United States Department of Agriculture

Food Safety and Inspection Service

CLG 1.00

FSIS Laboratory System Introduction, Method Performance Expectations, and Sample Handling for Chemistry

This chapter introduces the FSIS laboratory system and provides details for FSIS laboratory chemistry method performance expectations. Sample receipt, handling, preparation, and discard criteria are outlined along with tolerance and decision rules.

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Mission Statement—FSIS Laboratory System

The Field Service Laboratories (FSLs) support the mission of the United States Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS) in protecting public health by performing analyses of samples collected from meat, poultry, egg products, and *Siluriformes*. The FSLs are committed to integrity, accuracy, reliability, and timeliness of data. All laboratory employees are fully trained in the FSL quality management system, which is based on the current versions of the ISO/IEC 17025 standard and the AOAC/ALACC Guidelines for Laboratories Performing Microbiological and Chemical Analyses of Food, Dietary Supplements and Pharmaceuticals. Further, employees are trained to perform each laboratory method and to implement the FSIS laboratory quality system documents that describe the policies and procedures related to sample analysis. Ongoing assessment of employee and laboratory proficiencies are critical to the maintenance of the FSIS quality management system. These are achieved using quality assurance activities such as internal and external audits, the use of validated methods, and annual management reviews. Routine controls are also in place in each FSL such as negative and positive controls, third-party proficiency tests (PTs), and checks on data reporting. The FSIS FSLs strive for continual improvement and provide a standard of service that meets regulatory requirements. The FSLs have earned ISO 17025 accreditation to perform certain laboratory methods. Scopes of accreditation for each laboratory are available publicly and listed below. A2LA is currently the external accrediting body.

FSIS Field Service Laboratory	A2LA Certification Number
FSIS Eastern Laboratory (EL) Athens, Georgia	**1898.02** (Biological) **1898.03** (Chemical)
FSIS Midwestern Laboratory (ML) St. Louis, Missouri	**1898.04** (Biological) **1898.05** (Chemical)
FSIS Western Laboratory (WL) Albany, California	**1898.06** (Biological) **1898.07** (Chemical)

FSIS CORE VALUES

ACCOUNTABLE
FSIS holds itself accountable in fulfilling its regulatory mission and in serving the public interest.

COLLABORATIVE
FSIS actively promotes and encourages collaboration within our Agency and with our partners to prevent illness and protect public health.

EMPOWERED
FSIS employees are empowered with the necessary training tools, and techniques needed to make and carry out informed decisions to protect public health and promote food safety.

SOLUTIONS-ORIENTED
FSIS is committed to deploying creative, innovative, and effective evidence-based solutions to ensure that the United States food supply is safe.

Introduction

The Chemistry Laboratory Guidebook (CLG) contains chapters that provide approved regulatory methods and detailed protocols used by the USDA FSIS FSLs to screen and identify the presence of chemical hazards in meat, poultry, egg products, and *Siluriformes*. Regulatory justification for these commodities is granted under the authorities of the Poultry Products Inspection Act (PPIA), the Egg Products Inspection Act (EPIA), Federal Meat Inspection Act (FMIA), and Food Allergen Labeling and Consumer Protection Act (FALCPA). The FSIS FSLs test regulatory samples collected by FSIS inspection program personnel (IPP) as stipulated in the publicly available FSIS Annual Sampling Plan[1]. The FSIS laboratories include the Eastern Laboratory (EL) in Athens, GA; the Midwestern Laboratory (ML) in St. Louis, MO; and Western Laboratory (WL) in Albany, CA. Laboratory chemical analysis activities in support of the Annual Sampling Plan include monitoring of chemical residues such as veterinary drugs, pesticides, and environmental contaminants; conducting label verifications through allergen and food chemistry analysis; and ensuring the protection of food from chemical hazards such as metals and toxins. The CLG provides detailed protocols for the materials, equipment, preparation of reagents and standard solutions for chemical analysis, procedures for sample preparation, methods for chemical extraction of analytes of interest, parameters for analytical instrument settings, and method performance characteristics. The general overview for sample analysis using a CLG method is summarized in Figure 1.

