GOOD Test Portions: Guidance On Obtaining Defensible Test Portions

GOOD Test Portions

Laboratory Sampling Working Group
AAFCO, AFDO, and APHL
June 2018
http://www.aafco.org/Publications/GOODTestPortions

1800 S. Oak Street, Suite 100, Champaign, IL 61820-6974
http://www.aafco.org/

Lab sample.

Table of Contents

Continued

INTRODUCTION

Laboratory activities are focused on analytical methods, and often insufficient consideration is given to the selection of the test portion. Equal attention should be paid to the selection of the test portion, as it can be a very significant source of error in the laboratory. This document is intended to provide guidance to laboratories on the processes necessary to achieve a representative test portion.

Recognizing the need for guidance, in 2000 the Association of American Feed Control Officials (AAFCO) published *Guidelines for Preparing Laboratory Samples* (Thiex et al., 2000). Its purpose was to provide a general discussion of issues that need to be considered when a laboratory is tailoring laboratory sampling protocols for feed matrices and to provide example procedures based on the experiences of the members of the writing group. *Guidance on Obtaining Defensible Test Portions* (*GOOD Test Portions*) uses the concepts of *Guidance on Obtaining Defensible Samples* (*GOODSamples*; AAFCO, 2015) and is intended to replace the 2000 AAFCO publication. The scope has been expanded and now addresses laboratory sampling of all food materials (human food, animal food, and plant food[1]) and other agricultural and environmental materials. The objectives are to ensure confidence in the analytical result through the selection of a representative test portion and to increase awareness of the critical importance of Theory of Sampling in laboratory sampling protocols. Representative sampling requires control of all errors from selection of the primary sample through selection of the final test portion. The target audience is everyone with a stake in generating data to support defensible decisions.

This document is intended to be a companion document to *GOODSamples* and to be used in conjunction with *GOODSamples* rather than a stand-alone document. Reading, studying, and understanding *GOODSamples* is a prerequisite for understanding the concepts presented in this document, as the basic theory is discussed in greater detail therein and is not repeated in this guidance document. The types of error discussed in this document are described slightly differently than those presented in the *GOODSamples* document to better address the specific needs of the laboratory. As with *GOODSamples*, it is not intended to be a summary of best practices or current practices but rather to present a scientific, systematic approach for generating analytical results that meet project objectives. As with *GOODSamples*, *GOOD Test Portions* is not limited to food materials and is applicable across all industries in which laboratories select test portions for measurement of an analyte or characteristic.

Food samples are collected for a variety of purposes, including food safety, regulatory compliance, risk analysis, targeted or routine surveillance, or manufacturing process quality control. The absence of well-defined sample quality criteria to clarify sampling objectives will result in poor data quality and indefensible regulatory or risk-based decisions (e.g., safety, nutritional value, hazards present, specification limits).

This document serves as a guide to aid the laboratory in designing and implementing laboratory sampling protocols for food[1] samples received by the laboratory. Managers and laboratory personnel all play a role in the quality of laboratory sampling. Laboratories should have an established quality assurance system in place and should have a quality management system manual that addresses laboratory sampling in addition to the traditional topics. The laboratory should have written standard operating procedures related to laboratory sampling. In addition to written procedures, the laboratory should ensure that adequate facilities and environmental conditions, and proper equipment for laboratory sampling are in place to ensure the health and safety of laboratory personnel. Proper training, tools, and resources must be provided to the laboratory as they are critical to control error and ensure fit-for-purpose test results.

Laboratories and accrediting bodies are encouraged to consider laboratory sampling in their accreditation scopes. It is recommended that a method not be placed in a scope without addressing the laboratory sampling procedures for the method. This document is intended to be a resource for both laboratories and accrediting bodies.

This work was developed by a working group established under Cooperative Agreement No. U18FD004710 and comprises members of the Association of Public Health Laboratories (APHL), Association of Food and Drug Officials (AFDO), and the Association of American Feed Control Officials (AAFCO). The project was partially funded with federal funds from a federal program of $1.5 million.

[1]In this document, the term *food* includes human food, animal food (animal feed and pet food), and plant food (fertilizers and soil amendments) to be consistent with changes in US Food and Drug Administration use of the term.

Laboratory Sampling Working Group Members

Jo Marie Cook, contributor, Florida Department of Agriculture and Consumer Services, Tallahassee, FL

Heidi Hickes, contributor, Montana Department of Agriculture, Bozeman, MT

Lawrence Novotny, contributor, retired from South Dakota State University, Brookings, SD

Aaron Price, contributor, Canadian Food Inspection Agency, Ottawa, ON, Canada

Chuck Ramsey, subject matter expert and contributor, EnviroStat Inc., Vail, AZ

Yvonne Salfinger, contributor, consultant to AFDO and APHL, Denver, CO

Michele Swarbrick, co-chair and contributor, Minnesota Department of Agriculture, St. Paul, MN

Nancy Thiex, co-chair and contributor, AAFCO lead investigator, Thiex Laboratory Solutions LLC, Brookings, SD

Sharon Webb, contributor, University of Kentucky, Lexington, KY

Francis Pitard, technical reviewer, Francis Pitard Sampling Consultants LLC, Broomfield, CO

FASS Inc., Champaign, IL; technical editing, design, and layout

DEFINITIONS

Analyte integrity: The characteristic or concentration of the analyte of interest is maintained from selection of the primary sample through selection of the test portion.

Comminution: Reduction of particle size by crushing, chopping, grinding, etc.

Compositional heterogeneity (CH): The heterogeneity arising from differing composition of the analyte of interest among individual elements (e.g., particles) in a material.

Decision unit: The material from which a primary sample is collected and to which an inference is made.

Distributional heterogeneity (DH): The inherent heterogeneity arising from the nonrandom spatial or temporal distribution of elements in a material; heterogeneity inherent to the manner in which elements are scattered within the material. The greater the difference in composition between elements, the greater the possible distributional heterogeneity.

Element: The individual components that compose a material (e.g., particles or fragments for solid materials, molecules for liquids or gases, and particles or molecules for semisolids and slurries).

Finite element materials: Materials composed of elements that can be individually identified, individually selected at random, and individually tested.

Infinite element materials: Materials composed of elements that cannot be individually identified, individually selected at random, or individually tested.

Evidentiary integrity: The identity and authenticity of the evidence (test results).

Extraneous material: Material in the laboratory sample that is not part of the decision unit and must be eliminated or accounted for (i.e., removed at a nonselection process, avoided at a selection process and/or compensated by mathematical correction).

Global estimation error (GEE): Estimate of the total error in the entire process from primary sampling through final measurement. Global estimation error comprises total sampling error (TSE) and total analytical error.

Increment: A group of elements collected by a single operation of a sampling device and combined with other increments to form a sample. For some finite element materials, an increment may consist of a single element.

Inference: The estimation of a concentration or characteristic of a larger amount of material from data derived from testing a smaller amount of material.

Laboratory sampling: All manipulations performed on the laboratory sample after receipt and acceptance through selection of the test portion.

Laboratory sampling protocol: Detailed procedure(s) for obtaining a test portion from a laboratory sample. The protocol includes appropriate mass, number of increments, sample correctness, equipment, quality control, and procedures for maintaining evidentiary integrity necessary to meet the sample quality criteria.

Mass reduction process: Selection of a smaller mass or volume of material from a larger mass or volume.

Nonselection process: Manipulation of a sample (e.g., comminution, removal of extraneous material, removal of water), usually performed before a selection (e.g., mass reduction) process.

Probabilistic sampling: A sampling strategy in which all elements of a material have a non-zero, equiprobable chance of being selected.

Random error (imprecision): The tendency for repeated measurements to vary, typically expressed as a variance or standard deviation. The mean of a random error is zero. The major sources of random error in a selection process are fundamental sampling error and grouping and segregation error.

Sample: A mass or volume of a material selected from a larger mass or volume using principles of Theory of Sampling (TOS). The word *sample* should be used with a modifier as follows:

Primary sample: The material selected from a decision unit.

Laboratory sample: The material received by the laboratory.

Analytical sample: The material from which the test portion is selected.

Replicate samples: Multiple samples selected under comparable conditions.

Split samples: Equal portions obtained by dividing a primary, laboratory, or analytical sample in its entirety.

Composite sample: Multiple laboratory samples, multiple analytical samples, or multiple test portions combined solely for the purpose of analytical efficiency.

Sample correctness: Achieved when increment delimitation error and increment extraction error are controlled to a negligible level.

Sample quality criteria (SQC): A series of statements that clarify program technical and quality requirements to support defensible decisions. These statements include the question to be answered, definition of the decision unit, and desired confidence in the inference.

Selection process: The act of selecting a smaller mass or volume of material from a larger mass or volume. There are two types of selection processes: mass reduction and splitting.

Splitting: The division of a mass or volume of material into two or more equal portions.

Systematic error (bias): The tendency for a measurement to over- or underestimate the actual (true) value. The mean of a systematic error is not zero. The major systematic errors in laboratory sampling are AIE, CIE, EME, IDE, IEE, IWE, and MRE. (See *Total sampling error*.)

Test portion: The mass or volume of material selected from an analytical sample for a single test.

Theory of sampling (TOS): A set of principles that describe all errors contributing to total sampling error as well as techniques for estimation and mitigation of error to an acceptable level to meet sample quality criteria requirements.

Total sampling error (TSE): Error from all nonselection and selection processes that causes the concentration or characteristic of the test portion to deviate from the true concentration or characteristic of the decision unit. The major components of total sampling error are AIE, CIE, EME, FSE, GSE, IDE, IEE, IWE, and MRE:

Analyte integrity error (AIE): Systematic error due to changes in the concentration of the analyte or characteristic of interest.

Contamination introduction error (CIE): Systematic error due to the unintended introduction of the analyte of interest or an analytical interferent.

Extraneous material error (EME): Systematic error due to incorrect removal of extraneous material.

Fundamental sampling error (FSE): Random error due to compositional heterogeneity.

Grouping and segregation error (GSE): Random error due to distributional heterogeneity.

Increment delimitation error (IDE): Systematic error due to incorrect shape of the volume delimiting the increment resulting in nonequiprobable selection of elements.

Increment extraction error (IEE): Systematic error due to incorrect increment extraction (e.g., correctly delimited elements fail to become part of the increment).

Increment weighting error (IWE): Systematic error due to inconsistent or disproportional increment masses.

Mass recovery error (MRE): Systematic error due to loss or gain of mass.

Acronym/abbreviation	Definition
AIE	Analyte integrity error
CH	Compositional heterogeneity
CIE	Contamination introduction error
DH	Distributional heterogeneity
EME	Extraneous material error
FSE	Fundamental sampling error
GEE	Global estimation error
GSE	Grouping and segregation error
IDE	Increment delimitation error
IEE	Increment extraction error
IWE	Increment weighting error
MRE	Mass recovery error
QA	Quality assurance
QC	Quality control
RSD	Relative standard deviation
SQC	Sample quality criteria
TAE	Total analytical error
TOS	Theory of sampling
TSE	Total sampling error

EXPANSION OF *GOODSAMPLES* CONCEPTS FOR LABORATORY SAMPLING

Note: Information contained in this section applies to the concepts in *GOODSamples* specific to the laboratory setting. The reading and understanding of the *GOODSamples* document is necessary for application of *GOOD Test Portions*.

Sample Quality Criteria

Sample quality criteria (**SQC**) establish the question to be answered, the decision unit, and the desired confidence needed to support defensible decisions. Sample quality criteria apply to the entire measurement process, from selection of primary sample through a measurement on the test portion to making an inference to the decision unit. It is critical that the laboratory, program management, sampling, and quality assurance (**QA**) personnel are involved in the SQC process. Laboratory staff bring scientific expertise that is critical to the SQC process. Through communication, education, and negotiation on the part of all stakeholders, final SQC are developed.

If the SQC are not well understood by or communicated to laboratory staff, the laboratory results may not represent the decision unit as a result of inappropriate laboratory decisions such as incorrect compositing of the laboratory samples, choosing the incorrect analytes or method(s), or inappropriate laboratory sampling techniques (*GOODSamples*, AAFCO, 2015; section on SQC). Laboratories should never accept samples without established SQC and should be involved in the SQC process. If the laboratory was not involved in the SQC process, the laboratory needs to obtain the SQC before sample acceptance.

There are 3 major components of the SQC process.

1. What is the question?

    - ▷ What is the analyte(s) or characteristic of interest?
    - ▷ What is the concentration of concern (e.g., specification limit, action limit, label guarantee, detect or nondetect, defect)?
    - ▷ How will inference be made from the test results to the decision unit? See Figure 1.

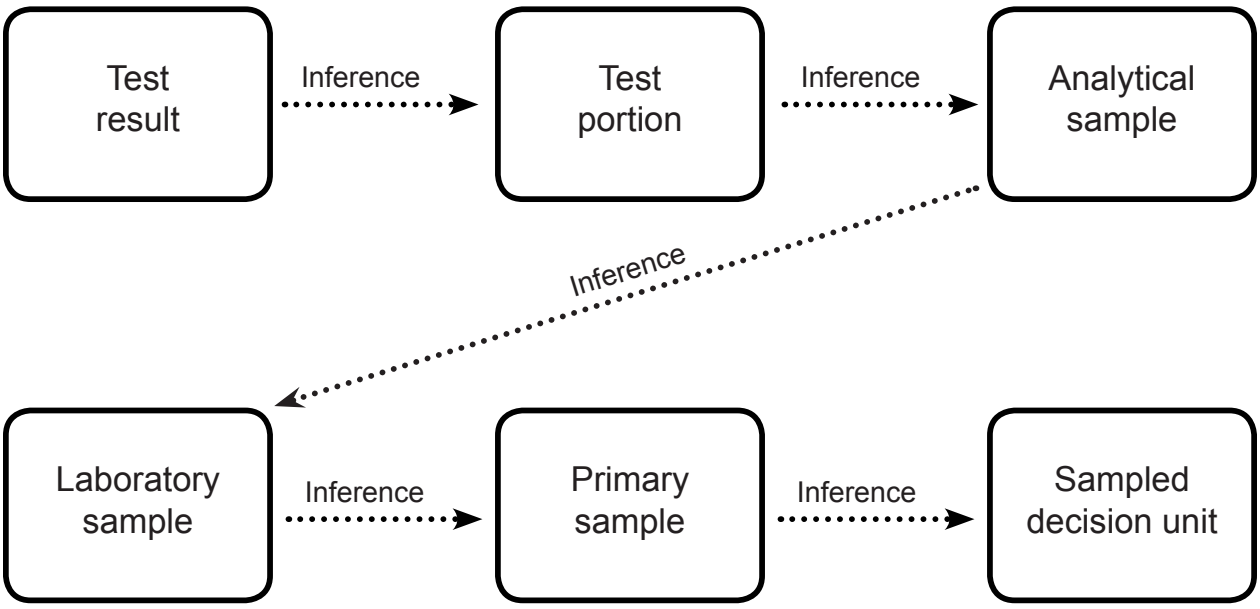


Figure 1. Example of probabilistic inference to a decision unit.
Adapted from Ramsey (2015) and reprinted with permission from J. AOAC Int.

2. What is the decision unit?
3. What is the desired confidence?

The laboratory has a unique role in developing the SQC. Critical input from the laboratory includes the following:

- Identify a fit-for-purpose test method to measure the analyte(s) or characteristic.
- Identify and resolve analyte integrity issues starting with primary sample selection.
- Verify that the laboratory has the needed capacity and capability for both laboratory sampling and testing.
- Identify potential sources of contamination and mitigation procedures.
- Participate in establishing procedures for estimation of global estimation error (**GEE**).
- Determine the mass required for all testing activities, including quality control requirements.
- Verify that subcontracted laboratories meet the requirements of SQC.

Verification of SQC upon Receipt of the Laboratory Sample

- If the laboratory was involved in the development of the SQC, the laboratory needs to verify that there were no changes to the SQC. If the laboratory finds that changes have been made to the SQC, it needs to verify that it has the capability and capacity to meet the requirements of the revised SQC.

- If the laboratory was not involved in the development of the SQC, the laboratory needs to obtain the SQC and verify that it has the capability and capacity to meet the requirements of the SQC.

- When a subcontracted laboratory that was not involved in the SQC process is used, it is critically important that the subcontracted laboratory's ability to meet the requirements of the SQC is evaluated and that the subcontracted laboratory understands the SQC requirements.

"If I had an hour to solve a problem I'd spend 55 minutes defining the problem and 5 minutes solving it." —Attributed to Albert Einstein

---

Material Properties

The material properties of the decision unit must be considered when developing laboratory sampling protocols. Material properties are described in *GOODSamples* and include the type of material elements and heterogeneity (AAFCO, 2015; Material Properties section).

Material Elements

The type of material elements (finite or infinite) will affect the laboratory sampling protocols. The laboratory may receive finite or infinite element materials. Comminution of a finite element material typically results in an infinite element material.

Heterogeneity

Heterogeneity is the root cause of error in sampling. There are 2 types of heterogeneity: compositional heterogeneity (**CH**) and distributional heterogeneity (**DH**). The magnitude and nature of CH and DH are unique to each material (see Figure 2) and dictate the sampling efforts. Compositional heterogeneity of a material is altered by comminution. Distributional heterogeneity is altered with any physical manipulation of the material (e.g., vibration causing segregation, pouring, comminution, or mixing).

Figure 2. Orange juice has many separate components with large distributional heterogeneity. The pulp falls quickly, the foam disperses slowly, and volatiles escape rapidly. © JoMarie Cook, Florida Department of Agriculture and Consumer Services, Tallahassee, Florida.

