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Quality-control materials in the USDA National Food and Nutrient Analysis Program (NFNAP)

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Abstract The US Department of Agriculture (USDA) Nutrient Data Laboratory (NDL) develops and maintains the USDA National Nutrient Databank System (NDBS). Data are released from the NDBS for scientific and public use through the USDA National Nutrient Database for Standard Reference (SR) (http://www.ars.usda.gov/ba/bhnrc/ndl). In 1997 the NDL initiated the National Food and Nutrient Analysis Program (NFNAP) to update and expand its food-composition data. The program included: 1) nationwide probability-based sampling of foods; 2) central processing and archiving of food samples; 3) analysis of food components at commercial, government, and university laboratories; 4) incorporation of new analytical data into the NDBS; and 5) dissemination of these data to the scientific community. A key feature and strength of the NFNAP was a rigorous quality-control program that enabled independent verification of the accuracy and precision of analytical results. Custom-made food-control composites and/or commercially available certified reference materials were sent to the laboratories, blinded, with the samples. Data for these materials were essential to ongoing monitoring of analytical work, to identify and resolve suspected analytical problems, to ensure the accuracy and precision of results for the NFNAP food samples.

Keywords Quality control (QC) · Reference materials · Food analysis · Food composition

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Introduction

The United States Department of Agriculture (USDA) Nutrient Data Laboratory (NDL) develops and maintains the USDA National Nutrient Databank System (NDBS), an in-house system for generating, storing, compiling, and disseminating data on the composition of foods. The USDA National Nutrient Database for Standard Reference (SR) is a food-composition database derived from the NDBS and released on the NDL web site [1], for use by scientists, the food industry, and the US public. The SR consists of values for more than 130 essential nutrients and other dietary components for over 7000 raw, processed, and prepared foods and food ingredients. It provides the basis for other database applications used for nutrition monitoring and research, and for data used in food policy development, trade, and nutrition education. To support these databases, the National Food and Nutrient Analysis Program (NFNAP) was initiated in 1997 at the NDL, with the goal of updating and expanding the food-composition data in the SR and adding special-interest databases for specific food components, for example flavonoids [2], proanthocyanidins [3], choline [4], and fluoride [5]. To date, nearly 900 food items have been sampled and analyzed for more than 100 nutrients and other dietary components as part of the NFNAP (Fig. 1).

The NFNAP is achieving these long-sought improvements in the SR by comprehensive revision of scientific concept and technical approach, including a "Key Foods" model [6, 7] to identify and prioritize foods and nutrients for analysis, probability based nationwide sampling of products [8–10], validated and documented analytical methods, and an all-encompassing analytical quality-control (QC) protocol. The NFNAP required the coordination of work among multiple sites to achieve the project goals from sample procurement to data dissemination (Fig. 2). This integrated approach was necessary because the USDA does not have an analytical facility dedicated to the receipt, processing, and analysis of the number of samples required for the NFNAP. Because the Food Analysis Laboratory Control Center (FALCC) at Virginia

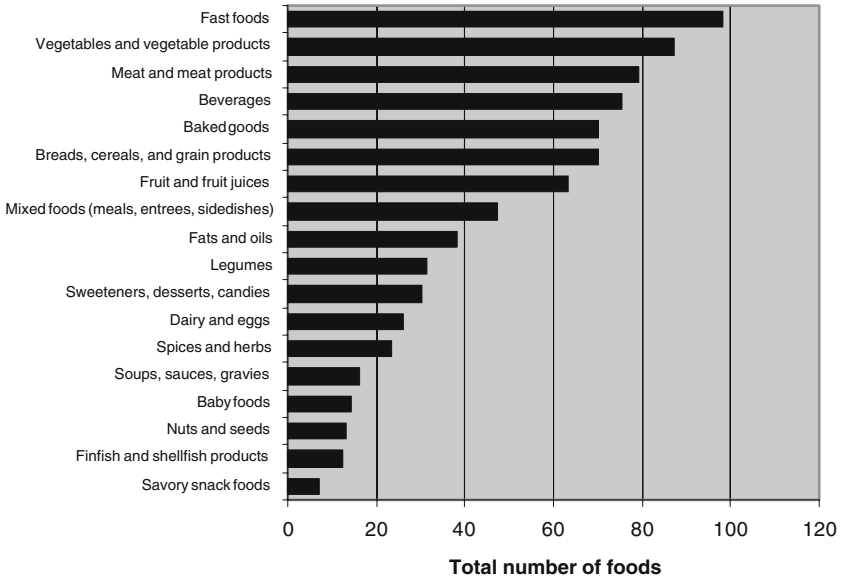

Fig. 1 Summary of foods and nutrients analyzed as part of the National Food and Nutrient Analysis Program

Food Components Assayed [a]	
Proximates (4)	Water soluble vitamins (7)
Protein	Amino acids (19)
Fat	Fatty acids (30+)
Ash	Choline (6)
Moisture	Cholesterol (1)
Carbohydrate fractions (14)	Phytonutrients (45+)
Fiber	Carotenoids
Sugars	Flavonoids
Starch	Isoflavones
Alcohol	Proanthocyanidins
Organic acids	Phytosterols
Minerals (10)	Methylxanthines (3)
Fluoride (1)	Total antioxidants [b]
Fat soluble vitamins (4)	

[a] Number of nutrients in the class analyzed is given in parentheses.
[b] Wu et al. [32]

Polytechnic Institute and State University in Blacksburg, Virginia, USA, had considerable experience in this sort of work [11, 12], they were selected by the NDL to fulfill this role in the project. Furthermore, because no single university/research facility could meet the analytical demands for all components and foods for the entire project (Fig. 1), the NDL implemented several contracts with commercial food-analysis laboratories and specific cooperative agreements with government and university laboratories to analyze food samples.

A fundamental goal of the NFNAP was that new data be both nationally representative of products currently available in the food supply and analytically accurate. The QC protocol included a selection process for choosing competent laboratories and ongoing assessment of each laboratory's analytical performance. The QC needs of the NFNAP were unique because of the number of laboratories involved, the analysis of many types of food, and the number of years the project was conducted. Although the Food and Drug Administration's (FDA) Total Diet Study (TDS) is a program of continuous nationwide sampling and analysis of commonly consumed foods [13, 14], the QC needs for that project differ significantly from those for the NFNAP. In the TDS, analyses are primarily for potentially toxic components (e.g. pesticides, heavy metals) and elemental composition, and assays are mostly performed in FDA laboratories. In the NFNAP, a wide range of components have been analyzed (Fig. 1). A critical component of the NFNAP QC program was the development of an extensive array of food-based control materials specifically for the NFNAP, which were used with commercially available certified reference materials. The objective of this paper is to discuss the development and use of these control materials to facilitate monitoring of the accuracy and precision of analyses conducted as part of the NFNAP. The importance of submitting independent, unmarked, food-specific control samples with test samples to contract laboratories to validate analytical results and provide comprehensive analytical quality control is illustrated.

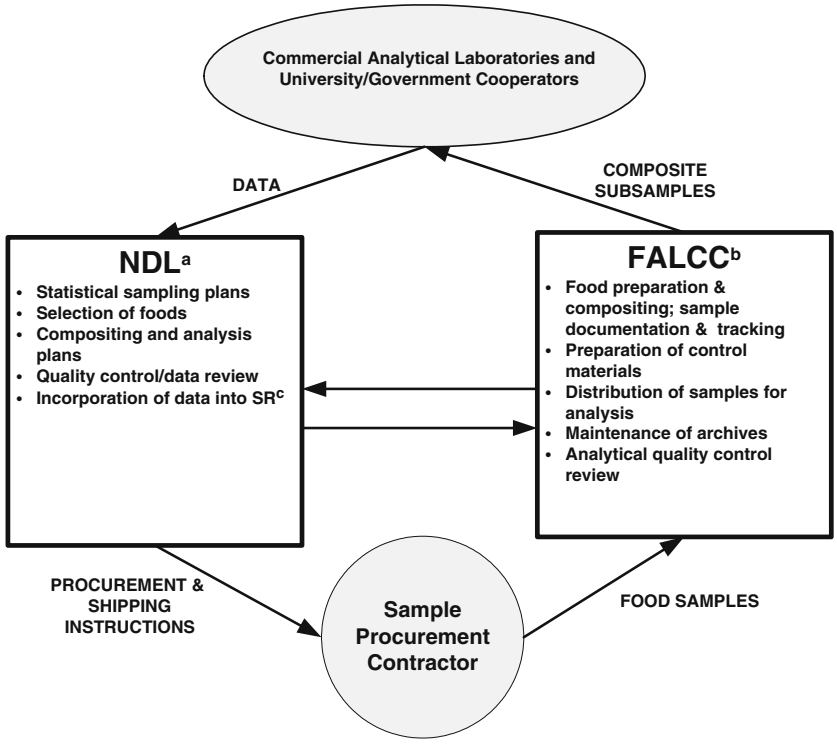

Fig. 2 Sample and data flow in the National Food and Nutrient Analysis Program

[a] USDA Nutrient Data Laboratory (Beltsville, MD, USA)
[b] Food Analysis Laboratory Control Center (Virginia Tech, Blacksburg, VA, USA)
[c] USDA National Nutrient Database for Standard Reference [1]

