Chapter 22

Validating Diet Composition by Chemical Analysis

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This chapter describes conceptual issues, selected methods, and a general approach to the field of chemical analysis of diets. Readers desiring more specific information on methods and procedures may contact our laboratory (Food Analysis Laboratory Control Center, KM Phillips, Director, Department of Biochemistry, 304 Engel Hall, Virginia Polytechnic Institute and State University, Blacksburg, VA 24060-0308; [540]231-9960 or kmpvpi@vt.edu).

Why Should Diets Be Chemically Analyzed?

Preliminary estimates of nutrient levels in research diets are made by calculation from food composition databases, but the diets themselves still must be chemically assayed. Why should this be, if composition data are available for the foods and nutrients of interest? This is a reasonable question that deserves a thoughtful response, especially because chemical analysis of diets can be expensive.

There are really three reasons for assaying the diets used in controlled diet studies. The first is to develop diets with the desired nutrient concentrations; the second is to verify, prior to feeding, that the prepared diets have the desired nutrient levels, and the third is to document the constancy of dietary composition over the course of the study. If experimental diets do not have the designed nutrient levels, and especially if the diets are not chemically distinguishable from each other, the study hypothesis will not have been subjected to a valid test.

Differences Between Calculated and Chemically Assayed Nutrient Values

Food Composition Databases and Individual Foods

The primary source of food composition data in the United States is the USDA *Nutrient Database for Standard Reference* ("USDA Database") (1), which is the origin of the values in most other widely used food composition tables, computer databases, and menu planning software. The USDA database was developed to provide average (or weighted average) food composition values applicable to the nation as a whole. The data are subject to many sources of variance in sample collection (such as varieties, brands, sea-

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sons, and locations) and to many other factors affecting nutrient content values, including plant cultivar and maturity, soil and water composition, feedstock for animals, and postharvest storage and processing/cooking. As a result, it is very likely that the nutrient content of a specific sample of food (as would be purchased for a feeding study) will differ from the average values published in the USDA (or other) database. The greater the naturally occurring nutrient variance in a food item, the greater the potential that the database average will not reflect the composition of a selected sample of that food. For example, in a report by the International Food Biotechnology Council (2), the ratio of the highest level to the lowest level of some common nutrients in commercial vegetables ranged from about 1.5 for potassium to 12.6 for sucrose in green beans to 15 for carotene in tomatoes. For most of the foods, for most of the nutrients, this ratio ranged from 2 to 5. Piironen, Varo, and Coivistoinen (3) found relative standard deviations (RSDs) of up to 50% in the vitamin E content of baked rye breads. Slover, Lanza, and Thompson (4) found RSDs as high as 28% for the total fat content of beef and up to 16% for the cholesterol content of fast foods. In this same study the range of cholesterol content in fast food hamburgers was 26.5 to 48.3 mg/100 g; for french fries the range was 7.2 to 16.4 mg/100 g.

This issue has been examined carefully for vitamin C. Vanderslice and Higgs (5) found the vitamin C content of several major food sources to vary by a factor of two. Consequently, for a later study of the bioavailability of vitamin C from fresh broccoli and oranges, crates of the products, each from a single cultivar and supplier, were assayed. The relative standard deviation of the vitamin C content of broccoli and oranges was, respectively, 16% and 14% among crates and 7% and 13% within crates (6), and these values differed considerably from those reported in the USDA database (Table 22–1).

As a result, the foods for this study were analyzed on a continuing basis and amounts in the diet were adjusted to ensure targeted levels of vitamin C. Had this not been done, errors and variance in vitamin C intake likely could have impaired the detection of biological effects.

The concentrations of some nutrients in some foods vary with the season of the year or from year to year, for example, fat and protein in soybeans (7), sodium, calcium, and zinc in tomatoes (8), and fatty acids in milk (9). Such variance may lead to changes in levels of experimental nutrients in diets during the course of a feeding intervention. This drift is of particular concern in long-term crossover studies, in which each subject serves as his or her own control.

The composition of the food supply also shifts with time because of changing agricultural production and food-processing practices. For example, the fat content of pork has been declining, presumably because of changes in the production operations of that industry (10). Likewise, there has been a general decrease in the amount of sodium added to processed foods in response to dietary recommendations to limit sodium intake. Some processed foods, such as certain brands of potato chips, may not be consistently made with the same oil, which causes variance in the fatty acid composition of different lots. Updates to food composition tables usually lag well behind these changes.

Assay methodology can also affect the validity of database values. Standard methods for particular nutrients are often applied to foods that are different from those for which the analytical procedure was designed and validated. If an assay method is faulty or inappropriate, data obtained by that method will be inaccurate. Analytical methods are continually being improved, and standard methods are being updated. Such improvements and revisions alter estimates of the composition of foods.

For instance, the studies of Marshall et al (11, 12) demonstrated that cholesterol values measured by gas-liquid chromatography (the current method of choice) were only about 68% of cholesterol values measured colorimetrically. Presumably these differences resulted from inclusion of plant sterols in the (now outdated) colorimetric assay results. Until recently this was the method by which most food da-

TABLE 22-1

Assayed Vitamin C Content of Broccoli and Orange Products Taken from Multiple Crates Within a Single Shipping Lot[1]

	Vitamin C Content			
	Assayed Value			USDA Database Value[3]
Sample	Mean (mg/100g)	Range (mg/100g)	RSD[2] (%)	Mean (mg/100g)
Broccoli, raw	121.2	88–163	15.5	93.2
Brocooli, cooked	80.2	55–121	19.0	62.8
Oranges, navel	75.9	65–86	14.0	57.3
Orange juice (frozen, reconstituted)	43.8	42–46	5.0	38.9

[1]Vanderslice JT, Higgs DJ (5):117–119.
[2]RSD = Relative standard deviation (= SD ÷ mean).
[3]US Department of Agriculture database as cited in (5).

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tabase values were generated. This issue is also raised by the work of Wills, Balmer, and Greenfield (13), who reported the fat content of a variety of foods determined by five commonly used methods; the fat content of peanut butter ranged from 38.7% to 51.8% and the fat content of soybean flour ranged from 15.8% to 19.5%.

Users of a food composition database often are not able to evaluate its credibility because many databases do not adequately document the quality or source of data. For example, Lurie et al (14) demonstrated that the published copper concentration values for more than half of the foods that are primary contributors of this element in the American diet are based on poor or limited analytical data.

In addition, some of the values in food composition databases are not obtained from direct assays but rather are imputed from "similar" foods or from the raw materials that go into a recipe for a food. The accuracy of values obtained by imputation may well be unacceptable for feeding studies. For commercial products, the 1990 Nutrition Labeling and Education Act (15) allowed and encouraged manufacturers to use an algorithm to adjust analytical values and thereby to derive nutrient label values for a food item. Each and every individual package of the product is also required to *meet or exceed* the labeled nutrient values for some nutrients, but for others, the law requires concentrations to *meet or be less than* the labeled nutrient values. Consequently, nutrition label data may substantially yet legally understate or overstate the actual nutrient content of an individual package of a food (16).

Food identification and preparation practices also result in inconsistencies between calculated and actual dietary nutrient levels. Nomenclature can be a significant problem because the same names may refer to different foods, menus, or products in various regions within a country or in different cultures (17). Even if a particular item is accurately portrayed in the database, if that food is misidentified by those preparing the diets, the calculated composition of the diet may be in error. Misidentifications are not uncommon in the kitchen, and the supervisory dietitian needs to be alert to this possibility. Furthermore, food preparation habits vary widely by cook, by kitchen, by culture, and by region. Recipes for prepared foods (eg, "meat loaf" and "lasagna") also can differ markedly and can considerably affect the composition of prepared foods. Cooking methods, times, and temperatures, trimming of meats, and peeling of fruits and vegetables can influence concentrations and oxidation states of food constituents such as vitamins, fatty acids, dietary fiber, starch, sugars, and cholesterol.

Examples of Calculated and Assayed Nutrient Content of Research Diets

The obvious question raised when planning whole food diets is, how large are differences between the calculated and actual nutrient contents likely to be? Table 22–2 summarizes some of the potential sources of variance and error in the nutrient content of experimental diets. Certainly, deviations will be affected by many factors other than those that influence the actual composition of individual foods, including the quality (ie, accuracy and completeness) of the particular food composition database used, the accuracy with which diets are coded for calculations, control of food procurement, the accuracy and precision of food measurement and preparation, the specific foods used, the duration of the feeding trial, and the particular nutrients studied. Overall nutrient deviations will likely vary, and probably decrease, as one moves from analysis of *individual foods* to analysis of *daily menus* and then *diet cycles*. Few data are available, however, to clarify this issue. Table 22–3 shows tentative qualitative estimates of expected deviations for selected nutrients, based on our own experience in analyzing experimental diets.

Our laboratory has validated diets for both the DELTA (Dietary Effects on Lipoproteins and Thrombogenic Activity) (18) and DASH (Dietary Approaches to Stop Hypertension) (19, 20) studies, two multicenter programs with rigorous diet design and diet composition quality control protocols. Figure 22–1 shows the assayed sodium content as a percent of the calculated level in 12 daily menus developed for the DASH study. In this case, the target sodium concentration was 3,000 mg/day, and the mean assayed content was 92% of target. If the menus had not been assayed, sodium in the diet would have been assumed to be 240 mg/day higher on average than it actually was. Furthermore, sodium in individual menus ranged from 55% to 112% of target, suggesting variable bias in calculated concentrations depending on the particular foods and menu.

Figure 22–2 shows analytical data from the prefeeding phase of the DELTA study. The mean assayed cholesterol content of 12 prepared menus was 87% of the calculated target of 300 mg/day. The menu-to-menu variability in cholesterol content illustrated by Figure 22–2 also suggests that the quality of data for this nutrient may vary by food. Similar results were obtained for total fat (21). In this study, validating menus prior to the feeding phase of the study allowed the investigators to eliminate menus that substantially deviated from target nutrient composition.

The State of Food Analysis Methodology

The state of the art in food analysis is less well developed than the more familiar clinical chemistry. For example, blood and body fluids are reasonably well-defined matrices in which analytes, such as cholesterol, are evenly distributed. Hence, dispensing uniform subsamples is straightforward. In addition, standard methods, commercial kits, and often automated systems are available for rapid determination of many blood or urine components. Concentrations of many analytes are defined by physiological limits, and standard samples for precise quality control are frequently available. Intra- and interlaboratory reference or calibration samples and systems are

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TABLE 22-2

Sources of Error and Variance in Nutrient Content of Experimental Diets

Source	Examples
Features of study design	Number of different diets Number of feeding periods Number of study sites Length of study/feeding periods Magnitude of difference in nutrient concentrations of treatment diets Number of menus and energy levels in diet cycle Nutrient levels that are intended *not* to differ Units of target nutrient concentrations (eg, % kcal; g/day)
Menu calculation and nutrient database	Variance across calculated menus vs calculated diets Number of different foods used Precision of weight specification for food items Experience of person calculating the diets Quality of data in the food composition database Completeness of database (number of missing and imputed values) Natural variance in food composition (database values are average values)
Food chemistry	Within-assay coefficient of variation, between-assay coefficient of variation Stability of nutrient during storage Lability of nutrient during cooking/food preparation Accuracy of lab (eg, ability to achieve accurate results for relevant standard reference material) Validity of analytical methodology (ie, reliable and accurate vs weak or problematic[1]) Homogeneity of composites Comparability of diet samples assayed to diets as consumed Assay quality control (eg, ability of quality control sample to monitor precision and accuracy of analysis system)
Food preparation	Experience of staff preparing foods. Food procurement protocol (eg, single lots vs multiple purchases) Standardization of preparation and cooking methods Accuracy and precision in weighing foods (including calibration of balances)

[1]See Table 22–4.

also available for laboratory calibration and certification. (Reference samples are chosen to have a background matrix similar to that of study samples, like food or plasma, and are well characterized for the analyte of interest.)

Food matrices are usually more complex than the biological fluids and tissues seen in a clinical laboratory. Not only are there hundreds to thousands of different compounds per cell type (as found in clinical samples), but a typical diet has components from numerous plant and animal sources combined with other pure and semipure ingredients. Many foods have active enzyme systems that, when released during food processing, may cause significant chemical transformations. Thermal degradation of some food components also occurs during processing and cooking. Food and diet samples are frequently heterogeneous in texture and composition, and nutrients are not usually distributed uniformly within the sample.

Another difference between food and clinical samples is that metabolite concentrations in clinical samples typically range from about 1 nMolar to 100 mMolar (a 100 million-fold range), whereas nutrient concentrations in foods usually range from 1 nMolar to 1 Molar (a billion-fold range). Thus, compared with clinical methods, the assays used for foods must be able to detect nutrient analytes over a much wider range of concentrations. This makes assays of foods yet more difficult, because analytical methods and quality control materials typically are developed and validated for specific and limited nutrient concentration ranges. If a particular sample has a higher or lower concentration of the analyte, then a different aliquot weight and/or dilutions will be required. Multiple quality control materials and considerable preliminary testing may also be necessary.

Nutrient assay methods are particularly matrix dependent. That is, the same method might yield different results depending on the overall composition of the food or diet. For instance, if acid hydrolysis is used to determine total fat in a sample that is high in carbohydrate, coextraction of the carbohydrate will give falsely elevated fat values (22, p. 95).

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TABLE 22-3

Sources of Error and Variance in Nutrient Content of Experimental Diets: Qualitative Estimates of Magnitude for Selected Nutrients[1]

Source	Fat/Fatty Acids	Sugars	Cholesterol	Vitamin E	Selenium
Number of feeding periods/duration of study[2]	M	M	M	M	M
Completeness of database	S	L	S	L	L
Natural variance in food composition[2]	M	M-L	M-L	L	L
Lability of nutrient during storage and cooking/food preparation (also see Table 22–7)	M[3]	L[4]	M[5]	L[5]	S-M[3]
Homogeneity of composites	L	M	M	M	M
Food procurement and preparation standards[2]	L	L	L	L	L
Accuracy and precision of weighing foods[6]	M	M	S-M	S	S
Experience of person calculating the diets	L	L	L	L	L

[1]S = small; M = moderate; L = large. Estimates are based on authors' experience.
[2]Likely interactive influence (number of feeding periods, natural variance in food composition, and food procurement and preparation standards).
[3]Potential loss during cooking (eg, to cooking container or cooking water).
[4]Degradation caused by fermentation or enzyme activity is a potential problem in some menus.
[5]Oxidation.
[6]Depends on how concentrated the nutrient is in its food source(s).

