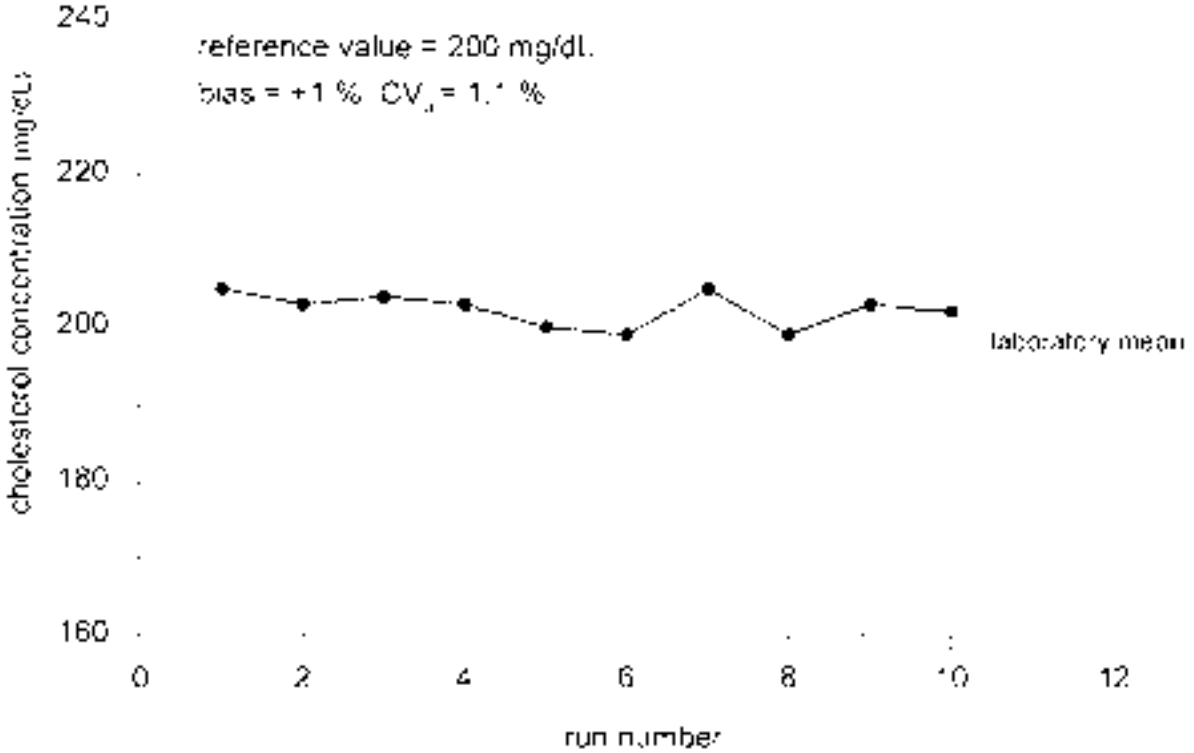

FIGURE 23-2. Analytical variation. The values represent the cholesterol concentration of a serum control pool with a reference value of 200 mg/dL as measured in a laboratory with a 1% positive bias. The laboratory mean is shown by the horizontal line at 202 mg/dL. CV_a, coefficient of analytical variation.

Postural and Postprandial Effects

The changes that can occur in lipid and lipoprotein concentrations when a standing participant assumes a sitting or recumbent position are shown in Table 23–2. The magnitudes of posture-related changes can vary among individuals, but on average, cholesterol concentration decreases approximately 5% when a standing participant sits and about 10% when a standing participant reclines (13). Similar changes are observed for HDL cholesterol. Triglyceride changes, however, are larger; triglycerides decrease almost 10% when a standing individual sits and almost 20% upon reclining. These changes begin to occur immediately, are about half-maximal after 5 to 10 minutes, and maximal after about 20 to 40 minutes. The changes are reversed over similar periods when the individual resumes the standing position (13).