Materials and methods

Approach to quality control of sample analyses

The basic system for in-house quality control of analytical assays in the NFNAP was adapted from that used in single laboratories (see Taylor [15] and Dux [16], for example) and extended to this application for multiple laboratories. As part of this model the NFNAP analytical QC program included blinded, matrix-matched control samples with each set of NFNAP food samples submitted for analysis. A suite of matrix-specific control composites (CC) was developed for the NFNAP. The rationale for the development of CC was the need to monitor precision and accuracy of data generated for the same type of samples over a number of years, laboratories, and possible changes in analytical methods or food formulation. These control data were critical to the NFNAP, which spanned several years and included multiple sampling of some foods. As far as possible, commercial reference materials (RM) with certified nutrient levels were used as samples for checking accuracy and linked with the use of the blinded control materials. The CC encompassed a broader range of foods materials than were available as RM, however, and were tailored to closely match the composition of the NFNAP samples being analyzed. Results for the RM and CC were evaluated on an ongoing basis, and quality-control charts and tolerance limits were developed for nutrients in these materials, according to standard procedures [15, 16] for the CC and certified limits for nutrient levels for the RM. Each participating laboratory was required to use in-house analytical quality-control materials (QCM) as part of a self-monitoring program and to submit these results with data for samples. Because in-house QCM are selected and assayed at the discretion of the laboratory, however, there was no guarantee the materials would be matched to the matrix of the samples being tested or run at the frequency needed for the NFNAP. Nor could data for in-house materials be used to compare results among laboratories. CC and RM could be included according to the level of validation needed for specific foods and nutrients on a batch-specific basis, and results for these materials enabled comparison of data across laboratories.

Several approaches were devised for monitoring the accuracy of the analytical data. The first and most straightforward situation occurred when an RM of a matrix similar to the samples to be assayed and with certified value(s) for the nutrient(s) to be analyzed was available. In practice, however, there was limited availability of RM with certified nutrient levels for the full range of foods and nutrients assayed in the NFNAP (Fig. 1; Table 1). Furthermore, the high cost of RM made the use of RM alone for monitoring all analyses impractical on a large-scale basis. Other techniques were therefore used to assess accuracy. For some components, tolerance limits for a given CC were established by obtaining data for the same nutrient from several laboratories

Table 1 Commercial reference materials used in the NFNAP

Reference Material[a]	Nutrient[b]	NFNAP Foods
AACC VMA 195[c]	Folate (3)[d]	Snack crackers, filled cookies, Native American breads and tortillas
(Fortified Ready-to-eat Cereal)	Minerals (3)	Snack crackers, filled cookies, Native American breads and tortillas
	Niacin (3)	Snack crackers, filled cookies, Native American breads and tortillas
	Proximates (5)	Snack crackers, filled cookies, Native American breads and tortillas
	Riboflavin (3)	Snack crackers, filled cookies, Native American breads and tortillas
	Thiamin (3)	Snack crackers, filled cookies, Native American breads and tortillas
AACC VMA 399[c]	Folate (5)	Bakery mix, yeast breads, egg noodles
(Fortified Ready-to-eat Cereal)	Minerals (4)	Bakery mix, yeast breads
	Niacin (7)	Bakery mix, yeast breads, fresh mushrooms
	Proximates (6)	Yeast breads, biscuits, egg noodles
	Riboflavin (3)	Bakery mix, yeast breads
	Thiamin (4)	Bakery mix, yeast breads
BCR CRM 121 (Wholemeal Flour)	**Folate** (5)	Cooked and ready-to-eat cereals, crackers, snack cakes, pies
BCR CRM 383 (Powdered Haricots Verts)	**Fiber** (3)	Cooked and ready-to-eat cereals
BCR CRM 421 (Milk Powder)	**Folate** (1)	Fluid milk
	Riboflavin (2)	Fluid milk
	Vitamin E (1)	Fluid milk
BCR CRM 431	**Niacin** (1)	Spices
(Lyophilized Brussels Sprouts Powder)	**Vitamin C** (6)	Fresh fruits and vegetables, spices
BCR CRM 485	**Carotenoids** (1)	Spices
(Lyophilized Mixed Vegetables)	**Folate** (20)	Fresh fruits and vegetables, canned fruit, spices
	Vitamin B6 (1)	Spices
BCR CRM 651 (Beer)	**Ethyl alcohol** (1)	Beer, wine
LGC 5002 (Wine)	**Ethyl alcohol** (1)	Beer, wine
LGC 5005 (Lager)	**Ethyl alcohol** (2)	Beer, wine
	Moisture (1)	Beer, wine
LGC 7017 (Sugar Confectionery)	**Sugars** (2)	Honey, corn syrup
LGC 7103 (Sweet Digestive Biscuit)	**Sugars** (1)	Molasses, maple syrup
NIST SRM 1544	**Fatty Acids** (4)	Canned meat entrees, salad dressings, vegetable oils, frozen meals, frozen pizza, spices, pies
(Fatty Acids and Cholesterol in a Frozen Diet Composite)	Proximates (8)	Canned meat entrees, salad dressings, vegetable oils, frozen meals, frozen pizza, spices, pies, butter, mayonnaise
	Cholesterol (14)	Canned meat entrees, salad dressings, vegetable oils, frozen meals, frozen pizza, spices, pies, eggs, mayonnaise, frozen dinners
NIST SRM 1546	*Choline* (2)	Canned meat entrees, frozen burritos, frozen pizza, frozen breaded chicken,
(Meat Homogenate)		cheeses, salad dressings, meat frankfurters
	Fatty Acids (8)	Cheeses, pie crust, crackers, snack cakes, beef, ham
	Folate (1)	Frozen breaded chicken
	Minerals (21)	Meat frankfurters, frozen breaded chicken, cheeses, Native American foods (oils, meat, fish, and eggs), eggs, imitation crabmeat, prepared poultry meat, canned meats, canned meat entrees, frozen meals, beef, ham
	Niacin (1)	Frozen breaded chicken, prepared poultry meat, canned meats, Native American foods (meat, fish, and eggs)
	Proximates (20)	Meat frankfurters, frozen breaded chicken, cheeses, eggs, Native American foods (fish and eggs), prepared poultry meat, canned meats
	Riboflavin (8)	Frozen breaded chicken, beef, ham
	Thiamin (1)	Frozen breaded chicken, prepared poultry meat, canned meats

Table 1 (continued)

