AAFCO
Association of American Feed Control Officials

Quality Assurance/Quality Control Guidelines For Feed Laboratories

supplement to

ISO/IEC 17025:2005
General Requirements for the Competence
of
Testing and Calibration Laboratories

including

AAFCO Accreditation Requirements

2014

Preface

The AAFCO Laboratory Methods and Services Committee deals with issues that affect feed laboratories. The Quality Assurance (QA) Subcommittee is assigned to provide guidelines to feed laboratories and the government entities to which they provide analytical services. The objective of the committee is to provide guidelines which are practical and affordable for the feed laboratories. Once implemented, these practices would assure that data of appropriate quality are generated by laboratories for feed programs, resulting in reliable and defensible analytical test results.

The Food Safety Modernization Act of 2011 (FSMA:2011) mandates that regulatory feed laboratories submitting test results to the Food & Drug Administration (FDA) in support of the Integrated Food Safety System (IFSS) be accredited to ISO/IEC 17025 although the FDA has yet to set an implementation date for compliance with FSMA:2011. To assist the feed laboratory community in their accreditation efforts the FDA provided funding to AAFCO to revise the Quality Assurance/Quality Control Guidelines for Feed Laboratories. This project was one of many multi-organization projects funded through a federal cooperative agreement program.[1]

AAFCO recommends that laboratories use these guidelines to design and implement a quality system that meet the needs of their laboratory and regulatory program. Laboratories are encouraged to study the accreditation requirements contained in Volume I, the guidance contained in Volume II, and the example documents and forms contained in Volume III of these guidelines to establish a quality system appropriate to their operations.

The guidance and examples offered in Volume II and Volume III represent one or more ways a laboratory may meet ISO/IEC 17025 requirements. There are certainly acceptable equivalent mechanisms a feed laboratory may utilize to meet the ISO/IEC 17025 “General Requirements for the Competence of Testing and Calibration Laboratories” standard.

Should any laboratory adopt portions or all of the guidance or examples as part of their documented quality program, the specific policies and practices should be detailed in the laboratory’s quality system. Once the adopted policies and practices are incorporated into a laboratory’s quality system, they become auditable requirements for that laboratory.

The first version of this manual was published in August 1998. This second version of this manual was published in 2007. The third version of this manual was published and adopted by the AAFCO Board of Directors in 2014.

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AAFCO Quality Assurance/Quality Control Guidelines for Feed Laboratories, 2014 Edition

Supplement to ISO/IEC 17025:2005
General Requirements for the Competence of Testing and Calibration Laboratories

ERRATA SHEET – February 2016

The following correction has been made to **VOLUME III Table 2 for Equipment, Calibration, Verification and Maintenance**

		Verification		**Maintenance**	
Equipment	**Calibration**	**Parameter checked**	**Minimum Frequency**	**Recommended**	**Minimum Frequency**
Water Purification System		Resistivity/conductivity	Weekly for Stills, Monthly for other systems, or as recommended by method	As recommended by manufacturer or per laboratory procedure	As recommended by manufacturer or per laboratory procedure

VOLUME I

Accreditation requirements as specified in ISO/IEC 17025:2005 “General Requirements for the Competence of Testing and Calibration Laboratories”

Additional AAFCO Requirements for Feed Laboratory Accreditation to ISO/IEC 17025

The following additional requirements are listed according to the corresponding section of ISO/IEC 17025.

4.6 Purchasing

State/government purchasing systems often approve or restrict vendors based on price, accounts receivable, or state-wide contract agreements. The laboratory must maintain its own list of quality critical approved suppliers based on its evaluation of the quality of goods or services received. At times this may be used to support state/government purchasing systems regarding why one vendor should be used over another.

5.4.1 Method Selection

Analytical methods are evaluated based on attributes such as accuracy, precision, specificity, sensitivity, detectability and practicality. Compromise between attributes is inherent in the selection of methods. However, any method selected for use must be appropriate to the requirements of the regulatory function and within the capabilities of the laboratory staff (fit for purpose). Depending on the documentation available on a method, varying degrees of method verification or validation are recommended before it is adopted for routine use.

5.4.2 In-House Method Verification of Standard and/or Official Methods

These include, but are not limited to, methods that have been collaboratively studied, approved and published by AOAC, AOCS, or ISO, as well as compendium methods published by USDA, FDA, EPA, AWWA, US-CFR and USP with a scope applicable to the intended use. When a laboratory chooses a standard or official method for use, something is already known about the method’s performance in other laboratories, but it is necessary to establish and document the method’s performance in-house. Laboratories should verify their own ability to achieve satisfactory performance of the method before any customer samples are analyzed. The type of method and its intended regulatory use will influence the details of the in-house verification. At a minimum, representative matrices and concentration ranges shall be included in the in-house verification.

5.7 Sampling

The laboratory shall ensure that sample integrity and representativeness is maintained for all processes under their control. Feed laboratories are, in many instances, not responsible for field sampling. As such, sampling considerations begin for the laboratory upon receipt of the laboratory sample.

When the laboratory is also the sampling entity, the laboratory shall have in place sampling plan(s) and sampling procedures that define the types of samples collected, sampling equipment and the process(es). Sample collection procedures used by the laboratory shall be based on appropriate scientific/statistical principles. Properly trained field staff should collect, document, preserve and ship samples using established guidelines.

The laboratory shall have documented procedures for sub-sampling and preparation to ensure that representative test portions are used for analyses. The laboratory shall have written procedures dealing with the preparation of analytical samples covering the range of sample types and analytical tests. The Laboratory sampling procedure(s) shall document the process to be used for reception, identification, processing (e.g. grinding, mixing), sub-sampling (if applicable), storage and disposal of feed samples received at the laboratory.

5.9 Proficiency Testing Samples / AAFCO Check Sample Program

Participating in proficiency testing schemes provides laboratories with an objective means of assessing and demonstrating the reliability of the data they are producing. Although there are several types of proficiency testing schemes, as described in ISO/IEC 17043 “Conformity assessment – General requirements for proficiency testing”, they all share a common feature: test results obtained by one testing laboratory are compared with those obtained by one or more other testing laboratories. Laboratories are encouraged to participate in the AAFCO Check Sample Program because of its broad inclusion of analytes in feed matrices.

VOLUME II

AAFCO Guidance for Compliance with ISO 17025

Table of Contents

Guidance for Compliance with ISO 17025

1. Scope

This Volume contains AAFCO quality assurance guidance for laboratories pursuing third-party accreditation to ISO/IEC 17025 compliant quality programs or laboratories implementing an ISO/IEC 17025 compliant quality program. AAFCO does not present this guidance as requirements for accreditation or compliance since individual laboratories should decide on the specific policies and practices appropriate for their operations. The Animal Feed Regulatory Program Standards (AFRPS) also recognize this guidance for feed laboratories supporting an Animal Feed Regulatory Program.

The guidance offered in this Volume represents one or more ways a laboratory may meet ISO/IEC 17025. There are certainly acceptable equivalent mechanisms a feed laboratory may utilize to meet the ISO/IEC 17025 "General Requirements for the Competence of Testing and Calibration Laboratories" standard.

Should any laboratory adopt portions or all of this guidance as part of their documented quality program, the specific policies and practices should be detailed in the laboratory's quality system. Once the adopted policies and practices are incorporated into a laboratory's quality system, they become auditable requirements for that laboratory.

1.1 Program Description

State, federal and other government officials of the fifty states, territories and Canada are charged with the responsibility of enforcing laws regulating the manufacture, labeling, distribution and sale of animal feeds and remedies within their jurisdiction. The philosophy regarding such feed regulation is based upon the protection of the consumers, as well as the regulated industry, the safeguarding of the health of man and animal and a provision for orderly commerce. To ensure that feeds meet label claims and pose no threat to health, laboratories are involved in the analysis of animal feed and feed ingredients. These laboratories provide analytical services to the governmental entities charged with the responsibility of administering law and regulation. They may be directly associated with the governmental entity or they may contract for services with the governmental entity. The relationship between government entities and laboratories is different in each jurisdiction, so this section will vary among laboratories and among jurisdictions.

2. Normative References

2.1 ISO/IEC 17025-2005: General Requirements for the Competence of Testing and Calibration Laboratories

2.2 CITAC/EURACHEM "Guide to Quality in Analytical Chemistry", 2002

2.3 EURACHEM/CITAC "Traceability in Chemical Measurement", 2003

2.4 EURACHEM/CITAC guide: Quantifying Uncertainty in Analytical Measurement, Third edition, (2012), S. L. R. Ellison and A. Williams (Eds). ISBN 978-0-948926-30-3. Available from www.eurachem.org.

2.5 EURACHEM "Accreditation for Microbiological Laboratories", 2002

2.6 Codex Alimentarius "Guidelines on Analytical Terminology", CAC/GL 72-2009

2.7 BIPM/OIML "International vocabulary of metrology – Basic and general concepts and associated terms (VIM)", BIPM JCGM 200:2012 or OIML V 2-200

2.8 IUPAC "Compendium of Chemical Terminology", IUPAC Gold Book

2.9 AOAC Guide: How to meet ISO 17025 Requirements for Method Verification 2008

3. Terms and Definitions

3.1 Definitions

Quality Assurance (QA) is an essential part of laboratory policy, which ensures that the feed laboratory consistently provides reliable and defensible analytical services. QA is designed to ensure appropriate laboratory quality control and quality assessment procedures are practiced and documented in an efficient and economic manner. The establishment of QA is the responsibility of the laboratory management.

Quality Control (QC) is the specific laboratory activities whose purpose is to measure and control the quality of the analytical data so it meets the needs of the feed program.

Quality Management is the overall system of laboratory activities whose purpose is to provide assurance that the overall quality control activities are being done effectively. It involves a continuing evaluation of the laboratory procedures and results and performance of individual methods.

Standard Operating Procedures (SOPs) are written procedures which describe routine laboratory activities in detail. SOPs are prepared for any routine activities that affect the overall quality and defensibility of analytical data. For Feed Laboratories, these activities include, but are not limited to, sample receiving and handling, analytical methods, standards preparation and calibration, instrument maintenance and calibration, laboratory safety, personnel training and analytical quality control (replicates, blanks, spikes, control samples, etc.).

4. Management Requirements

4.1 Organization

The quality system should include an organizational chart showing the relationships and the lines of communication within the laboratory. The chart should show the route by which information is exchanged between the feed control official (commissioner, program manager, etc.) and the laboratory. In addition, the laboratory should list the responsibilities of key personnel (e.g. Laboratory Director, Technical Manager, Sample Custodian, Quality Assurance Officer, Chemist, Microbiologist, Technicians) in the Quality Management System. This will be different for each laboratory, since each laboratory has a unique structure.

4.1.1 Ethics

The quality system should include a code of ethics which sets standards of behavior for each employee in the laboratory so that the reputation of the laboratory remains above reproach. The survival of the laboratory rests heavily on its reputation. This section again will depend on individual state statutes or organizational policies and vary from laboratory to laboratory. See AAFCO QA/QC Guidelines, Volume III; Attachment 1 for a brief summary of ethics resources.

4.2 Management System

A Feed Laboratory will find great benefit in a quality management system. The reduction of repeat analyses and corrections will help offset the cost of the quality program. The benefits are improved data quality, improved credibility of the laboratory, improved expertise of staff, improved staff morale, improved defensibility of data, and potential for sharing of data among agencies. There are costs associated with a quality program. Start-up costs may be significant, but costs tend to decline rapidly once the system is operational and functioning efficiently. Laboratories may adopt the AAFCO Guidance in accordance with their individual needs. This guidance represents the recommended policies and practices to assure data reliability and defensibility.

For a quality assurance program to be successful there must be a plan. A quality assurance plan is composed of three essential components:

- Prevention of Errors: Prevention requires an orderly program of planning and positive actions before or during analyses to ensure that analytical systems are functioning properly. Examples are quality control planning, training, calibration of instruments, instrument maintenance and frequent standardization of "standard solutions".
- Assessment: Assessment is a form of control that includes periodic checks on performance to determine precision and accuracy. Examples include analysis of duplicate and laboratory control samples, peer review of chart readings and calculations and validation of methodology.
- Correction: Correction is action taken to determine causes of quality defects and to restore proper functioning of the analytical system. Examples are trouble shooting to correct malfunctioning equipment, examination of additional check samples, re-evaluations of methodology and retraining.

4.2.1 Quality Assurance Objectives

Every quality assurance program must have a set of objectives. These must be clearly stated and supported by both management and staff. The objectives of a program will vary somewhat from one laboratory to another. The overall objective for quality assurance programs in Feed Laboratories is:

- To help ensure that analytical work will withstand legal scrutiny in regulatory actions.
- To maintain a continuing assessment of the accuracy and precision of data generated by analysts within the laboratory.
- To provide a measure of the accuracy and precision of analytical methods.
- To identify imprecise, inaccurate or otherwise problematic methods.
- To detect training needs within the laboratory.
- To identify facility or equipment needs.
- To provide adequate and appropriate record keeping and report writing.
- To provide a permanent record of instrument performance as a basis for validating data and projecting repair or replacement needs.
- To help ensure sample integrity and representativeness
- To upgrade the overall quality of laboratory performance.
- To maintain a continuing assessment of data comparability.
- To make a periodic assessment of data completeness.

4.3 Document Control

Documents are written policies and procedures which describe routine laboratory activities in detail. For Feed Laboratories these activities may include: sample receiving and handling, analytical methods, standards preparation and calibration, instrument maintenance and calibration, laboratory safety, personnel training and analytical quality control (replicates, blanks, spikes, control samples, etc.).

Document control should at a minimum include the following types of documents:

- Quality Manual,
- Policies,
- SOPs and Analytical Methods,
- Work Instructions,

- Template Forms, and
- Appropriate External Documents.

The use of controlled documents can benefit the laboratory in the following ways:

- Outline the critical aspects of a procedure and help to assure that these aspects are appropriately emphasized during the conduct of the procedure.
- Assure consistency among individuals who are performing a procedure. When controlled documents are in place, individuals do not have to rely on memory or word-of-mouth communication of procedures.
- Assure that appropriate documentation and data collection occur by outlining the records to be generated during the performance of a procedure.
- Assist in training individuals to clearly communicate the specific method for performing a procedure. This helps prevent misunderstandings.
- Assure that personnel perform work according to the most up-to-date standards or methods.
- Improve planning and organization. Preparing documents requires an individual to think through the process to be described.
- Assist in the effort to standardize, and this improves efficiency.

Properly written policies and procedures are a critical component of the laboratory. Like other components of a viable quality management system, documents can be approached in different ways, depending on the needs and requirements of a given program. Policies and procedures are written documents specifying the processes that must be followed to assure the quality and integrity of the laboratory activities. These documents should be detailed enough to adequately define the task it purports to describe and be general enough not to box the user into a situation where efficiency is lost or management prerogative is constrained.

For example, if one were to write a SOP describing the calibration of an analytical instrument that is used almost every day, and the SOP required daily calibration frequency, then that instrument would have to be calibrated daily, whether it was used that day or not. Alternatively, the SOP might have specified the calibration as daily when in use. Under the latter, the instrument would only be calibrated on days it was used, maximizing efficiency.

4.3.1 Preparation of Standard Operating Procedures

- Every laboratory should have SOPs in place. There are different ways to format an SOP. An example of how to write SOPs can be found in AAFCO QA/QC Guidelines, Volume III, Attachment 2A. When writing a SOP, the following structure may be considered (the order may vary from lab to lab):

- A unique name and/or lab ID number;
- History: This defines the age of a SOP and if it is a revision, why the revision was issued and what date it was approved.
- Purpose: This section simply states the purpose;
- Scope: This section defines the applicability;
- Responsibility: This section refers to who is responsible for implementation of the procedure;
- Frequency: Defines the interval at which the procedure described in the SOP will be applied such as day of use, weekly, monthly or yearly;
- Procedures: Specific step-by-step instructions required to successfully perform the tasks of the specific SOP.
- References: List all publications and sources.
- Approvals: All SOPs should have the typed names and signatures of the author(s), review staff and final approvers, as defined by the laboratory's quality management system.

- Analytical Method Documentation

  Before putting a method to routine use, the method should be fully documented. The procedure should be written in sufficient detail that a qualified analytical scientist can easily duplicate the method. Analytical procedures may be electronic to facilitate revisions. Policies to control quality system modifications and to maintain version identification should be planned and documented as part of the overall quality management system.

  The written documentation should include the following. (The order may vary from lab to lab.)

  - Method Identification (number, code, name)
  - Version number
  - Title
  - Effective Date
  - Approval Signatures
  - References
  - Statement of Scope
  - Basic Principles

- Safety Precautions
- Sample Preservation and Storage
- Quality Control Procedures
- Interferences
- Apparatus
- Reagents and Standards
- Procedure
- Calculations
- Comments

Refer to AAFCO QA/QC Guidelines, Volume III, Attachments 2 B, 2C and 2D for examples of analytical method formats.

4.3.2 External Documents

Procedures not produced by the laboratory, such as manufacturer's manuals and kit inserts, which are followed without modification.

4.3.3 List of Suggested SOPS and / or Work Instructions

The following is a list of suggested SOPs or Work Instructions. This is in no way a complete list of all SOPs that a laboratory should have. These SOPs have been divided into areas of interest.

