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**Journal of Regulatory Science**

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Regulatory Science

Journal of Regulatory Science 02 (2016) 1–8

# Taking and Testing GOODSamples: A Systematic Approach for Representative Sampling from Field to Test Portion

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**Abstract**

- The following manuscript provides a brief introduction to a systematic approach for representative sampling from field to test portion
- Sampling from field to test portion is a single measurement process
- Error is introduced during each mass reduction stage
- Error propagates as the square root of the sum of squares, so the largest error components have a proportionately greater impact
- A representative sample must be correct (unbiased) and have sufficiently small imprecision
- The systematic approach for developing a sampling protocol is based on two key inputs: sample quality criteria (SQC) and material properties
- This approach requires knowledge of the total error in the measurement process (global estimation error or GEE), from primary sampling through final measurement
- Error is estimated through quality control events
- If the GEE meets the requirements of the SQC, a confident inference can be made
- GOODSamples can be a valuable tool for the forage and feed communities to evaluate current practices and to develop new protocols

*Keywords:*
Sampling, sample, primary sample, laboratory sample, sample preparation, theory of sampling, sample quality criteria, global estimation error, representative sample

## 1. Background and Introduction

The United States Food and Drug Administration (US FDA) awarded a five-year cooperative agreement to the Association of Public Health Laboratories (APHL), Association of Food and Drug Officials (AFDO) and the Association of American Feed Control Officials (AAFCO) to support the implementation of The Food Safety Modernization Act (FSMA). One of the Specific Aims in the cooperative agreement is "Harmonized Policies and Procedures for Equivalency of Data". A task under this Aim is to establish a working group to develop harmonized policies and procedures for sample collection, shipment, analysis, storage and retention of food and feed materials. The Sampling and Sample Handling Working Group effort is led by AAFCO due to its history of recognition of sampling and sample preparation as critically important.

Currently, protocols for sample collection are at least as varied as the number of organizations that collect samples. This wide variety of sample collection techniques does not lend itself to data equivalency among organizations. The goal of the working group is to develop a common sampling strategy for sampling food and feed. With this common sampling strategy, data can be evaluated with respect to "fit for purpose" or, more aptly, "fit for decision" criteria for any organization, project or situation. The first audience for the resulting guidance document is regulatory food and feed programs and their associated laboratories, including management, inspectors, quality assurance officers and laboratory personnel; however it is applicable for all of the related or similar industries. The guidance document has been titled *Guidance on Obtaining Defensible Samples* or *GOODSamples* [1, 2].

All of the concepts briefly introduced in this manuscript are dealt with in greater detail in *GOODSamples*[1]and in much greater detail in the resources listed at the end of this manuscript. Please consult them to clarify concepts and provide additional rationale. All of the concepts apply equally to primary sample collection and to mass reduction stages carried out in the laboratory. Comments for primary sampling personnel and laboratory

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personnel are integrated throughout this manuscript.

## 2. Terminology

Sampling terminology is problematic! A key aspect of developing the sampling guidance document was to assess sampling terms and come to an agreement on key terms and definitions so that communication could be constructive. Terms were chosen to be consistent with theory of sampling (TOS), ISO standards, and AOAC INTERNATIONAL documents. It is strongly recommended that the terms in *GOODSamples* be adopted. Key terms from *GOODSamples* follow [1].
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- *Decision Unit*: Material from which sample is collected and inference made.
- *Global estimation error* (GEE): Total errors in the entire measurement process, from primary sampling through final measurement.
- *Increments*: 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.
- *Sample*: A portion of a material selected from a larger quantity of material. The word "sample" should only be used with a modifier:
    - *Primary sample*: The collection of one or more increments taken from a decision unit according to a sampling protocol.
    - *Laboratory sample*: The material received by the laboratory.
    - *Analytical sample*: Prepared from the laboratory sample and from which test portions are removed.
    - *Test portion*: The quantity of material taken for measurement.