Reference Material[a]	Nutrient[b]	NFNAP Foods
NIST SRM 1548a (Freeze-dried Typical Diet)	Fiber (4)	Frozen pancakes and waffles, Native American berry beverage, Native American corn meal, corn mush, tortillas, stews and tamales, spices
	Minerals (11)	Salad dressings, vegetable oils, frozen pancakes and waffles, fast foods (sandwiches, chicken nuggets, milk shakes, sodas), spices, beer, wine, mustard, frozen meals
	Proximates (15)	Salad dressings, vegetable oils, frozen pancakes and waffles, fast foods (sandwiches, chicken nuggets, milk shakes, sodas, pizza)
NIST SRM 1549 (Non-fat Milk Powder)	**Minerals** (3)	Fluid milk
NIST SRM 1563 (Cholesterol and Fat-Soluble Vitamins in Coconut Oil)	**Vitamin E** (2)	Mayonnaise
NIST SRM 1640 (Trace Elements in Natural Water)	**Minerals** (1)	Beer, wine
NIST SRM 1845 (Cholesterol in Whole Egg Powder)	**Cholesterol** (1)	Egg mix
NIST SRM 1846 (Infant Formula)	Folate (8)	Frozen pancakes and waffles, ready-to-eat cereals, fast food pizza
	Iron (1)	Toaster pastries
	Niacin (11)	Toaster pastries, fast food pizza
	Proximates (2)	Fluid milk, frozen pancakes and waffles, toaster pastries
	Riboflavin (5)	Toaster pastries, fast food pizza
	Thiamin (6)	Toaster pastries, fast food pizza ready-to-eat cereals
	Vitamin C (2)	Native American berry beverage, Latino beverages, lemonade, frozen chicken/rice entrees
NIST SRM 1946 (Lake Superior Fish Tissue)	**Fatty Acids** (2)	Imitation crabmeat, Native American meats, fish, eggs
NIST SRM 2383 (Baby Food Composite)	**Carotenoids** (12)	Frozen meals, frozen pizza, canned tomato products, dried fruit, orange juice, ketchup, pasta sauce, frozen vegetables, fresh fruits and vegetables, Native American corn meal and berry beverage, canned fruit, Latino beverages
	Cholesterol (1)	Eggs
	Choline (2)	Fresh fruits, frozen spinach, orange juice, canned tomato products, dried fruit
	Folate (7)	Frozen pot pies, frozen pizza, frozen burritos, orange juice, frozen vegetables
	Minerals (27)	Frozen meals, frozen pizza, frozen dinners, peanut butter, mayonnaise, orange juice, ketchup, pasta sauce, salsa, bottled drinking water, snack chips, salad dressing, popcorn, nuts, Native American stews, fast food pizza, meat baby foods
	Niacin (6)	Canned meat entrees, frozen pizza, frozen meals, orange juice, ketchup, pasta sauce, fast food coffee, beer, wine
	Pantothenic Acid (1)	Canned meat entrees, beer, wine
	Proximates (53)	Canned meat entrees, frozen meals, frozen pizza, canned tomato products, dried fruit, peanut butter, mayonnaise, orange juice, frozen burritos, salsa, frozen vegetables, snack chips, salad dressing, popcorn, Native American stews, spices, gelatin snacks, meat baby foods
	Retinol (2)	Native American stews
	Riboflavin (6)	Canned meat entrees, frozen meals, frozen pizza, orange juice, ketchup, pasta sauce, beer, wine
	Sugars (4)	Fast food sodas, beer, wine, yogurt
	Thiamin (6)	Frozen meals, frozen pizza, frozen cheese lasagna, orange juice, ketchup, pasta sauce, beer, wine
	Vitamin B12 (1)	Canned meat entrees, beer, wine
	Vitamin B6 (3)	Canned meat entrees, Native American stews, beer, wine
	Vitamin C (5)	Canned meat entrees, orange juice concentrate, frozen carrots, frozen spinach
	Vitamin E (3)	Peanut butter, mayonnaise, orange juice, ketchup, pasta sauce, spices

Table 1 (continued)

Reference Material[a]	Nutrient[b]	NFNAP Foods
NIST SRM 2384 (Baking Chocolate)	Proximates (3)	Granola bars, energy bars
NIST SRM 2387 (Peanut Butter)	**Fatty Acids** (2)	Coconut, vegetable oil spreads, pine nuts
	Vitamin E (1)	Vegetable oil spreads
NIST RM 8435 (Whole Milk Powder)	Proximates (2)	USDA commodity cheeses
	Riboflavin (1)	Whole milk
NWRI Calgary 93 (Drinking Water)	Fluoride (4)	Municipal water

[a]NIST, National Institute of Standards and Technology (Gaithersburg, MD, USA); BCR, Community Bureau of Reference, European Commission (Brussels, Belgium), obtained from RT Corporation (Laramie, WY, USA); LGC, Laboratory of the Government Chemist, Middlesex, UK, and purchased from RT Corporation; AACC, American Association of Cereal Chemists (St Paul, MN, USA); NWRI, National Water Research Institute, Environment Canada (Burlington, Ontario, Canada), obtained from LGC/Promochem (Middlesex, UK)
[b]Components in **bold** typeface are provided with certified values, those in regular typeface have reference values, and those in *italics* have information values. Certification levels for individual proximates, minerals, fatty acids and carotenoids may differ from the level indicated
[c]AACC Standard Reference Sample
[d]In parentheses are the number of times the RM was analyzed for the nutrient

using independent methods. Those with acceptable quality control that yielded similar results were used to provide a reasonable estimate of the true concentration of the nutrient. A final approach was to characterize components in a CC at a highly experienced research laboratory using methods explicitly validated for that matrix by use of recovery studies and thorough method validation. When none of these approaches was practical, the laboratory's in-house QC program was relied upon.

Procurement and use of commercial reference materials

The twenty-six reference materials (RM) indicated in Table 1 were purchased from commercial suppliers. Each RM was certified for one or more components of interest in the NFNAP. Each RM was stored according to the instructions on the accompanying certificate of analysis. RM were received in packages of different quantity of material and type of packaging. For materials supplied in ampoules, labels were removed and the samples were sent to laboratories in the original packaging. All other RM were repackaged to hide the identity of the material in the analytical sample stream and, for cost efficiency, to provide an appropriate quantity for a single analysis. For example, NIST 1546 (Meat Homogenate) is supplied in 85 g containers, and the material was repackaged into five smaller containers. Liquids were dispensed into bottles and solids into jars as described for the CC (section Preparation of control composites, below); each subsample was assigned a unique identification number in the same manner as NFNAP food composites and CC. During repackaging, careful precautions were taken to prevent contamination and to preserve the homogeneity and stability of nutrients. For example, each product was mixed thoroughly before subsampling, using scrupulously cleaned utensils (made of an appropriate material for avoiding contamination by analytes of interest. For example, no stainless steel was used for beverages to be analyzed for iron or trace elements), repackaged quickly in an appropriate environment, blanketed with nitrogen gas, and then immediately stored under the conditions specified in the certificate of analysis.

Design of NFNAP control composites

Design of control composites (CC) began with assessment of the types of food to be sampled and components to be analyzed (Fig. 1). The diverse nature of food types and wide-ranging expected concentration of components in these foods was a challenge when developing appropriate control composites. The NFNAP Key Foods List [6, 7] was useful in the process of formulating CC. Foods were grouped by matrix type according to moisture and macronutrient (e.g. fat, protein, carbohydrate) content and mineral and vitamin levels (and, to the extent possible, phytonutrient concentrations). When it was not possible to exactly match the composition of all foods in a group (e.g. if there were disparate nutrient levels among foods), intermediate nutrient concentrations were targeted for the CC. It was important to have CC that would be useful for monitoring several foods, rather than many CC that were each assayed a few times only. For example, a wide variety of vegetables (e.g. cruciferous vegetables, carotenoid-rich produce, starchy vegetables, leafy green vegetables, onions) were scheduled for analysis. Instead of preparing multiple control materials, a single mixed vegetable composite was designed to contain components and nutrient levels that would enable its use as a control for the several classes of vegetables to be sampled.

The goal for each CC was to prepare a homogeneous composite in which dietary components were uniformly distributed among all analytical subsamples and stable during storage. Easily homogenized and processed/cooked foods were usually selected to minimize the likelihood of additional loss of labile nutrients during storage. For the

vegetable CC, baby food green beans, baby food carrots, canned green peas, cooked winter squash, onion juice, canned spinach, canned asparagus, boiled white potatoes, and boiled green chili peppers were chosen. For particularly heterogeneous mixtures some foods were homogenized individually before mixing. For example, in the vegetable CC, the canned peas, spinach, asparagus, cooked squash, potatoes, and chili peppers were pre-homogenized.

The size of each composite was based on expected use, nutrient composition, and practical limitations such as freezer space and size of processing equipment. As the NFNAP progressed, it became apparent that production of large batches of CC (>400 subsamples) was most efficient, and some early control materials were replaced by use of larger CC that could span more food matrices, as described above. For example, a pasta/rice CC was replaced by the bread CC, the tomato CC was replaced with the vegetable CC; the orange juice CC and soda CC were replaced by a juice/cola CC. For each of these, initial parallel analysis of both control composites in the same batch was performed for each nutrient monitored by the material, to establish a cross-reference between results for the different CC.

Preparation of control composites

For each CC, a detailed written protocol was designed, reviewed, and tested before composite preparation. The protocol included a specific recipe to yield a target total mass and proportion of foods, and detailed methods for preparing, combining, and homogenizing the foods and dispensing aliquots of the composite. Precautions were included to control sources of contamination and prevent heterogeneity and separation during subsampling. An example and schematic diagram of the preparation of a typical mixed food CC has been given elsewhere [17].

