United States Department of Agriculture

Food Safety and Inspection Service

MLG 1.02

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

This chapter introduces the FSIS laboratory system and provides details for FSIS laboratory microbiology method performance expectations. Sample receipt, handling, preparation, and discard criteria are outlined.

Notice of Change

This chapter was revised for the following items:

- Updated Figure 1 to show the decreased time for reporting a screened negative result for *Campylobacter* samples from 3 days to 2 days and the decreased time for reporting all confirmed positive samples from Days 5-7 to Days 4-6.
- Clarified the method selection performance criteria for qualitative methods.
- Revised the sample discards section to describe resubmission of samples.
- Updated the contact information for inquiries.

Table of Contents

Mission Statement—FSIS Laboratory System

The FSIS Field Service Laboratories (FSLs) support the mission of the United States Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS) to protect public health by performing analyses of samples collected from meat, poultry, *Siluriformes*, and processed egg products. 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 consisting of documents that describe the policies and procedures related to their work. 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 accreditation to perform certain laboratory methods, and specific scopes of ISO 17025 accreditation for each laboratory are available publicly. Certificate numbers issued by the laboratory system's current accrediting body, A2LA, are as follows:

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 approaches they need to make and carry out informed decisions that 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 Nation's food supply is safe.

Introduction

The Microbiology Laboratory Guidebook (MLG) contains the protocols used by the USDA FSIS FSLs for the microbiological analysis of samples collected from meat, poultry, *Siluriformes*, and egg products, which are regulated under the authorities of the Poultry Products Inspection Act (PPIA), the Egg Products Inspection Act (EPIA), and the Federal Meat Inspection Act (FMIA). The FSIS FSLs test verification samples collected by FSIS inspection program personnel (IPP) as stipulated in the publicly available FSIS Annual Sampling Plan. 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 verification activities in support of the Annual Sampling Plan include testing for Shiga toxin-producing *Escherichia coli* (STEC, including *E. coli* O157:H7), *Listeria monocytogenes*, *Campylobacter* species, and *Salmonella* species. The MLG provides detailed protocols for the rapid screening, full confirmation, and isolation of these pathogens from verification samples. Further, the MLG contains protocols for the Whole Genome Sequencing (WGS) of pathogen isolates, examination of canned meat and poultry products, quantitative analysis of sanitary indicator organisms, the identification of certain bacterial toxins, and meat species determination. The general timeline for sample handling, testing, and reporting for regulated pathogens is summarized in Figure 1.