## 3. Global Estimation Error

Sampling is a process of making inference from analytical data through multiple mass reduction stages to a decision unit. There are currently various scenarios for organizational responsibility in the primary sample to test portion pathway. One organization may oversee the entire pathway. More commonly, the sampling activities are carried out by a separate organization from the laboratory activities. In either scenario, it must be recognized that there must be an accounting for the overall process from sampling through analysis. The laboratory itself is involved in a smaller scale "sampling" processes each time it selects a smaller mass from a larger mass (mass reduction). This may happen several times as the material moves through the laboratory workflow, with the final mass reduction stage being the selection of a test portion(s) for an analysis.

Both imprecision and bias errors are introduced in every stage of the measurement process. Generally, only analytical error is estimated while the larger error components associated with primary sampling and with laboratory sample preparation are unknown. Since error does not add directly, but propagates as the square root of the sum of squares, it follows that errors that are largest compared to others will have the greatest contribution to global estimation error (GEE), and mitigating the larger errors will have the most dramatic effect on lowering GEE.

*Eq.1:Global Estimation Error*= $\sqrt{(a^2 + b^2 + c^2 + ..... + n^2)}$
where *a, b, c, ., n* are individual imprecision errors for each sampling (mass reduction) stage and analysis.

## 4. What Is A Representative Sample?

A representative sample answers a question about a decision unit with an acceptable level of confidence.

- Imprecision is controlled by collecting an appropriate mass and number of increments to address heterogeneity
- Correctness (bias has been controlled to a negligible level) is achieved when every element in the decision unit has the same probability of being selected (equiprobable)
- Correctness is maintained when additional biases are not introduced during sample preparation and sample handling

A representative sample must:

- be correct, and
- have a sufficiently small imprecision

## 5. Sample Quality Criteria (SQC)

The framework for systematic scientific sampling consists of three components: Sample Quality Criteria (SQC), material properties and the TOS. The first of these three components, sample quality criteria (SQC) is a series of statements that clarify technical and quality needs as illustrated in Figure 1. The SQC answer the following questions:

1) What is the question to be answered?
    a. What information is sought?
        i. What is the analyte?
        ii. What is the level of concern?
    b. What type of data will be collected?
        i. Is a characteristic of the decision unit being evaluated?
        ii. Is an analyte concentration in the decision unit being sought?
    c. How is the inference going to be made?
        i. Direct inference (from a single result)?
        ii. Probabilistic inference (from a single result)?
        iii. Statistical inference e.g., average of multiple results, confidence interval?
2) What is the decision unit?

