BAM: Food Sampling/Preparation of Sample Homogenate

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Bacteriological Analytical Manual
Chapter 1
Food Sampling and Preparation of Sample Homogenate

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The adequacy and condition of the sample or specimen received for examination are of primary importance. If samples are improperly collected and mishandled or are not representative of the sampled lot, the laboratory results will be meaningless. Because interpretations about a large consignment of food are based on a relatively small sample of the lot, established sampling procedures must be applied uniformly. A representative sample is essential when pathogens or toxins are sparsely distributed within the food or when disposal of a food shipment depends on the demonstrated bacterial content in relation to a legal standard.

The number of units that comprise a representative sample from a designated lot of a food product must be statistically significant. The composition and nature of each lot affects the homogeneity and uniformity of the total sample mass. The proper statistical sampling procedure, according to whether the food is solid, semisolid, viscous, or liquid, must be determined by the collector at the time of sampling by using the *Investigations Operation Manual* (5). Sampling and sample plans are discussed in detail in ref. 6.

Whenever possible, submit samples to the laboratory in the original unopened containers. If products are in bulk or in containers too large for submission to the laboratory, transfer representative portions to sterile containers under aseptic conditions. There can be no compromise in the use of sterile sampling equipment and the use of aseptic technique. Sterilize one-piece stainless steel spoons, forceps, spatulas, and scissors in an autoclave or dry-heat oven. Use of a propane torch or dipping the instrument in alcohol and igniting is dangerous and may be inadequate for sterilizing equipment.

Use containers that are clean, dry, leak-proof, wide-mouthed, sterile, and of a size suitable for samples of the product. Containers such as plastic jars or metal cans that are leak-proof may be hermetically sealed. Whenever possible, avoid glass containers, which may break and contaminate the food product. For dry materials, use sterile metal boxes, cans, bags, or packets with suitable closures. Sterile plastic bags (for dry, unfrozen materials only) or plastic bottles are useful containers for line samples. Take care not to overfill bags or permit puncture by wire closure. Identify each sample unit (defined later) with a properly marked strip of masking tape. Do not use a felt pen on plastic because the ink might penetrate the container. Whenever possible, obtain at least 100 g for each sample unit. Submit open and closed controls of sterile containers with the sample.

Deliver samples to the laboratory promptly with the original storage conditions maintained as nearly as possible. When collecting liquid samples, take an additional sample as a temperature control. Check the temperature of the control sample at the time of collection and on receipt at the laboratory. Make a record for all samples of the times and dates of collection and of arrival at the laboratory. Dry or canned foods that are not perishable and are collected at ambient temperatures need not be refrigerated. Transport frozen or refrigerated products in approved insulated containers of rigid construction so that they will arrive at the laboratory unchanged. Collect frozen samples in pre-chilled containers.

Place containers in a freezer long enough to chill them thoroughly. Keep frozen samples solidly frozen at all times. Cool refrigerated samples, except shellfish and shell stock, in ice at 0-4°C and transport them in a sample chest with suitable refrigerant capable of maintaining the sample at 0-4°C until arrival at the laboratory. Do not freeze refrigerated products. Unless otherwise specified, refrigerated samples should not be analyzed more than 36 h after collection. Special conditions apply to the collection and storage of shucked, unfrozen shellfish and shell stock (1). Pack samples of shucked shellfish immediately in crushed ice (no temperature specified) until analyzed; keep shell stock above freezing but below 10C. Examine refrigerated shellfish and shell stock within 6 h of collection but in no case more than 24 h after collection. Further details on sample handling and shipment may be found in the *Investigations Operation Manual* (5) and the *Laboratory Procedures Manual* (3). The *Investigations Operation Manual* (5) contains sampling plans for various microorganisms. Some of those commonly used are presented here.

A. Sampling plans

1. *Salmonella* species

   a. Sample collection

      Because of the continuing occurrence of *Salmonella* in foods, sampling plans for these organisms have received the attention of committees of national and international organizations (6,7). Each of these committees has recommended varying the number of samples from a particular lot of food according to the

sampling category to which a food is assigned. Generally, the assignment to a sampling or food category depends on 1) the sensitivity of the consumer group (e.g., the aged, the infirm, and infants); 2) the possibility that the food may have undergone a step lethal to *Salmonella* during the manufacturing process or in the home; and 3) the history of the food. The selection of a sampling plan depends mainly on the first 2 criteria cited. The history of the food would be important in deciding whether to sample, i.e., whether there was a past history of contamination. For the *Salmonella* sampling plan discussed here, 3 categories of foods are identified.