Recent intake of a fat-containing meal has no measurable effect on total cholesterol (2), but can cause a marked transient increase in triglycerides, as well as smaller but significant transient decreases in LDL and HDL cholesterol (5–7). Triglycerides rise because chylomicrons are released to the circulation. Lipoprotein cholesterol decreases as the consequence of compositional changes that occur in the plasma lipoproteins as the chylomicrons are metabolized (5, 6). The magnitudes of the changes depend on the amount of fat ingested and are greater when the fat is administered in the form of a liquid mixture (5, 6) than when presented

TABLE 23-2

Postural Changes in Plasma Total Cholesterol, Triglycerides, and HDL Cholesterol[1]

	Decrease Relative to Standing Subjects (%)[2]	
Component	**Sitting**	**Reclining**
Total cholesterol	5	10
HDL cholesterol	7	8
Triglycerides	10	18

[1]Data from Bookstein L, Gidding SS, Donovan M, et al (11).
[2]Maximal changes are observed 20 min to 40 min after changing position.

in the usual form as a meal (7). Plasma triglyceride and lipoprotein cholesterol levels eventually return to baseline after the chylomicrons are removed from the circulation.

In the case of lipid and lipoprotein measurement, postural and postprandial changes are of sufficient magnitude that they can influence study findings if they are not taken into account. In order to minimize physiological variations from these sources, the participant is asked to fast before blood is drawn. As a matter of convenience for the patient, the NCEP Expert Panel on the Detection, Evaluation, and Treatment of Hypercholesterolemia in Adults (14) has recommended a fasting period of at least 9 hours. This is sufficient for *clinical* purposes but, as discussed by the NCEP Working Group on Lipoprotein Measurement (3), can produce some degree of systematic error in estimating fasting triglyceride and lipoprotein concentrations.

For *research* purposes, we recommend using a 12-hour fasting period. Water can be taken during this period, and required medications are generally not restricted. The posture used for blood sampling should be standardized. The sitting position is usually used, and the participant should be allowed to sit quietly for 5 or 10 minutes before blood sampling. When circumstances require drawing blood from a reclining participant, the same position should be used each time that participant is sampled.

Measurements in Serum vs Plasma

Lipoprotein concentrations can also differ depending on whether the measurements are made in serum or EDTA plasma (15, 16). EDTA (ethylenediaminetetraacetic acid) is the anticoagulant of choice for lipoprotein measurement, because it inhibits oxidative and other changes in the lipoproteins. Measurements in EDTA plasma, however, are 3% to 5% lower than in serum (15, 16). This is because of the osmotic effect of EDTA, which causes a slight shift of water from blood cells to the plasma. Anticoagulants such as citrate or oxalate, or additives such as fluoride, exert much larger osmotic effects and should not be used when lipids or lipoproteins are to be measured.

Lipid and lipoprotein measurements can be made in heparin plasma, however, because heparin exerts no significant osmotic effect when used in concentrations needed to prevent coagulation. Lipid and lipoprotein measurements made in heparin plasma are equivalent to those obtained in serum. For many clinical and research purposes, cholesterol, triglycerides, and HDL and LDL cholesterol can be measured either in serum or plasma. The two should not be used interchangeably, however; either serum or plasma should be used in any particular study.

Venous vs Capillary Samples

For research purposes lipid and lipoprotein measurements are best made in venous samples. Normal physiological variations observed in capillary (or whole blood) specimens appear similar to those in venous specimens (17). The analytical variation associated with capillary or whole blood measurements, however, is greater for venous measurements (17–19). This generates higher total variability for capillary and whole blood measurements, which may be particularly relevant for feeding studies in which the effects of dietary intervention may be modest.

The Comparative Magnitude of Physiological and Analytical Variation

Physiological variations cannot be eliminated, but they can be minimized by controlling preanalytical factors such as posture, fasting, and the time of day the sample is obtained. It is useful to have some idea of the average magnitude of normal physiological variation and the extent to which physiological variation itself can vary among individuals.

Normal physiological variation in an individual can be determined by measuring the component of interest on several occasions when the individual is in a steady state. For total cholesterol, the measurements would be made in serial specimens taken from the individual at least 1 or 2 weeks apart. The mean ($\overline{x}_s$) and standard deviation (SD) of the serial measurements is calculated and a coefficient of variation for that individual is derived:

$$CV_{spec,tot} = SD / \overline{x}_s \times 100$$

where $\overline{x}_s$ is the mean of the measurements in serial samples and $CV_{spec,tot}$ is the coefficient of *total variation* for specimens from that individual, which includes both physiological and analytical sources of variation. Physiological variation can be estimated by adjusting $CV_{spec,tot}$ for the analytical component of variance, as determined by the laboratory from the quality control measurements (2, 3). The adjusted value, CV_p, represents the coefficient of *physiological variation* for the participant and can be approximated fairly closely from the equation:

$$CV_p = [(CV_{spec,tot})^2 - (CV_a)^2]^{1/2}$$

where CV_a is the coefficient of *analytical variation* and is fairly constant with concentration.