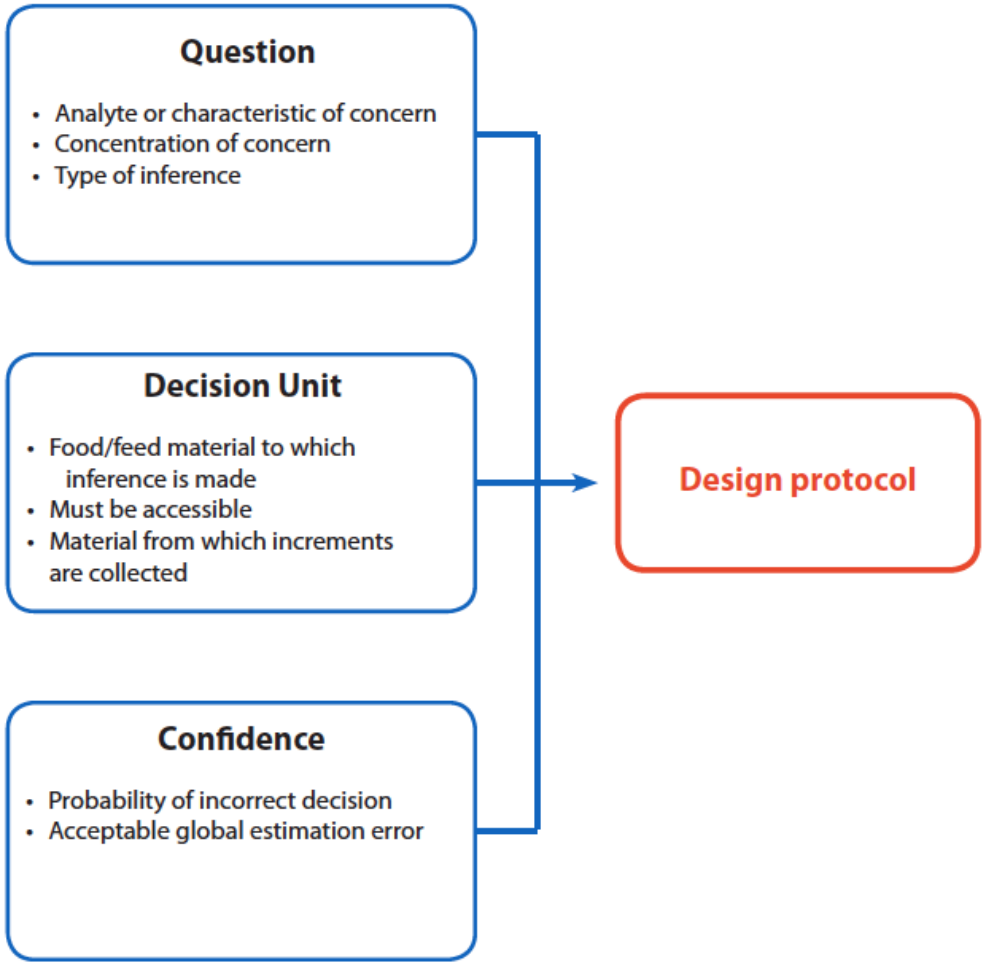


Figure 1: Sample quality criteria (SQC)

a. The choice of decision unit has a large impact on the sampling protocol. The scale of information drives the decision unit [3]. For example, bales from a single alfalfa field are loaded onto 10 trucks, each containing 100 bales. What information is desired?

   i. The average value of the entire field? If so, all 10 truckloads comprise a single decision unit.

   ii. The average value of each of the 10 trucks. If so, each of the 10 trucks is a decision unit (10 decision units).

3) What is the desired confidence in the inference?

   a. The greater confidence desired, the less GEE can be tolerated.

   b. The desired confidence is generally related to the risk and consequences associated with an incorrect decision.

## 6. Material Properties

The component of the framework for scientific sampling, material properties, refers to the intrinsic properties of the material that comprises the decision unit that must be considered when developing sampling protocols. Material properties include element type and heterogeneity.

Elements are the individual components (e.g., particles or fragments for solid materials, molecules for liquids, particles and molecules for slurries) comprising the decision unit. They can be either finite or infinite in nature. Finite element materials are materials composed of elements that can be individually identified and individually selected at random. Infinite element materials are materials composed of elements that cannot be individually identified nor individually selected at random. See Figure 2.

The second aspect of material properties that must be considered is the heterogeneity. Two types of heterogeneity exist: compositional heterogeneity and distributional heterogeneity. Compositional heterogeneity exists when the individual elements that make up the decision unit exhibit differing concentrations of the analyte of interest (e.g., alfalfa stem vs. leaf

Figure 2: Tomatoes representing a finite element material and flour representing an infinite element material.

tissue, corn vs. added mineral). Compositional heterogeneity always exists to some degree and cannot be altered without comminution.

Distributional heterogeneity results from non-random distribution of elements within the decision unit (e.g., settling of small, dense fines to the bottom of a container). Distributional heterogeneity always exists to some degree and can be altered with physical manipulation of the material (vibration, mixing, etc.).

## 7. Theory Of Sampling (TOS)

The third component of framework for systemic scientific sampling, the theory of sampling (TOS), is a systematic and scientific process for designing sampling protocols that meet the SQC. TOS provides techniques for mitigating and estimating error in sampling [4–6]. It is most commonly applied to infinite element materials elements since they must be selected in "groups" called increments. TOS describes the final sample mass (combination of all increments), how many increments need to be collected and dictates sample correctness.

