United States Department of Agriculture

Food Safety and Inspection Service

MLG 31.02

Isolating Bacteria from Food Animals for Antimicrobial Resistance Surveillance

This method describes the laboratory procedure for performing analysis of cecal content in support of National Antimicrobial Resistance Monitoring System (NARMS).

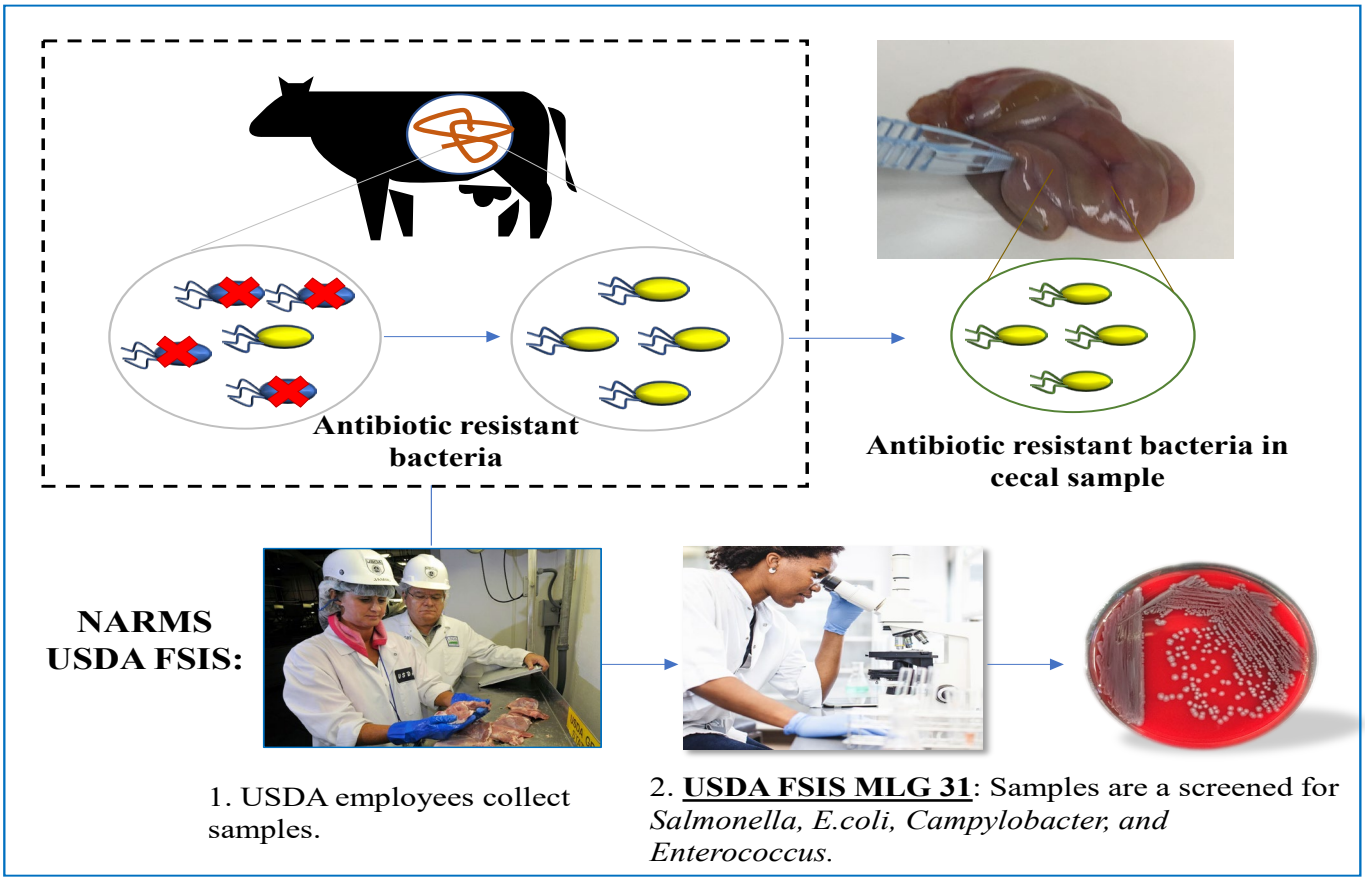
Notice of Change

- The method was revised to clarify the further characterization of *Campylobacter* species. Only *Campylobacter coli/jejuni/lari* will be carried to Whole Genome Sequencing (WGS) and Antimicrobial Susceptibility Testing (AST).
- This method revision also focuses on changes in selective media to add XLD agar plates and remove 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 method was revised to update the pathogen detection system used for *Salmonella* samples as part of modernization efforts.
- The revision also includes a biosafety chart to further explain safety precautions regarding MLG 31.

