Today, I will introduce you to some necessary tools for sampling for any food/agaricultural application, including microbiological testing, whether it be done as a preventative control or as a product monitoring activity.

The foundations for these tools are:
- Sample Quality Criteria
- Material Properties
- Theory of Sampling

These tools are required to yield "fit for decision" test results (representative sampling) through control of error in the measurement process.

Applicable for primary sampling or laboratory sampling.

A sample is representative only if:
- It is "correct" - systematic error is controlled to a negligible level
- Random error is within "fit for decision" criteria
- Lot of new terms

**Key Terms**
- **Decision Unit**: Material from which sample is collected and to which inference is made
- **Increment**: Individual portion of material collected by a single operation of a sampling tool and combined with other increments to form a primary sample.
- **Inference**: Estimating a concentration or characteristic about a larger amount of material from data derived from a smaller amount of material.
- Always use an adjective with "sample"!

**Sample Quality Criteria**
- What is the question?
- What is the decision unit?
- What is the desired confidence?
Sample Quality Criteria (SQC)

- A series of statements that clarify technical and quality needs to support defensible decisions (fit for purpose decisions)
- Three inputs: Question, Decision unit, Confidence

1. What is the question to be answered?
- What information is required?
  - What is the analyte?
  - What is the level of concern?
  - What type of data will be collected?
    - Characteristic of the decision unit?
    - Concentration of analyte(s) in the decision unit?
- How in the inference from the sample to the decision unit going to be made?
  - Direct inference (single result)?
  - Probabilistic inference (single result)?
  - Statistical inference?

2. What is the decision unit?
- The decision unit is the material from which the primary sample(s) is collected and to which the inference(s) is made.
- Maybe one or multiple decision units
- The decision unit establishes the scale of observation
  - Example: 10 pallets of 100 cases of 48 case tuna fish are present.
    - Is the average value of all 10 pallets of interest? All 10 pallets comprise a single decision unit (1)
    - Must each pallet be within spec? Each of the 10 pallets is a decision unit (10)
    - Must each case on every pallet be within spec? Each case is a decision unit (1000)
    - Must each can of tuna fish be within spec? Each can is a decision unit (48000)

2. Decision unit cont’d.
- Specific, definable, accessible
- Easy in the lab; difficult in the “field”
- Example coffee beans: individual bean, small package, shipping bag, or shipload of coffee beans.

3. What is the required confidence?
- If the risk related to an incorrect decision is high (e.g., people die), more confidence in the inference is required.
- To achieve more confidence in the inference, error must be controlled to a greater extent.
- To reduce error and increase confidence, may collect more increments and/or more mass and include more quality control.

Relationship among confidence, error & representativeness
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Material Properties
- Types of materials
- Types of heterogeneity
  - Compositional and distributional

Types of material elements – finite
- Finite Element Materials: consists of a finite number of discernible elements which can be individually identified and selected at random from the decision unit.

Types of material elements - infinite
- Infinite Element Materials: consists of a practically infinite number of indiscernible elements that cannot be individually identified nor collected individually.

Types of heterogeneity
- Compositional heterogeneity: exists when individual elements exhibit differing concentrations of the analyte of interest.
- Distributional heterogeneity: results from non-random distribution of elements.

Heterogeneity Considerations
- Compositional heterogeneity is a state of nature.
  - CH cannot be changed by mixing and moving of the material
  - Compositional heterogeneity can be changed by comminution (crushing, chopping, and grinding) the material
- Distributional heterogeneity is a transient state of nature.
  - DH can be changed by mixing and moving the material
  - Distributional heterogeneity is changed every time a material is stirred, blended, poured, piled, etc. In some cases it is increased (harder sampling problem) and in other cases it is decreased (easier sampling problem)

Theory of Sampling
Systematic & scientific procedure for designing sampling protocols to meet the QC.
Provides techniques for mitigating and estimating error
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Theory of Sampling (TOS)
- Developed by Pierre Gy from the 1950’s to the early 2000’s
- With finite element material you can use TOS or “classical statistics”
- TOS is essential for infinite element material
  - Individual elements that make up the decision unit cannot be individually selected at random—elements are selected in groups, called increments
- TOS describes
  - The final sample mass (combination of all increments)
  - How many increments need to be collected
  - The increment shape (correctness)

TOS
- SQC and Material Properties are inputs into TOS to determine the proper sampling protocol

+ Relationship of error to mass
- Presence of Compositional Heterogeneity (CH) leads to the Fundamental Sampling Error (FSE)
- FSE is related to the magnitude of compositional heterogeneity, mass and particle size distribution of the material.
  \[ s_{FSE} = \frac{\text{compositional heterogeneity} \times \text{diameter}^3}{\text{mass}_{sample}} \]
- To control FSE for a given CH, the mass must be increased, the diameter of the particles must be reduced or both. Note: particle size is cubed.