Compositional heterogeneity results in an imprecision error, Fundamental Sampling Error (FSE). FSE must be addressed with every mass reduction stage from primary sampling through selection of the test portion. FSE can be controlled to any level by collecting sufficient mass and/or reducing particle size.

$$\text{Equation 1 : } FSE^2 \propto \frac{Cd^3}{m_s}$$

C = sampling constant, unique for each material
d = diameter of 95% percentile of largest particles (cm), and
$m_s$ = mass of the sample (g).

Because of the relationship of FSE to particle size, mass and error, mass reduction must take the relationships into consideration. Any type of mass reduction without considering these relationships is unacceptable. The proper mass to collect is based on the heterogeneity of the material, so it is inappropriate to collect an identical mass as standard practice for all primary sampling situations.

Distributional heterogeneity leads to an imprecision error, Grouping and Segregation Error (GSE). GSE must also be addressed with every mass reduction stage from primary sampling through selection of the test portion. GSE is controlled to any level by collecting sufficient number of random increments. There is no simple calculation to determine the number of increments to collect, but three approaches can be used to reduce the GSE: reduce the FSE, increase the number of increments, and reduce the distributional heterogeneity of the material. The proper number of increments to collect is based on the heterogeneity of the material, so it is inappropriate to collect an identical number of increments as standard practice for all primary sampling situations. Mixing prior to sampling (such as prior to taking a test portion in the laboratory) may be effective, but only if the material particles have a relatively uniform shape, size and density. It is generally unacceptable to select a single, non-random increment as a test portion.

Bias errors are also addressed in TOS as the notion or condition of "correctness". Sample correctness is achieved when selection of elements at increment locations is equiprobable, and it is controlled by proper use of a correctly designed sampling tool. Once sample correctness is achieved with the primary sample, it must be maintained in subsequent mass reduction stages all the way to the test portion.

## 8. Tools

TOS mandates that sampling tools must be correctly designed and used properly. The correct tool shape is related to the dimensions of the decision unit. Tool shape and respective dimension that are most commonly used for infinite element materials are:

- Slices (cross stream cuts) for a one dimensional flowing stream
- Cylinders (similar to probe) for a two dimensional layer

Sampling tools should not only be correct, they should also:

- Be simple and reliable
- Be easy to decontaminate
- Be inert to the sample/analyte
- Collect increments of equivalent size

## 9. Evidentiary And Analyte Integrity

The purpose of evidentiary integrity is (1) to be able to tie a test result to a specific decision unit; (2) to demonstrate that the sample has not been adulterated or compromised during any step of the process from primary sample collection through generation of the analytical data; and (3) to assure that analyte integrity has been maintained. Analyte integrity is the assurance that physical, chemical, biological and/or radiological characteristics of interest in the decision unit have been maintained. Considerations for analyte integrity include preservatives, containers, holding times, sampling techniques and packaging and shipping procedures.

## 10. Laboratory Sampling And Preparation

As stated previously, all concepts apply equally for primary sampling and laboratory sampling activities. There are three important responsibilities for laboratories related to sampling:

- To respect the decision unit
- To ensure that analyte integrity is maintained during sample preparation and storage
- To obtain representative test portion(s) of the laboratory sample

Comminution (e.g., grinding) is a technique frequently used in laboratories to control FSE. A single type of particle size reduction equipment cannot handle all types of materials and it is imperative that laboratories have adequate equipment to handle the types of materials they will encounter. When evaluating comminution equipment, it is critical to ensure that:

- it is of sufficient capacity to process the laboratory sample,
- it will reduce the particle size sufficiently to control FSE, and
- it will produce a uniform shape and size to control GSE
- it can be sufficiently cleaned between materials.

