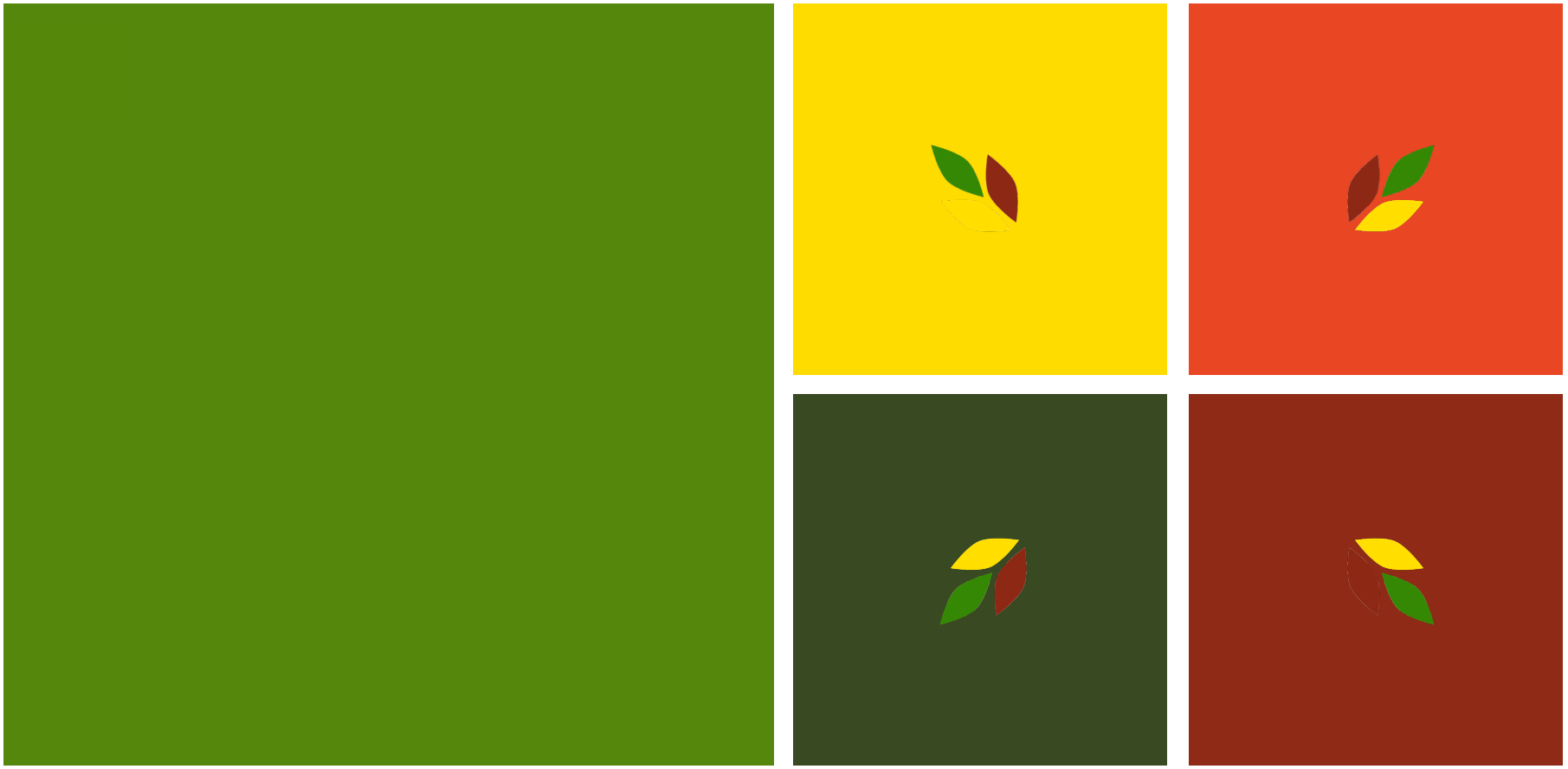


GOOD Test Portions

Guidance on Obtaining Defensible Test Portions (GOOD Test Portions)

Learning Objective

Tools, techniques, and considerations necessary for obtaining defensible test portions

Outline

- Review of GOODSamples
- Impact of Error on Data Quality
- Selection and Nonselection Errors
- Quality Control Measurements
- Equipment Selection
- Data Assessment
- Inference

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

Introduction

- GOODSamples and GOOD Test Portions are important
  - Sampling error is significant in the field and laboratory
  - Sampling error causes incorrect analytical results
  - Incorrect analytical results cause incorrect decisions
- Sampling quality control is important
  - Sampling error is typically much larger than analytical error
  - Little, if any, sampling quality control is currently performed
- Interpretation of data
  - Analytical results
  - Quality control results

Definitions

- Laboratory Sampling: All manipulations performed on the laboratory sample after receipt and acceptance through selection of the test portion. Includes selection and nonselection processes.
- Selection process: The act of selecting a smaller mass (or volume) sample from a larger mass (or volume) sample. There are two types of selection processes: mass reduction and splitting.
- Nonselection process: Manipulation of a sample (e.g. comminution, removal of extraneous material, removal of water), usually performed before a mass reduction process.
- Mass reduction: Selection of a smaller mass or volume of material from a larger mass or volume.

Definitions

- Splitting: The division of a mass or volume into two or more equal portions.
- Test Portion: The material subjected to an analytical test procedure.
- Laboratory Sampling Protocol: Detailed procedures for obtaining a test portion from a laboratory sample.
- Global Estimation Error (GEE) : The total error in the entire process from collection of the primary sample through the final measurement.

Three Subsampling Approaches

- Select entire Decision Unit (DU)
  - Analyze entire laboratory sample
  - No sampling error!
- Non-probabilistic subsampling
  - Take what is easiest or use your judgment in selection
  - Cannot make inference or determine error
- Probabilistic subsampling ← Focus
  - Make all laboratory sample available
  - Select material from random locations
  - Correct tools
  - Required for inference and error determination

Sample Types

- Primary Sample: The initial sample that is collected. The material collected from the Decision Unit.
- Laboratory Sample: The sample that is received by the laboratory. In many cases this will be the same as the primary sample.
- Analytical Sample: The material from which the test portion is selected.
- Replicate Sample: Multiple samples collected under the same conditions.
- Split Sample: The result of dividing material into equal portions.

GOODSamples Concepts

- Sample Quality Criteria (SQC) – the objectives
- Material properties – characteristics of material
  - Elements
  - Heterogeneity
- Sampling error – impacts confidence of analytical result
  - Random Errors
  - Systematic Errors
  - Blunders
- Maintaining sample integrity

GOODSamples Concepts cont…

- Theory of Sampling (TOS) – how to measure and control sampling error
  - Fundamental Sampling Error (FSE)
  - Grouping and Segregation Error (GSE)
  - Sample correctness
- Quality control – ensuring objectives are met
- Data evaluation – interpreting the analytical results
- Inference
  - Estimation of a concentration or characteristic of larger amount of material from the testing of a smaller amount of material

Sample Quality Criteria (SQC)

- What is the question?
  - What is the analyte or characteristic of concern?
  - What is the concentration of concern?
  - How will the inference be made?
- What is the Decision Unit?
- What is the required confidence?

Lab must be involved along with program staff in SQC process; lab brings scientific expertise.

What is the Question?