Table of Contents

Introduction

Antimicrobial resistance is a global health threat. Each year in the United States (US), antibiotic-resistant bacteria infect at least 2.8 million people. More than 35,000 people die from these types of bacterial infections (https://www.cdc.gov/drugresistance/index.html). The National Antimicrobial Resistance Monitoring System (NARMS) is a US public health surveillance system and includes the US Centers for Disease Control and Prevention (CDC), the Food and Drug Administration (FDA), the Department of Agriculture (USDA), and state and local health departments. These agencies collaborate to analyze samples from retail meats, food animals, and people. The data collected provides information about emerging bacterial resistance, the ways in which resistance is spread, and how resistance infections differ from susceptible infections caused by foodborne and other enteric bacteria.



Foodborne pathogens, which include antibiotic-resistant bacteria, can be found in an animal's intestinal tract. These pathogens can contaminate meat during the slaughter and processing of animals. The USDA Food Safety and Inspection Service (FSIS) veterinarians collect cecal samples in slaughter and processing establishments in support of the NARMS program (https://www.fsis.usda.gov/policy/fsis-directives/10100.1) and (https://www.fda.gov/media/101423/download). The samples are shipped to FSIS laboratories and analyzed for *Salmonella* spp, generic *Escherichia coli, Campylobacter* spp, and *Enterococcus* spp. Isolated bacterial targets are further characterized using Whole Genome Sequencing (WGS) (MLG 42) and Antimicrobial Resistance testing. The following method is used by USDA, FSIS as part of the NARMS Program. These laboratory procedures are for the isolation of *Salmonella*, generic *Escherichia coli*, *Campylobacter*, and *Enterococcus* from cecal samples of cattle (dairy and beef), swine (market hogs and sows), turkey and young chickens. The purpose of the cecal program is to identify potential sources of antimicrobial resistance in post-slaughter animal food sources prior to interventions, as compared to post-intervention samples collected for Hazard Analysis Critical Control Point (HACCP).

KEY DEFINITIONS

Antimicrobial: any agent (chemical or drug) that kills microorganisms or inhibits their growth.

Antibiotic: a drug prescribed for the treatment of a bacterial infection.

Antimicrobial Resistance (AMR): describes a characteristic of a microbe that is protected from the antibiotics and chemicals used to kill or inhibit microbial growth.

Antibiotic Resistance: a subset of AMR, describes bacteria that survive specifically in the presence of antibiotics.

Cecal: describes material obtained from the cecum, an intersectional pouch between the small and large intestines.

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 for microbes.

National Antimicrobial Resistance Monitoring System (NARMS): an interagency, collaboration between the CDC, USDA, FDA, and state and local health departments to monitor emerging antimicrobial resistance trends.

Safety Precautions

Follow CDC/NIH guidelines and use a Biosafety Level 2 (BSL-2) facility, engineering, and biosafety practices when handling cecal sample materials and live cultures (BMBL. 6th ed). The Safety Data Sheet (SDS) must be obtained from the manufacturer for the media, chemicals, reagents, and microorganisms used in the analysis. The personnel who will handle the material are to read the SDS prior to startup. See “Biosafety Chart” at the end of this chapter for more information.

Equipment, Supplies, Reagents, and Media

Table 1: Equipment and Supplies for MLG 31

Equipment or Supplies	Supplier	Purpose
Incubators, static 42 ± 1°C, 35 ± 2°C	General lab supplier	Incubation of primary enrichment
Centrifuge capable to hold 96-well PCR plates	General lab supplier	Prepare sample DNA
Pipet(s) and sterile filter tips for the 1µL, 5µL, 20µL 200µL and 30µL volumes	General lab supplier	Add and mix reagents
Neogen® Molecular Detection System (MDS)	Neogen®, Model # MDS100	Screen primary enrichment
PCR tube holder	Qualicon or equivalent	Prepare sample DNA
Heating block, 35 ± 2°C, 95 ± 3°C, 99°C	General lab supplier	Prepare sample DNA
Cooling block (2 – 8°C and 20 – 25°C	General lab supplier	Prepare sample DNA
Bruker® MALDI Biotyper	Bruker Inc.	Proteomic Confirmation
Vortex Mixer	General lab supplier	Mix reagents and tubes
Refrigerator (2 – 8°C)	General lab supplier	Store media and sample reserves
Gas Mixture (85% nitrogen, 10% carbon dioxide, and 5% oxygen)	General lab supplier	Provide microerobic conditions for cultivation of *Campylobacter.*
Balance, sensitivity to at least ± 0.1 g	General lab supplier	Weigh samples
Sterile inoculating loops	General lab supplier	Spread/streak plates
Anaerobic jar or equivalent container	General lab supplier	Microaerobic cultivation
Re-closable single-track zipper bag (1 gal and ½ gallon)	Uline, Catalog # S-1699	Secondary containment of enrichment bags to prevent leakage (optional)
AnaeroPack™ System (Mitsubishi Pack-MicroAero sachet) or equivalent product	Mitsubishi, Catalog # 10-05	Oxygen absorber-CO_2 generator for Campylobacter cultivation.
Transfer pipet (plastic)	General lab supplier	Isolate preparation
Stomacher bags with filters	General lab supplier	Primary bag for preparation and enrichment of cecal samples

