United States Department of Agriculture

Food Safety and Inspection Service

MLG 4.15

Isolation and Identification of *Salmonella* from Meat, Poultry, Pasteurized Egg, Siluriformes (Fish) Products and Carcass and Environmental Sponges

This method describes the laboratory procedure for performing the isolation and identification of *Salmonella* from meat, poultry, pasteurized egg, Siluriformes (Fish) products and carcass and environmental Sponges.

Notice of Change

- This method is revised to update sample preparations for *Salmonella* brine samples. A new method flow chart was also created for enhanced analyst understanding of the method.
- This method revision also focuses on changes in selective media to add XLD agar plates and removes BGS agar in the detection of *Salmonella*. XLD agar is well-established media that provides better fit-for-purpose identification of Salmonella colonies over BGS in the high-throughput setting of FSIS field service laboratories. This switch is also expected to reduce result reporting times for more difficult samples.
- The revision also includes a biosafety chart to further explain safety precautions regarding MLG 4.

Isolation and Identification of Salmonella from Meat, Poultry Pasteurized Egg, Siluriformes (Fish) Products and Carcass and Environmental Sponges Flow Chart

Day 1
Sample Prep

Sample receipt temperature of ≤15ºC is required for raw poultry and raw beef products.

Various Samples + Enrichment

Incubate BPW at 35°C for 18 – 24hrs (RTE), 20 – 24hrs (raw poultry). Incubate mTSB at 42°C for 15 – 24hrs (raw meat).

Day 2
Screening

Perform Neogen® Molecular Detection Assay 2 - *Salmonella*

For samples that screen positive:

Transfer 0.5 mL of enrichment broth to 10ml TT tubes.

Transfer 0.1 mL of enrichment broth to 10ml RV tubes.

Incubate all tubes at 42°C for 22 – 24hrs.

Day 3
Selective Media

Streak tubes of RV and TT to DMLIA plates. Streak RV to XLD plates.

Incubate at 35°C for 18 – 24 hrs.

Day 4
Isolation

Typical Colonies Observed

Pick typical colonies and streak to SBA plate. Incubate at 35°C for 16-24 hrs.

Re-incubate all XLD and DMLIA plates @ 35°C for 18 –24 hrs.

Non-typical Colonies or No Growth Observed

Re-incubate all XLD and DMLIA plates @ 35°C for 18 – 24 hrs.

Day 5
Confirmation

Examine SBA growth for purity.

Perform confirmatory tests using a single isolated colony.

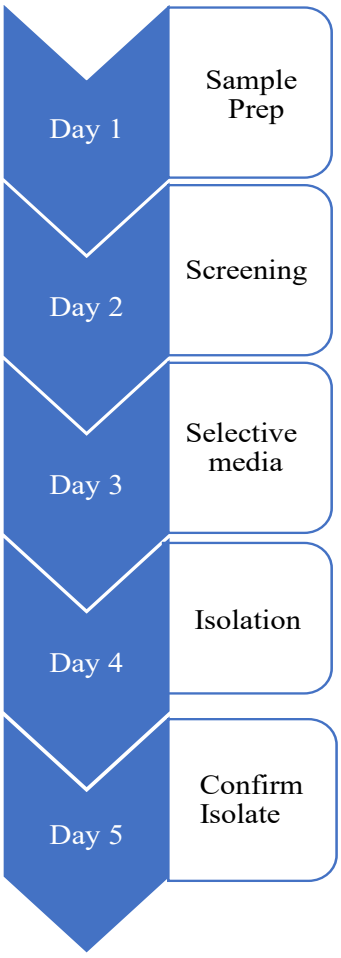
Test on Bruker® MALDI Biotyper

CONFIRMED POSITIVE Reported

Table of Contents

Introduction

The following methods describe the analysis of various meat, poultry, egg, sponge, & rinse samples, for *Salmonella*. They are not intended for the isolation and identification of *Salmonella enterica* serotype *typhi*. Successful isolation of *Salmonella* from food can be related to factors including food preparation procedures, the number of organisms present, sample handling after collection, and others. Competitive flora is one of the most important isolation factors with raw products. Each sample has its own unique flora contributing to isolation success. The method described below employs well - established media and tests for the isolation and identification of *Salmonella.*