- Administrative Procedures
  - How to Write an SOP
  - Analytical Methodology and Selection
  - Laboratory Chain of Custody
  - Records Retention and Records Disposal
- Equipment Maintenance
  - Refrigerators
  - Balances
- Equipment Calibration / Verification
  - Thermometers
  - Balances
  - Pipettes
  - Timers
- Glassware Selection and Maintenance
- Instrument Calibration and Frequency

- Instrument Maintenance, Operation and Troubleshooting
    - HPLC
    - Flame Atomic Absorption Spectrophotometer
    - Combustion Analyzer
- Laboratory Safety and Safety Training
- Method Validation for In-House and New Methods
- Purchasing / Ordering Procedures
    - Approved Supplier List
- Quality Control Requirements
- Sample Shipping to Other Laboratories
- Sample Receipt, Check-in and Storage
- Sample Preparation, Preservation and Holding Times
- Sample Storage, Archiving and Disposal
- Analytical Standards: Ordering, Receipt, Preparation and Documentation
- Personnel Training and Evaluation
- Waste Disposal
    - Disposal of Hazardous Waste

4.4 Review of Request, Tenders and Contracts

Valid analytical data can only be obtained with proper sample collection, sample preservation, transportation/shipment and application of the appropriate test methodology. To meet these criteria, the laboratory is encouraged to enter into a written agreement with the customer.

This agreement can be as simple as an analysis request form filled out by the laboratory or as extensive as a Quality Assurance Project Plan (EPA Guidance G-5). Example checklists for new requests are located in AAFCO QA/QC Guidelines, Volume III, Attachment 3A and 3B.

In some instances, the laboratory is also the sampling entity. The laboratory should have in place a sampling plan and sampling procedures that define the types of field samples collected, sampling equipment and how the lab will ensure representative sampling throughout the laboratory process from the receipt of the laboratory sample to the selection of a test portion.

4.5 Subcontracting of Tests and Calibrations

Refer to ISO/IEC 17025:2005.

4.6 Purchasing Services and Supplies

State/government purchasing systems often approve or restrict vendors based on price, accounts receivable, or state-wide contract agreements. The laboratory must maintain its own list of approved suppliers based on its evaluation of the quality of goods or services received. At times this might be used to support state/government purchasing systems why one vendor should be used over another. All goods and services should be evaluated regardless of whether they are deemed “quality critical” because it can be difficult to objectively define what is “quality critical”.

4.7 Service to Customer

Refer to ISO/IEC 17025:2005.

4.8 Complaints

Request for Splits and Referee Laboratories: A regulatory laboratory’s results are sometimes challenged or contested by a regulated organization. A request for a split sample is usually made to the Feed Control Official or the regulatory laboratory. The feed laboratory sends a split sample to the requester or another laboratory for analysis. The results of split sample analysis may indicate the reproducibility, or lack thereof, in the sub-sampling, sample preparation and or analysis. Records should be maintained to track and assess the results of these exchanges.

4.9 Control of Nonconforming Testing and/or Calibration Work

Refer to ISO/IEC 17025:2005.

4.10 Improvement

Refer to ISO/IEC 17025:2005.

4.11 Corrective Action

Refer to ISO/IEC 17025:2005.

4.12 Preventive Action

Refer to ISO/IEC 17025:2005.

4.13 Control of Records

Refer to ISO/IEC 17025:2005.

4.14 Internal Audits

Refer to ISO/IEC 17025:2005.

4.15 Management Review

Refer to ISO/IEC 17025:2005.

5. Technical Guidelines

5.1 General

Laboratories naturally seek to control the reliability and defensibility of their analytical results. Sometimes, however, the focus of quality management is too narrowly centered. The quality management system should be broadly focused on all laboratory processes including receiving, preparation, analysis, review, reporting and disposal of laboratory and analytical samples.

Sampling and sample submission practices vary among jurisdictions. In some states sampling functions are organized under separate, independent units while other states operate with a more integrated organization combining sampling, analysis and regulation. In any case, whatever the variation in organizational structure, it is imperative that there be close cooperation and mutual understanding among those collecting field samples, those analyzing laboratory samples and those engaged in resulting regulatory actions (data assessment and/or compliance). Without this cooperation, quality suffers, regardless of how dedicated individuals might be in any one phase of the process.

Such cooperation would include an agreement as to who is authorized to collect official samples, procedures for sample collection, procedures for sample submission and shipping, standardization of documentation and sample acceptance criteria based on data quality objectives. Sample preparation and analysis should be conducted according to established procedures and analytical methods to meet data quality objectives (fit for purpose). Pass/fail criteria should be based on regulatory requirements and risk assessment. Regulatory action should be based on statistically sound analytical science. Agreements and understandings between functional areas should be specific and in writing. The more independent the functional units, the more formal are the agreements that are needed.

5.2 Personnel

Training and the competency of lab personnel are integral parts of maintaining quality laboratory operations. It ensures that each staff member employs the knowledge and skill necessary to perform his/her job. With changes in technology and new measurement techniques, it is imperative that laboratory management provide and encourage continual training for all staff.

The following types of training are needed in all labs:

- Training of the new employee as evidenced by successful initial demonstration of assigned technical tasks/techniques;
- Ongoing competency as evidenced by periodic evaluation of assigned technical tasks, techniques, or data.
- Employees performing only a portion of a task or procedure need only be trained and evaluated on those steps.

5.2.1 Training Techniques

The following types of training are needed in all laboratories:

- Training of the new employee, and
- Ongoing professional training of current employees.

The following training techniques are used to increase the knowledge and skill level of the laboratory employees:

- On-the-job training including access to appropriate technical information,
- Attendance at lectures relating to the laboratory activities,
- Attendance at conferences, seminars and scientific meetings,
- Attendance at short courses (e.g., American Association of Feed Microscopists (AAFM), American Association of Oil Chemists Society (AOCS), AOAC INTERNATIONAL, AAFCO Workshop, American Association of Cereal Chemists (AACC),
- University or college instruction, and
- Specialized training by instrument manufacturers.

5.2.2 Training and Competency Records

The laboratory should maintain training and competency records for all employees. Data or other records generated in the lab should be kept as evidence of training and competency.

5.2.3 Training and Competency Evaluation

Evaluation of the analyst should be made to determine if training has been successful and the analyst is competent to perform the analysis. The use of materials of known composition can be used to evaluate training. Observation of analyst techniques in performing methods may also be appropriate.

5.3 Accommodations and Environmental Conditions

Refer to ISO/IEC 17025:2005.

5.4 Test and Calibration Methods and Method Validation

5.4.1 General

Every laboratory, no matter how limited its resources, must devote time and resources to the evaluation of existing and potential new methods. Failure to do so leads quickly to erosion of quality. Method evaluation ideally should consist of within laboratory work, followed by inter-laboratory collaboration. Inter-laboratory collaboration can be a very formal and defined activity, such as is used for the collaboratively validated methods of AOAC INTERNATIONAL (AOAC) or it could be an informal activity, such as sharing problem materials between several laboratories or cross checking results from a new method with check sample program results of an established method.

It is established AAFCO policy to maximize inter-laboratory collaboration. Section 11(f) of the AAFCO Model Bill states “Sampling and analysis shall be conducted in accordance with methods published by the AOAC or in accordance with other generally recognized methods”. When collaborated methods are not fit for purpose or are not available from AOAC or from organizations such as the International Organization for Standardization (ISO) and the European Committee for Standardization (CEN), it may be necessary to utilize literature methods, in-house methods or methods developed in other state laboratories.

5.4.2 Selection of Methods

Analytical methods are evaluated based on attributes such as accuracy, precision, specificity, sensitivity, detectability and practicality. Compromise between attributes is inherent in the selection of methods. However, any method selected for use must be appropriate to the requirements of the regulatory function and within the capabilities of the laboratory staff (fit for purpose). Depending on the documentation available on a method, varying degrees of method verification or validation are recommended before it is adopted for routine use.

In-House Method Verification of Collaboratively Validated Methods: These would include methods that have been collaboratively studied, approved and published by AOAC, AOCS, or ISO with a scope applicable to the intended use. When a laboratory chooses a fully collaborated method for use, something is already known about the method’s performance in other laboratories. However, it is necessary to establish and document the method’s performance in-house. The type of method and its intended regulatory use will influence the details of the in-house verification. Representative matrices and concentration ranges should be included in the verification. When collaborated methods are used, laboratories should verify their own ability to achieve satisfactory performance before reporting any data generated with the method(s).

AOAC International Guide “*How to Meet ISO 17025 Requirements for Method Verification*” 2008 provides an excellent overview on requirements for verification.

5.4.3 & 5.4.4 Laboratory Developed Methods and Non-Standard Methods

In-House Method Validation of Non-Collaborated Methods: When a method has not been put through the process of collaboration between laboratories, the laboratory should validate such methods. The validation should be as extensive as is necessary to meet the needs of the given application. The laboratory should record the results obtained, the procedure used and a determination as to whether the method is fit-for-purpose. In addition to the parameters noted in Section 5.4.2, the following should be considered in the validation protocol:

- Robustness or Ruggedness - There should be an in-depth investigation of the ruggedness of the method and there should be more investigation of the method's suitability to a range of feed matrices. Refer to *"Use of Statistics to Develop and Evaluate Analytical Methods"* by Grant Wernimont, AOACI, for an explanation of ruggedness testing.
- Collaboration - Consider initiating a formal collaborative study. Even a more limited Peer Verified AOACI collaboration reinforces the regulatory function.

5.4.5 Validation of Methods

For validation of non-standard methods, laboratory developed methods and standard methods used outside their intended scope, follow the validation criteria in EURACHEM Guide "*The Fitness for Purpose of Analytical Methods - A Laboratory Guide to Method Validation and Related Topics (1998)"*.

5.4.6 Estimation of Uncertainty of Measurement

Uncertainty of measurement is defined as a "non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand" (Refer to Section 2.7 for source). As a result, it is fundamentally important for a testing laboratory to have knowledge of the uncertainty of measurement of test results and to be able to provide this knowledge to their customer(s). A measurement's uncertainty is an indication of the quality of the analytical result or test method used. Measurement uncertainty values can allow comparison of analytical results within and between laboratories or with specifications and regulatory limits.

5.4.6.1 General Guidance

Procedures for estimating the uncertainty of measurement should be documented and applied to analytical methods requiring compliance to ISO 17025. Feed laboratories should address the following considerations in their procedures.

- Appropriate methods of evaluation must be used by laboratories.
  - ISO 17025 does not recommend one approach over another.
  - Any approach that uses statistically valid methodology and yields a reasonable estimate is as valid as another approach.

- Attempts to identify all significant components contributing to measurement uncertainty must be made.
    - An uncertainty component representing less than 1/3 of the largest uncertainty component has an insignificant impact on the total measurement uncertainty result.
    - However, the cumulative effect of several small uncertainty components may be significant and should not be ignored.
- When a well-recognized method specifies the limits to the values of the major uncertainty components, the laboratory is considered to have satisfied the requirements for reporting measurement uncertainty provided that the test method is followed without making any deviations.
- Method knowledge, experience and past performance data can be used to estimate the uncertainty of measurement.
- Determining measurement uncertainty through metrologically rigorous and statistically valid calculations can be useful, but is not always required.

Generally, there are two approaches that laboratories can use for the estimation of measurement uncertainty; rigorous consideration (bottom-up approach) and method performance (top-down approach). Rigorous consideration involves determining the uncertainty from each individual component of every identifiable source, calculating an uncertainty value for each component and combining these to yield an overall uncertainty result. It is the approach outlined in the ISO/IEC Guide 98-3 *“Guide to the Expression of Uncertainty in Measurement”* (GUM). It is a complex, bottom-up procedure that can be useful when quantifying individual uncertainty components. However, it can lead to an underestimate of the overall uncertainty since it is difficult to include all possible uncertainty contributions. In addition, results obtained from using the GUM approach may need to be verified by analytical data, e.g. inter-laboratory studies. The method performance approach involves the use of laboratory data (e.g. QC data, method validation reports, collaborative and inter-laboratory studies) to estimate the uncertainty of measurement. It is outlined in the EURACHEM/CITAC guide as well as in the VAM Project 3.2.1. It is a top-down approach which uses real analytical data from method runs to estimate the uncertainty. It can lead to an overestimation of the measurement uncertainty through inadvertently including certain components of the uncertainty budget more than once. Its advantage over the GUM is in the simplicity of the calculations and ability for laboratories to use existing analytical data. As a result, the procedures and calculations outlined in this QA/QC document are based on the method performance approach.

5.4.6.2 Procedure for Estimating Measurement Uncertainty

The following section outlines the steps that should be followed to estimate the uncertainty of measurement for an analytical method. AAFCO QA/QC Guidelines, Volume III, Attachment 4 provides a more detailed flowchart of these steps. A statistical model that forms the basis of this procedure can be found in AAFCO QA/QC Guidelines, Volume III, Attachment 5. Example estimates covering all of the steps below and using real laboratory data are in AAFCO QA/QC Guidelines, Volume III, Attachment 6.

- Step 1: Specify the measurand (true value = x).
- Step 2: Identify all significant uncertainty sources from the method.
- Step 3: Quantify the uncertainty components, $u(x_1)$, $u(x_2)$, $u(x_3)$, etc. See Section 5.4.6.4 below.
- Step 4: Convert the uncertainty components to standard deviations (standard uncertainty) as outlined in Section 5.4.6.5.
- Step 5: Calculate the combined standard uncertainty, $u(y)$ by following the equation in Section 5.4.6.6 below.
- Step 6: Calculate the expanded uncertainty, $U(y)$ as described in Section 5.4.6.7 and report.

5.4.6.3 Practical Considerations and Guidance

The uncertainty of measurement can be different for a given method at different concentrations. This is especially true when a method's scope includes a concentration range covering several orders of magnitude. The laboratory should be aware of the effect that the measurand concentration has on the measurement uncertainty. If necessary, the method uncertainty should be evaluated at selected concentration levels or ranges for a given analytical method.

When quantifying uncertainty components, the relative standard uncertainty, $u(x)/x$ should be determined. However, when uncertainty components are independent of analyte concentration, such as at low concentrations close to a method's quantitation limit, absolute standard uncertainties, $u(x)$ should be determined.

5.4.6.4 Quantifying Uncertainty Components

As described in Section 5.4.6.1 above, any approach that uses statistically valid methods to quantify uncertainty components is considered as valid as any other approach. As such, the array of available equations is too numerous to list here. Some example equations for estimating uncertainty components can be found within the references listed below.

- EURACHEM/CITAC guide: *Quantifying Uncertainty in Analytical Measurement*, Third edition, (2012), S. L. R. Ellison and A. Williams (Eds). ISBN 978-0-948926-30-3. Available from www.eurachem.org.
- ISO/TS 21748, *Guidance for the use of repeatability, reproducibility and trueness estimates in measurement uncertainty estimation*, 1st edition, 2004.
- NORDTEST Report TR 537, *Handbook for Calculation of Measurement Uncertainty in Environmental Laboratories*, Edition 2, 2004.
- VAM Project 3.2.1 Development and Harmonisation of Measurement Uncertainty Principles, *Part (d): Protocol for uncertainty evaluation from validation data*, Barwick, V.J., Ellison, S. L. R., 2000.
- CAEAL P19, *Policy on the Estimation of Uncertainty of Measurement in Environmental Testing*, Rev. 1.8.
- A2LA, *Guide for the Estimation of Measurement Uncertainty In Testing*, Adams, Thomas M., 2002.

5.4.6.5 Converting components to standard uncertainty, u(x)

Uncertainty components calculated from the example equations in AAFCO QA/QC Guidelines, Volume III, Attachment 6 are already in standard uncertainty form and no further adjustment is required. Uncertainty values from other sources must be expressed as standard uncertainties, that is, as standard deviations. The following table provides equations for calculating standard uncertainty when different data sets are available.

Table 1

Calculations for Standard Uncertainty, $u(x)$

Data used to determine $u(x)$	**Method for calculating $u(x)$**	**Equation**
Repeated single measurements	$u(x)$ equals the standard deviation of measurements	$u(x) = s(x)$
Average of measurements	$u(x)$ equals the standard deviation of the mean	$u(x) = s_{mean} = s(x) / \sqrt{n}$ where: n is the number of values
From a confidence interval (e.g. $\pm x$ at Z%)	$u(x)$ equals the confidence value divided by the appropriate point of the Normal distribution	$u(x) = x / t$ where: t is the t-value at a given level (Z) for ∞ degrees of freedom
From a range of data ($\pm x$) with no confidence level	where extreme values are likely, assume a rectangular distribution	$u(x) = x / \sqrt{3}$
From a range of data ($\pm x$) with no confidence level	where extreme values are unlikely, assume a triangular distribution	$u(x) = x / \sqrt{6}$

5.4.6.6 Estimating combined standard uncertainty, u(y)

The combined standard uncertainty $u(y)$ can be estimated by calculating the square root of the sum of the squares of all individual standard uncertainty components. The uncertainty components are typically comprised of the method precision, $u(P)$, the laboratory/method bias, $u(bias)$ and any other sources of uncertainty not covered by control sample analyses, $u(x_1)$, $u(x_2)$, $u(x_3)$, … $u(x_n)$.

$$u(y) = \sqrt{u(P)^2 + u(bias)^2 + u(x_1)^2 + u(x_2)^2 \ldots + u(x_n)^2}$$

Another way of estimating $u(y)$ is by using the reproducibility between laboratories (s_R) directly. This value can be obtained from a collaborative study or PT program. This estimate should be used only when the demand on uncertainty is low, since it will be an overestimate of a given laboratories uncertainty if their quality is high. Likewise, s_R can be an underestimate if matrix variations or non-homogeneity commonly impact results. The s_R value can be assumed to be an estimate of $\sqrt{u(P)^2 + u(bias)^2}$, so any sources of uncertainty not included with these components [$u(x_1)$, $u(x_2)$, $u(x_3)$, $u(x_n)$] should be included in the estimate of $u(y)$.

$$u(y) = \sqrt{s_R^{\,2} + u(x_1)^2 + u(x_2)^2 \ldots + u(x_n)^2}$$

5.4.6.7 Estimating expanded uncertainty, U(y)

The expanded uncertainty is an interval that is expected to contain a large fraction of the distribution of values reasonably attributed to the measurand. In order to determine the expanded uncertainty, the combined uncertainty must be multiplied by a coverage factor (k):

$$U(y) = k * u(y)$$

The choice of coverage factor that is typically chosen is $k = 2$. For a normal distribution this yields an interval containing 95% of the distribution of values.