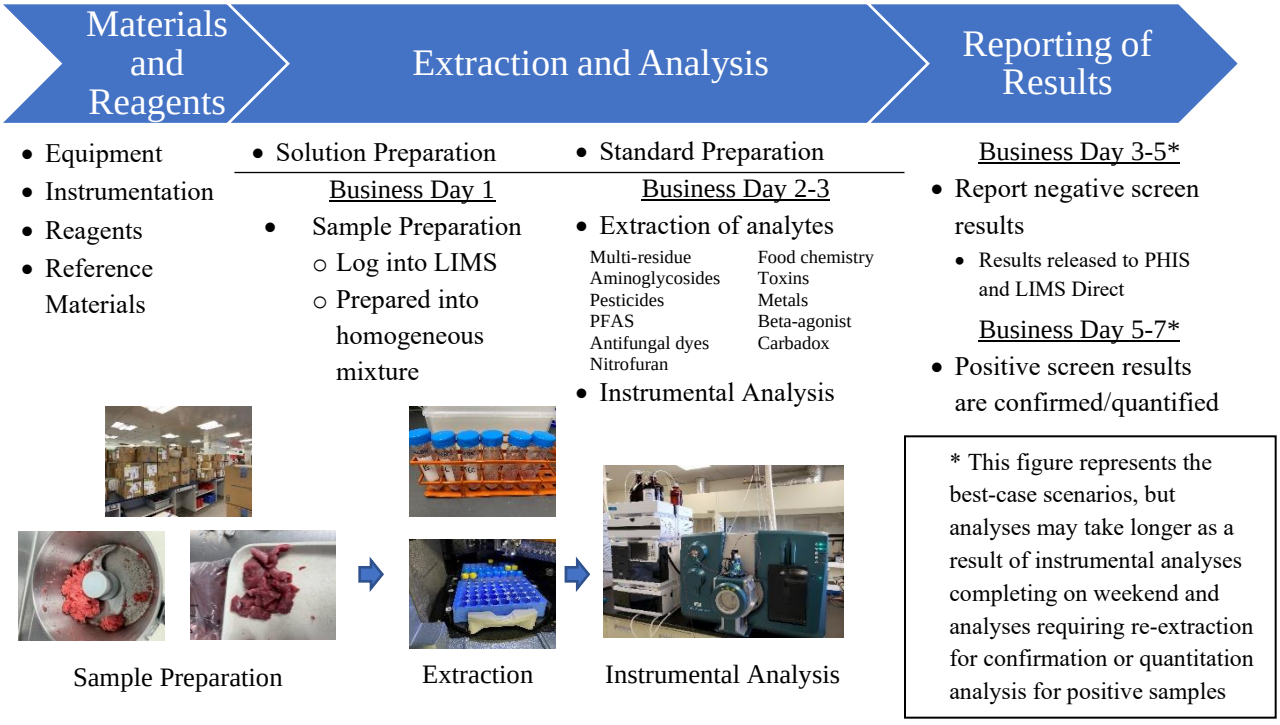

Figure 1: General overview of chemical analysis and reporting of results. Refer to CLG chapters for individual chemical analysis for details.

[1] https://www.fsis.usda.gov/science-data/sampling-program

Laboratory Information Management System (LIMS)

To ensure data integrity, the FSIS FSLs standardize inventory and data recording across the three laboratory locations using an integrated information system that minimizes paper records and manual data entry through automated synchronization with other Agency data systems. This system also standardizes sample traceability and data recording in the FSLs as well as laboratory results reporting to FSIS stakeholders. The LIMS is further used to inventory all reference materials, reagents, and consumables, while also providing documentation and records for calibration and maintenance events for equipment and instrumentation used in analyses. The LIMS application is highly customizable and allows full auditing of all transactions performed within the system. This system supports ISO 17025 and internal quality system requirements by maintaining full traceability of samples from receipt in the laboratory until reporting of results. Completed sample results are transmitted from LIMS to web-based portals, LIMS Direct and Public Health Information System (PHIS). LIMS Direct is a reporting interface that displays FSIS lab sample results directly from LIMS. PHIS is a comprehensive data analytic system to collect, consolidate, and analyze data from across FSIS. The IPP uses information from these systems to take appropriate action, passing or condemning tested animal product. FSIS strives to keep the turnaround time from sample collection to finalized results as short as possible while also maintaining the highest level of accuracy for the analyses.

