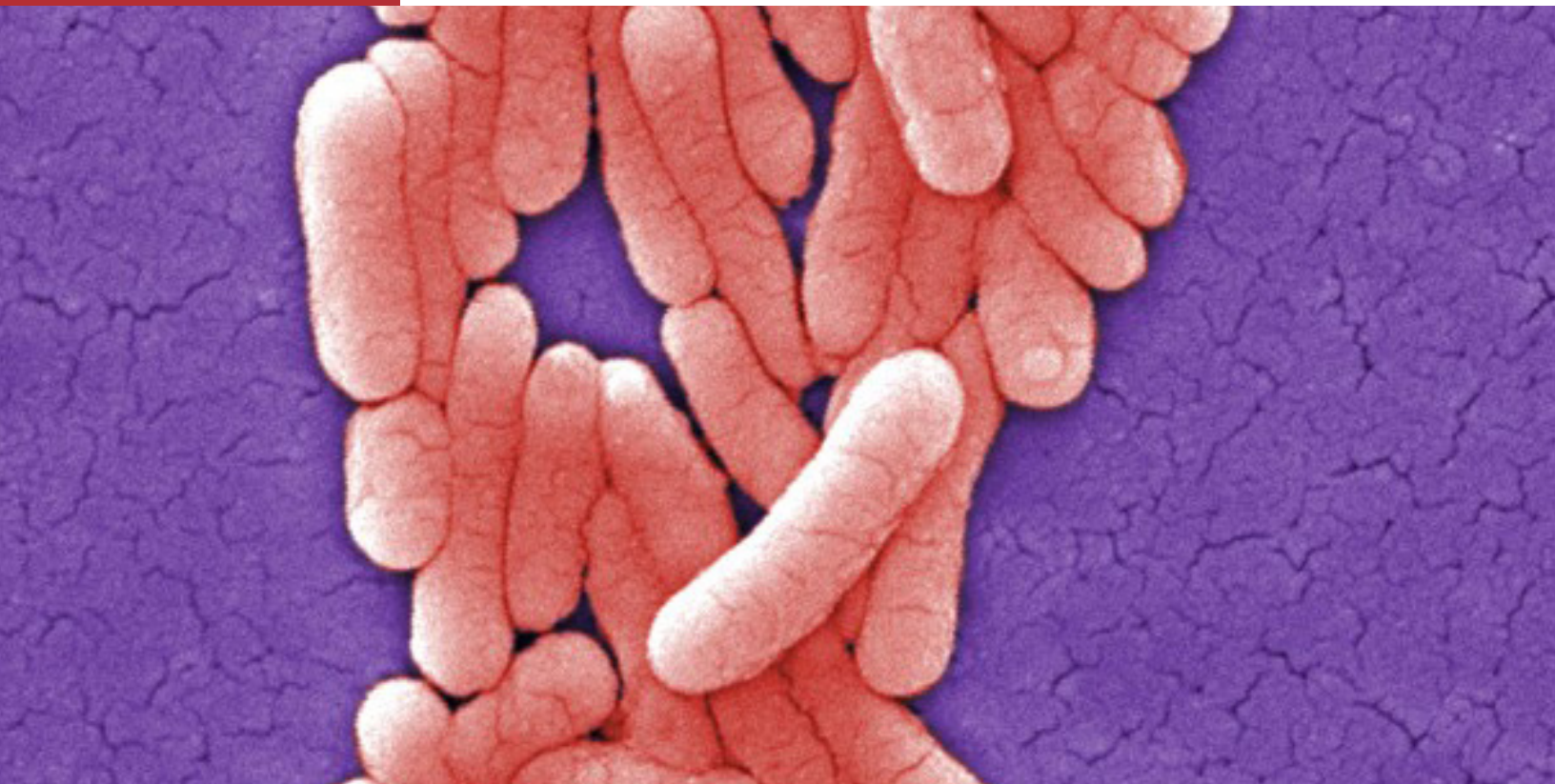


Isolation and Identification of *Salmonella* Species from Culture-Independent Diagnostic Test Positive Specimens



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Acknowledgements

Prepared by the APHL Salmonella Workflow Workgroup

Sandra Bandstra, MT (ASCP)

South Carolina Department of Public Health

David Boxrud, MS

Division of Foodborne, Waterborne, and Environmental Disease, Centers for Disease Control and Prevention