The methods described in this guidebook are for use by the FSIS laboratories. FSIS does not specifically endorse any of the mentioned test products and acknowledges that equivalent products are available for laboratory use. FSIS utilizes the following performance criteria when evaluating the suitability of an alternative laboratory method or product for a given analyte and sample matrix pair. A criteria value of 90% or greater is required of sensitivity, specificity, accuracy, and positive or negative predictive values. Performance criteria are relative to the reference cultural method for that analyte and sample matrix as outlined in the corresponding MLG chapter (https://www.fsis.usda.gov/news-events/publications/microbiology-laboratory-guidebook; click on MLG Chapter 1). Method validation is necessary to demonstrate the equivalence of alternative tests as detailed in the document titled "FSIS Guidance for Evaluating Test Kit Performance," Validation Studies Pathogen Detection Methods.pdf (usda.gov)

Another consideration is whether the examination is for routine monitoring or epidemiological purposes. The analyst will augment the method for epidemiological purposes with additional enrichment procedures and culture media, two temperatures of incubation, intensified selection of colonies from plates, and/or rapid screening methods.

Unless otherwise stated all measurements cited in this method have a tolerance range of ± 2%.

KEY FACT

Salmonella are gram-negative, rod-shaped bacteria that cause disease in humans. *Salmonella enterica* is often associated with foodborne illness and is subdivided into multiple species and serotypes. Specific serotypes are often linked to foodborne outbreaks. *S. enterica* is widely dispersed in nature and can be found in the intestinal tracts of domestic and wild animals.

Safety Precautions

CDC guidelines for the handling of Biosafety Level 2 organisms must be followed whenever live cultures are used. The Safety Data Sheet (SDS) must be obtained from the manufacturer for the media, chemicals, reagents, and microorganisms used in the analysis. The personnel handling the material will read the SDS prior to startup. See "Biosafety Chart" at the end of this chapter for more information.

Equipment, Reagents and Media

Table 1: Equipment and supplies for MLG 4

Equipment	Supplier	Purpose
Incubators, static 42 ± 1°C, 35 ± 2°C	General lab supplier	Incubation of primary enrichment
Water Bath, static 42 ± 0.5°C	General lab supplier	Incubation of secondary enrichment
Refrigerator (2 – 8°C)	General lab supplier	Store media and sample reserves
Heating block, , 99°C	General lab supplier	Prepare sample DNA
Balance, sensitivity to at least ± 0.1 g	General lab supplier	Weigh samples
Bruker® MALDI Biotyper	Bruker Inc.	Proteomic Confirmation
Biochemical test kit and system, GN cards VITEK® 2 system	*bioMérieux* Vitek, Inc.	Biochemical identification (optional confirmatory technique)
Sterile inoculating loops	General lab supplier	Spread/streak plates
Pipets and sterile filter tips for the 5uL, 20uL volumes	General lab supplier	Add and mix reagents
Transfer pipet (plastic)	General lab supplier	Isolate preparation
Stomacher bags	General lab supplier	Primary bag for preparation and enrichment
Blending/mixing equipment: Paddle blender or equivalent	General lab supplier	Mix samples
Cloth sampling Device	Fremonta, Cat. # MT-S100 or General supplier	Raw Beef Trim Sample Collection Device
Neogen® Molecular Detection System (MDS)	Neogen®, Model # MDS100	Screen primary enrichment
Cooling block (20 – 25°C)	General lab supplier	Prepare sample DNA
bioMérieux GENE-UP® PCR instrument	*bioMérieux* GENE_UP	Salmonella Quantification

Table 2: Kits and Reagents for MLG 4

Kits and Reagents	Supplier	Purpose
Bruker® MALDI Biotyper reagents	Bruker Inc. or General supplier	Proteomic Confirmation
Neogen® Molecular Detection System (MDS)Salmonella	Neogen®, Catalog #MDA2SAL96	Screen primary enrichment for *Salmonella* analyses
Crystal violet dye, 1% aqueous solution	General lab supplier	Sample Preparation
Calcium carbonate, sterile	General lab supplier	Sample Preparation
GENE-UP® QUANT *Salmonella* Test Kit	*bioMérieux* GENE_UP	Salmonella Quantification

Media formulations are available in MLG Appendix 1, Media and Reagents.

Media required for enrichment, plating, and preliminary confirmation tests:

a. Buffered peptone water (BPW)
b. Trypticase Soy Agar with 5% Sheep Blood (SBA)
c. Tetrathionate Broth (TT) (Hajna)
d. Nutrient agar slants
e. Rappaport-Vassiliadis Soya Peptone Broth (RV), Modified Rappaport Vassiliadis (mRV) broth, or Rappaport-Vassiliadis R10 broth
f. Xylose Lysine Deoxycholate agar (XLD agar)
g. Double Modified Lysine Iron Agar (DMLIA)
h. Saline (0.45%)
i. Modified Tryptone Soy Broth (mTSB)

QUALITY CONTROL

Lab Quality Control Procedures

Include at least one positive culture and one uninoculated medium control. Incubate the controls along with the samples and analyze them in the same manner as the samples. Confirm at least one positive control isolate with each sample set, when applicable.