+ Relationship of error to increments
- Presence of Distributional Heterogeneity (DH) leads to the Grouping and Segregation Error (GSE)
- GSE is related to the magnitude of distributional heterogeneity and the number of increments selected.
  \[ s_{GSE} = \frac{\text{distributional heterogeneity}}{\text{number of increments}} \]
- GSE is typically not measured, but controlled to less than 1/3 of the FSE so it becomes an relatively insignificant source of error. GSE controlled by selecting sufficient increments to reduce until GSE contribution is negligible.
  - Increments must be at random
  - Increments must be the correct shape
  - 50 increments generally sufficient

+ Relationship of error to sample correctness
- Control of systematic errors (e.g., increment delimitation error and increment extraction error) are collectively referred to as sample correctness.
- Sample correctness is a function of
  - Properly designed tools and equipment
  - Appropriate use of the properly designed tools and equipment
- Tools and equipment must ensure that all elements have an equiprobable chance of being selected
- Failure to adhere to principles of sample correctness result in systematic error (bias) that cannot be estimated – therefore critical to adhere to principles
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GOOD Test Portion Working Group Members
- Jo Marie Cook, FL Department of Ag & Consumer Services
- Heidi Hickes, MT Department of Agriculture
- Lawrence Novotny, SD State University, retired
- Aaron Price, Canadian Food Inspection Agency
- Chuck Ramsey, EnviroStat, Inc., Subject Matter Expert
- Yvonne Salfinger, AFDO & APHL
- Nancy Thiex, AAFCO
- Sharon Webb, University of KY Regulatory Services

GOOD Test Portions: Guidance on Obtaining Defensible Test Portions

Working Group Members
(AAFCO, AFDO, APHL, FDA)
- Anthony Adeuya, FDA ORA
- Lourdes Andujar, FDA ORA
- Linda Benjamin, FDA ORA
- Rula Bhikha, FDA ORA
- Don Burr, FDA ORA
- Jo Marie Cook, FL
- Andy Crawford, Consultant
- Dan Danielson, TN
- Qian Graves, FDA CFSAN
- Bill Hart, ANSI/ACLASS
- Greg Hoffman, Pfizer
- Gary Krinke, WI Hygiene
- Michael McLaughlin, FDA ORA
- Kenneth McManus, MD
- Sarah McMullen, FDA ORA
- Angela Montalbano, NY
- Lawrence Novotny, SD ret
- Eric Pittman, FDA ORA
- Lynn Post, FDA CVM
- Chuck Ramsey, EnviroStat
- Brian Ravitch, FDA ORA
- Yvonne Salfinger, Consultant
- Barb Schroeder, MN
- Thomas Scott, FDA ORA
- Richard Stephens, FL
- Nancy Thiex, Consultant

LABORATORY WORKFLOW
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Nonselection processes

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

Selection processes

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

Importance of relative size of error contributions

- Remember that sampling to test results is a single process
- Error propagates as the square root of the sum of squares
- So, largest errors have the greatest impact (by squared) on the total error

\[ GEE = \sqrt{PSE^2 + TSE^2 + TAE^2} \]

- If primary sampling error is 35%, laboratory sampling error is 20% and analytical error is 10%, GEE is 41.5%

\[ 35\% \times 20\% \times 10\% \]

The organization applies GOODSamples principles, estimates sampling error, buys new sampling tools (at a cost of $1500) and drops sampling error to 20%

If primary sampling error is 38%, laboratory sampling error is 20% and analytical error is 8%, GEE drops to 34.8%

\[ 38\% \times 20\% \times 8\% \]

It has been established that the order of magnitude of primary sampling is by far the greatest source of error, second is laboratory sampling and third is analytical error. To reduce error, resources need to be applied to reducing sampling errors. Risk for each step needs to be evaluated.

Importance of relative size of error contributions

- The lab gets a new VITEK and real time PCR, and drops analytical error to 5% (at a cost of $150,000).
- If primary sampling error is 35%, laboratory sampling error is 20% and analytical error is 5%, GEE drops to 40.6% (from 41.5%)

- The lab improves their sampling protocols and purchases a new splitter (cost of $16,000), and drops sampling error to 10%
- If primary sampling error is 35%, laboratory sampling error is 10% and analytical error is 5%, GEE drops to 37.7%

Primary and Laboratory Sampling Protocols – Validation and QC

- Must first understand the source of errors and how to mitigate them to develop fit for purpose protocols.
- Validation of protocols must establish
  - Within the tolerable error (within performance specifications?)
  - Confirm sample correctness (absence of systematic errors IDE and IEE)
  - Validate sufficient mass (to control FSE)
  - Validate sufficient number of increments (to control GSE)

Incorporate QC Checks
- Bias checks
- Replicates
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Nancy Thiex
Nancy.Thiex@gmail.com

Food Research Institute, UW-Madison
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