The data in Table 23–3 illustrate the 50th (median), 75th, and 95th percentiles for the CV_p values of lipids and lipoproteins as estimated by Kafonek, Derby, and Bachorik (4). The median CV_p for total cholesterol was 5%; those for HDL and LDL cholesterol were 7.1% and 7.8%, respectively, and that for triglycerides was about 18%. As is evident from the table, though, the CV_p values in many individuals were considerably higher.

Figure 23–3 illustrates the contribution of laboratory variation to the total variation observed in cholesterol values analyzed in serial samples from the same individual. The figure assumes a mean measured cholesterol concentration of 202 mg/dL, CV_a of 1.1%, and $CV_{spec,tot}$ of 5.1%. This corresponds to CV_p of 5.0%. The dotted line illustrates the variation that would be observed if normal physiological variation were zero, that is, if the participant's true choles-

TABLE 23-3

Coefficients of Physiological Variation (CV_p) for Lipids and Lipoprotein Cholesterol[1, 2]

	CV_p (%)		
Component	50th Percentile	75th Percentile	95th Percentile
Total cholesterol	5.0	9.0	14.0
HDL cholesterol	7.1	13.7	24.5
LDL cholesterol[3]	7.8	11.5	20.0
Triglycerides	17.8	26.3	43.6

[1]Data from Kafonek SD, Derby CA, Bachorik PS (4).
[2]128 participants, 3 specimens/participant taken over an average period of 20 weeks.
[3]Calculated using the Friedewald equation (20).

terol concentration were the same on each occasion. In the absence of physiological variation, fluctuations in the values reported in serial samples would be entirely attributable to measurement error and would be expected to fall within a 95% confidence interval of (202 ± 1.96 × 1.1) mg/dL, or 198 mg/dL to 206 mg/dL. On the other hand, the measured values, marked by the symbols and solid line, illustrate the variation that would be observed when the measurements include the contributions of both analytical and physiological sources of variation. The measured values would fall within a 95% confidence interval of (202 ± 1.96 × 5.1) mg/dL, or 181 mg/dL to 223 mg/dL. From the figure it can be appreciated that laboratory error, on average, would contribute relatively little to the observed differences in cholesterol values on different occasions. Indeed, it can be calculated for the present example that the analytical variance would contribute about 5% to the total variance of measurements made in serial specimens from the same individual.

Table 23–4 illustrates the expected contributions of laboratory error to the overall variance of the measurements for total cholesterol, triglycerides, and HDL and LDL cholesterol assuming median values for CV_p.