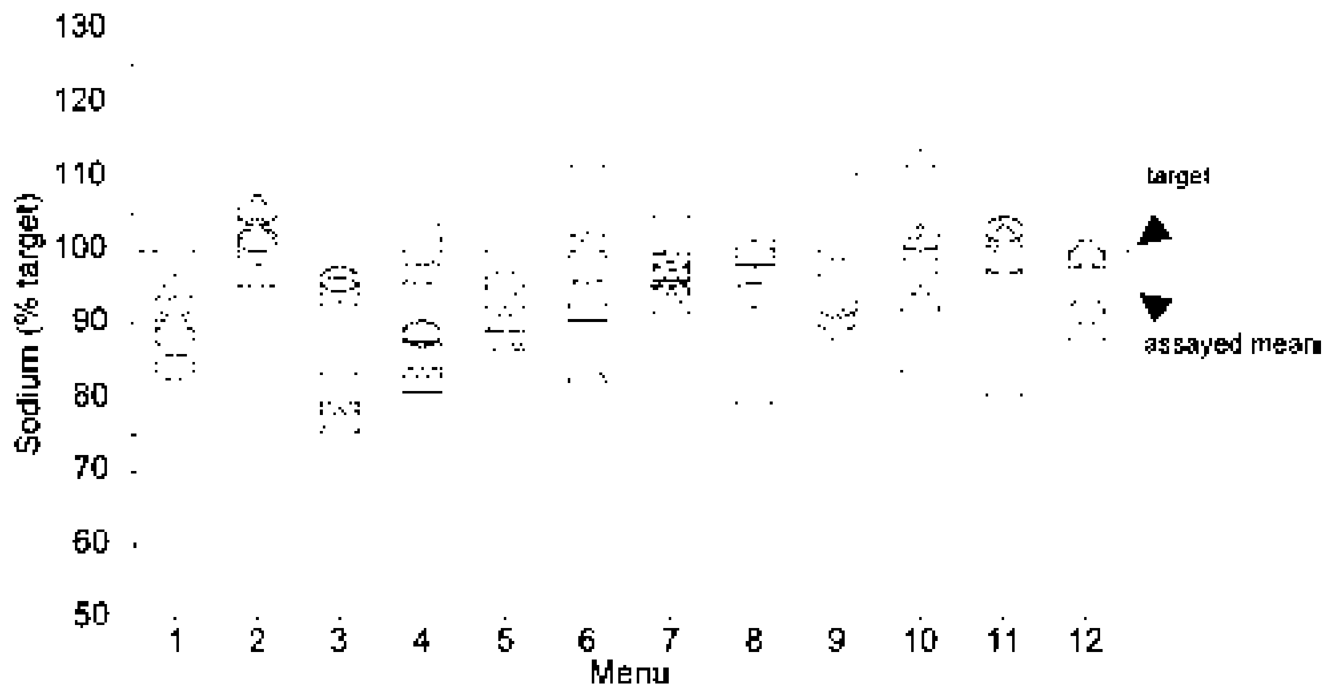

FIGURE 22-1. Sodium content of 12 daily menus developed for a controlled feeding trial with 3 experimental diets.[1, 2, 3]
[1]The target sodium content for all three diets was 3,000 mg/2,100 kcal.
[2]For each diet, each menu was prepared in duplicate and composited. Two aliquots from each composite were assayed. Datapoints show determinations on these individual aliquots (ie, two sodium values per composite). (Data from authors' laboratory.)
[3]Legend: Diet 1, △; Diet 2, □; Diet 3, ○.

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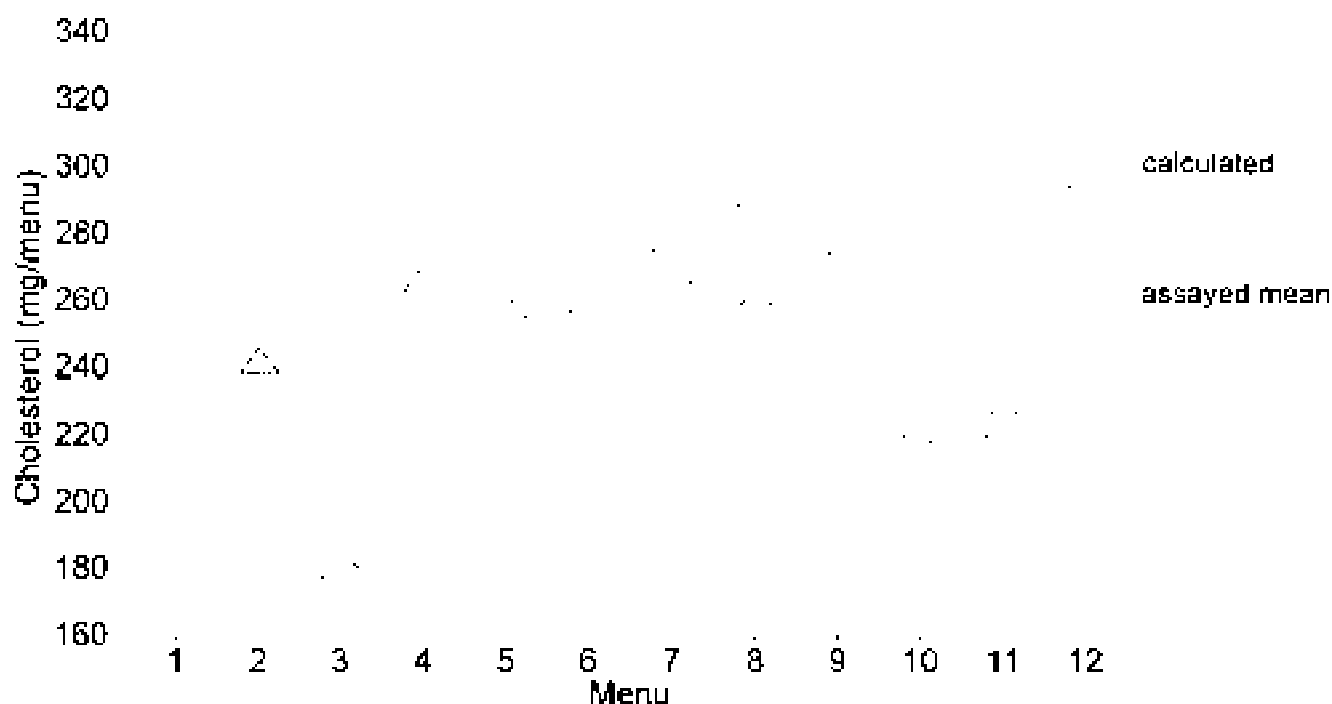

FIGURE 22-2. Cholesterol content of 12 daily menus developed for a controlled feeding trial with 3 experimental diets.[1, 2, 3]

[1]The calculated (target) cholesterol content for all three diets was 300mg/menu.

[2]For each diet, each menu was prepared in duplicate and composited. Datapoints show the cholesterol content of each composite (based on averaged data from analysis of duplicate aliquots). (Data from authors' laboratory.)

[3]Legend: Diet 1, △; Diet 2, □; Diet 3, ○.

In addition, methodology appropriate for analyzing a compound in a clinical sample usually is not applicable for use in food samples. Cholesterol in serum, for example, can be accurately determined using the enzyme cholesterol oxidase; however, if this same assay system is used to determine cholesterol in foods, the values obtained are falsely elevated because plant sterols are quantified in addition to cholesterol. These sterols are not commonly found in blood serum, but they are common components of plant foods. The use of cholesterol oxidase to determine cholesterol in diets would lead to approximately 20% overestimation of the actual levels.

It is therefore important to validate food assays in a matrix identical (or at least very similar) to that of the samples to be analyzed. This validation is complicated by a lack of standard reference materials for verifying the accuracy of nutrient measurements in different matrices, and mixed diets can vary widely in composition and physical properties. Although the National Institute of Standards and Technology (NIST) and the Association of Official Analytical Chemists (AOAC) are currently trying to rectify this deficiency (23), the production of reliable food analysis data require careful in-house validation of the accuracy and precision of methods in the matrices being analyzed.

The quality of current quantitative analytical methodology for various nutrients in foods is described in Tables 22–4 and 22–5. In general, *adequate* methods are those in which a good food analysis laboratory can obtain accurate and precise data on the nutrient content of all significant food sources of that nutrient. *Substantial* methods are those in which a good food analysis laboratory can obtain accurate and precise data on the nutrient content of many (but not all) significant food sources of that nutrient. *Conflicting* methods are those for which different methods yield different results, and there is no agreement among the experts as to which methods (if any) give accurate data. Where methodology is *lacking*, there is agreement among experts that none of the available methods gives accurate results for that nutrient in foods.

The field of food and diet analysis has not enjoyed the type of extensive methodology and instrumentation development that has occurred in clinical chemistry during the past two decades. Thus, there is virtually no automated methodology and almost a complete lack of commercial kits for assays of individual food components. Hence, most existing food analysis methods are still labor intensive. Output is low in terms of the number of assays per analyst and per instrument (frequently less than 10 per day), and unit labor costs are correspondingly high. Additional costs may be incurred because many assay methods have not been validated for use with the wide variety of matrices seen in diet and food analysis; such validations should be done prior to the use of the methodology.

All of these factors lead to the high costs that typically range from $10 to $100 per nutrient assay per sample for routine analyses. Startup costs can also be significant, depending on the nutrients being assayed and the diet matrices.

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TABLE 22-4

Criteria for Evaluating the State of Nutrient Analysis Methodology

State	Accuracy	Speed of Analysis	Cost per Analysis
Adequate	Excellent	Fast	Modest (<$100)
Substantial	Good	Moderate	Modest to high
Conflicting	Fair	Slow	High
Lacking	Poor	Slow	Unknown

TABLE 22-5

State of Methodology for Analysis of Specific Nutrients in Foods[1]

Nutrient Category	Adequate	Substantial	Conflicting	Lacking
Carbohydrates	—	Individual sugars Total dietary fiber Starch	Other fiber components	Resistant starch
Available energy	—	Bomb calorimetry	—	Calculated[2]
Lipids	—	Cholesterol Fatty acids: common (C12–C20)	Fat (total) Sterols Fatty acids: short chain (C4–C10), *trans*, omega-3	—
Mineral nutrients	Calcium Copper Magnesium Phosphorous Potassium Sodium Zinc	Iron Selenium Manganese	Arsenic Chromium Fluorine Iodine	Boron Cobalt Molbydenum Silicon Tin Vanadium Organic Species
Protein and amino acids	Nitrogen (total)	Amino acids (most)	Amino acids (some)	—
Vitamins	—	Niacin Riboflavin Thiamin Vitamin B-6 Vitamin E	Vitamin A Folate Vitamin B-12 Vitamin C Vitamin D Pantothenic acid Vitamin K	Biotin Choline
Other	—	—	Phytate Carotenoids Phytosterols Tocotrienols	Flavonoids Lignins Saponins

[1]Criteria for evaluating the state of nutrient analysis methodology are described in Table 22-4.
[2]Calculated from assayed proximate composition (water, fat, protein, ash, and carbohydrate by difference) and general Atwater factors (4 kcal/g for protein, 4 kcal/g for carbohydrate, 9 kcal/g for fat).

Diet Assay as Part of the Controlled Feeding Protocol

The diet assay component of a controlled diet study can be viewed as having two phases: *prefeeding validation* of the daily menus and *monitoring* of the diets as fed. In our experience the prefeeding validation is clearly the more important because it ensures that the desired nutrient levels are delivered to participants. Diet monitoring documents the nutrient levels fed and the degree of drift in the composition of the diets throughout the course of the study. If diet composition is validated prior to intervention *and* appropriate food procurement and preparation protocols are instituted to minimize subsequent nutrient variance (see Chapter 12, ‘‘Producing Research Diets,’’ and Chapter 13, ‘‘Delivering Research Diets’’), then drift is unlikely and the diets probably will meet design criteria throughout the study.

There are other advantages of having a well-designed diet assay component as an integral part of the feeding study

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protocol. First, the chemical data can provide valuable information for refining the design of subsequent studies, facilitating comparison of results from different investigations, and allowing the precise study of dietary components for which food composition data are lacking (eg, individual sugars, carotenoids, soluble dietary fiber). Second, a frozen archive of diet samples is a natural by-product of the sample preparation process. This archive can be a resource for retrospective studies, including assay of supplementary components that become of interest. Archived samples also might become extremely valuable for further characterization of the experimental diets if unexpected clinical endpoint results are obtained.

The Role of Food Analysts in Feeding Studies

The individuals responsible for chemical analysis of the diets or those with experience in quantitative chemical analysis of food composition should be involved in the overall planning of the diet intervention, as well as in designing various diets. As noted earlier, chemical assay of foods and diets is a specialized field; those with competence in clinical assays may, but do not necessarily, have sufficient expertise in the quantitative assay of diet and food components. Those experienced with food composition analysis will have the background required to suggest where differences between database values and chemically determined values might be a problem. They will appreciate the complexity of diet assays and can evaluate critical factors such as cost, turnaround time, precision of methods, normal nutrient levels in different foods, natural variations in food composition, and potential problems in diet assays. These analysts will frequently be able to suggest alternate approaches to diet design to maximize the accuracy and consistency of the diets delivered to participants.

Which Dietary Components Should Be Assayed?

At a minimum, assays should be planned for those nutrients fundamental to the experimental hypothesis and those nutrients known or suspected to influence the outcome variables. Additional assays may be required to obtain reference points necessary for the nutrient parameters. For example, if total fat will be calculated as a percent of total energy, total energy must be assayed in addition to total fat. The traditional measure of total energy requires ancillary determination of total weight, moisture, and ash. Alternatively, bomb calorimetry can be used to measure total energy, particularly for liquid formula diets. (See Diet Monitoring; also see Chapter 21, "Performance Improvement for the Research Kitchen.") Table 22–6 lists some typical calculated parameters for macronutrients and micronutrients and the corresponding assays needed.

It is also important to precisely define the analytes to avoid misunderstandings within the research team. For example, most chemists define analytes by their specific chemical structure, or sometimes by the assay methodology. In contrast, biomedical scientists may define analytes by their biological activity, which might effectively collapse a large number of individual components into a smaller number of categories. For example, "total saturated fatty acids" is actually the sum of multiple individually measured fatty acids. Similar disparities exist in the definitions of total carbohydrates, fiber, and energy.

As noted earlier, assay costs can be significant and can vary a great deal. Care must be taken not to raise the cost of the study by performing extraneous assays that are not central to the clinical investigation. The "turnaround time" from reception of the sample to the presentation of the results can also vary widely from assay to assay. Turnaround time can affect study timelines, and some assays may be inappropriate because the results may be obtained too late to be useful.

A Paradigm for Diet Analysis

A general paradigm for diet analysis is shown in Figure 22–3. It must be emphasized that this is a general approach, which must be adapted for the special concerns of a particular study. The phases of the diet analysis portion of a dietary intervention study are: (1) planning, (2) prefeeding diet validation, (3) diet monitoring, and (4) follow-up assays of the archived samples.

Planning

The plans for menu validation and diet documentation schemes will depend on the specific requirements of a given study and should be developed after the feeding protocol has been designed. The investigators should first assume that calculated and actual nutrient levels may differ and then think critically about the impact of any variance or inaccuracy in diet composition on the experimental hypotheses and biological measurements. Answering the following key questions during the planning phase will help the researchers determine the most appropriate analytical scheme for a particular study. Failure to address these issues early in the planning phase can lead to expensive mistakes later in the clinical study.