Total Sampling Error

There are 3 types of sampling errors—random error, systematic error, and blunders—which propagate to yield the total sampling error (**TSE**). The desired magnitude of the total sampling error is inversely proportional to the acceptable confidence (see Figure 3). Therefore, a complete understanding of errors and methods to mitigate errors is essential for designing a protocol to obtain a representative test portion.

Theory of Sampling (**TOS**) is a comprehensive approach to representative sampling and provides a system of categorization and minimization of error. It addresses all errors from the selection of the primary sample to the selection of the final test portion. As described in *GOODSamples* (AAFCO, 2015; section on TOS), the necessary inputs for TOS are SQC and material properties.

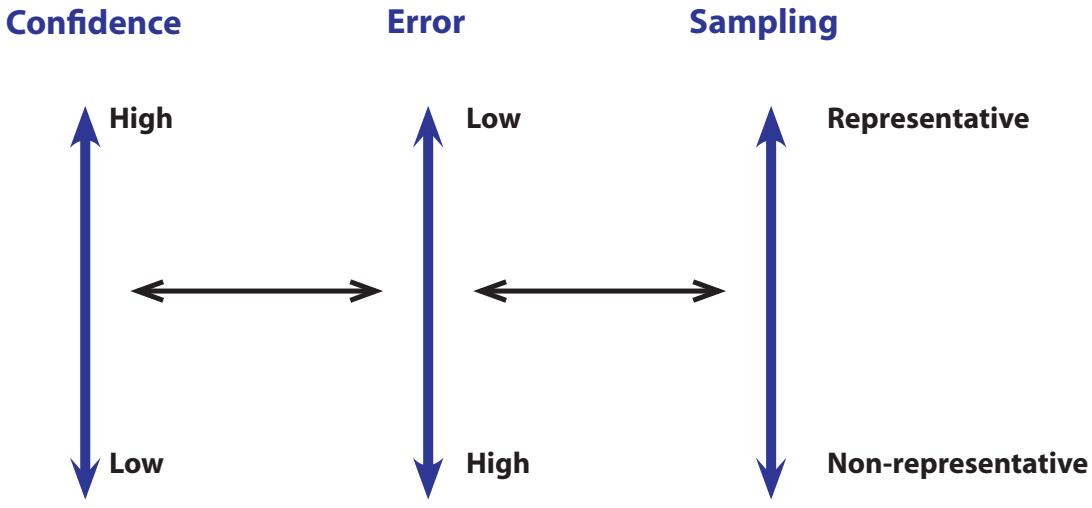


Figure 3. Relation between confidence, error, and representativeness (sampling). Adapted from Ramsey and Wagner (2015) with permission from J. AOAC Int.

Random Errors (Imprecision)—Random errors consist of fundamental sampling error (**FSE**) and grouping and segregation error (**GSE**), which arise from heterogeneity of the material. Fundamental sampling error is a function of particle size, mass, and CH. Fundamental sampling error remains after all other sampling errors are perfectly controlled. Grouping and segregation error is a function of the number of random increments and DH. Distributional heterogeneity is transient in nature and is affected every time a material is handled. Random errors also result from inconsistent methodology or operations. By definition, the mean of a random error is zero and the variance is non-zero.

Systematic Errors (Bias)—Systematic error results from increment delimitation error, increment extraction error, increment weighting error, extraneous material error, material recovery error, contamination introduction error, and analyte integrity error. Contrary to an analytical bias, these errors are never constant and are extremely difficult, if not impossible, to measure. Therefore, effort must be taken to ensure that all systematic errors are controlled using TOS to a point of being negligible throughout the entire laboratory sampling process. Systematic errors in sampling are generally inconsistent and manifest as random errors; therefore, systematic errors have both a systematic and random component. The random component of systematic errors cannot be reliably measured because of the transient nature of segregation. By definition, the mean and variance of a systematic error are both non-zero.

Blunders—Blunders are mistakes or accidents that result in loss of data or loss of data integrity and can occur at any point in the sampling process. Examples include breaking the sample container, mislabeling, transcription and transposition errors, incorrect recording, using an incorrect method or procedure, equipment failure, loss of evidentiary integrity, and so on. Blunders cannot be incorporated into TSE and must be prevented or eliminated, or the procedure must be repeated.

Maintaining Evidentiary Integrity

Evidentiary integrity must be maintained once the laboratory samples arrive at the laboratory; failing to do so can adversely affect the quality and evidentiary integrity of test results (AAFCO, 2015; Evidentiary and Analyte Integrity section). Traditionally, the purpose of evidentiary integrity in the laboratory has been to establish (1) trace-back information from the test result to receipt of the laboratory sample and (2) assurance that any sample or test solution has not been adulterated or compromised at any point from receipt through disposal. Evidentiary integrity may be demonstrated by documentation and procedures that are thoroughly discussed in other documents: the AAFCO *Quality Assurance Quality Control Guidelines for Feed Laboratories* (2014; section 5.8), APHL *Best Practices for Submission of Actionable Food and Feed Testing Data Generated in State and Local Laboratories* (2017), FDA *Investigations Operations Manual*, and International Organization for Standardization 6498:2012 *Animal feeding stuffs—Guidelines for sample preparation* (section 7.2).

In addition to the traditional interpretation, evidentiary integrity requires that the systematic errors, random errors, and blunders be sufficiently controlled to meet the SQC. This includes not only maintaining analyte integrity and sample correctness, as described in *GOODSamples*, but also controlling all other errors resulting from nonselection and selection processes as described in this document.

Evidentiary Integrity: Evidence that samples have been properly collected, processed, and stored in a manner to ensure that test result(s) can be traced to the decision unit and are a true representation of the decision unit (in legal terms, the identification and authentication of the evidence). Evidentiary integrity is demonstrated by documentation of trace-back (e.g., chain of custody forms); proper sampling procedures to ensure representivity (e.g., sample correctness); and processes to ensure analyte integrity is maintained.

LABORATORY SAMPLING

Introduction

Laboratory sampling consists of 2 major processes: the nonselection processes (e.g., comminution, removal of extraneous material, removal of water, use of a comminution aid such as dry ice) and the selection processes (i.e., selecting a smaller mass from the larger mass). Frequently, the term *sampling* refers only to the selection processes; however, in this document *sampling* refers to both the nonselection and selection processes.

GOODSamples (AAFCO, 2015) provides an overview of TOS, which is more extensively discussed in Pitard (1993). *GOODSamples* introduces the concepts of CH, DH, FSE, GSE, and sample correctness. The reader must be familiar with these concepts. Every nonselection and selection process contributes error to the TSE. Most laboratories are aware of the error contributions from chemical, microbiological, or physical methods of analysis and have these errors well characterized. However, the laboratory must also control and measure the errors associated with nonselection and selection processes. Because all errors affect confidence, integrity, and defensibility of the test result, it is imperative that errors from all sources are well characterized and controlled.

The principles of TOS must be strictly adhered to through all sampling processes to yield a test portion representative of the decision unit and meet the confidence established by the sample quality criteria. The principles of TOS define the following relationships:

- the relationship of error to mass,
- the relationship of error to increments, and
- the relationship of error to sample correctness.

Figure 4 illustrates the components of GEE, which include TSE and total analytical error (**TAE**). It further describes all components of TSE.

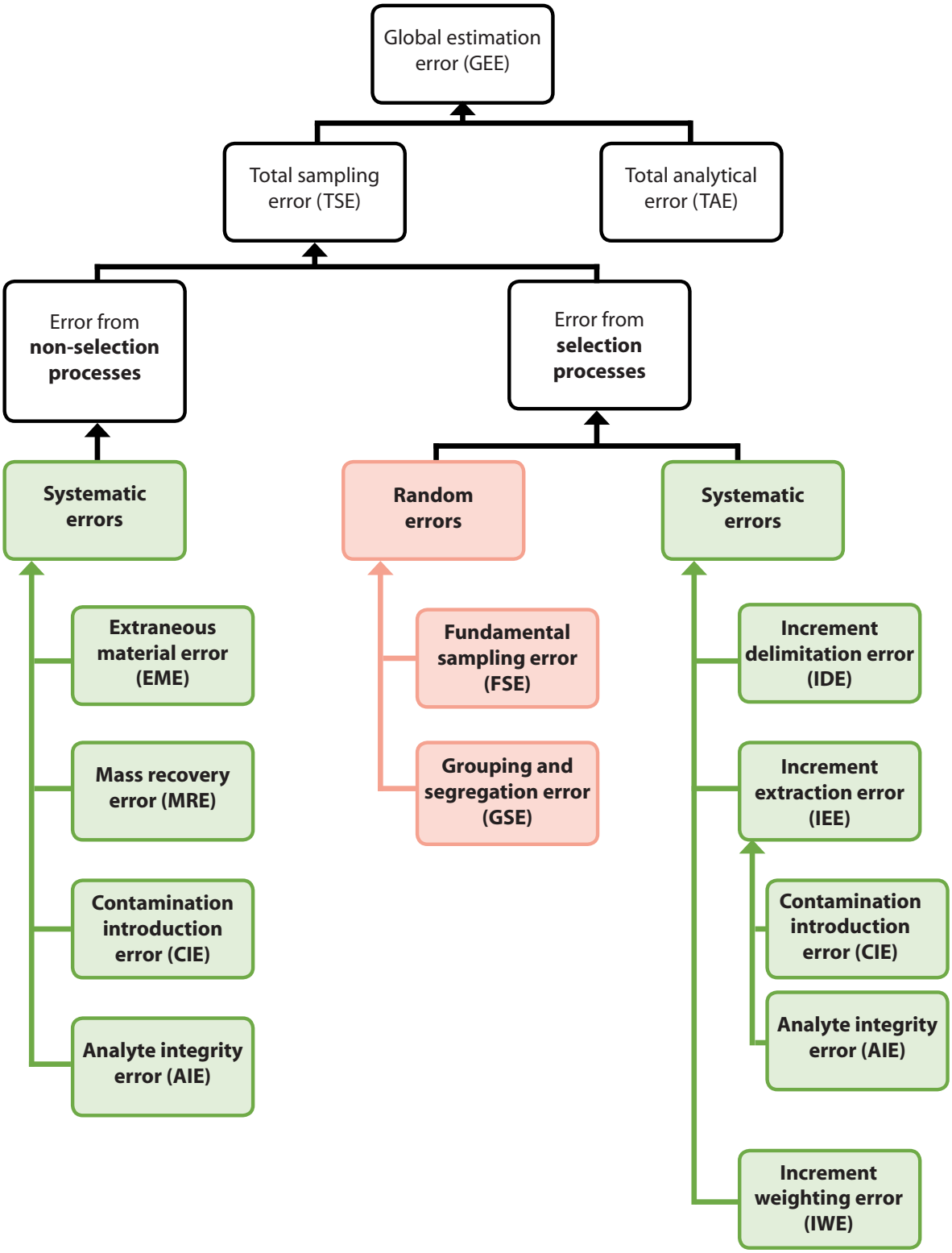


Figure 4. Categorization of errors contributing to total sampling error and relationship to global estimation error. Blunders are an additional type of error that result in loss of data. Blunders cannot be incorporated into global estimation error.

Nonselection Errors

Nonselection errors are generally due to negligence, inattention to detail, or a lack of understanding of error. Therefore, proper education and training are essential to understand and control nonselection errors. In TOS, nonselection errors are commonly referred to as preparation errors. All nonselection errors are systematic errors.

Systematic Errors

Extraneous Material Error—In some cases, extraneous material that is not part of the decision unit is accidentally or intentionally included in the laboratory sample. Because these materials are not part of the decision unit, they must be addressed (removed, avoided, or compensated) before nonselection and selection processes. Failure to adequately or properly address extraneous materials will result in extraneous material error (**EME**). Examples of extraneous materials may include dirt, rocks, metal fragments, shells, pits, skins, peels, moisture, liquid phase, solid phase, etc.

Mass Recovery Error—Failure to recover 100% of the material during a nonselection process results in mass recovery error (**MRE**). Any loss or gain of mass that affects the representativeness must be avoided. Examples include loss of fines or dust, loss of large particles, nonquantitative transfer of materials from equipment and containers, minor spillage at the balance, etc.

Contamination Introduction Error—Contamination introduced from external sources (e.g., the environment, the container, tools and equipment, or the analyst) results in contamination introduction error (**CIE**). The contaminant from an external source may be the analyte of interest or an interferent in the measurement of the analyte of interest.

Analyte Integrity Error—Analyte integrity error (**AIE**) is characterized by chemical, biological, physical, or radiological changes within the material that can result in either gain or loss of the target analyte(s). Analyte integrity error is typically controlled by preservation, holding times, and proper handling techniques. Examples of techniques to control AIE include controlling sterility, light, temperature, pH, humidity, etc. (*GOODSamples*, AAFCO, 2015).

Selection Errors

Selection errors consist of random errors and systematic errors.

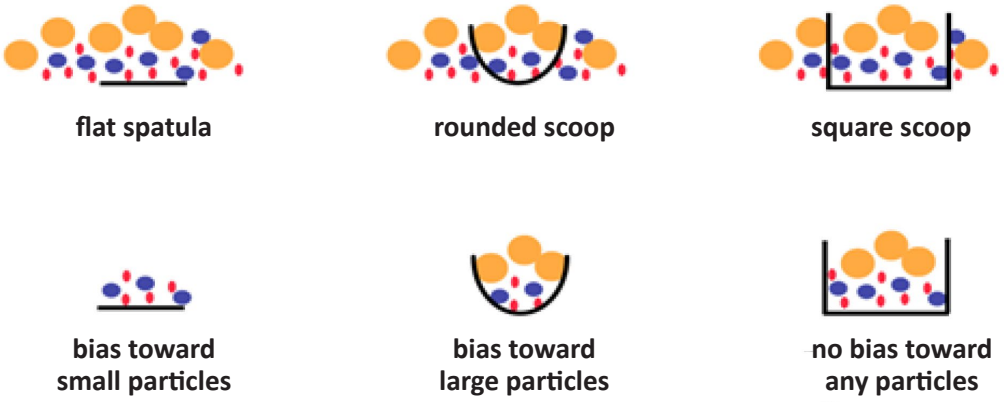
Random Errors

Fundamental Sampling Error—Fundamental sampling error is controlled through the selection of an adequate mass. Simply stated, the larger the mass resulting from the selection process, the smaller the variance of the FSE. Along with the mass, the FSE is a function of the shape factor, the granulometric factor, the maximum particle size, the mineralogical factor, and the liberation factor (see "Relationship of Error to Mass" on page 20). These factors are different for every material and analyte; therefore, an adequate mass must be uniquely established for each selection process.

Grouping and Segregation Error—Grouping and segregation error is controlled through the selection of a sufficient number of random increments. Note: Increments must always be selected at random locations. The variance of the GSE is a function of the number of increments and DH. Distributional heterogeneity is in constant flux, as it changes every time a material is manipulated. A minimum number of increments must be uniquely established for each selection process.

Systematic Errors

Increment Delimitation Error—Increment delimitation error (**IDE**) is a result of incorrect shape of the volume delimiting the increment. See Figure 5 for examples of increment delimitation error and refer to Pitard (1993) for more detail.



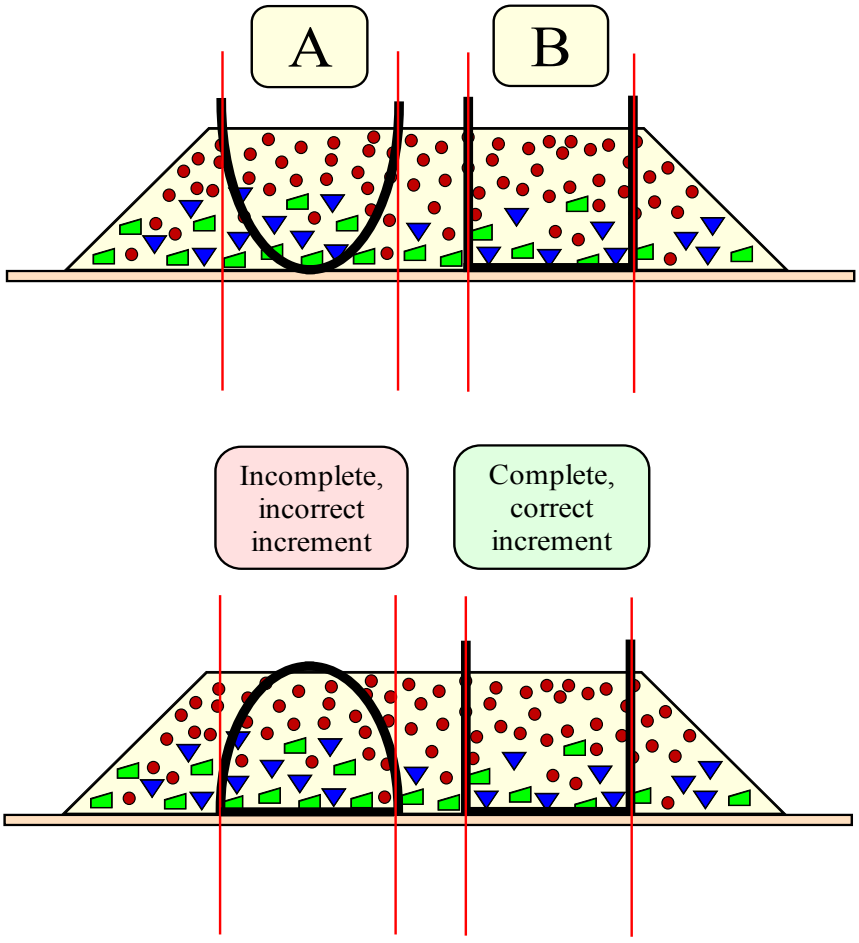


Figure 5. Illustration of increment delimitation error.
Upper image reprinted with permission from C. Ramsey, Envirostat, Vail, Arizona.
Lower image reprinted with permission from F. Pitard.