Table 2: Kits and Reagents for MLG 31

Kits and Reagents	Supplier	Purpose
Neogen® Molecular Detection System (MDS)	Neogen®, Catalog #MDA2SAL96	Screen primary enrichment for *Salmonella* analyses
Ethyl alcohol, 100% (USP grade only; non-denatured	General lab supplier	To dissolve Bolton broth selective supplements
Bruker® MALDI Biotyper reagents	Bruker Inc. or General supplier	Proteomic Confirmation

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. Modified Charcoal-Cefoperazone-Deoxycholate Agar (mCCDA)
c. Trypticase Soy Agar with 5% Sheep Blood (SBA)
d. Double strength blood free Bolton enrichment broth (2XBF-BEB) and Bolton broth selective supplements
e. Eosin Methylene Blue Agar (EMB)
f. Enterococcosel™ Broth
g. Enterococcosel™ Agar (ECA)
h. Tetrathionate Broth (TT) (Hajna)
i. Rappaport-Vassiliadis Soya Peptone Broth (RVS)

QUALITY CONTROL

Laboratory Quality Control Procedures

Include at least one positive culture and one un-inoculated 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. Use the appropriate quality controls for the pathogen being analyzed.

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

E. coli controls are to include a positive culture (sorbitol positive *E. coli* ATCC 25922) and an un-inoculated medium control.

Campylobacter controls are to include a positive culture (*Campylobacter jejuni* or *Campylobacter coli* ATCC strain) and an un-inoculated media control.

Enterococcus controls are to include a positive culture (*E. faecalis,* ATCC 33186) and an un-inoculated media control.

Preparing Cecal Samples

This procedure outlines the steps needed to prepare intestinal (cecal) samples from cattle (dairy and beef), swine (market hogs and sows), turkey, and young chickens.

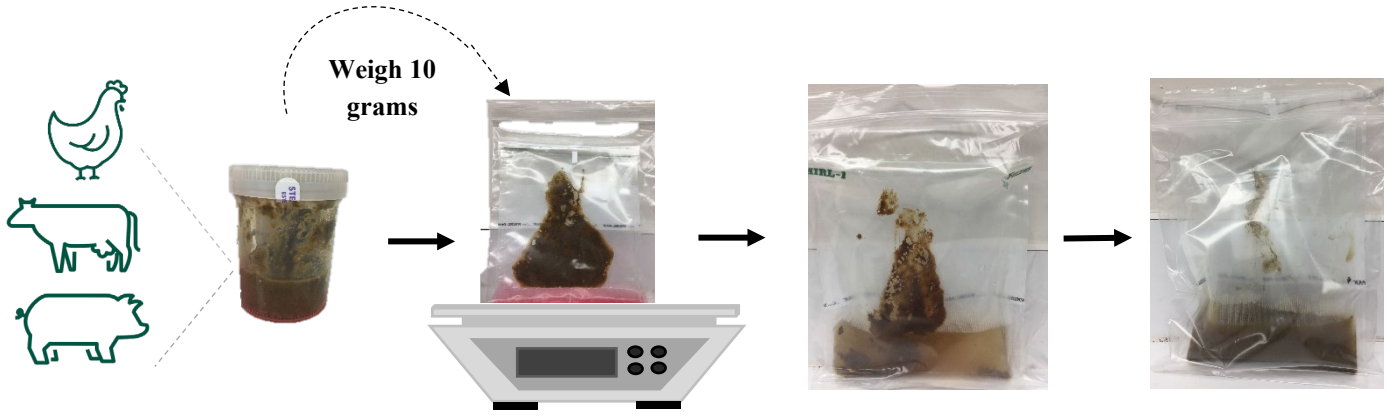


Fig 1: Overview of the steps for processing of cecal samples.

1. Insert the stomacher bag inside a secondary re-closable plastic bag to protect from spills.

2. Weigh 10 ± 2 g of cecal content into stomacher bag. If necessary, separate the cecal content from intestines prior to adding into the stomacher bag.

For chicken and turkey, do not use the intestinal lining as part of the weighed cecal content. Cut off the leading tip of the intestine with a scalpel and use forceps/tongue depressors to squeeze the cecal contents out of the intestines before depositing into the whirl-bag. Composite contents if multiple ceca are collected.

2. Close the stomacher bag and refrigerate at 2- 8°C for up to 8 days.

3. Add 90 ± 5 mL of BPW and close the stomacher bag.

4. Hand mix thoroughly. Use the cecal slurry to inoculate relevant selective medias for *Salmonella, Campylobacter*, *E. coli*, and *Enterococcus.*

NARMS: Isolating *Salmonella* from Cecal Samples

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.

This procedure outlines the steps to screen and isolate *Salmonella* from the cecum of cattle (dairy and beef), swine (market hogs and sows), turkeys, and young chicken. Enriched cecal 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.