Laboratory Reagents and Equipment

Reference Materials, Reagents, and Consumables:

Upon receipt of a standard, reagent, or consumable from the manufacturer, the contents of the package are verified against the packing slip. When it arrives at the laboratory, each new standard, reagent, and consumable is entered into LIMS for inventory tracking, assigned a unique inventory number, and labeled to identify contents and provide tracking information. If possible, reference materials are purchased from manufactures accredited to ISO Standard 17034.

All prepared solutions and prepared standards are assigned a unique reagent number and labeled. The preparation of solutions and standards is recorded in a log that documents information to ensure that calculations can be duplicated, and the source of the primary standard is traceable. Newly prepared standards are verified and reviewed against previously acceptable standards (if available) before sample results are reported. The old and new standards are analyzed according to the analytical methodology.

Consumables that require verification before use are usually defined in the methodology. The verification procedure outlined in the method is followed and documented. If the method does not specifically address verification before use, the laboratory will verify performance using method defined controls.

Upon expiration of a reference materials, reagent, or consumable, the item will either be disposed of according to local, state, and federal regulations or archived. If a standard is archived, it is for reference/identification use only.

Equipment and Instrumentation:

All laboratory equipment and instrumentation used in analyses that may have a significant effect on the results are inventoried with unique identification numbers in LIMS. All FSLs follow procedures to ensure that equipment is maintained and functioning properly for analyzing customer samples. Laboratory equipment is calibrated before being placed into service (at installation), at defined intervals and conditions, following repair, and after being stored or shipped. The LIMS system is used to document and record all maintenance and calibration events.

All weights and thermometers used to calibrate and verify performance of equipment are calibrated and traceable to a national standard such as the National Institute of Science and Technology (NIST). These reference standards are periodically re-calibrated by an accredited calibration service laboratory.

Companies contracted to perform equipment and instrumentation calibration provide the laboratory with a calibration certificate documenting traceability to a primary standard(s) or to a natural constant, measurement results, and measurement uncertainty. The service provider meets the requirements of ISO/IEC 17025 and is evaluated as a competent supplier.

Equipment that has been shown to be defective, outside specified limits, or gives suspect results is taken out of service and clearly labelled as such until repaired and shown by calibration or test to perform correctly. Any results produced using suspect or defective equipment are reevaluated. The laboratory initiates nonconforming work procedures to determine whether the suspect equipment posed any significant risk to results and what effect the departure had on previous tests.

Figures 2 and 3 are some examples of analytical instrumentation that the FSLs use.

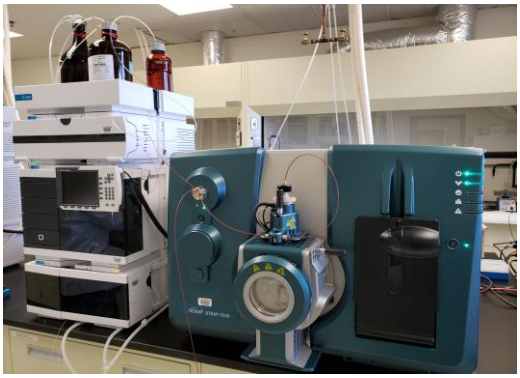

Figure 2: Agilent 1290 LC system coupled with Sciex 6500+ QTrap MS system. Photo courtesy of Sam Zipperer, USDA FSIS.

Figure 3: Agilent 7010 GC-MS System. Photo courtesy of Sarah Edwards, USDA FSIS.