Increment Extraction Error—Increment extraction error (**IEE**) is a result of improper removal of an increment even if the volume delimiting the increment is correct. An example is material falling out of a cylindrical probe as the increment is being removed. Increment extraction error also includes CIE and AIE. Note: TOS does not consider CIE and AIE to be errors from selection processes. However, due to the unique characteristics of food analytes, CIE and AIE need to be controlled in both nonselection and selection processes.

Contamination Introduction Error—Contamination introduced into the material during increment extraction from external sources (e.g., the environment, the container, tools and equipment, or the analyst) results in CIE. The contaminant may be the analyte of interest or an interferent in the measurement of the analyte of interest.

Analyte Integrity Error—Analyte integrity error is error occurring during increment extraction resulting from changes to chemical, biological, radiological, or physical characteristics from those of the decision unit. Analyte integrity error typically is controlled by appropriate sampling and handling techniques. Examples of introduction of AIE include loss of viability of the microbiological target, and volatilization, adsorption, or oxidation of the analyte during the selection process.

Increment Weighting Error—Increment weighting error (**IWE**) is a result of disproportional increments (in mass or volume) resulting in over- or underrepresentation of certain portions of the material being sampled. Disproportional increments can occur when flow rates change or slab cakes are not of uniform dimensions. In most cases in the laboratory, proportionate increments will be equal in mass or volume because flow rate and dimensions can easily be controlled. When increment selection is performed by manual techniques (e.g., using scoops or spatulas), consistency in increment mass or volume can be controlled by using a sampling tool that has the same capacity as the desired increment mass or volume (Figure 6).

Figure 6. Increments from a uniform 1- or 2-dimensional slab cake. (A) Equal/proportionate increments, resulting in control of increment weighting error (IWE). (B) Unequal/disproportionate increments, resulting in uncontrolled IWE. © Lawrence Novotny and Nancy Thiex (Thiex Laboratory Solutions LLC, Brookings, South Dakota).

Relationship of Error to Mass

Fundamental sampling error is related to the magnitude of CH, the mass selected, and the particle size distribution of the material. The relationship of error to CH, mass, and the maximum particle size is given by

$$s^2_{FSE} \propto \frac{\text{CH} \times \text{diameter}^3_{\text{max}}}{\text{mass}} \quad [1]$$

To control FSE for a given state of CH, the diameter of the particles must be reduced, the mass increased, or a combination of both. The estimation of mass and diameter is

relatively easy; however, estimation of CH is difficult and is dependent on the state of comminution.

As can be seen in Equation 1, because particle diameter is cubed, it has a larger effect on variance of FSE than mass. Therefore, comminution is a very effective technique for controlling FSE.

For more details on the applications of TOS equations to estimate the variance of FSE and example calculations, please refer to Appendix A.

Relationship of Error to Increments

Grouping and segregation error is related to the magnitude of DH and the number of increments selected. The relationship of error to DH and number of increments is as follows:

$$s^2_{GSE} \propto \frac{\text{DH}}{\text{number of increments}} \quad [2]$$

The equation for the variance of GSE is not as straightforward as the equation for the variance of FSE and is therefore not presented here. As a general practice, sufficient increments are selected to reduce GSE until its contribution to GEE is negligible. Research and experience have shown that approximately 50 increments are generally required to reduce GSE to a negligible level; however, if the DH is very large, the number of increments must be increased. If the DH is established to be low through experimentation or other quantitative measures (it cannot simply be assumed that DH is low), it may be possible to control GSE with as few as 10 increments.

Comminution is frequently used to control the variance of FSE and, as a secondary effect, can sometimes reduce the DH by reducing the range in particle sizes, thereby reducing the chances of segregation of fines from larger particles. However, if the analyte of interest becomes liberated during the comminution process, DH could be increased. When selecting comminution equipment, equipment that produces a more uniform particle size and shape is preferred. When evaluating selection techniques, those that provide the greatest number of correctly selected increments are preferred to control GSE.

Relationship of Error to Sample Correctness

Control of IDE and IEE are collectively referred to as *sample correctness*. Increment delimitation error occurs when all the elements of a material do not have an equal probability of being selected due to incorrect shape of the volume delimiting the increment (a function of tool design). Increment extraction error occurs when all elements in the material have an equal probability of selection but all correctly delimited elements do not become part of the selected increment (function of tool usage). Therefore, sample correctness is a function of (1) properly designed tools and equipment, and (2) appropriate use of the properly designed tools and equipment. It follows that tools, equipment, and techniques for mass reduction must ensure that all elements, regardless of chemical or physical (e.g., size or location) properties, have an equiprobable chance of being selected. Failure to adhere to the principles of sample correctness result in bias error that cannot be estimated; therefore, it is critical that sample correctness be adhered to at all times.

The state and dimension of the material impacts sample correctness and must be considered. Dimensions are discussed in *GOODSamples* (AAFCO, 2015). Materials fall on an infinite continuum from liquids to solids and can be broadly classified as either (1) liquids and slurries, or (2) semisolids and solids. When poured, a liquid or slurry will flatten to a uniform level, whereas a semisolid or solid will mound (Figure 7). Although these broad classifications are presented, the specific state of each material needs to be considered and addressed with respect to IDE and IEE.

Liquids and Slurries

Liquids can be single phase or multiphase, either with or without suspended solids. A slurry typically is characterized as a liquid with a large amount of suspended solids, although there is no specific scientific demarcation between liquids with suspended solids and slurries. From a sampling perspective, liquids with suspended materials and slurries are considered together.

Single-Phase and Multiphase Liquids Without Suspended Solids: Selection of material from a single-phase liquid without suspended solids (e.g., bottled water) is the easiest of all sampling selection scenarios. A single increment (aliquot) can adequately represent the single-phase liquid and sample correctness is not typically an issue. Selection of materials from multiphase liquids can be performed in the same manner as from a single-phase liquid if either of the following applies: (1) the multiphase liquid can be mixed to attain a single phase, or (2) the individual phases can be separated and sampled separately. If not, the material must

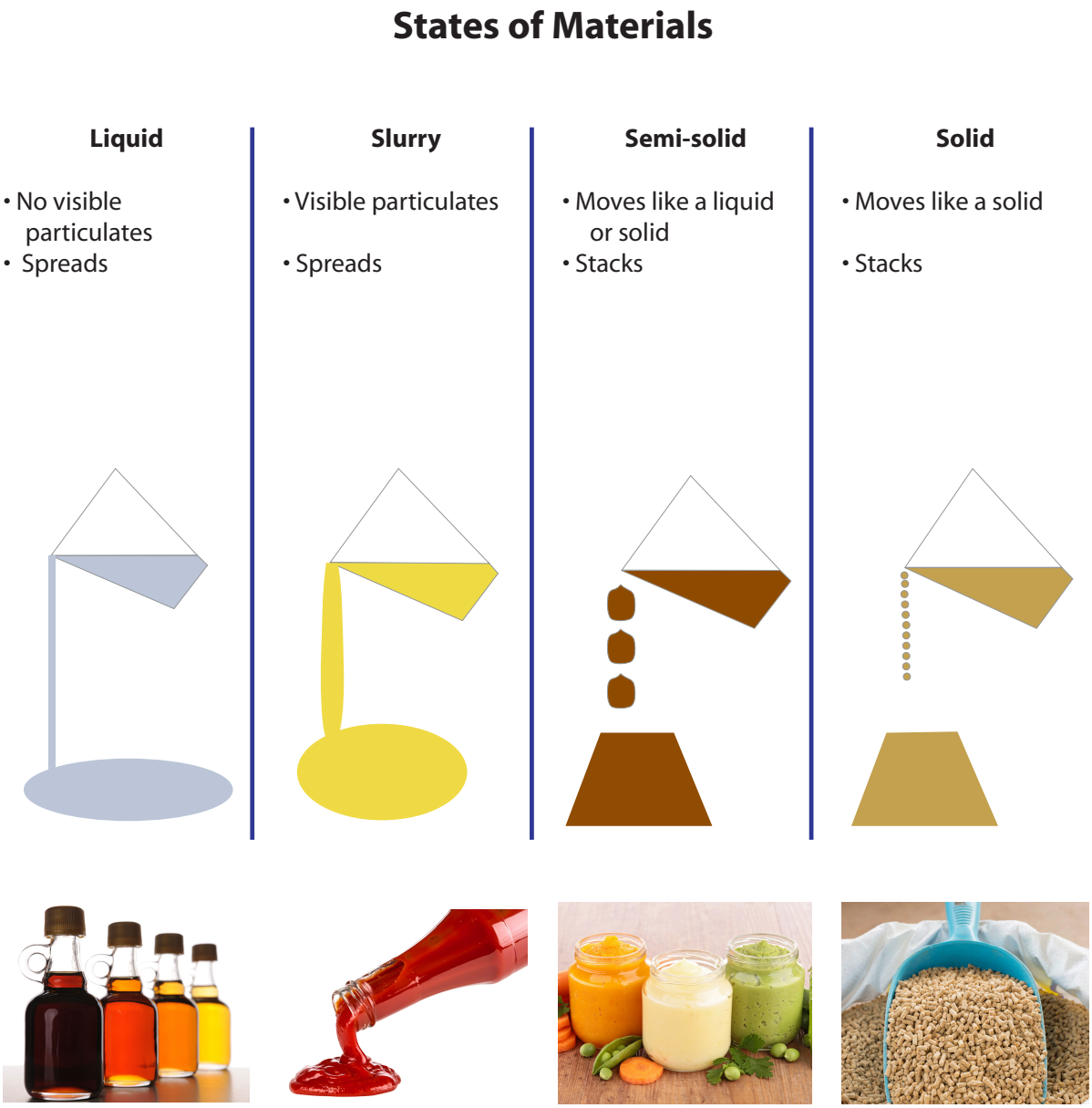


Figure 7. States of materials.
Modified from source: Oklahoma Cooperative Extension Service.

be sampled through the dimension of heterogeneity (i.e., vertically), and sample correctness must be considered and addressed (see Figure 8).

Liquids with Suspended Solids and Slurries: To achieve sample correctness, the preferential exclusion of particles due to their size or density must be avoided. The opening of the sampling device needs to be 3 times the size of the largest particle to ensure large particles are not excluded. For liquids, the particles typically are so small that this is not a critical factor; however, for slurries this may be a consideration. For example, slurries of fruit or vegetable material may contain skin fragments that are relatively large, so the opening of a sampling device would need to be 3 times the length of the longest dimension of the largest particles.

When material is removed from a liquid with suspended solids, the velocity of the sampled material changes as it enters the sampling tool. Larger/denser particles (due to their inertia) are less likely to be introduced into the sampling tool than the smaller/lighter particles (which have less inertia). Since suction causes a velocity change, use of any sampling tool that uses suction (e.g., pipette) is not recommended. One recommended approach that causes minimal disturbance is the use of an open tube (thief) or straw. When using such a tool, it must be *slowly* inserted into the liquid or slurry such that there is minimal disturbance, resulting in higher probability of equiprobable representation of all particles, regardless of size or density. It is also critical that the levels inside and outside the straw remain constant at all times to allow equiprobable selection of the vertical dimension. When using an open tube, it is important that the container has parallel sides to proportionally represent the top and bottom (see Figure 8). Another approach for low-viscosity liquids with suspended materials is the use of a churn splitter, which keeps solid particles in motion during the selection process (see Figure 9).

Note: The terms *homogenizer*, *homogeneous*, *homogenization*, *homogenizing*, and *homogenized* (any term with the root *homogen*) are commonly used in laboratories and are not specific to a type of force, equipment, or state of matter. They are not precise scientific terms, and a "homogeneous material" is an ideal state that is never achieved. All materials remain heterogeneous to some degree. These terms should not be used in protocols, standard operating procedures, or work instructions.

Correct delimitation:
Volumes proportionately represented

Incorrect delimitation:
Volumes disproportionately represented

Figure 8. Examples of correct and incorrect increment delimitation: (A) open tube in a multiphase liquid (olive oil and red wine vinegar) in a cylinder; (B) open tube in a multiphase liquid (olive oil and red wine vinegar) in an Erlenmeyer flask.
© Lawrence Novotny and Nancy Thiex (Thiex Laboratory Solutions LLC, Brookings, South Dakota).

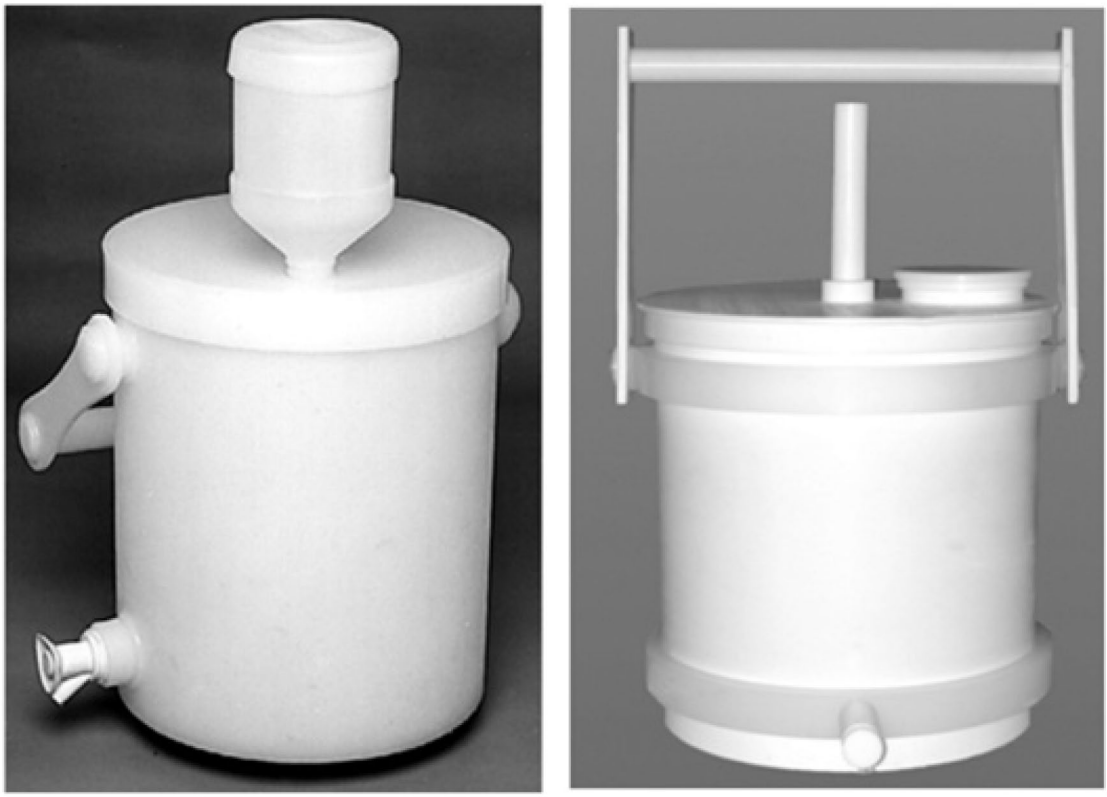


Figure 9. Churn splitter. Left image from Capel and Larson (1996). Right image from USGS National Field Manual.

Semisolids and Solids

As with liquids and slurries, to achieve sample correctness, the sampling device must provide for equipropable selection of all particles. Particle size, location, density, and other characteristics make some particles more difficult to represent. For a flowable material with a variety of particle sizes present, segregation can make it difficult to represent all particle sizes, especially the fines. Fine and dense particles often segregate to the bottom, tend to fall out of coring devices, and are often excluded when using scoops or spatulas to select from material spread on flat surfaces. To avoid preferential exclusion of the largest particles due to their size, the opening of the sampling tool needs to be 3 times the size of the largest particle. For nonflowable materials that are "sticky," segregation of fine particles may be less of a concern. In most cases, all the material must be removed from its container before the selection process. **The common practice of sampling solids directly out of the top of bottles should not be performed, as equiprobable selection is impossible, and sample correctness cannot be achieved.**

If a flowable material is poured into an elongated pile, a slice (increment) through the entire width and depth of the material is the correct increment shape to eliminate IDE. If a flowable or nonflowable material is shaped into a flattened pile of uniform thickness, a core through the entire depth is the correct increment shape to eliminate IDE. The entire slice or core must be recovered to avoid IEE. Taking a slice from a 1-dimensional flowable solid is accomplished with a square spatula with sufficiently tall sides to contain the slice, which can be difficult with large amounts of material. Taking a core from a 2-dimensional flowable solid is virtually impossible because the top portion is always selected but the very bottom portion is rarely selected or recovered properly. Taking a core from a nonflowable 2-dimensional solid is more easily accomplished because of the minimal segregation of the fine particles. The consequences are greatly mitigated when the particle size is uniform. Comminution to a more uniform particle size before using these selection techniques will mitigate issues with segregation, except in cases where the analyte becomes liberated. See the section titled Mass Reduction Techniques and Equipment on page 36 for further explanation.