- What are the key nutrients in the study (ie, those fundamental to the experimental hypothesis and those expected to affect biological measurements)?
- Are exact nutrient levels important, or is it more important to maintain the difference between nutrient levels among diets?
- If differences among diets are vital, how far apart are the nutrient levels that are being studied? What are the expected analytical variances for the assay of the key nutrients? Given these differences in nutrient levels, the normal variances in the foods in the diets, and the expected ana-

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TABLE 22-6

Assay Profiles for Selected Nutrient Parameters[1]

Nutrient Parameter	Usual Profile	Alternative Profile
Total intake of any nutrient (per menu)	Total food weight (g/menu) Nutrient concentration (g/100 g)	
Energy, total (per menu)	Total food weight (g/menu) Total fat (g/100 g) Moisture (g/100 g) Ash (g/100 g)	Total food weight (g) Bomb calorimetry (kcal or Kj)
Protein (% energy)	Protein (or nitrogen) (g/100 g) Total fat (g/100 g) Moisture (g/100 g) Ash (g/100 g)	Protein (or nitrogen) (g/100 g) Bomb calorimetry (kcal or kJ)
Carbohydrates, total (g/100 g)	Calculated by difference: Protein (or nitrogen) (g/100 g) Total fat (g/100 g) Moisture (g/100 g) Ash (g/100 g)	Assayed: Starch (g/100 g) Sugars (g/100 g) Fiber (g/100 g)
Fat, total (% energy)	Total fat (g/100 g) Moisture (g/100g) Ash (g/100 g)	Total fat (g/100 g) Bomb calorimetry (kcal or kJ)
Fatty acid (individual) (% total fat)	Total fat (g/100 g) Fatty acid (g/100 g)	

[1]These assay profiles are groups of distinct laboratory analyses that must be performed on aliquots from a single composited menu (which could represent one meal, one day, or several days). Additional data manipulations may be needed to generate final results. Examples include: difference calculations (for total carbohydrate), nitrogen-to-protein conversion factors; Atwater factors (for converting protein, fat, and carbohydrate content to energy); and adjustments between gross energy of combustion (by calorimetry) and physiological fuel value (Atwater factors). Energy may be expressed as kilocalories (kcal) or kiloJoules (kJ).

lytical variances, is it feasible that the proposed nutrient differences among the diets will actually be observed?
- Is a temporal relationship between nutrient intake and clinical measurements expected? If so, what unit of the diet (eg, meal, day, week) is significant?
- How much variability is expected in the levels of key nutrients in foods that make up the diet? For example: Are these nutrients susceptible to degradation? Does their concentration in food products have a high natural variance that cannot be controlled? Will foods be prepared at multiple sites?
- What is the scope and quality of available food composition data for the key nutrients? How much information is available about the variance of the nutrient content of the foods to be used in the diets?
- What is the value of definitive chemical data relative to the cost of chemical analyses?

Pilot Studies

Pilot studies should be considered at an early stage, because they can provide critical information for diet design. For instance, if the levels of different fatty acids are of interest and the main source of dietary fat is commercial oils, it would be useful to procure the oils to be used, assay for fatty acid concentrations, and use these data for diet design. In any case, once menus have been assayed and acceptable menus have been selected, ingredient specifications and preparation procedures should not be changed indiscriminately but only after careful discussion with input from all those involved in diet design, including those responsible for the chemical analysis of the diets.

Diet Validation

The purpose of diet validation is to evaluate by chemical analysis *prior to feeding* whether the prepared diets contain the key nutrients at the levels targeted for the intervention. In this way, unforeseen and correctable deviations from experimental design (and possible overlap of diet treatments during the intervention) are prevented. Our experience suggests that this validation usually is best done at the level of the daily menus. In some cases, it may be necessary to assay individual foods or meals. If assayed nutrient concentrations differ from design specifications, errant menus can be eliminated or reformulated and reassayed before being delivered to participants. Often, examination of the chemical data will reveal a possible source of deviation. Before any chemical assays are done, those responsible for diet preparation should have developed, specified, and standardized the ingredients and food preparation methods. Special attention should be given to the

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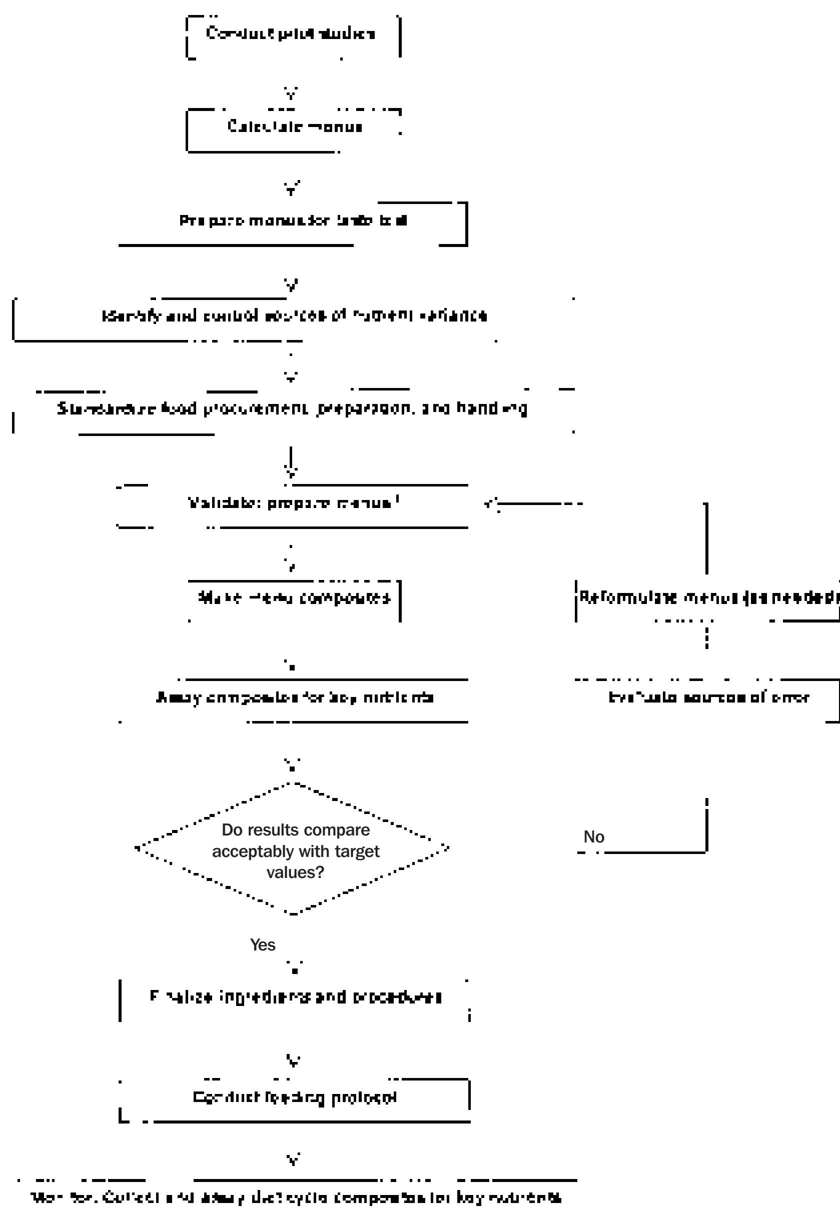

FIGURE 22-3. A scheme for validating and monitoring diet composition.

[1]For validation of diets for multicenter studies, menus should be prepared at 2 or more sites. In a four-site study, menus for 3 experimental diets were prepared as follows: diet 1, sites A and B; diet 2, sites B and C; diet 3, sites C and D (18).

primary sources of key nutrients, and the potential sources of variance should be identified and controlled.

Although prefeeding validation of individual menus is preferable, at least one menu cycle composite (ie, a composite containing all the food in one full rotation of menus) for each experimental diet should also be assayed for composition validation prior to feeding. (For multicenter studies we recommend collecting one of these cycles from each feeding site.) Assaying individual menus is the best way to prevent day-to-day overlap that can blur the distinctions between different dietary treatments. However, some investigators may choose to first check whether the entire menu cycle meets the design target. If not, having a set of frozen individual menus available for assay will allow the outliers to be identified. This is especially important if there is a large natural variance of the experimental nutrient levels in foods or differences in nutrient levels among diets are small.

Diet Monitoring

The bottom line for any feeding study is ensuring that actual levels of key nutrients fed to participants match the experimental design. Theoretically, diets that have been validated should meet the target composition specifications over the entire study and across all centers, if ingredients and preparation methods match those used in the prefeeding menu validation. In reality, errors or variance in preparation, substitution of ingredients, and seasonal or lot-to-lot variability

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in food composition are apt to occur, with the potential to cause drift in nutrient levels. The purpose of the diet monitoring is to document that the diets actually met the target specifications, and to document any changes in the diets consumed by participants over the course of the intervention.

The composition of diets as fed can be monitored by collecting and assaying exact replicates of foods eaten by the participants during the intervention. Although one might think it would be ideal to continually monitor each menu during the feeding trial, such intensive sampling can be expensive and not necessarily the best use of money and time. The study investigators should carefully evaluate the sampling plan for the diet monitoring, in the context of potential variance and the clinical measurements.

In the multicenter DELTA study (18, 24) daily menus for a given diet at a given site were collected and composited into individual diet cycle composites—one for each center and sampling period. Each field center sampled one diet energy combination during each menu cycle. In this fashion, we were able to document the composition of the diets across centers, calorie levels, and the duration of the study.

A less intensive sampling plan was used for a second DELTA feeding trial (25). We had found that once ingredient specifications and diet preparation protocols were fixed and menu compositions validated, we had little variance in the key nutrients (fatty acids, cholesterol and total fat) across centers, calorie levels, or time (18). Such information reassured reviewers and was a compelling argument that the study had been done in the intended manner. The goal is to undertake sufficient, but not excessive, sampling and assays in the diet monitoring phase. In general, we recommend that at least one menu cycle from each diet and feeding period be collected, composited, and assayed for monitoring purposes.

For certain limited applications, such as process control in the preparation of liquid formula diets composed of pure ingredients, bomb calorimetry might be used to monitor consistency of diet composition. (Also see Chapter 21, "Performance Improvement for the Research Kitchen.") However, because this technique measures total energy only, changes in the proportion of different nutrients may not be revealed. Also, although bomb calorimetry is not suitable for monitoring the specific nutrient composition of whole-food diets, it can provide useful information on the total energy content of formula diets or of other composited menu samples. In our experience, the total energy content of the diet (kcal/day or kcal/cycle) can vary even when the design targets for relative distribution of macronutrient calories are achieved consistently.

Follow-up Assays of Archived Samples

After the endpoint measurements are completed for a study, investigators often wish that they had information on the composition of diets for some nutrient not originally believed to be important to the study. Proper archiving of composited menu and diet samples can be an invaluable resource at such times. It is a relatively simple chore to archive composited samples if properly planned for at the beginning of the study. (See Storing Samples.)

Procedures for Chemical Analysis of Diet Samples

Obtaining dependable chemical measurements requires more than simply sending samples to a "black box" food analysis laboratory. Chemical measurements will be assumed to reflect the composition of diets as consumed, and the quality of the entire assay process directly impacts the reliability of the analytical data. Therefore, each phase of diet assay must be controlled and conducted with proficiency and documented if reliable results are to be obtained. This is especially important because any analytical value is generally regarded as the true value, regardless of the quality of the procedure by which it was generated. An overview of the diet assay process is shown in Figure 22–4. *An error at any stage will probably invalidate the results*. The probability of two errors canceling each other is quite small. If assays are performed indiscriminately or without proper quality control, the data will be noisy (imprecise) and misleading (inaccurate). When documentation is inadequate, the diet composition data may be questioned even if they are accurate.

This section will explain how reliable diet composition data can be obtained, from sample collection through evaluation of assay results. Particular emphasis will be placed on quality control of the analytical process. (Also see Chapter 23, "Laboratory Quality Control in Dietary Trials.") Key terms relating to diet assay are defined in Exhibit 22–1. Exhibit 22–2 provides a checklist for investigators preparing to chemically analyze diets.

Importance of Quality Control

Quality control (QC) has been defined as the "overall system of activities whose purpose is to control the quality of a product or service so that it meets the needs of users" (26). Within the controlled diet study, chemical assay of nutrients is part of overall quality control of the clinical results (the product of the study). Similarly, for the assay results to meaningfully reflect actual diet composition, quality control of the analytical process itself is crucial.

There are three basic goals of analytical QC: to minimize the variance of the measurements, to verify the accuracy of the measurements (lack of assay bias), and to document the precision and accuracy of the measurements. Repeated analyses of the same sample (ie, food composite) give an indication of overall assay variance. The total variance in a measured value for a sample is really the sum of actual variance in composition plus analytical variance. Because the goal of diet assay is to determine variance in diet

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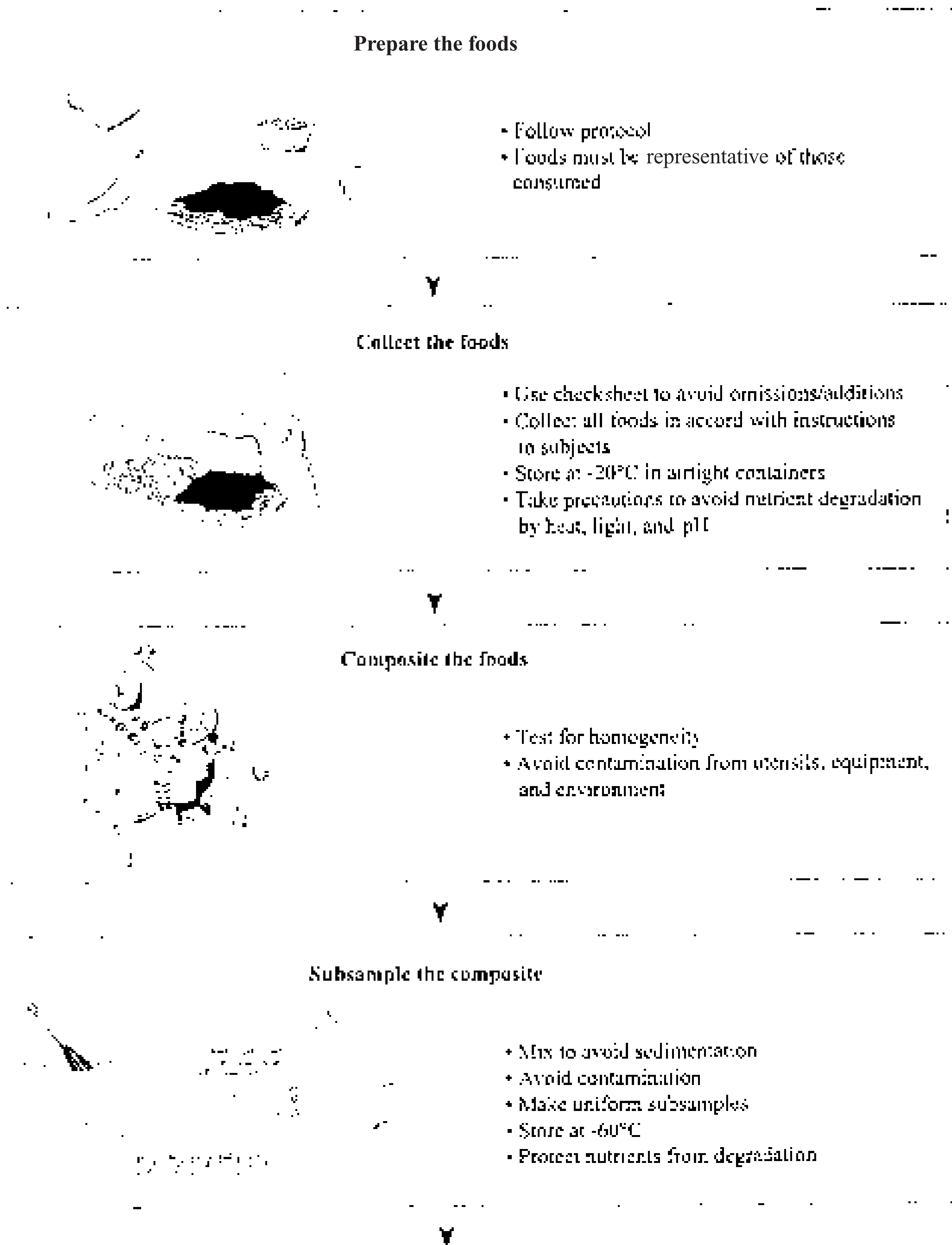

FIGURE 22-4. Overview of the diet assay process.[1]

[1]Illustrations by Karen Richardson, Virginia Polytechnic Institute and State University, Blacksburg, Va.