Salmonella controls are to include an H_2S+ positive, fluorescent strain of *Salmonella* (*Salmonella enterica* serotype Typhimurium, commercially available) and an uninoculated medium control.

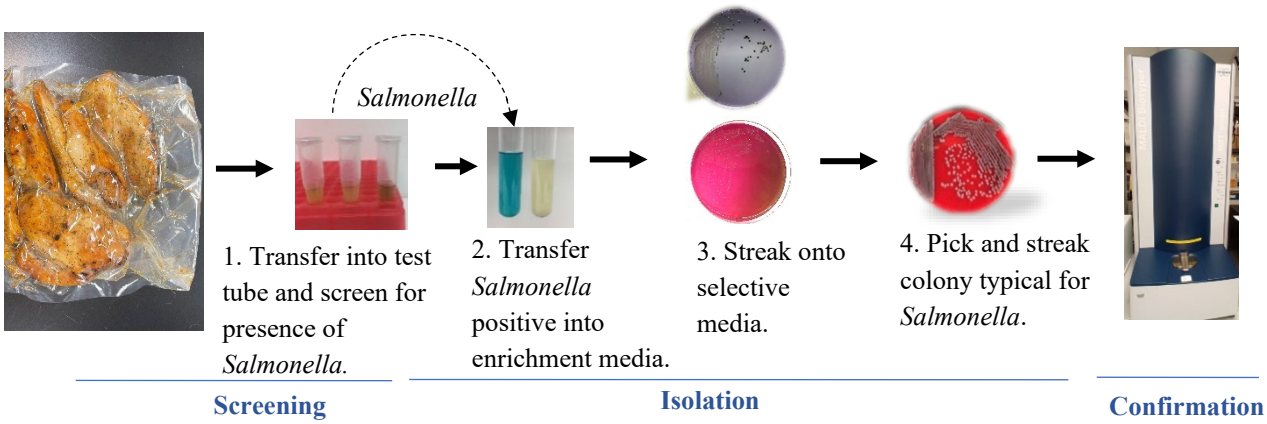
The positive control cultures will be inoculated into an appropriate enrichment broth at a low-level inoculum level, e.g., by preparing a test organism suspension in broth or saline equivalent in turbidity to a 0.5 McFarland standard. Using a 1µL loop, inoculate the broth or streak the plates to be tested. Alternatively, commercially prepared bacterial pellets will be used. Once the control cultures are started, incubate the controls along with the samples, and analyze them in the same manner as the samples. Confirm at least one isolate from the H_2S positive control sample. Confirmation of at least one colony from the H_2S negative control is required when confirming H_2S negative samples. In the absence of a positive test sample, control cultures will be terminated at the same point as the sample analyses.

Each step of the analysis requires the use of appropriate controls to verify that the results are valid. Biochemical kit and rapid test manufacturers will specify control cultures for use with their products. If not specified, quality control procedures for biochemical tests and test media will include cultures that will demonstrate pertinent characteristics of the product.

Preparing Samples

This procedure outlines the steps to screen and isolate *Salmonella* from meat, poultry, pasteurized egg products, whole bird and parts rinses and carcass and environmental sponges. Enriched samples are screened through Neogen® Molecular Detection System, a rapid screen technology. Samples that test positive are isolated through selective enrichment and plating media. Isolates are then confirmed by Bruker® MALDI Biotyper.

Figure 1: Overview of the steps for isolating *Salmonella* from meat, poultry, egg, sponge, and rinse samples.



Intact retail packages must be disinfected at the incision sites immediately prior to incision for sampling using an appropriate disinfectant, e.g., 3% hydrogen peroxide, ca. 70% ethanol or ca. 70% isopropanol. If the package does not appear to be clean, scrub gently using soapy water and rinse thoroughly prior to disinfection. Use a sterile sharp utensil for cutting the packaging. Aseptically pull the packaging away to expose the product for sampling.

For ready-to-eat (RTE) sausages in casing, the shell/casing is an integral part of the sample and must be free of pathogens and toxins. The casing is not to be disinfected since some casings are permeable and the disinfectant can be introduced into the core of the product. Consumers often slice through an inedible casing and then remove it thus any contamination on the surface of the casing could be transferred to the edible core of the product. Sample preparation and enrichment incubation times can vary by matrix and program. Refer to Table 1 and the following sample preparation sections.