Figure 10 provides illustrations for sample correctness of various sampling tools for a 1-dimensional material. The most common tools, the flat-blade spatula and the rounded-scoop spatula, are not appropriate for materials with a range of particle sizes. The most appropriate tool is the square-shaped spatula, which shows no bias toward particle size (see Figures 5 and 10). When a material is comminuted to a uniform particle size and shape, the choice of tool becomes less critical; however, IDE and IEE must still be considered.

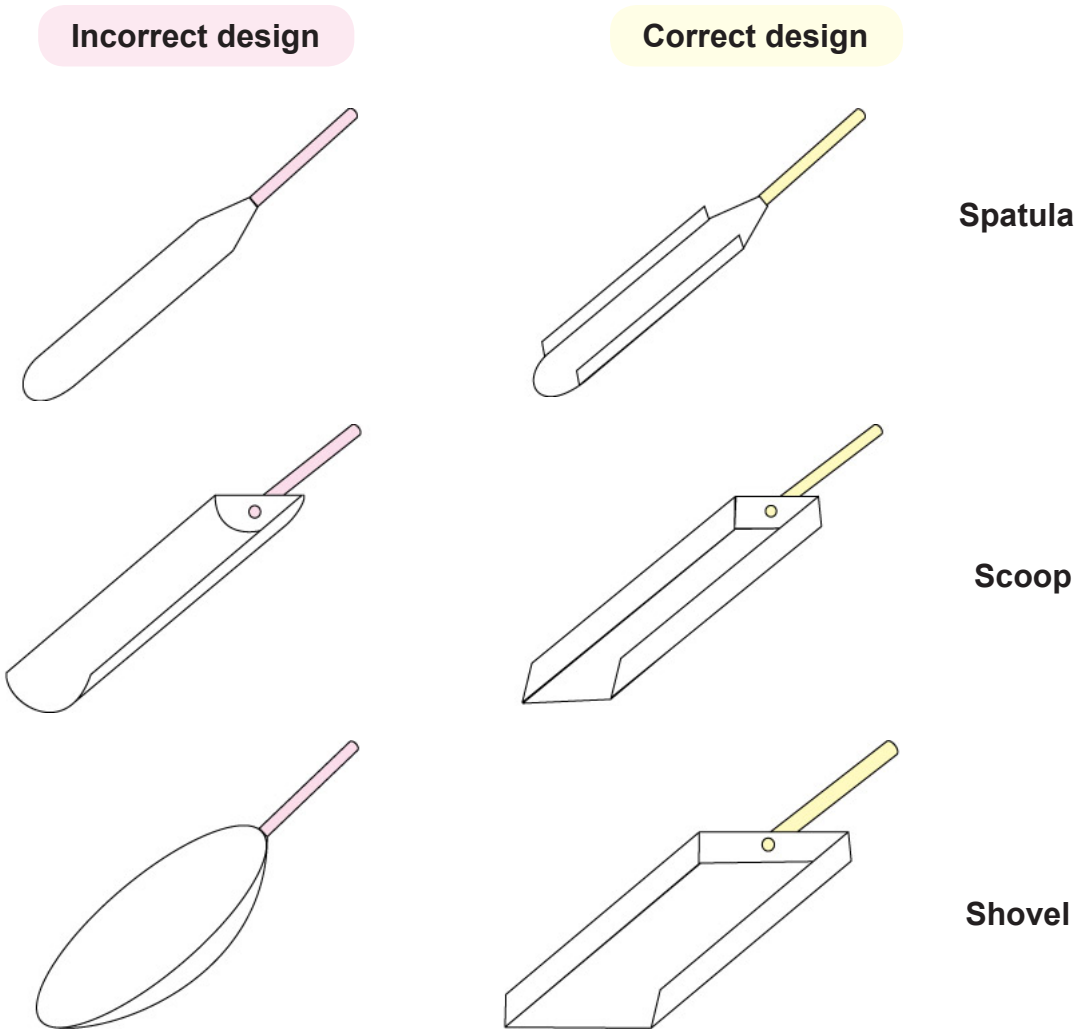


Figure 10. Incorrect and correct design of laboratory sampling tools. © Francis Pitard Sampling Consultants, Broomfield, Colorado. Reprinted with permission.

Nonselection Techniques and Equipment

Nonselection techniques include comminution, removal of extraneous material, preservation, and conditioning (e.g., freezing, drying). The majority of discussion in this document is related to comminution because of its importance in controlling the variance of FSE during subsequent selection processes.

Considerations for Comminution Equipment

Requirements for suitable comminution differ widely and depend on the physical and chemical characteristics of the material. Considerations for choosing communition equipment are listed on page 29.

Wear of the grinding plates or cutting edges of comminution equipment is inevitable and must be monitored. Wear can result in (1) undesirable particle size, particle shape, and particle size distribution; (2) contamination and other systematic errors; and (3) safety concerns. Equipment surfaces must be harder than the material being comminuted. If materials are cooled or embrittled with dry ice or liquid nitrogen for comminution, the hardness of the embrittled state should be considered. The cutting or crushing components are generally made of steel, stainless steel, titanium, tungsten carbide, agate, or hard porcelain.

There are multiple techniques for controlling undesirable heat generation during comminution. Some equipment is available with optional flow-through attachments that allow the continuous grinding of a large quantity of material while minimizing heat generation. Cooling the equipment and material before comminution (e.g., by freezing) or during comminution (e.g., by adding dry ice) may assist in controlling heat generation. To avoid excessive heat generation when comminuting a large quantity of material to a fine particle size, it may be beneficial to perform a coarse grind (e.g., 6-mm particle size) followed by a fine grind (e.g., 0.75-mm particle size).

It is impossible to comminute laboratory samples without loss of minute amounts of material resulting in negligible MRE. Loss of a significant amount of material during the comminution process results in unacceptable MRE and may be a source of contamination and potentially a safety hazard due to dust or contamination.

Uniformity of the final particle size and distribution is important to any comminution process. A wide range of particle sizes increases the potential of large DH. Some equipment or techniques, such as choppers, blenders, and hand tools, produce a wide range of particle sizes. With any comminution technique, especially those that do not

Considerations for Choosing Comminution Equipment

Initial properties of the material

- Material state
  - Liquids or slurries
  - Semisolids or solids
- Material hardness of solids (refer to Mohs' scale for hard materials or degree of softness for materials such as tissue, plant material, and moist feeds or foods)
- Flowability of the material
  - Fat or oil content of the material
  - Moisture content of the material
  - Sugar content of the material
  - Geometry of the particles
- Initial particle size of the material

Desired particle size characteristics

- Desired particle size
- Desired range of particle sizes

Capacity

- Initial mass of samples to be comminuted
- Number of samples to be comminuted

Control of systematic errors

- Control EME
  - Complete removal of extraneous material without removal of material associated with the decision unit
- Control MRE
  - Avoid loss of any material (e.g. fines, dust, volatiles)
  - Avoid adsorption of analyte
- Control CIE
  - All parts that come in contact with the sample should be inert
- Control AIE
  - Control of heat generation
  - Control of moisture change
  - Avoidance of chemical reactions during comminution
  - Maintaining sterility

Design considerations

- Can be cleaned easily
- Simple to operate
- Reliable
- Versatile in handling a wide range of materials

control the particle size directly (e.g., jaw crushers, blenders, hand tools), an evaluation of the resulting particle size should be made, especially the largest particle size(s). The evaluation can be done by sieving, or with other types of particle size analysis.

Comminution Equipment

Several attempts have been made to categorize comminution equipment by particle size of the product, whereas others have attempted to classify comminution equipment by action. One author stated that "clear unambiguous classification of equipment is not possible" (Lowrison, 1974). No industry standard classifications nor standard vocabulary have been adopted, and manufacturers have often either defined categories around their specific equipment or defined categories based on a feature of their models.

In this document, equipment is grouped by 2 major physical forces of action: crushing and cutting. Cutting equipment uses knives, blades, or rotors to slice material into smaller pieces. Crushing equipment applies pressure against material to break it into smaller fragments. Some equipment uses both types of force, but one of the forces is the predominant force.

A variety of techniques can be employed to facilitate comminution. These include removal of moisture or embrittlement by freezing. Moisture removal is accomplished by "drying" at various temperatures ranging from freeze-drying to ambient room temperature to oven drying. Embrittlement is accomplished prior to comminution by freezing or by adding dry ice or liquid gases, such as liquid nitrogen. In some cases, dry ice can be added directly to the material during comminution.

Below are descriptions of the various types of equipment in common use. ***Note:*** A list of commonly used comminution equipment titled "Examples of Comminution Equipment" is available at http://www.aafco.org/Portals/0/SiteContent/Laboratory/Comminution_Equipment.pdf.

Crushing Equipment

Jaw crushers: Crushers operate by compressing material in a chamber between 2 strong breaking jaws—1 stationary and 1 moving—made of hardened steel, sometimes with a coating such as tungsten carbide. The jaws are located between thick panels that form a duct that tapers down toward the adjustable discharge gap. The tapering enables a progressive crushing action to occur from the fill hopper to the discharge gap. The gap between the crushing jaws adjusts to control and ensure reproducible maximum particle size. These devices can accommodate large initial particle sizes and therefore can be used in the reduction of coarse materials.

Disc mills: Disc mills pulverize material between a stationary disc and a rotating grinding disc. Discs can be smooth or have teeth (those with teeth are termed burr mills). Material is gravity fed to the center of the stationary disc and is progressively ground finer as it moves until it is discharged at the outer edge of the discs. Final particle size is set by adjustment of a gap setting. Disc mills are used for soft to medium-hard and fibrous materials in either a continuous or a batch mode.

Impact mills: Impact mills have a fast-moving part that collides with a stationary part, compressing and fracturing the material. Although generally used on a larger scale, laboratory models are available. Rotary hammers include the swing hammer, which is designed to break up relatively large pieces. In these mills, further reduction occurs with subsequent impact with the casing or screen. A typical impact mill is a hammer mill.

Ball mills: Ball mills are also referred to as rod, jar, or pebble mills. Ball mills pulverize by impact using hard balls inside an enclosed grinding jar or bowl. Material is placed in a bowl (or jar) with grinding balls and rotated. Throughput and milling efficiency can be affected by sizes and shapes of grinding jars, the rotation speeds, the cycle time, and the number, weight, and size of balls added to the grinding jar. The biggest limitation with ball mills is that cycle time is generally very long compared with other techniques. Ball mills can be used to comminute wet or dry materials.

Planetary mills: Planetary mills use a 2-way planetary action to rapidly comminute using high impact, resulting in a very narrow particle size range. Material to be comminuted is placed in a bowl (or jar) with grinding balls and placed on a rotating platform. In planetary action, balls rotate opposite to the direction of the bowl platform rotation and centrifugal forces alternately add or subtract. Grinding balls roll halfway around bowls and then are thrown across the bowls to impact opposite walls at high speed. Comminution is further intensified by interaction of balls. High-energy planetary action gives a narrow particle size range in shorter cycle times than conventional ball mills with gravity tumbling. This type of mill can be used for soft to hard or brittle materials, for mixing, and for emulsification of suspensions and pastes.

Vibrating cup mills or ring and puck mills: Vibrating cup or ring and puck mills use high-impact energy to reduce particle size. Inside a bowl, a disc or a disc and ring set are accelerated by centrifugal force or vibrated. These mills are frequently used for hard materials, such as agricultural soils.

Cryogenic mills: Cryogenic mills use a bath of liquid nitrogen and a solenoid to comminute. The solenoid magnetically shuttles a steel impactor back and forth between 2 stationary end plugs inside a vial. The material is placed inside the vial, and the vial is immersed in a cryogenic bath of liquid nitrogen. The liquid

nitrogen embrittles the material, making it easy to be pulverized by the impactor. A limitation of some cryogenic mills is the small capacity; however, larger capacity mills are available. Advantages are the ability to handle a wide variety of higher moisture, soft and fibrous materials and the ability to produce a very fine final particle size compared with other comminution techniques.

Mortar grinders/mortar mills: Mortar mills are an automated version of the traditional mortar and pestle. A graded pestle is connected to a variable-speed overhead motor, and the material is crushed between the grinding bowl and the grinding arm (or pestle). Mortar mills can be used for both wet and dry grinding. The longer the grinding time, the smaller the final particle size.

Combination grinder/cutting (cross-beater) mills: Cross-beater mills use a combination of cutting/shearing and crushing forces to reduce the particle size. Materials are fed through a hopper directly into the center of the grinding chamber, where they are caught by the cross-beater and crushed between the impaction plates of the cross-beater and the toothed grinding insert. Particle size is reduced until the particles are small enough to fall through a sieve.

Tissue grinders: Tissue grinders consist of a pestle that fits snugly inside a tube. The movement of the pestle inside the tube creates the grinding action. The components are made of glass, plastic, Teflon, or stainless steel. The pestle can be either hand or motor operated. A limitation of some tissue grinders is a small capacity.

Paddle/peristaltic/bag blenders: With paddle, peristaltic, or bag blenders, the material is placed in a sealed plastic pouch and inserted between 2 paddles. One paddle moves in a pummeling motion against the other paddle, pulverizing or liquefying the material in the pouch. They are commonly used to macerate materials for microbiological analysis. Materials containing bones or sharp or protruding objects that can puncture the plastic pouch should not be comminuted this way.

Hand tools: Hand tools such as hammers, mallets, and mortars and pestles can be used to break up large blocks or chunks into smaller pieces. Final particle size and uniformity is operator dependent. Hand tools can be used to comminute material to a particle size to be fed into the opening of other comminution equipment.

Cutting/Shearing Equipment

Cutting/shearing mills: Cutting mills use blades or rotors to shear or cut the material. For many of these mill types, flow-through devices are available that allow for continuous comminution of a large quantity of the material. The final particle size is controlled by the size of the screen openings. Cutting mills can be subcategorized by (1) rotating blades and stationary blades or (2) rotating blades and either a sieve screen or an abrasive grinding ring.

Mills with rotating blades and stationary blades: Material is fed between the stationary blades in the housing and the rotating blades on the rotor until it is fine enough to pass through the sieve into the collecting receptacle. Cutting mills are designed to handle dry, tough, medium-hard, soft, elastic, and fibrous materials.

Mills with rotating blades and screen/grinding ring: Material is cut between the rotating blades and a sieve screen or grinding ring. Frequently, these mills combine crushing and cutting/shearing forces from high-speed rotors to rapidly grind soft to medium-hard and fibrous materials. These types of mills are commonly known as centrifugal mills, cyclone mills, or rotor mills.

Choppers: Choppers use rotating knives or blades to cut or chop semi-moist, soft, or fibrous materials. Based on their cutting operation, choppers can be subcategorized as knife choppers or bowl choppers.

Knife choppers: Material is augured to a stationary die or fed to a stationary edge, against which knives or blades rotate to provide the comminution. The particle size is regulated by the diameter of the holes in the die (as in a meat chopper) or the rate of feeding the material across the edge (as in a laboratory forage chopper) and the speed of the rotating knives (in both types).

Bowl choppers: Blades rotate at high speed inside an enclosed bowl. The blades can be arranged in the same plane or at different planes or angles to facilitate comminution depending on the size of the bowl. Depending on the characteristics of the material, the final chopped product will vary in consistency and particle size.

Blenders: Blenders use blades rotating at high speeds inside a container to comminute materials. Blenders can be classified into 2 types, stationary and immersion.

Stationary blenders: The rotating blades, which are at the bottom of the container, are small relative to the total volume of the container. The container is designed to propel the material into a vortex so that it repeatedly encounters the blades.

Immersion blenders: The rotating blades are inside a shaft (generally referred to as the stator), which is attached to a motor. The blade assembly (commonly referred to as the probe) is inserted into a container and moved through the material, or the probe can be fixed to a stand and the container moved.

Hand tools: Manual comminution can be performed with hand tools such as scissors, clippers, pruning shears, knives, paper cutters, saws, and salad choppers. The size and uniformity of the final particles is operator dependent. Hand tools can be used to comminute material to a particle size to be fed into the opening of other comminution equipment.

Mixing Techniques and Equipment

Mixing techniques include the following motions, either manual or automated: stirring, shaking, rolling, vortexing, blending, figure 8 motion, and churning. Many laboratories use these techniques routinely before a selection process in an attempt to reduce DH; however, the action is more of an illusion than an effective technique. In fact, mixing may actually increase DH by promoting segregation of materials with different particle sizes, shapes, and densities and mixing has no impact on CH. For materials in which fines do not readily segregate, appropriate mixing may reduce DH. Mixing is not effective when a container is full or almost (e.g., two-thirds) full.

For many automated mixing techniques (e.g., cone blenders), the DH of the material can be quite low during the mixing operation; however, the DH can increase when the mixing action is stopped and increase even further upon removal of the material from the equipment. To minimize segregation upon removal, the material needs to be of uniform particle size, shape, and density. No comminution equipment will produce a completely uniform particle size and shape; however, some equipment will produce a better particle size distribution (cutting/shearing mills with screens) than others (blenders).

The appropriate approach to control DH is selection of multiple increments, which is effective for all materials. If mixing is used, it should be demonstrated that it reduces DH and should be evaluated with a performance test (see ISO, 2012). Mixing never negates the need for selection of multiple increments.

Selection Techniques and Equipment

A selection process is the physical selection of a smaller mass from a larger mass. Selection processes may occur at many points, beginning with the selection of the primary

sample and culminating with the selection of the test portion. To control FSE, a selection process may be preceded by a nonselection process. Selection processes can be divided into 2 categories:

- Selection of a smaller mass from a larger mass (mass reduction)
- Division of a mass into 2 or more equal portions (splitting)

Considerations for choosing selection equipment and tools are listed in the sidebar below. Note: Even though the division of a mass into 2 or more equal portions is technically mass reduction, the term *mass reduction* is reserved for the first category (selection of a representative smaller mass from a larger mass) and the term *splitting* is reserved for creating multiple equal portions.

