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Food Safety and Inspection Service
Office of Public Health Science
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Laboratory Guidebook
Notice of Change

Chapter new, **revised**, or archived: MLG 4.09

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

Effective Date: 01/02/2017

Description and purpose of change(s):

FSIS Laboratories extended this method to Ready-to-Eat (RTE) Siluriformes (fish).

The FSIS Laboratories validated a 1:4 enrichment broth to sample ratio (1 part sample in 3 parts enrichment broth) for the analysis of RTE products for the presence of *Salmonella* (MLG 4 and 4C).

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 may be available for laboratory use. Method validation is necessary to demonstrate the equivalence of alternative tests as detailed in the document titled "FSIS Guidance for Evaluating Test Kit Performance" available on the FSIS website.

United States Department of Agriculture
Food Safety And Inspection Service, Office of Public Health Science

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4.1 Introduction

This method describes the analysis of various meat, poultry and Siluriformes (fish) products, sponge and rinse samples, and egg products for *Salmonella*. It is not intended for the isolation and identification of *Salmonella* Typhi.

Success in isolating *Salmonella* from any food can be related to a number of factors including food preparation procedures, the number of organisms present, sample handling after collection, etc. With raw samples, the competitive flora may be the most important factor. It varies from sample to sample and from one kind of matrix to another.

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

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

4.2 Safety Precautions

Salmonella are generally categorized as Biosafety Level 2 pathogens. CDC guidelines for manipulating Biosafety Level 2 pathogens should be followed whenever live cultures of *Salmonella* are used. A Class II laminar flow biosafety cabinet is recommended for procedures in which infectious aerosols or splashes may be created. 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 should read the SDS prior to startup.

4.3 Quality Control Procedures

4.3.1 Method Controls

A *Salmonella* spp. H_2S positive culture and an uninoculated media control must be used from the start of the analysis. A H_2S-negative *Salmonella* spp. positive culture shall also be included either from the start of the analysis or limited to sets containing screen positive samples, from the differentiation and confirmation steps starting with streaking to BGS and either DMLIA or XLT4 agar plates. To facilitate identification of control isolates, the laboratory may use strains of uncommonly found serogroups or tagged cultures such as those that visibly fluoresce under ultraviolet (UV) light to differentiate QC strains from true contaminants. *S.* Abaetetuba is suggested as a readily available, H_2S-positive culture that is not commonly found in meats or meat products. *S.* Choleraesuis is typically negative for H_2S production. These cultures may be obtained from ATCC. Other serotypes may be found that have H_2S-negative strains. The positive control cultures should be inoculated into an appropriate matrix at a low inoculum level, e.g., by preparing a test organism suspension in broth or saline equivalent in turbidity to a McFarland 0.5 standard. Using a 1ul loop, inoculate the broth or streak the plates to be tested. Alternatively, commercially prepared bacterial pellets may 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 may be terminated at the same point as the sample analyses.

4.3.2 Specific Procedure Controls

The biochemical and serological tests require the use of appropriate controls to verify that the results are valid. *Salmonella* 'O' antisera should be tested with QC control cultures or sera before initial use, and with a saline control for each test. Biochemical kit and rapid test manufacturers may specify control cultures for use with their products. If not specified, quality control procedures for biochemical tests and test media should include cultures that will demonstrate pertinent characteristics of the product.

4.4 Equipment, Reagents, Media and Test Kits

Not all of the materials listed below may be needed. Media and reagents specific to the selected biochemical test method may be needed in addition to the materials listed below.

4.4.1 Equipment

a. Sterile tablespoons, scissors, forceps, knives, glass stirring rods, pipettes, petri dishes, test tubes, bent glass rods ("hockey sticks") as needed
b. Blending/mixing equipment: Paddle blender, Sterile Osterizer-type blender with sterilized cutting assemblies, and blender jars or equivalent and adapters for use with Mason jars;
c. Sterile plain, clear polypropylene bags (ca. 24" x 30 - 36"), or Whirl-Pak™ type bags, or equivalent
d. Incubator, 35 ± 2°C
e. Incubator or water bath, 42 ± 0.5°C
f. Water bath, 48-50°C
g. Glass slides, glass plate marked off in one inch squares or agglutination ring slides
h. Balance, 2000 g capacity, sensitivity of 0.1 g
i. Inoculating needles and loops
j. Vortex mixer
k. VITEK® 2 Compact System, or equivalent