Foods for CC were procured primarily at retail outlets in the Blacksburg (Virginia, USA) area. Beef and chicken baby food were obtained from a single lot for each of these CC and were generously donated by Beech-Nut (St Louis, MO, USA). Two-hundred to a thousand subsamples (20 to 25 g each) were usually generated for each CC, although as few as 34 were prepared for composites that served as controls for limited nutrients (e.g. caffeine in coffee) or specific samples (e.g. bottled water). Homogenization methods were tailored to the type of matrix. Some uniform products (e.g. peanut butter, water, beef baby food, chicken baby food) were subsampled directly from a single lot. Uniform liquids (e.g. soft drinks and oils) were stirred thoroughly; salad dressings were homogenized with a hand blender (Cuisinart, East Windsor, NJ, USA). All other foods were processed with a 6-L or 25-L Robot Coupe industrial food processor (Robot Coupe USA, Jackson, MS, USA). Foods included in the milk chocolate, snack food, and cake control composites were frozen in liquid nitrogen before homogenization to facilitate blending. Immediately after homogenization, subsamples were dispensed among 30-mL glass jars with Teflon-lined lids (Qorpak, Bridgeville, PA, USA) or 125-mL high-density polyethylene bottles (for the soda, water, and juice/cola control composites), sealed under nitrogen, and stored protected from light at −60±5°C. Frequent stirring was performed throughout the dispensing process to avoid sedimentation or separation of the homogenate.

The homogeneity of each composite was assessed by visual inspection and systematic analysis of selected nutrient(s) in subsamples drawn from throughout the dispensing sequence. Nutrients most representative of the uniformity of the entire matrix and the least expensive assays possible were chosen. Moisture was the primary component evaluated in high-moisture composites, because sedimentation or separation was assumed to be the most probable cause of potential heterogeneity among subsamples. Total fat, ash, and minerals (Na, K, Mg, Fe, Ca) were also evaluated in selected composites. Moisture in orange juice, tomato, peanut butter, vegetable, and mixed food control composites was analyzed using a CEM LabWave 9000 Moisture/Solids Analyzer (CEM Corporation, Matthews, NC, USA) as described elsewhere [17] or by drying under vacuum [18] for salad dressing CC, bread CC, pasta/rice CC, snack food CC, cheese CC, and cake CC. Ash was analyzed using a muffle furnace [19, 20]. Total lipid and minerals were analyzed as described elsewhere [12, 21]. Each nutrient was assayed in triplicate in each subsample (jar) selected for analysis. The variance in nutrient concentration between and within subsamples was evaluated by analysis of variance (ANOVA), using the "ANOVA: One-Way" tool of Corel Quattro Pro 8 software (Corel Corporation, Ontario, Canada).

The uniformity of the beef baby food CC, which was repackaged directly from the retail jars, was assayed for each of the proximates minerals, fatty acids, amino acids, vitamin A, vitamin E, thiamin, riboflavin, niacin, folic acid, pantothenic acid, vitamin B-6, and vitamin B-12. Two aliquots of the material were sent to a contract laboratory for analysis of each component.

Studies of the stability of selected nutrients, including folate and vitamin C, were also conducted. Presentation of these data is beyond the scope of this manuscript, but some of the results have been published [17, 22]. These studies included verification that the sample storage procedure would maintain stable moisture content for at least 5 years [17]; this was an important consideration, because nutrient levels were determined on a fresh mass basis.

Distribution of control materials

Each batch of samples sent to a laboratory to be analyzed for a specific nutrient included at least one QC sample, either a control composite or reference material, or both. Utilization of these QC materials was based on the level of validation needed for specific foods and nutrients. When nutrients from a food were identified as significant contributors to the diet on the basis of the Key Foods process, as described elsewhere [6, 7], a reference material was included in the batch whenever possible.

Review of data for quality-control materials

Data received from laboratories for CC and RM were thoroughly examined by a quality control review committee composed of five members (with a quorum of three) with expertise in a wide range of nutrients, foods, and analytical methods. In addition, when reviewing results for a particular food, the committee met with other NDL staff with significant experience with the composition of that product.

After receiving data from a laboratory for a particular analytical batch, the results were compiled for review, and organized by food matrix and nutrient. Results for unmarked quality-control materials (RM and CC), and data for the laboratory's in-house QCM and results for the NFNAP samples were evaluated. The in-house QCM values were compared with the target values reported by the laboratory. Results for each nutrient in each CC and RM were compiled by nutrient and linked to laboratory and assay batch. As data for CC and RM were accumulated, the overall and intra-laboratory means and CVs were calculated. A fundamental criterion for the CC measurements was precision. Without precision, estimates of nutrient concentrations with acceptable uncertainty could not be established for samples on the basis of a single analysis of the control material included in each assay batch.

Data for any replicate analyses were reviewed for variability. In general, CVs for CC and RM data or sample replicates that exceeded 10% for nutrients were flagged for further evaluation and possible re-analysis. When at least 15 values had been collected with acceptable precision for a given nutrient in a CC, a mean and tolerance limits (±3 times the standard deviation of the mean) could be established to identify future outliers. CC results were also linked to those for the same nutrient in any RM sent with the same batch of food samples, to provide a measure of the accuracy of results for the CC. The results for RM were compared with the acceptable range based on the certified value plus or minus the measure of uncertainty. Criteria for accepting data for RM depended on several factors. Assayed values were usually expected to fall within the established target range. If, however, the certified value was based on definitive analysis and/or had a very narrow confidence interval that did not incorporate acceptable limits for analytical variability, values falling narrowly outside the tolerance limits were sometimes considered acceptable. For example, if the certified range was narrower than the acceptable variability for the assay (usually within ±5%), the acceptable range was expanded. Also, if the concentration of a nutrient was close to detection limits, greater variability was allowed, on the basis of the work of Horwitz, as described by Garfield et al. [23]. Finally, the significance of a nutrient in the food being evaluated affected the criteria for acceptability of the data; for example, if the concentration of a nutrient was expected to be nutritionally insignificant in a particular food, on the basis of current knowledge of nutrient composition for the same or similar foods, and the assayed nutrient concentration was consistent with that expectation, resources were not expended to evaluate the accuracy and precision of those data.

In situations where nutrient values in CC could not be referenced to those in certified RM with a similar matrix and all laboratories were using similar methods, estimating nutrient concentrations was more difficult. Systematic bias associated with the methods would be difficult to recognize for any one laboratory. Further reliability was gained in some cases in which a standard method (e.g., AOAC [24]) was used to assay a food for which the procedure was specifically tested in collaborative studies.

The analytical results for food samples with which the unmarked CC and RM were analyzed were also considered as part of the data QC process. These data were compared with the nutrient concentrations reported in similar foods within the same analytical run, if possible, and with food-composition data from SR [1] or other sources, for example McCance and Widdowson [25] and the published literature. Although not used as a criterion for acceptance or rejection of analytical results, significant deviations from expected values prompted additional quality-assurance checks, including verification of data entry by the analytical laboratory. Knowledge of changes in food formulations was also a consideration when comparing analytical data with existing data.

Results and discussion

Homogeneity of control composites

Table 2 describes the major control composites used in the NFNAP. Representative results for verification of the homogeneity of control composites are summarized in Table 3. These data confirmed the uniformity of the CC composition for analytical samples taken throughout the subsampling sequence; the overall coefficient of variation (CV) was usually less than 1% and there was no statistically significant differences among aliquots taken throughout the dispensing sequence. For example, data for moisture in the 18 jars of the vegetable CC that were assayed in triplicate revealed no difference among jars and within jars (P=0.39), and the overall CV was 0.075%. Both analysis of variance results and the CV for all aliquots were evaluated to determine suitable homogeneity on a composite-specific basis. For example, although the P-value was significant (<0.05) for moisture in the cake CC and cheese CC, the overall CVs of only 0.02% and 0.41%, respectively, were well within acceptable analytical limits for the level of moisture (40 to 43 g/100 g) in these materials. Similarly, the relatively high CV of 12.67% for moisture in the snack food CC was acceptable given the extremely low moisture content (<1.5 g/100 g).

For the beef baby food CC assayed at a qualified contract laboratory, the CV was less than 6% for most components and there was no detectable difference for several nutrients, including total fat, ash, and phosphorus. These results,

coupled with subsequent results from multiple contract laboratories, confirmed the homogeneity of these nutrients in all subsamples of the CC.