Considerations for Choosing Selection Equipment and Tools

Properties of the material

- Material state (liquid, slurry, semi-solid, solid)
- Flowability of the material
- Particle size of the material

Capacity

- Mass of initial sample and final mass(es)
- Throughput

Dimension of the material

- 1-dimensional
- 2-dimensional
- 3-dimensional

Control of systematic errors

- Control increment delimitation error
- Control increment extraction error
- Control contamination introduction error
- Control analyte integrity error
- Control increment weighting error

Design considerations

- Can be cleaned easily
- Simple to operate
- Reliable
- Allows collection of increments of equivalent/proportional mass or volume

Mass Reduction Techniques and Equipment

The laboratory must be alert to the requirements of sufficient mass, increments, and sample correctness and potential for other systematic errors for each mass reduction. All mass reduction techniques are operator dependent, and proper training is critical.

Mass Reduction Techniques for Solids and Semisolids

Mass reduction techniques for nonflowable solids and semisolids are limited to 1- and 2-dimensional slab cakes. Mass reduction techniques for flowable solids include 1- and 2-dimensional slab cakes and splitting techniques (described beginning on page 41).

1-Dimensional slab cake—A slab is formed by creating an elongated pile as the entire sample is poured out of the container. The surface on which the material is poured typically is covered with an inert sheet (e.g., aluminum foil or paper). The material can be poured in a single pass or in a back-and-forth motion. Each increment is taken by removing a slice across the entire width and depth of the 1-dimensional slab cake at each increment location (Figure 11). Increments must be of proportional mass or volume (to control IWE) and taken at systematic random locations along the entire length of the slab cake. If the slab cake is of uniform thickness and width, all increments should be the same mass to control IWE. The technique is disruptive to the slab cake, especially for small slab cakes. As needed, the slab cake is re-formed perpendicular to the original slab cake to collect additional increments (Figure 11B). It is critical that the tool be a square scoop with parallel sides tall enough and long enough to select the entire width and height of the 1-dimensional slab cake. A 1-dimensional slab cake works well for smaller amounts of material.

2-Dimensional slab cake—A slab cake is formed by flattening the entire sample, using a straight edged tool, to a depth typically no more than 1 cm. The surface on which the material is poured typically is covered with an inert sheet (e.g., aluminum foil or paper) before forming the cake. The final shape (rectangular, circular, or irregular) of the slab cake is not critical; however, a uniform depth is critical. The slab cake should be formed with minimal manipulation of the material to avoid segregation of fines to the bottom. Each increment is taken by removing an entire vertical column of the material at the increment location (Figure 12). Technically, it is impossible to remove a perfectly vertical column from a slab cake because the tool will always select more material from the top than from the bottom (generating IDE and IEE); however, if the material has been comminuted to a uniform size and shape particle, the error may be mitigated. Increments of equal or proportionate mass (to control IWE) are taken at systematic random locations across the entire surface of the slab cake. There is no

ideal scoop shape for a 2-dimensional slab cake; however, with appropriate comminution and use of a tool that emulates a vertical column, this method can be effective.

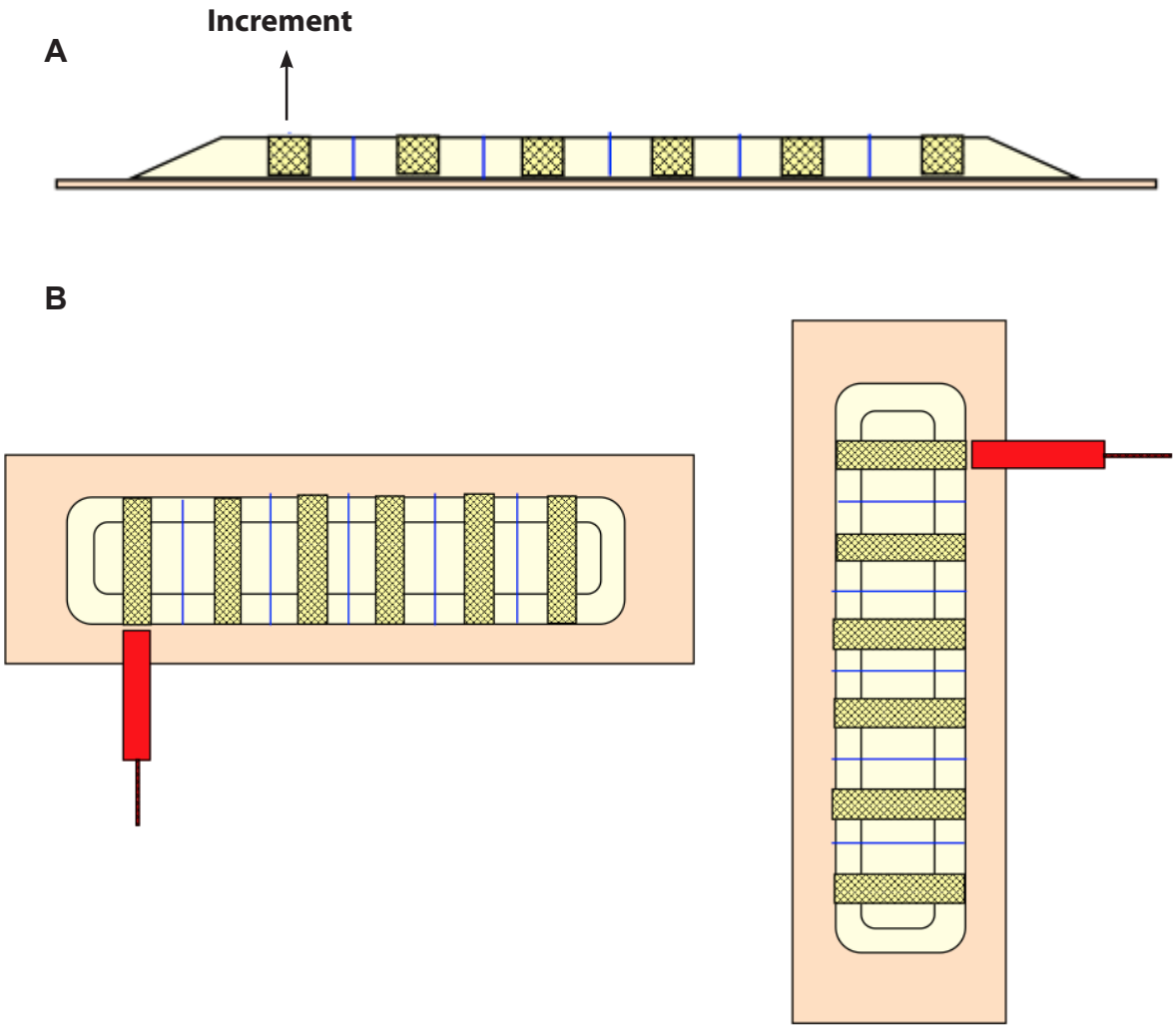
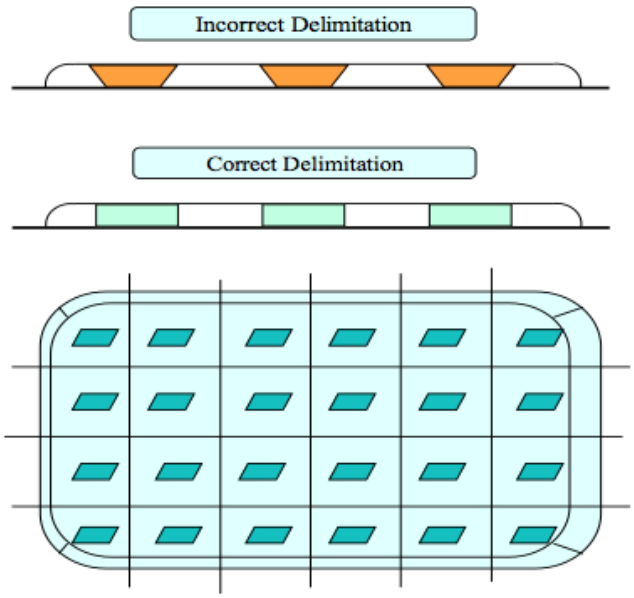


Figure 11. (A) Side view and (B) top view of 1-dimensional slab cake. Each shaded portion is one increment. In a 1-dimensional slab cake, correct delimitation is not difficult. As needed, reform the slab cake and repeat until the desired number of increments is obtained. © Francis Pitard Sampling Consultants, Broomfield, Colorado. Reprinted with permission.

A



B

C

Figure 12. (A) Two-dimensional slab cake illustration showing incorrect and correct delimitation. In a 2-dimensional slab cake, correct delimitation is difficult. © Francis Pitard Sampling Consultants. Reprinted with permission. (B) Two-dimensional slab cake of soil analytical sample. Photo © Diane Anderson (Agriculture & Priority Pollutants Laboratories Inc., Clovis, California), 2017. (C) Two-dimensional slab cake of hemp analytical sample. Photo: New York State Department of Agriculture and Markets, Albany, New York.

CAUTION

Caution must be used when implementing a 2-dimensional selection process, as control of IDE is extremely difficult, if not impossible, to achieve. Before implementing a 2-dimensional technique, it must be optimized for the specific material. No generic 2-dimensional technique can be applied across different materials; each material must have a specific protocol.

Mass Reduction Techniques for Liquids and Slurries

The challenge in mass reduction of liquids and slurries is the presence of solids in the liquid. The easiest liquid to mass reduce is a single-phase liquid without solids or a liquid in which solids are present but not part of the decision unit (i.e., solids are extraneous material).

Single-phase liquid without solids—The mass reduction technique for single-phase liquids without solids is straightforward: simply pour or remove an increment with any tool. Only 1 increment needs to be selected.

Multiphasic liquids without solids—In some cases, the individual phases of a multiphasic liquid can be mixed to temporarily achieve a single phase. For these materials (e.g., milk), adequately mix, then mass reduce as a single-phase liquid. It is criticial that the phases remain completely mixed until selection is completed. It is recommended that mixing protocols be developed and validated for each multiphasic liquid. When the phases cannot be mixed or it is not desirable to mix the phases, 2 common techniques can be utilized. One technique is complete separation of the phases and mass reduction of each phase individually as a single-phase liquid. The mass reduced portions from each phase could be proportionately recombined, or each portion could be tested separately. The second technique is the selection of a cylindrical core throughout the entire depth of all phases (from a container having vertical walls; see Figure 8). This is

No brainers: Some common laboratory practices should rarely, if ever, be used: shaking and stirring solids (especially in a full container) when followed by a selection process that uses only a few increments; pipetting a liquid or slurry with suspended material (even if mixing immediately prior to or during the selection process); shaking a liquid containing solids to mix immediately before pouring off a test portion.

most easily accomplished with an open tube; however, care must be taken that the same amount of material is collected at each point in the vertical dimension. Before the tube is removed, the top end of the tube is capped and the vacuum is created to allow containment of the increment during removal. Only 1 increment needs to be selected because DH, by definition, exists only in the vertical dimension.

Liquids with solids—Liquids with suspended solids represent one of the most difficult of all mass reduction scenarios when the analyte of interest is contained in or adsorbed onto the particles. The challenge with mass reduction of a stationary liquid with suspended solids is that the velocity of the liquid changes as it enters the sampling tool. The change in velocity results in nonequiprobable selection of the elements. Mixing before or during selection does not alleviate this bias.

There are several acceptable techniques for mass reduction of liquids with suspended solids. One technique is to separate the solids from the liquids (using centrifugation or filtration), test separately, and combine the results mathematically. The second technique is the use of a churn to keep the the suspended solids in motion during the selection process (see Figure 9). The vertical motion allows for equiprobable selection of the larger and smaller particles.

A third technique is the use of an open tube of sufficient diameter inserted into the material so slowly that is does not perturb the liquid or the suspended solids. Before the tube is removed, the top end of the tube is capped and the vacuum is created to allow containment of the increment during removal. The increment, when removed, must be the same height as the liquid in the straight-walled container. If not the same height, material has escaped and the bottom portion is underrepresented (resulting in IEE). This technique is applicable only for liquids in which all the solids can be suspended, as particles that have settled will not be adequately represented. Only 1 increment needs to be selected.

Slurries—Relatively thin slurries can be mass reduced with an open tube if all the solid materials can be suspended. The diameter of the tube is dependent on the particle size of the solids and the flowability of the material into the tube. Relatively thick slurries have less issue with preferential particle size selection. Thick slurries generally can be mixed to reduce DH. If the slurry does not readily flow, it could be sampled as a semi-solid. When mass reducing all slurries, care needs to be taken that separation of liquids from solids does not occur during the selection process.

Splitting Techniques and Equipment

There are 3 basic types of splitting: rotary splitting, fractional shoveling, and stationary splitting. The distinguishing feature among splitting techniques is the number of increments in each split. All other things being equal, the splitting technique that delivers the most increments per split will have the lowest GSE. With all splitting techniques, the entire mass must be split. Minimum mass, sample correctness, and other systematic errors need to be controlled. Splitting techniques are not amenable to all materials.

Generally, a laboratory sample should be comminuted in its entirety before splitting to control FSE of the splits. When an unprocessed or "reserve" sample is required (e.g., microscopy or filth, visual examination, weed seed screen, regulatory purposes, logistics), it is critical that the mass of the primary sample be increased to control the FSE of the splits.

A study by Allen and Kahn (1970) is often cited as one of the first to compare the error among splitting techniques. The estimated error is presented in Table 1 to illustrate the relationship between number of increments and error. For food products, larger errors would be expected; however, the relationship between error and number of increments would be maintained. It is obvious from Table 1 that coning and quartering should never be used as a selection technique.

Table 1. Results from split of sand mixture containing mass fractions of 60% coarse sand and 40% fine sand ($P = 0.06$)[1]

Technique	No. of increments	RSD[2]	Estimated maximum error, %
Coning and quartering	2	6.81	22.7
Stationary splitting	10–12	1.01	3.4
Rotary splitting	>100	0.125	0.42
Random variation		0.076	0.25

[1]Adapted from data published by Allen and Kahn (1970).
[2]RSD = relative standard deviation.

In another study on mixtures of wheat, rape seed, and glass (Peterson et al., 2004), 17 techniques were characterized and ranked for systematic error, random error (repeatability), mass recovery, user dependency, operation time, and ease of cleaning. The article concludes with recommendations for use of various rotary splitters and 2 well-designed stationary splitters, each with more than 30 chutes (>15 increments).

To summarize, more increments lead to less error in splitting by reducing the GSE. In general, any technique utilizing less than 15 to 20 increments per split is strongly discouraged unless the DH is established to be low. Techniques utilizing more than 30 increments are recommended. Techniques utilizing several hundred increments are strongly encouraged.

Rotary Splitter

A rotary splitter consists of a feeding hopper (to maintain continuous flow), chute, and motorized rotating receptacles (Figure 13). With each rotation, an increment is delivered to each receptacle, allowing for hundreds of increments per split and resulting in lower GSE per split than other splitting techniques. The technique is not as operator dependent as other splitting techniques, but care still needs to be exercised. This technique applies only to flowable solids.

Design criteria, in addition to general criteria, specific for rotary splitters:

- Cutting edge should be radial from center (pie shaped).
- Splitter should be capable of producing increments of equivalent mass (Figure 14).
- Splitter should be capable of maintaining constant speed during operation.
- Drop from the feeding chute to receptacles should be minimal to prevent dust formation.

Proper use of a rotary splitter:

- Use a hopper and chute to feed the material into the rotary splitter. Hand feeding results in uneven feeding rates and therefore nonuniform splitting.
- Adjust the feeding rate so that the material flows slow enough that each receptacle contains hundreds of splits.
- Feed the entire mass of material through the splitting device. If the receptacles fill during the process, they need to be replaced with empty receptacles so that the process can continue until all the material is split. The receptacle contents can then be combined to create the split.

Figure 13. Examples of rotary splitters, ranging from large-capacity floor model to medium-capacity bench top to micro-capacity bench top.
Rotary splitter image source (left): Sepor, Wilmington, California; medium-capacity model image (center) printed with permission from Heidi Hickes (http://analyticallab.mt.gov); micro-capacity model source (right): Quantachrome, Boynton Beach, Florida.

- After splitting, weigh (or visually inspect) each receptacle to ensure contents are approximately the same mass or volume of material. If the masses or volumes are not equal, determine the cause, correct, and then recombine and resplit. Unequal volumes are typically the result of clogging, which happens when material bridges (Figure 14).
- Monitor splitter to ensure correct operation.

Because the final mass of each split is a function of the mass of the starting material and the number of receptacles, the split mass obtained may not be the desired mass. Multiple splits can be combined to form a larger mass split. Alternatively, one or more splits can be combined and resplit to obtain a smaller mass split.

Fractional Shoveling

With the fractional shoveling technique, increments are selected from the material and placed into individual receptacles to form the splits. With this technique, any number of splits (from 2 to n) can be formed. The increment mass needs to be adjusted so that each split has an adequate number of increments (≥30). Increments need to be sequential across all receptacles and repeated in sequence until all the

Equal splits from a rotary splitter

Unequal splits from a rotary splitter

Figure 14. Equal and unequal splits. Unequal splits are never acceptable.

material is used (Figure 15). Increment size must be consistent throughout the entire splitting process (controlling IWE), and sample correctness must be maintained. Controlling IWE, maintaining sample correctness, and avoiding MRE are the limitations of this technique, and great care must be exercised, as it is very operator dependent. With this technique, it is tedious to collect hundreds of increments per split. Generally 30 to 50 increments are sufficient to control GSE, depending on sample quality criteria. This technique works for semisolids and flowable and nonflowable solids; however, for flowable solids the rotary splitter is preferred.

