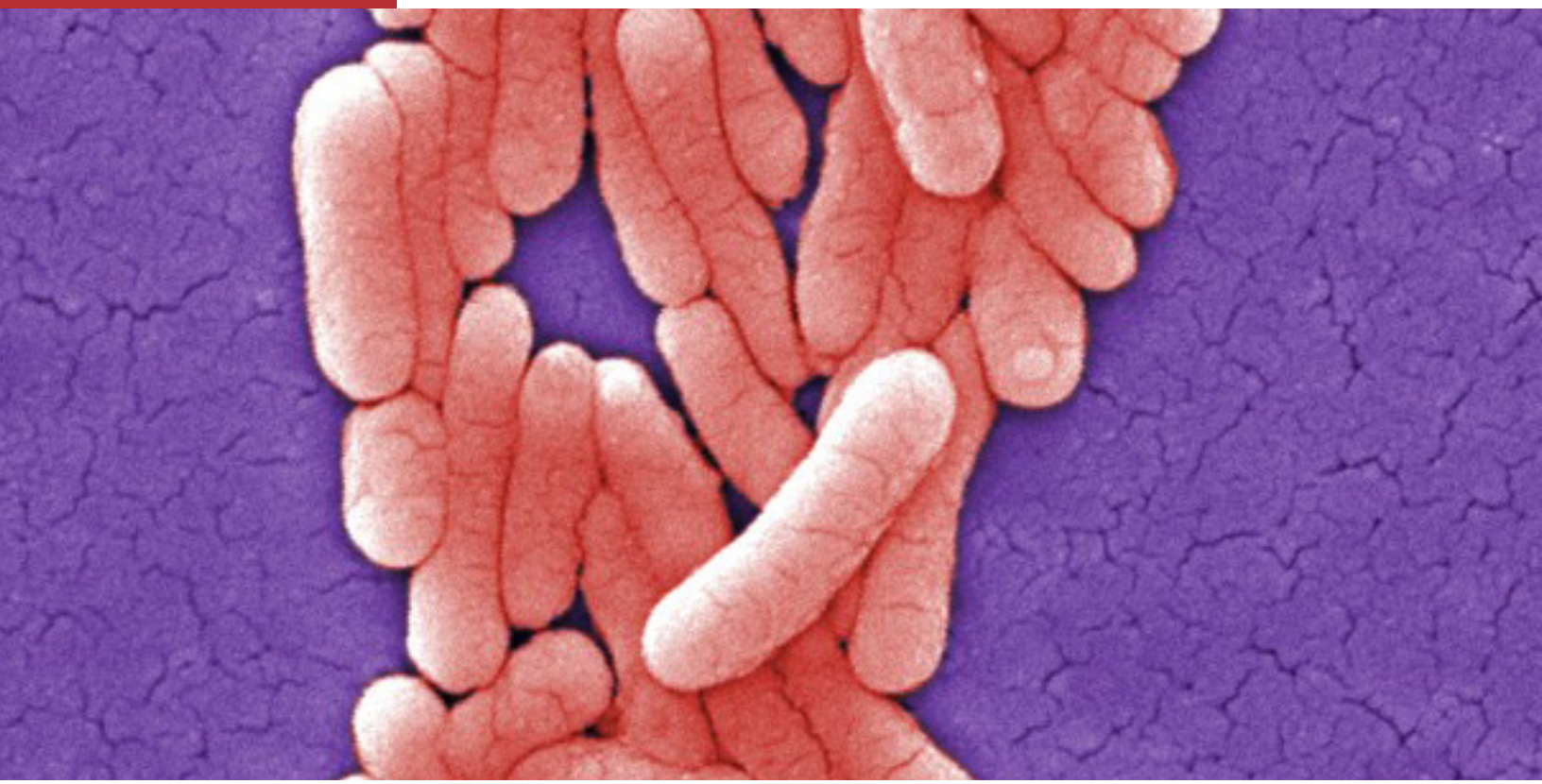
Isolation and Identification of *Salmonella* Species in Public Health Laboratories



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APHL ASSOCIATION OF PUBLIC HEALTH LABORATORIES®

Contents

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 temperature[7], 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 possible[1].