KEY FACT

Sample temperature of ≤15°C is required upon receipt for raw poultry, raw pork and raw beef products.

Disinfect the surface of intact sample package(s) prior to opening.

FSIS Regulatory programs require *Salmonella* testing in concurrence with STEC for raw beef products. mTSB serves as a universal media for culturing STEC and *Salmonella* from a single sample.

Table 3. Sample Preparation and Enrichment Guide

Product	Sample Preparation		Incubation
	Portion Size	Enrichment Amount determined by volume or weight	Cultural or rapid screen
Ready-to-Eat Meat, Poultry and Siluriformes Products	325 ± 6.5 g	975 ± 19.5 mL BPW	35 ± 2°C for 18 – 24 hr.
Raw Poultry Products	325 ± 32.5 g	1625 ± 32.5 mL BPW	35 ± 2°C for 20 – 24 hr.
Raw Meat and Raw Beef Mixed Products	325 ± 32.5 g	975± 19.5 mL mTSB	42 ± 1°C for 15 – 24 hr.
Poultry Carcass and Environmental Sponges	1 sponge pre-moistened with 10 mL buffer	50 ± 1 mL BPW to bring total volume to 60 mL*	35 ± 2°C for 20 – 24 hr.
Raw Beef Product Cloth Samples	1 cloth pre-moistened with 25 mL buffer	Bring total volume to 200 ± 4 mL mTSB	42 ± 1°C for 15 – 24 hr.
Meat Carcass and Environmental Sponges	1 sponge pre-moistened with 10 mL buffer	50 ± 1 mL mTSB to bring total volume to 60 mL*	42 ± 1°C for 15 – 24 hr.
Whole Bird and Parts Rinses	30 ± 0.6 mL sample rinse fluid	30 ± 0.6 mL BPW	35 ± 2°C for 20 – 24 hr.
Pasteurized Liquid, Frozen or Dried Egg Products	100 ± 2 g	900 ± 18 mL BPW	35 ± 2°C for 18 – 24 hr.
Fermented Products	325 ± 6.5 g + 10 g of sterilized calcium carbonate	2925 ± 58.5 mL BPW with 1 mL of a 1% aqueous solution of crystal violet per liter	35 ± 2°C for 18 – 24 hr.
Dried Products (Breading Mix, Dehydrated Sauce, Soup Mix, Dried Milk)	325 ± 6.5 g	2925 ± 58.5 mL BPW	35 ± 2°C for 18 – 24 hr.
Environmental Aqueous chilling solutions samples	500 ± 2 mL	225 ± 5 mL (or 225 ± 5 g) BPW	35 ± 2°C for 20 – 24 hr.

* Or maintain a 1:6 ratio for different project buffer volumes, e.g., 25 mL buffer + 125 mL enrichment.

RTE Meat, Poultry, Siluriformes Products and Pasteurized Egg Products

Follow additional program requirements for preparing sample and sub-sample composites. Outbreak samples will require a different sample preparation. Follow customer specifications.

Using a sterile scalpel, knife, spoon, or other tool, cut small pieces from representative sites of submitted product to prepare a composite sample portion. While multiple packages of a product are usually submitted, for large products, a single package will be submitted.

For multi-component RTE products, follow the appropriate sample preparation instructions listed below:

- If the meat, or poultry component is separate and distinct from other non-meat ingredients, analyze only the representative meat/poultry portion of the RTE product. Examples include products with the meat/poultry portion separate from any vegetable/dessert component, or fajita kits with meat/poultry, onions/peppers, and tortillas in three separate internal packages/bags within an outer package.
- When meat, or poultry, is combined with other ingredients to form the product (e.g., beef stew containing vegetables, potatoes, etc.), analyze representative meat/poultry portions in combination with other ingredients.
- Weigh the composite sample into a large sterile bag.
- Add ambient temperature sterile BPW. Stomach approximately two minutes.
- Incubate at 35 ± 2°C for 18 – 24 hr.
- Proceed to the section entitled "Rapid Screening *Salmonella* Test Procedure" for use of the rapid screen or refer to the section entitled "Selective Enrichment and Plating" to continue the cultural analysis.

Raw Poultry Products

Figure 2: A raw poultry sample (Photo Credit: LQARCS)

If the sample is not already ground, it is best to mince it with sterile scissors or leave it whole (e.g., chicken wings). Add BPW. Stomach, or hand massage until clumps are dispersed.