Fractional shoveling has the following advantages:

- It can be implemented in the laboratory or field.
- It does not involve extra equipment (e.g., splitters).
- It has minimal clean-up and decontamination requirements.
- Any number of splits can be generated.

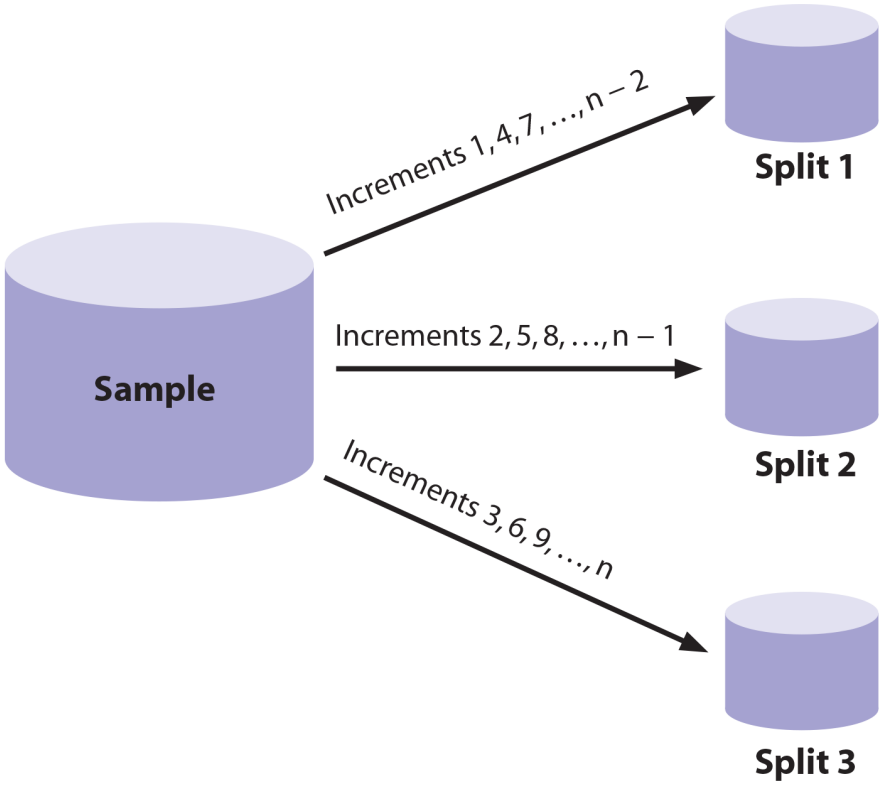


Figure 15. Fractional shoveling.

The size or mass of each increment is determined by the number of splits to be made, the initial mass, and the number of increments needed for each split. Toward the end of the splitting process there can be a small amount of fines remaining to be split. The increment size must be reduced so that the fines are equally apportioned among the splits.

Stationary Splitters

Stationary riffle splitters consist of a hopper with a series of equal alternating, inclined angle chutes (or riffles) that empty into 2 receptacles (Figure 16). They can be gated or ungated. The gated riffle splitter has a closed hopper that is manually opened to allow the material to flow into the chutes. With the ungated riffle splitter, the material flows immediately into the chutes when delivered to the hopper. Although this technique is prevalent, most stationary splitters found in laboratories are inadequate due to the insufficient number of increments (<30 chutes = <15 increment/split). Stationary splitters that have an adequate number of chutes (≥30) may either be too large for a laboratory setting or have chutes that are too narrow for

Figure 16. Stationary splitter. Source: James Cox and Sons, Colfax, California.

many applications. Stationary splitters are prone to operator error and misuse and must be used with care. In rare situations where DH is low and the tolerable error is large, a splitter with fewer than 30 chutes may provide an adequate split; however, stationary splitters are inappropriate for most food applications. This technique applies only to flowable solids.

Safety Considerations

Hazards associated with laboratory nonselection and selection processes include exposure to toxicants or irritants via dermal contact, inhalation or ingestion, and hazards associated with use of electrical equipment and sharp moving parts. Employees must demonstrate awareness of their work surroundings and follow all laboratory safety policies.

Safety considerations for both nonselection and selection processes are as follows:

- Minimize exposure to toxicants or irritants (e.g., antibiotic drugs, mycotoxins, heavy metals, microbiological contaminants, pesticides) via dermal contact, inhalation, or ingestion. Safety equipment, such as biological safety cabinets or fume hoods, and personal protective equipment (e.g., gloves, respirators, laboratory coats) may be needed as determined by risk assessment.

- Work in an appropriately ventilated area (e.g., down draft tables, fume hoods, biosafety cabinets). For dry materials, use a vacuum cleaner with HEPA filter to clean the hood area, mills, and work area.
- Segregate work areas to minimize worker exposure and potential laboratory contamination.
- Wear appropriate personal protective equipment (e.g., gloves, laboratory coat, eye and ear protection, respirators and dust masks).
- Ensure adequate training on proper use of equipment.
- Keep equipment and work surfaces clean and uncluttered.
- Check that all electrical equipment is properly grounded and maintained.
- Do not wear loose apparel that may get caught in moving equipment.
- Optimize equipment to minimize dust generation.

Additional safety considerations for nonselection (comminution) processes are:

- Keep hands and fingers away from moving equipment parts during operation.
- Open a mill only after it has completely stopped.
- Check to see that safety interlocks on all equipment are operating properly.
- Ensure that guards and shields are on equipment and in proper working order.

Minimizing test portion mass is often done in an attempt to reduce exposure to solvents, reagents, etc. Attempts to miniaturize methods often compromise the representativeness of the test portion. Any reduction in selection mass, and especially reduction in test portion mass, should be carefully validated to ensure the reduced mass is sufficient to control the variance of the FSE.

QUALITY ASSURANCE AND QUALITY CONTROL

Quality assurance and quality control (**QC**) are critical components of defensible data; however, they are traditionally overlooked for laboratory sampling. Laboratory sampling generally introduces substantially greater error than analytical error, and therefore must not be ignored in the overall laboratory quality system. In some cases the QA and QC will be similar to what is incorporated for analytical methods, and in other cases it will be fundamentally different. In this document, QA and QC for protocol development and validation are addressed under QA, whereas QA and QC for daily production activities are addressed under QC.

Quality Assurance

When protocols for laboratory sampling are being developed, it is critical to ensure that the scope of application is well defined and that tolerable error has been established. This is not unlike method development for analytical methods. The validation must demonstrate that the protocol meets the desired scope of application and tolerable error previously established.

Validation of a Laboratory Sampling Protocol

Validation of sampling protocols must confirm sample correctness and validate sufficient mass and sufficient number of increments to meet the error criteria. There are two approaches to validation. The first approach is to determine error utilizing TOS. The second approach is one in which error is determined experimentally from test results. The number of increments is usually only validated experimentally, while sufficient mass can be validated utilizing TOS or experimentally.

The TOS approach for validating mass is described in Appendix A. For this approach, detailed knowledge of the material properties is needed so that the FSE equations can be utilized. The TOS approach can be very effective when used within a manufacturing facility to carefully plan sampling for specific materials (for example, a Type A feed drug formulation or a food nutritional supplement). TOS can also be used to estimate mass for an experimental validation.

The remainder of this chapter will be devoted to a discussion of experimental approaches. Experimental validations for laboratory sampling can be designed in several ways. No single experimental validation method is recommended because every analyte, type of material and laboratory situation is different. Some options for experimental valida-

tion include whole sample extraction, use of materials containing a known amount of the analyte of interest, use of materials containing a known amount of surrogate for the analyte of interest, and use of tracers.

Whole sample extraction—An option for validation of laboratory sampling protocols is to compare the result obtained from testing the selected portion to the result obtained when testing the entire unselected (remaining) portion. If the unselected portion is too large for a single extraction process, multiple extractions may be performed and the multiple extracts proportionately combined to obtain a single test result to represent all the unselected material. If two results compare, the selection process is valid.

Materials with a known amount of analyte of interest or surrogate—A second option is to perform the protocol to be validated on known materials containing the analyte of interest. These materials can be certified reference, fortified, or incurred. When using certified reference or fortified material, it is critical that the materials mimic routine samples. Surrogates that mimic the behavior of the analyte of interest (e.g., radiolabeled compounds, isotopes) are an alternative to the analyte of interest to facilitate measurement.

Tracers—A third option is the use of tracers that are unrelated to the analyte of interest (e.g., sand; Microtracers™, a trademark of Micro-Tracers Inc., San Francisco, CA) but that can provide useful information. The limitation for this option is that the analyte of interest may or may not behave as the tracer behaves. Caution needs to be exercised when extrapolating results to routine samples.

Validation materials must be carefully chosen to match materials in the scope of the application as closely as possible. Certified reference materials for sampling are not readily commercially available; however, fortified materials can be created in the laboratory. Surrogates, tracers, or fortified materials are unique in that they can be incorporated at a single point or at multiple points. Replication is used to establish random error from the point of incorporation forward (see Figure 17). Incorporating replication at multiple selection processes allows isolation of error for a specific selection process. Identification and quantification of all systematic errors is resource intensive or impossible; therefore, when developing protocols, the focus needs to be toward the elimination of systematic errors at their source.

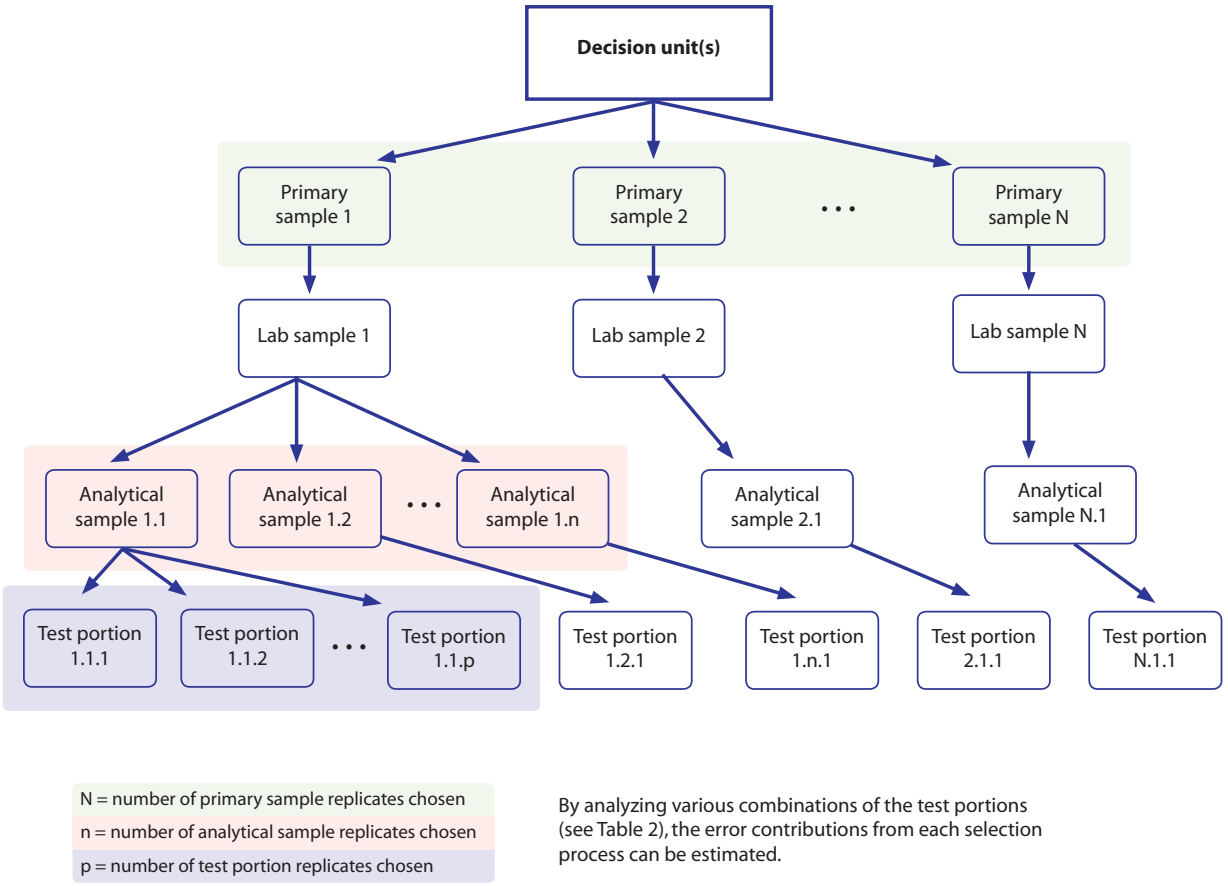


Figure 17. Estimating error contributions from selection processes.

Table 2. Equations to estimate error contribution for specific selection processes

Error estimate	Relative standard deviation (RSD) calculation[1]	Error calculation[2]
Estimate of overall GEE (TSE + TAE)	RSD of 1.1.1, 2.1.1, …, N.1.1 = RSD_{ps}	$GEE = RSD_{ps}$
Estimate of overall TSE	RSD of 1.1.1, 2.1.1, …, N.1.1 = RSD_{ps}	$TSE = \sqrt{RSD_{ps}^2 - TAE^2}$
Error contribution from selection of the primary sample (ps)	RSD of 1.1.1, 2.1.1, …, N.1.1 = RSD_{ps}	$E_N = \sqrt{RSD_{ps}^2 - RSD_{as}^2}$
Error contribution from selection of the analytical sample (as)	RSD of 1.1.1, 1.2.1, …, 1.n.1 = RSD_{as}	$E_n = \sqrt{RSD_{as}^2 - RSD_{tp}^2}$
Error contribution from selection of the test portion (tp)	RSD of 1.1.1, 1.1.2, …, 1.1.p = RSD_{tp}	$E_p = \sqrt{RSD_{tp}^2 - TAE^2}$

[1]RSD is expressed as a decimal (part of 1) for all error calculations.
[2]To express calculated error as %RSD, multiply calculated GEE, TSE, E_N, E_n and E_p by 100.

Note: TAE can be determined by replicating the test on a single test portion. This is possible when the test portion is solubilized and the entire test solution is not consumed in a single test.

Example of estimating error from selection of an analytical sample using Figure 17 and Table 2: A laboratory wishes to estimate the random error contribution associated with selection of the analytical sample for arsenic in rice.

- Observing the principles of TOS, multiple analytical samples are prepared (1.1, 1.2, ..., 1.n)
- Observing the principles of TOS, replicate test portions are selected from a single analytical sample (1.1.1, 1.1.2, ..., 1.1.p) and analyzed for arsenic. The relative standard deviation (**RSD**) of the results from these test portions is 7%
- Observing the principles of TOS, single test portions are selected from each of the analytical samples (1.1.1, 1.2.1, ..., 1.n.1) and analyzed for arsenic. The RSD of the results from these test portions is 14%

Using Equation 3 (page 52), the random error contribution associated with selection of the analytical sample can be calculated as:

$$\mathrm{RSD}_n, \% = \sqrt{0.14^2 - 0.07^2} \times 100 = 12\%$$

Verification of Laboratory Sampling Protocol

Once validated, a sampling protocol can be periodically verified through a series of performance tests. Performance tests can be devised to verify a nonselection or selection process, including carryover, final particle size distribution, and contamination and cleaning procedures. Proficiency testing, although readily available for analytical performance, is not readily available for sampling processes at the time of this writing.

Performance tests can be used to evaluate new equipment or changes in protocols by comparing results with those obtained from previous equipment or procedures. Performance tests can also be used to determine whether a new analyst is adequately trained for laboratory sampling.

The frequency of performance tests needs to be adequate to monitor changes in personnel, equipment, or protocols. With the accumulation of results over time, reasonable performance expectations can be determined for personnel, equipment, and protocols. The equipment normally used for production (e.g., not the newest screen or best mill) must be used for the performance test. Environmental conditions must also be considered and match the working environment. The objective is to ensure that all equipment is performing and analyst techniques are performed within established performance criteria.

Examples of performance tests are provided in ISO (2012). Tests should be modified to conform closely to the materials being sampled and analyzed. A well-chosen set of validation materials that represent a worst-case scenario will increase confidence that routine samples can be processed with error no greater than that determined by the performance test.

Quality Control

Quality control events are a series of checks to monitor and measure error during routine nonselection and selection processes. There are QC events for random errors and for some systematic errors. For systematic errors for which there are no QC events (increment delimitation error, increment extraction error, and increment weighting error), great care needs to be taken to ensure that they have not been introduced into the laboratory sampling process.

Random Error

Quality control replicates are used to monitor random error. Using more replicates improves the error estimation. The error can be monitored at any selection process to provide an estimate of the random error from that point forward. By performing replication at multiple selection processes, it is possible to isolate error from an individual process through subtraction (see Figure 17). The error contribution from any selection process equals the random error starting from that process forward minus the random error starting from the subsequent process forward. To perform the subtraction, the error needs to be expressed as a decimal portion of 1 (e.g., 15% RSD = 0.15):

$$\mathrm{RSD}_n, \% = \sqrt{E_n^2 - E_{n+1}^2} \times 100 \qquad [3]$$

where E_n = error (as a decimal) from process n.

The procedure for replication at any selection process is to collect each replicate using the same tool and selecting the same number of increments but selecting increments at different random locations.