- What is the analyte or characteristic of concern?
  - Handling, containers, aseptic, time
- What is the concentration or characteristic of concern?
  - Sampling technique
  - Preservation
- How will the inference(s) be made?
  - Probabilistic
  - Use of statistics
  - Type I and Type II

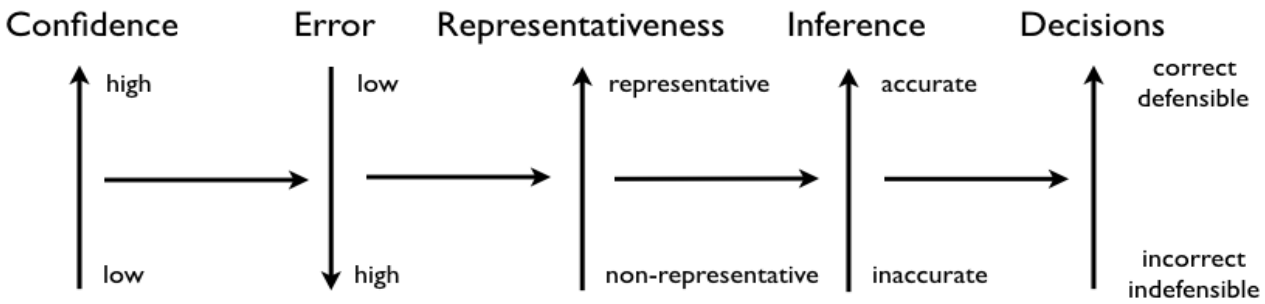
What is the Decision Unit (DU)?

- The Decision Unit is the material from which the primary sample(s) is collected and to which the inference(s) is made
  - What are the spatial boundaries?
  - What are the temporal boundaries?
  - What are the potential extraneous materials?
    - Pits, seeds, skin, gravel, dirt, moisture, etc.
- There may be one Decision Unit or there can be many
- May be thought of as the scale of decision making

This is the most important part of sampling, yet many people do not even consider it as part of their objective.

What is the Required Confidence?

- Related to the probability you made an incorrect decision
- Related to the consequences of making an incorrect decision



Everything is related, but it all begins with the control of error.

Sample Quality Criteria

- Entire organization involved
  - Primary sample collection
  - Laboratory
  - Quality control
  - Decision maker
- Not a hard process, just a new process for some
- It is nothing more than planning how to make defensible decisions
  - Control and mitigation of error
  - Measurement of error

Material Properties

- Finite element vs Infinite element
- Heterogeneity is the root cause of error in all sampling
  - Compositional Heterogeneity (CH)
  - Distributional Heterogeneity (DH)
- The magnitude and nature of the material properties are unique to each material and dictate the sampling efforts

Material properties are the unique physical, chemical, biological and radiological properties that make each type of material different to sample.

Finite vs Infinite

- Finite element material
  - The Decision Unit is made of elements that can be individually identified, selected at random, and interrogated.
  - Example: watermelons
- Infinite element material
  - The Decision Unit is made of elements that are so small and numerous that they practically cannot be individually identified, selected at random, or interrogated.
  - Example: flour
- Material that has been comminuted is typically considered infinite element material

Heterogeneity

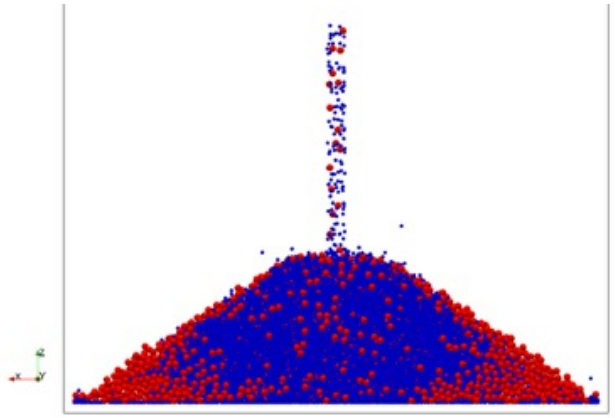
- Compositional Heterogeneity is the difference in concentration (or characteristic) between the elements that make up the Decision Unit *without* regard to position or time
- Distributional Heterogeneity is the difference in concentration (or characteristic) between the elements that make up the Decision Unit *with* respect to position or time
- Heterogeneity always exists

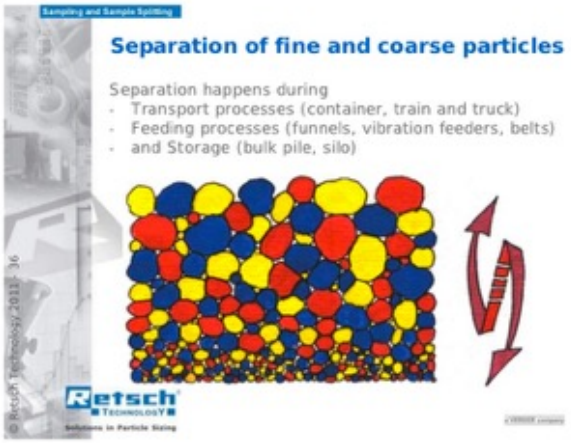
Heterogeneity

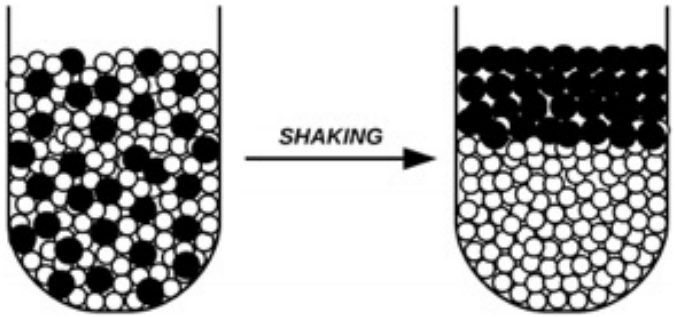
Orange juice has many separate components with large Distributional Heterogeneity. The pulp falls quickly, the foam disperses slowly, and volatiles escape rapidly.

Without Compositional Heterogeneity there can be no Distributional Heterogeneity.

Heterogeneity in the Laboratory







GOOD Test Portions

Mixing

- Does it impact Compositional Heterogeneity?
- Does it impact Distributional Heterogeneity?
- What type of materials mix well?
- Not a viable method to reduce Distributional Heterogeneity in most cases
- Mixing never creates a homogeneous state

Good Test Portions recommends removal of the term homogeneous from all protocols. It is an incorrect term and leads to a false sense of security.

GOOD Test Portions

Integrity

- Analyte integrity
  - The identity and concentration of the analyte of interest is the same throughout the entire measurement process, as it was in the Decision Unit prior to sample collection
- Evidentiary integrity
  - The ability to trace an analytical result to the Decision Unit with the assurance that the analyte integrity has been maintained
- Integrity is maintained by a system of procedures, photos, and paperwork. Chain of Custody (CoC) is part of maintaining integrity
- Organizations may have different integrity procedures

Sampling Error

- Random errors
  - Sometimes called precision or imprecision errors
  - Average of all random errors is zero
- Systematic errors
  - Sometimes called bias errors
- Blunders
  - Sometimes called gross errors

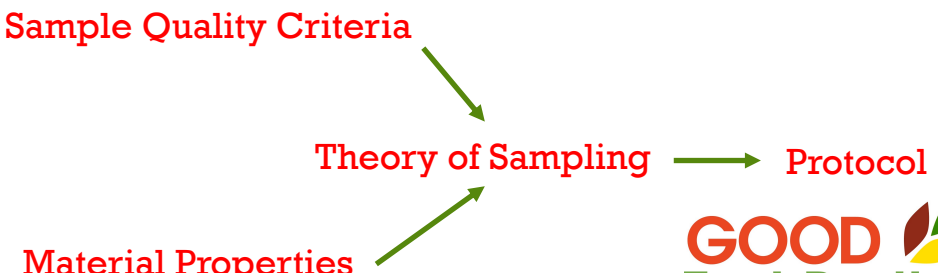
Errors cause the results from the analytical test to be different than the true result for the test portion. This leads to incorrect inference and ultimately to incorrect and indefensible decisions.