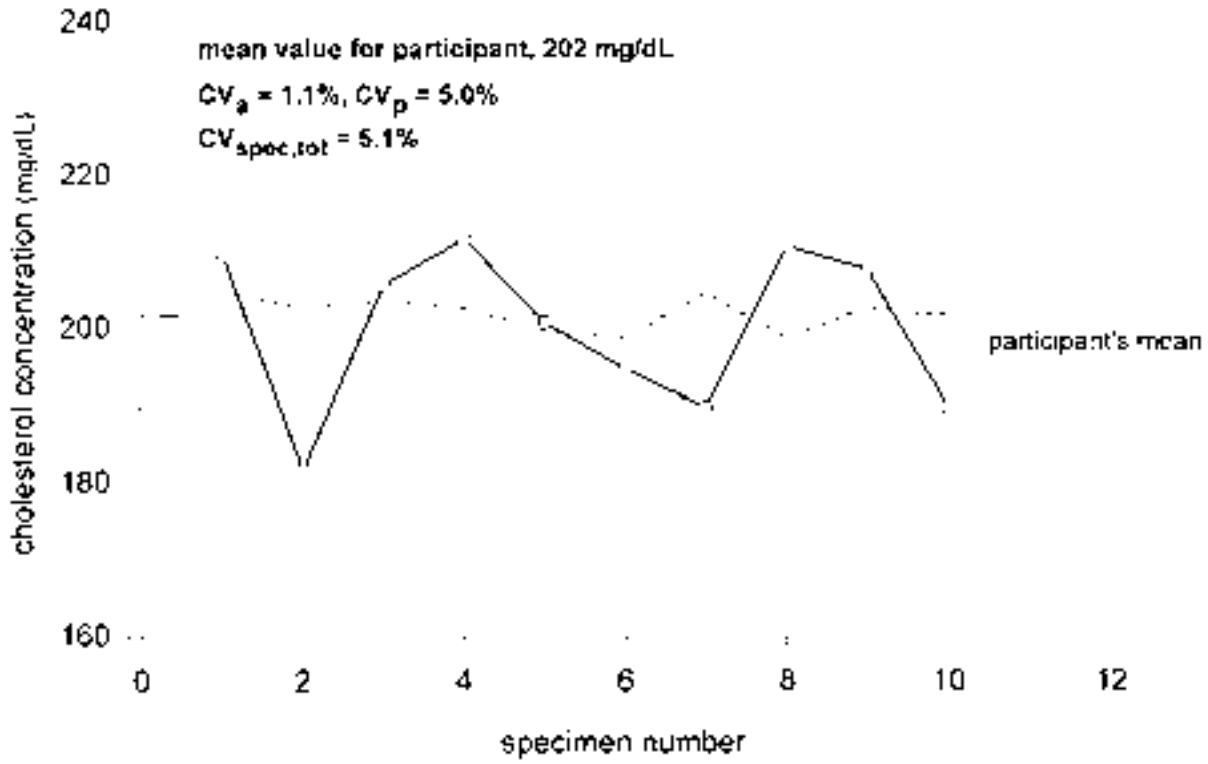

FIGURE 23-3. Serial measurements in 10 specimens from a single individual (o–o). Also shown (.....) is the expected contribution of the analytical component of variation to the overall variation of the serial measurements, ie, the variation that would be observed if physiological variation were zero. The figure assumes the coefficients of variation indicated. CV_a, CV_p, and $CV_{spec,tot}$, coefficients of analytical, physiological, and total variation, respectively.

TABLE 23-4

Contribution of Analytical Variance to Total Variance of Lipid and Lipoprotein Cholesterol Measurements in Participants with Median CV_p Values

Component	% CV_p[1]	% Variance Contributed by Analytical Variation[2]
Total cholesterol	5.0	4.6
HDL cholesterol	7.1	8.0
LDL cholesterol	7.8	14.6
Triglycerides	17.8	1.2

[1]Data from Kafonek SD, Derby CA, Bachorik PS (4).
[2]Assumes CV_a values as follows: total cholesterol, 1.1%; HDL cholesterol, 2.1%; LDL cholesterol, 3.1%; triglyceride, 2.0%, as observed in one of the authors' (PSB) laboratories.

Response to Treatment

The effects of normal physiological variation discussed earlier assume that the participant is in the steady state. Participation in any study, however, can be expected to alter the steady state to a greater or lesser extent, depending on how much the participant must change his or her usual, day-to-day routine. For this reason, a ''run-in'' period should be used to allow the participants to reach the new steady state before taking any measurements. Measurements should then be made in two or more serial specimens taken at least 1 or 2 weeks apart and the values averaged to estimate the participant's baseline lipid and lipoprotein concentrations.

After treatment is begun, it should be continued long enough for the participant to reach a posttreatment steady state. For dietary studies, this may require 6 or 8 weeks. It is then advisable to make posttreatment measurements in two or more specimens, again taken 1 or 2 weeks apart. The measurements are averaged to provide an estimate of the posttreatment value. Tables 23–5 through 23-8, respectively, illustrate the $CV_{spec,tot}$ values to be expected for the mean cholesterol, triglyceride, and HDL and LDL cholesterol concentrations determined from measurements in 1 to 10 serial specimens. $CV_{spec,tot}$ values are calculated using the estimates for median and 75th percentile CV_p values shown in Table 23–3. The $CV_{spec,tot}$ values indicated may be of use for estimating the size of the study population needed for dietary intervention studies.

It should be pointed out that for each component, most of the reduction in $CV_{spec,tot}$ is achieved after the first three to four serial specimens. The number of serial specimens to be used for any particular study, however, will be influenced by several factors. These include the purpose of the measurements, the number of participants in the study, the magnitudes of the minimum changes the investigator wishes to detect, and the logistics and cost of the measurements.