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), Gelatin, Galacturonate, 4-Methylumbelliferyl-β-D-glucorinide (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 loop
- 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.
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 TET[1]. 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.
   - 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. 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. After *Salmonella* is identified, immediately perform whole genome sequencing to identify the serotype and subtype the isolate. 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 reaction of *Salmonella* spp.). The following biochemicals can be used for additional subspeciation of *Salmonella* spp.: Malonate, Lactose, Dulcitol, Salicin, Sorbitol, Mucate, Tartrate, ONPG, Gelatin, 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.

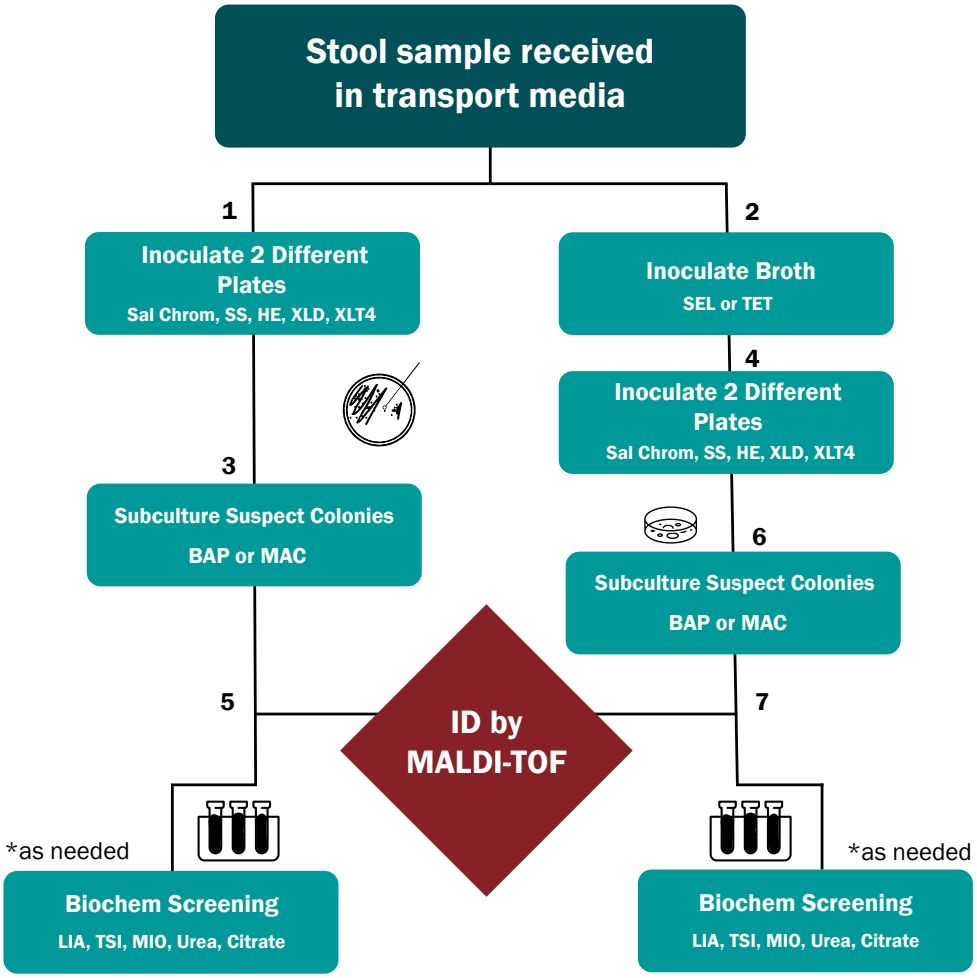
References

1. *Manual of Clinical Microbiology*, 13th edition, 38: 746-759. Washington, DC: ASM Press. Dekker J, Faron M, Humphries R, Buchan B, Ledeboer N. 2023.
2. Development of an Enteric Bacterial Enrichment Broth and Its Performance for Isolation of Clinically Significant Bacterial Pathogens from Stool. Open Journal of Medical Microbiology, 11, 1–17. Horseman T, Lustik M, Fong K. 2021.
3. Evaluation of Statens Serum Institute enteric medium for detection of enteric pathogens. Blom M, Meyer A, Gerner-Smidt P, Gaarslev K, Espersen F. J Clin Microbiol. 1999 Jul;37(7):2312-6.
4. Performance of the chromID Salmonella Elite chromogenic agar in comparison with CHROMagar™ Salmonella, Oxoid™ Brilliance™ Salmonella and Hektoen agars for the isolation of *Salmonella* from stool specimens. Martiny D, Dediste A, Anglade C, Vlaes L, Moens C, Mohamed S, Vandenberg O. Diagn Microbiol Infect Dis. 2016 Oct;86(2):128-30.