The measurement uncertainty should be reported as an expanded uncertainty in the following format:

X (units) $\pm$ $U(y)$ (% or units)

where: X = the measured result

Reporting the result in this form means that the “true value” is within $U(y)$ (units or %) of the measured result, 95% of the time.

5.5 Equipment

Refer to Table 2 “Equipment, Calibration, Verification and Maintenance, Recommended Practices for Feed Laboratories” in AAFCO QA/QC Guidelines, Volume III.

5.6 Measurement Traceability

For this guidance document AAFCO considers analytical chemical standards (reference materials per ISO 17025), certified reference materials, and consensus materials as material requiring traceability to SI units where possible.

The integrity of chemical standards and reagents is a crucial factor in the successful analysis of samples. The lab should develop guidelines for source, storage, preparation, expiration date and documentation on all standard materials. Documenting the standards is a great assistance in tracing sources of error in analysis and defending the reliability of data.

Sources and Traceability: The accuracy of results produced will be only as accurate as the reference standards and working standards used for the calibration process. The laboratory should acquire and use standards supplied by the National Institute of Standards and Technology (NIST), United States Pharmacopeia (USP), the manufacturer, ATCC, supply houses or other equivalent sources, which will attest to the authenticity and concentration or amount of the standard. When possible the standard should be traceable to SI Units of measurement. The traceability should be documented throughout the preparation and use of the standard. Standards should be replaced when outdated. An individual should be designated to monitor these activities.

- Standards Receipt and Storage: The laboratory should develop SOPs that track the date of receipt, date of opening, storage, use, expiration and disposal of reference standards. Proper storage of standards and working solutions should be clearly defined in written methods. Microbiological organisms are stored appropriately or per the manufacturer's instructions. For example, Vitamin A standards should be stored in a freezer while metals standards can be maintained at room temperature.
- Standards Logbook: A standards logbook should be maintained to document the preparation of stock standards and working standards. Information tracked in the logbook would include name/lot/source/purity of standard, preparation concentration(s), initials of analyst, date prepared, initials of reviewer and date reviewed. The logbook may be a hardcopy or electronic document provided all the required information is included.
- Preparation of Standards: Preparation of standards, including stocks, intermediate solutions and working standards should be properly recorded so that traceability is maintained and errors in the preparation process can be identified. The preparation of standards should be clearly explained in written procedures. Weighing should be performed on calibrated balances and dilutions should be made using calibrated glassware, such as volumetric class A flasks and pipettes. The procedure must be maintained to achieve the number of significant figures required for the method. Labeling of standard containers should include name and concentration of the standard, date made and initials of the analyst. Supervisory or peer review should also be noted by initialing and dating the preparation log.
- Standards Disposal: Any standard that exceeds the expiration date or is of questionable quality should be disposed of in accordance with laboratory protocols for proper disposal of laboratory waste. Microbiological waste will be placed in biohazard bags and sterilized before disposing.

5.7 Sampling

5.7.1 Sample Collection and Submission to the Laboratory

Sample collection procedures should be experimentally validated based on appropriate statistical methods and be specific to particular feed matrices. Sampling procedures should be documented in writing and should address the factors to be controlled to ensure the validity of the analytical results.

AAFCO provides guidelines for field and laboratory sampling that are available on the AAFCO website: http://www.aafco.org/.

The laboratory should work together with the customer in the establishment of sampling and sample handling procedures. There should be mutual establishment of sampling schedules, sampling frequency, shipping protocols and the design of appropriate forms and documentation. Such cooperation benefits efficiency and quality. Properly trained field staff should collect, document, preserve and ship primary/field samples using established guidelines. Deviations from the sampling plan should be documented and approved by the customer and the laboratory. These changes to the sampling plan should be communicated to those involved in the sampling, receipt and analysis of the feed materials.

5.7.2 Sample Submission

Primary/field samples and related documentation, including completed field chain of custody forms, should be shipped or transferred to the laboratory at the same time. In the case of hazardous materials, use the appropriate form of transportation. Example collection and submission forms are found in AAFCO QA/QC Guidelines, Volume III, Attachments 7A and 7B.

5.8 Handling of Test and Calibration Items

The over-all objective of quality assurance requires that all types of samples be handled in the laboratory in such a way as to maintain integrity. The specific sample receiving, log-in, preparation and storage procedures will vary somewhat among laboratories, but their overall objective will be the same. These following sections should describe the respective procedures used in the laboratory.

5.8.1 Sample Receipt and Sample Acceptance

Sample receiving guidelines for the laboratory should be documented in detail. Although there are many common elements, this process will vary somewhat among laboratories. For example, chain of custody procedures may be routine in some laboratories and employed in others only when there are potential legal ramifications.

A sample custodian should be designated by the laboratory. The sample custodian’s responsibilities include receipt of samples, reviewing sample submission forms, recording tracking information (logging in samples), and proper storage and disposal of analytical samples. Upon arrival, each laboratory sample should be inspected for condition and identification. Submission documentation should be checked to ensure that it accurately reflects the identification number or code, size, labels and any other information submitted with laboratory samples. A copy of a product label is very useful for verifying claims, lot number and manufacturer. Laboratory samples should be inspected for intact container, signs of tampering, etc.

In the event that an incoming laboratory sample does not meet the established guidelines, there should be a written policy on the proper course of action. Rejection of laboratory samples, re-sampling or correction of documentation errors should not be decided upon subjectively, but should be based upon written policies established by the program and laboratory managers that are clearly understood and agreed upon by those collecting and receiving the laboratory samples.

5.8.2 Chain of Custody

Chain of Custody is a legal term that refers to the ability to guarantee the identity and integrity of the samples (or data) from collection through reporting of the test results. A secure chain of custody, combined with the use of proper analytical methods and techniques, is necessary for defensibility of data and to ensure acceptance of data by other agencies or jurisdictions.

Custody of a sample means that it is:

- Only in the possession or in view of authorized person(s) after being in his/her physical possession, or
- It is retained in a secured area with restricted access after being in one's physical possession, or
- Stored in a secured area, with access restricted to authorized personnel only.

Chain of custody procedures should be employed when the analytical results will be used for enforcement purposes or when the results may be challenged in a hearing or court of law or it is established by the program. The purpose of chain of custody procedures is to provide evidence that a sample has not been tampered with or misidentified and to provide a traceable path from the primary/field sample to resulting analytical data. Chain of custody records document all transfers from the time the field sample is collected to the time all regulatory action associated with the test results is completed and final disposal of the laboratory and analytical samples and associated records has occurred.

Since a field chain of custody form tracks the primary sample from the time of collection in the field to the time of receipt of the sample at the laboratory, when the primary/field sample is transferred to the laboratory, the person transferring will sign and date the field chain of custody or transfer form. When the laboratory sample is received and accepted at the laboratory, the receipt is documented. Some laboratories use a separate transfer form for this documentation. All documentation should accompany the samples. The mode of shipment/delivery should be noted on the chain of custody form.

Once in possession of the laboratory, the laboratory must have the means to track and document the custody of the laboratory and related analytical samples from receipt to disposal. This can be done using laboratory chain of custody forms, in paper or electronic form. Paper forms will have spaces for the analysts and sample custodian to sign and date. Whenever an official seal is broken or a transfer occurs between sample custodian and analyst or between the analysts or storage locations, such action is documented on the laboratory chain of custody form. Each state feed lab should prepare forms with these guidelines in mind. Please see AAFCO QA/QC Guidelines, Volume III, Attachments 8A, 8B and 8C for examples of these forms.

5.8.3 Sample Log-in

Procedures detailing the sample log-in process should be documented. In all laboratories, after the sample custodian has received and accepted laboratory samples and related documents, he/she logs the laboratory samples into the laboratory logbook or LIMS (Laboratory Information Management System). They may be logged in manually using a logbook or electronically using a computer database. The log-in procedure should address the following items:

- Assignment of a unique sequence of laboratory sample numbers.
- Labeling of the laboratory samples with the assigned sample numbers.
- Entry of field sample identification number.
- Entry of pertinent sample information which may include the manufacturer's name and address, dealer's name and address, brand and/or description, collection date, sampler's identification or name, lot size, receipt date or other information.
- Entry of analytes to be tested and their associated label guarantees when given.
- Restricted access to log-in, editing and updating of files.

5.8.4 Sample Preparation

The laboratory should have documented procedures for sub-sampling to ensure that representative test portions are used for analyses. Quality analytical work requires appropriate procedures be documented and followed regarding the preparation of laboratory samples for analysis. These procedures are designed to ensure that test portions taken for analysis are representative of the total material received. They are also designed to achieve the objective of a representative test sample in a safe and precise manner. AAFCO provides sampling guidelines for the field and laboratory that are available on the AAFCO web site.

Throughout the sample preparation processes, care must be taken to avoid cross contamination by carefully cleaning equipment used between samples, handling materials in order of increasing analyte concentration and/or labeling potentially contaminative material to alert personnel of possible contamination hazards. The laboratory should write procedures dealing with the preparation of specific feed types. Types will vary among states, but may include the following:

- Dry feeds,
- Liquid feeds,
- Feed Ingredients and grains (DGs, wheat, corn, oats, etc.),
- Canned pet foods,
- Texturized or molasses coated feeds,
- Solidified molasses based feeds,
- Oilseeds or high fat feeds
- Mineral mixes,
- Feed and grains for mycotoxin analysis,
- High moisture feeds and/or forages.

Areas which should be addressed within the sample preparation procedures, or in separate documents, include:

- Work flow
- Grinding room environment (humidity, dust removal)
- Grinder/equipment operation,
- Grinder/equipment maintenance,
- Prevention of unintended contamination,
- Safety considerations,
- Mass reduction procedures (subsampling).

5.8.5 Sample Storage and Disposal

Sample storage and disposal protocols need to be documented by the laboratory, and practiced as documented. The objectives of the storage and disposal protocols are to ensure that:

- Laboratory samples and analytical samples are stored within the laboratory under conditions that assure that they will not be contaminated or tampered with;
- Laboratory samples and analytical samples are stored under conditions that assure that analytical integrity is maintained (i.e.,

not degraded by light, heat or other environmental conditions, bacterial growth is controlled, etc.);

- Laboratory samples and analytical samples are stored under conditions that assure physical integrity (loss due to breakage, unintended disposal, etc.)
- Location of a laboratory or analytical sample is known at all times;
- Laboratory and analytical samples are stored/disposed of in a manner that is environmentally safe, poses no danger to laboratory personnel and is consistent with local waste disposal regulations, after holding time(s) have expired. Holding times should be mutually established with the client taking into consideration all relevant factors (stability of the analyte, regulatory disposition, programmatic needs, storage conditions and space, etc.)

5.9 Ensuring the Quality of Test and Calibration Results

The quality control checks are a systematic in-house approach to ensure the production of high quality data. The objectives of these control checks are as follows:

- To provide reliable and defensible analytical results;
- To provide a measure of the precision and accuracy of the analytical methods;
- To monitor the accuracy and precision of the analyst;
- To identify problematic methods which can be flagged for further research;
- To detect training needs within the laboratory;
- To provide a permanent record of instrument performance which is used for validating data and projecting instrument repair or replacement needs;
- To monitor the effectiveness of the laboratory performance and the quality assurance program.

Table 3, Quality Control Checks for Analytical Procedures in AAFCO QA/QC Guidelines, Volume III, offers guidelines for the types of quality control a lab should use in analyses and when samples pass or fail the QC criteria established by the laboratory.

5.9.1 Definitions of Controls to Monitor Bias / Systematic Error / Accuracy:

- *Laboratory Reagent Blank*: A sample consisting of reagent(s) without the target analyte or sample matrix, introduced into the analytical procedure at the beginning and carried through all steps to determine contamination or interferences introduced by the laboratory environment, glassware, reagents, equipment or instruments.

- *Laboratory Control Sample (LCS)*: Materials which are very similar to those being analyzed and which have a known composition. Whenever possible, they should be traceable to an outside source (AAFCO, NIST, etc.). Control samples are analyzed with each batch of samples to check instrument performance, review laboratory techniques and calculations and monitor analyst performance. Control Sample results are monitored by plotting control charts (see 5.9.5).
- *Control Plates for Microbiological Plating Methods*: A negative and positive control should be analyzed with each batch.
- *Calibration and Calibration Check*: A calibration is the analysis of a series of calibration standards to establish a relationship between the standards and instrument response. The levels of the calibration standards should bracket the range of expected sample measurements. The calibration and the instrument performance are checked with independent calibration check standard(s).
- *Matrix Spike:* A matrix spike is a type of Laboratory Control Sample and is prepared by adding a known amount of analyte(s) to a test portion which is similar to those being analyzed. It is preferred that the material being spiked not contain any of the analyte(s). Matrix spikes are analyzed to determine the effect of the matrix on a method's recovery. Matrix spike recoveries are monitored by plotting control charts (see 5.9.5.).
- *Standard Addition Method*: Standard addition (or spiking) is when analysis is done by adding the standard material to the test portions themselves. This technique is useful in situations such as:
    - When the matrix is either unknown or so complex that an external calibration standard cannot be used with confidence.
    - When the chemistry is complex or highly variable for the sample pretreatment or assay.
    - When the assay depends on highly precise instrumental conditions that are difficult to control.
- *Internal Standard: An internal standard is a substance that* is not normally found in the sample matrix, has similar chemical and physical properties to the analyte and has an easily measured signal that does not interfere with the response of the analyte. In addition, the response of the internal standard should not be affected by other components of the sample. A known or fixed amount of the internal standard is added to test portions of the unknowns and standards alike. The responses of the analyte and

internal standard are determined and the ratio of the two responses is calculated. The response ratio (analyte to internal standard) depends only on analyte concentration. A plot of this response ratio as a function of analyte concentration yields a calibration curve. The concentration of the internal standard should be such that the responses of the analyte and internal standard are of the same order of magnitude to minimize error in calculating the ratio. The internal standard method is used to correct for matrix effects, extraction inefficiencies and instrument variability.

5.9.2 Definitions of Controls to Monitor Variance/Random Error/ Precision:

- *Duplicate Analysis*: Analysis of two test portions of the same analytical sample analyzed within a batch. The results from duplicate analysis are used to evaluate analytical precision within the batch. Duplicate analysis results are monitored by plotting relative percent difference (RPD) control charts. See Section 5.9.5 *Control Charts*.
- *Laboratory Control Sample (LCS)*: Samples of known analyte concentration which are very similar to those being analyzed and which are analyzed with each batch of samples over a period of time. The LCS can also be used to monitor batch to batch or day to day variance. Results are monitored by plotting percent recovery control charts. See Section 5.9.5 *Control Charts*.
- *Matrix Spike:* A matrix spike is a type of Laboratory Control Sample and is prepared by adding a known amount of analyte(s) to a test portion which is similar to those being analyzed. It is preferred that the material being spiked not contain any of the analyte(s). Matrix spikes are analyzed to monitor the day to day or batch to batch variance of the method. Matrix spike recoveries are monitored by plotting control charts (see 5.9.5.).

5.9.3 Quality Control Checks for Analytical Procedure

Quality control checks should be established for each analytical method. Quality control checks including applicability, acceptance criteria and corrective action are summarized in Table 3, Quality Control Checks for Analytical Procedures in AAFCO QA/QC Guidelines, Volume III.

5.9.4 Repeat Analysis

Unacceptable Quality Control:
The laboratory should implement procedures when QC results are non-conforming. The procedure for corrective action should start with an investigation to determine the root cause of the problem. Where corrective action is needed, the laboratory will identify potential

corrective actions. The action most likely to eliminate the problem should be implemented.

Repeat Analysis for Potentially Violative Samples:

When analytical quality controls are acceptable, but the result for a feed component does not meet the guaranteed analysis within an acceptable tolerance, the lab may repeat the analysis before reporting the result.

Analytical Variations (AVs) based on the AAFCO feed check sample program are guidelines for helping control officials make routine decisions on the acceptability of products. These AVs can also be used by the laboratory as a guideline or as a starting point to determine what analysis needs to be repeated. Usually, if the result is not within 2/3 of the AV of the guarantee, repeat analysis is needed.

Another way to determine which analysis should be repeated is to use coefficients of variation for in-house laboratory control samples to establish acceptance criteria for each analyte. Whatever system is used, the in-house criteria for repeats should be tighter than the program criteria for allowed variations for violative samples.