Samples for Chemistry Analysis: General Considerations

Sample Collection:

Samples are collected by Inspection Program Personnel (IPP) according to FSIS Directive 10800.1[2] and the collected tissue is then shipped overnight by the contracted carrier to the appropriate FSL.

Sample Receiving:

Sample boxes are sorted and transported to designated sample box opening areas by laboratory personnel, as shown in Figure 4. Each laboratory has controlled procedures to verify sample delivery. Sample identity, acceptability, and analysis requirements are confirmed after opening the sample box. All samples are uniquely identified on a sample form or other acceptable documentation and then logged into the laboratory's Laboratory Information Management System (LIMS) database.

Sample Discards:

All sample boxes and samples are inspected upon receipt, as shown in Figure 5. The criteria for assessing the suitability of samples for analysis depend on factors including, but not limited to, Office of Public Health Science (OPHS) policies, analyses being conducted, and container integrity. For example, if it is determined that the integrity of the sample, security seal, or shipping container is compromised (e.g. sample is leaking or rancid, seal broken), if the sampling form or supplemental sampling information is missing or incomplete, or if ineligible product has been submitted for the requested sampling project, the sample may be discarded.

FSIS laboratory personnel make every effort to avoid discarding samples. These efforts include soliciting additional information from sample collectors or sample resubmission (where applicable).

- When an unacceptable sample is received, the laboratory will contact IPP to ask them if they would like to resubmit a sample from the same production lot under the same form or have the sample discarded. A reply is expected within three business days.

[2] https://www.fsis.usda.gov/policy/fsis-directives/10800.1

- When a sample is received without a form or missing information and the form number can be obtained, the laboratory is to contact IPP within 24 hours. The contact will:
    - Request the appropriate missing or updated information or documentation (e.g., completed form, nutritional label).
    - Inform IPP that the missing or updated information or documentation must be provided within 48 hours of sample receipt to avoid a discard.

Sample Log In:

All samples that meet acceptability criteria are recorded using the Laboratory Information Management System (LIMS). Samples are provided a unique identifier, and information such as sample tracking numbers and assigned analyses is entered into LIMS.

Sample Preparation:

The target tissue (tissue analyzed for screening analysis) is separated from the other tissues for preparation. The other tissues received are placed in cold storage to ensure sample integrity. As shown in Figure 6, the target tissue is cut into small chunks while avoiding large amounts of fat and connective tissue. The sample is then ground into a homogenous mixture, as displayed in Figure 7. Once the sample is prepared, the sample is bagged, labeled, and transferred to the chemistry section for analysis.

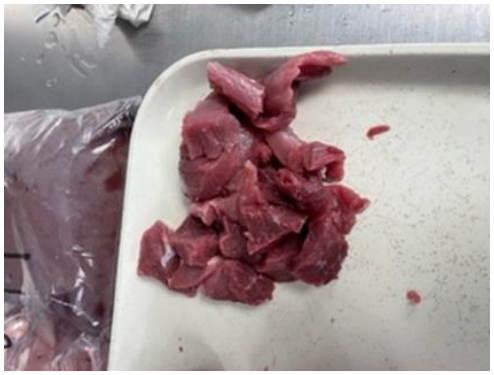

Figure 6: Lean muscle sample with connective tissue removed. Photo courtesy of Hue Quach, USDA FSIS.

Figure 7: Homogenized sample. Photo courtesy of Hue Quach, USDA FSIS

Minimum Level of Applicability (MLA)

The FSIS CLG lists the analytical methods currently available to the agency and describes the analytical process and performance characteristics of each method. One such performance element is the Minimum Level of Applicability (MLA). FSIS defines the MLA as the lowest level at which a method has been successfully validated for a residue in each matrix (meat or poultry). FSIS will generally not report or act on any analytical results below the applicable MLA. Each CLG method clearly states the MLAs for the chemistry analytes that pertain to that method.