Systematic Error

Unlike systematic error for analytical procedures, systematic error for laboratory sampling is generally impossible to measure in routine production without elaborate and expensive techniques. Some ideas are presented here; however, the reader of this document should develop fit-for-purpose techniques.

Extraneous material error: Extraneous material error occurs when the extraneous material is not removed in total or when some of the decision unit is removed along with extraneous material. A qualitative measure may be to compare, either visually or by weighing, the separated extraneous material as performed by multiple analysts. It would be impossible to quantify this in terms of an error; however, if the removal process is incorrect or inconsistent, error will result.

Contamination introduction error: Contamination introduction error from equipment and the environment can be evaluated by processing material that contains no analyte and interpreted as QC blanks. Swabs and swipes of equipment can be processed as QC blanks.

Mass recovery error: Mass recovery error can be determined by visual inspection of equipment for incomplete removal of material or weighing of material before and after processing. While actual mass lost may be determined, error introduced by the loss of this material cannot be quantified.

Analyte integrity error: Analyte integrity error cannot be quantified; however, some techniques may detect this type of error. The easiest technique is to measure splits at multiple points in time and observe for trends. Other techniques such as use of radiolabeled compounds, use of fortified or known incurred materials, or comparison with a previously validated protocol may also provide opportunities to monitor analyte integrity error.

The pesticide community has published several techniques to estimate error for validation, verification, or routine work, including use of fortified materials and radiolabeled compounds.[1]

[1]Maestroni et al. (2000); Fussell et al. (2002, 2007a, 2007b).

LABORATORY SAMPLING PROCESSES

This section describes the integration of the nonselection and selection processes from the laboratory sample through selection of the test portion. There are hundreds of possible scenarios for generating a test portion from a laboratory sample, and different pathways can be equivalent. These scenarios range from a single process in which the laboratory sample becomes the test portion(s) (and is used in its entirety) to scenarios in which there are multiple nonselection and selection processes (see Figure 18). The number of processes is a function of the SQC and the equipment that a laboratory has available. For instance, a laboratory may not have the equipment to finely comminute a large amount of material efficiently and may choose to perform a coarse comminution, followed by a selection process, followed by a second comminution yielding the desired particle size. The final particle size is the same, but the number of processes differs. A nonselection process may consist of multiple steps—for example, removal of extraneous material, conditioning, and comminution. In some cases, multiple selection processes may occur without an intervening nonselection process—for example, generating a split with a rotary splitter followed by selection of a test portion from one of the splits. The greater the number of nonselection and selection processes, the greater the opportunity for introduction of error, so the fewest processes is preferred. An efficient approach would be to comminute the entire laboratory sample and select the test portion from the resulting material (1 nonselection process and 1 selection process).

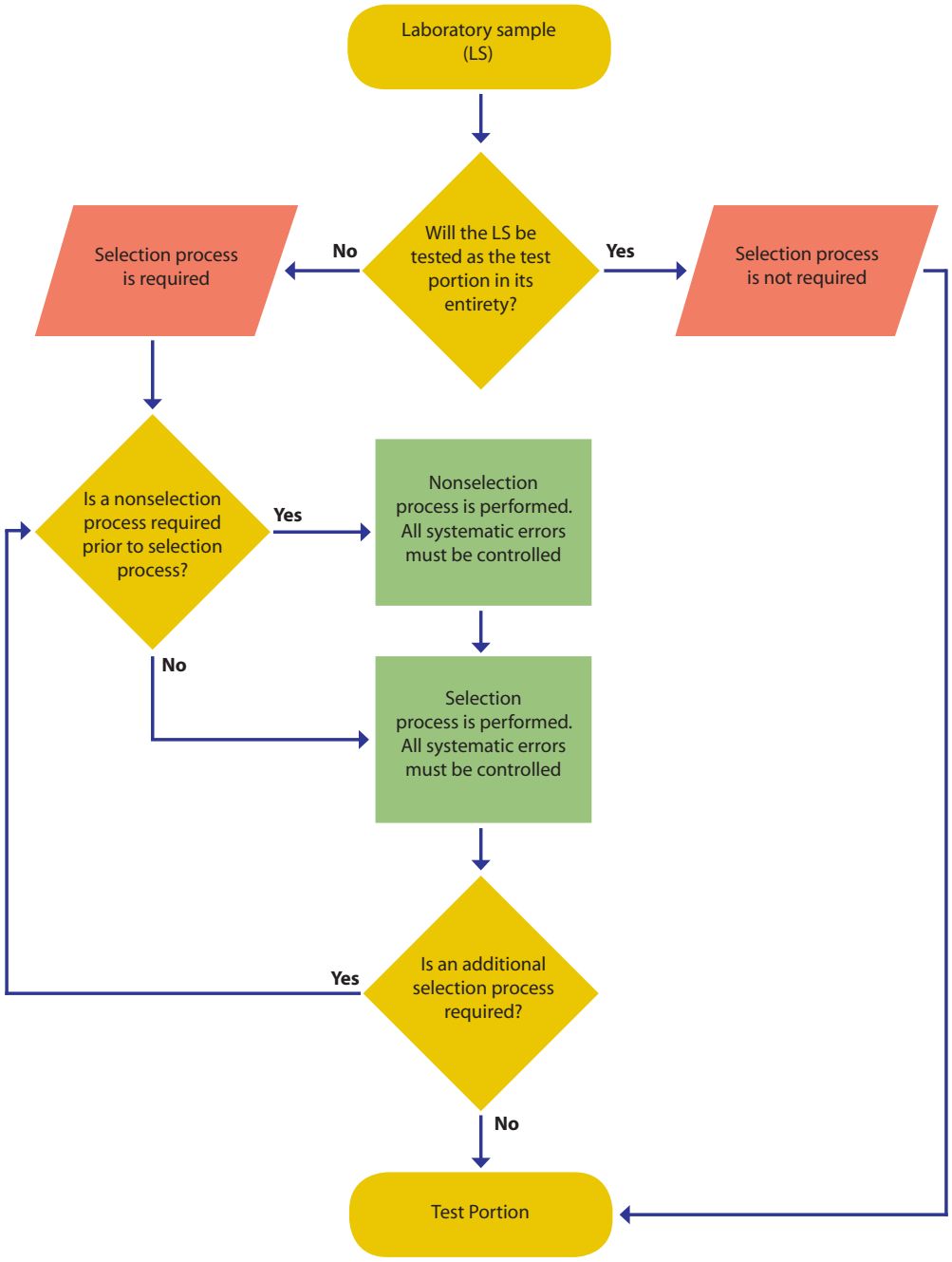


Figure 18. Flowchart illustrating sampling workflow in the laboratory. Multiple non-selection and selection processes are performed as necessary to achieve sample quality control.

Special Considerations for Selecting the Test Portion

Selecting the test portion is the final selection process. This process is especially critical due to the small mass selected, and careful consideration is needed to ensure that the test portion is representative. Commonly, laboratories are less conscious of the possible introduction of errors during test portion selection. The following steps are recommended:

- Select an appropriate number of proportionate/equal increments at random. Never take only 1 increment unless the material is a single-phase liquid.
- Do not attempt to obtain an exact weight, such as 1.00 g. Instead, target a range, such as 1.0 ± 0.2 g to minimize nonequiprobable selection of particles.
- Avoid IWE when selecting increments (see Figure 6). IWE is commonly introduced when selecting unequal increments to obtain an exact weight (e.g., starting with a large increment and ending with tapping a few particles off a spatula).
- Ensure that the correct tool is used to avoid bias toward one particle size (see Figures 5 and 10).
- Do not scale down test portion mass without validating impact on the variance of FSE.

DATA ASSESSMENT AND INFERENCE

Data Assessment

Data assessment is the process of evaluating the data quality to determine whether it meets the requirements of the SQC. Data quality assessment includes review of documentation, evaluation of quality control data, estimation of the GEE, and evaluation of analytical data with proximity to the specification limit or detection limit.

Documentation should

- show that correct protocol(s) were followed and note any exceptions or modifications,
- demonstrate that evidentiary integrity was maintained, and
- provide evidence that systematic errors (EME, MRE, IDE, IEE, CIE, AIE, and IWE) were controlled to a sufficient extent that their contributions are negligible.

Quality Control Evaluation and GEE

- Quality control blanks should demonstrate the absence of contamination (CIE) below a critical level.

- The GEE should be below a 35% RSD and meet the requirements of the SQC. When the RSD exceeds 35%, the range of results that falls within 3 standard deviations not only includes zero, but it is over 100% error (3 × 35% = 105%), and the analytical result becomes practically impossible to distinguish from zero. Furthermore, at a 35% RSD, we enter the domain where the data are not normally distributed, resulting in underestimation of the true concentration, and nondefensible data.

- With consideration to GEE and the specification limit, determine the confidence of making a defensible decision. In other words, establish that the result ± the GEE does not include the specification limit. For a more thorough discussion, see *GOODSamples* (AAFCO, 2015).

Each organization must establish protocols for evaluating error and making defensible decisions, including actions to take if error is excessive. When the error is excessive, the test result cannot be used for defensible decision making. Simply retesting until a desirable (e.g., passing, non-violative) result is obtained is not a scientifically valid action. Prior to sampling, thought should always be given to the sources of error and

techniques for mitigating the error (e.g., more mass, more increments, choice and use of tools and equipment). Due to the nature of the propagation of error, errors that are relatively small compared with other errors have little effect on the GEE; therefore, mitigating the largest errors will have the most dramatic effect on lowering GEE.

Inference

Inference is the process of estimating or inferring a concentration or characteristic of a decision unit based on test results from sample(s) collected from a decision unit. There are many inferences throughout the measurement system. Every time a selection process is performed, there is an inference from the smaller mass to the larger mass from which it originated. The laboratory inference is from the test result to the laboratory sample; however, the ultimate inference is from the test result(s) to the decision unit and possibly from sampled decision units to unsampled decision units (see Figure 19). Inference is discussed thoroughly in *GOODSamples* (AAFCO, 2015) and in Ramsey (2015). In current practice, laboratories typically take responsibility for making inference to the laboratory sample; however, because the true goal is to make inference to the decision unit, the laboratory must be involved in the SQC process. This may necessitate a paradigm shift throughout the entire organization.

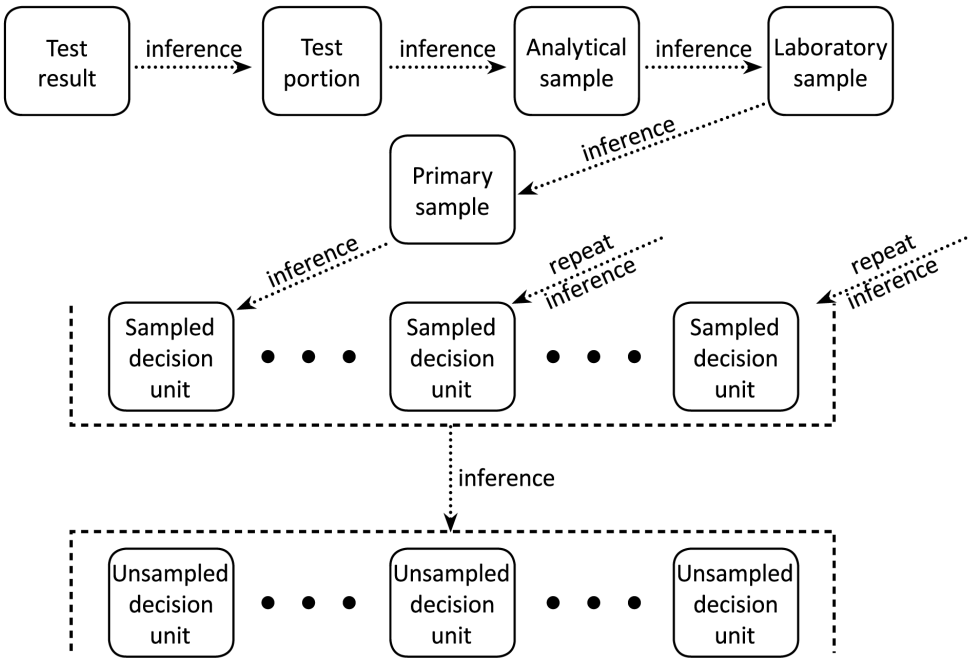


Figure 19. Examples of probabilistic inference to a decision unit and to unsampled decision units. Adapted from Ramsey (2015) with permission from J. AOAC Int.

TRAINING

Laboratory sampling is a skill that must be learned as any other laboratory skill. An informal process of passing down knowledge is totally insufficient. Due to the large sources of error in laboratory sampling, it is imperative that the laboratory training program include training and training verification to ensure competency in laboratory sampling. Areas of training may include, but are not limited to, the following:

- Appropriate personal protective equipment and appropriate safety practices
- Sample quality criteria
- Theory of sampling
- Material properties (DH, CH)
- Causes and control of systematic error (EME, MRE, IDE, IEE, CIE, AIE, and IWE)
- Causes and control of random error (FSE and GSE)
- Choice, use, and maintenance of comminution tools and equipment
- Choice, use, and maintenance of splitting tools and equipment
- Choice, use, and maintenance of mass reduction tools and equipment
- Maintaining analyte integrity (control of AIE)
- Maintaining evidentiary integrity
- Quality assurance and quality control
- Data assessment

APPENDIX A

Introduction

Equations based on theory of sampling (TOS) have been developed to relate the variance of fundamental sampling error (FSE) to sample mass. The derivations of these equations have been thoroughly presented and discussed in papers and the references provided, and because the equations are fairly complex, attempts at using them can only be made after thorough study to understand the variables and assumptions. However, once understood, the equations can be very useful in estimating the mass necessary to control the FSE to meet sample quality control (SQC).

Fundamental sampling error is controlled through selection of sufficient mass. At a minimum, it is necessary that sufficient mass be collected to represent all particles sizes. In addition, sufficient mass must be collected to represent the analyte of interest. To control FSE, the mass requirements (a continuum from least to greatest) can generally be described as follows:

(1) The least mass is required when the analyte of interest is perfectly correlated with a particle size. Representing all the particles of the material would therefore represent the analyte of interest. With food materials, this is not a common occurrence.

(2) Greater mass is required when the analyte of interest is not perfectly correlated with particle size. There may be some correlation between particle size and the analyte of interest; however, not all particles may have the analyte of interest or some of the analyte of interest may be liberated. This is a common case when sampling food materials.

(3) The greatest mass is required when the analyte of interest is liberated from the host material and exists as relatively few particles of highly concentrated analyte. This is a common case when sampling food materials.

It is interesting to note that determining the variance of the FSE is easiest to calculate in cases (1) and (3) above. The difficulty with case (2) above is calculation of the liberation factor (l), which is problematic.

Cautions when applying Appendix A:

- ▷ The theory of sampling requires serious study effort to understand and implement. A basic understanding of TOS can be gained through training courses or study of the references provided.
- ▷ The results obtained for variance of FSE using equations assume that all other errors are controlled.
- ▷ All approaches should be evaluated experimentally with actual data to verify assumptions.

Equations

The equation for variance of FSE to represent the analyte of interest is

$$s^2_{FSE} = \left(\frac{1}{m_S} - \frac{1}{m_L}\right)\frac{cflgd^3}{m_S} \qquad [A1]$$

where

c is the mineralogical factor (expressed in g/cm^3);

f is the particle shape factor (dimensionless) describing the deviation from the ideal shape of a cube (for a cube, $f = 1$; for spherical particles, $f \approx 0.5$; for flattened particles, $f < 0.5$; for elongated particles, $f > 1$);

g is the granulometric factor (dimensionless) describing the range of particle sizes in the material to be sampled: for a wide size distribution, $g \approx 0.25$; for perfectly uniform sized particles, $g = 1$; for materials calibrated (sized) between two consecutive screen sizes, $g \approx 0.55$; for naturally calibrated materials (e.g., beans, wheat), $g \approx 0.75$;

l is the liberation factor (dimensionless), $0 \le l \le 1$, describing the degree of liberation of the analyte of interest from the host material (for fully liberated analytes, $l = 1$);

d is the largest particle diameter (expressed in cm), defined as the square mesh screen that retains 5% of mass of the material;

m_S is the mass (expressed in g) of the selected portion (sample mass);

m_L is the total mass (expressed in g) from which m_S is selected; and

s is the standard deviation (relative).

Equation A1 can be simplified when the analyte of interest is liberated analyte ($l = 1$) and the mass of the selected portion is less than 10% of the total mass:

$$s^2_{FSE} = \frac{cfgd^3}{m_S} \qquad [A2]$$

For a given state of comminution, d^3 does not change and the grouping of all the constants $cfgd^3$ is referred to as the constant factor of compositional heterogeneity (IH_L). The IH_L makes it easier to relate mass to error as the other factors can be described with this constant. When the mass of the selected portion is less than 10% of the total mass, IH_L is given by

$$IH_L = cflgd^3 \qquad [A3]$$

When the mass of the liberated material is less than 10% of the total mass, the mineralogical factor, c, for the liberated material of interest can be estimated by

$$c \approx \frac{\lambda_m}{a_L} \qquad [A4]$$

where

a_L is the proportion of the liberated material to the entire mass, and
λ is the density of the liberated material (expressed in g/cm^3).