Incubate at 35 ± 2°C for 20 – 24 hr.

Raw Meat and Raw Beef Mixed Products

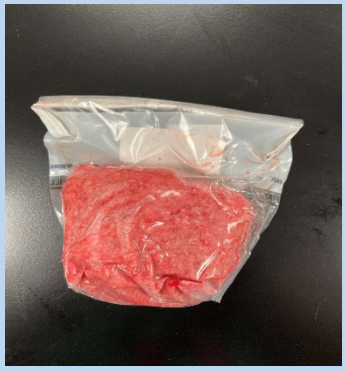


Figure 3: A raw beef sample (Photo Credit: LQARCS)

Add the mTSB at a 1:4 dilution, e.g., 325 ± 32.5 g sample with 975 ± 19.5 mL mTSB broth. Stomach, or hand massage until clumps are dispersed.

Incubate at 42 ± 1°C for 15 – 24 hr.

Raw Beef Product Cloth Samples

Figure 4: A raw beef cloth sample (Photo Credit: LQARCS)

For raw beef cloth samples that are premoistened with 25 mL of buffer, tare the balance with a dry cloth then bring total volume of sample up to 200 mL ± 4 mL with mTSB for enrichment. Aseptically manipulate the cloth to ensure it is submerged into the mTSB before stomaching and incubation. Mix well.

Incubate at 42 ± 1°C for 15 – 24 hr.

Carcass Sponges and Environmental Sponges

Figure 5: An environmental contact sponge (Photo Credit: LQARCS).

For poultry carcass or environmental sponges, add 50 mL BPW to the sample bag containing a sponge moistened with 10 mL. Mix well.

Incubate at 35 ± 2°C for 20 – 24 hr.

For meat carcass or environmental sponges, add 50 mL of mTSB to the sample bag containing a sponge moistened with 10 mL. Mix well.

Incubate at 42 ± 1°C for 15 – 24 hr.

Whole Bird and Parts Rinses

Figure 6: A whole bird and parts rinse (Photo Credit: LQARCS).

For whole bird and parts rinses:

Use 30 ± 0.6 mL of the sample rinse fluid obtained above for *Salmonella* analysis. Add 30 ± 0.6 mL of sterile BPW and mix well.

Incubate at 35 ± 2°C for 20 – 24 hr.

Pasteurized Liquid, Frozen, or Dried Egg Products

Figure 7: Liquid whole egg products (Photo Credit: LQARCS)

For Pasteurized liquid, Frozen, or Dried Egg product:

Mix the liquid sample with a sterile spoon, spatula, or by shaking. Transfer the liquid egg product into a sterile polypropylene bag.

Add sterile BPW and mix well by shaking or stomaching.

With dried egg samples, gradually add BPW to the sample. Add a small portion of the sterile BPW and mix to obtain a homogeneous suspension. Add the remainder of the BPW. Mix until a lump-free suspension is obtained.

Incubate at 35 ± 2°C for 18 – 24 hr.

Fermented Products

Figure 8: A fermented RTE sample (Photo Credit: LQARCS).

For fermented products that contain Lactic Acid Starter Culture, follow the procedure for RTE foods except:

Blend by hand massaging or stomach the sample with 10 ± 0.2 g of sterilized calcium carbonate.

Use BPW that contains 1 mL of a 1% aqueous solution of crystal violet per liter.

Incubate at 35 ± 2°C for 18 – 24 hr.

KEY FACT

Fermented products can be distinguished based on ingredients list with typical identifications such as lactic acid starter culture.

Dried Products (Breading Mix, Dehydrated Sauce, Dried Soup Mix)

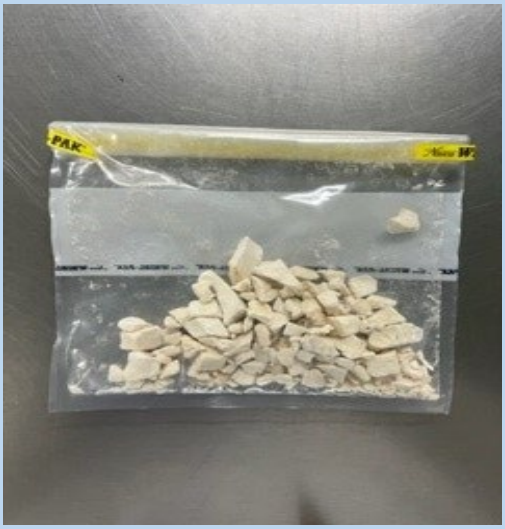


Figure 9: A dried RTE product. (Photo Credit: LQARCS).