Theory of Sampling (TOS)

- Theory of Sampling allows for the control and mitigation of sampling error to meet the SQC
- The development of TOS started in the late 1800s, but the formalization of TOS started in the 1950s by Pierre Gy (1924-2015)
- Mainly applied in the mineral industry, but it is applicable and has been applied to food and feed
- Purpose of TOS is to
  - Identify errors
  - Measure errors
  - Control errors

Theory of Sampling cont…

- Inputs to TOS are Material Properties and SQC
- The required sample mass is a function of the magnitude of the Compositional Heterogeneity and the tolerable error
- The required number of increments is a function of the magnitude of the Distributional Heterogeneity and the tolerable error
- Sample correctness is the control of systematic errors
  - Correct tool design
  - Correct tool use
  - Proportionate sampling



GOOD Test Portions

What is an Increment?

- The mass/volume collected from a single operation of a sampling tool
- The individual portions of material that are combined to form a single sample
- A sample can be made up of a single increment (material from a single point) or of many increments (material from multiple points)
- The term *Multi Increment Sample* (MIS) is used to distinguish between a sample with a single increment from a sample with multiple increments

Relationships

$$s^2_{FSE} \propto \frac{CH}{m_s} d^3$$

Where:
FSE = variance of the Fundamental Sampling Error
CH = Compositional Heterogeneity
m_s = sample mass
d^3 = diameter of the largest particles

$$s^2_{GSE} \propto \frac{DH}{n_{inc}}$$

Where:
GSE = variance of the Grouping and Segregation Error
DH = Distributional Heterogeneity
n_{inc} = number of increments

In the Laboratory

- Selecting increments at random to control GSE is not difficult
  - Can empty sample container
  - Can manipulate entire sample
- Obtaining sufficient mass to control FSE is more of a challenge due to small mass in many analytical methods
  - Fewer reagents and smaller vessels required for analysis
  - Pollution prevention
  - Throughput
- Controlling FSE error to make defensible decisions cannot be compromised
  - More mass
  - Comminution

Comminution

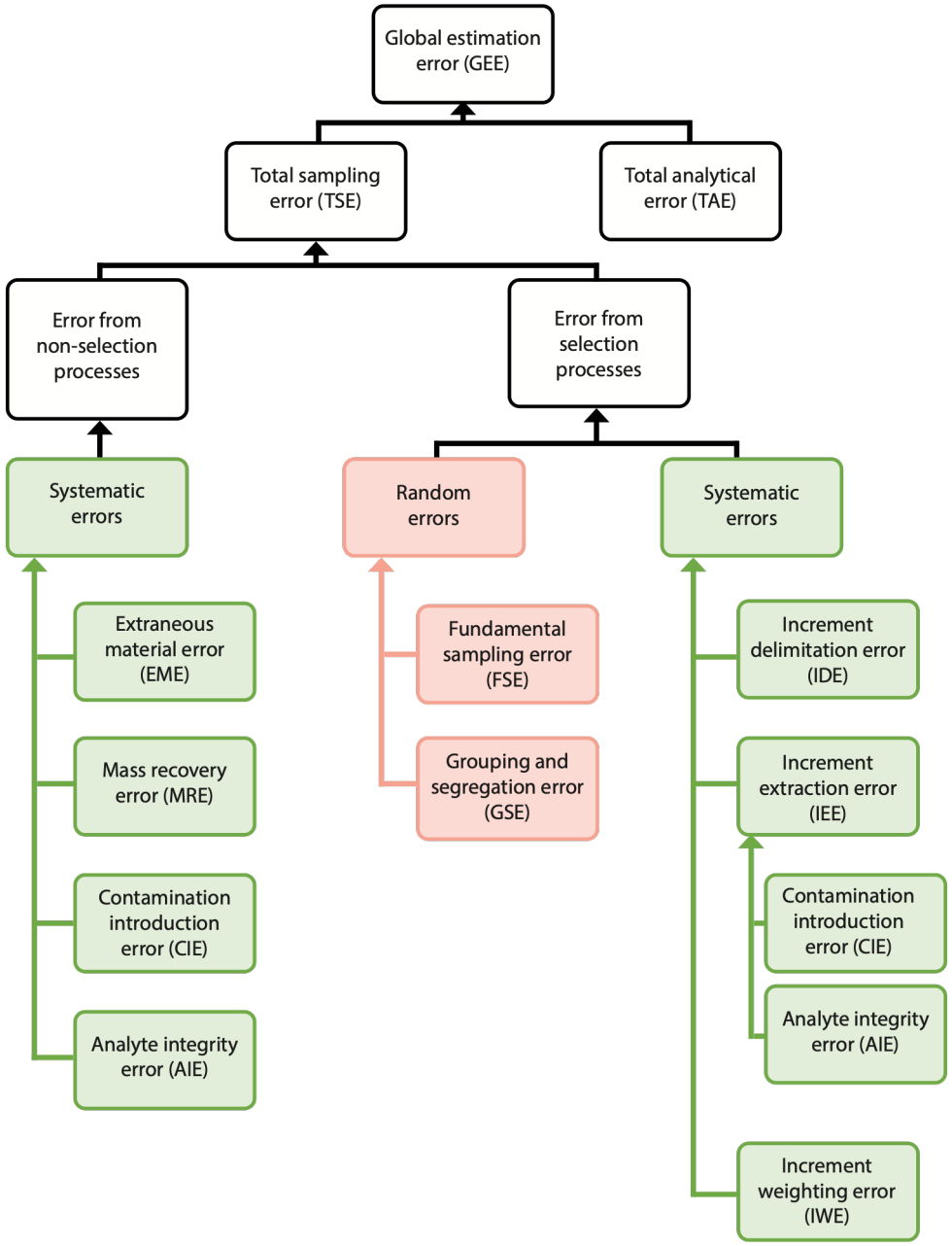
- Controls FSE directly

$$s_{FSE}^2 \propto \frac{CH}{m_s} d^3$$

- Helps with other error as well
  - GSE as less chance of segregation with correct comminution
  - Sample correctness as elements more uniform in nature
- Need to represent all sample or at minimum a representative portion
- Not all materials can be comminuted
  - Analyte integrity
  - Physical characteristics of material

Sampling Errors

- Nonselection errors
- Selection errors
- Relationship of error to mass
- Relationship of error to increments
- Relationship of error to sample correctness


Global estimation error (GEE)
Total sampling error (TSE)
Total analytical error (TAE)
Error from non-selection processes
Error from selection processes
Systematic errors
Random errors
Systematic errors
Extraneous material error (EME)
Mass recovery error (MRE)
Contamination introduction error (CIE)
Analyte integrity error (AIE)
Fundamental sampling error (FSE)
Grouping and segregation error (GSE)
Increment delimitation error (IDE)
Increment extraction error (IEE)
Contamination introduction error (CIE)
Analyte integrity error (AIE)
Increment weighting error (IWE)

Random Errors – Selection Process

- Fundamental Sampling Error (FSE)
  - Function of particle size, mass, and Compositional Heterogeneity
- Grouping and Segregation Error (GSE)
  - Function of number of increments, and Distributional Heterogeneity
- Relationships
  - Relationship of error to mass
  - Relationship of error to increments

Controlling Random Errors

- Fundamental Sampling Error
  - Increase sample mass
  - Comminution
    - Some types of comminution are more effective than others
    - Not all material can be comminuted without loss of analyte
- Grouping and Segregation Error
  - Increase number of increments
  - Control Distributional Heterogeneity
    - Can be very difficult to accomplish
    - Changes every time a material is manipulated

Systematic Errors – Selection Process

- New systematic error terms introduced
- Systematic errors in sampling are impossible to estimate (unlike systematic errors in analysis)
- Causes of errors and practices to control errors

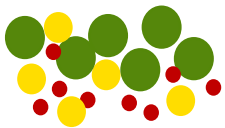
Sample Correctness

- Equiprobable selection of all the particles/molecules at the random increment location
- Tools and techniques should not discriminate toward or against a selection of a particle/molecule based on its
  - Size
  - Shape
  - Location
  - Density
  - Static charge
  - Chemical or biological property
  - Etc.