Method Selection and Implementation

CLG method documents are designed to provide laboratory analysts with complete instructions and useful references to facilitate training, performance, quality assessment, and interpretation of data for chemistry methods utilized in the FSIS laboratories. FSIS recognizes that other laboratories may use other appropriate methods and equipment.

FSIS does not endorse or approve methods for use by the food industry, and the inclusion of a particular method in the CLG should not be considered an endorsement; it is simply the current approved method in use. Similarly, the mention of specific brand or trade names for products, equipment manufacturers, media, chemicals, or reagents associated with methods contained in the CLG does not constitute endorsement by FSIS. The Agency acknowledges that equivalent products may be available for laboratory use. As these method chapters are primarily designed to instruct FSIS analysts on how to perform analyses in FSIS laboratories, it is important to use names of actual products used in FSIS laboratories for clarity.

FSIS laboratories perform a thorough method evaluation when an alternative method or process is being considered, or if a significant change to an accepted method may affect its performance characteristics. Further, when the scope of an accepted method is extended to a new type of food sample (matrix), analyte, or level, the method is evaluated using those new conditions. FSIS considers the following performance criteria and definitions as goals when evaluating the suitability of a qualitative or quantitative CLG method for a given analyte and sample matrix pair.

Actual criteria used for the evaluation depend on method capability and laboratory needs.

1. **False Negative Rate**
    - The ratio of false negatives found divided by true positives present, expressed as a percentage.
        - $False\ Negative\ Rate = \frac{False\ Negative \times 100}{True\ Positives}$
2. **False Positive Rate**
    - The ratio of false positives found divided by true negatives present, expressed as a percentage.
        - $False\ Positive\ Rate = \frac{False\ Positives \times 100}{True\ Negatives}$
3. **Limit of Detection (LOD) or Method Detection Limit (MDL)**
    - The lowest amount of analyte that can be reliably observed or found in the sample matrix by the method used.
        - $LOD = t \times S$

            Where:

            t = Student's t value appropriate for a 99% confidence level.

            s = Standard deviation estimate with n-1 degrees of freedom.

- Must be below the method MLA

4. **Limit of Quantitation (LOQ)**
   - The smallest measured amount of analyte in a sample that can be reliably quantified with a specified degree of accuracy.
     - $LOQ = 3.3 \times LOD$

       Where:
       LOD = limit of detection
     - Must be below the method MLA.
5. **Ruggedness**
   - The ability of an analytical procedure to resist changes in results when subjected to minor changes in environmental and procedural variables, laboratories, personnel, etc.
     - Acceptable limits are determined that meet laboratory needs when factors are found to affect method performance.
6. **Range**
   - Analyte concentration in a sample for which an analytical method can detect an analyte with the desired sensitivity and selectivity rates.
     - Correlation coefficients (r) of > 0.995 across the validated range.
7. **Recovery**
   - The amount of analyte quantified by the analytical method, expressed as a percentage of the amount known to be present in the sample.
     - $Recovery = \frac{|A-B| \times 100}{B}$

       Where:

       A=amount quantified by method

       B=amount known to be in the sample
     - Acceptable recovery values follow published guidelines from credible scientific organizations (FDA, Codex Alimentarius, or AOAC).

8. **Repeatability**
    - Closeness of the agreement between the results of successive measurements of the same measurand carried out under the same conditions of measurement.
        - $RSD_r < 2^{1-0.5 \log C} \times 0.67$

          Where:

          C = concentration of the analyte as mass fraction and

          0.67 reflects that RSD_r values are usually between one half to two thirds that of $RSD_{R.}$

          RSD_r = Relative Repeatability Standard Deviation.
        - Data acceptability is based on FDA and AOAC guidelines, to include the modified Horwitz equation.
9. **Reproducibility**
    - Closeness of the agreement between the results of measurements of the same measurand carried out under changed conditions of measurement.
        - $RSD_R < 2^{1-0.5 \log C}$

          Where:

          C = concentration of the analyte as mass fraction

          RSD_R = Relative Reproducibility Standard Deviation.
        - Data acceptability is based on the modified Horwitz equation.
10. **Specificity or Selectivity of 90% or greater at the MLA**
    - The probability that the method will classify a test sample as negative, given that a test sample is a known negative.
        - $Specificity = 100 - false\ positive\ rate$
        - Interfering materials must be documented for qualitative analysis and interfering materials from the sample matrices do not contribute >3 % to the analytical total for quantitative analysis
11. **Sensitivity of 90% or greater at the MLA**
    - The probability that the method will classify a test sample as positive given that a test sample is a known positive.
        - $Sensitivity = 100 - false\ negative\ rate$

Additional performance characteristics may be reviewed as needed. Critical parameters such as ranges for weights and temperature measurements are also identified during the evaluation process.

Regulatory Results and Decision Rules

Residues:

FSL results for chemical residues are compared to levels established by the Food and Drug Administration (FDA) or Environmental Protection Agency (EPA). The established levels for the different tolerances set by the FDA can be found in the 21 CFR and the tolerances set by the EPA can be found in the 40 CFR. The established levels ensure that meat, poultry, and egg products tested are safe and wholesome and do not contain levels of a chemical compound that would render the product in question adulterated under the FMIA, PPIA, or EPIA.

As described in Figure 8, if the results are below the MLA then the result is reported as "Not detected". If the results are above the MLA during screening then the result is recorded as detected and is a "Presumptive positive." The FSLs conduct additional testing to confirm and/or quantitate the presence of the residue, and a violation occurs if a chemical residue exceeds the established level. In situations involving quantitation, if the result is above the MLA but below the tolerance, the result is reported as "Detected, non-violative." However, if the result is above the tolerance, then the result is reported as "Detected, violation." In situations involving confirmation, if the result is confirmed above the MLA, then the result is reported as "Detected, violation." If additional testing must be performed at a FSL other than the one that originally received the sample, a portion of the sample is shipped to the appropriate FSL and the same procedures for sample collection, sample receiving, and sample preparation are followed.