Weigh the product into a sterile polypropylene bag.

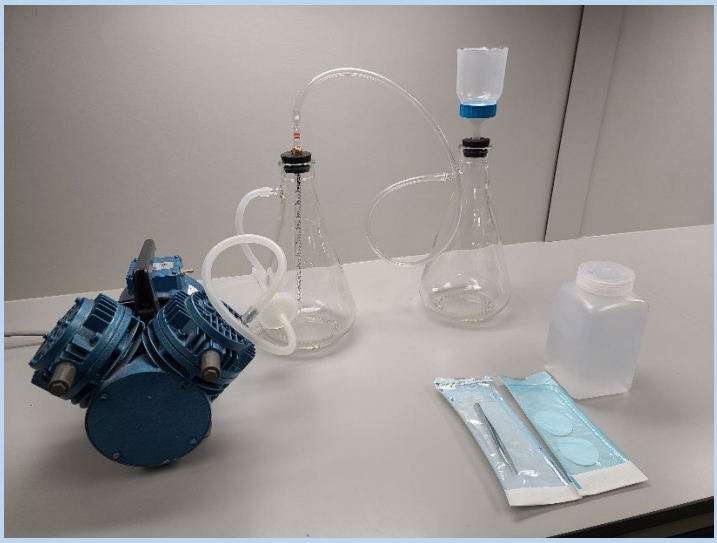
Add a small portion of the ambient temperature sterile BPW and mix to obtain a homogeneous suspension. Add the remainder of the BPW. Mix until a lump-free suspension is obtained.

Incubate at 35 ± 2°C for 18 – 24 hr.

KEY FACT

Dried products such as soup mixes require a sample or broth dilution greater than 1:4 because of physical difficulties encountered by absorption of broth by the dehydrated product.

Environmental Aqueous Chilling Solutions Samples



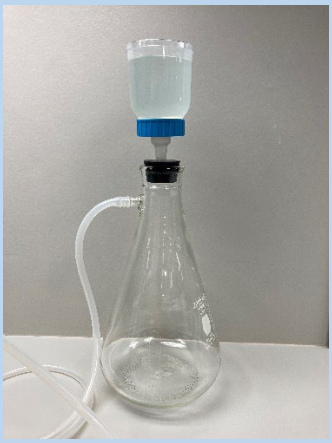


Figure 10: Example photos of how to set up for a brine sample (Photo Credit: Leo Gude and Sherre Chambliss).

For environmental aqueous chilling solutions and surface rinse solutions such as water, brine, and propylene glycol solutions, see below instructions.

Briefly:

Pour 500 ± 2 mL of sample solution into a sterile filter bag. Filter the solution by pouring it through a glass fiber filter and a 0.45 µm hydrophobic grid membrane filter in a vacuum filter system. When the sample has been completely filtered, aseptically remove both the glass fiber filter and the hydrophobic membrane filter, and transfer them back to the used filter bag. These filters can be easily clogged by particulates. Therefore, it may be necessary to replace the filters during the filtration process. If more than one filtration is required, transfer all filters used into original filter bag.

Add 225 ± 5 mL (or 225 ± 5 g) of BPW to the bag containing the filters. Stomach 2 ± 0.2 minutes. Ensure that the filters are submerged.

Incubate the homogenate at 35 ± 2°C for 20 – 24 hr.

Rapid Screening *Salmonella* Test Procedure

Following incubation, perform the rapid screen using the current Neogen® Molecular Detection System (MDS) System, or equivalent rapid screen technology. Follow the user guide for preparing reagents, performing the remainder of the assay, and reading the results.

Samples that are rapid screen-negative will be reported as negative. All positive samples will continue to cultural analysis.

For samples with rapid screen results that are considered inconclusive, the laboratory will investigate. Based on the findings, the laboratory must:

- repeat the rapid screen analysis from the lysate step or
- prepare new rapid screen lysate tubes and repeat the analysis or
- analyze the samples culturally.

In analytical runs where the positive control results are NOT positive, all samples are affected, and an investigation shall be performed. Based on the findings the laboratory must:

- repeat the rapid screen analysis from the lysate step or
- prepare new rapid screen lysate tubes and repeat the analysis or
- analyze all the samples culturally.

In analytical runs where the sterility control results are positive, perform an investigation as all samples are affected. Based on the finding the laboratory must:

- identify the root cause, evaluate sterility of media,
- analyze sterility sample culturally, add an additional sterility sample to act as a process control.

