

Projects for FERN CAP Initial Application

Mandatory Projects

(All laboratories will participate in the following projects for the FY25 CAP year.)

1. FSIS Microbiology Laboratory Guidebook (MLG) methods (including screen and confirmation) in FERN Laboratories

Goals:

- At least two FSIS MLG methods using approved matrices or product types validated accompanied with data for review by LQARCS.
- At least two FSIS MLG methods on the laboratory's scope of ISO 17025 accreditation at the end of 5-year CAP cycle.
- Demonstrate readiness to assist FSIS in identifying the presence of the following analytes in confirmed positive isolates: *Salmonella*, STEC, *Listeria monocytogenes* or *Campylobacter*.
- Analyze FSIS meat and meat products when requested and provide results to FSIS.

The laboratory will develop the ability to conduct and demonstrate validation of at least two FSIS MLG methods and add them to their scope of ISO 17205 accreditation. One method must be added by the end of the third year, with the second by the fifth year. The matrices or product types of interest will include FSIS tested products.

A workgroup will be formed to organize the activities needed to complete these projects.

FSIS MLG methods include:

- *Salmonella*
[Salmonella MLG 4.14.pdf](#)
- STEC (all 7 *E. coli* O groups)
[STEC MLG 5C.03.pdf](#)
- *Listeria monocytogenes*
[L. monocytogenes MLG 8.13.pdf](#)
- *Campylobacter*
[Campylobacter MLG 41.07.pdf](#)

If the laboratory is unable to use the current FSIS screening and confirmation technologies (3M™ or Bruker MALDI Biotyper® System), the laboratory may substitute technology that FSIS has previously validated and determined to be appropriate (e.g., BAX® System or VITEK® 2). Acceptance of the method(s) will be determined by LQARCS.

2. Food Defense Surveillance

The laboratory participating in the FSIS FERN Cooperative Agreement Program (CAP) will perform ongoing Food Defense Surveillance of FSIS regulated products. The laboratory will review the list of FSIS products or analyses and submit a 1-year plan for surveillance in the proposal response. The laboratory will pick at least four different product types to test throughout the year. The laboratory can decide how to pair the analyte or target and product(s) for each month.

The laboratory is to report all findings in the FSIS FERN Communication, Collaborative, and Management (CCM) Portal **monthly** using the activation procedures and documents issued by the FSIS Laboratory Quality Assurance, Response and Coordination Staff (LQARCS) Contaminants and Threat Agents Group (CTAG). The laboratory is to acquire samples from retail locations, preferably using established state processes for retail collection. Sample collections are to follow appropriate chain-of-custody guidelines. The laboratory can also receive samples directly from FSIS.

This project will address requirements of National Security Memorandum – 16 (NSM-16) to increase testing and diagnostic surge capacity and to standardize diagnostic and reporting protocols to facilitate timely information sharing. This activity also supports the FSIS mission of ensuring the safe and wholesome nature of meat, poultry, and egg products within the United States.

This project supports the following cooperative agreement objectives:

- Maintain laboratory response capability for food defense purposes
- Demonstrate ongoing laboratory proficiency and readiness

Surveillance Plan

Please submit the laboratory's surveillance plan using the table below.

Microbiology Analytes: SET, Ricin/Abrin, *B. anthracis*, *Yersinia pestis*. Others as directed by CTAG for FSIS special emphasis projects. The sampling plan will include 15 samples per month to cover 45 samples per analysis (SET, Ricin or Abrin, *B. anthracis* and *Yersinia pestis*) for the year. The laboratory has flexibility to choose when to analyze SET, Ricin or Abrin, *B. anthracis*, or *Yersinia pestis* throughout the year.

Chemistry Analytes: CHE.0006 (poison toxin screen), CHE.0008 (poison toxin screen), CHE.0009 (metals screen). Others as directed by CTAG for FSIS special projects. Methods for analyzing the 15 samples per month will be included in the plan.

Radiochemistry Analytes: Gamma emitters, Gross alpha, Gross beta, Strontium, Americium. Twenty samples per quarter will be included in the plan.