Food Category I. - Foods that would not normally be subjected to a process lethal to *Salmonella* between the time of sampling and consumption and are intended for consumption by the aged, the infirm, and infants.

Food Category II. - Foods that would not normally be subjected to a process lethal to *Salmonella* between the time of sampling and consumption.

Food Category III. - Foods that would normally be subjected to a process lethal to *Salmonella* between the time of sampling and consumption.

In certain instances, it may not be possible to fully conform to the sampling plan. Nonetheless it is still important to ascertain whether or not Salmonella is present in the suspect food. Therefore, the analyst should still try to analyze as many analytical units as is required for the food of interest, i.e., 60 analytical units for Category I foods, 30 analytical units for Category II foods, and 15 analytical units for Category III foods. Individual 25 g analytical units may be combined into 375 g composites as described above unless otherwise indicated in Chapter 5, the OMA, or if the composited samples are not fully wettable. Below are examples of situations that might confront the analyst.

1) The number and weights of the sample units is correct.

Each sample should be mixed to ensure homogeneity before withdrawing a 25 g analytical unit. The analytical units can be composited (fifteen 25 g units into a 375 g composite), unless otherwise indicated in Chapter 5 or in the OMA. Samples should be preenriched at a 1:9 sample-to-broth ratio.

2) The number of sample units is correct, but several of the sample units have been damaged and are unusable.

For example, fifteen 1 lb bags of pasta have arrived for testing, but 5 of the bags are torn and unusable. In this case, the analyst should only sample from the 10 intact bags. The contents of each intact bag should be mixed to ensure homogeneity before the analytical units are withdrawn. Since the analyst needs one 375 g composite, ten 37.5 g analytical units,

from the remaining 10 intact bags, should be used to form the composite. The composite should be combined with its preenrichment medium at a 1:9 sample-to-broth ratio (375 g sample/3375 ml preenrichment) as directed in Chapter 5 or the OMA.

3) The number of sample units is incorrect, but the total weight of the sample unit(s) is greater than what would be necessary to perform the sample analysis.

For example, a single 10 lb wheel of cheese has arrived for testing. Since cheese is a Category II food, thirty 25 g analytical units must be analyzed. These analytical units should be taken randomly from a wide variety of locations around the wheel. If *Salmonella* is present in a food, then the odds of detecting it will be enhanced if two 375 g composites are analyzed rather than a single 25 g analytical unit, as would be the case if the analyst were to treat the entire wheel as a single sample.

4) There is less sample available than is necessary to form the required number of composites.

For example, an 8 oz (226.8 g) bag of almonds has arrived for testing. Almonds are a Category II food. Category II foods require thirty 25 g analytical units (750g), so it is impossible to analyze the amount of almonds required by the sampling plan. In this case, the analyst should analyze all of the almonds at a 1:9 sample-to-broth ratio (226.8g sample/2041 ml preenrichment medium).

If, in the above example, the total weight of the almonds had been less than 2 composites (750 g), but more than 1 composite, then the analyst should analyze both a whole and a partial composite. The analytical units comprising these composites should be taken randomly from a wide variety of locations in the lot of almonds. Both composites, should be preenriched at a 1:9 sample-to-broth ratio.

This sampling plan applies to the collection of finished products under surveillance and/or for determination of compliance for regulatory consideration. It also applies to the collection of factory samples of raw materials in identifiable lots of processed units and/or finished products where regulatory action is possible. It does not apply to the collection of in-line process sample units at various stages of manufacture since those samples do not necessarily represent the entire lot of food under production. The actual techniques involved in sampling are covered in the *Investigations Operation Manual* (5).

A sample unit consists of a minimum of 100 g and is usually a consumer-size container of product. Take sample units at random to ensure that a sample is representative of the lot. When using sample containers, submit a control consisting of one empty sample container that has been exposed to the same conditions as those under which the sample was collected. Collect more than one sample unit from large institutional or bulk containers when the number of sample units required exceeds the number of containers in the lot. A sample unit will consist of more than one container when containers are smaller than 100 g (e.g., four 25 g containers could constitute a sample unit).