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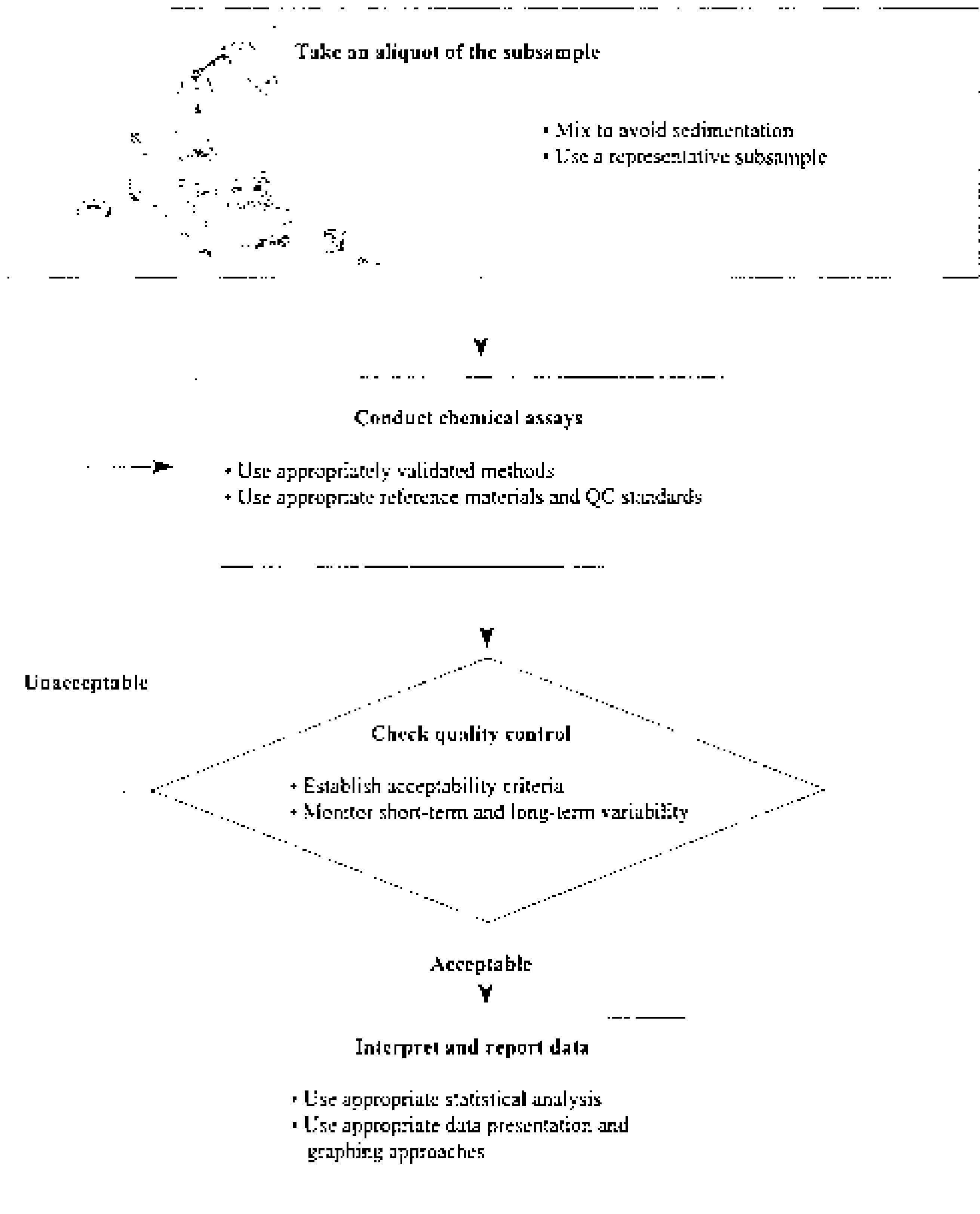

FIGURE 22-4. Continued

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EXHIBIT 22-1

Glossary of Analytical Chemistry Terms

Accuracy: The degree of agreement of a measured value with the true or expected value of the quantity of concern.[1]
Aliquot: A measured amount of composite taken for a specific assay.
Archive sample: A subsample placed in long-term storage for study documentation.
Bias: A systematic error in a method or a deviation in the system caused by some artifact or idiosyncrasy of the assay process.[1]
Composite: A homogenized mixture of foods.
Homogeneity: The degree of uniformity of the distribution of analyte(s) throughout the food composite.
Matrix: The food or food composite, with characteristic physical and chemical properties, in which a nutrient analyte occurs.
Method validation: The process of verifying that a given (standardized) method yields results of acceptable precision and accuracy for a given analyte in specified concentration range in the matrices of interest.
Precision: The degree of mutual agreement characteristic of independent measurements as the result of repeated application of the process under specified conditions.[1]
Quality control (QC): The entire system by which accuracy and precision of data are achieved to meet the end use of the data.
Quality control chart: A graph that is used to evaluate precision, accuracy, and drift of the measurement system, for example, a plot of the assayed value of the control sample vs time.
Quality control material (QCM): A material with nutrient levels and matrix similar to food composite samples to be analyzed, and of adequate homogeneity and stability to monitor the precision of the measurement process.
Standard reference material (SRM): A substance for which one or more properties are established sufficiently well to calibrate instrumentation or to validate a measurement process.[2]
Subsample: A portion of the total composite.

[1]Taylor JK (26):7.
[2]Taylor JK (26):159.

EXHIBIT 22-2

Checklist for Planning the Diet Assay Component of a Feeding Study

_______ Decide which nutrients to assay.
_______ Develop a sampling plan for validation and monitoring.
_______ Establish the time lines and budget for all diet assay activities.
_______ Evaluate storage space.
_______ Determine susceptibility of analytes to degradation or contamination and measures for prevention.
_______ Select and document assay methods.
_______ Develop appropriate forms for documenting samples, procedures, and data and for maintaining a complete audit trail.
_______ Gather materials and develop standard procedures for collection, shipping, compositing, and assay.
_______ Perform pilot tests for composite homogeneity.
_______ Procure the appropriate food-based control material(s).
_______ Establish appropriate criteria for precision of assay data for each nutrient.
_______ Select food analysis laboratory.
_______ Validate in-house assays.
_______ Set quality control standards for assay values.
_______ Establish quality control charts for each assay.

composition (the measured value is the estimate), analytical variance must be known and ideally it should be minimized.

Determining the accuracy of an assay is more difficult. Most quantitative analysts believe that although accuracy can be disproved, it cannot be proven. Estimates of assay bias are frequently made by analyzing reference samples of known composition and comparing the results with known values. If the results differ consistently, the assay has bias. However, even if the results for a reference material agree, the result for a particular unknown sample still might be wrong because the unknown sample is not identical to the reference sample.

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Analytical errors can arise at any point in the entire diet assay process. *Sampling errors* occur when the aliquots that are assayed are not representative of the original sample. For food composites, sampling errors include nonrepresentative collection of food samples; loss or degradation of nutrients during food collection, storage, or assay; contamination of samples; and composite heterogeneity. *Method bias* contributes to analytical error when different methods designed to measure the same component differ in accuracy and precision and are used interchangeably. Examples of this are the difference in cholesterol determined by gas chromatography and by colorimetry (11); in total fat measured by acid hydrolysis, chloroform/methanol extraction or as the sum of triacylglycerols (22); or in *trans* fatty acids measured by infrared spectrophotometry vs gas chromatography (27). *Measurement error* comprises all factors involved in the chemical determination, such as imprecision in weighing, dilution, extraction, detection, and calculation (28). *Reporting errors* include omissions, transposition of data values, or sample misidentification.

Given the myriad sources of analytical error, proper quality control measures are *essential* to the generation of believable analytical values. The following summarize key aspects of quality (discussed below in more detail).

- Carefully collecting foods, preparing composites, and subsampling composites for analysis.
- Using suitable and validated assay methods.
- Implementing assay quality control and using reference materials.
- Maintaining adequate documentation of samples, methodology, quality control procedures, and results.
- Appropriately evaluating (ie, reviewing and interpreting) analytical data and calculations.

Collecting, Compositing, and Subsampling Diets

Collecting Diet Samples

There are two overriding concerns in food collection: the foods sampled for assay must strictly replicate those consumed by participants, and no nutrient loss or degradation should occur after collection. No special treatment should be afforded "lab" samples during preparation. The foods must be procured from the same sources and prepared, handled, and heated in exactly the same manner as for participants. Inedible portions (eg, apple cores, banana peels, chicken bones, wrappers) must be removed. Generally, water and nonnutritive beverages (coffee, tea, diet sodas) are not included in food samples for assay but may be analyzed separately at the discretion of the investigator (12). For example, tap water could be a significant source of minerals in a trace element study. Usually foods can be collected and stored frozen (at −20°C) in clean, airtight containers prior to homogenization (however, see Table 22–7 for considerations for particular nutrients). A well-tested food collection protocol that has been used in our laboratory for some time is provided in Exhibit 22–3.

If the assayed samples do not accurately represent the food in the menu or diet, if nutrient loss or gain occurs after collection, or if composite aliquots are heterogeneous, then the analytical data will not represent the actual diet composition no matter how accurate and precise the chemical measurements. The following general precautions will minimize errors throughout sample collection, composite preparation, and storage:

- Use carefully cleaned and dried containers and utensils.
- Wear powder-free gloves when manipulating samples.
- Minimize sample handling and transfer.
- Minimize temperature fluctuations (eg, repeated freeze-thaw or exposure to freezer automatic defrost cycles).
- Limit time exposure to temperatures in excess of 4°C or extended storage at greater than −20°C.
- Protect samples from contact with extraneous materials.
- Maintain a clean, climate-controlled laboratory environment.

Additional nutrient-specific safeguards may be necessary. (See Table 22–7 for typical causes of sample alteration along with measures for prevention.) An experienced food chemist or the food chemistry literature can be consulted to determine appropriate criteria for other nutrients.

For prefeeding menu validation, all traces of foods should be included in the composite, because the goal at this stage is to verify calculated nutrient levels. Each item is weighed into the collection container or directly into the food processor bowl if the composite is to be prepared immediately. The weight of each item in the composite is recorded on a checksheet. During the feeding intervention, when the goal is to document the nutrient levels as consumed, diet samples should be collected with the same technique used by participants. For example, if subjects are instructed to wipe down and consume all residues of food with bread or muffin, this same procedure should be employed for the assay sample. In the ideal scenario, the extra foods (for the assay composite) are plated and served to one or more additional "participants," with food handlers unaware that the meals are not to be eaten. These "participants" collect rather than consume the foods. In this way, selection biases such as choosing poorer cuts of meat or damaged/mishandled foods for the laboratory sample can be avoided.

The foods should be collected at the same time the corresponding items are consumed by the participants; longer storage, even under refrigeration, can lead to microbiological spoilage or chemical deterioration of nutrients. In addition, the foods collected for the assay sample should be documented in the same manner as participants' menus, for example with a tray assembly checklist. (See Chapter 18, "Documentation, Record Keeping, and Recipes.") Any known deviations from the food preparation protocol must be logged on this or a separate standard form. Deviations include ingredient substitutions, weight differences, and

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EXHIBIT 22-3

Procedure for Assembling Foods for Composites

The following is a general procedure for collecting and storing daily menu samples. Modification may be needed for specific foods and/or nutrients (see text and Table 22-7).

A. MATERIALS

Prepared foods from menus (prepared exactly as for consumption by participants)
Airtight food collection containers[1]
Stainless steel spatula(s)
Cryogenic marker[2]
Disposable fat-free powder-free gloves[3]
Refrigerator (0°C to 4°C)
Freezer (−20°C or lower)

B. GUIDELINES FOR FOOD PREPARATION

- Procure the foods from the same sources and prepare, handle, and heat the foods exactly as specified by the menu and recipes. For example, reconstitute dehydrated foods (eg, mashed potatoes) and prepare and cook composite foods (eg, casseroles) and other cooked items (eg, meats) according to the recipe/menu.
- Reconstitute beverage mixes (nondiscretionary) according to menu instructions (ie, as if the drink were to be consumed) before adding to the food collection.
- Remove inedible portions (eg, apple cores, chicken bones, wrappers) when food is collected for analysis.
- Do not include discretionary/*ad libitum* beverages (eg, water, coffee, tea, diet sodas) in the menu samples for assay. (It may be necessary to analyze ad lib beverages for some studies; in most cases they should be assembled as separate samples.)
- For any portion-controlled items: for diet validation, weigh out the exact amount specified by the menu; for monitoring, collect the portion-controlled serving as specified by the menu.
- Protect samples from contact with extraneous materials and maintain a clean environment.
- Use carefully cleaned and dried containers and utensils and wear powder-free gloves to handle and collect foods.
- Include all traces of prepared/weighed foods specified by the menu in the menu collection (because the goal at the diet validation is to verify calculated nutrient levels).
- Record and report any known deviations from the menu preparation protocol. Deviations include ingredient substitutions, weight differences, preparation differences, brand name differences, etc. (This information will be used to evaluate any discrepancies between assayed and calculated nutrient levels.)
- Make sure that each diet sample container is clearly labeled with sample identification information, using a cryogenic marker.

C. TOTAL MENU COLLECTION

1. Assemble all foods from the breakfast menu. *Include* milk and juices but *not* ad lib beverages (eg, coffee, tea, water, diet soft drinks).
2. Retrieve a food collection container and label it, using the cryogenic permanent marker, with the menu number and diet description, date, and your initials (and any other key information).
3. Wear clean, fat-free, powder-free gloves and using a clean stainless steel spatula, scrape *all* of the food into the container. If bread or a muffin is a part of the meal being collected, set it aside and use it to scrape the plate, then add it to the collection container.
4. Completely seal the container and place it in the refrigerator (0°C to 4°C) until collection of total menu is complete (24 hrs or less).
5. Repeat steps 1 through 4 for lunch, dinner, and snacks, adding foods into the same container.
6. After all foods have been collected in the container, completely seal the container and place it in the freezer (−20°C or lower).

[1]For example, Rubbermaid™, available from Consolidated Plastics Co (Twinsburg, Ohio): 12-cup rectangular size (#0040) for 2,000-kcal menu; 19-cup square size (#0016) for 3,000-kcal menu.
[2]For example, Nalgene Cryoware™ markers, available from Fisher Scientific (Atlanta, Ga), catalog #13–382–52.
[3]For example, Sup-pli Line antistatic powder-free vinyl gloves, from Fisher Scientific (Atlanta, Ga), catalog #11–393–85B.