Targeted Food Defense Surveillance for FY24 Plan

	FSIS Product	Number of Microbiology Analyses (include name of each analysis)	Number of Chemistry Analyses (include name of each analysis)	Number of Radiochemical Analyses (include name of each analysis)
October				
November				
December				
January				
February				
March				
April				
May				
June				
July				
August				
September				

Any subsequent changes to an approved surveillance plan will require prior approval from the assigned Project Officer. Issues with purchasing of matrices and sample kits are possible reasons to change the plan during the year.

3. Special Event Testing

This project involves the testing of samples associated with National Special Security Events (NSSEs) (e.g., Super Bowl, Kentucky Derby, other high-profile events) as determined by FSIS. The laboratory will participate in a minimum of two events per year. The laboratory is to budget for 10 – 15 samples per event. The FSIS Inspection Program Personnel (FSIS inspectors) will ship samples directly to the laboratory, along with sample forms. Samples are to be maintained following the appropriate chain-of-custody guidelines. The laboratory will complete analyses in two or more disciplines and report results within the stipulated reporting window, typically one week from receipt of samples. Analytical results will be reported to CTAG using the FSIS FERN CCM Portal and activations specific for the event. The laboratory will be responsible for returning extra supply boxes (shippers), gel coolants, and packing supplies to the appropriate FSIS Field Service Laboratory.

This project supports the following cooperative agreement objectives:

- Maintain laboratory response capability for food defense purposes
- Demonstrate on-going laboratory proficiency and readiness

4. FERN Wide Proficiency Testing (PTs)

FSIS FERN cooperative agreement laboratories will participate in FERN-wide proficiency testing, which is made available to all FERN laboratories. The laboratory will participate in PT events according to the discipline awarded.

Additional Projects

Projects should be written for completion within one award year; laboratories are expected to propose at least one project per discipline (microbiology, chemistry, radiochemistry). Projects of interest to the laboratory and applicable to FSIS FERN, but not included in the following list, can also be submitted for consideration.

Microbiology Projects

1. Ricin

Goals:

- Verify in two FSIS matrices the FERN Ricin method using RCA120 or any other available positive control.
- Demonstrate readiness to assist FSIS in screening food or enrichment broths for the presence of ricin.
- Report results for 30 samples in addition to the retail surveillance.

Laboratories are to verify the FERN ELISA ricin method for use in their lab using a positive control. Suppliers have stopped producing the current positive control (RCA120) product so obtaining it is difficult. Only labs that have a stock of RCA120, other ricin toxin controls, or can acquire a control at the time of proposal submission can write to this project. A workgroup will be formed to organize activities for this project.

2. *Clostridium botulinum* toxin

Goal:

- Demonstrate readiness to assist FSIS in screening meat and meat product isolates or enrichment broths for the presence of *C. botulinum* toxin by extending the *C. botulinum* MALDI method to two FSIS approved matrices.

The laboratory must obtain antibodies from the University of California to perform the *C. botulinum* MALDI assay. A material transfer agreement (MTA) will be necessary for this acquisition. The purchased antibodies will be used to demonstrate capability in conducting the *C. botulinum* MALDI assay.

The CTAG is seeking at least three laboratories to perform this project. If the laboratory has previously obtained antibodies, the appropriate supplies, and demonstrated capability with the method, this laboratory can continue to work with MALDI by conducting a matrix extension to two FSIS approved matrices.

3. Staphylococcal Enterotoxin (SET)

Goal:

- Validate a PCR method for SET for as many serotypes as possible in at least two FSIS approved matrices.

The laboratory is to validate a PCR method for SET in two FSIS approved matrices. This PCR method will be used to complement the current FERN method for SET. The method will be applicable to as many SET serotypes as possible. The method to be validated can be obtained from previous FERN projects or other state labs.

4. *Bacillus cereus* detection in FSIS Matrices

Goals:

- Implement the FSIS MLG 12 method into the laboratory system.
- Provide matrix extension validation data for at least 2 FSIS approved matrices.
- Report results for 30 samples in addition to the retail surveillance samples.