The numbers of sample units to be collected in each food category are as follows: Food Category I, 60 sample units; Food Category II, 30 sample units; Food Category III, 15 sample units. Submit all samples collected to the laboratory for analysis. Advise the laboratory in advance of perishable sample shipments.

b. Sample analysis

The laboratory will analyze each sample for the presence of *Salmonella* according to methods described in this manual, or in *Official Methods of Analysis* (2). Take a 25 g analytical unit at random from each 100 g sample unit. When a sample unit consists of more than one container, aseptically mix the contents of each container before taking the 25 g analytical unit. To reduce the analytical workload, the analytical units may be composited. The maximum size of a composite unit is 375 g or 15 analytical units. The minimum number of composite units to be tested for each food category is as follows: Food Category I, 4 composite units; Food Category II, 2 composite units; Food Category III, one composite unit. For each 375 g composite, the entire amount of 375 g is analyzed for *Salmonella*.

Keep the remainder of the sample unit in a sterile container for compliance requirements as per section 702(b) of the Federal Food, Drug, and Cosmetic Act as amended through February, 1993. Refrigerate perishable samples and samples supporting microbial growth. An analytical control is required for each sample tested. The sampled lot is acceptable only if analyses of all composite units are negative for *Salmonella*. If one or more composite units are positive for *Salmonella*, the lot is rejected, provided that the analytical control is negative for *Salmonella*. A lot will not be resampled unless the environmental control for *Salmonella* is positive. For all samples positive for *Salmonella*, determine the somatic group. See Chapter 5 for information on further handling of these cultures. Recommendations for regulatory action may be based on the identification of the *Salmonella* somatic group and will not require definitive serotyping before initiation of regulatory action.

c. Imports.

These sampling plans apply to imported food products intended for human consumption.

d. Classification of food products for sampling purposes

Foods that have been classified into the 3 categories described above for regulatory sampling are listed in the categories according to the Industry Product Code sequence and nomenclature (4). Listing does not necessarily mean that these products are probable sources of *Salmonella*. **Food Category I**. - Foods that would not normally be subjected to a process lethal to *Salmonella* between the time of sampling and consumption and are intended for consumption by the aged, the infirm, and infants. **Food Category II**. - Foods that would not normally be subjected to a process lethal to *Salmonella* between the time of sampling and consumption. Examples are as follows:

Industry Product Code

2	**Milled grain products not cooked before consumption (bran and wheat germ)**
3	Bread, rolls, buns, sugared breads, crackers, custard- and cream-filled sweet goods, and icings
5	Breakfast cereals and other ready-to-eat breakfast foods
7	Pretzels, chips, and other snack foods
9	Butter and butter products, pasteurized milk and raw fluid milk and fluid milk products for direct consumption, pasteurized and unpasteurized concentrated liquid milk products for direct consumption, dried milk and dried milk products for direct consumption, casein, sodium caseinate, and whey
12	Cheese and cheese products
13	Ice cream from pasteurized milk and related products that have been pasteurized, raw ice cream mix and related unpasteurized products for direct consumption
14	Pasteurized and unpasteurized imitation dairy products for direct consumption
15	Pasteurized eggs and egg products from pasteurized eggs, unpasteurized eggs and egg products from unpasteurized eggs for consumption without further cooking
16	Canned and cured fish, vertebrates, and other fish products; fresh and frozen raw shellfish and crustacean products for direct consumption; smoked fish, shellfish, and crustaceans for direct consumption
17	Meat and meat products, poultry and poultry products, and gelatin (flavored and unflavored bulk)

20-22	Fresh, frozen, and canned fruits and juices, concentrates, and nectars; dried fruits for direct consumption; jams, jellies, preserves, and butters
23	Nuts, nut products, edible seeds, and edible seed products for direct consumption
24	Vegetable juices, vegetable sprouts, and vegetables normally eaten raw
26	Oils consumed directly without further processing; oleomargarine
27	Dressings and condiments (including mayonnaise), salad dressing, and vinegar
28	Spices, flavors, and extracts
29	Soft drinks and water
30	Beverage bases
31	Coffee and tea
33	Candy (with and without chocolate; with and without nuts) and chewing gum
34	Chocolate and cocoa products
35	Pudding mixes not cooked before consumption, and gelatin products
36	Syrups, sugars, and honey
37	Ready-to-eat sandwiches, stews, gravies, and sauces
38	Soups
39	Prepared salads
54	Nutrient supplements, such as vitamins, minerals, proteins, and dried inactive yeast