Results of quality-control sample analyses

The total number of assays performed on the NFNAP CC and commercial RM to date are summarized in Tables 1 and 4. Over the course of the NFNAP, more than 100 individual components have been analyzed (Fig. 1), many at three or more laboratories. Discussion of all data is beyond the scope of this presentation. Examples have been selected to illustrate key points regarding the use of these quality-control materials to validate the accuracy and precision of nutrient data for NFNAP food samples.

Accuracy of analytical data

Evaluation of the accuracy of the analytical data using an RM with a certified value for the nutrient is illustrated in Fig. 3 by results for cholesterol determined in pizza samples. Each of three laboratories analyzed the same pizza composites and control materials and obtained different cholesterol concentrations, despite reporting use of the same standard analytical method. From the data for pizza samples alone it was not possible to determine which values were accurate. Results for the RM [NIST SRM 1544 (Fatty Acids and Cholesterol in a Frozen Diet Composite); Table 2] fell within the certified range for Laboratory C only, however, suggesting that data for the pizza samples assayed at Laboratory C were the most accurate.

Table 5 illustrates results from parallel analysis of a CC and a related RM to establish tolerance limits for nutrient concentrations in the CC. In this example, data for protein, fat, riboflavin and palmitic acid in the beef baby food CC and in NIST SRM 1546 (Meat Homogenate) analyzed at five laboratories are summarized. For these nutrients (and others not shown), the analyzed values determined by the three laboratories that analyzed the RM were within the certified or reference concentration range; there was, furthermore, excellent agreement among all four facilities for nutrient concentrations assayed in the CC (with the exception of iron from Laboratory D and riboflavin from Laboratory E).

The use of data for nutrient concentration from several laboratories using independent methods to establish tolerance limits for control materials is illustrated in Fig. 4 by the results for selenium (Se) in the beef baby food CC, mixed food CC, snack food CC, and NIST SRM 1546 (Meat Homogenate). The rationale for this approach was that it is unlikely that all methods would have the same systematic bias, should any bias exist. Two of the laboratories (A and D) used a hydride generation/atomic absorption spectrometry (HG/AA) method [26] whereas

Table 2 Key NFNAP control composites[a]

Composite Name	Composition
Beef baby food	Commercial beef baby food
Chicken baby food	Commercial chicken baby food
Vegetable	Baby food green beans, baby food carrots, canned green peas, cooked winter squash, onion juice, canned spinach, canned asparagus, boiled white potatoes, boiled green chili peppers
Mixed food	Canned chili (with and without beans), canned spaghetti (with and without meat), canned beef stew, pot pies (chicken, turkey), beef & bean burritos, low-fat cheese lasagna, lasagna with meat, sausage pizza, pepperoni pizza, vegetable pizza, cheese pizza, fish sticks
Bread	White bread, 100% whole wheat bread, French bread, Italian bread, seven-grain bread
Starchy vegetable	Fat-free canned refried beans, boiled white potatoes, baby food sweet potatoes, baby food corn
Snack food	Potato chips, pretzels, cheese puffs, corn chips, crackers
Salad dressing	Regular ranch-style salad dressing
Cheese	Swiss cheese, cheddar cheese, low-moisture part-skim mozzarella cheese, provolone cheese, Monterey jack cheese
Oil	Peanut oil
Cake	Chocolate cake, pancakes
Peanut butter	Creamy peanut butter
Water	Bottled water
Soda	Carbonated cola and lemon-lime sodas
Chocolate milk	Whole chocolate milk
Milk chocolate	Milk chocolate candy bars
Juice/cola	Cola syrup, grape juice cocktail, pulp-free orange juice, distilled water, 10% juice drink (vitamin C fortified)
Pasta/rice	Instant white rice (cooked), extra long grain white rice (cooked), orzo (cooked)
Tomato	Canned tomato puree, canned tomato sauce
Orange juice	Pulp-free orange juice

[a]The following CC were relatively small composites that were not frequently used and/or were replaced by other CC: coffee, dry soup, condensed soup, American cheese, parmesan cheese, fat-free salad dressing, regular and low-fat salad dressing, cooked macaroni and cheese, cookie

Table 3 Representative data from verification of the homogeneity of control composites

Control composite[a]	Total subsamples dispensed	Component assayed	Number of subsamples assayed[b]	ANOVA *P*-value[c]	Overall mean (g/100 g)	Range (g/100 g)	Overall CV (% of mean)
Vegetable	792	Moisture	18	0.389	89.26	89.12–89.39	0.075
Mixed food	408	Moisture	3	0.349	63.50	63.23–63.67	0.20
		Total Lipid	6*	0.944	7.08	7.01–7.15	0.65
Snack food	144	Moisture	3	0.149	1.13	0.88–1.30	12.67
		Total Lipid	3*	0.436	26.64	26.48–26.78	0.44
Bread	336	Moisture	3	0.182	34.12	33.18–35.04	1.68
Cake	432	Ash	6	0.467	1.84	1.80–1.88	1.43
		Total Lipid	6*	0.076	9.19	8.93–9.52	2.10
		Moisture	8	0.017[e]	42.45	41.95–42.94	0.02
Pasta/rice	144	Moisture	3[d]	0.638	69.90	69.53–70.43	0.47
Tomato	144	Moisture	3	0.918	89.06	88.97–89.14	0.10
Orange juice	96	Moisture	3	0.611	88.04	87.91–88.13	0.08
Cheese	240	Moisture	6	0.025[f]	39.56	39.29–39.89	0.41

[a]See Table 2
[b]Three replicates (two when marked*) assayed per subsample, with subsamples drawn at equal intervals from the beginning, middle, and end of the dispensing sequence
[c]For analysis of variance (ANOVA) (α=0.05) between and within-jar results for aliquots drawn from the beginning, middle, and end of the dispensing sequence, as described in the text
[d]For two subsample jars, replicates=2
[e]Control materials for the assay were bread CC (in-house CV=1.22, n=33) and tomato CC (in-house CV=0.07, n=9)
[f]Control materials for the assay were a mixed food composite (in-house CV=0.18, n=63) and American cheese CC (in-house CV=1.09, n=21)

the third (F) used isotope-dilution gas chromatography–mass spectrometry (ID-GC–MS) [27], which is regarded as a definitive method. There was good agreement for all matrices, making it possible to establish target values and tolerance limits to facilitate use of the CC to monitor the accuracy of Se results for NFNAP samples. The mean Se values for beef baby food CC from the laboratories were virtually identical (4.68 compared with 4.63 μg/100 g, n=24 and n=12, respectively), but the CVs were different (12% compared with 4%) because of differences in the limits of quantitation (LOQ) (3 μg/100 g for HG compared with 0.2 μg/100 g for ID-GC–MS). A target value of 4.66 μg/100 g with a tolerance range of ±1.20 was determined using all the data and making some allowance for the higher LOQ of the more conventional HG/AA method. Although confidence in such an estimate will always be less than that in a certified value for an RM, data such as this example for Se provided a reasonable evaluation tool for several nutrients within the resources available.

Figure 5 is an example of tolerance limits for an analyte in a CC that were established at a research laboratory. In this example, beta-sitosterol, campesterol, and stigmasterol in the oil CC were analyzed at a research laboratory using carefully validated methods monitored with appropriate recovery studies [28]. Data for NFNAP vegetable oil samples run at Laboratories A and H with the oil CC, yielding the values shown, could then be regarded as accurate via the link to the validated sterol concentrations in the oil CC.

Even when there was no specific measure of accuracy, laboratories were expected to be able to replicate results for a CC from assay to assay. For example, in the early part of NFNAP there were no RM certified for amino acids. One laboratory was disqualified from amino acid analysis because of the high variability of their results for a CC; the samples that had originally been sent to that laboratory were therefore re-analyzed at other laboratories. Precision was good for results for the same CC from the other laboratories and there was agreement among those laboratories.