If circumstances (e.g., a power outage or equipment failure) do not allow testing using the rapid screen system, the laboratory shall continue cultural analysis of all samples by proceeding with isolation and purification steps as per MLG 4, section entitled “Selective Enrichment and Plating Media”.

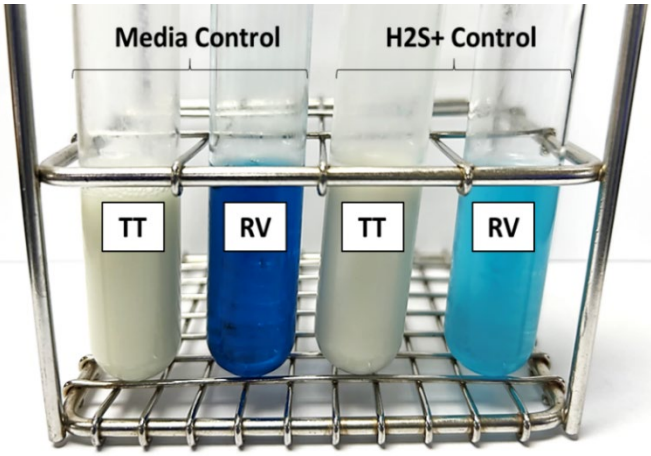
Selective Enrichment and Plating Media

Transfer 0.5 ± 0.05 mL of sample into 10 mL TT broth (Hajna) broth and 0.1 ± 0.02 mL into 10 mL RV broth. Incubate at 42 ± 0.5°C for 22 – 24 hr. or in a water bath at 42 ± 0.5°C for 18 – 24 hr.

Carefully mix contents of tube by vortexing or equivalent means. Streak TT/RV to DMLIA agar plates using a 10 µL loopful of inoculum. Streak RV to XLD using 10 µL loopful of inoculum. Streak the entire agar plate for isolation.

Incubate at 35 ± 2°C for 18 – 24 hr.

Figure 2: Examples of TT (Hajna) and RV broths inoculated with media control are shown on the left and broths inoculated with *Salmonella* are shown on the right.

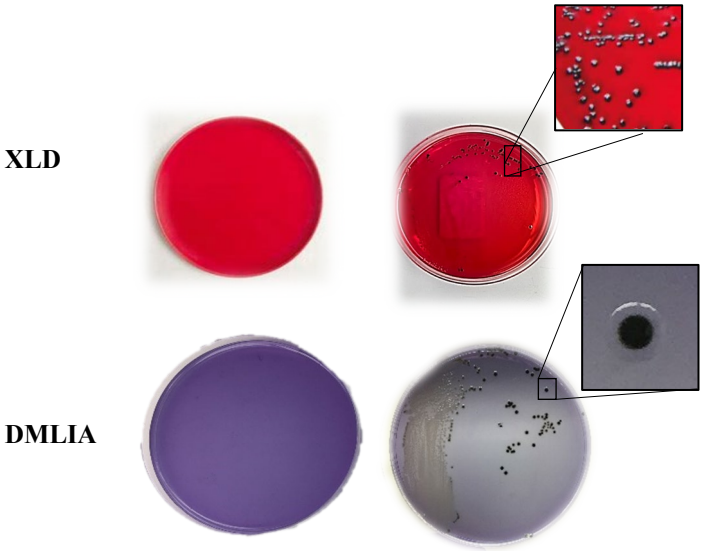


Examination of and Picking Colonies from Plating Media

Examine the XLD and DMLIA plates for the presence of colonies meeting the description of suspect *Salmonella* colonies:

- XLD: Select colonies that are red with black or dark centers.
- DMLIA: Select purple colonies with (H_2S positive) or without (H_2S negative) black centers. Since *Salmonella* typically decarboxylate lysine and ferment neither lactose nor sucrose, the color of the medium reverts to purple.

Figure 11: Example of XLD and DMLIA. Control plates without *Salmonella* are shown on left. Plates streaked with *Salmonella* are shown on right. Magnification shows isolated colonies.



Select well-isolated colonies that meet the description for *Salmonella* spp. from a XLD and DMLIA plates and streak onto SBA. Pick at least one representative colony of each morphology if available from each plating media type in preparation for isolate confirmation. Before any sample is reported as *Salmonella* negative, pick at least three total typical colonies from each plating media, if available. A representative of each typical colony type must be picked from each plate type before reporting the sample as *Salmonella* negative. Pick only from the surface and center of the colony. Avoid touching the agar because these highly selective media suppress growth of many organisms that are viable.