When repeat analysis is required to confirm a violative (excessive/deficient) analyte, the laboratory should perform appropriate quality control analyses for many analytes, especially for drugs, vitamins or minerals/elements. Spiking into the sample, performing standard additions, analyzing the same or similar matrix or analyzing other quality control samples may define possible problems with the method.

If all of the analyses indicate no problem with the methods utilized for a given sample, the lab can report the analytical results. On the other hand, if a problem is indicated, the lab should investigate and solve the problem before reporting the result. When reporting analytical results, unless agreed upon with the customer, the lab should report the average or median of acceptable results, or the most representative result of the data set. Using these guidelines, the lab can assure that the analytical results are defensible and when appropriate, eliminate repeat analysis.

Allowed Variations for Violative Samples:

A tolerance above or below the label guarantee accounting for sampling and analytical variations should be established to determine if products are in compliance with guarantees. These tolerances should be established by control officials in communication with laboratory management. AAFCO AVs are an excellent starting point. However, AAFCO AVs should be compared to laboratory performance for specific analytes as measured by the coefficient of variation to validate their acceptability for the program.

5.9.5 Control Charts

A control chart is a graphic representation of an analytical result obtained as a function of date or batch number. They are very useful for visualizing trends. Where feasible, control charts should be maintained and used for each analytical procedure and be the basis for corrective and preventative actions when they indicate potential problems with methods. The control charts should be constructed by the analysts at the time of the analysis and used immediately to determine the acceptability of the results generated. Constructing control charts long after the analysis date only demonstrates historical control and is of minimal value for determining immediate run or batch acceptability. The control chart may include the following: laboratory name, analyte, method of analysis, date(s), concentration range and analyst(s). Control charts can be constructed by hand or using a commercial QC or statistical software such as SAS, NWA Quality Analyst or a spreadsheet program such as Microsoft Excel.

Several types of control charts can be used. The simplest type is the Shewhart chart. It is the easiest to construct and interpret. This is the most useful for evaluating a single value. Two alternatives to the Shewhart control chart are the cumulative sum (or cusum) control chart, and the exponentially weighted moving average (EWMA) control chart. They have the advantage of responding very quickly to small shifts in the procedure and are useful for evaluating trends. However, "rules" can be developed for detecting nonrandom patterns and applied to Shewhart charts to make them more useful for detecting systematic patterns. Instructions for constructing Shewart charts are provided in this section.

5.9.5.1 Control Charts for Accuracy (X Chart)

- Control Limits for Reference Materials or Laboratory Control Samples: A standard reference material or control sample is analyzed with each batch of 10-20 samples or, if a large number of samples is run in a batch, one control sample is analyzed for each 10 or 20 unknowns (rate of 5% - 10%). For example, a batch of 5 mineral mix and 12 mixed feed samples should contain a mineral mix control sample and a mixed feed control sample. After a control sample has been analyzed 15 to 20 times, the mean concentration and standard deviation of the mean are calculated and control limits are determined. See AAFCO QA/QC Guidelines, Volume III, Attachment 9A for an example Accuracy Control Chart.

  The X-chart is appropriate for any analysis where reference control samples are available. Generally it is used for monitoring nitrogen (crude protein), fiber, fat, minerals, drugs and many other analytes in feeds.

- Control Limits for Matrix Spike Sample Recovery:

  The spiked-sample control chart is similar to the X-chart, but instead of using a reference material or control sample, an unknown sample or a blank sample is spiked with a known amount of the analyte. It is important that the analyses are run on the same or similar matrix or the variability in the recovery will be incorrectly estimated. After spike recoveries have been determined on 15 - 20 samples, the mean percent recovery and the standard deviation of the percent recovery are calculated. Control limits can be determined and a representative control chart can be constructed. See AAFCO QA/QC Guidelines, Volume III, Attachment 9B for an example Recovery Control Chart.

5.9.5.2 Quality Control Limits for Precision (RPD or $\overline{R}$-Chart):

- A duplicate sample is analyzed with each batch of 10-20 samples or, if a large number of samples are run in a batch, one duplicate is analyzed for each 10 or 20 unknowns (rate of 5% - 10%). For each pair of duplicates, the difference (or range) is calculated. Because the difference (range) will depend on the concentration to some extent, duplicate analysis should be plotted as relative percent difference (RPD) or with samples of similar concentration for range only. After 15 or 20 duplicate analyses are run, an average range may be calculated for use as control limits. See AAFCO QA/QC Guidelines, Volume III, Attachment 9C for an example Precision Control Chart.

5.9.6 Proficiency Testing Samples / AAFCO Check Sample Program

The AAFCO Feed Check Sample Program is a proficiency testing/check sample series available by subscription each year from this organization. Each participant receives a series of samples during the program year. Samples include commercially manufactured feeds, ingredients, and pet foods that are carefully ground (if required) and blended to insure uniformity of composition of all collaborators' packets. The series normally includes a wide variety of feeds containing drugs and antibiotics currently in use at both premix and finished feed levels. Minerals and vitamins are included in some samples and usually one sample annually is a mineral-vitamin premix or supplement.

The results from all the participating laboratories are statistically analyzed, summarized and reported back to each laboratory. These data are extremely useful to the laboratory for evaluating methods and laboratory performance over a variety of sample matrices (or types) and provides check samples to be used as controls in your QA/QC program for each set of feeds analyzed. Additional information is available at the AAFCO Check Sample Program website:

http://www.aafco.org/. For additional proficiency testing providers, including internationally accredited programs, laboratories are encouraged to search the EPTIS Database founded by the European Union and hosted by the German Federal Institute for Materials Research and Testing. (http://www.eptis.bam.de, last access in November 2013). The database includes more than 1500 proficiency testing schemes from more than 150 providers in the Americas and Europe with increasing listings in Africa and Asia-Pacific.

5.9.7 Handling of Analytical Data

By nature, the process of acquiring raw data and subsequent data reduction for the final reported result is a complicated process. It is dependent on factors such as testing, assignment of clear lines of responsibility for various parts of the process, communication between all involved parties when modifications and updates are made and a basic understanding of the details of the process by those involved.

The raw data and final results should be archived according to established guidelines consistent with programmatic needs. It is important that these processes be clearly defined, understood by all laboratory personnel and well documented.

- *Raw Data*: Raw data can be acquired in a variety of ways such as manual recording of observations, electronic recording of instrument response such as spectra and direct computer acquisition and calculation. In each case, raw data should be archived to be accessible for review. All manual recording should be done in permanent, indelible ink.

  The laboratory should have policies on the appropriate maintenance and archiving of accurate laboratory notebooks. The laboratory may give consideration to appropriate computer backup and archiving. More sophisticated processes of direct computer acquisition of data may involve electronic archiving of whole instrument runs rather than just data of individual samples and the process of archiving and indexing becomes more complex. Laboratories should be alert to the fact that instrument software upgrades will frequently have an impact on archiving and data recall.

- *Data Reduction*: Data reduction is the process of converting raw data into a form suitable for reporting. Data reduction usually takes two forms: manual and automated. The process of data reduction should be clearly documented in the method. The analyst's worksheets and lab notebooks should have manual calculations completely recorded for each sample so that they may be reviewed and reconstructed by a second analyst, supervisor and/or data reviewer. Automated data reduction includes the input of data by the analyst or direct computer acquisition where an electronic signal is transmitted to a computer. The process for automated data reduction must be precisely stated in the method so that if necessary the calculations can be done by hand.

- *Data Review*: Before final reporting, it is recommended that data be reviewed independently of the originating bench chemist or microbiologist. A peer chemist, microbiologist and/or supervisor should review the data. The review process should take into consideration the results of in house QC checks, the performance of standard curves relative to historical norms, indicators of instrument performance and stability and expected agreement between independent replicate results. Initials or signature authenticates that a review has taken place.
- *Software Verification*: Manual calculations, based on documented SOPs, should be done to validate the accuracy of the software utilized for automated data reduction. Automated systems should be checked with a set of challenge data.

5.10. Reporting the Results

When all individual analyses are complete and processed, administrative review and interpretation of the data should occur before the final official laboratory report is submitted to the feed official and/or manufacturer. Automating the reporting process can reduce errors of transcription. Reporting software should undergo the same rigorous testing and validation required of the software utilized for data reduction. Each report should include at least the following information, unless the laboratory has valid reasons for not doing so.

The laboratory report / database should include:

- date received at the laboratory,
- analytes of interest,
- method reference,
- complete product description when available,
  - product name
  - lot number
- collection date and inspection number,
- laboratory number,
- date analysis completed,
- results,
- signature (handwritten or electronic),
- a space for lab remarks and
- date reported

- where relevant, a statement to the effect that the results relate only to the items tested or calibrated.

Optional items that can be reported include:

- Manufacturers name and address,
- Label claims,
- Retailer or facility name and address,
- Inspector name and:
- Whether or not the sample met label claims.

VOLUME III

AAFCO QA/QC Feed Laboratory Guidelines Tables and Attachments

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ATTACHMENT 1 (Section 4.1.1)

Ethics Resources

An Ethics Policy typically addresses professional integrity and code of conduct for employees. Although many organizations provide ethics guidance or model policies, none have been uniformly adopted across scientific disciplines. Some jurisdictions (international, national/federal, state/territorial, tribal, and local) have codified ethics and/or codes of conduct in law. In addition, laboratories may operate in locations or organizations affected by collective bargaining, contractual agreements, or organizational policies which may address professional integrity and code of conduct.

Laboratories are encouraged to fully research their jurisdictional laws or requirements, organizational policies, relevant agreements and professional associations' guidance when forming their ethics policy.

The references cited below are not intended to be a comprehensive list, but are offered for laboratories as potential resources for research of ethics guidance, law and policies.

Standards of Ethical Conduct for Employees of the Executive Branch, U.S. Office of Government Ethics, 5 CFR Part 2635, As amended at 76 FR 38547- July 1, 2011 (http://www.oge.gov/, last accessed 13 November 2013)

Values and Ethics Code for the Public Sector, Government of Canada, Treasury Board of Canada Secretariat (http://www.tbs-sct.gc.ca, last accessed 19 November 2013)

Standards of Conduct for the International Civil Service, United Nations (http://www.unesco.org, last accessed on 18 November 2013)

Australian Public Service Values and Code of Conduct, Australian Public Service Commission, Public Service Act of 1999, As amended through 2013 (http://www.apsc.gov.au, last accessed 09 December 2013)

ATTACHMENT 2 A (Section 4.3.1)

Title: Format and Content of Standard Operating Procedures (SOP)

Table of Contents

Approval Signatures	
Author	Date
Supervisor	Date
Technical Manager	Date
Quality Manager	Date

1. Purpose

To standardize the writing and format of standard operating procedures (SOPs).

2. Scope

Preparation of all SOPs utilized will follow the format described in this SOP.

3. References and Attachments

3.1

4. Specific Procedure

Note: These are the minimum requirements for standard operating procedure. Some programs may require additional information be included to meet the program requirements.

4.1 Header, or header and footer containing:

- Title and number* must appear on every page, Can be omitted on first page as it has title and number section.
- Effective date,
- Revision number,
- Page notation,
- ”Section”
- Filename path (network)

4.2 Title Page

- Descriptive Title
- Table of Contents
- Signature and Approval
  - ➢ Name of author,
  - ➢ Name of supervisor,
  - ➢ Name of technical Manager,
  - ➢ Name of Quality Assurance Manager or Officer.
- Description of changes and revisions

4.3 Purpose and Scope

4.4 References and Attachments

4.5 Procedure: (All instructions are must do. Any devisations must be documented.) The following are incorporated into this document.

- How it is done,
- References to policies, work instructions and/or analytical methods

4.6 Responsibilities

5. Responsibilities

ATTACHMENT 2 B (Section 4.3.1)

Test Method Name	Revision: 1.0 Effective Date:	Page 1 of 1 Replaces: N/A

1. **Purpose & Applicability** –
    1.1. This method is used for the determination of ANALYTE(S) in MATRICES in the concentration range of CONCENTRATION LIMITS WITH UNITS. OPTIONAL: INCLUDE SUMMARY OF ANALYTICAL PRINCIPLE.
    1.2. This method applies to analysis performed by the LABORATORY NAME.

2. **References** –
    2.1. PRIMARY REFERENCE(S)
    2.2. ADDITIONAL REFERENCE(S)

3. **Procedure** –
    3.1. Equipment, Reagents, and Supplies
        3.1.1.
        3.1.2.
    3.2. Solutions
        3.2.1.
        3.2.2.
    3.3. Sample Preparation
        3.3.1.
        3.3.2.
    3.4. Determination
        3.4.1.
        3.4.2.
    3.5. Calculations
        3.5.1.
        3.5.2.
    3.6. Supplementary Considerations (AS NEEDED)

4. **Waste Disposal** –
    4.1. LIST SPECIAL CONSIDERATIONS or statement “There are no special waste disposal requirements associated with this SOP beyond standard treatment of laboratory reagents.”

5. **Special Safety Considerations** –
    5.1. LIST SPECIAL CONSIDERATIONS or statement “There are no special safety considerations associated with this SOP beyond general laboratory safety guidelines.”

6. **QA/QC** –
    6.1.
    6.2.
    6.3.

7. **Documentation** –
    7.1. This method is readily available to LIST STAFF WITH ACCESS.
    7.2. This method is maintained by LIST PERSON IN CHARGE OF CONTROLLED DOCUMENT in the Laboratory SOP Manual and revised as necessary. Superseded revisions are maintained in the Archived Laboratory SOP Manual.

ATTACHMENT 2 C (Section 4.3.1)

TABLE OF CONTENTS

1. Introduction

1.1 Scope

1.2 Definition

1.3 Principles

2. Safety and Environmental Considerations

2.1 The following chemicals have the potential to be highly toxic or hazardous. Safe handling practices are detailed in the MSDS (Material Safety Data Sheet) for each chemical. Hazards and safety precautions are summarized below.

Chemical	Hazard	Safety Precautions
	•	•
	•	•
	•	•
	•	•

2.2 Toxicity and carcinogenicity of other chemicals used in this method have not been fully established. Regard each chemical as a potential health hazard.

2.3 Minimize personal exposure by wearing a lab coat, safety glasses, and impervious gloves when conducting this analysis.

2.4 Minimize the release of chemicals to the environment by working in a fume hood when handling chemicals that produce noxious fumes, and by properly disposing of spent samples.

3. Sampling

In some cases the laboratory provides the samplers/investigators. Other labs has divisions or clients outside their facility that perform this function.

4. Apparatus and materials

4.1

4.2

5. Reagents and Standard

5.1

5.2

6. Sample Preparation

6.1 *Detailed preparation can be listed here or the lab can reference a separate procedure.*

7. Procedure

7.1 *Step by step instruction to complete the analysis.*

8. Calculations

8.1 *Include all calculations including quality control.*

8.2

9. Critical Control Points

9.1 *Highlight the critical control points that may affect the outcome of the analysis.*

10. Quality Control Criteria

10.1 *Define what quality control is required for the method, e.g. blanks, duplicates, spikes*

11. System Maintenance

11.1

12. Reference

12.1

13. Responsibilities

13.1

ATTACHMENT 2 D (Section 4.3.1)

Approvals

Approved by: ____ ______________
Date

Quality Assurance Review by: __________________________ ______________
Date

1. SCOPE AND PURPOSE:

This method measures LIST ALL ANALYTES in foods, animal feeds, food and feed ingredients, and physiological samples.

2. PRINCIPLE:

2.1. BRIEF DESCRIPTION OF HOW TEST IS RUN
2.2. IF MULTIPLE TEST CODES ARE LISTED IN THE HEADER, EXPLAIN HOW EACH IS USED
Known Interferences: LIST. IF NONE, OMIT THIS SECTION.

3. PRECISION:

Records of method precision based on Method Validation and/or known control summaries are located in the QA Master file for this test method. Assay precision may vary with test matrix and analyte level. Terms used to describe method precision are defined in NPSOP3000, Test Method Validation.

4. REFERENCES:

4.1. PRIMARY REFERENCE - SEE PROTOCOL FOR FORMAT
4.2. LIST ALL SECONDARY REFERENCES

5. DEFINITIONS:

5.1. LIST ALL DEFINITIONS AND ACRONYMS NOT IN COMMON USAGE

6. SAFETY PRECAUTIONS:

Established laboratory safety requirements, as outlined in the Chemical Hygiene Plan, must be observed at all times, including the use of eye protection in designated laboratory work areas.
6.1. USE STANDARD STATEMENTS FROM QA
6.2. USE STANDARD STATEMENTS FROM QA

7. SUPPLIES:

7.1. APPARATUS:
    7.1.1. LIST APPARATUS INCLUDING SIZE, SPECIFICATIONS, SOURCE

7.2. CHEMICALS:
    7.2.1. LIST. INCLUDE SPECIFICATIONS (PURITY) AND SOURCE. INCLUDE INSTRUCTIONS FOR PREPARATION FOR PREP OF REAGENTS AND REF STANDARDS

8. PROCEDURE:

8.1. SAMPLE PREPARATION:
    See NPSOP5100, Laboratory Handling and Preparation of Samples for Testing
    8.1.1. INCLUDE ANY INSTRUCTIONS SPECIFIC TO METHOD (i.e., kneading, mixing, slurrying) THAT SHOULD BE PERFORMED PRIOR TO STARTING TEST.
8.2. APPARATUS PREPARATION:
    8.2.1. CLEANING, SETUP, CALIBRATION, SUITABILITY CHECKS, WARM-UP PERIOD AS REQUIRED

8.3. DETERMINATION:
    8.3.1. STEP-BY-STEP INSTRUCTIONS

9. CALCULATIONS:

9.1. Manual: DETAILED MANUAL CALCULATIONS. DEFINE VARIABLES AND SOURCE OF ALL NUMBERS
9.2. Computer: DETAILED INSTRUCTIONS INCLUDING ALL INPUTS

10. RECORDS:

List all values or observations that should be recorded for this test.