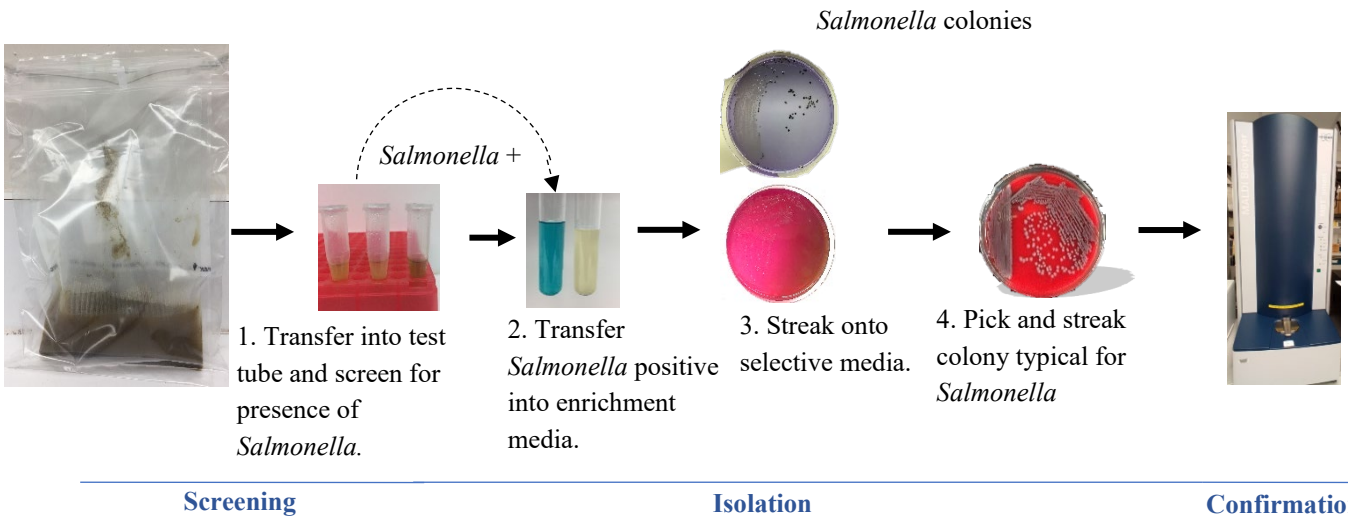


Fig 2: Overview of the steps for isolating *Salmonella* from cecal samples.

1. Incubate samples at 35 ± 2°C for 18-24h.

2. Remove samples from incubator and 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.

II. Isolating *Salmonella*

1. For screen positive samples and the uninoculated media control, transfer 0.5 ± 0.05 mL of sample into 10 mL TT broth (Hajna) and 0.1 ± 0.02 mL into 10 mL RV broth.

2. Incubate at 42 ± 0.5°C for 22-24 hours.

3. 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 for each plate. Streak the entire agar plate for isolation.

4. Incubate XLD and DMLIA plates at 35 ± 2°C for 18-24 hours.

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

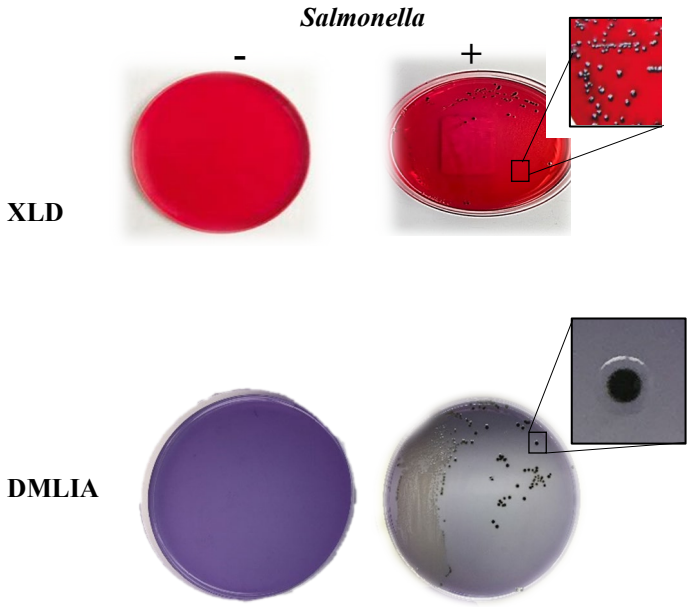


Fig 3: Example of XLD and DMLIA. Control plates without *Salmonella* are shown on left. Plates streaked with *Salmonella* are shown on right. Magnification shows isolated colony.

- XLD: Select colonies that are red colonies with black centers. (Fig 3: XLD and DMLIA).
- DMLIA: Typical colonies are purple with (H_2S-positive) or without (H_2S-negative) black centers. Since *Salmonella* typically decarboxylates lysine and ferments neither lactose nor sucrose, the color of the medium reverts to purple (Fig 3: XLD and DMLIA). Avoid picking colonies from a DMLIA plate with rusty-brown growth as this may be *Proteus*.

6. Select 2 well-isolated colonies that meet the description for *Salmonella* spp. from a XLD or DMLIA plate and streak onto SBA.