If there are typical colonies on a plate that are not well isolated, pick from the typical colonies and streak directly to a new set of selective agar plates. Alternatively, transfer typical colonies into a tube of TT broth (Hajna) or RV broth and incubate overnight, then streak to selective agars.

Incubate XLD and DMLIA plates for an additional 18 – 24 hr. at 35 ± 2°C. Incubate SBA plate for 16 – 24 hr. at 35 ± 2°C.

Reexamine initially negative plates and pick colonies as above. After 48-hr incubation, plates with no typical colonies are discarded as negative. Plates with colonies undergoing confirmation testing must be stored at 2 – 8°C until testing is complete. If suspect *Salmonella* colonies do not confirm, reexamine the plates from which they were picked, and if appropriate, select colonies again for confirmation following the section entitled "Examination of and Picking Colonies from Plating Media".

KEY DEFINITIONS

Bruker® Matrix-Assisted Laser Desorption Ionization (MALDI) Biotyper (MBT): A method of ionization for mass spectrometry, commonly applied to the analysis and identification of biomolecules.

Confirming *Salmonella* and Further Analysis

Perform confirmatory tests using a single isolated colony from the SBA plate. Commercially available test systems such as Bruker® MALDI Biotyper or validated equivalent systems are to be employed. Refer to the manufacturer's instructions for the use of the instrument, preparation of reagents, and troubleshooting guidance.

This method allows for the use of each available preparation method (direct, extended direct, and tube extraction) as needed to identify organisms. Refer to the manufacturer's documentation for a full list of organism coverage and thresholds.

The *Salmonella* isolate will be further characterized using Antimicrobial Susceptibility Testing (AST), and Whole Genome Sequencing (WGS).

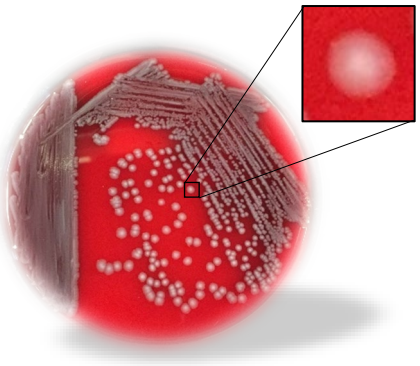


Figure 4: Example of an SBA plate streaked with *Salmonella.* Magnification shows isolated *Salmonella* colony.

Confirmation Criteria

A genus-level identification requires a minimum score of 1.70; and a species-level identification requires a minimum score of 2.00 when using the Bruker® MALDI Biotyper.

Sample *Salmonella* Enumeration Procedure (Poultry Rinsates and Raw Poultry)

Screen positive *Salmonella* samples are prepared for enumeration using DNA extractions. *Salmonella* PCR enumeration is performed concurrently with confirmatory *Salmonella* positive samples using the *bioMérieux* GENE-UP® PCR instrument with the GENE-UP® QUANT *Salmonella* Test Kit. DNA extractions are stored at ≤ -20°C until final isolate confirmation. The DNA extraction is used to determine the concentration of *Salmonella* spp. in the original, un-enriched poultry rinsate or raw poultry sample. Refer to manufacturer's instructions for use of the instrument, preparation of reagents, and troubleshooting guidance.

Biosafety Chart

SAFETY INFORMATION AND PRECAUTIONS

1. Recommended protective equipment: Nitrile or latex gloves, lab coat, safety glasses.
2. Hazards

Procedure Step	*Hazard*	*Recommended Safety Procedures*
Preparing samples with Primary Enrichment- Disinfecting intact retail packages with ethanol	Ethanol is acutely toxic by oral, dermal, or inhalation means. Highly flammable.	Wear gloves when using ethanol and avoid breathing the fumes directly. Avoid handling ethanol around heat or open flame.
Preparing samples with Primary Enrichment- Spraying down work area with bleach	Bleach is corrosive to the skin with prolonged exposure and may cause skin irritation with short-term exposure. Serious eye damage or eye irritation can occur if accidentally sprayed near eyes.	Wear gloves, safety glasses, and a lab coat when using bleach to prevent dermal or eye exposure.

Appendix: Alternative Methods

FSIS laboratories can elect to use biochemical confirmation methods (VITEK® 2) for reasons including: Bruker® MALDI MBT is unavailable, interruption in reagent supply chain, or results comparison.

To biochemically confirm isolates, inoculate appropriate VITEK® 2 cards (if using VITEK® 2 Compact) or equivalent.

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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:

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This method has been validated, reviewed, approved, and deemed suitable and fit for purpose for use in the USDA FSIS Field Service Laboratories.

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