Record	How often	Where Recorded	Recorded by
WHAT	WHEN	WHERE	RESPONSIBILITY

11. METHOD QUALITY CONTROL PLAN:

11.1. Method {List control samples, duplicates, blanks, limits, linearity checks, etc.}

Points to Check	Checked by	How often?	Specification	Record Check	Action if out of specification

11.2. Apparatus (List balance checks, oven temp checks, instrument checks, etc.)

Points to Check	Checked by	How often?	Specification	Record Check	Action if out of specification

12. RECORDKEEPING:

12.1. LIST OF RECORDS WHERE DATA IS RECORDED AND RETAINED

13. RELATED DOCUMENTS:

13.1. LIST SOPS OR METHODS RELEVANT TO THIS METHOD

14. REVISION HISTORY:

Copies of previous versions are maintained in the QA Master File.

Revision Date	Replaces	Description of Change

ATTACHMENT 3 A (Section 4.4.1)

Laboratory Project Manager: ____

A. Contact Information

Customer Name: ____

Customer Telephone: ____

Customer E-mail: ____

Customer Agency/Division/Address: ____

____

B. Project Specific Information

1. Type:

Survey: [ ] Regulatory [ ] Non-Regulatory

Investigation: [ ] Regulatory [ ] Non-Regulatory [ ] Criminal

[ ] Other (explain) ____

2. Sample Information:

Analyte(s) of Interest: ____

____

Number of Samples: ____

Amount of Sample Required: ____

Frequency of Submission: ____

Who will be Submitting Samples: ____

Submission Forms to be Used: ____

Sample Documentation Needed: ____

Chain of Custody Requirements: ____

Sample Container: ____

Sample Shipment Conditions: ____

Preservatives to be Used: ____

Sample Matrix: ____

Storage Requirements: ____

Time Required to Maintain Sample after Analysis: ____

Final Sample Disposition Requirements: ____

3. Technical Requirements:

Analytical Method to be Used: ____
Is the Method on Laboratory's Scope of Accreditation? ______Yes ______No
Does the customer require the work to be performed under accredited conditions? ______Yes ______No

Analytes: ____

Desired Reporting Limits or Method Detection limits: _____________________________________

Quality Control to be Included: ____

Quality Control Frequency: ____

Level of Method Validation and Confirmation: ____

Other Quality System Parameters Required: ____

4. Data Reporting:

Electronic or Paper Report Required: ____

Direct Data Entry into Client's Database Required: ____

Individual Sample Reports or Summary: ____

Content of the Report: ____

Should the report include the Measurement of Uncertainty: ______Yes ______No

Who Receives Copies of the Final Report: ____

Retention Schedules for Final Reports and Analytical Data: _________________________________

Reviewed By: ____

Approval Date: ____

ATTACHMENT 3 B (Section 4.4.1)

Request for Analytical Services

Date:		Received by:	
Client:		Phone number:	
1) Sample Matrix:			
2) Number of Samples expected:			
3) Analysis requested:		3b) Detection Limit Required	
4) Are these samples for regulatory action? *(circle one)*	Yes	No	
5) Start date			
6) Results needed by? *(turn around)*			
5) When will samples arrive?			
6) How will they be delivered and time expected?			
7) Is chain of custody required? *(circle one)*	Yes	No	
8) Type of report *(circle one)*	Electronic	Paper	Other *(describe)*
9) Notify Lab of sample delivery, turnaround and analyses required			
10) Comments			

ATTACHMENT 4 (Section 5.4.6)

Flow Chart Outlining the Uncertainty Estimation Process[1]:

STEP 1

SPECIFY THE MEASURAND

Write out a statement of what is being measured and define the scope of the test. Determine if there are any demands from the customer that measurement uncertainty must meet, e.g. expanded uncertainty must be less than +/-10%.

STEP 2

IDENTIFY UNCERTAINTY SOURCES

Identify all sources of uncertainty and determine their significance (see section 2.11.1 for guidance on what components are significant). These might include, but are not limited to:

- transportation, storage, handling
- sample preparation, e.g. grinding
- laboratory environment, e.g. temperature
- analytical method, such as:
  - volumetric glassware and pipettes
  - extraction/clean-up
  - different analysts/operators
  - instrument calibration
  - sample weighing
  - injection volume
  - peak integration
- sampling
- standard purity

STEP 3

QUANTIFY UNCERTAINTY COMPONENTS

These will include the uncertainty due to precision, $u(P)$, to laboratory and/or method bias, $u(bias)$, and any sources identified in step 2 that are not covered by laboratory control samples (e.g. sampling, sample preparation, storage). An estimate (best guess) may be required to determine these components.

STEP 4

CONVERT COMPONENTS TO STANDARD UNCERTAINTY

Convert all uncertainty components to standard deviations, that is, standard uncertainty, $u(x)$. Confidence intervals and similar distributions should also be converted to standard uncertainty.

STEP 5

CALCULATE COMBINED STANDARD UNCERTAINTY

This is achieved by taking the square root of the sum of the squares of all the standard uncertainty components.

e.g. $u(y) = \sqrt{[\ u(P)^2 + u(bias)^2 + u(x_1)^2 + u(x_2)^2 + \ldots\ u(x_n)^2\ ]}$

STEP 6

CALCULATE EXPANDED UNCERTAINTY

The expanded uncertainty, $U(y)$ is defined as $U(y) = k * u(y)$, where k is a coverage factor. The expanded uncertainty yields an interval which is expected to contain most of the values that are reasonably attributed to the measurand. Typically $k = 2$, which corresponds to a confidence level of 95%.

---

[1] EURACHEM/CITAC "Qualifying Uncertainty in Analytical Measurement" 3rd Edition, 2012

ATTACHMENT 5 (Section 5.4.6)

A Model for Estimating the Uncertainty of Measurement:[1, 2]

$y = x + (\delta + B) + e$

where: y = the measurement result of a sample
x = expected value for y
δ = method bias
B = laboratory bias
[the uncertainty for δ and B are combined to $u(bias)$]
e = random error from the precision of the method, $u(P)$

It should be noted that for empirical methods, the method bias (δ) is by definition zero, and only bias effects from measurement standards and laboratory bias (B) should be considered.

From the above model, an estimate of the uncertainty of measurement can be determined using the following equations:

1) When in-house validation studies, quality control or inter-laboratory comparison data is available:

$$u(y)^2 = u(P)^2 + u(bias)^2 = s(R_w)^2 + u(R_m)^2$$

where: $s(R_w)^2$ = the estimated variance of e under within-laboratory reproducibility conditions – intermediate precision
$u(R_m)^2$ = the estimated variance of laboratory and method bias (if applicable) – from recovery studies

2) When collaborative study or PT data exists:

$$u(y)^2 = s_L^2 + s_r^2 = s_R^2$$

where: s_R^2 = the estimated variance under reproducibility conditions
s_L^2 = the estimated variance of B (if once method is used by all laboratories) or of B and δ (if several different methods have been used)
s_r^2 = the estimated variance of e

---

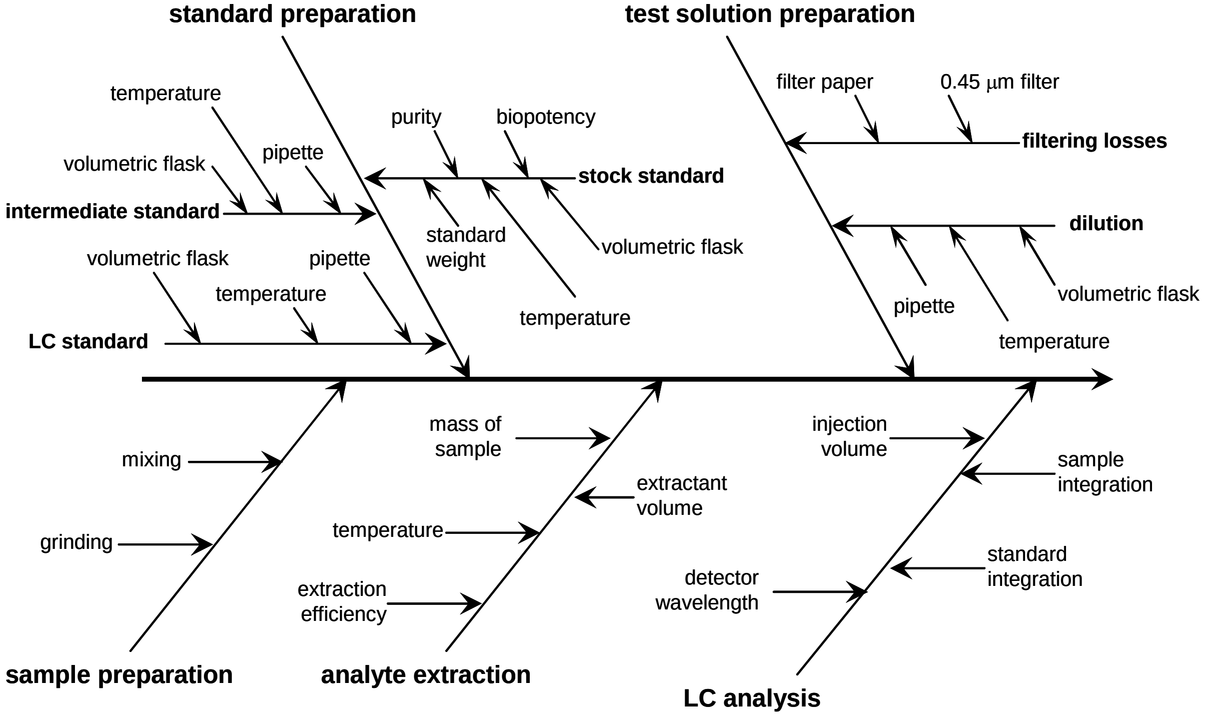
[1] Nordtest Report TR537 2004-02: Handbook for calculation of measurement uncertainty in environmental labs, 2nd edition

[2] ISO21748:2004(E): Guidance for the use of repeatability, reproducibility and trueness estimates in measurement uncertainty estimation, 1st edition

ATTACHMENT 6 (Section 5.4.6)

Example 1: Estimate of measurement uncertainty for monensin analysis in feed at medicated levels.

Identification of Significant Uncertainty Sources:



Introduction:

The method scope covers many matrices over a wide concentration range. Data from QC spike samples and validation test samples will be used to estimate the uncertainty components arising from the analytical method. Because QC and validation data cover a wide time period, uncertainty components from lab glassware, different analysts, different instruments, standard preparations, etc. are taken into account in the precision and bias estimates. However, the sample preparation (mixing and grinding) step is not taken into account by laboratory samples; therefore a separate estimate of the uncertainty due to sample preparation will be included.

An estimate for the combined measurement uncertainty will be determined from the following values:

- Uncertainty due to the precision of the method, $u(P)$
- Uncertainty due to the bias (from recoveries), $u(bias) = u(R_m)$
- Uncertainty due to sample treatment, $u(H)$

Combined Uncertainty, $u(y) = \sqrt{u(P)^2 + u(R_m)^2 + u(H)^2}$

In order to combine the uncertainty components according to the above equation, each component must be in standard uncertainty form.

Uncertainty due to precision:

5 samples (different matrices and concentrations) were analyzed at least 4 times over many analysis runs. The RSD_{pooled} for the data (see following precision table) is given below and will be used as the estimate for $u(P)$.

$$RSD_{pooled} = \sqrt{\frac{(4-1)\times 0.0455^2 + (4-1)\times 0.0468^2 + (4-1)\times 0.0084^2 + (5-1)\times 0.0121^2 + (4-1)\times 0.0096^2}{(4-1)+(4-1)+(4-1)+(5-1)+(4-1)}}$$

$$u(P) = RSD_{pooled} = 0.0294$$

Uncertainty due to bias:

A QC spike has been analyzed with each run for nearly two years. The resulting recovery and uncertainty results, $u(R_m)$ for the QC spike sample (see following bias table for data) will be used to estimate the uncertainty due to bias.

$$\overline{R}_m = \frac{102.4}{100} = 1.024 \qquad u(\overline{R}_m) = \overline{R}_m \times \sqrt{\left(\frac{0.706^2}{46\times 102.4^2}\right) + \left(\frac{0.82}{100}\right)^2} = 0.00846$$

Test to see if the recovery is significantly different from 1:

$$t = \frac{|1-1.024|}{0.00846} = 2.852 \qquad k = 2 \text{ (95\% confidence level)}$$

The recovery is significantly different than 1, since $t > k$. Therefore, a correction to $u(R_m)$ must be included.

$$u(\overline{R}_m)' = \sqrt{\left(\frac{1-1.024}{2}\right)^2 + 0.00846^2} = 0.0147$$

Uncertainty due to sample preparation:

Duplicate samples coming from different bottles have been analyzed for over a year. The standard deviation of the duplicates will be used as the estimate of uncertainty in sample homogeneity (see the following table for raw data).

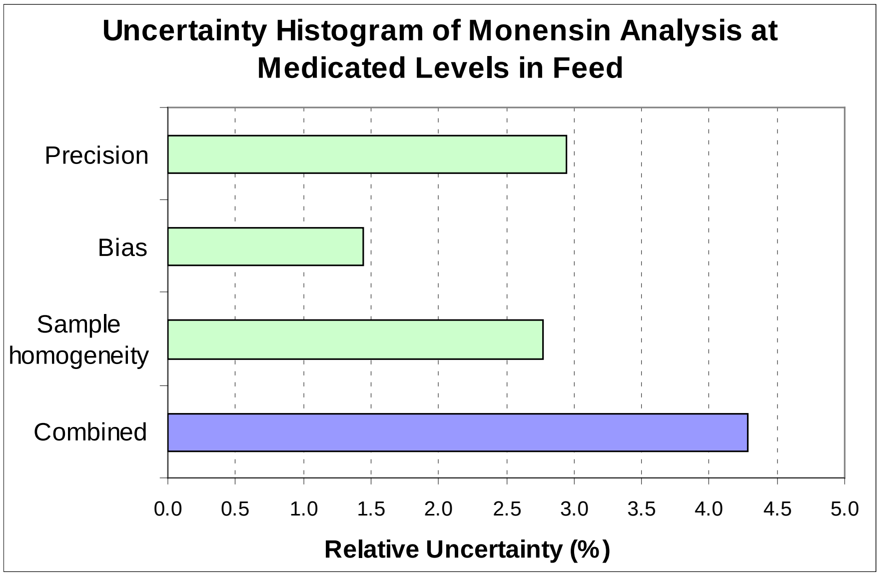
where: R_i = the range between the i^{th} duplicate pair
X_i = the mean between the i^{th} duplicate pair
n = the number of duplicate pairs

$$u(H) = s_{dup} = \sqrt{\frac{\sum_{i=1}^{n} (R_i / \overline{X}_i)^2}{2n}} = \sqrt{0.04140/54} = 0.028$$

Combined Uncertainty:
The relative standard uncertainties will be combined to obtain the relative standard uncertainty of the method.

$$\frac{u(y)}{y} = \sqrt{\left(\frac{u(P)}{P}\right)^2 + \left(\frac{u(R_m)}{R_m}\right)^2 + \left(\frac{u(H)}{H}\right)^2}$$

Description	Value, (x)	Standard uncertainty, $u(x)$	Relative standard uncertainty, $u(x)/x$
Precision	1	0.0294	0.0294
Bias	1.024	0.0147	0.0144
Sample treatment	1	0.028	0.028
Combined	N/A	N/A	**0.043**



Expanded Uncertainty:
The combined relative uncertainty of the method is multiplied by a coverage factor, $k = 2$ in order that the uncertainty estimate covers the 95% confidence level.

$$U(y) = u(y) * k = 0.043 * 2 = \mathbf{0.086}$$

Therefore, the expanded uncertainty is 0.086, which means that the true value lies within an interval of +/- 8.6% of the reported value, 95% of the time.