7. Incubate the SBA plates at 35 ± 2˚C for 16 - 24 hours (Fig 4).

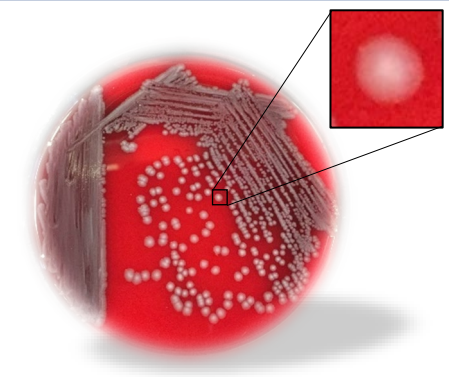


Fig 4: Example of sheep blood agar (SBA) streaked with *Salmonella.* Magnification shows isolated *Salmonella* colony.

III. Confirming *Salmonella* and Further Analysis

1. Perform confirmatory tests using a single isolated colony. 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, 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.

Confirmation Criteria

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

2. NARMS *Salmonella* requires a genus level identification to move forward for further characterization analysis.

3. Streak the confirmed *Salmonella* isolate onto a second SBA plate. Incubate the SBA for 16-24 h at 35 ± 2°C.

4. The confirmed *Salmonella* isolate is further characterized using Antimicrobial Susceptibility Testing (AST) and Whole Genome Sequencing (WGS).

NARMS: Isolating generic *E. coli* from Cecal Samples

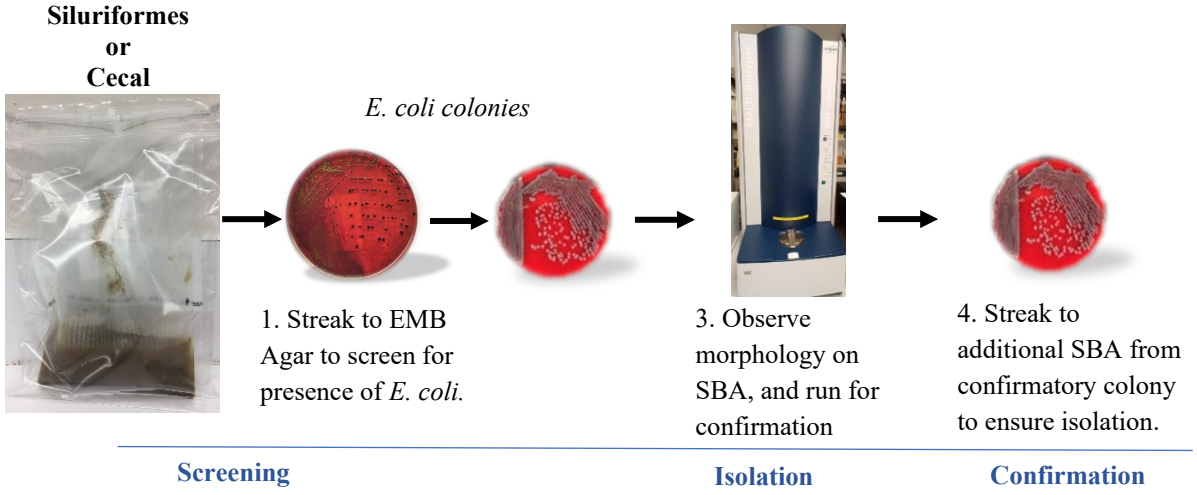


FIG 5: Overview of the steps to isolate *E. coli* from cecal and Siluriformes samples.

KEY FACT

E. coli are gram-negative, rod-shaped bacteria predominant in the normal flora of vertebrates. Although numerous serotypes of *E. coli* have been identified to cause foodborne illness in humans, generic *E. coli* are used as indicator organisms to detect emerging antibiotic resistance in gram negative bacteria. The term "generic *E. coli*" refers to *E. coli* naturally present in the gut which pose small foodborne disease risk.

This following procedure describes the analysis of intestinal (cecal) samples from cattle (dairy and beef), swine (market hogs and sows), turkey, goat, lamb, and young chickens for generic *E. coli*.

I. Screening for generic *E. coli* from Cecal Samples

1. Using a 10 µl loop, streak cecal BPW enrichment from the filtered stomacher bag to one EMB agar plate. Incubate at 42 ± 1°C for 18-24 hours.

II. Isolating generic *E. coli*

1. Typical colonies on EMB agar plates are black with or without a metallic green sheen.

2. Streak a typical, well isolated colony from EMB to SBA plate. Incubate 16-24 hours at 35 ± 2°C.

If a typical colony is not well isolated, re-streak the typical colony from the initial EMB to another EMB plate for further isolation.

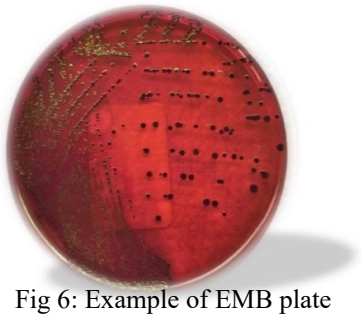


Fig 6: Example of EMB plate streaked with *E. coli* with black colonies with metallic green sheen.