4.4.2 Reagents

a. Crystal violet dye, 1% aqueous solution
b. Butterfield's phosphate diluent
c. Saline, 0.85%
d. Saline, 0.85% with 0.6% formalin for flagellar antigen tests
e. Calcium carbonate, sterile
f. *Salmonella* polyvalent O antiserum and *Salmonella* individual O grouping sera for groups A-I (antisera for further O groups are optional)
g. *Salmonella* polyvalent H antiserum, Slide Agglutination H Antisera from Statens Serum Institut (SSI), or Oxoid *Salmonella* Latex Test (Unipath Company, Oxoid Division, Ogdensburg, NY) or equivalent

h. Additional reagents as needed for biochemical tests: e.g. GN cards for VITEK® 2 Compact System

4.4.3 Media

a. Buffered peptone water (BPW) or Modified Tryptone Soya Broth (mTSB)
b. TT broth (Hajna)
c. Modified Rappaport Vassiliadis (mRV) broth, Rappaport-Vassiliadis R10 broth, or Rappaport-Vassiliadis Soya Peptone Broth (RVS)
d. Brilliant green sulfa agar (BGS; contains 0.1% sodium sulfapyridine)
e. Xylose lysine Tergitol™ 4 agar (XLT4) or Double modified lysine iron agar (DMLIA)
f. Triple sugar iron agar (TSI)
g. Lysine iron agar (LIA)
h. Trypticase soy broth (TSB) or Tryptose broth
i. Trypticase soy agar (TSA)
j. Nutrient agar slants
k. Nutrient broth, semi-solid
l. Tryptic soy agar with 5% sheep blood agar
m. Additional media as needed for biochemical tests

4.5 Sample Preparation

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. A sterile scalpel may be helpful for cutting the packaging. Aseptically pull the packaging away to expose the product for sampling.

Note: For Ready-to-Eat (RTE) sausages in casing, the shell/casing is an integral part of the sample and should be free of pathogens and toxins. The casing is not to be disinfected since some casings are permeable and the disinfectant may be introduced into the core of the product. In addition 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 may vary by matrix and program. Refer to Table 1 and the following sample preparation sections.

Table 1. Sample Preparation and Enrichment Guide

Product	Sample Preparation		Incubation
	Portion Size	Enrichment Amount determined by volume or weight	Cultural or PCR rapid screen
Ready-to-Eat Meat, Poultry and Siluriformes Foods	325 ± 6.5 g	975 ± 19.5 ml BPW	35 ± 2°C for 18-24 h
Raw Poultry Products	325 ± 32.5 g or 25 ± 2.5 g	1625 ± 32.5ml BPW or 225 ± 4.5 ml BPW	35 ± 2°C for 20-24 h
Raw Meat and Raw Beef Mixed Products	325 ± 32.5 g or 25 ± 2.5 g	975± 19.5 ml mTSB or 75 ± 1.5 ml mTSB	42 ± 1°C for 15-24 h
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 h
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 h
Whole Bird and Parts Rinses	30 ± 0.6 ml sample rinse fluid	30 ± 0.6 ml BPW	35 ± 2°C for 20-24 h
Pasteurized Liquid, Frozen or Dried Egg Products	100 ± 2 g	900 ± 18 ml BPW	35 ± 2°C for 18-24 h
Raw Siluriformes Products	25 ± 2.5 g	225 ± 4.5 ml BPW	35 ± 2°C for 22-26 h
Fermented Products	325 ± 6.5 g + 10 g of sterilized calcium carbonate	2925 ± 58.5 ml of BPW with 1 ml of a 1% aqueous solution of crystal violet per liter	35 ± 2°C for 18-24 h
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 h

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

4.5.1 Ready-to-Eat Meat, Poultry and Siluriformes Foods

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

Include representative portions from each submitted package to achieve the analytical sample portion. Using a sterile scalpel, knife, spoon, chisel 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 may be submitted.

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

If the meat, poultry or Siluriformes 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, poultry, or Siluriformes 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.

a. Weigh the composite sample into a large sterile bag (or sterile blender jar if required by the customer or sample type).

b. Add ambient temperature sterile BPW. Blend or stomach approximately two minutes.

c. Incubate at 35 ± 2°C for 18-24 h.

d. Proceed to Section 4.6 to continue the cultural analysis or refer to MLG 4C for use of the BAX® PCR Assay.