Tables of Raw Data:

Data used to determine uncertainty in sample treatment:

Sample	duplicate	duplicate 2	range	mean
OTT-FE-2005-0339	50.6	50.5	0.1	50.55
OTT-FE-2005-0225	28.1	29.5	-1.4	28.8
OTT-FE-2005-0224	29.9	30.3	-0.4	30.1
OTT-FE-2005-0170	62.5	58.1	4.4	60.3
OTT-FE-2005-0115	31.1	30.5	0.6	30.8
OTT-FE-2005-0056	26.3	28.0	-1.7	27.15
OTT-FE-2004-1701	30.9	28.3	2.6	29.6
OTT-FE-2004-1627	103.5	105.4	-1.9	104.45
OTT-FE-2004-1626	33.0	32.1	0.9	32.55
OTT-FE-2004-1592	49.8	49.6	0.2	49.7
OTT-FE-2004-1490	75.6	81.5	-5.9	78.55
OTT-FE-2004-1354	10.0	10.7	-0.7	10.35
OTT-FE-2004-1321	19.8	19.7	0.1	19.75
OTT-FE-2004-RU-0075	70.5	69.4	1.1	69.95
OTT-FE-2004-1176	75.3	77.4	-2.1	76.35
OTT-FE-2004-1134	27.5	26.6	0.9	27.05
OTT-FE-2004-1083	20.0	20.3	-0.3	20.15
OTT-FE-2004-1079	25.8	26.7	-0.9	26.25
OTT-FE-2004-1067	19.7	19.1	0.6	19.4
OTT-FE-2004-1004	13.8	13.6	0.2	13.7
OTT-FE-2004-1003	28.6	28.1	0.5	28.35
OTT-FE-2004-0968	23.4	22.4	1.0	22.9
OTT-FE-2004-0886	102	103	-1.0	102.5
OTT-FE-2004-0853	11.47	10.91	0.6	11.19
OTT-FE-2004-0458	510	516	-6.0	513
OTT-FE-2004-0181	11	10.9	0.1	10.95
OTT-FE-2003-1907	46.8	47.8	-1.0	47.3

Data used to determine precision:

Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	Std dev
OTT-FE-2002-0420	23.2	24.2	25.4	25.6		24.6	1.12
OTT-FE-2004-0213	44.1	41.1	39.4	41.5		41.5	1.94
OTT-FE-2004-RU-0038	31.8	31.9	31.3	31.6		31.7	0.265
OTT-FE-2004-1627	102	103	103	103.5	105.4	103	1.25
OTT-FE-2005-RU-0020	381	388	384	389		386	3.7

Data used to determine bias:

Date	Recovery	Date	Recovery	Date	Recovery
AD 06JAN2004	102.6	AD 11MAI2004	101.5	AD 04FEV2005	103.4
AD 13JAN2004	102.2	AD 14MAI2004	101.1	AD 18FEV2005	103.2
AD 15JAN2004	102.3	AD 01JUIN2004	101.1	AD 08MARS2005	102.8
AD 15JAN2004	103.6	JL 15 JUNE 04	101.2	AD 31MAR2005	101.7
AD 09FEV2004	102.6	AD 06AOUT2004	102.8	AD 01APR2005	101.1
AD 24FEV2004	102.2	AD 11AOUT2004	103.0	AD 14AVR2005	102.3
AD 02MARS2004	103.0	AD 31AOUT2004	102.4	AD 11MAY2005	102.3
AD 03MARS2004	102.1	JL 21 SEPT 2004	103.4	AD 13MAY2005	102.0
AD 23MARS2004	102.1	LP 12 NOV 2004	102.6	AD 25MAY2005	102.8
AD 01AVRIL2004	102.7	LP 17 NOV 2004	102.6	AD JUNE15/2005	102.3
AD 15AVR2004	102.0	AD 09DEC2004	103.4	AD 28JUIN2005	101.3
AD 20AVR2004	101.5	AD 07JAN2005	103.1	AD 11JULY2005	101.9
AD 22AVR2004	102.8	AD 13JAN2005	102.7	LP 15 AUG 2005	101.2
AD 28AVR2004	102.7	AD 14JAN2005	103.2	AD 02SEPT2005	102.9
AD 30AVR2004	103.0	AD 17JAN2005	103.5	LP 09 SEPT 2005	101.9
AD 04MAI2004	102.9				

Average of recovery values = 102.4
Standard deviation of recovery values = 0.706
Number of recovery values = 46

Estimate of uncertainty in the amount of spike added:

$$u(m_{spike})^2 = u(spike_{vol})^2 + u(spike_{conc})^2$$

where: $u(spike_{vol})$ = the uncertainty in the pipet used to spike the blank
$u(spike_{conc})$ = the uncertainty in the concentration of the spiking solution

$$u(spike_{vol}) = \sqrt{\left(\frac{100 \times B}{S \times \sqrt{3}}\right)^2 + R^2} = \sqrt{\left(\frac{100 \times 0.012}{1 \times \sqrt{3}}\right)^2 + 0.35^2} = 0.78$$

where: B = pipet bias (mL)
S = pipet size (mL)
R = pipet repeatability (%)

$$u(spike_{conc}) = \frac{\text{uncertainty of standard purity}}{t\text{ value at given confidence}} = \frac{0.5}{1.96} = 0.26$$

$$u(m_{spike}) = \sqrt{0.78^2 + 0.26^2} = 0.82$$

Example 2: Estimate of measurement uncertainty of zearalenone analysis in animal feed and feed ingredients (including cereal grains).

Identification of Significant Uncertainty Sources:

1) Method uncertainties - Preparation of standards
    - standard purity
    - weighing
    - glassware, volumetrics and pipettes
    - repeatability
    - intermediate time factor

   - Operations
    - sample weighing
    - extraction/clean-up efficiency
    - glassware, volumetrics and pipettes
    - instrument calibration
    - injection volume
    - peak integration
    - different analysts
    - different instruments
    - repeatability
    - intermediate time factor
2) Sample Preparation – the laboratory riffles the sample in half, one half is ground and split into two bottles, one of which is analyzed.

Introduction:

A collaborative study was conducted with 13 participating laboratories each analyzing 9 samples covering a range of matrices and concentrations (corn, barley, wheat, DDG, swine and dairy feeds at 0.08 – 0.28 ppm). The calculation of between laboratory reproducibility (s_R) from the collaborative study will be used to estimate the method uncertainty component. Since the data obtained from the collaborative study covers many laboratories, uncertainty components from lab glassware, different analysts, different instruments, standard preparations, etc. are taken into account. However, the sample treatment (grinding) step is not accounted for by the inter-laboratory data and an estimate of the uncertainty due to sample treatment will be included.

An estimate for the combined measurement uncertainty will be determined from the following values:

- Uncertainty due to method reproducibility, s_R
- Uncertainty due to sample treatment, $u(H)$

Combined Uncertainty, $u(y) = \sqrt{s_r^{\,2} + u(H)^2}$

In order to combine the uncertainty components according to the above equation, each component must be in standard uncertainty form.

<u>Uncertainty due to method reproducibility:</u>

9 samples were analyzed each by 13 different laboratories as part of a collaborative study. The relative s_R result from each sample will be pooled to yield an estimate of the method reproducibility. The raw data is summarized in the collaborative study table below. Using the between laboratory reproducibility value (s_R) in the uncertainty budget may lead to an overestimate of our laboratories uncertainty since s_R uses data from

many laboratories. However, the demand on the uncertainty estimate is low, so the use of s_R has been deemed acceptable.

$$\text{relative } s_{R\ pooled} = \sqrt{\frac{\sum_{i=1}^{k}\left[s_{R(i)}{}^2 \times (n_i - 1)\right]}{\sum_{i=1}^{k}(n_i - 1)}} = \sqrt{\frac{2.478515}{105}} = 0.154$$

Uncertainty due to sample treatment:

Duplicate samples from 2 different bottles have been analyzed over several years. The standard deviation of the duplicates, s_{dup} will be used as the estimate of uncertainty due to sample treatment (see the following table for raw data).

where: R_i = the range between the i^{th} duplicate pair
X_i = the mean between the i^{th} duplicate pair
n = the number of duplicate pairs

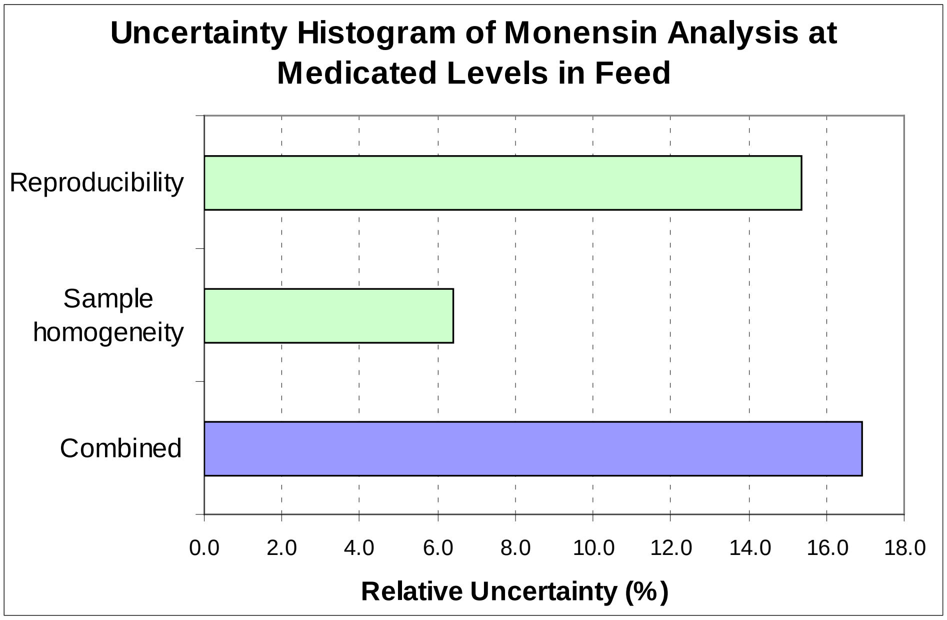
$$u(H) = s_{dup} = \sqrt{\frac{\sum_{i=1}^{n}(R_i / \overline{X}_i)^2}{2n}} = \sqrt{0.31364 / 76} = 0.064$$

Combined Uncertainty:

The relative standard uncertainties will be combined to obtain the relative standard uncertainty of the method.

$$\frac{u(y)}{y} = \sqrt{s_{r(pooled)}{}^2 + \left(\frac{u(H)}{H}\right)^2}$$

Description	Value, (*x*)	Standard uncertainty, *u*(*x*)	Relative standard uncertainty, *u*(*x*)/*x*
Reproducibility	1	0.154	0.154
Sample treatment	1	0.064	0.064
Combined	N/A	N/A	**0.167**



Expanded Uncertainty:

The combined relative uncertainty of the method is multiplied by a coverage factor, $k = 2$ in order that the uncertainty estimate covers the 95% confidence level.

$$U(y) = u(y) * \ k = 0.16653 * 2 = \mathbf{0.333}$$

Therefore, the expanded uncertainty is 0.333, which means that the true value lies within an interval of +/- 33.3% of the reported value, 95% of the time.

Tables of Raw Data:

Data used to determine between laboratory reproducibility:

Target Amount	Mean Result	relative sample s_R	# of labs
0.161	0.166	0.133073	13
0.111	0.115	0.177302	13
0.120	0.122	0.151216	13
0.0765	0.0796	0.149074	13
0.281	0.273	0.13751	12
0.142	0.134	0.165794	13
0.279	0.250	0.133536	12
0.115	0.120	0.196974	12
0.202	0.189	0.124808	13

Data used to determine uncertainty in sample treatment:

Sample	duplicate	duplicate 2	range	mean
OTT-FE-2001-1277	0.411	0.406	0.005	0.4085
OTT-FE-2001-1359	0.637	0.601	0.036	0.619
OTT-FE-2001-1470	0.44	0.46	-0.020	0.45
OTT-FE-2001-RU-	0.23	0.26	-0.030	0.245
OTT-FE-2001-RU-	0.197	0.193	0.004	0.195
OTT-FE-2002-0225	0.155	0.142	0.013	0.1485
OTT-FE-2002-1128	0.161	0.179	-0.018	0.17
OTT-FE-2002-1813	0.14	0.13	0.010	0.135
OTT-FE-2003-0981	0.167	0.170	-0.003	0.1685
OTT-FE-2003-1021	0.139	0.146	-0.007	0.1425
OTT-FE-2003-1023	0.113	0.110	0.003	0.1115
OTT-FE-2003-1072	0.138	0.142	-0.004	0.14
OTT-FE-2003-1083	0.229	0.230	-0.001	0.2295
OTT-FE-2003-1122	0.173	0.161	0.012	0.167
OTT-FE-2003-1194	0.124	0.119	0.005	0.1215
OTT-FE-2003-1304	0.115	0.105	0.010	0.11
OTT-FE-2003-1379	0.283	0.259	0.024	0.271
OTT-FE-2003-1426	0.235	0.275	-0.040	0.255
OTT-FE-2003-1457	0.270	0.259	0.011	0.2645
OTT-FE-2003-1528	0.150	0.143	0.007	0.1465
OTT-FE-2003-1975	0.141	0.136	0.005	0.1385
OTT-FE-2003-1994	0.193	0.185	0.008	0.189
OTT-FE-2003-1995	0.345	0.352	-0.007	0.3485
OTT-FE-2003-2048	0.108	0.106	0.002	0.107
OTT-FE-2004-0046	0.160	0.166	-0.006	0.163
OTT-FE-2004-RU-	0.136	0.143	-0.007	0.1395
OTT-FE-2004-0366	0.166	0.152	0.014	0.159
OTT-FE-2004-0683	0.113	0.114	-0.001	0.1135
OTT-FE-2004-0799	0.218	0.233	-0.015	0.2255
OTT-FE-2004-0859	0.525	0.553	-0.028	0.539
OTT-FE-2004-1216	0.106	0.104	0.002	0.105
OTT-FE-2004-1318	0.238	0.280	-0.042	0.259
OTT-FE-2004-1358	0.165	0.159	0.006	0.162
OTT-FE-2004-1598	0.458	0.453	0.005	0.4555
OTT-FE-2004-1610	0.123	0.131	-0.008	0.127
OTT-FE-2004-1611	0.101	0.147	-0.046	0.124
OTT-FE-2004-1656	0.370	0.330	0.040	0.35
OTT-FE-2004-1716	1.02	0.938	0.082	0.979

Example 3: Estimate of measurement uncertainty for potentiometric titration of sodium chloride in animal feed.

Significant Sources of Uncertainty:

Feed Sampling	10 cores are used to create the composite sample	
Sample Preparation	grinding the sample	
	rifling the ground portion into 2 bottles	
Analytical Method	Standardization of Titrant	standard purity
		standard mass
		accuracy of titrator
		repeatability of triplicate titrations
	Test Solution Preparation	sample mass
		glassware (pipettes and volumetrics)
		Extraction efficiency
	Test solution analysis	different analysts
		accuracy of titrator
		repeatability of triplicate titrations
		intermediate time factor

Introduction:

The method scope covers many matrices over a wide concentration range. The client has indicated that field sampling be included in the uncertainty estimate. Data from PT samples will be used to estimate the analytical method uncertainty components. Because this data covers a wide time period, uncertainty components from lab glassware, different analysts, repeatability, standard purity, etc. are taken into account in the precision and bias estimates. However, the field sampling and sample preparation (grinding and rifling) steps are not taken into account by the PT samples. As a result, an estimate of the uncertainty from these other components will be made.

An estimate for the combined measurement uncertainty will be determined from the following values:

- Uncertainty due to the precision of the method, $u(P)$
- Uncertainty due to the bias (from recoveries), $u(bias) = u(R_m)$
- Uncertainty due to sampling, $u(S)$
- Uncertainty due to sample prepartation, $u(H)$

Combined Uncertainty, $u(y) = \sqrt{u(P)^2 + u(R_m)^2 + u(S)^2 + u(H)^2}$

In order to combine the uncertainty components according to the above equation, each component must be in standard uncertainty form.

Uncertainty due to precision:

2 AAFCO samples (different matrices and concentrations) were each analyzed 30 times over several years and the standard deviation of the results determined (see the following precision table). The RSD_{pooled} for the two sets of data is given below and will be used as the estimate for $u(P)$.

$$RSD_{pooled} = \sqrt{\frac{(30-1)\times 0.008164^2 + (30-1)\times 0.007331^2}{(30-1)+(30-1)}}$$

$$u(P) = RSD_{pooled} = 0.00776$$

Uncertainty due to bias:

The AAFCO check sample has been analyzed each month for nearly two years. The resulting recovery (compared to the consensus value) and uncertainty results, $u(R_m)$ for this sample will be used to estimate the uncertainty due to bias (see following bias table for data). The consensus value and resulting standard deviation of the consensus value will be used as the check samples certified value and its corresponding uncertainty.

$$\overline{R}_m = \frac{0.9963}{1.00} = 0.9963 \qquad u(\overline{R}_m) = \overline{R}_m \times \sqrt{\left(\frac{0.02104^2}{22\times 0.9963^2}\right) + \left(\frac{0.0061}{1.00}\right)^2} = 0.007554$$

Test to see if the recovery is significantly different from 1:

$$t = \frac{|1-0.9963|}{0.007554} = 0.530 \qquad k = 2 \text{ (95\% confidence level)}$$

The recovery is not significantly different than 1, since $t < k$. Therefore, a correction is not necessary and $u(\overline{R}_m)$ =0.0076.

Uncertainty due to sample preparation:

The allowable limit between re-tests for a given sample under repeatability conditions is +/- 0.5%, as given in the quality control section of the method. Therefore, the result from a second test portion must be within +/- 0.5% of the result from the first test portion of the same sample, when analyzed in the same analysis run. Differences from the sample preparation step (ie. grinding and rifling) will contribute significantly to the difference between the results from two test portions (test solution preparation and analysis will also contribute to the difference, but to a lesser extent). Since there is insufficient real data from duplicates to estimate the sample preparation uncertainty component, the repeatability limit of +/- 0.5%, converted to a standard uncertainty, will be used to estimate $u(H)$.

$$u(H) = \frac{0.005}{\sqrt{6}} = 0.002041$$

Since the confidence level of the limit (0.5%) is unknown, but extreme values are unlikely, the limit can be divided by $\sqrt{6}$ to obtain a standard uncertainty.