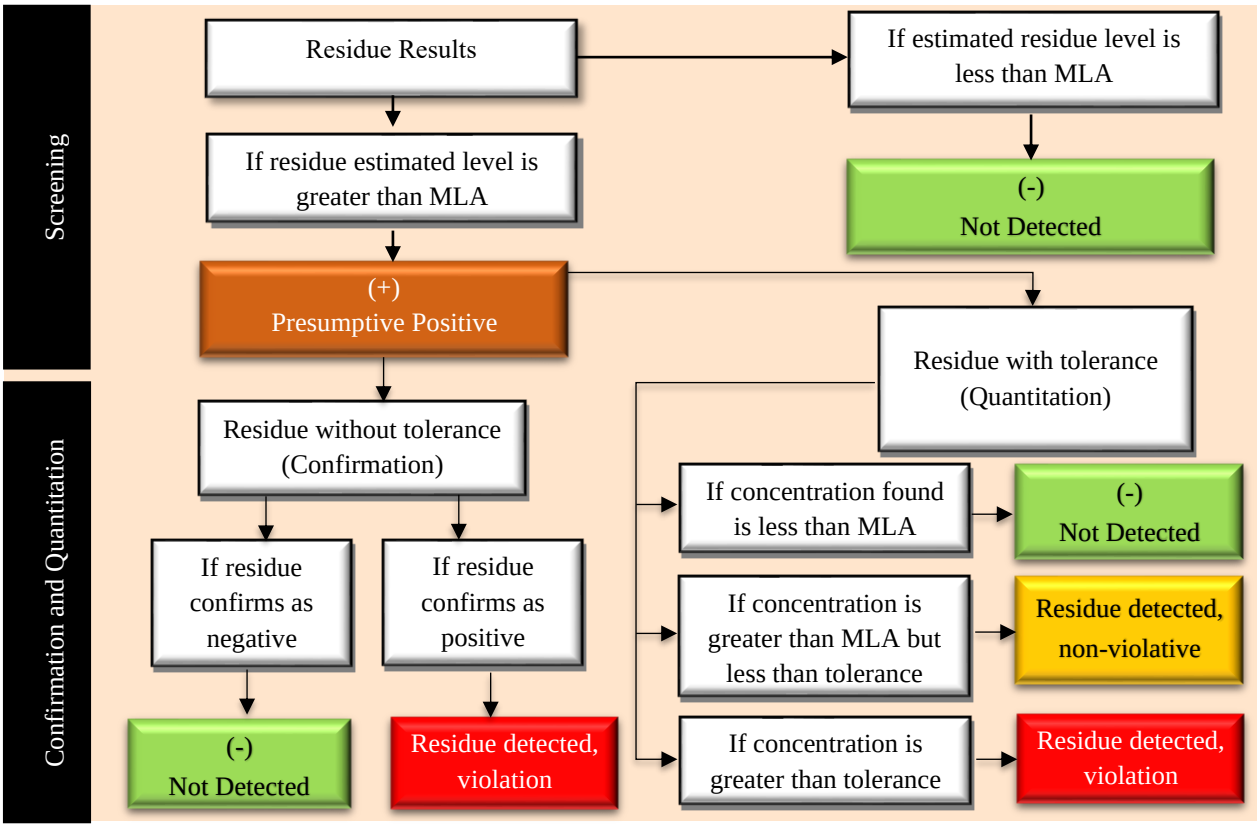

Figure 8: Chemical residue decision rules

Label Verification - Allergens and Food Chemistry:

Under the Federal Meat Inspection Act (FMIA), the Poultry Products Inspection Act (PPIA), and the Egg Products Inspection Act (EPIA), under which the Food Safety and Inspection Service (FSIS) operates, all ingredients used to formulate a meat, poultry, or egg product must be declared in the ingredients statement on product labeling. A product is misbranded under the FMIA, PPIA, or EPIA when it contains ingredients that are permitted but are not declared on product labeling. The guidance for misleading labels or practices is found in 9 CFR.

FSL testing helps to verify the voluntary addition of allergen statements (e.g. "contains" statements) on meat and poultry product labels immediately following the ingredients statement. FSL results for allergen screening and confirmation are compared to FDA-established levels in accordance with the Food Allergen Labeling and Consumer Protection Act (FALCPA). If the results are above the MLA during allergen screening, then the result is reported as detected and is a "Presumptive positive." The FSL then conducts confirmatory testing for the specific allergen, and if the result is confirmed, the result is to be reported as a violation due to product mislabeling.

Additionally, the agency verifies label claims through food chemistry analysis, to include measurement of salt, sodium, fat, moisture, and protein content. In accordance with FSIS Notice 26-19, sodium and fat analysis are conducted through exploratory sampling to verify industry compliance with labeling regulations and related 9 CFR requirements. Measurement of moisture, protein, and salt content is conducted through compliance sampling as determined by inspection program personnel (IPP) for amenability of the products.

Chemical Hazards:

In accordance with 9 CFR, FSLs conduct chemical hazard analysis to determine the presence of foreign materials in products to be consistent with the Hazard Analysis and Critical Control Point (HACCP) regulations, FMIA, and PPIA. FSL results for heavy metal and toxin analysis ensure that only wholesome, unadulterated products are eligible to bear the mark of inspection and to enter commerce.

If chemical hazards such as a toxin or heavy metal are detected during screening, FSIS laboratories are to conduct confirmatory analysis. Confirmed results in samples may lead to an evaluation by the Health Hazard Evaluation Board (HHEB) and the Recall Committee. In accordance with FSIS Directive 8091.1, the HHEB and the Recall Committee are to evaluate the public health risk of potential human health hazards associated with meat, poultry, or egg products.

Contact Information and Inquiries

Inquiries about methods can be submitted through the USDA website via the "Ask USDA" portal at https://ask.usda.gov or please contact:

Chemistry Section

Laboratory Quality Assurance, Response, and Coordination Staff

USDA/FSIS/OPHS

950 College Station Road

Athens, GA 30605

OPHS.LQAD@usda.gov