III. Confirming generic *E. coli* and Further Analysis

1. Perform confirmatory tests using a single isolated colony. 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.

2. 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.

Confirmation Criteria

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

3. NARMS *E. coli* requires a genus and species-level identification to move forward for further characterization analysis.

4. Streak the confirmed *E. coli* colony onto a second SBA plate using the same colony to ensure isolation. Incubate the SBA plate for 16-24 h at 35 ± 2°C. Check for consistent colony morphology across the plate.

5. The confirmed *E. coli* isolates are further characterized using Antimicrobial Susceptibility Testing (AST) and Whole Genome Sequencing (WGS).

NARMS: *Enterococcus* from Cecal Samples

KEY FACT

Enterococcus are gram-positive bacteria with spherical and ovoid shape often observed as pairs or chains. They can survive harsh environments and are ubiquitous in nature including gastrointestinal tracts, meats, and food animals. *Enterococcus* are opportunistic pathogens that can cause disease in humans but are more commonly used to track antibiotic resistance in gram-positive bacteria.

This following procedure describes the analysis of intestinal (cecal) samples from cattle (dairy and beef), swine (market hogs and sows), turkey, and young chickens for *Enterococcus*.

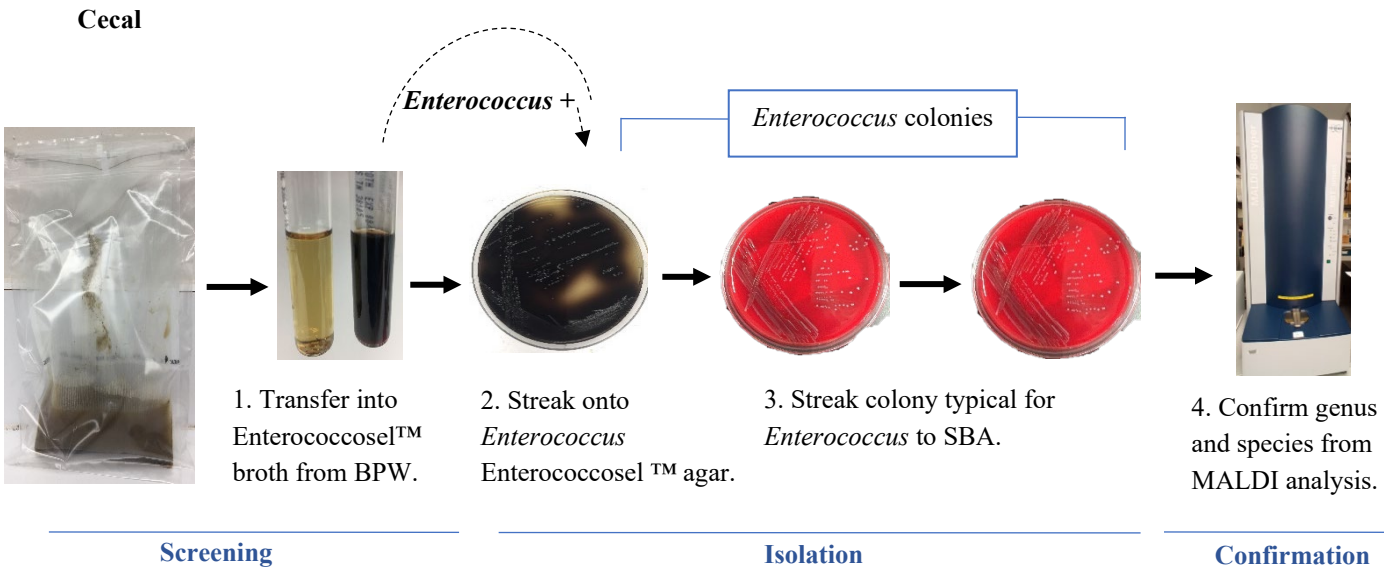


Fig 7: Overview of the steps for isolating *Enterococcus* from cecal samples.

I. Screening for *Enterococcus* from Cecal Samples

1.Add 0.5 mL of enriched BPW sample to 3 mL of Enterococcosel™ Broth. Incubate tubes with loosened caps at 35 ± 2°C for 2-24 hours (Fig 8).

3. Streak 1 µL of enrichment from all Enterococcosel™ broth tubes to Enterococcosel™ agar (ECA) for isolation. Incubate plates for 18-24 hours at 35 ± 2°C.

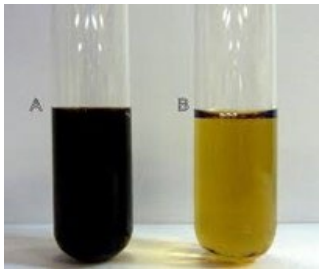


Fig 8: Typical EC Broth tubes for *Enterococcus* exhibit a black liquid.