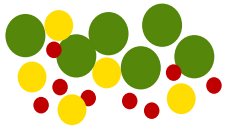
Sample Correctness

- Sample correctness is the sum of the systematic errors from the selection process
- Sample correctness is control of IDE, IEE, IWE
  - IDE occurs when all elements of a material do not have an equiprobable chance of being selected (function of tool design)
  - IEE occurs when all elements have an equal probability of being selected, but the correctly delimited elements do not become part of the increment (function of tool usage)
  - IWE occurs when the increments are not of a consistent or proportional mass

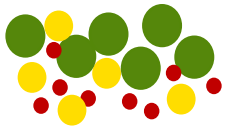
Sample Correctness



flat spatula



rounded scoop



square scoop

bias toward
small particles

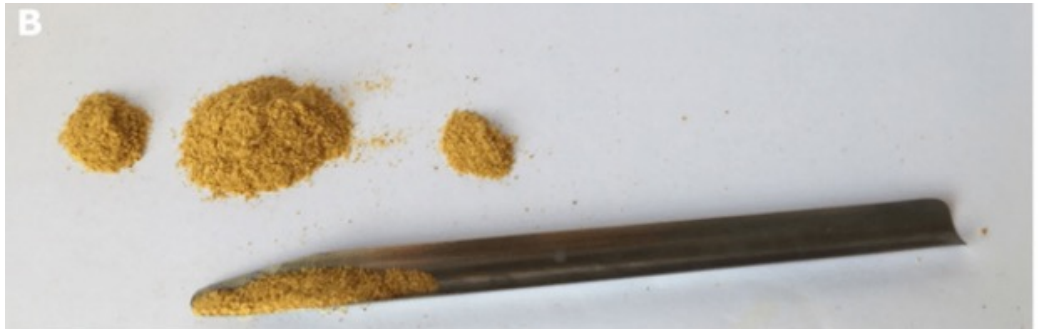
bias toward
large particles

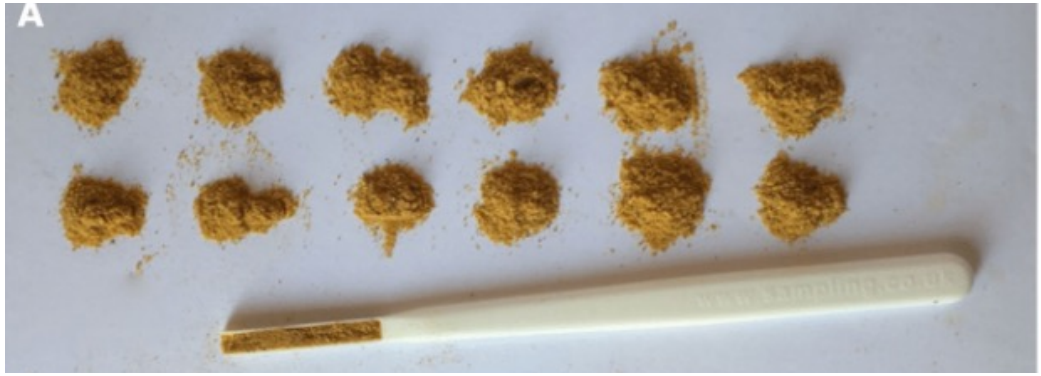
no bias toward
any particles

Increment Extraction Error

- Removal of all the desired elements
  - Do not take extra material
  - Do not lose material
    - Fall out of tool
    - Stick in or on tool
- Contamination Introduction Error (CIE)
  - Tool/container not clean
  - Person not clean
- Analyte Integrity Error (AIE)
  - Analyte
  - Interferent

Increment Weighting Error





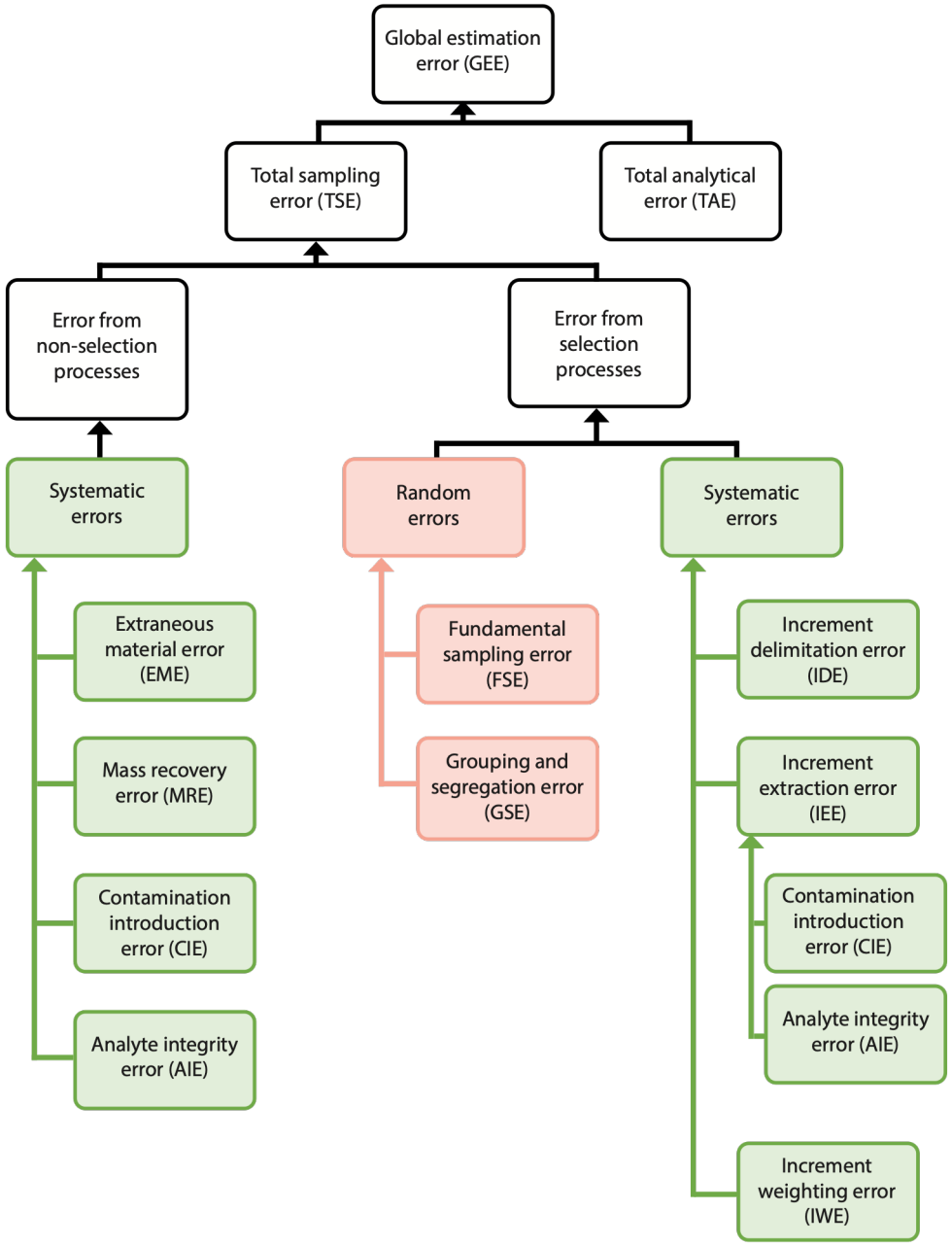
Do you see any issues?