The Evidentiary Chain

Laboratory quality control is only one of the issues to be confronted in planning a study. The specimens usually pass through a series of sequential steps that are accomplished by different individuals and in different locations, and procedural mistakes can occur before the specimens reach the laboratory. Specimens can be misidentified, mishandled, or misdirected. As a consequence, a specimen may be lost; the analysis may be delayed to the point that changes have occurred in the analyte of interest; data collection and transmission may be delayed, preventing their timely use by the researcher or physician; or the analytical results for one participant might be identified as originating from another.

Although these problems can be minimized by proper planning, development of detailed study protocols, adequate staff training, and appropriate pilot studies, it is nonetheless important to document that established procedures are actually being followed. This is particularly important in long-term studies during which staff turnover can occur and incoming staff may not have received adequate training by their predecessors. It is therefore necessary to develop a system for tracking specimens through the various steps beginning with sample collection and ending with the laboratory report. This system should provide procedures for documenting the date each step is accomplished and the identity of the staff member performing that task. The steps that should be well documented include: specimen collection and preparation; temperature and length of storage before shipment; when and by whom the specimens were shipped to the laboratory; date specimens are received by the laboratory and by whom; the condition upon arrival; dates of analysis and identities of technicians performing each analytical run; and dates of data transmission.

A reliable way of identifying specimens must also be provided. One fairly straightforward approach is to develop, in advance, a master list of study numbers that can be assigned to the participants as they are enrolled. The study coordinating center or the laboratory can then provide multiple copies of computer-generated labels to be used for the specimen collection tubes, transport vials, and log sheets that accompany the specimens when they are shipped. Specimen identification information should include, as a minimum, the participant identification number and specimen collection date, and this information should be transmitted by electronic means to minimize transcription and data entry errors. Participants should not be identified by name. Information that links the specimen to a particular individual should be

TABLE 23-5

Observed Coefficient of Total Variation for Mean Cholesterol Concentrations in Serial Specimens

	$CV_{spec,tot}$[1] at	
Number of Specimens	50th Percentile CV_p[2]	75th Percentile CV_p[2]
1	5.1	9.1
2	3.7	6.5
3	3.1	5.3
4	2.7	4.6
5	2.5	4.2
6	2.3	3.8
7	2.2	3.6
8	2.1	3.4
9	2.0	3.2
10	1.9	3.1

[1]$CV_{spec,tot}$, or observed total CV, includes both physiological and analytical components of variation. CV_p, coefficient of physiological variation; CV_a, coefficient of analytical variation.
[2]Values shown assume CV_a = 1.1% and are calculated for median (5.0%) and 75th percentile (9.0%) estimates for CV_p. Based on data from the Working Group on Lipoprotein Measurement (3).

TABLE 23-6

Coefficient of Total Variation for Mean Triglyceride Concentrations in Serial Specimens

	$CV_{spec,tot}$[1] at	
Number of Specimens	50th Percentile CV_p[2]	75th Percentile CV_p[2]
1	17.9	26.4
2	12.7	18.7
3	10.5	15.3
4	9.1	13.3
5	8.2	11.9
6	7.5	10.9
7	7.0	10.1
8	6.6	9.5
9	6.3	9.0
10	6.0	8.6

[1]$CV_{spec,tot}$, or observed total CV, includes both physiological and analytical components of variation. CV_p, coefficient of physiological variation; CV_a, coefficient of analytical variation.
[2]Values shown assume CV_a = 2.0% and are calculated for median (17.8%) and 75th percentile (26.3%) estimates for CV_p. Based on data from the Working Group on Lipoprotein Measurement (3).

safeguarded and should be made available to staff on a need-to-know basis only, and staff should be instructed on the general principles of confidentiality of patient results.

The study protocols serve to document how the study is conducted. They should include procedures that minimize the effects of physiological variations; specify proper specimen collection, storage, and transportation procedures; describe how laboratory performance will be monitored; and indicate the frequency and format of the laboratory reports, and how study data will be transmitted. To facilitate the development of these protocols, the study team should include investigators with the appropriate laboratory expertise.

Finally, provisions should be made for easy communication between the laboratory and other components of the study. This is particularly important when specimens will be sent from a remote specimen collection site(s). Occasional problems will arise that need to be addressed quickly. For example, a shipment may be delayed in transit, a specimen may be identified incorrectly, or its integrity may be compromised during shipment. Formal procedures should also be put in place to follow up all such verbal communications with written documentation.