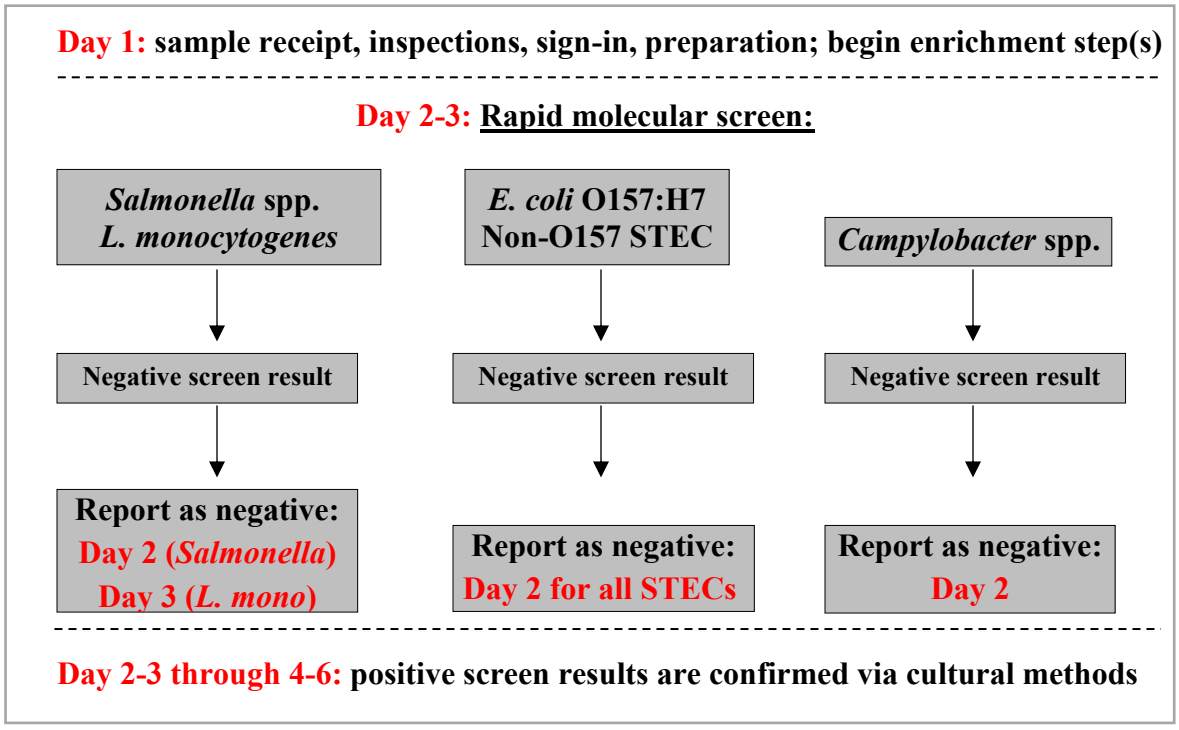

Figure 1. Timeline for pathogen testing and reporting results. Refer to the MLG chapters for individual pathogen details.

Method Selection and Implementation

FSIS MLG methods are designed to provide FSIS laboratory analysts with full instructions and a useful reference to facilitate training, performance, quality assessment, and interpretation of data. FSIS recognizes that laboratories associated with the regulated industry and public health government sister agencies may use other methods and equipment to test for food-borne pathogens.

FSIS does not endorse or approve methods for use by the food industry, and the inclusion of a particular method in the MLG should not be considered an endorsement; it is simply the current method being used by FSIS. Similarly, the mention of specific brand or trade names for products, equipment manufacturers, media, chemicals, or reagents associated with methods contained in the MLG 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 the actual products used in FSIS laboratories.

FSIS microbiological detection methods typically have an enrichment process, followed by a rapid screening step to identify negatives and potential positives, followed by cultural isolation on various selective media to isolate the organism, then the laboratory performs a final confirmation process. Analysts perform genetic characterization on positive sample isolates using Whole Genome Sequencing (WGS). Following characterization via WGS, sequencing data is made publicly available and bacterial isolates are curated and archived.

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 fitness for purpose or performance characteristics. Further, when the scope of an accepted method is extended to a new type of food sample (matrix) or sample from a food-producing environment, the method is evaluated in the new sample type.

For qualitative detection methods, FSIS utilizes the following performance criteria and definitions when evaluating the suitability of an alternative laboratory method for a given analyte and sample matrix pair:

- **Sensitivity of 90% or greater**
- **Specificity of 90% or greater**
- **Accuracy of 90% or greater**
- **Positive Predictive Value of 90% or greater**
- **Negative Predictive Value of 90% or greater**

Sensitivity is the conditional probability that the selected samples will test positive using an alternative method given that the samples tested positive using an accepted reference method. Also described as the **true positive** rate. The sensitivity of an alternative method can be calculated using the following equation, based upon the term definitions in Table 1:

$$\frac{a}{(a+c)}$$

Specificity is the conditional probability that the selected samples will test negative using an alternative method given that the samples tested negative using an accepted reference method. Also described as the **true negative** rate. The specificity of an alternative method can be calculated using the following equation, based upon the term definitions in Table 1:

$$\frac{d}{(b+d)}$$

Taken together, **Sensitivity** and **Specificity** describe the **Accuracy** of an alternative method: **Accuracy** is the probability that the alternative method will provide the correct result when compared to the results from the accepted reference method. To calculate the overall accuracy of an alternative method, use the following equation, based upon the term definitions in Table 1:

$$\frac{(a+d)}{(a+b+c+d)}$$

Positive Predictive Value (PV+) is the conditional probability that a sample will test positive using the accepted reference method given that the alternative method returned a positive result. The PV+ of an alternative method can be calculated using the following equation, based upon the term definitions in Table 1:

$$\frac{a}{(a+b)}$$

Negative Predictive Value (PV-) is the conditional probability that a sample will test negative using the accepted reference method given that the alternative method returned a negative result. The PV- of an alternative method can be calculated using the following equation, based upon the term definitions in Table 1:

$$\frac{d}{(c+d)}$$

Table 1. Term definitions for true/false positives and true/false negatives.

	Reference Method Positive	**Reference Method Negative**
Alternative Method Positive	**a** **True Positive (TP)**	**b** **False Positive (FP)**
Alternative Method Negative	**c** **False Negative (FN)**	**d** **True Negative (TN)**

Additional performance characteristics may be reviewed as needed, including exclusivity and inclusivity panels, ease of use, and measurement uncertainty. Critical parameters such as ranges for weights, incubation times, and temperature measurements are also identified during the evaluation process. Method validation is necessary to demonstrate the equivalence of alternative test kits as detailed in the guidance document available on the USDA FSIS website titled "FSIS Guidance for Evaluating Test Kit Performance." In addition to the full evaluation of updates to laboratory methods, the FSIS laboratory system applies these same criteria when evaluating commercial media, test kits, or laboratory equipment.