Systematic Errors – Nonselection Process

- Extraneous Material Error (EME)
  - Material that is in the sample but not part of the Decision Unit
- Mass Recovery Error (MRE)
  - Loss of material due to spillage, entrapment in processing equipment, etc.
- Contamination Introduction Error (CIE)
  - Sample contamination from outside the sample
- Analyte Integrity Error (AIE)
  - Changes in concentration from initial primary sampling collection through selection of the final test portion

Blunders

- Mistakes or accidents in the lab
- Analyte and/or evidentiary integrity is lost
- Blunders cannot be incorporated into a Global Estimation Error (GSE) calculation and must be prevented/eliminated or the procedure must be repeated
- Examples
  - Switch samples
  - Break sample container
  - Incorrect dilution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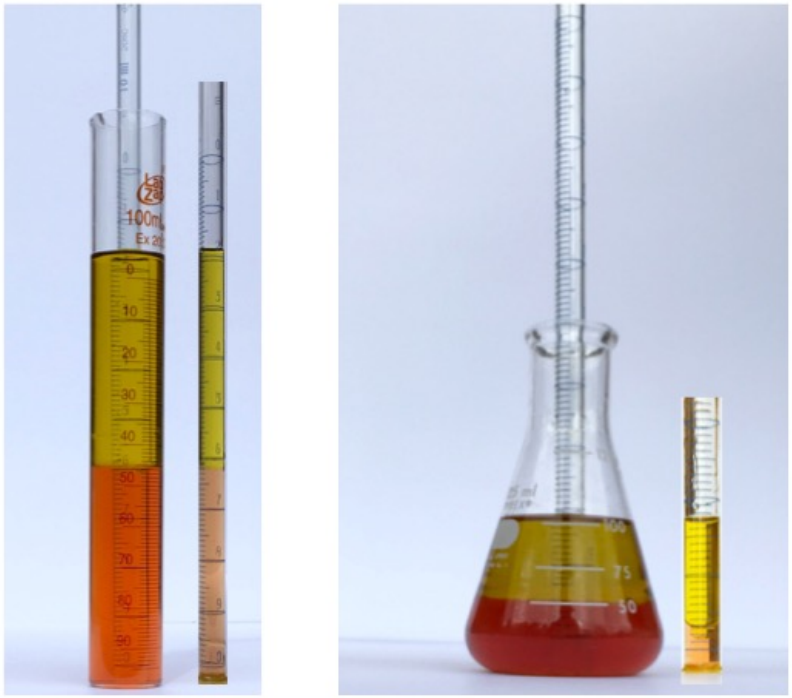
Example of Increment Delimitation Error



Quality Assurance and Quality Control

- Quality Assurance System
  - Validation of a laboratory sampling protocol
  - Verification of laboratory sampling protocol
- Quality Control Events
  - Random error
    - Replication
    - At any stage of the measurement process
  - Systematic error
    - Contamination
    - Carryover
    - All other systematic errors are difficult

Two Types of Inference

- Type I inference
  - From the analytical result(s) to a specific Decision Unit
  - There may be one or many Type I inferences in an investigation
  - Most common type of inference, especially when all the Decision Units can be sampled
- Type II inference
  - From sampled Decision Units to unsampled Decision Units
  - Useful when there is a desire to make inference to all the Decision Units, but not all the Decision Units can be sampled
  - Very common when
    - Not a single Decision Units can fail the criteria
    - A specified percent of Decision Units can fail the criteria

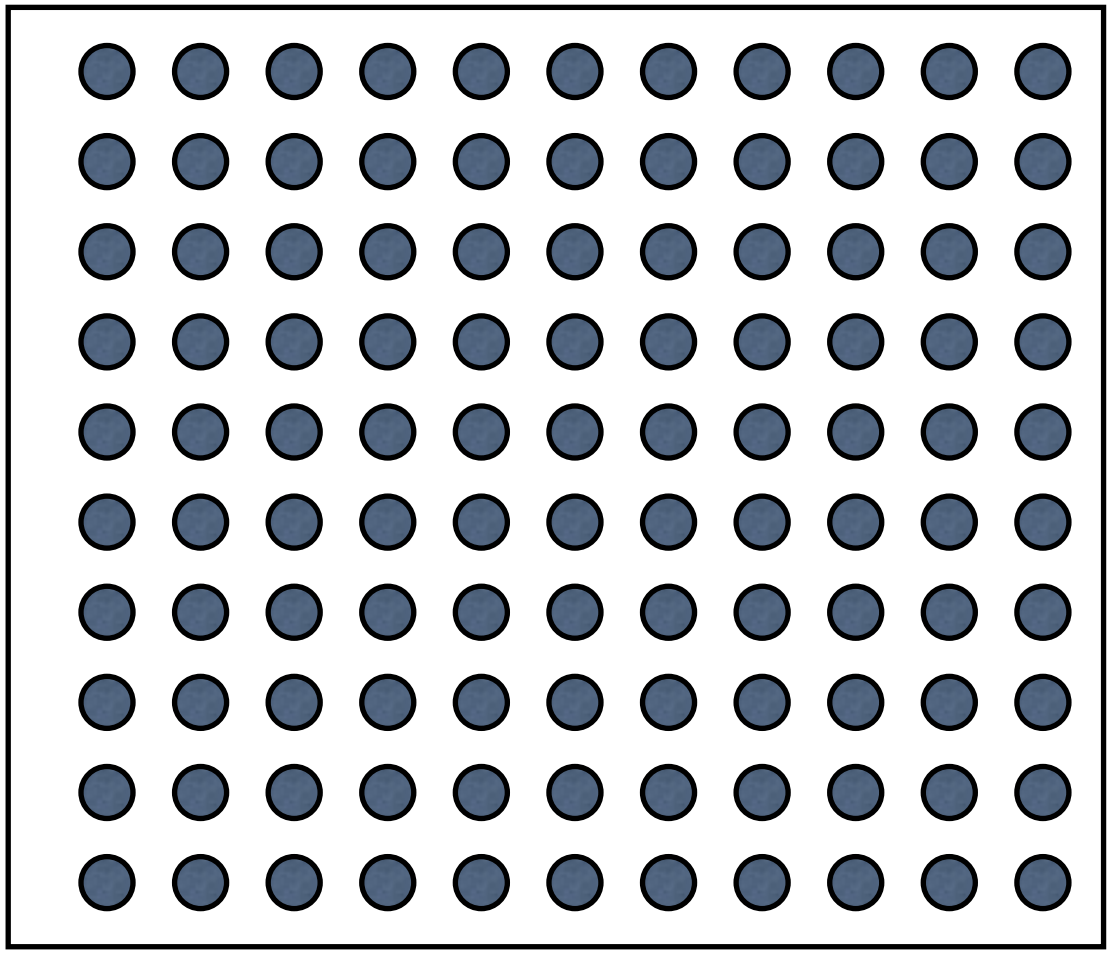
Example Type I Inference

- There are seven truckloads of grain to be sampled
- The Decision Unit is one truckload of grain
  - There are seven DUs
- The laboratory receives seven samples and analyzes each sample
- There are seven Type I inferences made, one for each truck

Example Type II Inference

- 7,000 trucks of grain are to be sampled
- The Decision Unit is a truckload of grain
  - There are 7,000 DUs
- The nature of the investigation does not warrant the resources to sample and analyze each of the 7,000 DUs
- A subset of trucks (DUs) are chosen at random to sample
- From this subset of trucks that are sampled and analyzed, we can make an inference to the trucks that were not sampled