II. Isolating *Enterococcus*

1. Examine the ECA plates for the presence of colonies meeting the description for suspect *Enterococci* colonies. Typical colonies are tiny and translucent with brownish black to black zones (Fig 9).

2. Pick one well-isolated, typical colony from the Enterococcosel™ plate and streak for isolation to SBA.. *Enterococcus* colonies tend be circular and tiny but are highly variable in appearance on SBA. Incubate for 16-24 hours at 35 ± 2°C.

3. To ensure the isolation of a single *Enterococcus* species, pick one well-isolated, typical colony from the initial SBA and streak to a fresh SBA plate for isolation. This is important since there are frequently several species of *Enterococcus* present within individual samples.

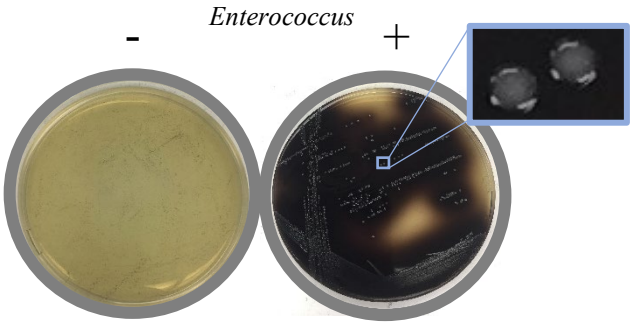


Fig 9: Example of *Enterococcus* on ECA plates. Shown on the left is a plate without growth. Shown on the right is a plate with typical *Enterococcus* growth with magnified colonies.

III. Confirming *Enterococcus* and Further Analysis

1. Perform confirmatory tests using an isolated colony. 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.

Confirmation Criteria

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

2. NARMS *Enterococcus* requires a genus and species-level identification to move forward for further characterization analysis.

3. Incubate the SBA and check for consistent colony morphology across the plate. Streak the confirmed *Enterococcus* colony to SBA. Incubate the SBA plate for 16-24 h at 35 ± 2°C.

4. The confirmed *Enterococcus* isolates are further characterized using Antimicrobial Susceptibility Testing (AST) and Whole Genome Sequencing (WGS).

NARMS: Isolating *Campylobacter* from Cecal Samples

SAMPLE PREPARATION PARAMETERS

Microaerobic Incubation

To obtain and maintain microaerobic conditions during incubation, use either sachets or gas tanks.

- Use two sachets of the AnaeroPack™ System (Mitsubishi Pack-MicroAero sachet) for a 7.0-L jar container.
- Use one sachet for a 2.5-L jar container.
- Use gas tanks for the appropriate gas mixture (85% nitrogen, 10% carbon dioxide, and 5% oxygen).

Maintaining a microaerobic atmosphere throughout testing activities is critical to avoid *Campylobacter j/c/l* die-off. Work quickly to provide the proper growing environment and when reading prepared slides.

KEY FACT

Campylobacter are gram-negative, spiral, uniflagellate, microaerophilic bacteria that cause foodborne illness. The two most frequently occurring *Campylobacter* species that are of clinical significance/concern for human consumption of meat and meat products are *C. jejuni* and *C. coli*. These two *Campylobacter* species are mainly isolated from intestinal tract of poultry and from poultry products.

The following procedure describes the analysis of intestinal (cecal) samples from cattle (dairy and beef), swine (market hogs and sows), turkey, and young chickens for *Campylobacter*.

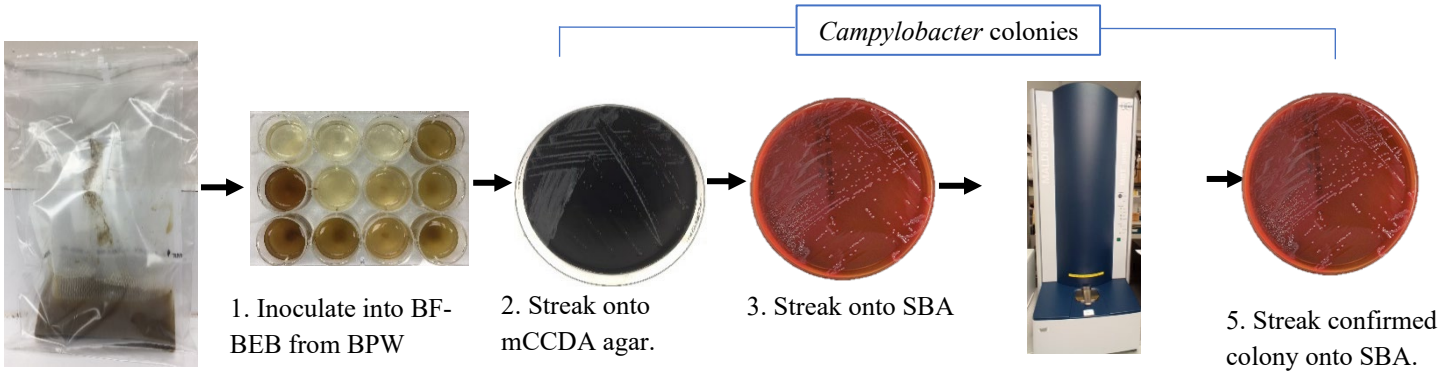


Fig 10: Overview of the steps for isolating *Campylobacter* from cecal samples.