Comparison of data among laboratories

Figure 6 shows data for a CC that enabled monitoring of the continuity of results over time and of multiple laboratories for the same food matrix. In this example, the vegetable CC was included as a blinded control of mineral analysis of a variety of fresh fruits and vegetables submitted to four laboratories over the course of two years. The produce samples included seasonal replication, so it was important to dissociate laboratory bias from any actual difference in food composition. Results from three laboratories were in good agreement but those from the fourth (Laboratory B) were clearly lower. The sodium data for fruits and vegetables from Laboratory B were rejected. Further inquiry revealed that Laboratory B had changed method and equipment after it had been qualified for mineral analysis but before the time these samples were analyzed. This situation again emphasizes the importance of using external control materials to monitor long-term service laboratory performance as particularly important in the NFNAP. Commercial laboratories are sometimes

Table 4 Summary of the number of data points for nutrients assayed in key NFNAP control composites[a]

Control Composite[b]	Proximates	Sugars	Fiber	Minerals	B vitamins	Vit C	Vit D	Vit E	Vit K	Retinol	Folate	Amino acids	Cholesterol	Fatty acids
Beef Baby Food	52	14	9	46	39	11	6	8	7	10	14	28	45	27
Bread	29	21	22	24	22	6		5	7	1	10	15	5	15
Cake	9	7	8	8	7	3		3	4		7	6	4	7
Chocolate	2	3	2	3	1	1			1		1	2	5	4
Cheese	7	6		6	7	1	1	3	3	1	1	6	2	7
Chocolate Milk	5	5	3	5	5	4	4	2	2	3	3	5	3	4
Juice/Cola	8	8	8	7	8	8			1		5	3		
Mixed Food	38	23	21	24	28	19	4	8	12	7	15	22	29	23
Oil	2			2	1			1		1			2	2
Peanut Butter	19	4	3	15	5	2		6	3		4	4	1	11
Salad Dressing	10	6	6	8	7	5		6	5	3	7	6	9	10
Snack Food	9	4	4	11	9	4		1	6		8	4		5
Soda	11	10	2	10	6	5		1	1		3	2		
Starchy Vegetable	6	6	5	10	7	7	2	1	1	1	6	5	1	3
Vegetable	66	10	10	67	63	67	1	2	6		35	4	1	3
Water	3	2		5										

[a]Shading of boxes is used to highlight the relative number of values, with unshaded indicating fewer than 10, lightly shaded corresponding to 10 to 15, medium intensity to 16 to 30, and darkest shading to more than 30 data points.
[b]See Table 2.

purchased by other laboratories, resulting in a change in analytical facilities and personnel, and university laboratories also may change staff during a study spanning several years.

Detection of outlying values

Figure 7 illustrates how data for a CC were used to distinguish outlying values that were not detected by a laboratory's in-house QCM. In this example, whole-egg powder was used as a control for analysis of cholesterol in frankfurters with which an unmarked sample of the beef baby food CC was submitted. Figure 7 shows results for the CC were well outside the tolerance limits of 38 to 47 mg/100 g for two assay batches, but values for the egg powder in-house QCM (~2000 mg/100 g) were well within the target range. The tolerance limits for the in-house QCM were set by the laboratories themselves whereas the tolerance limits for the beef baby food CC were determined by taking the overall mean ±2 times the standard deviation of values from all laboratories after eliminating statistical outliers. The NFNAP samples from the errant batches were re-assayed.

A second situation in which a CC served as a valuable control occurred when the level of a nutrient was detectable in foods being tested but the concentration in the laboratory's in-house QCM was below the limit of detection. For example, baked products (bagels, pretzels, English muffins, cupcakes) were submitted for analysis of

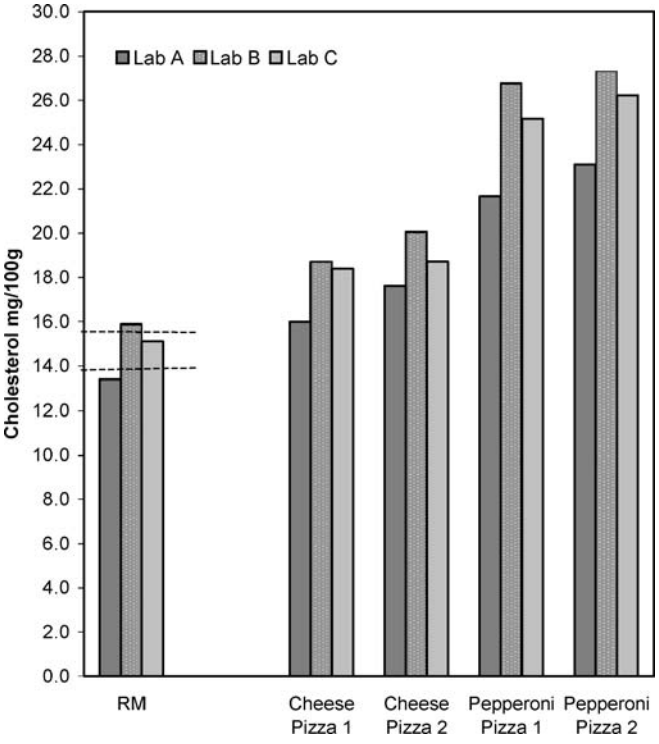

Fig. 3 Comparison of cholesterol results for a certified mixed food reference material (RM) [NIST SRM 1544 (Fatty Acids and Cholesterol in a Frozen Diet Composite); Table 2)] and for two cheese and two meat pizza samples analyzed by three different laboratories all reporting use of the same method [33]. *Dashed lines* indicate the certified range for cholesterol in the RM

Table 5 Results for proximates in NIST SRM 1546 (Meat Homogenate) and beef baby food control composite assayed at independent laboratories

A. Analytical results compared with certified nutrient levels in NIST SRM 1546-Meat Homogenate[a,b]

Nutrient	Certificate mean (range)	Certification level	Units	Laboratory A	Laboratory C	Laboratory E
Protein	14.9 (13.9–15.9)	Reference	g/100 g	15.0±0.7 (15)	15.0 (1)	14.6±1.2 (5)
Fat	21.0 (19.6–22.4)	Reference	g/100 g	21.2±1.7 (20)	20.4 (2)	20.8±4.7 (4)
Zinc	1.85 (1.69–2.01)	Reference	mg/100 g	1.94±0.07 (17)	–	1.87 (2)
Iron	1.14 (1.04–1.24)	Certified	mg/100 g	1.07±0.07 (19)	–	1.10 (1)
Riboflavin	0.200 (0.141–0.259)	Reference	mg/100 g	0.203±0.057 (14)	–	–
Palmitic acid (16:0)	4.56 (4.17–4.95)	Certified	g/100 g	4.73±0.49 (12)	–	–

B. Laboratory results for beef baby food control composite[b,c]

Nutrient	Units	Laboratory A	Laboratory C	Laboratory D	Laboratory E
Protein	g/100 g	12.7±0.6 (11)	12.8±0.7 (4)	12.3±0.2 (5)	12.3±0.7 (4)
Fat	g/100 g	7.3±0.3 (17)	7.2±0.3 (8)	7.4±0.7 (7)	7.3 (2)
Zinc	mg/100 g	2.77±0.12 (13)	2.50±0.53 (8)	2.60±0.49 (3)	2.45 (1)
Iron	mg/100 g	1.59±0.14 (13)	1.65±0.56 (8)	2.68±0.78 (3)	1.4 (1)
Riboflavin	mg/100 g	0.117±0.025 (15)	0.126±0.025 (9)	0.118±0.029 (3)	0.157 (2)
Palmitic acid (16:0)	mg/100 g	1.74±0.08 (10)	1.80±0.07 (6)	1.74±0.08 (3)	–

[a]National Institute of Standards and Technology, Gaithersburg, MD, USA
[b]Values shown are mean±standard deviation, with number of replicate analyses given in parentheses. – indicates no data
[c]See Table 2

sugars, including maltose, and the laboratory employed a sweet cracker as an in-house control. The maltose content of that material was below the laboratory's detection limit, however. Results for maltose in the baked goods were nearly twice the concentrations expected on the basis of existing data [25]. An analytical error was suspected because the maltose level of 3.45 g/100 g reported for the bread CC was also almost twice the concentration previously reported by several laboratories (1.60 g/100 g, SD 0.2, n=13). When the sample batch was re-assayed, data for the samples were also lower and more typical for these types of product (e.g. for bagels, 2.66 to 7.53 g/100 g compared with 3.81 to 18.84 g/100 g).