Single Decision Unit

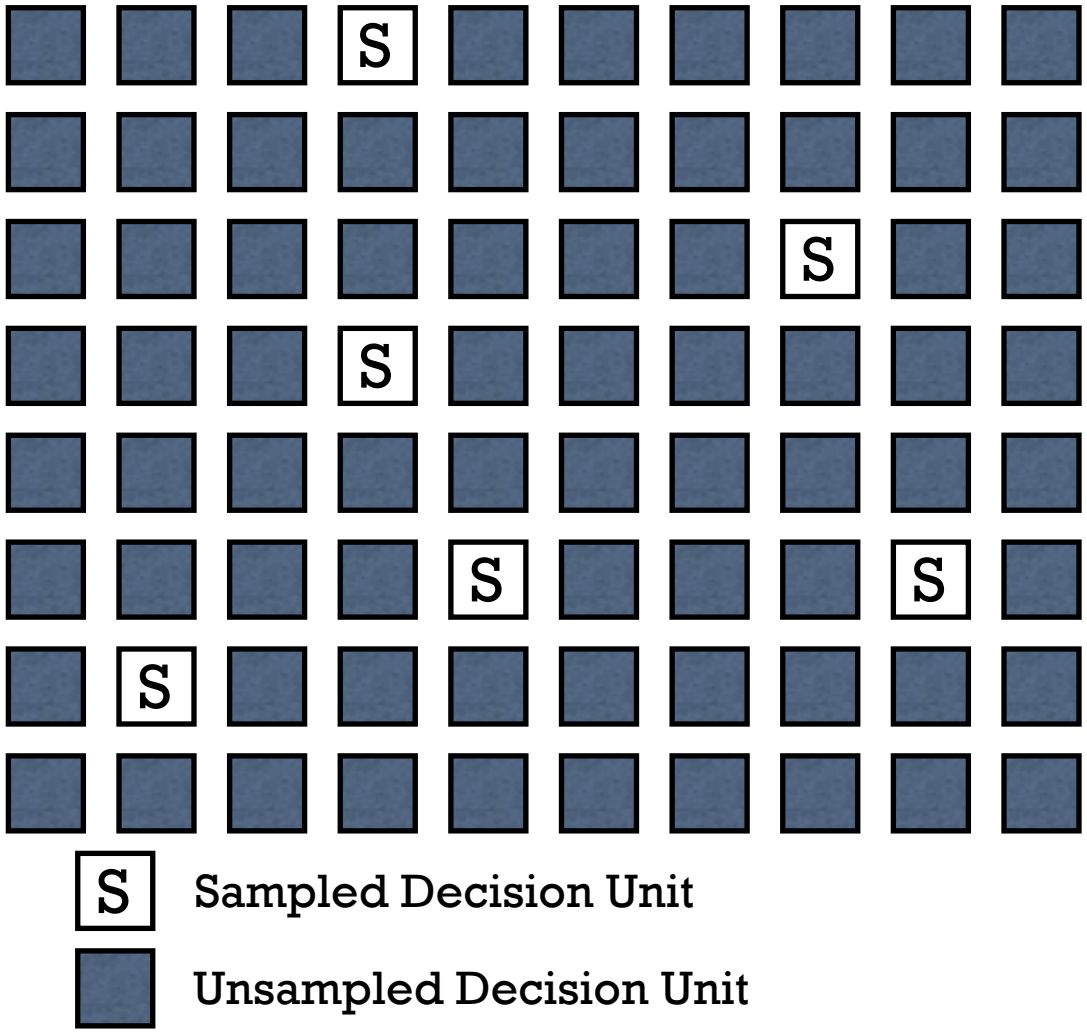


increments inside Decision Unit

Permission Chuck Ramsey, EnviroStat, Inc.

GOOD Test Portions

80 Decision Units



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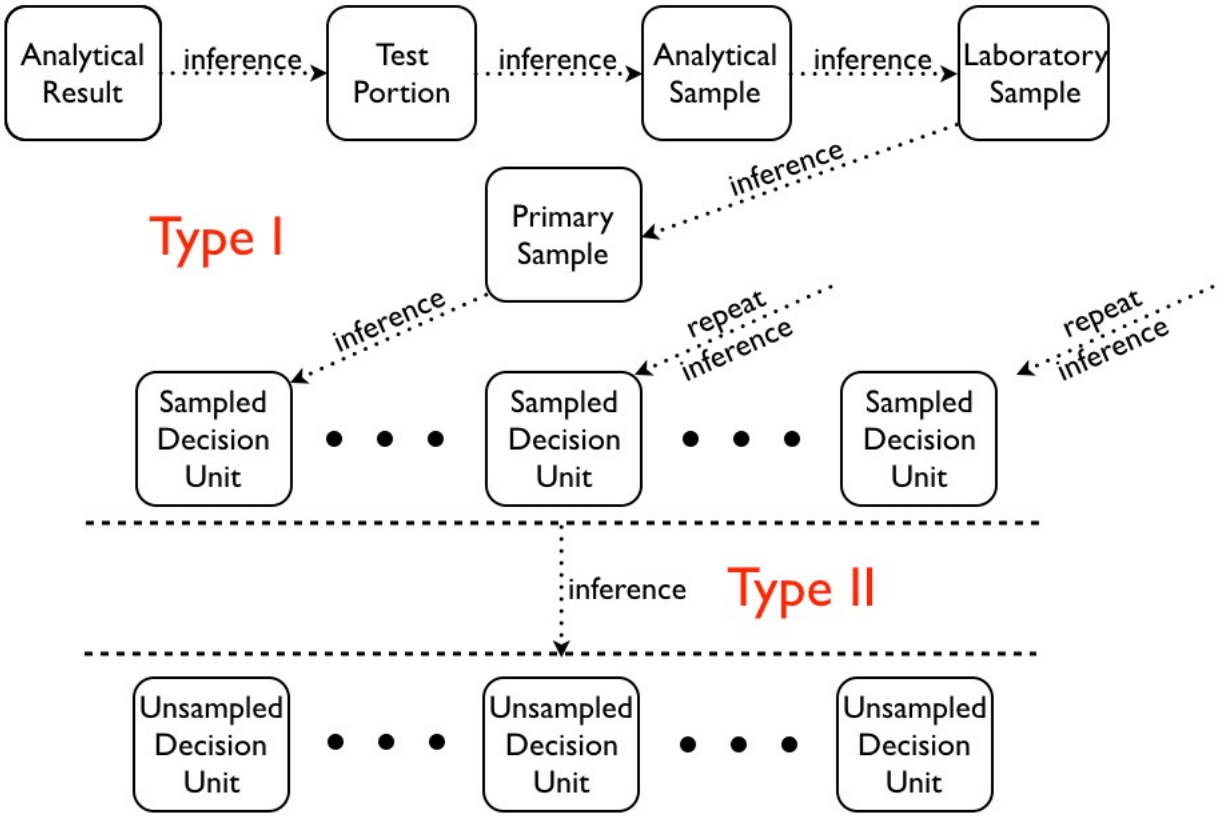
Inference