The laboratory will implement and verify FSIS MLG 12, Examination of Meat and Poultry Products for *Bacillus cereus*, in FSIS regulated food matrices. The goal is to be prepared to run this method on suspect food samples. The laboratory will need to provide method verification data following guidance from FSIS LQARCS. The matrix extension will follow FERN validation guidelines found in FERN-ADM.00008.01, “FERN Validation Guidelines for FERN Chemical, Microbiological and Radiological Methods.” In that document, the laboratory will follow Table 4, Food Matrix Extension Protocol for Microbiology Methods, Matrix extension level 2. The laboratory will extend the method to at least 2 FSIS approved matrices with preference for ready-to-eat foods. The extension data must be submitted to LQARCS for approval.

5. *Clostridium perfringens* detection in FSIS Matrices

Goals:

- Implement the FSIS MLG 13 method into the laboratory system.
- Provide matrix extension validation data for at least 2 FSIS approved matrices.
- Report results for 30 samples in addition to the retail surveillance samples.

The laboratory will implement and verify FSIS MLG 13, Examination of Meat and Poultry Products for *Clostridium perfringens*, in FSIS approved food matrices. The goal is to be

prepared to run this method on suspect food samples. The laboratory will need to provide method verification data following guidance from FSIS LQARCS. The matrix extension will follow FERN validation guidelines found in FERN-ADM.00008.01, “FERN Validation Guidelines for FERN Chemical, Microbiological and Radiological Methods.” The laboratory will follow Table 4, Food Matrix Extension Protocol for Microbiology Methods, Matrix extension level 2. The laboratory will extend the method to at least two FSIS approved matrices with preference for ready-to-eat foods. The extension data must be submitted to LQARCS for approval.

6. *Clostridium botulinum* PCR Sample Screening

Goals:

- Demonstrate on-going capability to detect *C. botulinum* toxin genes for toxin serotypes A, B, E, and F.
- Demonstrate readiness to assist FSIS in screening meat and meat product isolates or enrichment broths for the presence of *C. botulinum* toxin genes.
- Ensure up-to-date PCR methods for *C. botulinum* toxin gene detection.

The focus of this project will be the detection of the genes encoding toxin serotypes A, B, E, and F, the most common food-associated *C. botulinum* toxin serotypes. The laboratory is to have a PCR method for detection of these genes. The goal of the project will be to validate the use of the PCR method to screen FSIS matrices for the presence of the toxin genes. The validation study will follow the guidelines found in FERN-ADM.00008.01, “FERN Validation Guidelines for FERN Chemical, Microbiological and Radiological Methods,” to validate the method in two approved matrices. The laboratory should follow the information in Table 3 (p. 11) for Level 1 validation and the Protocol 3 (p. 26) for the validation samples. The use of cloned or synthetic targets instead of actual *C. botulinum* strains is acceptable. The laboratory will focus validation studies on FSIS matrices such as cans, pouches, or dried meat products. The laboratory can choose to validate the method first in the enrichment broth described in MLG Chapter 10.01, Examination of Canned and Aseptically Processed, Hermetically Sealed Meat and Poultry Products, as one of the required two matrices. The laboratory that has a validated PCR method in place can perform a matrix extension to two FSIS approved products as described above. The laboratory will follow Table 4, Food Matrix Extension Protocol for Microbiology Methods, Matrix extension level 2.

Extraneous Materials/Filth Testing Project:

This is a high priority project for FSIS.

The laboratory will verify FSIS extraneous materials/filth procedures and analyze FSIS regulated product matrices. The laboratory will analyze samples for suspected contamination upon FSIS request.

Goals:

- Verify that the participating laboratories can analyze samples for acidic digestion, alkaline digestion, alkaline phosphatase, macroscopic examination, and microscopic examination (methods available upon request).

- Provide regular, on-going sampling and testing of FSIS regulated products, up to 60 samples per year (15 per quarter). These samples are to be done in addition to the samples in the Food Defense Surveillance project. The laboratory is to obtain all samples.
- Provide a sample analysis of extraneous materials or filth upon FSIS request.
- Perform analysis on one set of proficiency samples (PT) provided by USDA/FSIS/Eastern Laboratory/Pathology Section.
- Provide one set of extraneous materials or filth PT samples to the USDA/FSIS/Eastern Laboratory/Pathology Section for analyses.