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TABLE 22-7

Precautions to Minimize Deleterious Effects of Sample Preparation and Storage on the Chemical Composition of Diet Samples[1]

Constituent	Partial Changes	Significance of Change	Precautions
Water	Loss (dehydration)	Affects composition of composite as analyzed. Affects calculated total energy.	Report nutrients on dry weight basis. Determine moisture immediately after measuring total (wet) composite weight.
	Changes in distribution in food	Affects homogeneity of composite especially when freezing/thawing	Mix composites thoroughly before/while taking aliquots. Thaw frozen subsamples completely and thoroughly mix before/while taking aliquots.
	Uptake (hydration)	Important if composite is lyophilized	Keep samples in sealed containers. Devise sampling operations to minimize water uptake. Report nutrients on dry weight basis, measuring water in the assayed aliquot at the same time samples are weighed for nutrient assay.
All organic constituents (including protein)	Microbial degradation	Changes in overall composition	Store at low temperature (<0°C).
	Enzymatic degradation (autolysis)	Losses and gains of nutrients	Endogenous enzymes may have to be inactivated.
Fat	Separation	Heterogeneity of composite	Thaw to room temperature. Thoroughly mix before taking aliquots.
	Oxidation	Destruction of polyunsaturated fatty acids	Store at ≤–30°C in sealed, air-free containers, preferably flushed with nitrogen or argon gas. Antioxidants may prevent oxidation in some samples.
	Contamination from handling containers	Falsely elevated values	Use thoroughly cleaned containers. Wear clean gloves when handling containers.
Sugars	Caramelization at elevated temperatures	Losses from decomposition	Avoid elevated temperatures (>60°C). Analyze fresh or freeze-dried samples.
	Conversion of sucrose to mannitol	Loss of sucrose	Keep sample frozen.
Starch	Retrogradation	Increases resistance to enzymatic attack and decreases starch measured by enzymatic methods	Work on freshly prepared sample with minimal storage. Do not dry samples.
Inorganic constituents	Contamination of original sample by soil, water, storage container	Falsely elevated values	Wash containers and utensils carefully and rinse with distilled deionized water.

(*continued*)

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TABLE 22-7

Continued

Constituent	Partial Changes	Significance of Change	Precautions
Inorganic constituents	Contamination of sample by dust, processing equipment and other metallic sources in laboratory	Falsely elevated values	Use containers cleaned according to strict protocol for trace element analysis (including acid washing). Protect samples from dust contamination in laboratory. Acid wash all containers and utensils. Store subsamples in glass acid-washed containers.
Fat-soluble vitamins	Oxidation	Loss	Store at low temperature ($< -20°C$). Protect from light and O_2. Store samples in dark containers, flushed with nitrogen or argon gas.
Water-soluble vitamins			
Thiamin	SO_2 degradation	Destruction	Exclude SO_2.
Riboflavin	Oxidation Photodegradation	Extensive loss	Protect from light and oxygen. Store at low temperature, in the dark, under nitrogen or argon gas.
Niacin	Microbial activity	Loss and/or synthesis	Store at low temperature ($<0°C$).
Vitamin B-6	Microbial activity	Loss and/or synthesis	Store at low temperature ($<0°C$).
Folates	Enzymatic deconjugation Oxidation	Loss	Inactivate deconjugase enzymes immediately. Protect with ascorbate.
Vitamin B-12	Microbial activity	Loss and/or synthesis	Store at low temperature ($<0°C$).
Vitamin C	Enzymatic oxidation	Loss	Analyze fresh if possible. Extract immediately into metaphosphoric acid.
	Catalysis by trace metals	Loss	Avoid metallic contamination.

[1]Adapted from: Greenfield H, Southgate DAT, *Food Composition Data* (New York: Elsevier; 1992).

preparation differences. This information will be used to evaluate any discrepancies between assayed and calculated nutrient levels. (See Evaluating the Analytical Data.)

Containers used to collect diet samples must be clearly labeled with sample identification information, using a cryogenic marker and labels that are water resistant and adhesive at $-20°C$ (eg, Nalgene Cryoware™ markers and Poly Paper computer labels from Fisher Scientific, Atlanta, Ga). To preserve the composition of collected foods, samples should be stored immediately at $-20°C$ or lower in airtight containers. These conditions will hinder microbial and enzymatic degradation and moisture loss. Additional precautions should be taken for preservation of specific nutrients (Table 22–7). Some components require assay of fresh material and/or addition of stabilizers during composite preparation and storage to prevent nutrient degradation; others may be susceptible to degradation during freeze-thaw, such as sucrose (29). In these instances, appropriate adjustments to the protocol must be made.

If samples are shipped off-site (eg, to the food analysis laboratory) for homogenization, care must be taken to maintain the integrity of the foods and prevent loss during shipment. Foods should be frozen solid at $-20°C$ or lower and shipped on dry ice in a sealed, insulated cooler to prevent thawing during transit. Each shipment should include a transfer form to document which specific samples were shipped, the sample weights, and the condition of samples.

At the receiving end, samples should be inspected immediately upon receipt for signs of thawing or damage, and the condition of samples should be recorded. Weighing con-

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tainers before and after shipment will ensure the absence of leakage during transit. Any procedural deviations (eg, open or damaged containers, thawed foods, absence of labels, missing samples) should be documented on the sample transfer form, which is then returned to the shipping facility. Clearly adulterated samples should be documented and discarded.

Preparing Composites

The purpose of homogenization is to prepare a uniform slurry from a collection of whole foods with no nutrient gain or loss in the process. Whole diets are heterogeneous and variable mixtures of individual foods in which nutrients are unevenly distributed across widely varying concentration ranges and matrices. Homogenizing the menu or diet samples into a food composite thus is possibly the most critical step in the assay of diets.

A *composite* is a uniform mixture of the foods constituting the unit of the diet to be assayed and is basically a slurry of small particles of these foods. A *menu composite* comprises all foods served in a given day, and a *diet cycle composite* includes all foods from one full rotation of menus. If food is lost or if nutrients are altered during composite preparation or are not uniformly distributed, assay values will not represent nutrient levels in the original foods. Composite preparation can be done in-house or at the food analysis laboratory, but in either case it must be performed carefully by trained personnel.

A typical menu composite has a volume of 2 to 3 liters, and a week's menus will add up to 20 liters or more. Chemical assays are usually performed on small aliquots (1 mL to 10 mL) taken from the larger volumes of these mixtures. Therefore, it is critical that the homogenate is uniform, so that assayed aliquots are representative of the entire composite. Otherwise, no matter how accurate and precise the measurements, the values will be meaningless with respect to the original material.

It can be difficult to prepare a uniform homogenate of a menu or diet sample. The homogenization is affected by many factors, including the types, proportion, and texture of different foods; fat levels and types of fat; water content; and the presence or absence of emulsifiers, all of which vary widely from food to food, menu to menu, and diet to diet. No standard method of homogenization can guarantee acceptable results for all foods or mixtures of foods. For multicenter studies, it might seem that preparing composites at each feeding site would reduce the cost of shipping samples for analysis, because in most cases only a small part of the whole homogenate is used for all the assays. It is best, however, to prepare all composites at a single location to prevent site-to-site variability at this critical stage.

For most diets and nontrace element nutrient assays, menu and diet cycle composites can be prepared using a stainless steel batch food processor to yield a composite with acceptable homogeneity for 3-g to 5-g analytical aliquots (50% to 80% moisture). Nutrients susceptible to degradation or contamination (refer to Table 22–7) require modification of the basic procedure and/or equipment. For example, trace element analysis for chromium and nickel requires titanium blades and nylon-coated utensils to prevent contamination by elements (eg, chromium) from stainless steel; in the analysis of vitamin C (ascorbic and dehydroascorbic acid), citric or metaphosphoric acid must be added during homogenization (30). Temperature control also is critical during homogenization. Prolonged exposure to elevated temperatures (generally >4°C) potentiates microbial growth and/or nutrient degradation. Even brief exposure to high heat from the processor motor can cause breakdown of some nutrients.

Immediately after homogenization, the composite should be dispensed into sample storage jars. (Although most storage jars are made of plastic, the choice of material for these jars and any other storage container should be made in light of the technical requirements of the protocol. Among the factors that should be considered are size, completeness of seal, resistance to freezing, exclusion of light, and whether any undesirable components can leach into the sample.)

Lichon and James (28) reviewed alternate procedures for preparing food composites. In principle, the less processing the foods are subjected to during homogenization, the more likely the samples will represent the foods as consumed. For this reason, it is recommended that samples be assayed in the fresh or frozen-thawed state. However, in the case of certain components (such as sucrose) further treatment (such as lyophilization) is necessary or preferable to stabilize the nutrient (29). In these instances, these extra processing steps would be carried out only for the aliquots destined for assay of that nutrient.

Preparing Subsamples and Analytical Aliquots of Composites

The terms *subsample* and *aliquot* are often used interchangeably. Specifically, however, a subsample is any portion of the total diet composite; an aliquot is a measured amount of composite taken for a specific assay (see Exhibit 22–1). The use of subsamples considerably reduces multiple freeze-thaw cycles and external contamination of the food composites. For most studies, 4 subsamples (15-g to 25-g jars) per analyte for assays and an additional minimum of 5 subsamples for the study archive are adequate. At the point of subsampling the food composite, the composite temperature should be between 20°C and 25°C; lower temperatures result in congealing of fat and concentrating of solutes; higher temperatures contribute to nutrient loss caused by chemical and enzymatic reactions.

Subsampling should be accomplished rapidly and the subsamples immediately frozen to avoid microbial or enzymatic degradation of nutrients. Frozen samples must be thawed and thoroughly mixed prior to taking aliquots for assays. Portioning the total food composite into subsamples with continual stirring prevents sedimentation. Again, specific considerations may be required for labile nutrients (Table 22–7). If samples are lyophilized, they must be reblended (and at the same time protected from excess heat)

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after freeze-drying because the process of lyophilization can cause stratification of food components.

Taking aliquots of composites for analysis is a crucial step in an assay, the significance of which is often overlooked by untrained personnel. Typical diet composites are prone to sedimentation and fat separation. Thus, if a composite is not adequately mixed prior to dispensing, assayed aliquots will not be representative of the original material.

Composite Homogeneity

Composites must be homogeneous because settling or incomplete blending can be a source of assay bias (ie, from the analysis of unrepresentative samples). The homogeneity of a composite can be evaluated by assaying a range of aliquots, drawn across the entire subsampling procedure. A suggested sampling plan is shown in Figure 22–5. Moisture content is a useful indicator of homogeneity, and the distribution of key nutrients for a given study should be checked as well. Replicate aliquots from each subsample are assayed, and the standard deviations of the replicate values are calculated for within and among subsamples. A composite can be considered homogeneous for purposes of the assay, if (1) the overall variance for replicate values is acceptable based on the end use of the data, and (2) the variance among subsamples does not exceed variance within subsamples (as determined by a statistical analysis of variance).

A pilot study to check composite homogeneity is recommended. If composites appear heterogeneous, additional blending is recommended. If a composite is still not of acceptable homogeneity and cannot be further blended, one way to improve the confidence of assay data is to analyze a greater number of subsamples (ideally drawn from across the subsampling process) and obtain a mean value based on multiple replicates. This will increase the cost of analysis. Alternatively, one can analyze larger aliquots of the composite if allowed by the assay procedure. It is not necessary to validate the homogeneity of each diet composite when different diets to be assayed are composed of similar foods.

Storing Samples

Proper storage of composited diets is important to retain the original composition. The proximate composition (moisture, ash, protein, total fat), fatty acids, and cholesterol of composited diets appear to be stable for at least 3 years at −60°C when samples are packaged and stored as described earlier (Holden JM, USDA, Beltsville, MD; unpublished data). The lability of other nutrients (Table 22–7) should be considered as necessary. Each jar and its lid should be labeled for identity using a cryogenic marker.

Before investigators initiate the study, they should ensure that adequate freezer space is available for sample storage, and alternate freezer space should be identified for emergency use. Freezers fail and power outages do occur with distressing frequency, so it is desirable to have alarm-wired freezers that are visually inspected *and* electronically monitored for temperature fluctuations. Protocols should be put in place to minimize the damage that might occur if these problems arise.

Assay Methods

Methods must be carefully chosen and validated, performed by trained analysts, and undergo continuous quality control

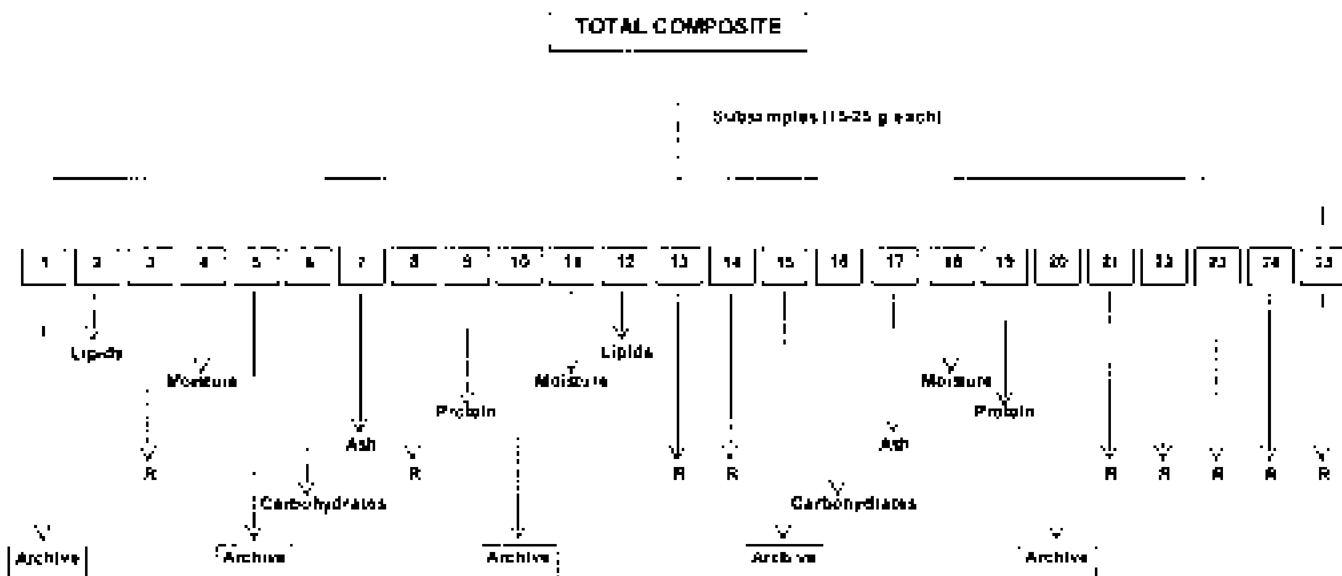

FIGURE 22-5. Distribution of composite subsamples for nutrient assays: a sample scheme.[1, 2]

[1]Subsamples are numbered in chronological order of dispensing from the total composite.

[2]R = Reserve sample (for repeat assays, if needed).

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if accurate, precise, and *meaningful* results are to be obtained. These concerns are pertinent whether assays are performed in-house or by an outside laboratory. Most clinical investigators subcontract assays to an experienced food analysis laboratory. By understanding the issues involved in assay methodology, validation, and quality control, investigators will be better prepared to select and interact with the food analysis laboratory and evaluate the resulting data.

Choosing Methods

Most laboratories use "standard" methods or modifications thereof. Standard methods exist for many nutrients and are often the first resource. Such methods are tested and published by several organizations, for example: the Association of Official Analytical Chemists (31), the American Association of Cereal Chemists (32), the American Oil Chemists' Society (33), and the International Union of Pure and Applied Chemistry (34). Excellent discussions of methodology for a wide range of nutrients can be found in Sullivan and Carpenter (35) and Greenfield (36; pp. 81-126). Sullivan and Carpenter (35) also summarize current methods accepted for nutrition labeling in the United States.

Note, however, that standard methods are just that: *standardized* to a set protocol, but not necessarily *accurate* for all possible food matrices or collaboratively studied for precision and accuracy. Currently accepted standard methods vary widely in these qualities (Tables 22–4 and 22–5). Most methods have been verified for only a limited range of matrices. Use of a nonvalidated assay system can result in significant bias, described next.