Therefore, substituting for c,

$$IH_L = \frac{\lambda_m}{a_L} fgd^3 \qquad [A5]$$

When the selected mass (a_L) is <10% of the entire mass, the sample mass to control variance of FSE can easily be obtained by the following relationships:

$$m_S = \frac{IH_L}{s^2_{FSE}} \qquad s^2_{FSE} = \frac{IH_L}{m_S} \qquad [A6]$$

The equation for the variance of FSE for particle size representativeness is

$$s_{FSE}^2 = \left[\frac{1}{m_S} - \frac{1}{m_L}\right] f \cdot \lambda \left[\left(\frac{1}{a_{Lc}} - 2\right) d_{FLc}^3 + \sum_x d_{FLx}^3 \cdot a_{Lx}\right] \quad [A7]$$

where

f, d, m_S, m_L, and s are as described above;

d_{FLc} is the average particle size of the fraction of interest;

d_{FLx} is the average of each of the other particle size fractions except d_{FLc};

λ is the density of the material (expressed in g/cm^3);

a_{Lc} is the proportion of d_{FLc} to the entire mass; and

a_{Lx} is the proportion of d_{FLx} to the entire mass.

When the mass of the selected portion is less than 10% of the total mass, Equation A7 can be simplified to

$$s_{FSE}^2 = \frac{f\lambda}{m_S}\left[\left(\frac{1}{a_{Lc}} - 2\right) d_{FLc}^3 + g d^3\right] \quad [A8]$$

Equation A8 can be used to represent any size class. For examples that follow, the largest size class (largest particles) are the size class of interest; therefore, $a_{Lc} = 0.05$. If a_{Lc} is small (<0.25) and d_{FLc} is not much different from d, Equation A8 can be further simplified to:

$$s_{FSE}^2 = \frac{f\lambda}{m_S}\left(\frac{1}{a_{Lc}} - 2\right) d^3 \quad [A9]$$

Examples for Calculating Fundamental Sampling Error

The following examples are cases where the analyte of interest is liberated. A liberated constituent is one in which the analyte of interest is liberated (or separated) from a host material. When sampling a material with a liberated analyte, two FSE must be considered: (1) the FSE in representing the liberated analyte of interest, and (2) the FSE in representing the particle sizes of the host material. The minimum mass to control the variance of FSE will be controlled by the maximum of either (1) the mass needed to represent the liberated analyte of interest or (2) the mass needed to represent the particle sizes of the host material.

Caution needs to be exercised in applying equations without first understanding the assumptions and limitations. For the following examples, it is assumed for any selection process (mass reduction) that the mass selected is small compared with the total mass. Only two mass reduction processes are considered in these examples: selection of the primary sample and selection of the test portion.

Example 1

A feed product with a density of 0.75 g/cm^3 has a pure vitamin added at the rate of 0.03%. The density of the vitamin is 0.95 g/cm^3. The vitamin particles are rounded and calibrated between two consecutive screen sizes. The maximum particle size for the feed product and the vitamin is 1.5 mm.

The characteristics of the vitamin are as follows:

- density is 0.95 g/cm^3
- particles are rounded
- granulometric factor is 0.55
- upper diameter is 1.5 mm
- concentration of the active ingredient in the vitamin is 100%

The characteristics of the feed product to which the vitamin is added are as follows:

- density is 0.75 g/cm^3
- particles are rounded
- upper diameter is 1.5 mm
- concentration of vitamin in the feed product is 0.03%

If 250 g is selected from a 20-kg container as a primary sample and then comminuted to 0.75 mm before selection of a 1.0-g test portion, what is the total variance of FSE?

Two calculations are needed to estimate the variance of FSE: (1) estimation of FSE based on the liberated vitamin, and (2) estimation of FSE based on the maximum particle size.

Estimation of FSE based on liberated vitamin:

The granulometric factor, density, and particle size are for the liberated vitamin. Using Equation A4,

$$c \approx \frac{\lambda_m}{a_L} \approx \frac{0.95}{0.0003} \approx 3{,}167$$

The variance of the FSE (using Equation A2) for selection of a primary sample is

$$s^2_{FSE} = \frac{cfgd^3}{m_S} = \frac{3{,}167 \times 0.5 \times 0.55 \times 0.15^3}{250} = 0.012$$

The variance of the FSE for selection of a test portion (Note: once a calibrated material is comminuted, the granulometric factor becomes 0.25) is

$$s^2_{FSE} = \frac{cfgd^3}{m_S} = \frac{3{,}167 \times 0.5 \times 0.25 \times 0.075^3}{1} = 0.167$$

$$s_{FSE\ total} = \sqrt{0.012 + 0.167} = 42\%$$

Estimation of FSE based on the maximum particle size:

Determine the variance of FSE for the primary sample, using Equation A9:

$$s^2_{FSE} = \frac{f\lambda}{m_S}\left(\frac{1}{a_{Lc}} - 2\right)d^3 = \frac{0.5 \times 0.75}{250}\left(\frac{1}{0.05} - 2\right)0.15^3 = 0.00009$$

Because the FSE to represent the particle sizes (0.00009) is much smaller than the FSE to represent the liberated vitamin (0.012), the mass to represent the liberated vitamin will be the minimum mass. The total error of 42% is unacceptable and the largest source of error is in the selection of the test portion. Options can be explored to control the FSE in selection of the test portion.

Exploring options to control the variance of FSE in the laboratory:

Various combinations of comminution and test portion mass can be evaluated to determine the best approach to control the variance of the FSE in the laboratory. Two combinations follow:

(a) The entire 250-g laboratory sample is comminuted to 0.1 mm before selection of the 1.0-g test portion. Using Equation A2, calculate FSE:

$$s_{FSE}^2 = \frac{clfgd^3}{m_S} = \frac{3{,}167 \times 1 \times 0.5 \times 0.25 \times 0.01^3}{1} = 0.0004$$

$$s_{FSE} = \sqrt{0.012 + 0.0004} = 11\%$$

Once the FSE for selection of the test portion is controlled, collecting a larger primary sample would be the most efficient approach to further reduce total FSE.

(b) The entire 250-g laboratory sample is comminuted to 0.75 mm (due to logistical constraints of comminuting finer) and the test portion is increased to 10 g. Using Equation A2, calculate FSE:

$$s_{FSE}^2 = \frac{clfgd^3}{m_S} = \frac{3{,}167 \times 1 \times 0.5 \times 0.25 \times 0.075^3}{10} = 0.017$$

$$s_{FSE} = \sqrt{0.012 + 0.017} = 17\%$$

Note: If the laboratory sample is not comminuted before selection of a 1.0-g test portion, the FSE is extremely large. Using Equation A2, calculate FSE:

$$s_{FSE}^2 = \frac{clfgd^3}{m_S} = \frac{3{,}167 \times 1 \times 0.5 \times 0.55 \times 0.15^3}{1} = 2.94$$

$$s_{FSE} = \sqrt{0.012 + 2.94} = 172\%$$

If the laboratory sample is not comminuted, then the *entire* laboratory sample can be used as the test portion. In that case, the variance of the FSE contribution from laboratory sampling would be zero because no selection process is performed. The total FSE would simply be the FSE contribution from the primary sample (s_{FSE} = 11.0%).

Example 2

Various scenarios are evaluated for selection of a test portion from a granola for an active ingredient incorporated into the granola via a liberated supplement.

The characteristics of the supplement are as follows:

- density is 1.3 g/cm^3
- particles are rounded
- upper diameter is 0.8 mm
- granulometric factor is 0.25
- concentration of the active ingredient in the supplement is 10% (10% purity)

The characteristics of the granola to which the supplement is added are as follows:

- density is 0.9 g/cm^3
- particles are rounded
- upper diameter is 0.9 cm
- concentration of active ingredient in granola is 20 ppm

In this example, a different approach (Equation A5) is illustrated for estimation of sample mass. Because the supplement is not pure analyte of interest, a purity factor (a_L/ purity) is used when calculating the mineralogical factor c.

$$IH_L = fgd^3\left(\frac{\lambda}{a_L}\right) = 0.5 \times 0.25 \times 0.08^3\left(\frac{1.3}{0.000020/0.1}\right) = 0.42 \text{ grams}$$

The mass of sample required to control FSE to 10% to represent the analyte of interest in the granola can be calculated using Equation A6:

$$m_S = \frac{IH_L}{s^2_{FSE}} = \frac{0.42}{0.1^2} = 42 \text{ grams}$$

The mass of sample required to control the FSE to 10% to represent the particle sizes of the granola can be calculated using Equation A9:

$$m_S = \frac{f\lambda}{s^2_{FSE}}\left(\frac{1}{a_{Lc}} - 2\right)d^3 = \frac{0.5 \times 0.9}{0.1^2}\left(\frac{1}{0.05} - 2\right)0.9^3 = 590 \text{ grams}$$

In this example, more mass is needed to represent the particle sizes of the granola (590 g) than to represent the concentration of the liberated supplement (42 g) in the granola. If it is desired to reduce the mass of the test portion below 590 g, comminution must be performed. If all the granola is comminuted to 1 mm before selection of the test portion, what is the mass required to control the FSE to 10% (using Equation A9)?

$$m_S = \frac{f\lambda}{s_{FSE}^2}\left(\frac{1}{a_{Lc}} - 2\right)d^3 = \frac{0.5 \times 0.9}{0.1^2}\left(\frac{1}{0.05} - 2\right)0.1^3 = 0.81 \text{ grams}$$

The mass required to control the FSE for the analyte of interest in the liberated supplement does not change (still 42 g) because the comminution did not affect the particle size of the supplement (there is still the same number of supplement particles per unit mass). The mass required to represent the particle sizes of the granola decreases to 0.81 g; however, 42 g will be needed to achieve an FSE of 10%. If a test portion mass of 42 g is undesirable and if the material cannot be comminuted finer than 1 mm, another possible option to control the FSE is to purchase an alternative supplement with a smaller maximum particle size.

If an alternative supplement can be purchased with a maximum particle size of 0.4 mm, what is the required mass of the test portion to control FSE to 10%?

Use Equation A5 to calculate IH_L and Equation A6 to calculate mass:

$$IH_L = fgd^3\left(\frac{\lambda}{a_L}\right) = 0.5 \times 0.25 \times 0.04^3\left(\frac{1.3}{0.000020/0.1}\right) = 0.052 \text{ grams}$$

$$m_S = \frac{IH_L}{s_{FSE}^2} = \frac{0.052}{0.1^2} = 5.2 \text{ grams}$$

With procurement (or manufacture) of a supplement with a 0.4 mm maximum particle size, the test portion mass can be reduced from 42 g to 5 g.

Calculate the FSE for the following protocol for sampling the granola based on the original characteristics:

- Selection of a 1-lb (454-g) primary sample from a 25-kg bag
- Comminution of the entire 1-lb primary sample to 1 mm
- Selection of a 30-g test portion from the comminuted material

Based on previous calculations, we know that the mass required to represent the particle sizes determines the required sample mass for the primary sample. The required mass to select a representative test portion is determined by the liberated supplement.

Using Equation A9, the error to represent the particle sizes of granola in selection of the primary sample is:

$$s_{FSE}^2 = \frac{f\lambda}{m_S}\left(\frac{1}{a_{Lc}} - 2\right)d^3 = \frac{0.5 \times 0.9}{454} \times 18 \times 0.9^3 = 0.013$$

Using Equation A6, the error to represent the analyte of interest in the test portion is:

$$s_{FSE}^2 = \frac{IH_L}{m_S} = \frac{0.42}{30} = 0.014$$

The FSE from both sampling stages is:

$$FSE = \sqrt{0.013 + 0.014} = 16\%$$

If the FSE of 16% is too large to meet the SQC, and since the FSE from each sampling stage is approximately equal, both need to be addressed to significantly effect FSE. To reduce the FSE, one or more of the following options is available:

- ▷ increase the primary sample mass,
- ▷ comminute the primary sample to a finer particle size,
- ▷ increase the test portion mass.

Final Considerations and Caution for Examples

The examples provided here are not exhaustive; they simply demonstrate the power of TOS and how TOS can be useful when designing sampling protocols. No reference has been made to SQC, quality control, other sampling errors etc., which are critical for development of a sampling protocol. A reader desiring to perform calculations based on TOS needs a greater understanding of TOS than presented here to understand appropriate applications and assumptions. In addition, SQC, material properties, grouping and segregation error, and sample correctness need to be considered in the development of any sampling protocol. It becomes obvious that the examples cannot be used as a template to design a specific sampling protocol.

REFERENCES

AAFCO (Association of American Feed Control Officials). (2014). AAFCO *Quality Assurance Quality Control Guidelines for Feed Laboratories* (section 5.8). AAFCO, Champaign, IL.

AAFCO (Association of American Feed Control Officials). (2015). *Guidance on Obtaining Defensible Samples: GOODSamples*. AAFCO, Champaign, IL. https://www.aafco.org/Publications/GOODSamples.

Allen, T., & Kahn, A. A. (1970). Critical evaluation of powder sampling procedures. *Chem. Eng.* **238**, 108–112.

APHL (Association of Public Health Laboratories). (2017). *Best Practices for Submission of Actionable Food and Feed Testing Data Generated in State and Local Laboratories*. APHL, Silver Spring, MD. https://www.aphl.org/aboutAPHL/publications/Documents/FS-2017Jun-Best-Practices-Food-Feed-Data.pdf.

Capel, P. D., & Larson, S. J. (1996). Evaluation of selected information on splitting devices for water samples. Water-Resources Investigations Report 95-4141. USGS (US Geological Survey), Sacramento, CA. https://pubs.usgs.gov/wri/1995/4141/report.pdf.

FDA (Food and Drug Adminstration). (2018). *Investigations Operations Manual*. FDA, Washington, DC. https://www.fda.gov/ICECI/Inspections/IOM/default.htm.

Fussell, R. J., Addie, K. J., Reynolds, S. L., & Wilson, M. F. (2002). Assessment of the stability of pesticides during cryogenic sample processing. 1. Apples. *J. Agric. Food Chem.* **50**, 441–448.

Fussell, R. J., Hetmanski, M. T., Colyer, A., Caldow, M., Smith, F., & Findlay, D. (2007a). Assessment of the stability of pesticides during the cryogenic processing of fruits and vegetables. *Food Addit. Contam.* **24**, 1247–1256.

Fussell, R. J., Hetmanski, M. T., MacArthur, R., Findlay, D., Smith, F., Ambrus, A., & Brodesser, P. J. (2007b). Measurement uncertainty associated with sample processing of oranges and tomatoes for pesticide residue analysis. *J. Agric. Food Chem.* **55**, 1062–1070.

ISO (International Organization for Standardization). (2012). Animal feeding stuffs—Guidelines for sample preparation. Standard no. 6498. ISO, Geneva, Switzerland.

Maestroni, B., Ghods, A., El-Bidaoui, M., Rathor, N., Jarju, O. P., Ton, T., & Ambrus, A. (2000). Testing the efficiency and uncertainty of sample processing using ^{14}C-labelled chlorpyrifos: Part I and Part II. Pages 49–72 in *Principles and Practices of Method Validation*; Fajgelj, A., Ambrus, Ä., eds. Royal Society of Chemistry, Cambridge, UK.

Peterson, L., Dahl, C. K., & Esbensen, K. H. (2004). Representative mass reduction in sampling—A critical survey of techniques and hardware. *Chemom. Intell. Lab. Syst.* **74**, 95–114.

Pitard, F. F. (1993). *Pierre Gy's Sampling Theory and Sampling Practice*. 2nd ed. CRC Press, Boca Raton, FL.

Ramsey, C. A. (2015). Considerations for inference to decision units. *J. AOAC Int.* **98**, 288–294. https://doi.org/10.5740/jaoacint.14-292.

Ramsey, C. A., & Wagner, C. (2015). Sample quality criteria. *J. AOAC Int.* **98**, 265–268. doi: https://doi.org/10.5740/jaoacint.14-247.

Thiex, N., L. Novotny, C. Ramsey, G. Latimer, L. Torma, & Beine, R. (2000). *AAFCO Guidelines for Preparing Laboratory Samples*. Association of American Feed Control Officials, Oxford, IN.

USGS (US Geological Survey). (2017). Chapter A2, Selection of equipment for water sampling in National Field Manual. USGS (US Geological Survey), Reston, VA. https://water.usgs.gov/owq/FieldManual/Chapter2/Chapter2_V3-1.pdf.

Photo credits

Front cover: farm—iStock.com/Bestgreenscreen, kibble—iStock.com/candy_vandy, pelleted feed—iStock.com/domnicky, pizza—iStock.com/danilsnegmb, olive oil—iStock.com/ddukang, bacon—iStock.com/Bestfotostudio, dog food—iStock.com/touc, beef—iStock.com/robynmac, vitamins—iStock.com/Creativeye99, egg—iStock.com/robynmac, canned meat—iStock.com/beemore, carrot—iStock.com/pixitive, volumetric flask—iStock.com/studiocasper, orange juice—iStock.com/lleerogers, peanut butter—iStock.com/Saturated, beans—iStock.com/fcafotodigital, strawberry—iStock.com/photomaru, cheese—iStock.com/robynmac, fish—iStock.com/robynmac, lettuce—iStock.com/natalie-claude, milk—iStock.com/Jewelsy, bread—iStock.com/OLEKSANDR PEREPELYTSIA, tomato—iStock.com/anna1311, soil—iStock.com/aristotoo, fertilizer—iStock.com/tumdee.

Inside front cover: puppy—iStock.com/cmannphoto.

Page 16: blender—iStockphoto.com/Robert Daly.

Page 23: maple syrup—istock.com/Saturated, ketchup—istock.com/Grafner, baby food—istock.com/margouillatphotos, grain—istock.com/vaakim.

GOOD Test Portions

Published by FASS Inc.
1800 South Oak Street, Suite 100,
Champaign, Illinois 61820
(217) 356-3182
http://www.fass.org