I. Screening for *Campylobacter*

1. Add 1 mL of 2X BF-BEB and 1 mL of the of the BPW/sample preparation to a labeled well of a 36-well tissue culture plate or polystyrene test tube.

3. Incubate each plate or test tube for 29-31 hours at 42 ± 1.0°C in a sealed container containing the appropriate microaerobic conditions.

4. Streak 30 µl from the well or test tube to mCCDA agar plates. Place the agar plates into a sealed container applying the appropriate microaerobic conditions. Incubate plates at 42 ± 1.0°C for 22-24 h.

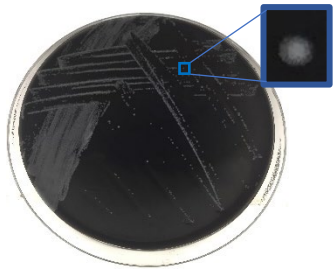


Fig 11: Example of *Campylobacter* growth on mCCDA agar plate.

Examining Plates

After incubation on mCCDA plates, examine mCCDA plates for typical colony types (Fig 11). Typical colonies on mCCDA are round to irregular with smooth edges. They can have thick translucent off white/caramel growth to spreading, film-like transparent growth, translucent or mucoid, glistening, flat or slightly raised, and may vary in size.

II. Additional Isolation

1. Streak typical colonies onto SBA and incubate at 42 ± 1°C for 24-48 h. After incubation, examine SBA plates. Typical *Campylobacter* colonies appear as shown in Figure 12. To ensure purity in the case of mixed populations, restreak isolated colonies onto SBA and incubate at 42 ± 1˚C for 24-48 h.

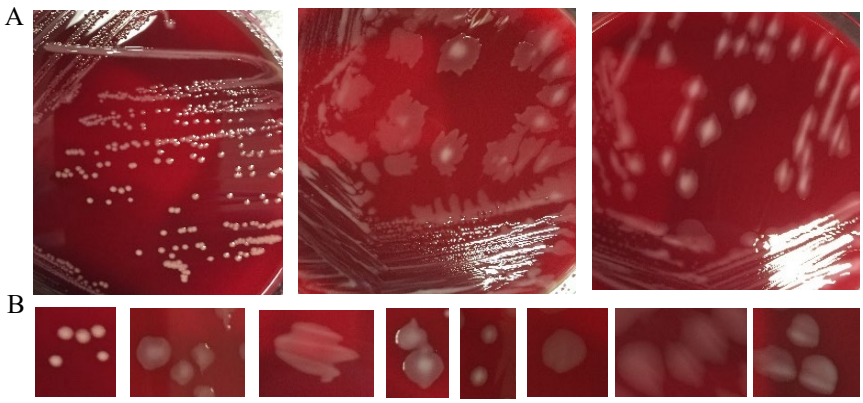


Fig 12: Examples of *Campylobacte*r growth on SBA. (A) SBA zoomed to show morphology. (B) Examples of *Campylobacter* colonies.

III. Confirming and Further Analysis

1. Perform confirmatory tests using an isolated colony. 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.

Confirmation Criteria

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

2. NARMS *Campylobacter* requires a genus and species-level identification to move forward for further characterization analysis.

3. Streak the confirmed *Campylobacter* colony to SBA. Incubate the SBA plate for 16-24 h at 42 ± 1°C under microaerobic conditions. Check for consistent colony morphology across the plate.

4.The confirmed *Campylobacter* isolate is further characterized using Antimicrobial Susceptibility Testing (AST) and Whole Genome Sequencing (WGS).

WGS and AST will only be conducted on *C. jejuni/coli/lari* species. Long term storage is maintained for all *Campylobacter* species obtained during analysis.

Biosafety Chart

Safety Information and Precautions

1. Recommended protective equipment: Nitrile or latex gloves, lab coat, and 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.

References

MLG 41 Title: Isolation, Identification and Enumeration of *Campylobacter jejuni/coli/lari* from Poultry Rinse and Sponge Samples.

MLG 4 Title: Isolation and Identification of *Salmonella* from Meat, Poultry, Pasteurized Egg, and Siluriformes (Fish) Products and Carcass and Environmental Sponges.

Bruker® MALDI Biotyper Users Guide. Proteomic isolate confirmation testing.

The National Antimicrobial Resistance Monitoring System: Manual of Laboratory Methods https://www.fda.gov/media/101423/download.

CDC About Antibiotic Resistance. https://www.cdc.gov/drugresistance/about.html.

Centers for Disease Control and Prevention and National Institutes of Health (CDC/NIH). 2020. Biosafety in Microbiological and Biomedical Laboratories, 6th ed. U.S. Government Printing Office, Washington, D.C. also found at the CDC internet site.

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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