For novel constituents, the scientific literature is a source of methodology. A selected listing of food analysis journals is given in Exhibit 22–4. Books on methodology for specific nutrients are also available. An appropriate or adaptable method, and possibly an expert laboratory willing to perform the assays on a contractual basis, can usually be located by contacting the authors of articles, abstracts, or books.

Accuracy, precision, cost, quality control, and turnaround time all affect the choice of an ideal method. For each method the investigator should be aware of strengths and limitations and the inherent potential for bias. To minimize variance from analytical bias, it is imperative that for a given nutrient the *same method* is used throughout the study and that all assays are performed at the *same laboratory.*

Method Bias

Often there is more than one standard method for a given nutrient. For example, total fat can be determined by various gravimetric methods: acid hydrolysis (eg, AOAC methods 922.06, 925.12, 925.32, 935.38, 935.39D, 945.44, 948.15, 950.54) (31); Soxhlet (ether) extraction (AOAC methods 920.39B, 920.39C) (31); chloroform/methanol extraction (AOAC methods 983.23) (31); or as the sum of fatty acids measured by gas chromatography (AOAC methods 969.33, 963.22) (31).

The FDA Nutrition Labeling and Education Act (NLEA) (15) defines total fat as the sum of fatty acids C4 to C24 expressed as triglycerides, yet currently there is no corresponding validated methodology for measuring total fat in foods. Because traditional gravimetric total fat methods are known to measure more than fatty acids as "total fat" (22), it can be expected that newer methods standardized to the NLEA definition of total fat will yield different (and in most cases, lower) values for total fat.

There are also several different standard methods utilized to assay *trans* fatty acids, including direct gas chromatography (GC) (AOAC method 985.21) (31), infrared spectrophotometry (AOAC method 965.34) (31), combined GC-infrared spectrophotometry (27), and silver ion thin-layer chromatography or silver ion high-performance liquid chromatography (HPLC) combined with gas chromatography (GC) (37). There are demonstrated biases among different methods. For example, direct GC alone yields values for the total C18:1 *trans* fatty acid content of whole diet composites and food oils that are 5% to 25% lower than values obtained using GC combined with silver ion HPLC (37).

Another source of bias is interlaboratory bias, in which the "same" method yields consistently different results at different laboratories. Interlaboratory variance is well known among chemists (38). Table 22–8 summarizes differences in selected nutrients analyzed in a mixed diet composite at independent laboratories (39). These data illustrate the difference in composition that would be observed simply by sending aliquots to different laboratories. In this study the

EXHIBIT 22-4

Journals Reporting Food Analysis Methods and Results

Journal of Agricultural and Food Chemistry, American Chemical Society (Washington, DC)
Journal of the American Oil Chemists Society, American Oil Chemists Society (Champaign, Ill)
Journal of the Association of Official Analytical Chemists International, Association of Official Analytical Chemists International (Gaithersburg, Md)
Food Chemistry, Elsevier Science Ltd (Oxford, UK)
Journal of Food Composition and Analysis, Academic Press, Inc (San Diego, Calif)
Journal of Food Lipids, Food and Nutrition Press, Inc (Trumbull, Conn)
Journal of Food Science, Institute of Food Technologists (Chicago, Ill)

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TABLE 22-8

Nutrient Levels in a Total Diet Composite Assayed at Five Commercial Laboratories[1]

A. Assayed Component per 100 g of Diet Composite[1]

Component		Laboratory				
		A	B	C	D	E
Moisture	(g/100 g)	64.1	64.8	64.8	65.6	65.9
Protein	(g/100 g)	7.8	7.8	7.6	7.4	7.8
Ash	(g/100 g)	1.1	1.2	1.3	1.5	1.3
Total Fat	(g/100 g)	5.2	5.2	5.9	5.6	5.9
Cholesterol	(mg/100 g)	45.8	21.7	25.0	16.9	16.5
Sodium	(mg/100 g)	238.9	219.4	244.4	241.7	258.3
Potassium	(mg/100 g)	188.9	—	197.2	205.6	179.2
Calcium	(mg/100 g)	63.9	58.3	63.9	55.6	60.3

B. Assayed Components per Menu[2]

Component		Laboratory				
		A	B	C	D	E
Moisture	(g)	1,154	1,166	1,166	1,181	1,186
Protein	(g)	140	140	137	133	140
Ash	(g)	20	22	23	27	23
Total Fat	(g)	94	94	106	101	106
Cholesterol	(mg)	824	391	450	304	297
Sodium	(mg)	4,300	3,949	4,399	4,351	4,649
Potassium	(mg)	3,400	—	3,550	3,701	3,226
Calcium	(mg)	1,150	1,049	1,150	1,001	1,085

[1]Holden JM. USDA, Beltsville, MD. Unpublished data, 1994.
[2]Values in Part B are derived from values in Part A.

key nutrients were total fat and cholesterol. Clearly, the values determined for these components varied from laboratory to laboratory. Total fat calculated as grams per daily menu ranged from 93.6 g to 106.2 g.

In the absence of standard reference materials to determine the accuracy of the measurement systems, it is difficult to assess which value is ''correct.'' The previous example underscores the importance of using methods validated for the samples at hand and instituting rigid quality control, including analysis of a food-based control material with each assay. Furthermore, although reputable commercial laboratories routinely employ adequately tested methods, standard methodology does not guarantee accurate results across all food matrices.

The significance of method bias will depend on how the analytical data are used—that is, are the exact nutrient levels of primary concern, or are the differences in the nutrient levels among diets more important? Take, for example, a design in which difference in the nutrient levels among diets is the most important factor, and three diets are studied at levels of 26%, 30%, and 37% of energy as total fat. In this case, the primary parameter is *difference* in fat content; therefore, bias is less critical, and the key concern is consistency throughout the study.

Validating Methods

Validating a method means confirming that the assay measures the concentration of the analyte with acceptable accuracy and precision, in the specific sample type or types to be tested, at a given laboratory (*accuracy* and *precision* are defined in Exhibit 22–1).

Following a standard written method does not guarantee that a given laboratory will obtain acceptable results. The laboratory performing assays must demonstrate that acceptable results can be obtained *in that laboratory* for the relevant nutrient levels *in the appropriate matrices*. Characteristics of a valid method include:

- Produces the same results as a previously accepted method over probable concentrations of the analyte in the matrices to be analyzed.
- Achieves quantitative recovery of pure analyte standards in a total assay.
- Achieves quantitative recovery of analyte standards added to the matrices to be assayed (ie, method of ''standard additions'').
- Yields a result for appropriate standard reference material(s) within the certified range.
- Has a level of precision for replicate assays (>5 replicates)

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of the analyte in the sample matrices to be assayed that is acceptable for the purpose of the study.
- Is free of major sources of interference.
- Has a known limit of detection (LOD) in the matrices to be assayed, and that LOD is acceptable for the study.
- Has a known analytical range in the matrices to be assayed, and that analytical range is acceptable for the study.
- Produces acceptable results with the method when performed by more than one analyst and more than one laboratory.
- Has a built-in quality control protocol.

Detailed discussions can be found in DeVoe (40), Garfield (41), and Dux (42). Even after the laboratory has demonstrated proficiency, the investigator is strongly advised to include blinded control samples along with the diet composite samples submitted for analysis to validate each individual data set.

Assay Quality Control

Assay quality control is the implementation of a system to ensure that the accuracy and precision of chemical measurements meet requirements for the end use of the data. A full discussion of quality control and the statistical treatment of analytical measurements is beyond the scope of this text. Taylor (26), Dux (42), Garfield (41), as well as Chapter 23, ''Laboratory Quality Control in Dietary Trials,'' can help investigators to establish quality control protocols for any in-house assays.

When assays are performed out-of-house, the investigator must still implement quality control measures, and in a sense they are even more important without knowledge of the entire assay system. There are four basic components to quality control of measurements made at an outside laboratory, including use of appropriate control samples with study samples; implementing quality control charts and appropriate standards for precision of measurements; selecting a reputable laboratory that follows Good Laboratory Practice Standards (43); and comprehensively documenting samples, procedures, and data. Other specific components of quality control and quality assurance should be addressed internally by the food analysis laboratory.

Control Materials

A food-based *quality control material* (QCM) is a homogeneous composite consisting of food(s) similar in type and having nutrient concentrations comparable to those in samples to be assayed. Additionally, analyte concentrations in a QCM are well-characterized and known to be stable for the duration of the study. The purpose of the QCM is to ensure the absence of deviations in the routine measurement processes.

Prior to the study, the mean and tolerance limits for the concentration of each key nutrient in the QCM should be established by performing a series of assays using the methods that will be used for the study. (See Quality Control Charts and Standards for Analytical Precision.) Subsequently, an aliquot of the QCM is assayed with each batch of samples, or approximately every 15 samples in a continuous system. A value for the QCM outside the tolerance limits indicates a possible shift in the measurement system; this suggests that results for other samples analyzed in the same batch may be invalid.

Credible laboratories analyze an in-house QCM and/or reference material with each assay run. If the laboratory analyses are done under contract, it also is necessary to include a blinded sample of externally procured, matrix-matched QCM with each batch of diet samples to check consistency of results over time and appropriate handling of samples and data. It is particularly important that at least one of the routinely employed in-house or external QCMs have a matrix and nutrient composition that is similar to the mixed diet composites generated by a particular study. In the worst case, significant errors in the system may not be detected unless a food-based QCM is used.

This point is illustrated in Figure 22–6, which shows data for two QCMs used in the determination of total dietary protein assayed as Kjeldahl nitrogen. Each assay run was conducted using both QCMs. All of the *nitrogen* values for the *nonfood* control material, ammonium oxalate, were well within the acceptable limits (Figure 22–6a). For three of the assay runs (P035, P036, and P037), however, *protein* values for the *food-based* control material were unacceptably low (Figure 22–6b). Review of the data revealed that a mistake had been made in the algorithm that was used to convert assayed nitrogen content to calculated protein content. This calculation step was routine for the food-based control material and for the experimental diet samples but was not necessary for the nonfood control material. Had only the ammonium oxalate control material been used, the mistake would not have been detected, and the reported protein values for the experimental diet would have been erroneously low.

An appropriate food-based QCM is: matrix matched (ie, composed of foods comparable to study samples), with nutrients at concentrations similar to those in samples; homogeneous; stable for the duration of the study; and characterized for key nutrient concentrations. There are a few commercially available mixed-food standard reference materials (SRMs) that have been certified for the concentrations of selected nutrients, and these are sometimes used as QCMs (23). However, because *standard reference materials* are rigorously characterized, they are quite expensive. Most commercial SRMs are freeze-dried and thus present a different matrix than the wet-diet composite. For many nutrients, no mixed-food standard reference material exists. However, an acceptable QCM need not be as rigorously characterized as an SRM because its main purpose is to monitor the precision over time of an assay system that has been validated for accuracy.

One simple way to obtain a QCM for a given diet trial is to prepare a composite of study menus. Sullivan and Carpenter (35) have also discussed the preparation of in-house

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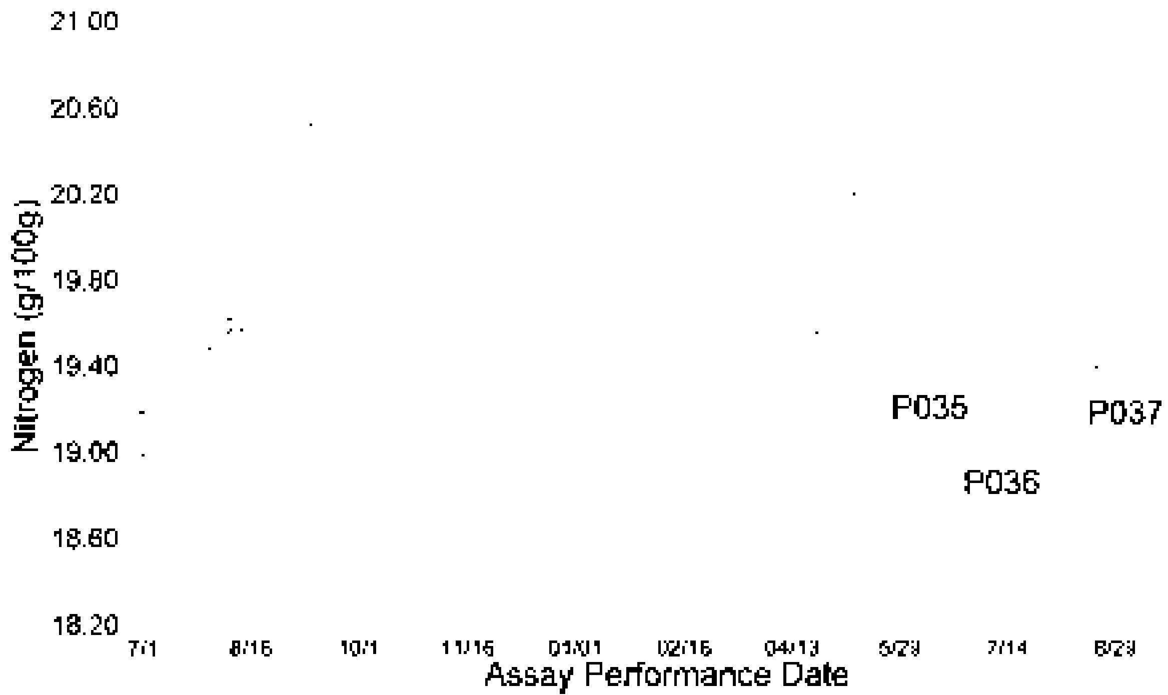
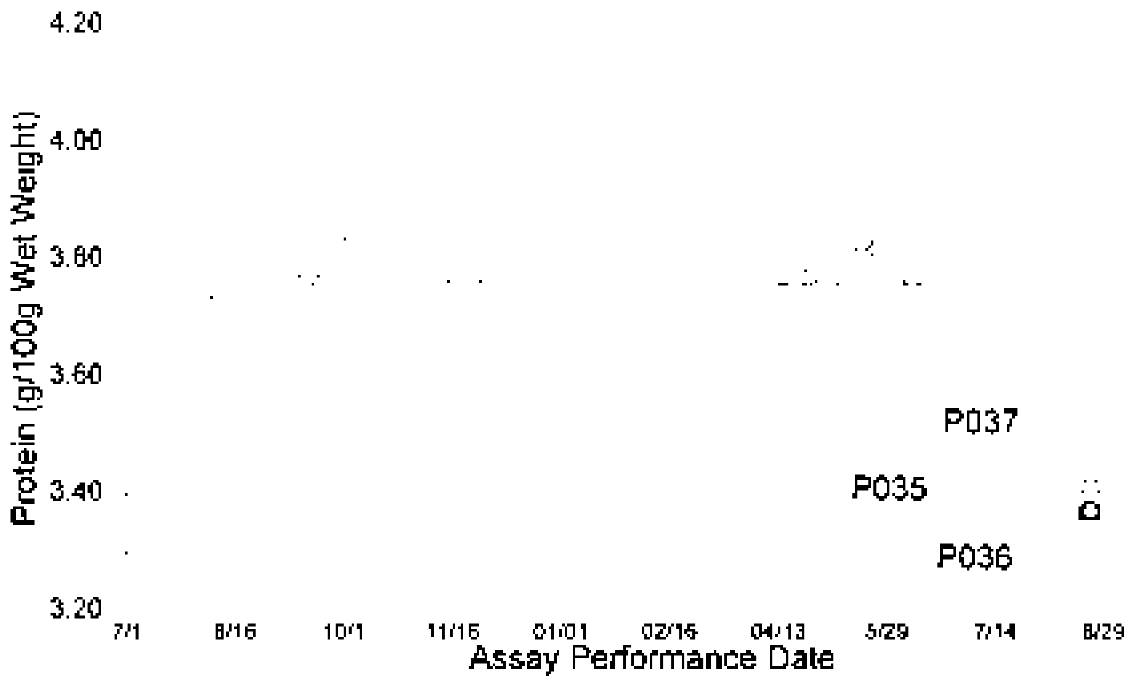


A. Nitrogen content of nonfood quality control material (ammonium oxalate).



B. Protein content of food-based quality control material (mixed-diet composite).

FIGURE 22-6. Quality control charts for protein assay by the Kjeldahl nitrogen method.[1, 2, 3]

[1]Each oval represents the assay value for one quality control sample (data from authors' laboratory).