Chemistry Projects

1. Meals-Ready-To-Eat (MRE) Testing

This is a high priority project for FSIS.

Goals:

- Expand testing of toxic chemicals and heavy metals in MREs.
- Utilize validated methods that analyze the toxic chemical and heavy metals analytes listed in FERN-CHE.0006, FERN-CHE.0008, FERN-CHE.0009, CLG-SAZCN1.03, and CLG-TM4.01.
- Provide testing capacity and capability for the FSIS laboratory system.

The laboratory is to expand their scope of testing to include toxic chemical and heavy metal analysis in meals-ready-to-eat (MREs). The laboratory agrees to test up to 50 MRE samples per year for FSIS. To analyze MRE samples, the laboratory will demonstrate capability for use of the following validated methods: FERN-CHE.0006, FERN-CHE.0008, FERN-CHE.0009; and upon request, CLG-SAZCN1.03 (cyanide/azide) and CLG-TM4.01 (thallium and arsenic).

Heavy metals of interest and reporting levels for MREs

Metal	Reporting Level (ppb)
Aluminum	24
Arsenic	100
Barium	3.6
Boron	4.8
Cadmium	10
Chromium	3.6
Cobalt	25
Copper	3
Iron	30
Lead	25
Manganese	200
Molybdenum	50

Nickel	6
Selenium	500
Strontium	3
Thallium	50
Vanadium	3.6
Zinc	30

Results are to be submitted to CTAG in a timely manner, typically no more than six days from sample receipt. Samples can be batched to meet the same timeframe. The CTAG will report results in the appropriate format established by FSIS. If a positive result is obtained, the laboratory is to follow the guidelines listed in FSIS Directive 10,000.1. If applicable, FSIS can use the final results for future validation studies.

A laboratory with ISO 17025 accreditation for meat and meat product testing is preferred for this project.

2. Detection of diacetoxyscirpenol, T2 toxin, and diisopropylfluorophosphate

Goals:

- Extend the scope and validate current methods to known toxic chemicals.
- Increase surveillance for diacetoxyscirpenol, T2 toxin, and diisopropylfluorophosphate.
- Close gap of FERN screening capabilities for toxic chemicals with revised validated method.

The laboratory is to perform a method extension using FERN-CHE.0008 for detection of:

- (1) diacetoxyscirpenol and T2 toxin, or
- (2) diisopropylfluorophosphate, or
- (3) both 1 and 2.

The extension study is to be conducted in accordance with the guidelines listed in FERN-ADM.0008.01, "FERN Validation Guidelines for FERN Chemical, Microbiological and Radiological Methods," and follow the protocols listed in Appendix 3, Protocol 1, Section B. The extension study will include two FSIS approved matrices. The purpose is to detect T2 toxin, diacetoxyscirpenol, and diisopropylfluorophosphate at detection levels similar to the analytes already validated in FERN-CHE.0008 (approx. 1ppm).

3. Mixed Standard and Challenge Test Activity

Goals:

- Provide a reliable mixed standard solution for use by the FERN CAP Laboratories in surveillance, PTs, and challenge samples to conduct FERN-CHE.0008.
- Add T2 and diacetoxyscirpenol to the mixed standard solution.
- Demonstrate FERN CAP Laboratory proficiency and preparedness through a set of challenge test samples.

The laboratory is to create and supply mixed standard solutions for the FERN-CHE.0008 LC/MS method. With the pending method extension occurring to expand the scope of FERN-CHE.0008, the laboratory is to add T2 toxin and diacetoxyscirpenol to the standard mixture. The laboratory is required to perform a stability study on each batch as an on-going check for the validity of the solutions as related to the FERN-CHE.0008 LC/MS method. The laboratory will perform a challenge test (CT) once a year that incorporates the mixed standard in the analysis. The mixed standard will be shipped to other participating FSIS FERN CAP laboratories twice a year.

4. Rodenticide (Super Warfarin) Methodology

Goals:

- Develop and validate a new method for detection of super-warfarins using FSIS approved products (one raw product and one ready-to-eat product).
- Increase surveillance to rodenticide (super warfarin).
- Close gap of FERN screening capabilities for rodenticides.