When selecting comminution (e.g., grinding) equipment, consider the following:

- physical and chemical properties of the material,
- initial maximum particle size of the material,
- final desired particle size and the range of permissible particle sizes,
- needed capacity and throughput,
- inertness to analyte of interest,
- complete sample recovery,
- ease of cleaning, disinfecting, and sterilization.

A common laboratory preparation practice is splitting of samples for mass reduction. As with any form of mass reduction, minimum mass to control FSE must be a primary consideration and sufficient mass must be available so that the final reduced mass still has acceptable FSE. A second consideration is choosing a technique that provides sufficient increments to control the GSE (more increments results in lower GSE). Common splitting techniques used for this form of mass reduction are: rotary splitting, fractional shoveling, stationary riffle splitting and coning and quartering [7]. Rotary splitting is by far the most precise because it selects more increments than other techniques followed by fractional shoveling, stationary riffle splitting and lastly, coning and quartering. A third consideration is the correctness of the increment selection. Coning and quartering is a very poor mass reduction method and is strongly discouraged due to the large error it generates. The common practice of arbitrarily splitting an unground laboratory sample for the purpose of analytical efficiency without first reducing particle size to control FSE is very questionable.

Finally, laboratories should be equally concerned with validating sample preparation procedures as with validating analytical methods, especially given that the error associated with sample preparation procedures is greater than error associated with most analytical procedures [8]. AAFCO published *Guidelines for Preparing Laboratory Samples* in 2000 [9] is available for purchase at http:// www.aafco.org/Publications/QA-QC-Guidelines-for-Feed-Laboratories. It was adopted and republished by ISO as ISO 6498:2012 [10] . The AAFCO document is currently under revision to ensure compliance with *GOODSamples*, and will be available in late 2016 free of charge.

## 11. Quality Control

Quality control is a tool to assess data quality that is widely implemented in laboratories but seldom implemented in sampling or sample preparation. This absence of quality control from sampling activities is a practice that needs immediate attention. Quality control is used to estimate global estimation error, to determine if a process is in control and to validate a method or protocol.

Quality control checks for bias are blanks. Blanks can be used to check for contamination from containers, the environment or carryover from tools and equipment.

Quality control checks to estimate imprecision are replicates. Replicates can be implemented at multiple points to sort out error contributions form various mass reduction stages (see Figure 3). GEE can be estimated from data resulting from replicated primary samples. Replicating test portions from the same analytical sample provides an estimate of the imprecision associated with selection of the test portion and the test (no information about the preceding stages).

## 12. Inference

Inference is the process of estimating (or inferring) a concentration or characteristic about a decision unit based on a sample(s) collected from that decision unit [11]. Inference is an important aspect of the sampling process often overlooked and misunderstood. Inference occurs at every stage of mass reduction in the primary sample to test portion pathway. Two forms of inference are presented and discussed in *GOODSamples*: estimating the average analyte concentration in a decision unit