[2]_________ Mean, - - - - - Mean ± 3 SD.

[3]P035, P036, and P037 represent three assay runs performed with both quality control materials. Multiple samples of food-based QCM were analyzed in run P037.

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control materials. The minimum total amount of QCM that should be prepared can be estimated as

(Total number of composites)
× (Total number of components to be assayed per composite)
× (Number of replicate assays per component)
× (1 QCM subsample per 15 replicate assays)
× (25 grams per QCM subsample)
× 5
= grams QCM required.

The "safety factor" of 5 allows for assays to establish control limits, plus reruns and supplemental assays. However, it is preferable to prepare as much excess QCM as storage and handling allow because unused samples can always be discarded but the exact material cannot be replicated.

For the QCM to be a useful tool for monitoring assay precision, its homogeneity and stability are essential. Because total variance in the QCM measurements comprises both sample heterogeneity and analytical variability, the more homogeneous the QCM, the greater the ability to detect real variance in the assay system. Sample-to-sample variance for the QCM must be lower than the acceptable variance for study samples; otherwise, meaningful deviations in the assay system will be undetectable.

Homogeneity of the QCM can be assessed as described previously (see Composite Homogeneity). If necessary, to optimize uniformity of the QCM, foods that are difficult to composite (eg, fresh vegetables, leafy greens, nuts, and raisins) can be prehomogenized. Alternatively, substitutions can be made with commercially available homogeneous foods of like composition—for example, chicken baby food for whole chicken pieces or breadcrumbs for sliced bread—while maintaining calculated levels of key nutrients. Moisture loss, contamination, and degradation of the key nutrients in QCM samples must also be prevented for the material to be an effective monitoring tool. Again, Table 22–7 lists precautions for preserving labile nutrients. If stability is uncertain, it is best to be extra cautious and store the QCM at −60°C or lower, protect it from freeze-thaw cycles, and limit exposure to light and oxygen by storing in dark containers capped tightly under nitrogen or argon.

Quality Control Charts and Standards for Analytical Precision

A quality control chart (QC chart) is a plot of the assayed values of the QCM vs assay date (see Figure 22–6). A QC chart is specific to a given method and control material. The QC chart depicts assay performance through a period of time and allows the detection of (meaningful) drift or isolated deviations in measurements. QC charts can be used by the investigator to monitor the proficiency of the laboratory and document any assay variance.

Control limits (ie, an acceptable range for the QCM value) are established and then are used to evaluate data from a given assay. For food analysis, typical control limits are ± 3 times the standard deviation of the mean, approximating the 99% confidence interval (26, pp. 131-132; 43, pp. 19-20). A detailed discussion of establishing and evaluating control limits can be found in Taylor (26).

The mean, standard deviation, and control limits are calculated from a set of preliminary assays (15 or more) of the control material using fixed methodology. It is best for these measurements to span the most possible sources of variance (eg, different analysts, time, new batches of reagents) expected across the duration of the study. To establish a control chart for subcontracted assays, 5 samples of the QCM can be sent on a minimum of three separate occasions as far apart in time as possible.

Details of the statistics and interpretation of QC data are beyond the scope of this discussion, and the reader is referred to one or more textbooks on the subject (26, 41, 42). The concern for the clinical investigator evaluating the QCM control chart is detecting gross shifts in the assay system that might compromise interpretation of the diet composition results. This can be done by assuming that the control limits established from preliminary assays are representative of routine assay precision. If a subsequent value for the QCM is outside the ± 3 SD limits, it is relatively certain that the discrepancy is real, and all samples in that batch should be reassayed. Although the premise is that any factor causing a deviation in the QCM value equally affects other samples in the batch, which may not be true, the risk of falsely rejecting data is small relative to the chance of otherwise accepting errant values. If reassay yields a control value out of range, further analyses should be stopped until the reason for the deviation is investigated and resolved.

Another criterion for the control chart is that all values should be normally distributed about the mean. A rule of thumb is that more than 7 consecutive values on the same side of the mean suggests a statistically significant shift in the system (26, pp. 136-137). Sometimes assay systems will drift slightly up and down over time for no identifiable reason, and this can be considered part of overall analytical variance. Although these shifts may be *statistically* significant, the practical importance of such deviations will be determined by their magnitude and the corresponding impact on the use of the data in a particular study. No general methods have been published for determining acceptable limits for assay drift based on end use of the data and setting corresponding QC criteria, and this is a subject for future study. Although use of quality control samples allows documentation of assay performance, it is incumbent upon the clinical investigator to interpret the significance of any deviations in the context in which the sample data are used.

Replicate assays offer yet another data control measure. It is wise to plan for each composite to be analyzed in replicate (ie, duplicate or preferably triplicate, and occasionally higher). These replicate assays are used to detect and account for isolated, sample-specific, assay errors and/or sample heterogeneity. The investigator should be sure that the food

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TABLE 22-9

Key Components of Documentation for Diet Assays

Component	Details Requiring Documentation
Sample identification information	Source Study Description (including details of food preparation procedure) Date Weight
Sample collection information	Date Location Procedures used (including name of person collecting sample) Weight
Sample storage information	Date Location Temperature
Subsampling information	Procedure Date Parent sample
Sample preparation	Detailed description of compositing Description of other procedures performed
Assay procedures performed	Detailed description of each procedure used, including thawing and taking aliquots
Quality control information associated with sample value(s) (eg, QC chart)	Source or description of quality control materials, reference standards, and calibration standards Assay results for these materials
Shipment of samples	Sample identification Sample weights Condition of samples (eg, temperature) at time of shipping and time of receiving
Reported values	Source of data Original sample identification Procedures used

analysis laboratory understands the need for replicates. Commercial laboratories often base the per-sample assay cost on the assumption of a single determination. Single analysis always runs the risk of error because of sample heterogeneity or an isolated error in the assay procedure, despite an acceptable batch QCM value and other data from the laboratory regarding the general precision of the assay. Nonetheless, replicate assays done by a commercial laboratory can be expensive, so the investigator must balance the potential for variance and its impact on the study against cost.

These concerns will be greater in long-term studies or in those requiring high precision. Again, a statistician can be of assistance in this domain. Doing blinded reruns on about 5% of the samples is a good practice that ensures the reproducibility of results for individual composites over the long term.

When a composite is assayed in replicate, we recommend drawing the replicate aliquots from different subsamples (ie, storage jars) that are separated from one another chronologically in the composite subsampling process. Because it is impractical to confirm the homogeneity of every composite and settling during composite subsampling is the most likely source of any heterogeneity, assaying replicates in this fashion serves to maximize the chance of detecting any differences. It also renders the mean a better estimate of the true overall composition of the composite. An example of such an assay sampling plan is illustrated in Figure 22–5.

Documentation

Thorough documentation is essential to provide an audit trail linking reported values for a sample to details of the sample description, sample handling, assay methodology used, and associated data (eg, values for quality control samples run in the same assay batch, values for the same sample determined in different assays). It is beyond the scope of this chapter to comprehensively discuss each component of documentation. Interested readers are referred to Good Laboratory Practice Standards for further information (43). A list of key components of documentation for diet assays as part of a well-controlled diet study is given in Table 22–9.

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EXHIBIT 22-5

Laboratories That Analyze Nutrients in Foods

Covance Laboratories
3301 Kinsman Boulevard
Madison, WI 53704
Phone: (608) 241-4471
Web site: http://www.covance.com

Eurofins Scientific
2394 Route 130
Dayton, NJ 08810
Phone: (800) 841-1110
Web site: http://www.eurofins.com

Food Analysis Laboratory Control Center (FALCC)
Department of Biochemistry
Virginia Polytechnic Institute and State University
Blacksburg, VA 24061-0308
Phone: (540) 231-9960
Web site: http://www.vt.edu

Lancaster Laboratories
2425 New Holland Pike
Lancaster, PA 17605-2425
Phone: (717) 656-2308
Web site: http://www.lancasterlabs.com

Pennington Food Analysis Laboratory
Pennington Biomedical Research Center
Louisiana State University
6400 Perkins Road
Baton Rouge, LA 70808-4124
Phone: (225) 763-2500
Web site: http://www.pbrc.edu

Ralston Analytical Laboratories
2RS Checkerboard Square
St Louis, MO 63164
Phone: (800) 423-6832
Web site: http://www.ralstonanalytical.com

Southern Testing and Research Labs
3809 Airport Drive
Wilson, NC 27896
Phone: (252) 237-4175
Web site: http://www.strlabs.com

USDA Food Composition Laboratory
Building 161, Room 102
Beltsville Agricultural Research Center
Agricultural Research Service, USDA
Beltsville, MD 20705
Phone: (301) 504-8356
Web site: http://www.usda.gov

Disclaimer: This list is provided for the information of readers and is not intended to be comprehensive. Mention of these laboratories does not constitute endorsement by National Heart, Lung, and Blood Institute, The American Dietetic Association, or the authors.

Choosing a Food Analysis Laboratory

The quality of the food analysis laboratory and its product (assayed nutrient levels) will directly affect conclusions about the composition of diets fed to participants. Possibilities for a contract laboratory include government, university, or commercial facilities (see Exhibit 22–5). Certain analyses (for example, *trans* fatty acids, carotenoids) are not standard offerings of most commercial laboratories, and the best approach is to determine (eg, from a literature search) who are the experts in the field and obtain from them recommendations for a subcontractor.

The responsibility for method validation and assay quality control rests with the laboratory itself. Individual laboratories vary widely in quality. Usually, it can be assumed that a reputable commercial laboratory employs well-tested methods. However, the investigator should still request documentation about the exact assay methods used. Although an established method may be in use, this does not necessarily mean the method has been validated for the particular mixed-food composite matrix or for the expected nutrient levels in diet samples from a given study.

The food analysis laboratory should be provided a listing of constituent foods in composite samples to allow the chemist to verify specific methods. The laboratory should also be given the expected concentration range for each nutrient to be assayed so that analytical concentration limits (ie, weight of nutrient per gram of analytical aliquot) can be estimated.

A high-quality laboratory would answer affirmatively to the questions listed in Exhibit 22–6, which test for practices basic to well-conducted chemical analyses. Other pertinent issues are the general reputation of the laboratory, the number of years it has been in business, the usual turnaround time, ability to provide documentation, and potential for flexibility in working with the study and providing continuing support. The relative importance of these factors will be affected by the number of samples, the nature of the analytes, and the duration of the study. Obviously cost is an important consideration, but cost concerns should never compromise data requirements. If accuracy and precision criteria are stringent and standard reference materials are available for analytes, these samples can be sent to the laboratory to test performance. Once a laboratory has been chosen for a given assay, all assays should be performed at that laboratory to eliminate potential bias and interlaboratory variance.

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EXHIBIT 22-6

Questions for Assessing the Quality of a Food Analysis Laboratory[1]

_______ Do you use standardized, written methods?
_______ Will you provide descriptions of your methods, any modifications of standard methods, and your internal laboratory quality control procedures?
_______ Do you assay each sample at least in duplicate? Are values for replicate assays reported?
_______ Do you participate in collaborative check sample (ie, reference sample or ''round robin'') programs when available? Is your performance in these programs within acceptable limits? Can you provide documentation of performance, if requested?
_______ Do you use standard reference materials to validate each method?
_______ Do you use internal standards for chromatographic assays?
_______ Do you develop and implement food-based control materials to monitor the accuracy and precision of all assays in your laboratory?
_______ Are control materials matrices matched to the samples being assayed?
_______ Are summary statistics available for control materials?
_______ Is the control material assayed with each batch of samples?
_______ For each sample value reported, is the control material identity and corresponding assayed value reported?
_______ What is the cost of replicate assays of each nutrient? Do you have any additional costs or service charges? Who pays for shipping?
_______ What is the average time from receipt of the sample to the issuance of an assay report? What is the longest time it will take you to produce an assay report?
_______ How should we prepare the samples for your analysts?

[1]Courtesy of Holden JM, USDA Nutrient Data Laboratory, Beltsville, Md.

EVALUATING THE ANALYTICAL DATA

The first step in evaluating analytical data is to assess the corresponding quality control material data for each assayed value (using a QC chart) and the precision of replicate values, and to reject any data that do not meet the quality control criteria established for the study. Next, assayed nutrient levels are converted to the required units, if necessary. Normally assay values are reported on an as-received basis: for example, grams per 100 g wet weight. The clinical investigator will usually be interested in the nutrient density of the diet, in certain units (eg, g per day, % of kcal). The data can then be compared to the target diet composition and tolerance limits.

For diet validation, if analyzed nutrient concentrations agree with the diet design, no changes should be made to the menus that were assayed. If the analyzed concentration of one or more nutrients deviates meaningfully from the calculated target in a given menu or diet, there are several possible reasons: error(s) in food preparation, analytical error(s), or a true difference in the actual and targeted compositions. Because detection of true differences is the goal of diet analysis, the first step in evaluating any discrepancy is to rule out the other sources of error. Food preparation deviations are suspected when analyses of replicate composites yield different results. Food preparation records should be reviewed to make sure that items were procured and prepared according to specifications and that portions were weighed correctly. In our studies, we have been able to identify the source of some significant deviations in the composition of prepared menus in this manner (44).

If a preparation error is found, the menu can be prepared again and assayed. Analytical error will be minimized if procedures are properly validated and if composite preparation and assays are performed with strict quality control as outlined in this chapter. The quality control chart can be examined to determine whether assay drift might explain the deviation. Samples of off-target composites can also be reassayed to double-check the data values.

If the actual nutrient concentrations in prepared diets truly deviate from design, there are several possible courses of action. If individual menus were assayed, those erring from target composition can be adjusted and reassayed, or eliminated from the menu cycle. If the diet cycle composite was analyzed, then individual menus (either archived when the cycle composite was prepared or prepared fresh) can be assayed, and those found to differ from target composition can be adjusted (recalculated and cooked) and reassayed, or they can be dropped from the final set of menus.

Finally, there are several other considerations in evaluating the raw analytical nutrient data. First, variance in *moisture content* will cause variance in nutrient values as percent of wet weight (g/100 g as received), which is the usual unit in which raw data are reported. For example, if the parameter of interest is total fat per day, a 2,000-g daily menu containing 60 g of fat would have an assayed total fat content of 3.0 g/100 g. If an extra 100 g water (with no nutritional value) were inadvertently added, the assayed total fat content

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would be lower (2.8 g/100 g), even though total fat (60 g) and energy in the menu were unchanged. This difference is corrected when the assayed weight percent of fat is multiplied by total food weight to yield total grams/menu.

Nutrients can also be expressed as percent of dry weight. Errors, bias, and/or variance in the moisture assay will, however, be incorporated into the variance of a nutrient value on a dry-weight basis. The higher the moisture content of the sample, the greater the impact of any error in the moisture measurement. Lyophilized samples are of particular concern. Although they have a low water content, these materials tend to be hygroscopic and the moisture level can fluctuate significantly. Therefore, data for freeze-dried samples should always be reported on a dry-weight basis, and moisture must be assayed in an aliquot of each sample, obtained from the same container, at the same time an aliquot of that material is weighed for the nutrient assay.