Eddrenna Brown

South Carolina Department of Public Health

Tabatha E. East, MS

Tennessee Department of Health

Ray Erickson, MS

Minnesota Department of Health

Allison Gennety

Association of Public Health Laboratories

Greg Goodwin, MS

South Carolina Department of Public Health

Charlotte Lane, MS

Division of Foodborne, Waterborne, and Environmental Disease, Centers for Disease Control and Prevention

Kirsten Larson, MPH

Association of Public Health Laboratories

Karim Morey, MS, M (ASCP)

Oregon State Public Health Laboratory

Robin Rasnic, MLS (ASCP)

Tennessee Department of Health

Sharon Shea, MHS, MT (ASCP)

Associate of Public Health Laboratories

Amanda (Jo) Williams-Newkirk, PhD

Division of Foodborne, Waterborne, and Environmental Disease, Centers for Disease Control and Prevention

Susan Van Duyne, MA

Division of Foodborne, Waterborne, and Environmental Disease, Centers for Disease Control and Prevention

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Purpose

A recommended workflow for the culture and isolation of *Salmonella* species from human feces.

Background

The following is a procedure for the culture and isolation of *Salmonella* spp. from human feces. *Salmonella* is one of the most common etiologic agents of bacterial diarrhea. Sensitive and specific laboratory methods for the isolation, identification, serotyping and subtyping of *Salmonella* are key to monitoring and control programs. Culture-independent diagnostic tests (CIDTs) have impacted the ability of public health laboratories (PHLs) to obtain bacterial isolates to perform additional characterization to identify and prevent foodborne illness outbreaks. In recent years, PHLs have received an increasing volume of specimens that tested positive for foodborne pathogens using CIDTs at the clinical laboratory. In this instance, the PHL needs to perform culture to isolate the pathogen so the isolated bacteria can be characterized by whole genome sequencing (WGS) or other methods. The goal of this document is to provide PHLs with methods to isolate and identify *Salmonella* spp. from stool specimens as efficiently as possible.

Fecal specimens are the preferred laboratory samples for diagnosis of infectious diarrhea. However, the recovery of enteric pathogens from feces is often complicated by multiple factors, including prior antibiotic treatment, transport stress, intermittent shedding of pathogens in the feces and a low inoculum of *Salmonella* bacteria in relation to other enteric flora. These factors necessitate the use of culture algorithms that employ selective enrichment and the use of selective/differential media.

Selective enrichment suppresses fecal flora while allowing the target pathogen to grow. Selective media can also utilize various phenotypic characteristics to preliminarily differentiate potential pathogens from fecal flora.

Specimen Collection and Transport

The preferred specimen is fresh stool collected in transport media such as Cary-Blair (CB). Unpreserved stool and fecal or rectal swabs submitted in transport media may also be acceptable. Stool samples without preservative should be considered for rejection if transit time exceeds limits stated by the receiving laboratory (often two hours after specimen collection). Rectal swabs are not a preferred sample and should only be utilized when the patient cannot produce a fecal sample. The rectal swab should be examined after collection; fecal matter should be visible on the swab. If fecal matter is not visible on the swab, the swab should not be submitted for culture.

Suspect *Salmonella* specimens collected in modified CB-preservative or CB swabs can be shipped at room temperature⁷, however, if extreme temperatures are anticipated, ice packs should be used. Ideally, specimens should be received as quickly as possible, not exceeding four days since collection, as isolate recovery may decline. At the PHL, culture for *Salmonella* should begin as soon as reasonably possible. Specimens may be held at 2–8°C until culture is completed. If the unpreserved sample cannot reach the laboratory within the specified time (generally two hours) it is recommended that the raw stool cultures be stored frozen at less than -15°C and rectal swabs be frozen at -70°C until testing is possible¹.

Materials and Supplies

Media

- Use a minimum of two of the following media: Hektoen Enteric agar (HE), Xylose-Lysine-Deoxycholate agar (XLD), Xylose Lysine Tergitol 4 agar (XLT-4), Salmonella CHROMagar, Salmonella-Shigella agar (SS)
- Less-Selective Media: Blood agar (BAP), MacConkey agar (MAC)
- Enrichment broths: Either Selenite (SEL) or Tetrathionate (TET)
- Biochemicals for screening: Triple Sugar Iron (TSI), Lysine Iron agar (LIA), Motility, Indole, Ornithine (MIO), Citrate, Urea

- Biochemicals for identification (if not using MALDI-TOF): Malonate, Lactose, Dulcitol, Salicin, Sorbitol, Mucate, Tartrate, Ortho-nitrophenyl-β-D-galactopyranoside (ONPG), Galacturonate, 4-Methylumbelliferyl-β-D-glucuronide (MUG).
- Some laboratories use the API-20E or other automated identification methods in place of individual biochemicals.
- Before using media, review the manufacturer's instructions to determine appropriate incubation times and organisms for media quality control.
- It is important to review the manufacturer's instruction for use of media, broths and biochemicals.