4.5.2 Raw Poultry Products

a. Unless the exact sample portion is submitted, weigh the product into a sterile polypropylene bag, sterile blender jar, or other sterile jar. Note: If the sample is not already ground, in some cases it may be best to mince it with sterile scissors or leave it whole (e.g. chicken wings) to avoid jamming blender blades with skin or connective tissue.

b. Add the BPW. Stomach, blend or hand massage until clumps are dispersed.

c. Incubate at 35 ± 2°C for 20-24 h.

d. Proceed to Section 4.6 to continue the cultural analysis or refer to MLG 4C for use of the BAX® PCR Assay.

4.5.3 Raw Meat and Raw Beef Mixed Products

a. Unless the exact sample portion is submitted, weigh the meat into a sterile polypropylene bag, sterile blender jar, or other sterile jar.

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

c. Incubate at 42 ± 1°C for 15-24 h.

d. Proceed to Section 4.6 to continue the cultural analysis or refer to MLG 4C for use of the BAX® PCR Assay.

4.5.4 Carcass Sponges and Environmental Sponges

a. For poultry carcass or environmental sponges, add the BPW to the sample bag containing a sponge moistened with 10 ml of buffer to bring the total volume to 60 ml. Mix well. If the project calls for a larger volume of buffer, adjust the volume of enrichment broth to a 1:6 ratio. Incubate at 35 ± 2°C for 20-24 h.

b. For meat carcass or environmental sponges, add the mTSB to the sample bag containing a sponge moistened with 10 ml of buffer to bring the total volume to 60 ml. Mix well. If the project calls for a larger volume of buffer, adjust the volume of enrichment broth to a 1:6 ratio. Incubate at 42 ± 1°C for 15-24 h.

c. Proceed to Section 4.6 to continue the cultural analysis or refer to MLG 4C for use of the BAX® PCR Assay.

4.5.5 Whole Bird and Parts Rinses

Due to differences between sample types/sizes (e.g. chicken vs. turkey carcasses; size of parts), follow instructions given in the specific project protocol.

a. For chicken carcasses:
Aseptically drain excess fluid from the carcass and transfer the carcass to a large sterile bag.

Pour 400 ml (or other volume specified in program protocol) of collection medium into the cavity of the carcass contained in the bag.

Rinse the bird inside and out with a rocking motion for one minute (ca. 35 RPM). This is done by grasping the carcass in the bag with one hand and the closed top of the bag with the other. Rock with a reciprocal motion in about an 18-24 inch arc, assuring that all surfaces (interior and exterior of the carcass) are rinsed.

b. For chicken parts:
Add the weight of the specific part plus the volume of collection medium specified by the program protocol into the sterile bag.

Rinse the parts with the broth assuring that all surfaces are rinsed.

c. Transfer the sample rinse fluid to a sterile container.

d. 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.

e. Incubate at 35 ± 2°C for 20-24 h.

f. Proceed to Section 4.6 to continue the cultural analysis or refer to MLG 4C for use of the BAX® PCR Assay.

NOTE: If analyses other than *Salmonella* are to be performed, the carcass may be rinsed and dilutions made directly from the collection rinse.

4.5.6 Pasteurized Liquid, Frozen, or Dried Egg Products

a. Mix the liquid sample with a sterile spoon, spatula, or by shaking.

b. Weigh the liquid egg product into a sterile polypropylene bag, sterile blender jar, or other sterile jar.

c. Mix the inoculated BPW well by shaking, stomaching, or blending.

Note: If a special sample or specification requires a sample size other than 100 g, the ratio of egg sample to BPW is to be maintained at 1:10.

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

e. Incubate at 35 ± 2°C for 18-24 h.

f. Proceed to Section 4.6 to continue the cultural analysis or refer to MLG 4C for use of the BAX® PCR Assay.

4.5.7 Raw Siluriformes (Fish) Products

Follow program requirements for preparing sample and sub-sample composites.

a. Weigh the tissue into a sterile bag, sterile blender jar, or other sterile jar.

b. Add the BPW. Stomach or blend, as required, for approximately two minutes or shake thoroughly.

c. Incubate at 35 ± 2°C for 22-26 h.

d. Proceed to Section 4.6 to continue the cultural analysis or refer to MLG 4C for use of the BAX® PCR Assay.

4.5.8 Fermented Products

Follow the procedure for RTE foods in Section 4.5.1 except:

a. Blend/stomach the sample with 10 ± 0.2 g of sterilized calcium carbonate.

b. Use buffered peptone water that contains 1 ml of a 1% aqueous solution of crystal violet per liter.