These examples illustrate how in-house QCM that do not match the matrix and/or nutrient concentration of samples submitted for analysis may fail to detect problems with the sample assay or a particular analytical batch. Differences between extractability, stability, concentration, and the presence of components that interfere with isolation or quantitation of the analyte may exist for different food products. For example, analysis of fatty acids in an oil does not require extraction of the fat, so if an oil is used as a control for analysis of fatty acids in foods such as sandwiches, nuts, and meats, results for the control sample will not reflect any deficiency in extraction of fat from the matrix. Similarly, other components in a food can interfere with extraction or separation of the analyte; pH differences or the presence of inhibitory components affect the performance of assays involving enzymatic digestion (e.g. folate analysis [29]). Also, disparity in nutrient concentration that puts samples in a different range of an analytical calibration curve relative to the control material may lead to biased error in quantitation and also a different limit of detection. When dry milk powder is used as a control for whole milk, for example, the nutrient concentrations are vastly different, and laboratories do not always adjust the sample size to take into account the different moisture content of dried and undried samples.

Commercial laboratories typically do not use a comprehensive suite of QC materials to match samples in each analytical batch; frequently, the in-house QCM does not match characteristics of samples submitted for analysis. The in-house QCM used by laboratories for selected

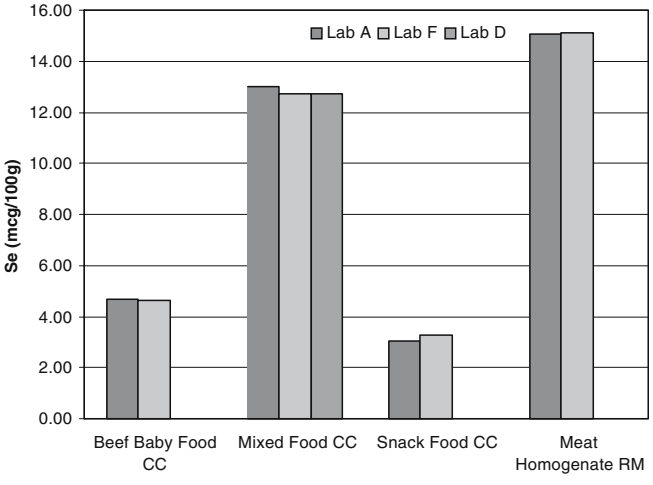

Fig. 4 Selenium in beef baby food, mixed food, and snack food control composites (CC) and meat homogenate reference material (RM) (NIST SRM 1546; Table 1) determined by two independent methods: Laboratories A and D: hydride generation/atomic absorption spectrometry [26]; Laboratory F: isotope-dilution gas chromatography–mass spectrometry [27]

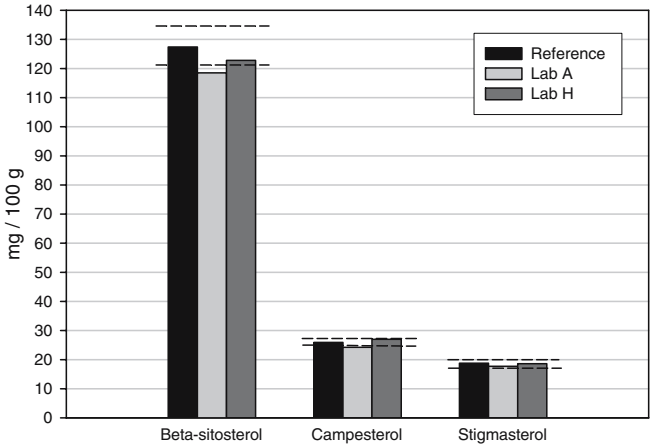

Fig. 5 Phytosterols in oil control composite assayed at three laboratories. *Dashed lines* indicate validated mean ±5% determined by Laboratory I

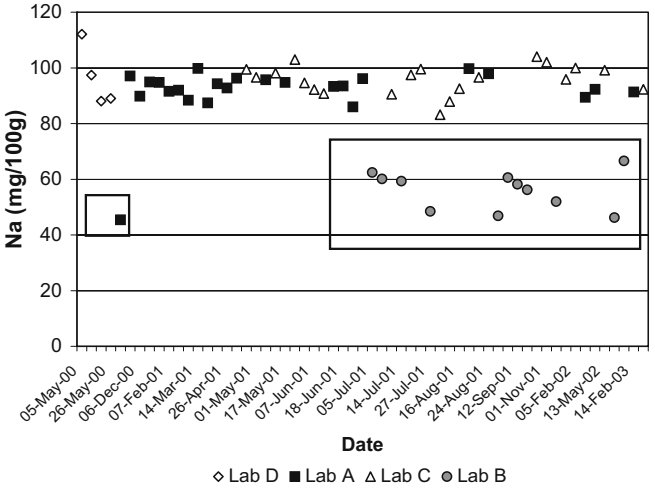

Fig. 6 Sodium assayed in vegetable control composite over a two-year period at four laboratories. Values within the boxes, and data for associated samples, were rejected

NFNAP samples are summarized in Table 6, with the corresponding CC that were used as unmarked external controls. Although these examples are only a subset of the many assays run for the NFNAP, they are representative of the overall collection of data. It is clear that in-house control materials frequently do not match sample type, and were, in fact, often strikingly different, for example (Table 6) when the same control materials were used for amino acid, moisture, and mineral assays of three very different food matrices (peanut butter, asparagus, and chocolate milk).

Conclusions

The specific array of control composites developed for the NFNAP, with commercially available reference materials, met the unique analytical quality control needs for this multi-year program. Data for these samples, along with other quality-assurance and quality-control measures implemented, support the accuracy and precision of food-composition data incorporated into SR [1]. The extensive archive of CC samples is also a resource for linking results from the NFNAP to subsequent studies. Another valuable use of CC data would be estimation of limits of analytical uncertainty for single-assay values obtained for a given sample of a food. For example, it was typical for a particular food that single samples from several regional or brand name composites were analyzed, and results for a given nutrient were averaged. Although this procedure provides a reliable estimate of the overall mean uncertainty of the nutrient concentration in the product, the uncertainty in the average value includes both analytical and sampling variability, precluding comparison of results among composites. Such comparisons would be useful for evaluating differences among samples (e.g. because of supplier, brand, or growing region for a plant product). Data for a CC of the same matrix assayed under the same conditions could theoretically be extrapolated to represent overall assay uncertainty for that matrix, saving the cost of multiple assays of individual composites that would be necessary for a valid statistical evaluation. Such a system is the subject of additional research.

The NFNAP quality-control data also emphasize the need for careful monitoring of routine analyses performed by commercial laboratories. This concern is particularly relevant to the common practice of submitting samples for single analysis without appropriate reference or control materials. Although contract laboratories report the use of standard methods and were pre-qualified for the NFNAP for many assays by achieving acceptable results for commercial reference materials, it was practically impos-

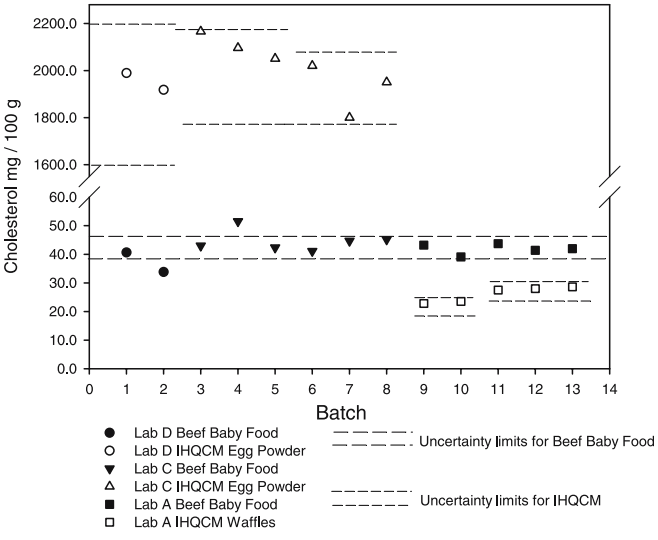

Fig. 7 Results for beef baby food control composite (CC) submitted with frankfurter samples for analysis of cholesterol and in-house quality-control materials (IHQCM) for Laboratories B and C (whole egg powder) and Laboratory A (waffles). *Short dashed lines* indicate laboratory in-house tolerance ranges; *dashed lines* show tolerance limits for the CC

Table 6 Representative examples of sample matrices, laboratory in-house control materials (in-house QCM), and NFNAP control composites (CC) used for nutrient assays at commercial laboratories