Other Supplies

- Inoculating loops
- Applicator swabs
- Supplies for MALDI-TOF

Culture and Identification Workflow

(See [Appendix A](#))

Day 0 — Stool specimen collected in transport media is received at the PHL.

1. Inoculate two of the following selective media: HE, XLD, Salmonella CHROMagar, SS, XLT4 (Step 1 on workflow diagram). See Appendices B and C for a description and pictures of *Salmonella* growth on the selective media.
 - A recommended strategy to better detect atypical *Salmonella* isolates is to pair CHROMagar Salmonella with one of the other plate options (HE, SS agar, XLD, or XLT4). Some unusual *Salmonella* morphologies such as H₂S negative and lactose or sucrose fermenting *Salmonella* will appear atypical on HE, SS, XLD, and XLT-4 but should appear typical on CHROMagar Salmonella. While CHROMagar Salmonella does well with the atypical morphologies previously listed, it is ineffective at identifying *Salmonella* with weak C8 esterase production. Note: CHROMagar is more expensive than the other media.
2. Inoculate one of the following enrichment medias: SEL, TET (Step 2 on workflow diagram).
 - Use of an enrichment media is critical to increase sensitivity.
 - *S. Typhi* grows better in SEL compared to TET¹. If *S. Typhi* is suspected, consider using SEL.

Day 1

1. Pick at least one of each suspicious morphology from selective media to BAP or MAC plates (Step 3 on workflow diagram).
2. Inoculate two of the following selective media: HE, XLD, Salmonella CHROMagar, SS, XLT4 (Step 4 on workflow diagram) from enrichment broth.

Day 2

1. Pick colonies to TSI, LIA, MIO, citrate, and urea, as well as perform identification by MALDI-TOF as needed (Step 5 on workflow diagram).
 - If *Salmonella* is identified by MALDI-TOF, there is no need to continue isolation and identification for *Salmonella* from enrichment broth.
 - Some PHLs have elected to not perform TSI, LIA, MIO, citrate, and urea and instead rely solely on MALDI-TOF results. TSI, LIA, MIO, citrate and urea can help identify presumptive typhoidal *Salmonella*.
 - Additional information about MALDI-TOF and biochemicals are found in the [Additional Comments](#) section.
2. From the selective media inoculated from enrichment media, pick at least one of each suspicious morphology from selective media to BAP or MAC plates (Step 6 on workflow diagram) and continue identification as indicated on Day 3.

Day 3

1. Pick colonies to TSI, LIA, MIO, citrate, and urea, as well as perform identification by MALDI-TOF (Step 7 on workflow diagram).
 - Additional information about MALDI-TOF and biochemicals are found in the Additional Comments section.

Additional Comments

Some *Salmonella* strains may display atypical morphology or biochemical reactions. Laboratories should consider isolation and identification of *S. Typhi* when developing laboratory testing workflows.

Identification by MALDI-TOF is preferred over biochemicals because MALDI-TOF is a low-cost option which is cheaper to run and faster compared to biochemicals. MALDI-TOF is not able to reliably identify the species, so biochemical supplementation may be needed to correctly identify presumptive typhoidal serotypes if whole genome sequencing (WGS) is not performed. The current MALDI-TOF Vitek MS library does not correctly identify *Salmonella bongori*, additional biochemicals (Appendix E) or other methods may be needed to identify *S. bongori*. If a laboratory does not have access to a MALDI-TOF, the following biochemicals can be used for presumptive *Salmonella* (not including subspecies) identification: TSI, LIA, MIO, citrate, and urea (see Appendix D and Appendix E for typical reactions of *Salmonella* spp.). The following biochemicals can be used for additional subspeciation of *Salmonella* spp.: Malonate, Lactose, Dulcitol, Salicin, Sorbitol, Mucate, Tartrate, ONPG, Galacturonate, MUG (see Appendix E for typical biochemical reactions of *Salmonella* spp.).