Selection of commercial products for use in the FSIS FSLs involves extensive market research by the FSLs and the FSIS Office of Public Health Science (OPHS), and may require a formal procurement process, including a public Request for Proposals (RFP) from interested vendors.

Microbiological Samples: General Considerations

Sample Receiving: Most microbiology samples are sent to the FSLs by overnight courier in insulated sample boxes containing coolant packs to preserve sample integrity. Sample boxes are sorted and transported to designated sample box opening areas by laboratory personnel. Each laboratory has controlled procedures to verify the 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. The criteria for assessing the suitability of microbiological samples for analysis depend on factors including, but not limited to, Office of Public Health Science (OPHS) policy decisions, analyses being conducted, temperature of sample, and container integrity. All samples being analyzed for the presence of microorganisms are handled aseptically.

Sample Discards: All sample boxes are inspected upon receipt. 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), or an incorrect product has been collected, the sample may be discarded or a replacement sample from the same lot may be resubmitted to the laboratory. Rinse samples are discarded as this product cannot be recollected from the same lot. Raw microbiology samples received at a temperature > 15°C cannot be analyzed as warmer temperatures can allow competing bacteria to outgrow the targets. *Campylobacter* analysis cannot be performed if the sample is received at a temperature < 0°C, as target bacteria may be damaged by temperatures that are too cold. The most frequent causes for discarded samples include sample receipt temperatures outside of the acceptable temperature range, sample container leaking, seal integrity compromised, and ineligible product being submitted for the requested sampling project. **FSIS attempts to reduce the numbers of discarded sample by verifying the following criteria prior to sample submission:**

- A complete, signed copy of the sampling form is included, as well as any supplemental information required for the sampling project (e.g., product label).

- The sample shipper has been properly packed according to the relevant Directive or Notice.
- The collected product is eligible for the requested sampling program and has been collected according to the procedures in the relevant Directive or Notice.
- The sample seal has been applied according to Directive 7355.1.
- The sample is shipped as soon as possible after collection, preferably the same day.

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

Sample Preparation: Samples with weight specifications are weighed and processed aseptically according to the sampling protocol and placed in the proper sterile container for the next processing step. To ensure a representative laboratory test sample is prepared, portions are selected and/or excised (cut) from multiple locations in the sample received in the laboratory. Sterile instruments are used for cutting, removing, and manipulating all samples. The remainder of the sample is placed in an appropriate sterile container, closed to preserve the sterility and integrity of the sample and held in a secure location as a reserve. The sample reserve is held for additional testing as needed and must be held according to the sampling protocol. When weighing is complete, the area is cleaned and disinfected; cutting implements are sterilized. Each method in this Guidebook contains additional safety, quality control, and sample preparation instructions.

Laboratory Reagents and Equipment

Reagents and Equipment: All chemicals, media, immunoreagents, and commercial test kits used on samples are within current shelf expiration dates and subjected to quality control and quality assurance procedures to ensure their proper performance for their intended purpose. All instrumentation is maintained and properly calibrated to ensure correct performance during use in all methods. Positive and negative controls are always to be used, as specified for a given procedure. All analytical results, test controls, quality assurance and quality control procedures, instrument maintenance programs, and any observed laboratory deviations are documented, controlled, and secured.

Range of Measurements: Although all of the methods described in this guidebook have exact numerical values given for performance parameters such as weight and volume measures, pH, and time and temperature to achieve optimum results, it should be clearly understood that an acceptable range exists where results can be achieved without compromising the integrity of the method. For any given method, unless otherwise stated within the text of this MLG, the following allowable ranges are acceptable:

Weight and volume measures: ± 2%
pH: ± 0.2 units
Time: hours ± 1 hour; minutes ± 2%
Temperature: ± 1.0°C

Laboratory Information Management System (LIMS)

To ensure data integrity, the FSIS FSLs standardize 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 standardizes sample traceability and data recording in the FSLs, and laboratory results reporting to FSIS stakeholders. Further, the LIMS application in use by the FSIS FSLs is highly customizable and allows full auditing of all transactions performed within that 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.

Contact Information and Inquiries

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

Microbiology Section
Laboratory Quality Assurance, Response, and Coordination Staff
USDA/FSIS/OPHS
950 College Station Road
Athens, GA 30605
OPHS.LQAD@usda.gov