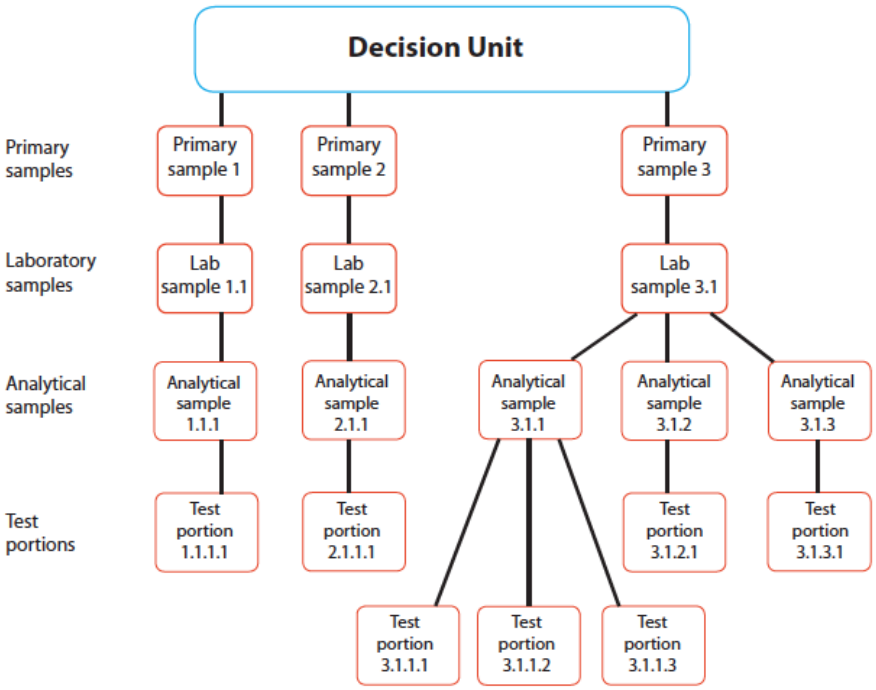


Figure 3: Levels of replication using triplicates.

and estimating a percentage of decision units that have some characteristic or concentration.

## 13. Data Quality Assessment

The final step in the scientific and systematic process is data quality assessment. It includes review of documentation, evaluation of quality control and estimation of the GEE. The documentation should support the premise that the correct protocol(s) was followed. Evaluation of quality control blanks should support the absence of contamination below a critical level. Evaluation of quality control replicates should indicate that they are within an acceptable range. The GEE should be below 35%, and meet SQC. The proximity of the actual concentration and GEE to the specification limit need to be examined to determine if a defensible decision can be made.

## 14. Conclusion

A shortcoming of current practices is a lack of knowledge of the error in the entire measurement system and in reported data. In addition, there is no systematic process to ensure that sampling protocols meet project objectives. *GOODSamples* address both of these shortcomings. It not only provides a systematic process to develop sampling protocols to meet project objectives, but it also provides a mechanism to evaluate existing protocols to determine if they meet the intended objectives. *GOODSamples* also provides a system for estimating the error in the entire measurement system (GEE), which is critical to data integrity. Implementing *GOODSamples* leads to equivalency of data and defendable and cost effective decisions related to feed nutrition and feed safety. *GOODSamples* is a valuable tool for the forage and feed communities and addresses the limitation of current practice.

The sampling principles described in this manuscript as applied to food and feed were recently published in the Journal of AOAC INTERNATIONAL in a Special Guest Editor series [12–24].

## 15. Highlights

- This manuscript provides a brief introduction to a systematic approach for representative sampling from field to test portion
- Sampling from field to test portion is a single measurement process
- Error is introduced during each mass reduction stage
- Error propagates as the square root of the sum of squares, so the largest error components have a proportionately greater impact
- A representative sample must be correct (unbiased) and have sufficiently small imprecision
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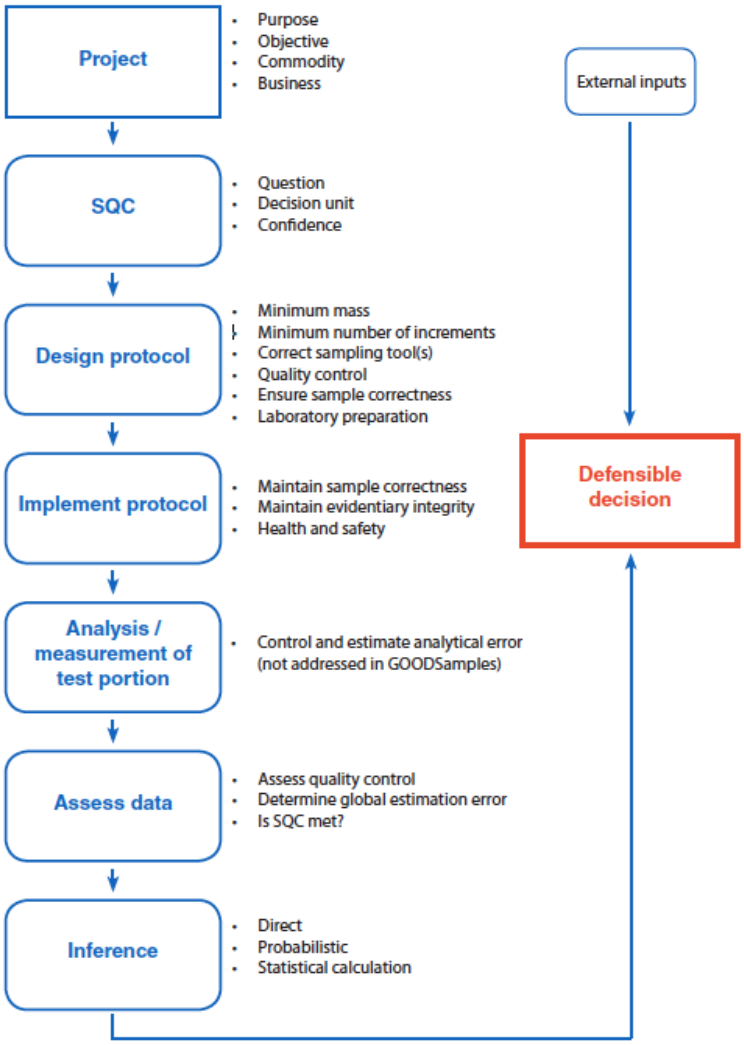