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GOOD Test Portions

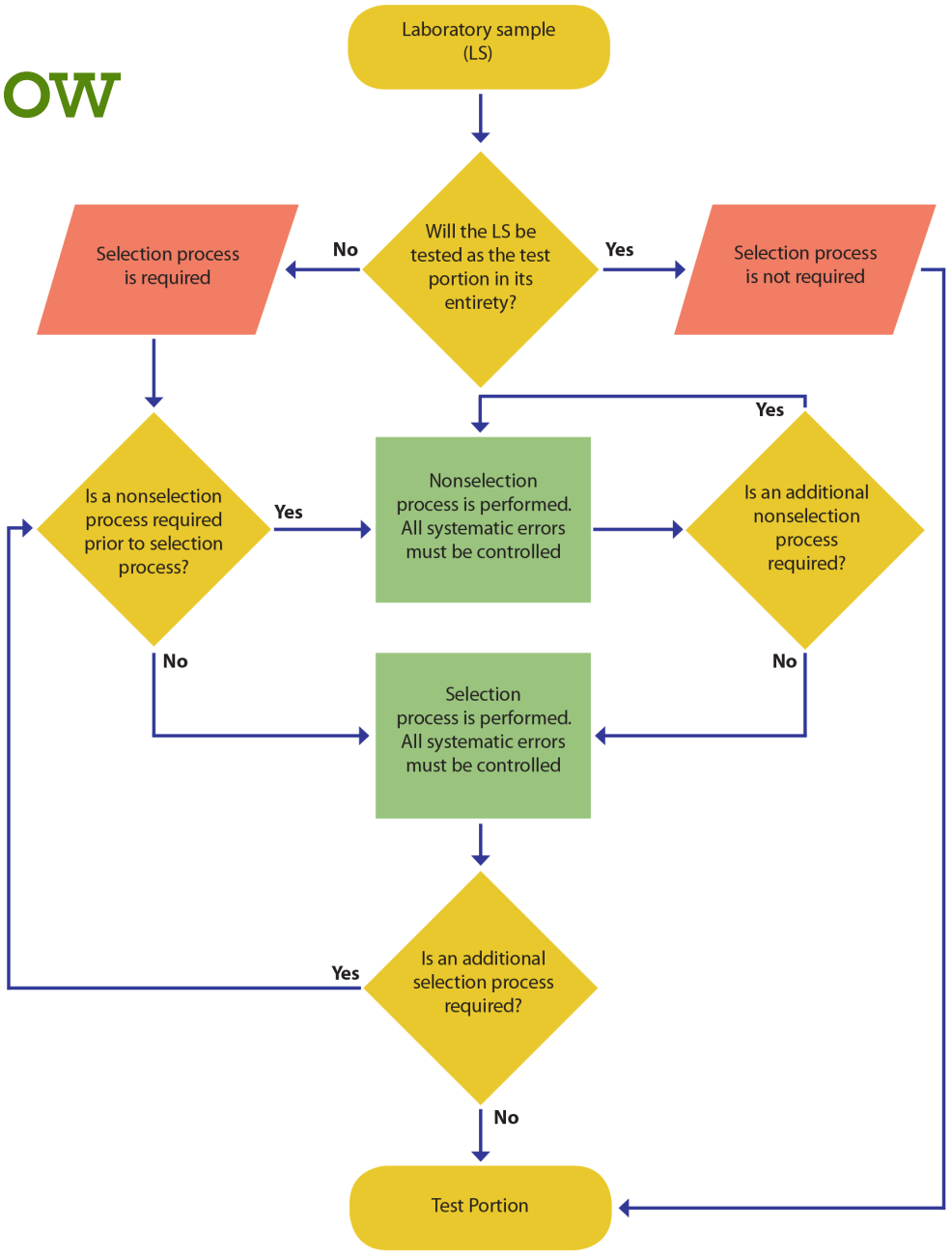
Data Assessment

- Sample Quality Criteria
- Documentation
- Quality control
  - Random errors
  - Systematic errors
- Global Estimation Error
- Inference

Data Assessment

- Need estimate of GEE
  - Replication for random errors
  - Need to check that systematic errors are in control
- Compare GEE with the tolerable error from the SQC
  - SQC states the minimum error or confidence required
  - If the GEE exceeds the SQC, then the data is not of sufficient quality to make a decision
- If the GEE exceeds the tolerable error
  - Identify the source of error and correct it
  - Reperform the sampling and analysis as necessary (after errors have been mitigated)

Workflow


Laboratory sample (LS)
Will the LS be tested as the test portion in its entirety?
No
Yes
Selection process is required
Selection process is not required
Is a nonselection process required prior to selection process?
Yes
No
Nonselection process is performed. All systematic errors must be controlled
Is an additional nonselection process required?
Yes
No
Selection process is performed. All systematic errors must be controlled
Is an additional selection process required?
Yes
No
Test Portion

Laboratory Sampling Processes

- Select an appropriate number of proportionate/equal increments at random. Never take one increment unless the material is a single phase liquid without suspended material
- Do not attempt to obtain an exact weight
- Avoid IWE when selecting increments
- Ensure the correct tool is used to avoid bias toward one particle size
- Do not scale down test portion mass without validating impact on the variance of FSE

Equipment Design

- Robust
- Inert
- Safe
- Easy to clean and maintain
- Equiprobable
  - Correct design
  - Easy to use
  - Equal increments

Equipment Design

- Flowability
- Capacity
- Heat generation
- Material hardness
- Material particle size
- Material losses

Equipment

- Selection Process
  - Spatulas
  - Riffler
- Nonselection Process
  - Mills
  - Knife mills
  - Crushers
  - Choppers
  - Blenders
  - Hand tools

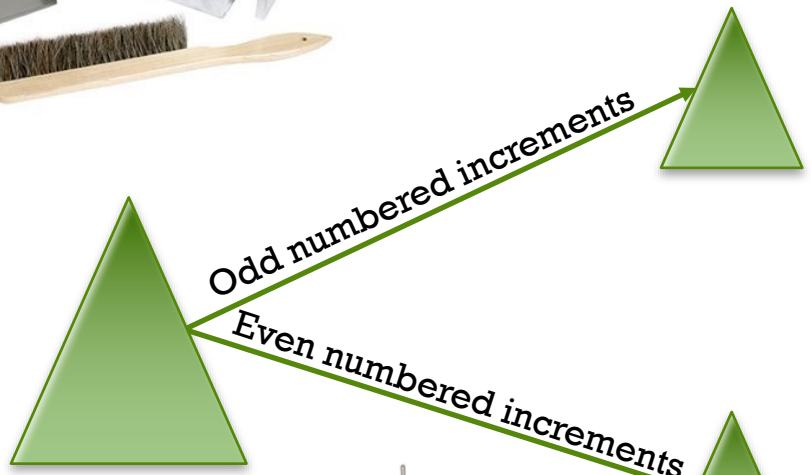
Courtesy of Jo Marie Cook
Florida Dept of Ag

Mass Reduction Technique (Selection Process)

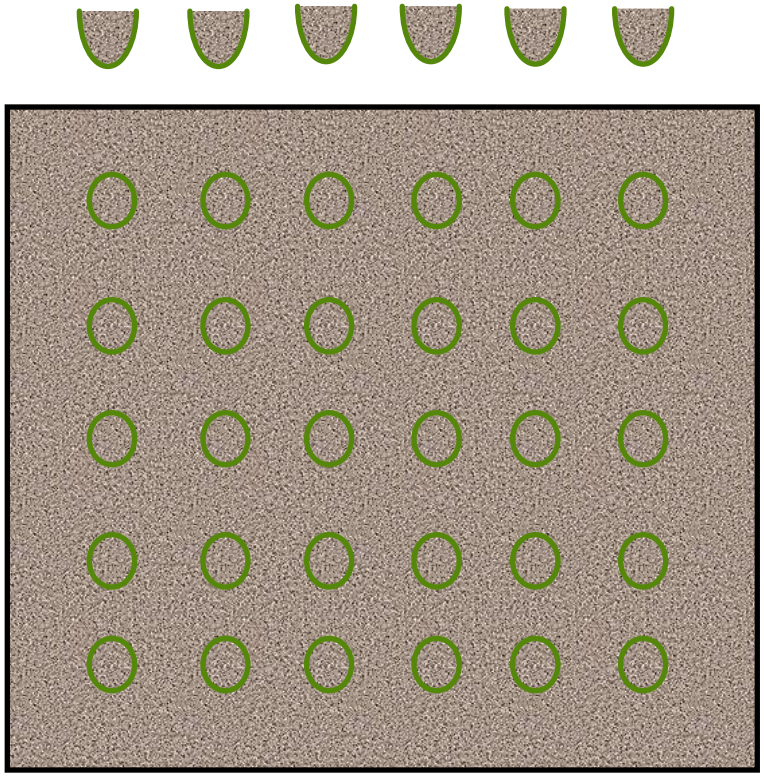
- Stationary riffler
- Rotary riffler
- Alternate shoveling
- Slab cake
  - Two dimensional
  - One dimensional

Splitting

- Stationary splitters
  - Not recommended
  - Very prone to operator error
  - Not enough increments
  - Difficult to clean and maintain
- Rotary splitters
  - "Correct"
  - Need flowable material
- Manually
  - Alternate shoveling



Two Dimensional Slab Cake



side view

top view

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Composites

- A composite sample is a combination of individual samples that are combined solely for the purpose of analytical efficiency
- The term is used incorrectly in many cases
- A composite sample is not used to represent a material
- While the act of collecting many increments to form a sample may appear in some aspects to mimic the compositing technique, they are fundamentally very different
- Compositing in agriculture has been used since the late 1800s

One of the First Composites

EXPERIMENT STATION

OF THE

KANSAS STATE AGRICULTURAL COLLEGE,

MANHATTAN.