4.5.9 Dried Products (Breading Mix, Dehydrated Sauce, Dried Soup Mix, and Dried Milk)

a. Weigh the product into a sterile polypropylene bag, sterile blender jar, or other sterile jar.

b. 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.

c. Incubate at 35 ± 2°C for 18-24 h.

d. Proceed to Section 4.6 to continue the cultural analysis or refer to MLG 4C for use of the BAX® PCR Assay.

Note: Dried products such as soup mixes may require a sample/broth ratio greater than 1:10 because of physical difficulties encountered by absorption of broth by dehydrated product.

4.5.10 Most Probable Numbers (MPN) Determination

Follow MPN instructions given in the specific project protocol or see MLG Appendix 2, Most Probable Number Procedure and Tables.

4.6 Selective Enrichment and Plating Media

a. Transfer 0.5 ± 0.05 ml of sample into 10 ml TT broth (Hajna) broth and 0.1 ± 0.02 ml into 10 ml mRV broth.

b. Incubate at 42 ± 0.5°C for 22-24 h or in a water bath at 42 ± 0.5°C for 18-24 h.

c. Carefully mix contents of tube by vortexing or equivalent means. Streak to BGS and either DMLIA or XLT4 agar plates using a 10 µl loopful of inoculum for each plate. Streak the entire agar plate with a single sample enrichment.

d. Incubate at 35 ± 2°C for 18-24 h.

e. Select typical colonies.

4.7 Examination of and Picking Colonies from Plating Media

4.7.1 Picking Colonies

a. After the recommended incubation interval, examine the selective-differential agar plates and controls for the presence of colonies meeting the description for suspect *Salmonella* colonies. Pick well-isolated colonies.

- BGS. Select colonies that are pink and opaque with a smooth appearance and entire edge surrounded by a red color in the medium. On very crowded plates, look for colonies that give a tan appearance against a green background.
- XLT4. Select black colonies (H_2S-positive) or red colonies with (H_2S-positive) or without (H_2S-negative) black centers. The rim of the colony may still be yellow in 24 h; later it should turn red.
- 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.

b. Pick at least one typical isolated colony from any of the plates. (NOTE: Before any sample is reported as *Salmonella* negative, pick at least three total typical colonies, 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 may be viable.

c. 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 mRV broth and incubate overnight, then streak to selective agars.

d. Incubate all plates for an additional 18-24 h at 35 ± 2°C.

e. Reexamine initially negative plates and pick colonies as above. After 48-hour incubation, plates with no typical colonies may be discarded as negative. Plates with colonies undergoing confirmation testing should 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, re-pick colonies for confirmation following Section 4.7.1.b.

4.7.2 Screening Media

a. Inoculate TSI and LIA slants in tandem with a single pick from a colony by stabbing the butts and streaking the slants in one operation. If screw cap tubes are used, the caps must be loosened. Incubate at 35 ± 2°C for 24 ± 2 h.

Note: The same colony may be used to streak a plate used for subsequent biochemical testing described in Section 4.9.

Examine TSI and LIA slants as a set. Note the colors of butts and slants, blackening of the media, and for TSI slants presence of gas as indicated by gas pockets or cracking of the agar. Note also the appearance of the growth on the

slants along the line of streak. A typical control on LIA should produce a purple butt with (H_2S-positive) or without (H_2S-negative) blackening of the media. A typical control on TSI should produce a yellow butt and red slant, with (H_2S-positive) or without (H_2S-negative) blackening of the media.

Discard, or re-streak for isolation, any sets that show "swarming" from the original site of inoculation. Discard sets that show a reddish slant in lysine iron agar. Isolates giving typical *Salmonella* spp. reactions and isolates that are suggestive but not typical of *Salmonella* spp. should be confirmed by a combination of biochemical and serological procedures. Refer to Table 2 for a summary of TSI-LIA reactions.

Note: TSI and LIA slants may be held at 2-8°C for up to 96 hours. Prior to any subsequent testing, fresh TSI and LIA slant(s) must be inoculated and incubated per instructions in a (above).

b. The motility testing in the last column of the table is optional. Refer to "Edwards and Ewing's Identification of Enterobacteriaceae" (Ewing, 1986) for additional information.

4.8 Serological Tests

Molecular serotyping may be performed to further characterize isolates in lieu of serological testing.

4.8.1 Somatic (O) Antigen Agglutination Tests

Isolates are tested with polyvalent O antiserum reactive with serogroups A through I + Vi.

Following a positive reaction using polyvalent O antiserum, test the isolate using individual *Salmonella* antisera for O groups A through I + Vi. Additional individual O groups may be tested, but testing for O groups A through I + Vi should encompass the majority of the *Salmonella* serotypes commonly recovered from meat and poultry products.