The laboratory is to develop and validate a method for the detection of a variety of rodenticide (super-warfarin) targets that includes brodifacoum, bromadiolone, chlorophacinone, difethiolone, diphacinone, and others as appropriate. The method will be separate from other currently conducted FERN chemistry methods and will specifically target the applicable rodenticides. The validation of the method is to be conducted in accordance with the guidelines in FERN-ADM.0008.01, "FERN Validation Guidelines for FERN Chemical, Microbiological and Radiological Methods," and follow the parameters listed in Table 2 and protocols listed in Appendix 3, Protocol 1. The validation is to involve two FSIS approved matrices to include one raw product and one ready-to-eat product. The level of rodenticide to be evaluated with the method will need to be appropriate for screening and comparable to current FERN screening methods.

5. Expand Analyte Scope for FERN-CHE.0006, Drug and Poisons Screen using Acidic and Basic Extraction with GC-MS

Goals:

- Expand the FERN-CHE.0006 method to known toxic chemicals.
- Increase surveillance to analytes listed in CLG-TOX1 and other state programs.
- Close gap of FERN screening capabilities for toxic chemicals.

The laboratory is to conduct a method extension of toxic chemicals to FERN-CHE.0006. The method extension will include aldrin, diazinon, fentanyl, carfentanil, terbuphos, tetramine, and endrin that are currently listed in CLG-TOX1. Additionally, the method extension will include other known toxins that are evaluated by surveillance programs conducted by state programs. All method extension work is to be conducted in accordance with the guidelines in FERN-ADM.0008.01 and follow the protocols listed in Appendix 3, Protocol 1, Section B.

The validation is to involve two FSIS matrices and all analyte levels will be comparable to the current levels listed in FERN-CHE.0006. The laboratory can also propose additional analytes with justification and approval by CTAG.

6. Phthalates in Processed Meat Products

Consumption of processed foods such as pre-packaged hamburgers, chicken nuggets, frozen pizza, and frozen burritos have been associated with phthalate exposure in preliminary studies. This project aims to build upon initial method validation work for these compounds completed by CAP labs in FY 23 and FY 24. The laboratory will participate in a workgroup to determine sound phthalate methodology, discuss issues, and compare results and successes. The laboratory will provide an expected timeline of work to be accomplished during the award year and indicate time to validate the agreed upon method.

This project addresses the following objectives:

- Validate the agreed upon method for phthalate analysis in processed meat products.
- Demonstrate that the method provides supportable analytical data for phthalates in processed meat products by testing additional matrices.

Radiochemistry Projects:

1. Alpha and beta emitters

Goals:

- Extend the current method to include the following matrices: hot dogs, breaded chicken, sausage, pre-packaged barbequed meats.
- Address further the gap in FERN for detecting alpha and beta radioactivity, simultaneously.
- Increase capacity and capability of cooperative agreement laboratories.

The method entitled “Rapid and simultaneous detection of alpha/beta radioactivity in food by solid phase extraction liquid scintillation counting” provides a radioanalytical method for triaging foods that can be contaminated with anthropogenic alpha and beta radioactivity. This method will provide an initial assessment of radioactivity levels and ascertain whether quantitative analysis is required. Current matrices validated for this method include 85% lean ground beef, pork bacon, lamb, turkey, and chicken tissue. The proposed project is to conduct a matrix extension to include the following products: hot dogs, breaded chicken, sausage, and pre-packaged barbequed meats. This extension will be a continuation of adding product types to the multi-laboratory validated method developed by FDA. Matrix extensions will follow Level 2, FERN-ADM.0008.01 Validation Guidelines found in Table 5, p.13.

2. Radiostrontium Matrix Extension

Goals:

- Extend the current method to include the following matrices: hot dogs, breaded chicken, sausage, pre-packaged barbequed meats.
- Increase capacity and capability of cooperative agreement laboratories.

This is a matrix extension of FERN-RAD.0007.00, Total Radiostrontium in 70% Lean and 93% Lean Ground Beef Matrices. Currently this method is validated in ground beef and raw ground chicken. The method will be extended to additional FSIS matrices. Matrix extensions will follow Level 2, FERN-ADM.0008.01 Validation Guidelines found in Table 5, p.13