Samples	Nutrient	Laboratory In-House QCM	NFNAP CC[a]	Laboratory
Peanut butter	Amino acids	Corn	Peanut butter	C
	Moisture	Barium chloride		
	Minerals	Cat food		
Asparagus	Amino acids	Corn	Vegetable	C
	Moisture	Barium chloride		
	Minerals	Cat food		
Chocolate milk	Amino acids	Corn	Chocolate milk	C
	Moisture	Barium chloride		
	Minerals	Cat food		
Breadcrumbs	Moisture	Smoked sausage	Bread	D
Watermelon	Sugars	Graham Crackers	Vegetable	B
	Fat	Cheese		
	Minerals	Infant formula		
	Starch	Corn		
Fast food breakfast sandwiches	Fatty acids	Oil	Mixed food	A
	Fat	Dog food		
	Fiber	Bran cereal		
	Starch	Wheat starch		

[a]See Table 2 for composition of control composites

sible, because of the lack of certified RM for all nutrients and matrices and the high cost of RM and assays, to verify the accuracy and precision of all analyses at all laboratories, for all nutrients and all matrices. Facilities may also change equipment, analysts, methods, etc., so batch-specific validation of data is essential. It became clear as the study progressed that commercial laboratories performed consistently well in the analysis of certain nutrients in a wide range of foods, whereas the accuracy and/or precision of results for others were much less reliable. These latter examples included primarily more "complicated" assays such as those requiring chromatographic separation of complex mixtures (e.g. carotenoids) or for which performance is particularly affected by matrix composition, nutrient stability, nutrient concentration, sample size, and/or the occurrence of the analyte in different forms (e.g. free or bound, conjugated, encapsulated) in different foods (e.g. folate [29, 30]); phytosterols in vegetable oils compared with nuts, seeds, and whole grain [28]; fat in flaxseed [31]. The latter examples required analysis to be performed by experienced research laboratories, for example university and government facilities, which focused on methods for specific analytes within the area of their research expertise. Also, increasing interest in nutrients such as *trans* fatty acids and the related conjugated linoleic acid isomers in ruminant meats and in new products containing enriched nutrients or novel ingredients demand ongoing attention to development of appropriate control and reference materials and validated analytical methods to generate accurate food-composition data.

Overall NFNAP findings suggest further research is necessary to achieve more reliable data for "problem" nutrients. It is, however, important to realize that achieving the ultimate goal of 100% accuracy and high precision for 100% of foods 100% of the time is virtually impossible, and that the cost of the work increases in proportion to the level of validation, and these practical concerns must be balanced against the intended use of the results. Reliable methods are simply not currently available for some components in all foods (e.g. folate, Vitamin D). Research to evaluate the reliability of individual nutrient analyses in different food matrices based on the extensive NFNAP data set is in progress.

Meanwhile, users of food-composition data and, particularly, of commercial food analysis services, need to be cognizant of the analytical QC requirements and include external check samples to adequately assess the accuracy and precision of nutrient data reported.

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References

1. U.S. Department of Agriculture, Agricultural Research Service. (2005). USDA National Nutrient Database for Standard Reference, Release 18. Nutrient Data Laboratory, on the World Wide Web, http://www.ars.usda.gov/Services/docs.htm?docid=8964

2. U.S. Department of Agriculture, Agricultural Research Service. (2003). USDA Database for the Flavonoid Content of Selected Foods. Nutrient Data Laboratory, on the World Wide Web: http://www.nal.usda.gov/fnic/foodcomp/Data/Flav/flav.html
3. U.S. Department of Agriculture, Agricultural Research Service. (2004). USDA Database for the Proanthocyanidin Content of Selected Foods. Nutrient Data Laboratory, on the World Wide Web: http://www.nal.usda.gov/fnic/foodcomp/Data/PA/PA.html
4. U.S. Department of Agriculture, Agricultural Research Service. (2004). USDA Database for the Choline Content of Common Foods. Nutrient Data Laboratory, on the World Wide Web: http://www.nal.usda.gov/fnic/foodcomp/Data/Choline/Choline.html
5. U.S. Department of Agriculture, Agricultural Research Service. (2004). USDA National Fluoride Database of Selected Beverages and Foods. Nutrient Data Laboratory, on the World Wide Web: http://www.nal.usda.gov/fnic/foodcomp/Data/Fluoride/Fluoride.html
6. Haytowitz DB, Pehrsson PR, Holden JM (2002) J Food Comp Anal 15:183–194
7. Haytowitz DB, Pehrsson PR, Holden JM (2000) J Food Comp Anal 13:425–433
8. Pehrsson PR, Haytowitz DB, Holden JM, Perry CR, Beckler DG (2000) J Food Comp Anal 12:379–389
9. Perry, CR, Pehrsson PR, Holden J (2003) Proceedings of the American Statistical Association, Section on Survey Research Methods [CD-ROM], Alexandria, VA, American Statistical Association, San Francisco, CA
10. Perry CR, Beckler DG, Pehrsson PR., Holden J (2001) Proceedings of the 2000 Joint Statistical Meetings, American Statistical Association, Section on Survey Research Methods, Indianapolis, IN, pp. 267–72
11. Dennis BH, Champagne C, Ershow A, Karmally W, Kris-Etherton PM, Phillips KM, Stewart KK, Stewart PW, Van Heel N, Wang C-H, Windhauser M, Farhat-Wood A, for the DELTA Investigators (1998) J Amer Diet Assoc 98:766–776
12. Phillips KM, Stewart KK, Karanja NM, Windhauser MM, Champagne CM, Swain JF, Lin P-H, Evans MA, for the DASH collaborative research group (1999) J Amer Diet Assoc 99: S60–S68
13. Pennington JAT, Capar SG, Parfitt CH, Edwards CW (1996) J Assoc Off Anal Chem Int 79:163–170
14. U.S. Food and Drug Administration. (2004) Total Diet Study. Retrieved July 1, 2005 from the Center for Food Safety and Applied Nutrition Home Page on the World Wide Web: http://www.cfsan.fda.gov/~comm/tds–toc.html
15. Taylor JK (1987) Quality assurance of chemical measurements. Lewis Publishers, Chelsea, MI
16. Dux JP (1986) Handbook of quality assurance for the analytical chemistry laboratory. Van Nostrand Reinhold, New York, NY
17. Phillips KM, Simpkins AH, Amanna KR, Wolf W, Stewart KK, Clark S, Kim KP, Beecher GR, Holden J (2001) Fresenius J Anal Chem 370:297–302
18. Association of Official Analytical Chemists (1990) Official methods of analysis of the Association of Official Analytical Chemists, 15th Edition. Method: 934.01. Association of Official Analytical Chemists, Washington, DC
19. Association of Official Analytical Chemists (1984) Official methods of analysis of the Association of Official Analytical Chemists, 14th Edition. Method 31.012. Association of Official Analytical Chemists, Washington, DC
20. Association of Official Analytical Chemists (1984) Official methods of analysis of the Association of Official Analytical Chemists, 14th Edition. Method 31.013. Association of Official Analytical Chemists, Washington, DC
21. Phillips KM, Tarragó-Trani MT, Grove TM, Grün I, Lugogo R, Harris, RF, Stewart KK (1997) J Amer Oil Chem Soc 74:137–142
22. Phillips KM, Wunderlich KM, Holden JM, Exler J, Gebhardt SE, Haytowitz DB, Beecher GR, Doherty RF (2005) Food Chem 94:587–595
23. Garfield FM, Klesta E, Hirsch J (2000) Quality assurance principles for analytical laboratories. AOAC International, Gaithersburg, MD, pp. 119–120
24. Horwitz W (2000) (ed) AOAC Official Methods of Analysis, 17th edition. Association of Official Analytical Chemists International, Gaithersburg, MD
25. Food Standards Agency (2002) McCance and Widdowson's The Composition of Foods, Sixth Summary Edition. The Royal Society of Chemistry, Cambridge, UK
26. Association of Official Analytical Chemists (1995) Official methods of analysis of AOAC International, 16th Edition. Method: 986.15. AOAC International, Gaithersburg, MD
27. Reamer DC, Veillon C (1981) Anal Chem 53:2166–2169
28. Phillips KM, Ruggio DM, Ashraf-Khorassani M (2005) J Food Lipids 12:124–140
29. Aiso K, Tamura T (1998) J Nutr Sci Vitaminol (Tokyo) 44:361–370
30. Martin JI, Landen WO Jr, Soliman AG, Eitenmiller RR (1990) J Assoc Off Anal Chem 73:805–808
31. Daun JK, Thomas R (2005) J Am Oil Chem Soc 82:767–769
32. Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL (2004) J Agric Food Chem 52:4026–4037
33. Association of Official Analytical Chemists (2000) Official methods of analysis of AOAC International, 17th Edition. Method: 944.10. AOAC International, Gaithersburg, MD