Use growth from either the TSI or LIA slant. Test first with polyvalent O antiserum. Include a saline control with each isolate. If there is agglutination with the saline control alone (auto-agglutination), identify such a culture by biochemical reactions only. If the saline control does not agglutinate and the polyvalent serum does, test the culture with *Salmonella* O grouping antisera.

Typically, a positively identified isolate and/or control is identified by the individual O group result, but an isolate can be identified by the poly group if it tests as positive for multiple individual O groups. The isolate is noted as an auto-agglutinator if it reacts with saline.

Occasionally, an isolate will be recovered which is typical of *Salmonella* biochemically and is serologically poly H-positive, but is non-reactive with any of the available O group antisera. Report these isolates as "*Salmonella* non A-I + Vi" or "*Salmonella* O group beyond I" if no further testing is performed. Further tested isolates are reported by their specific serotype.

4.8.2 Flagellar (H) Antigen Agglutination Tests

The Oxoid *Salmonella* Latex Test, SSI H Antisera for Slide Agglutination, or equivalent, may be used for H antigen agglutination testing. Follow the manufacturer's instructions for performing the test.

Alternatively, use growth from either the TSI or LIA slant to inoculate a tube of trypticase soy broth or tryptose broth. Incubate at 35 ± 2°C overnight. Add an equal amount of saline containing 0.6% formalin and let sit one hour. Remove one ml to each of two 13 x 100 mm test tubes. To one of the tubes, add *Salmonella* polyvalent H serum in an amount indicated by the serum titer or according to the manufacturer's instructions. The other tube serves as an autoagglutination control. Incubate both tubes at 48-50°C in a water bath for up to 1 hr without mixing or shaking during incubation. Record the presence or absence of agglutination.
If desired, use Spicer-Edwards pooled serum or H typing serum. The specific procedure may be found in "Edwards and Ewing's Identification of Enterobacteriaceae" (Ewing, 1986).

4.9 Biochemical Procedures

Commercially available biochemical test kits, including automated systems may be used for biochemical identification. Alternatively, use traditional methods of biochemical identification. Refer to AOAC Official Method 967.27 or "Edwards and Ewing's Identification of Enterobacteriaceae", 4th Edition, for biochemical reactions of *Enterobacteriaceae* and for fermentation media and test procedures.

For some biochemical test kits, i.e. the VITEK® 2 Compact System, streak a TSA + 5% sheep blood agar plate from either the TSI or LIA slant. Incubate 16-24 h at 35 ± 2°C. Some commercial biochemical test systems may require streaking to other non-selective media prior to inoculation of their test kit. Follow manufacturer's instructions.

Note: Plates may be stored up to 96 hours at 2-8°C. Stored plates must be streaked to a new plate and incubated per instruction prior to use.

Table 2. Potential *Salmonella* Reactions Requiring Biochemical Analysis. Additional culture work may be required and other factors should be considered before discarding any sample.

Triple Sugar Iron Agar			Lysine Iron Agar		Polyvalent Sera		Further Testing/ Disposal
Butt	Slant	H_2S	Butt	H_2S	O	H	
Y	R	+	P	+	+	+	B. & M. T.
Y	R	+	P	+	+	-	B. & M. T.
Y	R	-	P	-			B. & M. T.
Y	R	-	Y	-	+	+	B. & M. T.
Y	R	-	Y	-	-	-	B. & M. T.
Y	R	+	Y	+/-			B. & M. T.
Y	Y	-	Y or P	-			Discard
Y	Y	+	P	+			B. & M. T.
NC	NC						Discard

Y = Yellow; R = Red; P = Purple; B. & M. T. = Perform biochemical testing, e.g., VITEK® 2 Compact System, and optional motility tests;
NC = No change in color from uninoculated medium.

4.10 Culture Storage and Maintenance

For short-term (no longer than 3 months) storage, inoculate a nutrient agar slant, incubate at 35 ± 2°C overnight and then store at 2-8°C. For long-term storage, lyophilize cultures or freeze using cryo-beads, i.e. Cryostor™ or equivalent.

Maintain "working" *Salmonella* stock cultures on nutrient agar slants or equivalent. Transfer stocks monthly onto duplicate nutrient agar slants, incubate overnight at 35 ± 2°C, and then maintain them at 2-8°C. Use one of the slants as the working culture. Use the other slant for sub-culturing to reduce the opportunity for contamination.

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