The recommendations made here are based on the best information and data available for isolation and identification of *Salmonella* in PHLs. Additional studies to reduce the amount of media needed to detect *Salmonella* in PHLs would be greatly appreciated and may have additional cost reductions with minimal impact on the percentage of specimens that grow *Salmonella* spp. Please contact entericreferencelab@cdc.gov or foodsafety@aphl.org with any comments or questions.

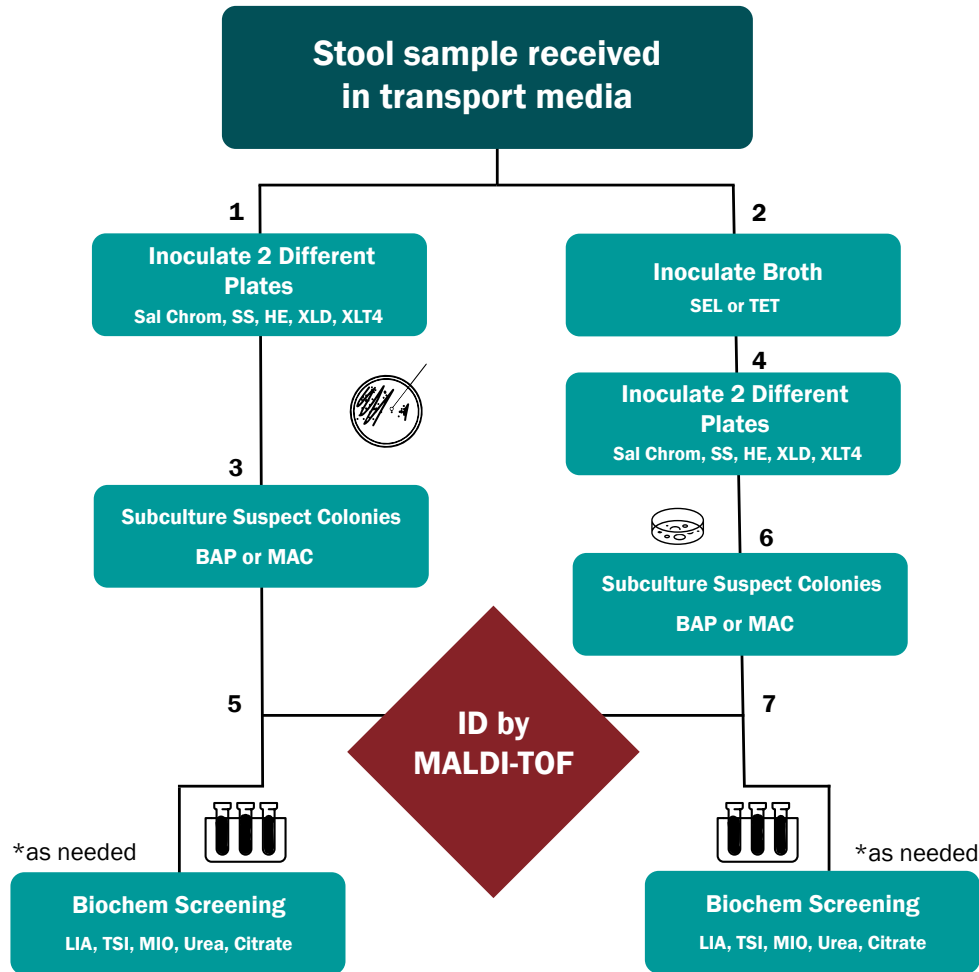
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Appendix A

Flow Diagram for Isolation and Identification of *Salmonella*

Salmonella Workflow



Appendix B*

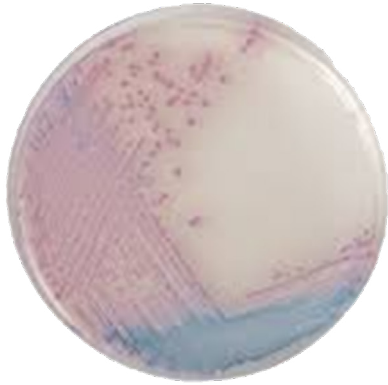
Typical Morphology Description of *Salmonella* Species on Selective Media

Media	<i>Salmonella</i> spp.
Salmonella Shigella (SS)	Colorless colonies with black centers
Hektoen Enteric (HE) Agar	Clear greenish blue colonies with black centers
Xylose Lysine Deoxychocolate (XLD) Agar	Colonies range in colors from clear to pink/red with black centers; colonies may appear yellow after 24 hours. Paratyphi A colonies will typically look pink to red.
Salmonella CHROM agar	Mauve colonies
XLT-4 Agar	Black or red colonies with a black center; colonies may appear yellow after 24 hours. Paratyphi A colonies will typically look pink to red.