Food Category III: Foods that would normally be subjected to a process lethal to *Salmonella* between the time of sampling and consumption. Examples are as follows:

Industry Product Code

2	Whole grain, milled grain products that are cooked before consumption (corn meal and all types of flour), and starch products for human use
3	Prepared dry mixes for cakes, cookies, breads, and rolls
4	Macaroni and noodle products
16	Fresh and frozen fish; vertebrates (except those eaten raw); fresh and frozen shellfish and crustaceans (except raw shellfish and crustaceans for direct consumption); other aquatic animals (including frog legs, marine snails, and squid)

18	Vegetable protein products (simulated meats) normally cooked before consumption
24	Fresh vegetables, frozen vegetables, dried vegetables, cured and processed vegetable products normally cooked before consumption
26	Vegetable oils, oil stock, and vegetable shortening
35	Dry dessert mixes, pudding mixes, and rennet products that are cooked before consumption

2. Aerobic plate counts, total coliforms, fecal coliforms, *Escherichiacoli* (including enteropathogenic strains), *Staphylococcus* spp., *Vibrio* spp., *Shigella* spp., *Campylobacter* spp., *Yersinia* spp., *Bacilluscereus*, and *Clostridium perfringens*

    a. Sample collection

    From any lot of food, collect ten 8-oz subsamples (or retail packages) at random. Do not break or cut larger retail packages to obtain an 8-oz subsample. Collect the intact retail unit as the subsample even if it is larger than 8 oz.

    b. Sample analysis.

    Analyze samples as indicated in current compliance programs.

B. Equipment and materials

1. Mechanical blender. Several types are available. Use blender that has several operating speeds or rheostat. The term "high-speed blender" designates mixer with 4 canted, sharp-edge, stainless steel blades rotating at bottom of 4 lobe jar at 10,000-12,000 rpm or with equivalent shearing action. Suspended solids are reduced to fine pulp by action of blades and by lobular container, which swirls suspended solids into blades. Waring blender, or equivalent, meets these requirements.
2. Sterile glass or metal high-speed blender jar, 1000 ml, with cover, resistant to autoclaving for 60 min at 121°C
3. Balance, with weights; 2000 g capacity, sensitivity of 0.1 g
4. Sterile beakers, 250 ml, low-form, covered with aluminum foil
5. Sterile graduated pipets, 1.0 and 10.0 ml
6. Butterfield's phosphate-buffered dilution water (Rll (/food/laboratory-methods/bam-r11-butterfields-phosphate-buffered-dilution-water)), sterilized in bottles to yield final volume of 90 ± 1 ml
7. Sterile knives, forks, spatulas, forceps, scissors, tablespoons, and tongue depressors (for sample handling)

C. Receipt of samples

1. **The official food sample is collected by the FDA inspector or investigator.** As soon as the sample arrives at the laboratory, the analyst should note its general physical condition. If the sample cannot be analyzed immediately, it should be stored as described later. Whether the sample is to be analyzed for regulatory purposes, for investigation of a foodborne illness outbreak, or for a bacteriological survey, strict adherence to the recommendations described here is essential.
2. **Condition of sampling container.** Check sampling containers for gross physical defects. Carefully inspect plastic bags and bottles for tears, pinholes, and puncture marks. If sample units were collected in plastic bottles, check bottles for fractures and loose lids. If plastic bags were used for sampling, be certain that twist wires did not puncture surrounding bags. Any cross-contamination resulting from one or more of above defects would invalidate the sample, and the collecting district should be notified (**see** C-5, below)
3. **Labeling and records.** Be certain that each sample is accompanied by a completed copy of the Collection Report (Form FD-464) and officially sealed with tape (FD-415a) bearing the sample number, collecting official's name, and date. Assign each sample unit an individual unit number and analyze as a discrete unit unless the sample is composited as described previously in this chapter.
4. **Adherence to sampling plan.** Most foods are collected under a specifically designed sampling plan in one of several ongoing compliance programs. Foods to be examined for *Salmonella*, however, are sampled according to a statistically based sampling plan designed exclusively for use with this pathogen. Depending on the food and the type of analysis to be performed, determine whether the food has been sampled according to the most appropriate sampling plan.
5. **Storage.** If possible, examine samples immediately upon receipt. If analysis must be postponed, however, store frozen samples at -20°C until examination. Refrigerate unfrozen perishable samples at 0-4°C not longer than 36 h. Store nonperishable, canned, or low-moisture foods at room temperature until analysis.
6. **Notification of collecting district.** If a sample fails to meet the above criteria and is therefore not analyzed, notify the collecting district so that a valid sample can be obtained and the possibility of a recurrence reduced.