Uncertainty due to sampling:

Samples submitted for testing are a composite of 10 sample cores. A report on the *precision of samples and analyses of fertilizers and feeds* was produced by S. R. Miles and F. W. Quackenbush in 1950 and published in the journal AOAC. Their report gives the precision due to sampling for several feed constituents and is summarized in the table below. The values shown in column 2 represent the sampling precision from 10 composites at the 95% confidence level.

Constituent	Sampling precision	Deviation from guarantee	Relative deviation from guarantee	Mean result[1]	Relative sampling precision[2]
Protein	0.31	+1.9	1.08	23.75	0.0131
Fat	0.03	+0.5	1.09	5.56	0.0054
Fibre	0.11	-1.6	0.78	7.27	0.0151

[1] The mean result is determined by dividing the deviation from guarantee by the relative deviation from guarantee minus one.

[2] The relative sampling precision is determined by dividing the sampling precision by the mean result.

From the above table, the largest relative sampling precision will be used to estimate the uncertainty component due to sampling. In order to convert this value to a relative standard deviation, the report states that it must be divided by 1.65. The converted value can be used directly as the standard uncertainty for $u(S)$.

$$u(S) = \frac{0.0151}{1.65} = 0.00917$$

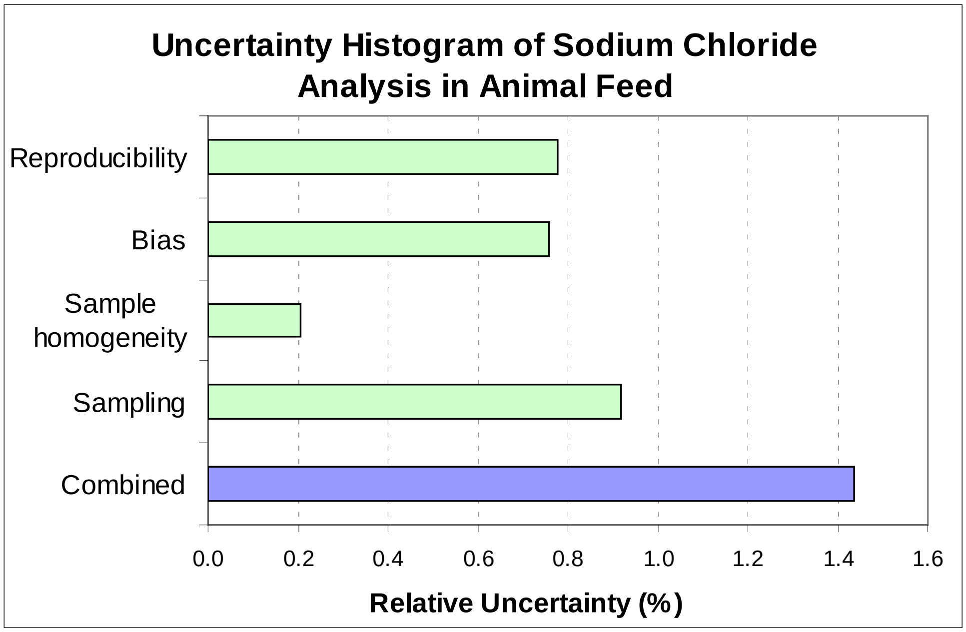
Combined Uncertainty:

The relative standard uncertainties will be combined to obtain the relative standard uncertainty of the method.

$$\frac{u(y)}{y} = \sqrt{\left(\frac{u(P)}{P}\right)^2 + \left(\frac{u(R_m)}{R_m}\right)^2 + \left(\frac{u(H)}{H}\right)^2 + \left(\frac{u(S)}{S}\right)^2}$$

Description	Value, (*x*)	Standard uncertainty, *u*(*x*)	Relative standard uncertainty, *u*(*x*)/*x*
Precision	1	0.0078	0.0078
Bias	0.996	0.0076	0.0076
Sample treatment	1	0.0020	0.0020
Sampling	1	0.0092	0.0092
Combined	N/A	N/A	**0.0143**

Note: The uncertainty due to sample treatment could be omitted from the calculation of combined uncertainty since it is less then one-third of the largest uncertainty component and makes an insignificant contribution to the overall uncertainty.



<u>Expanded Uncertainty:</u>
The combined relative uncertainty of the method is multiplied by a coverage factor, *k* = 2 in order that the uncertainty estimate covers the 95% confidence level.

$$U(y) = u(y) * k = 0.014348 * 2 = \mathbf{0.0287}$$

Therefore, the expanded uncertainty is 0.0287, which means that the true value lies within an interval of +/- 2.87% of the reported value, 95% of the time.

Tables of Raw Data:

Data used to determine precision:

Analysis date	Result	
2001/07/10	1.7843	Sample
2001/07/10	1.8030	2001-26 (n=24)
2002/09/29	1.8255	
2002/09/29	1.8032	
2002/10/30	1.7955	Grand AVG
2002/12/11	1.7936	1.79199
2002/12/20	1.7940	
2003/01/16	1.7999	SD ±
2003/02/27	1.7965	0.04312
2003/09/04	1.8069	
2003/04/22	1.8038	
2003/05/27	1.8127	
2003/07/09	1.8098	
2003/07/21	1.7903	
2003/09/11	1.7738	
2003/10/08	1.7856	
2003/11/04	1.7756	
2003/11/19	1.7871	
2003/12/12	1.7867	
2004/01/19	1.7949	
2004/02/17	1.8044	
2004/03/19	1.7877	
2004/04/21	1.7992	
2004/06/01	1.7903	
2004/07/07	1.8041	
2004/07/13	1.7584	
2004/10/05	1.8067	
2004/11/01	1.7781	
2004/12/03	1.7929	
2004/12/22	1.7599	
Average	**1.7935**	
Std Dev	**0.0146**	
RSD	**0.008164**	

Analysis date	Result	
1999/02/08	2.8368	Sample
1999/02/08	2.8827	9831 (n=23)
1999/03/09	2.8054	
1999/03/09	2.8351	
1999/03/09	2.8340	Grand AVG
1999/04/09	2.8192	2.8368
1999/05/07	2.8219	
1999/05/26	2.7949	SD ±
1999/06/22	2.8049	0.0671
1999/06/22	2.8643	
1999/07/14	2.8186	
1999/09/22	2.8263	
1999/10/07	2.8067	
1999/11/02	2.8288	
1999/11/15	2.8028	
1999/11/26	2.8196	
1999/11/26	2.8145	
1999/11/26	2.8116	
1999/12/02	2.8200	
2000/01/10	2.8252	
2000/01/10	2.7967	
2000/02/28	2.7969	
2000/02/28	2.8133	
2000/03/10	2.7934	
2000/04/28	2.7918	
2000/05/31	2.8182	
2000/07/25	2.8112	
2000/08/08	2.7882	
2000/10/04	2.7972	
2000/10/04	2.8258	
Average	**2.8169**	
Std Dev	**0.0207**	
RSD	**0.007331**	

Data used to determine bias:

Feed matrix	Consensus value	Relative dev. of consensus value	# of labs	Recovery value
Cattle Conditioner	1.0390	0.0038	36	1.000
Milk Replacer	2.2778	0.0050	19	1.007
Stocker Starter, Medicated	0.8072	0.0074	32	0.988
Calf Starter, Medicated	0.7932	0.0067	33	0.991
Pig Starter, Medicated	0.6469	0.0055	33	0.967
Pig Starter, Medicated	0.7164	0.0042	35	0.994
Complete Pig Wormer	0.5165	0.0062	44	0.972
Pig Starter, Medicated	0.8402	0.0048	31	0.995
Show Broiler, Medicated	0.4057	0.0092	38	0.979
Pig Grower, Medicated	0.5776	0.0060	37	0.985
Pig grower, med.	0.5329	0.0062	33	1.074
Multi-species dewormer	1.2058	0.0045	34	0.996
Pig started, med.	0.7631	0.0060	34	0.997
Pig starter	0.5664	0.0061	34	0.976
Beef Starter	0.6942	0.0091	36	0.991
Chicken crumble, med.	0.5232	0.0072	36	1.002
Pig started, med.	0.5877	0.0070	33	1.000
Cattle pellets, med.	5.3171	0.0033	38	0.989
Broiler starter, med.	0.4809	0.0079	32	0.987
Milk replacer	2.4292	0.0044	23	1.005
Lamb balancer	3.6237	0.0033	41	1.013
Calf Starter, Medicated	1.0508	0.0045	29	1.012
	pooled value	**0.0061**	**mean**	**0.9963**
			std dev	**0.0210**

ATTACHMENT 7 A (Section 5.7.2)

State Department of Agriculture Division Address Telephone **SAMPLE COLLECTION REPORT**	COMMERCIAL FEED	Inspection Number Sample Type

NAME ON LABEL/GUARANTOR			DATE OF SAMPLE	INSPECTOR SAMPLE NO.
Address			PRODUCT NAME	
City	State	Zip	LOT NO.	INVOICE NO. INVOICE ATTACHED” ☐ Yes ☐ No
NAME OF MANUFACTURER/PRODUCER			ORIGINAL SHIPMENT (lbs, tons, etc.)	ORIGINAL SHIPMENT DATE
Address			DESCRIPTION OF LOT SAMPLED: ☐ BAG ☐ BULK ☐ CAN	
City	State	Zip	SIZE OF LOT SAMPLED: (tons, lbs, bags, etc.)	
SAMPLE SECURED FROM			SAMPLE METHOD: ☐ Stream _______________ ☐ Probe ________________ ☐ Liquid ________________ ☐ Bag Purchase __________	
Address	Telephone No. (include area code)		REASON FOR SAMPLE COLLECTION: ☐ Routine ☐ Requested by _______________ ☐ Other ____________	
City	State	Zip	COMMENTS:	
CUSTOMER				
Address				
City	State	Zip		

NOTICE OF INSPECTION. FOR THE PURPOSE OF DETERMINING COMPLIANCE WITH:

☐ State Commercial Feed Law and Rules with related portions of the Federal Food, Drug, Drug and Cosmetic Act and Regulations (FFDCA). Authority for entry, inspection and sampling is found in Statute § XXX.

I AM SATISFIED THIS SAMPLE REPRESENTS THE PRODUCT DESCRIBED

SIGNED ____ TITLE ____

INSPECTOR’S SIGNATURE _______________________ TITLE ___________________ COUNTY ________________

Analyte	**Claim**	**Analyte**	**Claim**	**Analyte**	**Claim**
Protien, crude	min.	Cu		CTC	
EqCP	max.	Fe		DECO	
Fat, crude	min.	Mg		LAS	
Fiber, crude	max.	K		LINCO	
ADF	max.	Se		MON	
Moisture	max.	Zn		OTC	
Ash	max.	LYS	min.	PYT	
NaCl (salt)		Vit. A		SMZ	
Ca		AMP		STZ	
P		ARSAN		TIAM	
Na		CDX		TYL	

CUSTOMER-FORMULA FEED SAMPLE: Attach Formula and Labels for Ingredients

DRUG SOURCE = ____________________________% or ____________________________________grams per pound

ATTACHMENT 7 B (Section 5.7.2)

REQUEST FOR ANALYSIS

Laboratory Name
Address 1
Address 2
City, State Zip/Post Code
Phone No

Customer: ____________________ Alt. Invoice: ____________________
Submitter: ____________________ Address ____________________
Address: ____________________ ____________________
City, State ZIP ____________________ ____________________
Phone ____________________ Phone: ____________________
Fax ____________________ Fax: ____________________

Lab Use Only

Sample Type:	
Feed	
Fertilizer	
Lime	
Pesticide	
Seed	
Food & Dairy	
Inorganic Lab	
Other	

Delivery Method:	
Hand	
Mail	
Courier (attach receipt)	

Sample Description	**Test / Guarantee**	**Requested Analysis**														**Lab Number**

Comments:

I request the above analysis be performed & understand I may be required to pay the associated fees

Relinquished/
Submitted by: Date: Time:

Received
for Lab by: Date: Time:

ATTACHMENT 8 A (Section 5.8.2)

CHAIN OF CUSTODY

Page:	of:

Collected From:	Collected Date:
Sample Description:	Collected Time:
Location:	

Collected By:

Name:	Signature:
Position:	

Transfer of Possession

#	Date / Time	Location of Sample Transfer	Transfer From	Transfer To
1	Date:		Name:	Name:
			Position:	Position:
	Time:		Signature:	Signature:
2	Date:		Name:	Name:
			Position:	Position:
	Time:		Signature:	Signature:
3	Date:		Name:	Name:
			Position:	Position:
	Time:		Signature:	Signature:
4	Date:		Name:	Name:
			Position:	Position:
	Time:		Signature:	Signature:

ATTACHMENT 8 B (Section 5.8.2)

State Department of Agriculture
Laboratory Division
Street Address
City, State ZIP+4
Phone
Fax

CHAIN-OF-CUSTODY

(Sample Transfer Form)

Sample ID(s):	Sample Type:
Reason for Transfer:	
Sample ID(s):	Sample Type:
Reason for Transfer:	
Sample ID(s):	Sample Type:
Reason for Transfer:	
Sample ID(s):	Sample Type:
Reason for Transfer:	

Relinquished By (Facility Name):	
Signature:	Date/Time:
Received By (Facility Name):	
Signature:	Date/Time:
Relinquished By (Facility Name):	
Signature:	Date/Time:
Received By (Facility Name):	
Signature:	Date/Time:

ATTACHMENT 8 C (Section 5.8.2)

SAMPLE TRANSFER FORM

1. STORAGE LOCATION		2. NAME OF PRODUCT	3. SAMPLE NO.
A.	C.		
B.	D.	4. NAME AND ADDRESS OF RESPONSIBLE FIRM	CR$_X$/DEA SPL SPLIT SAMPLE

5. DATE SAMPLE RECEIVED	6A. BY WHOM RECEIVED	6B. DIST/DIV ☐ ☐	7. DATE RECORDS REC'D

8. **METHOD OF SHIPMENT**	A. PERSONALLY FROM	C. SHIPPED FROM
	B. VIA PP BUS FREIGHT AIR	D. B/L NO.

9. **DESCRIPTION OF SHIPMENT**	A. SHIPPING CONTAINERS ☐ ☐	NUMBER ☐	TYPE ☐	CONDITION
	B. SAMPLE PACKAGES	NUMBER	SIZE, TYPE, ETC.	CONDITION
	C. SEAL INSCRIPTION	COPY IN FULL		CONDITION

10. SAMPLE DELIVERY				**11. SAMPLE RETURNED**			
DATE	AMOUNT	FROM	TO	DATE	AMOUNT	TO	FROM

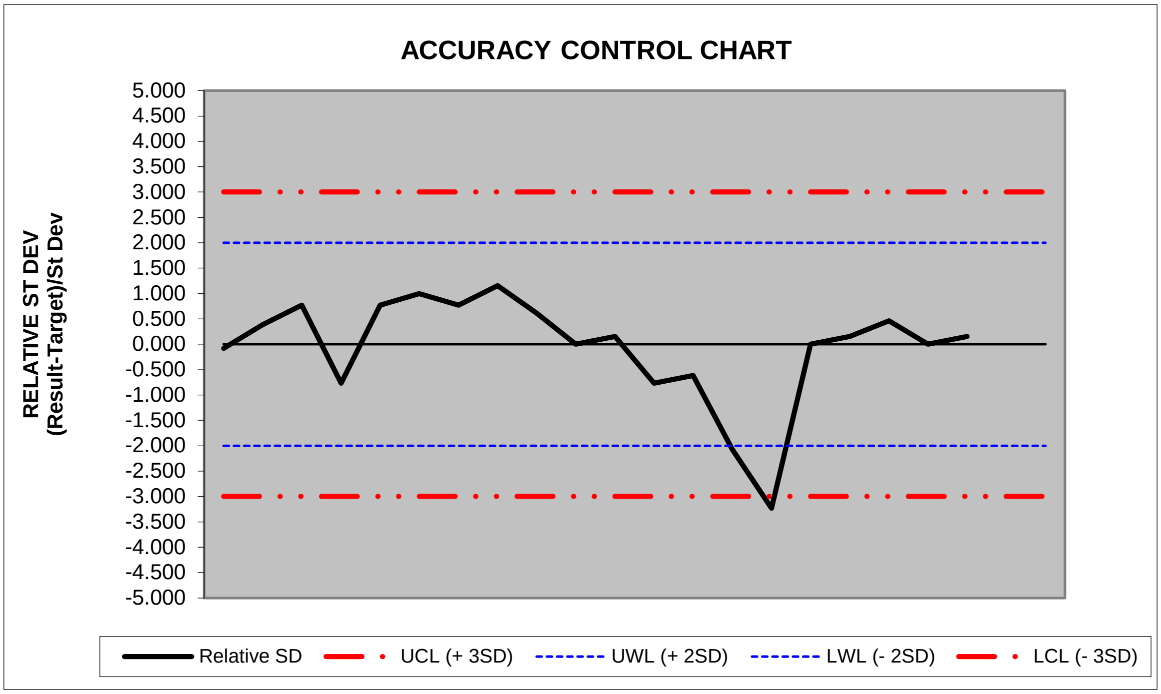
12. **SAMPLE DISPOSITION**	A. DATE SDN	B. DATE DESTROYED	C. DESTRUCTION METHOD	D. AMOUNT DESTROYED	E. BY WHOM	F. REASON