Provisions should also be made to monitor compliance with the study protocols, ensure that specimens move through the system on schedule, and collect and transmit study data, including quality control data, in a timely manner. Required turnaround times should be decided upon, although they may vary according to the complexity of the test, stability of the analyte, and the urgency of the report. For example, nonesterified fatty acid levels change during

TABLE 23-7

Coefficient of Total Variation for Mean HDL Cholesterol Concentrations in Serial Specimens

	$CV_{spec,tot}$[1] at	
Number of Specimens	50th Percentile CV_p[2]	75th Percentile CV_p[2]
1	7.4	13.9
2	5.4	9.9
3	4.6	8.2
4	4.1	7.2
5	3.8	6.5
6	3.6	6.0
7	3.4	5.6
8	3.3	5.3
9	3.2	5.0
10	3.1	4.8

[1] $CV_{spec,tot}$, or observed total CV, includes both physiological and analytical components of variation. CV_p, coefficient of physiological variation; CV_a, coefficient of analytical variation.
[2] Values shown assume CV_a = 2.1%, and are calculated for median (7.1%) and 75th percentile (13.7%) estimates for CV_p. Based on data from the Working Group on Lipoprotein Measurement (3).

TABLE 23-8

Coefficient of Total Variation for Mean LDL Cholesterol Concentrations in Serial Specimens

	$CV_{spec,tot}$[1] at	
Number of Specimens	50th Percentile CV_p[2]	75th Percentile CV_p[2]
1	8.4	11.9
2	6.3	8.7
3	5.5	7.3
4	5.0	6.5
5	4.7	6.0
6	4.4	5.6
7	4.3	5.3
8	4.1	5.1
9	4.0	4.9
10	4.0	4.8

[1] $CV_{spec,tot}$, or observed total CV, includes both physiological and analytical components of variation. CV_p, coefficient of physiological variation; CV_a, coefficient of analytical variation.
[2] Values shown assume CV_a = 3.1%, and are calculated for median (7.8%) and 75th percentile (11.5%) estimates for CV_p. Based on data from the Working Group on Lipoprotein Measurement (3).

storage, and the analysis should be initiated on the day the specimen is collected, if possible. There is less urgency with cholesterol, triglycerides, or HDL or LDL, provided the specimens are properly handled and stored.

Documentation of the methods and procedures used in studies, particularly in large studies, is important for a number of reasons. First, long-term studies may not be completed by the same individuals who initiated them. Written documentation is essential to ensure that procedures are not inadvertently changed in ways that could introduce systematic errors affecting the interpretation of the data. Second, data analysis may begin years after the study started, and accurate documentation of methods and procedures will be required when publishing the findings. Finally, the data analysis for large studies may proceed for many years after the formal termination of the study. These analyses may be conducted by individuals who took no actual part in the planning or conduct of the investigations. The valid interpretation of such add-on analyses will depend on the availability of proper study documentation.

Specimen Banking

The issue of specimen banking should be addressed at the outset of the study. Can the specimen be discarded after it has been analyzed and the data have been finalized? Should aliquots of the specimens be collected for long-term storage? If so, how many should be prepared from each participant, from how many participants should they be collected, and

how long should these samples be retained? Are storage facilities available or can they be acquired? What is the cost of maintaining a specimen bank, and what will be done with the specimens when the study has concluded?

In general, any remnants of the particular aliquots used for the primary measurements should be discarded because they can be expected to suffer some degree of evaporation or other changes during the period of handling and storage required to make the primary measurements. If specimens are to be banked, separate aliquots should be prepared specifically for this purpose. A number of factors must be considered. These include the number of aliquots to be stored, the likely availability of sufficient specimen volumes, the stability of components that may be of interest in the future, and the availability of storage space. Considerable investments of time and resources are made recruiting, characterizing, and randomizing the study population. The availability of stored specimens from the study populations can be useful if certain measurements are to be delayed because of logistical, technical, or economic constraints. They also can afford particularly cost-effective opportunities to make additional measurements as new components of interest may emerge, because the study population will have already been characterized. It is therefore common practice, particularly in large studies, to provide for the permanent storage of one or more aliquots of each specimen. This practice is worthwhile for dietary studies, and should be considered in the planning phase of the study.

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