Second, all errors or variance in component analytical values will be *additively* incorporated into calculated nutrient parameters (eg, total energy calculated from proximates, nutrients as grams/day or percent of energy). Errors may or may not cancel. Therefore, the precision of calculated parameters will generally be lower than that of assayed values. Furthermore, if analytical nutrient data are converted from weight percent to total weight per day (week, etc), the absolute magnitude of the effect of any analytical deviation will be directly related to the total sample weight. For example, if the total menu weighs 1,500 g or 3,000 g, respectively, differences in assayed cholesterol concentrations of 15 mg/100 g vs 17 mg/100 g in a given composite will translate to differences of 30 mg (225 mg/100 g vs 255 mg/day) or 60 mg (450 mg/100 g vs 510 mg/day).

Budget Considerations

Budgets for chemical analysis of research diets are not based solely on assay costs. Aside from expenses typical of any analytical laboratory operation, funds also must be available to purchase basic food supplies and to prepare and collect the individual foods or meal samples. For validation samples, each daily menu for each dietary treatment is prepared in duplicate. For monitoring samples, meals and snacks are prepared for at least one extra participant for each dietary treatment for one or more menu cycles. Such costs must also be anticipated when planning pilot studies.

As a guide for budget preparation, cost components of chemically analyzing diet composition as part of a controlled feeding trial are outlined in Exhibit 22–7. This summary presumes samples will be collected and composited on site, with aliquots shipped to a food analysis laboratory for assays.

Conclusion

A key goal of well-controlled feeding studies is to produce and deliver experimental menus that consistently meet the diet design criteria. Diet validation *prior to feeding* ensures that menus contain the targeted nutrient levels. When this prefeeding chemical validation is combined with appropriate and standardized food procurement, handling, and preparation protocols to maintain consistency of diet composition across time (and sites, if the study is multicenter), delivery of the desired diets is likely.

Diet monitoring assays can document what was actually fed to participants. However, by the time the monitoring results are available, the feeding periods usually are finished. Also, it is impractical to sample and analyze enough samples to determine whether a deviation in composition assayed in a single monitoring sample represents an isolated error (in that particular sample), a consistent deviation in the diet composition (which deserves correction), or variance in composition (cycle-to-cycle and/or sample-to-sample). Therefore, the bulk of resources allotted to chemical assays should be used for validating diets prior to feeding and controlling diet preparation during intervention.

This chapter has presented procedures for the implementation of diet assays and has outlined the key components involved in obtaining reliable data from analysis of diet composites. Chemical assay of diets in an intervention study should be considered a control measure, similar in purpose to compliance checks. Although most clinical investigators routinely document their efforts and degree of success in encouraging high compliance, assaying diet composition is a less familiar concept. Both processes, however, serve to ensure desired nutrient intake. Given the limitations of food composition databases and the unpredictability of nutrient variance in prepared diets, prepared experimental diets should be chemically analyzed to definitively link dietary nutrient concentrations with biological measurements. The resulting enhancement of confidence in the validity of the study outcomes suggests that diet analysis should be routinely incorporated into protocols for well-controlled feeding studies.

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References

1. US Department of Agriculture. *Nutrient Database for Standard Reference, Release 12*. Nutrient Data Laboratory Home Page http://www.nal.usda.gov/fnic/foodcomp. Riverdale, Md: Agricultural Research Service; 1996.
2. International Food Biotechnology Council. Variability in the composition of traditional foods: nutrients, microorganisms, and toxicants. In: Biotechnologies and food: assuring the safety of foods produced by genetic modification. *Reg Toxicol Pharmacol*. 1990;12(3):S11-S78.

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EXHIBIT 22-7

Cost Components for the Chemical Analysis of Diet Composites[1]

LABOR[2]
Designing sampling and analysis plans for diet validation and monitoring
Preparing quality control material
Preparing extra menu/food samples for diet validation and monitoring
Preparing composites
Shipping composite samples for analysis
Receiving, processing, analyzing, and reporting nutrient data
Performing quality control review of analytical data
Maintaining documentation and audit trails
Assaying food samples and quality control samples for nutrients
Establishing and performing data analysis and quality control program

EQUIPMENT
Industrial batch food processors for preparing composites: 25 L or larger for menu cycles (7 to 8 daily menus); 6 L for individual daily menus
Analytical balance
Other laboratory equipment as needed

SUPPLIES
Containers for food collection
Jars for composite subsamples
Insulated coolers for shipping samples
Dry ice
Supplies for food collection (see Exhibit 22-3)
Supplies for preparing food composites
Chemicals and other laboratory supplies

FOOD[3]
Prefeeding diet validation samples
Diet monitoring samples
Quality control material

STORAGE FACILITIES
Refrigerator
Freezer (−20°C) (for storing uncomposited samples)
Ultra-low temperature freezer (−60°C) (for short-term storage and archival storage of homogenized samples)

ADMINISTRATIVE EXPENSES
Computer with software and printer
System for logging and tracking samples and associated information, including computer and software
Shipping menus to off-site composite homogenization facility; composited samples to food analysis laboratory

QUALITY CONTROL MATERIAL (QCM) ANALYSES (TO ESTABLISH QUALITY CONTROL CHARTS)
Preparation (food, labor)
Storage
Containers
Assay charges

[1]Cost formulas vary greatly among research units. Some may need to determine per-assay costs that incorporate many categories of expenses. Others will separate labor, equipment, supplies, etc. Overhead rates, fringe benefits, and space and utility charges also vary.
[2]Labor costs are specified as tasks. Job categories and wages or salaries for individuals performing these tasks will vary among laboratories.
[3]Food costs for validation samples will be higher if the daily menu for each diet is prepared and collected in duplicate. Additional costs will be incurred if pilot studies or analyses of individual food items are planned. Food costs for diet monitoring will be determined by the number of extra samples prepared (eg, the food cost for at least one extra participant for each diet cycle and each diet treatment collected).

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3. Piironen V, Varo P, Coivistoinen P. Stability of tocopherols and tocotrienols in food preparation procedures. *J Food Comp Anal*. 1988;1:152–158.
4. Slover HT, Lanza E, Thompson RH Jr. Lipids in fast foods. *J Food Sci*. 1980;45:153–159.
5. Vanderslice JT, Higgs DJ Vitamin C content of foods: sample variability. *Am J Clin Nutr.* 1991;54(Suppl): 1323S–1327S.
6. Vanderslice JT, Higgs DJ. Vitamin C content variability in samples: implications for dietary studies. In: *Proceedings of the 15th National Nutrient Databank Conference*. Ithaca, NY: The CBORD Group, Inc; 1991:117–119.
7. Hurburgh CR Jr. Long-term soybean composition patterns and their effects on processing. *J Am Oil Chem Soc*. 1994;71:1425–1427.
8. Clarke RP, Merrow SB. Nutrient composition of tomatoes homegrown under different cultural procedures. *Ecol Food Nutr.* 1979;8:37–46.
9. Baer RJ. Alteration of the fatty acid content of milk fat. *J Food Protection*. 1991;54:383–386.
10. Lee SM, Buss DH, Holcombe GD, Hatton D. Nutrient content of retail cuts of beef, pork, and lamb: preliminary results. *J Hum Nutr Diet*. 1995;8:75–80.
11. Marshall MW, Clevidence BA, Thompson RH Jr, Judd JT. Cholesterol and plant sterols in controlled mixed diets: two analytical methods vs food table values. *J Food Comp Anal*. 1989;2:2–12.
12. Marshall MW, Clevidence BA, Thompson RH Jr, Judd JT. Problems in estimating amounts of food cholesterol. Part 2: three methods for self-selected mixed diets. *J Food Comp Anal*. 1989;2:228–237.
13. Wills RBH, Balmer N, Greenfield H. Composition of Australian foods. 2: Methods of analysis. *Food Tech Austr.* 1980;32:198–204.
14. Lurie DG, Holden JM, Schubert A, Wolf W, Miller-Ihli NJ. The copper content of foods based on a critical evaluation of published analytical data. *J Food Comp Anal*. 1989;2:298–316.
15. US Congress. Public Law 101–535 (Nutrition labeling, CFR 21, 1993). Nutrition Labeling and Education Act of 1990. *Code of Federal Regulations*. Vol 21. Washington DC: US Government Printing Office; 1993.
16. Steinke FH. Nutrient composition data uses and needs of food companies. In: Rand WM, Windham CT, Wyse BW, Young VR, eds. *Food Composition Data: A User's Perspective*. Avon, UK: The Bathe Press, United Nations University; 1987:97–100.
17. Foote D. Food composition data and clinical dietetics. In: Uses and Abuses of Food Composition Data. *Food Tech Austr.* 1990;42:S8-S9.
18. Dennis BH, Stewart P, Wang CH, Champagne C, Windhauser M, Ershow A, Karmally W, Phillips K, Stewart K, Van Heel N, Farhat-Wood A, Kris-Etherton PM. Diet design for a multicenter controlled feeding trial: the DELTA program. *J Am Diet Assoc*. 1998;98:766–776.
19. Sacks FM, Obarzanek E, Windhauser MM, Svetky LP, Vollmer WM, McCullough M, Karanja N, Lin P-H, Steele P, Proschan MA, Evans MA, Appel LJ, Bray GA, Vogt TM, Moore TJ, for the DASH Collaborative Research Group. Rationale and design of the Dietary Approaches to Stop Hypertension Trial (DASH): a multicenter controlled-feeding study of dietary patterns to lower blood pressure. *Ann Epidemiol*. 1995;5:108–118.
20. Appel LJ, Moore TJ, Obarzanek E, Vollmer WM, Svetky LP, Sacks FM, Bray GA, Vogt TM, Cutler JA, Simons-Morton D, Lin P-H, Windhauser MM, Lin PH, Karanja N, for the DASH Collaborative Research Group. A clinical trial of the effects of dietary patterns on blood pressure. *N Engl J Med*. 1997;336:1117–1124.
21. Phillips KM, Stewart KK, Champagne CM, Holden JM, for the DELTA Investigators. Assay of menus as part of a multicenter clinical feeding trial: comparison of calculated and assayed fat content. *Proceedings of the 108th Annual Meeting of AOAC International,* Portland, Ore, September 12–15, 1994; Abstract 14–022. Gaithersburg, Md: Association of Official Analytical Chemists International, Inc; 1994.
22. Carpenter DE, Ngeh-Ngwainbi J, Lee S. Lipid analysis. In: Sullivan DM, Carpenter DE, eds. *Methods of Analysis for Nutrition Labeling*. Gaithersburg, Md: Association of Official Analytical Chemists International, Inc; 1993;85–104.
23. Wolf WR. Reference materials. In: Sullivan DM, Carpenter DE, eds. *Methods of Analysis for Nutrition Labeling*. Gaithersburg, Md: Association of Official Analytical Chemists International, Inc; 1993:111–122.
24. Stewart KK for the DELTA Investigators. Diet composition documentation in a multicenter clinical feeding trial. *FASEB J*. 1995;9(3):A289.
25. Phillips KM for the DELTA Investigators. Diet composition validation and monitoring as part of a multicenter clinical feeding trial of Step 1 Diet. *FASEB J*. 1996;10(3):A262.
26. Taylor JK. *Quality Assurance of Chemical Measurements*. Chelsea, Mich: Lewis Publishers, Inc; 1987.
27. Ratnayake WMN. Determination of *trans* unsaturation by infrared spectrophotometry and determination of fatty acid composition of partially hydrogenated vegetable oils and animal fats by gas chromatography/infrared spectrophotometry: collaborative study. *J Assoc Off Anal Chem Int*. 1995;78:783–802.
28. Lichon MJ, James DW. Homogenization methods for analysis of foodstuffs. *J Assoc Off Anal Chem*. 1990; 73:820–825.
29. Li BW, Schuhmann PJ, Wolf WR. Chromatographic determinations of sugars and starch in a diet composite reference material. *J Agric Food Chem*. 1985;33:531–536.
30. Vanderslice JT, Higgs DJ, Hayes JM, Block G. Ascorbic acid and dehydroascorbic acid content of foods-as-eaten. *J Food Comp Anal*. 1990;3:105–118.

eat right. American Dietetic Association

Source: "Well-Controlled Diet Studies in Humans, A Practical Guide to Design and Management", American Dietetic Association, © 1999.

31. Association of Official Analytical Chemists. *Official Methods of Analysis*. 15th ed. Gaithersburg, Md: Association of Official Analytical Chemists International, Inc; 1990.
32. American Association of Cereal Chemists. *Approved Methods*. 9th ed. St Paul, Minn; American Association of Cereal Chemists; 1995.
33. American Oil Chemists Society. *Official Methods and Recommended Practices*. 4th ed, Vols 1 and 2. Champaign, Ill: American Oil Chemists Society; 1996.
34. Paquot C, Hautfenne A, eds. *Standard Methods for the Analysis of Oils, Fats, and Derivatives*. 7th ed. Boston: International Union of Pure and Applied Chemistry, Blackwell Scientific Publications; 1987. (This is one of a multivolume series comprising analysis methods for a large variety of compounds.)
35. Sullivan DM, Carpenter DE, eds. *Methods of Analysis for Nutrition Labeling*. Gaithersburg, Md: Association of Official Analytical Chemists International, Inc; 1993: 85–104.
36. Greenfield H, Southgate DAT. *Food Composition Data: Production, Management and Use*. New York, NY: Elsevier Science Publishers Ltd; 1992.
37. Phillips KM, Lugogo RD, Harris RF, Tarrago-Trani MT, Bailey JA, Stewart KK. Direct determination of trans-octadecenoic acid in diet composites using a combination of silver ion-high performance liquid chromatography and capillary gas chromatography. *J Food Lipids*. 1997;4:173–188.
38. Wernimont GT. *Use of Statistics to Develop and Evaluate Analytical Methods*. Spendley W, ed. Gaithersburg, Md: Association of Official Analytical Chemists, International, Inc; 1985.
39. Holden JM, Davis CS, Wolf WR, Beecher GR. Variability in nutrient measurements by five commercial laboratories. *Proceedings of the 108th Annual Meeting of AOAC International*. Portland, Ore, September 12–15, 1994. Vol 77; Number 3; abstract number 14–024. Gaithersburg, Md: Association of Official Analytical Chemists International, Inc.
40. DeVoe JR, ed. *Validation of the Measurement Process*. Washington, DC: American Chemical Society; 1977.
41. Garfield FM. *Quality Assurance Principles for Analytical Laboratories*. Gaithersburg, Md: Association of Official Analytical Chemists International, Inc; 1991.
42. Dux JP. *Handbook of Quality Assurance for the Analytical Chemistry Laboratory*. New York, NY: Van Nostrand Reinhold; 1986.
43. Garner WY, Barge MS, Ussary JP, eds. *Good Laboratory Practice Standards*. Washington, DC: American Chemical Society; 1992.
44. Stewart KK, Phillips KM, Champagne CM, Dennis BH for the DELTA Investigators. Menu validation in the DELTA study: the use of total nutrient weights to determine sources of variance and errors. In: Dietary Assessment Methods, Part X (Food Consumption Patterns). *Proceedings of the Second International Conference on Dietary Assessment Methods*. Boston, Mass, January 22–24, 1995, Willett W, ed. *Am J Clin Nutr*. 1995;65(4)(Suppl):1366S.

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