Figure 4: Overview of the *GOODSamples* approach for defensible decisions

- Error is estimated through quality control events
- If the GEE meets the requirements of the SQC, a confident inference can be made
- GOODSamples can be a valuable tool for the forage and feed communities to evaluate current practices and to develop new protocols

## 16. Acknowledgment

The authors would like to acknowledge APHL, AAFCO, AFDO, FDA leads, and all the workgroup members involved in the effort to develop national sampling guidelines. Working Group members are: Rina Bhikha, FDA ORA, Long Beach, CA; Donald Burr, FDA ORA, Rockville, MD; Jo Marie Cook, Florida Department of Agriculture and Consumer Services, Tallahassee, FL; Andy Crawford, Consultant to AAFCO, Crawford Science Consulting, Hacienda Heights, CA; Dan Danielson, Tennessee Department of Agriculture, Nashville, TN; Qian Graves, FDA CFSAN, College Park, MD; William Hirt, ANAB, Alexandria, VA; Gregory Hoffman, Zoetis, Kalamazoo, MI; Lourdes Andujar, FDA ORA, Aguada, PR; Michael McLaughlin, FDA ORA, Rockville, MD; Kenneth McManus, MD Department of Agriculture, Annapolis, MD; Sarah McMullen, FDA ORA, FDA Southeast Regional Laboratory, Atlanta, GA; Angela Montalbano, New York State Department of Agriculture and Markets, New York, NY; Lawrence Novotny, retired from South Dakota State University, Brookings, SD; Charles Ramsey, EnviroStat, Inc., Windsor, CO; Yvonne Salfinger, Consultant to AFDO and APHL, Denver, CO; Barb Schroder, Minnesota Department of Agriculture, St. Paul, MN; Thomas Scott, FDA ORA WEACA, Winchester, MA; Richard Stephens, Florida Department of Agriculture and Consumer Services, Tallahassee, FL; Nancy Thiex, Consultant to AAFCO, Thiex Laboratory Solutions, LLC, Brookings, SD.

This publication was supported by Grant No. U18FD004710-01 from the US FDA. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of FDA, AAFCO, APHL or AFDO. This

project was 100% funded with federal funds from a federal program of $1.3M.

The publication has also been presented in the 2016 Southwest Nutrition Conference Proceedings and in the Southwest Nutrition Conference on February the $18^{th}$ and $19^{th}$ 2016 in Tempe, Arizona, USA.

## 17. Article Information

The article was received January the 5th, 2016, in revised form January the 31st, 2016 and available on-line May the 5th, 2016.

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