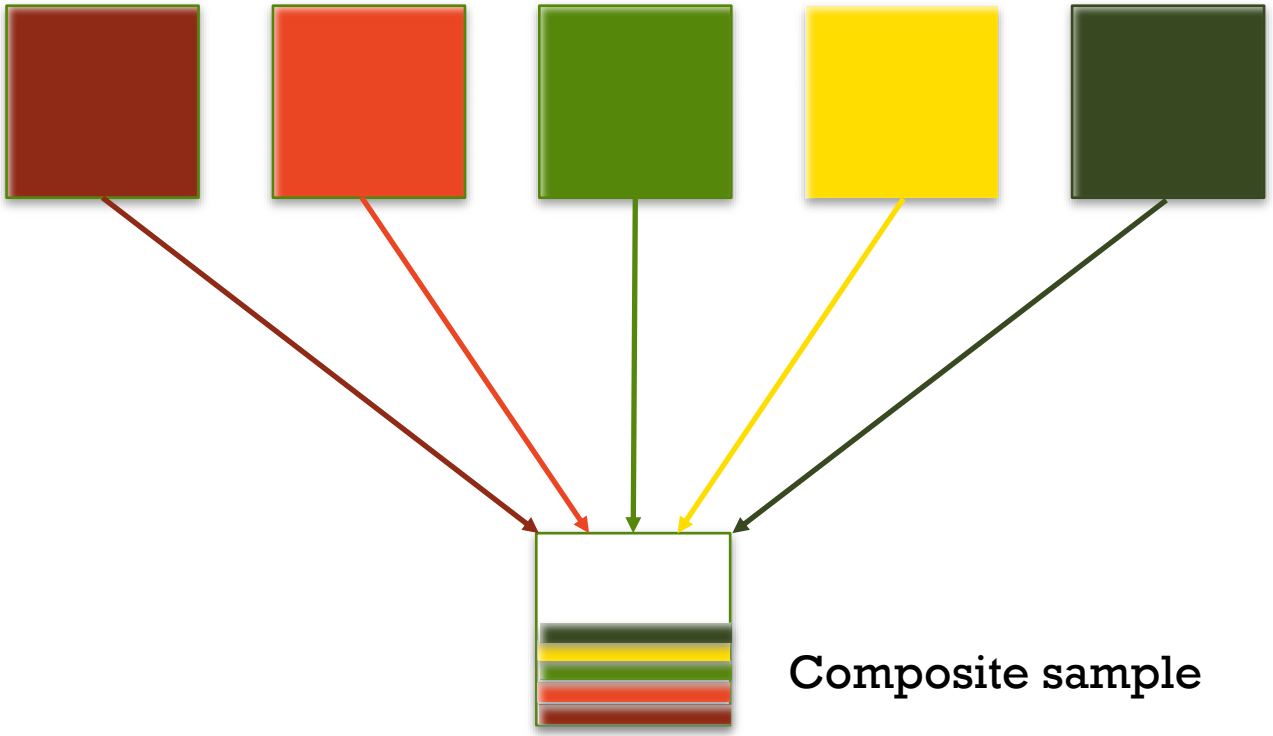
BULLETIN No. 125—MAY 1904.

(Issued September 1904.)

A composite sample of eight milkings just before the 1st and 15th of each month is made, which forms a part of the regular herd record. On May 10, 1899, a large herd of cows was put on green feed, and individual testing was done for seven days previous and for a number of days after the changing of the feed. This special test ran through the time of the regular test, and the comparison shown in the following table is between the average of the individual tests and the composite tests for the same milkings. The samples were taken by the milkers with no thought of the comparison being made. In taking the sample the milk was poured from the milking-pail into another pail, and a portion dipped out for the composite sample, using a dip-

GOOD Test Portions

Example of Composite



A representative amount from each individual sample are combined to form the composite sample.

Composite Example

- Chemistry Example
  - Regulatory limit is 10 parts per million (ppm)
  - Five samples are composited into one sample
  - If the concentration is below 2 ppm, then none of the original samples could have had a concentration above 10 ppm
  - If the concentration is above 2 ppm and below 10 ppm, then it is possible that one of the original samples has a concentration above 10 ppm and the five individual samples need to be reanalyzed
- Biological Example
  - Regulatory limit is the detection of a pathogen
  - If the pathogen is detected in the composite sample, then at least one of the original samples contained a pathogen

Composite

- Cautions
  - Need to have detectability in the composite sample
  - May have to re-analyze the original samples if violative
  - Not all materials are easy to composite
- Benefits
  - Allows analysis of many samples without increased analytical cost
  - Saves both time and money
    - Great for outbreak response
    - Great when tests are expensive or limited

Training

- Appropriate PPE and appropriate safety practices
- SQC
- TOS
  - Causes and control of systematic errors
  - Causes and control of random errors
- Data assessment
  - Interpretation of analytical results (including sampling error)
  - Inference to Decision Unit
- Equipment selection and use

Effects of Poor Laboratory Sampling

- Data is highly variable
  - Rejection of high or low values
  - Desire to retest/resample high or low values
  - Perform root cause investigation
  - Incorrectly determine material is out of specification
- Data is biased low or high
  - Incorrect analytical result
  - Repeat testing reconfirms incorrect result
  - Confidence increases even though inference is incorrect

This cannot be allowed to happen. You cannot retest out of a poorly designed sampling protocol.

Achieving Defensible Sampling

- Sample Quality Criteria
- Scientifically valid sampling protocol
  - More than just random locations
  - Address all sampling errors
- Valid quality control
  - Type
  - Amount
- Data assessment
- Inference

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Steps in Developing Defensible Sampling Protocol

- Determine Sample Quality Criteria
  - Question
    - Average, detection, identification
    - Level of concern (or detection)
    - How inference will be made
  - Decision Unit
    - Spatially
    - Temporally
    - Extraneous material

Steps in Developing Defensible Sampling Protocol cont…

- Determine Sample Quality Criteria (continued)
  - Confidence
    - Probabilistic
    - Statistical
- Determine material properties
  - Finite vs Infinite
  - Compositional Heterogeneity
  - Distributional Heterogeneity
    - Space
    - Time

Steps in Developing Defensible Sampling Protocol cont…

- Minimize/control Fundamental Sampling Error
  - Sample mass
  - Comminution
- Minimize/control Grouping and Segregation Error
  - Collection of increments
  - Mixing for some materials
- Maintain sample correctness
  - Equiprobable selection of all particles
  - Control EME, MRE, CIE, AIE, IWE
- Eliminate blunders

Steps in Developing Defensible Sampling Protocol cont…

- Develop sampling protocol
  - Mass
  - Increments
  - Comminution
  - Tools and techniques
    - Nonselection process
    - Selection process
  - Containers
  - Cleaning
  - Type and amount of quality control

Summary

- This has been a very brief overview of GOOD Test Portions
- Study GOODSamples and GOOD Test Portions
- Appendix A of GOOD Test Portions has some example calculations
- Study reference documentation in GOODSamples and GOOD Test Portions
- Additional training

GOOD Test Portions

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ENVIROSTAT Training in Sampling for DEFENSIBLE DECISIONS

GOOD Test Portions

Funding

This presentation was supported by the Cooperative Agreement No. U18FD006244 from the U.S. Food and Drug Administration (FDA). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of FDA. This project was funded with federal funds from a federal program.

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