D. Thawing

Use aseptic technique when handling product. Before handling or analysis of sample, clean immediate and surrounding work areas. In addition, swab immediate work area with commercial germicidal agent. Preferably, do not thaw frozen samples before analysis. If necessary to temper a frozen sample to obtain an analytical portion, thaw it in the original container or in the container in which it was received in the laboratory. Whenever possible, avoid transferring the sample to a second container for thawing. Normally, a

sample can be thawed at 2-5°C within 18 h. If rapid thawing is desired, thaw the sample at less than 45°C for not more than 15 min. When thawing a sample at elevated temperatures, agitate the sample continuously in thermostatically controlled water bath.

E. Mixing

Various degrees of non-uniform distribution of microorganisms are to be expected in any food sample. To ensure more even distribution, shake liquid samples thoroughly and, if practical, mix dried samples with sterile spoons or other utensils before withdrawing the analytical unit from a sample of 100 g or greater. Use a 50 g analytical unit of liquid or dry food to determine aerobic plate count value and most probable number of coliforms. Other analytical unit sizes (e.g., 25 g for *Salmonella*) may be recommended, depending on specific analysis to be performed. Use analytical unit size and diluent volume recommended for appropriate *Bacteriological Analytical Manual* method being used. If contents of package are obviously not homogeneous (e.g., a frozen dinner), macerate entire contents of package and withdraw the analytical unit, or, preferably, analyze each different food portion separately, depending on purpose of test.

F. Weighing

Tare high-speed blender jar; then aseptically and accurately (± 0.1 g) weigh unthawed food (if frozen) into jar. If entire sample weighs less than the required amount, weigh portion equivalent to one-half of sample and adjust amount of diluent or broth accordingly. Total volume in blender must completely cover blades.

G. Blending and diluting of samples requiring enumeration of microorganisms

1. **All foods other than nut meat halves and larger pieces, and nut meal**. Add 450 ml Butterfield's phosphate-buffered dilution water to blender jar containing 50 g analytical unit and blend 2 min. This results in a dilution of 10^{-1}. Make dilutions of original homogenate promptly, using pipets that deliver required volume accurately. Do not deliver less than 10% of total volume of pipet. For example, do not use pipet with capacity greater than 10 ml to deliver 1 ml volumes; for delivering 0.1 ml volumes, do not use pipet with capacity greater than 1.0 ml. Prepare all decimal dilutions with 90 ml of sterile diluent plus 10 ml of previous dilution, unless otherwise specified. Shake all dilutions vigorously 25 times in 30 cm (1 ft) arc in 7 s. Not more than 15 min should elapse from the time sample is blended until all dilutions are in appropriate media.
2. **Nut meat halves and larger pieces**. Aseptically weigh 50 g analytical unit into sterile screw-cap jar. Add 50 ml diluent (G-l, above) and shake vigorously 50 times through 30 cm arc to obtain 10^{0} dilution. Let stand 3-5 min and shake 5 times through 30 cm arc to resuspend just before making serial dilutions and inoculations.

3. **Nut meal**. Aseptically weigh 10 g analytical unit into sterile screw-cap jar. Add 90 ml of diluent (G-l, above) and shake vigorously 50 times through 30 cm arc to obtain 10^{-1} dilution. Let stand 3-5 min and shake 5 times through 30 cm arc to resuspend just before making serial dilutions and inoculations.

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