ATTACHMENT 9A (Section 5.9.5)

ACCURACY CONTROL CHART

(Use for Reference Sample of Known Analyte Concentration & Uncertainty)

Date	Test Result	Target/Ref Value	St Dev	Result Rel St Dev	In Control?
10/1	2.54	2.55	0.13	-0.077	YES
10/2	2.60	2.55	0.13	0.385	YES
10/3	2.65	2.55	0.13	0.769	YES
10/4	2.45	2.55	0.13	-0.769	YES
10/10	2.65	2.55	0.13	0.769	YES
10/11	2.68	2.55	0.13	1.000	YES
10/15	2.65	2.55	0.13	0.769	YES
10/16	2.70	2.55	0.13	1.154	YES
10/17	2.63	2.55	0.13	0.615	YES
10/21	2.55	2.55	0.13	0.000	YES
10/22	2.57	2.55	0.13	0.154	YES
10/23	2.45	2.55	0.13	-0.769	YES
10/24	2.47	2.55	0.13	-0.615	YES
10/29	2.28	2.55	0.13	-2.077	WARNING
10/30	2.13	2.55	0.13	-3.231	NO
11/6	2.55	2.55	0.13	0.000	YES
11/7	2.57	2.55	0.13	0.154	YES
11/8	2.61	2.55	0.13	0.462	YES
11/8	2.55	2.55	0.13	0.000	YES
11/8	2.57	2.55	0.13	0.154	YES

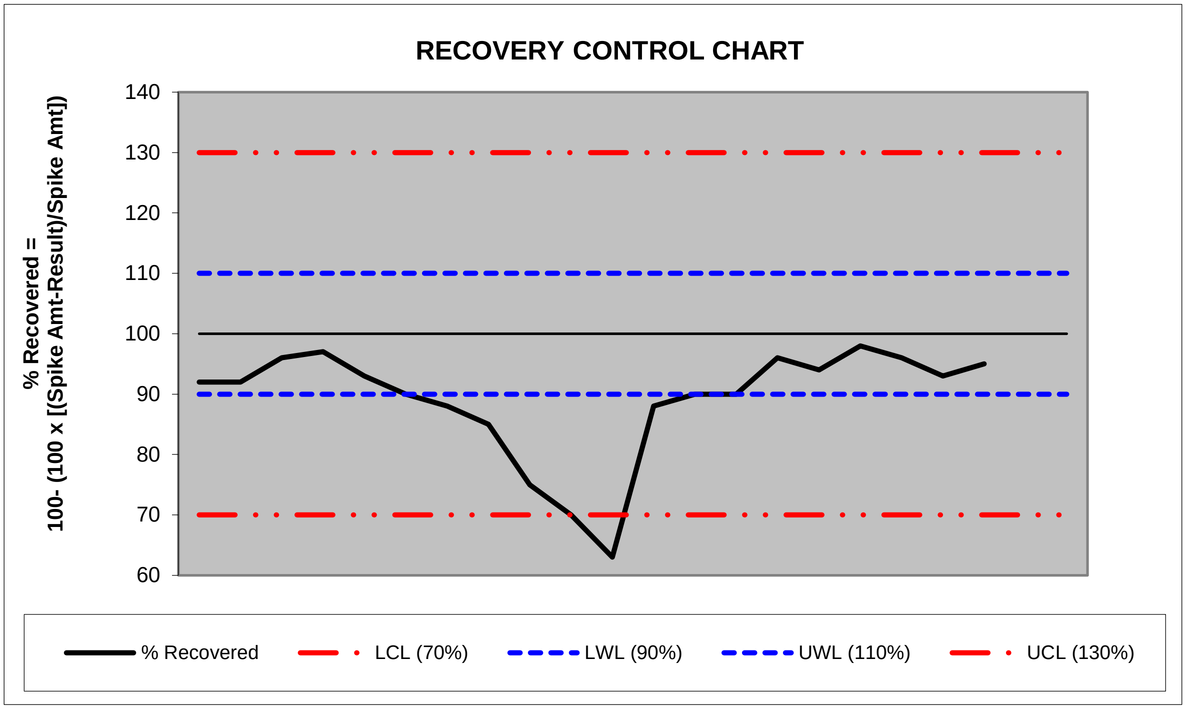


ATTACHMENT 9B (Section 5.9.5)

RECOVERY CONTROL CHART

(Use for Spiked Samples)

Date	Result	Spike Amount	% Recovered = 100 - (100 x [(Spike - Result)/Spike Amt])	In Control?
10/1	92	100	92	YES
10/2	92	100	92	YES
10/3	96	100	96	YES
10/4	97	100	97	YES
10/10	93	100	93	YES
10/11	90	100	90	YES
10/15	88	100	88	WARNING
10/16	85	100	85	WARNING
10/17	75	100	75	WARNING
10/21	70	100	70	WARNING
10/22	63	100	63	NO
10/23	88	100	88	WARNING
10/24	90	100	90	YES
10/29	90	100	90	YES
10/30	96	100	96	YES
11/6	94	100	94	YES
11/7	98	100	98	YES
11/8	96	100	96	YES
11/8	93	100	93	YES
11/8	95	100	95	YES



ATTACHMENT 9C (Section 5.9.5)

PRECISION CONTROL CHART

(Use for Duplicate Samples)

Date	Result 1	Result 2	RPD = 100 x { |Rslt 1-Rslt 2|/Avg Rslt }	In Control?
10/1	2.54	2.75	7.94	WARNING
10/2	2.75	2.78	1.08	YES
10/3	2.78	2.97	6.61	YES
10/4	2.97	2.80	5.89	YES
10/10	2.80	2.77	1.08	YES
10/11	2.77	2.73	1.45	YES
10/15	2.73	2.77	1.45	YES
10/16	2.77	2.43	13.08	NO
10/17	2.63	2.55	3.09	YES
10/21	2.55	2.57	0.78	YES
10/22	2.57	2.45	4.78	YES
10/23	2.45	2.47	0.81	YES
10/24	2.47	2.50	1.21	YES
10/29	2.50	2.41	3.67	YES
10/30	2.41	2.35	2.52	YES
11/6	2.35	2.31	1.72	YES
11/7	2.31	2.24	3.08	YES
11/8	2.24	2.31	3.08	YES
11/8	2.31	2.35	1.72	YES
11/8	2.35	2.54	7.77	WARNING

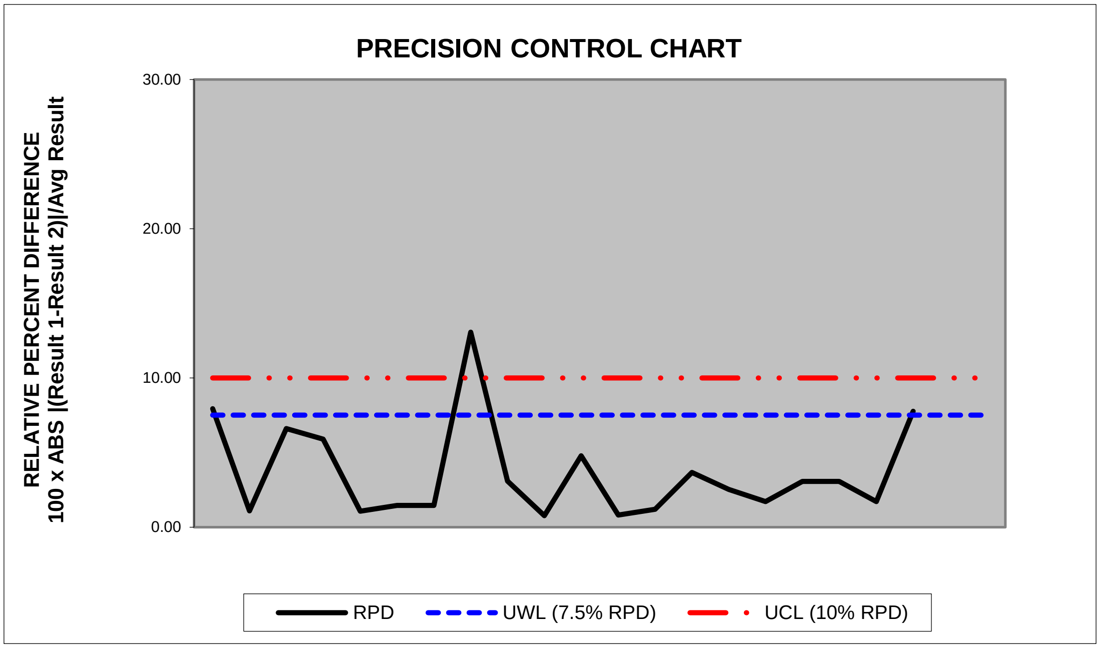


Table 2

Equipment, Calibration, Verification and Maintenance

Recommended Practices for Feed Laboratories

		Verification		Maintenance	
Equipment	**Calibration**	**Parameter checked**	**Minimum Frequency**	**Recommended**	**Minimum Frequency**
Autoclaves		Accuracy of temperature sensing system, uniformity and stability of temperature	At installation (or initial use), annually	Service, Clean as needed	Every 6 months or recommended by manufacturer or per lab procedures;
Automated and proprietary instrumentation and methods		Refer to instrument manufacturer's instructions	As recommended by manufacturer or per laboratory procedure	Refer to instruments manufacturer's instructions	As recommended by manufacturer or per laboratory procedure
Balances	Calibrated with reference weights, performed annually	Mass measurement	Day of use with internal calibration or reference weight	Service	Annually
				Clean as needed	Each use
Bath, Evaporator, Concentrators, Blocks - including water, oil, sand, beads and dry		Temperature	Thermometer verified annually	Clean as needed	For Quality critical process, temperatures are recorded with day of use.
Centrifuge				Service, Clean as needed	As recommended by manufacturer or per laboratory procedure

Table 2

Equipment, Calibration, Verification and Maintenance

Recommended Practices for Feed Laboratories

		Verification		Maintenance	
Equipment	**Calibration**	**Parameter checked**	**Minimum Frequency**	**Recommended**	**Minimum Frequency**
Water Purification System		Resistivity/conductivity	Weekly or as recommended by method	As recommended by manufacturer or per laboratory procedure	As recommended by manufacturer or per laboratory procedure
Digestors, Open Vessel (hot block, Kjeldahl)		Temperature and uniformity	Annual	Refer to instruments manufacturer's instructions	
Heating Block (used for PCR, CHARM)		Temperature	Thermometer verified annually	Refer to instruments manufacturer's instructions	
Dispensing equipment, vial fillers		Mass measurement/volume	At installation and day of use at each volume		
Freezers		Accuracy of temperature sensing system, uniformity and stability of temperature	At installation (or initial use), annually	Clean, defrost	As scheduled by lab
		Temperature	Day of use		
Hydrometer, reference		One point calibration using standard of known specific gravity	Every 2 years		
Hydrometer, working		One point calibration to reference hydrometer	Annually		

Table 2

Equipment, Calibration, Verification and Maintenance

Recommended Practices for Feed Laboratories

		Verification		Maintenance	
Equipment	**Calibration**	**Parameter checked**	**Minimum Frequency**	**Recommended**	**Minimum Frequency**
Incubator		Accuracy of temperature sensing system, uniformity and stability of temperature	At installation (or initial use), annually	Clean	As scheduled by lab
		Temperature	Day of use		
Laminar flow hoods		Service	Annually	Clean, sanitize	Each use
Microscope		Calibrate stage micrometer	At installation	Service	Annually
				Clean lens	Each use
Ovens and furnaces		Accuracy of temperature sensing system, uniformity and stability of temperature	At installation (or initial use), annually	Clean	As scheduled by lab
		Temperature	Day of use		
Petri dishes		Sterility	Check each lot		

Table 2

Equipment, Calibration, Verification and Maintenance

Recommended Practices for Feed Laboratories

		Verification		Maintenance	
Equipment	**Calibration**	**Parameter checked**	**Minimum Frequency**	**Recommended**	**Minimum Frequency**
pH meters, ion selective, and related conductivity equipment		pH reading with standard buffers or appropriate solutions	Bracketing anticipated range of use; before use in anticipated range	Clean electrodes	Each use
Pipets, disposable		Cleanliness, sterility (for microbiology applications), mass measurement/volume	All parameters each lot		
Refrigerators		Accuracy of temperature sensing system, uniformity and stability of temperature	At installation (or initial use), annually	Clean	As scheduled by lab
		Temperature	Day of use		
Safety cabinets and laminar airflow cabinets (if used for culture or sterility work)		Open medium control (sterility check; magnehelic gauge check), airflow	At installation; quarterly	Service	Annually
				Clean, sanitize	Each use
Special incubation equipment				Clean, sanitize	Each use

Table 2

Equipment, Calibration, Verification and Maintenance

Recommended Practices for Feed Laboratories

		Verification		Maintenance	
Equipment	**Calibration**	**Parameter checked**	**Minimum Frequency**	**Recommended**	**Minimum Frequency**
Spectrophotometer		Blank Readings	Day of use	Clean	As recommended by manufacturer or per laboratory procedure
		Wavelength	At installation by manufacturer		
Still, DI and RO Units		Conductivity	Weekly	Clean	As recommended by manufacturer or per laboratory procedure
Thermocouples, reference		Boiling water & ice point (or other temperatures indicative of typical working range)	Annually		
Thermometer reference Recognized national or international	Critical points on scale, Annually				
Thermometer, working		Specific points against national or international reference thermometer	Annually		
Timers (quality critical)		National Time Standard	Annually		

Table 2

Equipment, Calibration, Verification and Maintenance

Recommended Practices for Feed Laboratories

		Verification		Maintenance	
Equipment	**Calibration**	**Parameter checked**	**Minimum Frequency**	**Recommended**	**Minimum Frequency**
Volumetric delivery devices; mechanical pipets and mechanical burets	As recommended by Manufacturer	Accuracy and precision using mass of water or by spectrophotometric method	Every 6 months	Clean	As scheduled by lab
Volumetric glassware Non-class A - pipets, burets, and volumetric flasks		Accuracy and precision using mass of water or by spectrophotometric method	Upon receipt, manufacturer's Certificate of Graduation Accuracy for Class A or Class A designation on glassware may be accepted.		
Water activity meter		Water activity of known solutions	Day of use		
Weights, reference	Re-calibration for accuracy from recognized national or international calibration units. Every 5 years.				
Weights, working		Verify against reference weights	Annually		

Table 3

Quality Control Checks for Analytical Procedures

Quality Control	Purpose	Frequency	Acceptance Criteria	Corrective Action
Laboratory Reagent Blank	As required by method. Used to check for contamination and/or interferences.	Minimum one per sample set.	This is method dependent. For example, a blank response of less than 1/2 method limit of quantitation.	High Blank: 1. Check for instrument or equipment contamination 2. Check for reagent contamination 3. Repeat blank determination (and analysis) after determining the source of contamination. Negative Blank: (i.e. inverted response) 1. Check instrument performance/interference. 2. Check for reagent interference. 3. Repeat blank determination (and analysis) after determining the source of interference.
Laboratory Control Sample	Applicable to most methods when material is available. Used to monitor method and analyst performance.	Minimum one per sample set.	Analytical result within the control limits.	1. Review calculations and laboratory techniques. 2. Check instrument performance. 3. Investigate possible sources of cross contamination and error. 4. Repeat analysis.
Control Plates for Microbiological Assay	Drugs and Antibiotics, Used to check media sterility and organism viability.	Minimum one per sample set.	Negative control: no growth Positive control: growth	1. Growth in negative control indicates contamination. 2. No growth in positive control indicates issue with media or organism. 3. Repeat analysis.
Matrix Spike	Drugs, Antibiotics, Vitamins, Micronutrients, Contaminates and other: Used to check method performance.	One per sample set, Potentially violative samples, problem samples.	Recoveries as established for each method/matrix.	1. Investigate possible problems with process. 2. Check instrument performance. 3. Repeat analysis.

Table 3

Quality Control Checks for Analytical Procedures

Quality Control	Purpose	Frequency	Acceptance Criteria	Corrective Action
Duplicate Analysis	All methods when possible. Used to check on method and analyst precision.	One per sample set.	Relative range measurements within those established by inter-laboratory method performance study or intra-laboratory studies.	1. Review calculations. 2. Check instrument performance. 3. Check sample preparation procedure. 4. Repeat analysis in duplicate. 5. Repeat the set. Repeat samples in duplicate if problem cannot be identified.
Calibration check	Most instrumental methods. Used to verify calibration & standards, and establish instrument performance.	Full calibration prior to analysis. Calibration check every nth sample, depending on method.	Established for each method.	1. Check instrument performance. 2. When applicable, check standard preparation 3. Prepare new calibration curve and calibration check controls with fresh standards.
Standard Addition	Used to check for interference and instrument performance, i.e., linearity, in spectrophotometric methods such as atomic absorption, ion specific electrode methods, and chromatographic methods.	Each sample, if used in calculation of results. As needed when used to check for matrix interference, and for problem samples.	As established for each method/matrix.	1. Check instrument performance. 2. Reevaluate the method for the matrix. 3. Re-analyze preferably with method modification
Internal Standard	As applicable and defined by methods e.g. Sulfamethazine, Amino Acids, Vitamin A.	Each sample.	As established for each method.	1. Check instrument performance (proper calibration line fitting). 2. Re-inject sample solution. 3. Check internal standard solution. 4. Repeat analysis.