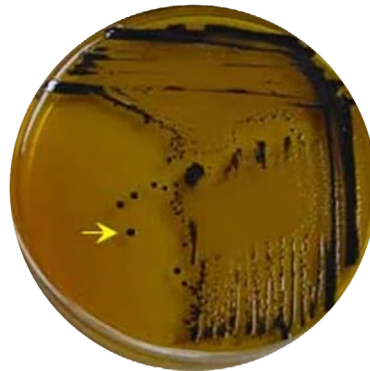
* Rare serotypes, such as Typhi and Paratyphi A, may produce little to no H₂S so colonies may not have a black center.

Appendix C

Morphology of *Salmonella* on Selective and Differential Media



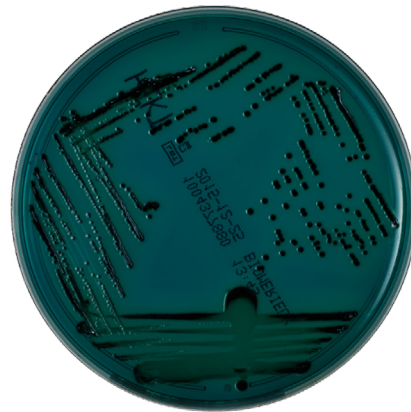
1. *Salmonella* on Salmonella CHROMagar



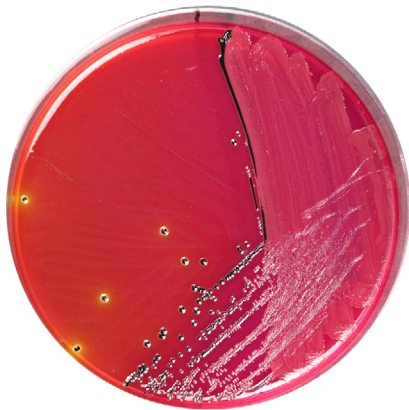
2. *Salmonella* on Salmonella Shigella (SS) Agar



3. *Salmonella* on XLD Agar



4. *Salmonella* on HE Agar



5. *Salmonella* on XLT4 media at 24hrs



6. *Salmonella* on XLT4 media at 48hrs

Appendix D

Comparative Phenotypic Profiles of *Salmonella* Species, *S. Typhi*, and *S. Paratyphi A*

Biochemical	<i>Salmonella</i> (majority)	<i>S. Typhi</i>	<i>S. Paratyphi A</i>
Citrate	+	-	-
TSI (slant)	K	K	K
TSI (butt)	A	A	A
TSI (H ₂ S)	+	Trace amount	-
TSI (gas)	G	No gas	G
LIA (slant)	K	K	K
LIA (butt)	K	K	A
LIA (H ₂ S)	+	Trace amount	+
LIA (gas)	G	-	-
MIO (Motility)	+	+	+
MIO (Indole)	-	-	-
MIO (Ornithine)	+	-	-
Urea	-	-	-

*K= alkaline, A= acidic, G= gas production. For more visuals, view [Appendix V](#) from WHO GFN.

Appendix E*

Typical Results of Biochemicals for *Salmonella* Species and Subspecies

Substrate	<i>Salmonella enterica</i>						<i>Salmonella bongori</i> (formerly ssp. V)
	ssp.* I	ssp. II	ssp. IIIa	ssp. IIIb	ssp. IV	ssp. VI	
Dulcitol	+	+	-	-	-	V	+
Galacturonate	-	+	-	+	+	+	+
Lactose	-	-	-(75%)	+(75%)	-	V	-
Malonate	-	+	+	+	-	-	-
Mucate	+	+	+	-(70%)	-	+	+
MUG	V	V	-	+	-	V	-
ONPG	-	-	+	+	-	V	+
Salicin	-	-	-	-	V*	-	-
Sorbitol	+	+	+	+	+	-	+
Tartrate	+	-	-	-	-	-	-

ssp. – subspecies

(+) – >90% positive

(-) – <10% positive

V – variable

* – varies by serovar (Kauffman White table, 2007, 9th edition)

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7700 Wisconsin Avenue, Suite 1000
Bethesda, MD 20814

Phone: 240.485.2745

Fax: 240.485.2700

www.aphl.org

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