5. An evaluation of the performance of XLD, DCA, MLCB, and ABC agars as direct plating media for the isolation of *Salmonella enterica* from faeces. Nye K, Fallon D, Frodsham D, Gee B, Graham C, Howe S, Messer S, Turner T, Warren R. J Clin Pathol. 2002 Apr;55(4):286-8.
6. Comparison of Xylose Lysine Deoxycholate Agar, Hektoen Enteric Agar, Salmonella-Shigella Agar, and Eosin Methylene Blue Agar with Stool Specimens. Appl Microbiol. 1969 Sep;18(3):393-5.
7. Submission of Enteric Pathogens from Positive Culture-Independent Diagnostic Test Specimens to Public Health, Association of Public Health Laboratories Interim Guidelines. 2016.
8. Antigenic Formulae of the *Salmonella* Serovars 9th ed. (WHO Collaborating Centre for Reference and Research on *Salmonella*, 2008). Grimont P, Weill F.

Appendix A

Flow Diagram for Isolation and Identification of *Salmonella*

Salmonella Workflow



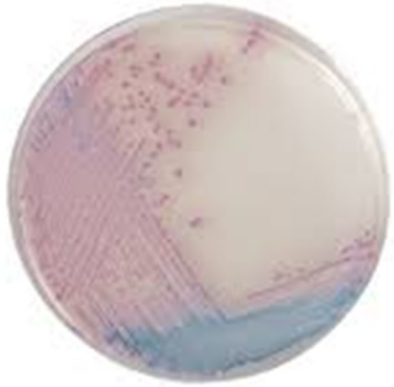
Appendix B

Morphology Description of *Salmonella* Species on Selective Media

Media	*Salmonella* spp.
Salmonella Shigella (SS)	Colorless colonies with black centers
Hektoen Enteric (HE) Agar	Clear greenish blue colonies with black centers
Xylose Lusine Deoxychocolate (XLD) Agar	Colonies range in colors from clear to pink/red with black centers
Salmonella CHROM agar	Mauve colonies
XLT-4 Agar	Black or red colonies with a black center

Appendix C

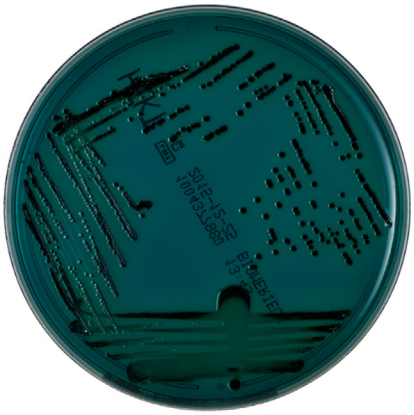
Morphology of *Salmonella* on Selective and Differential Media



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 agar

Appendix D

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

Biochemical	*Salmonella* (majority)	*S.* Typhi	*S.* Paratyphi A
TSI (slant)	K	K	K
TSI (butt)	A	A	A
TSI (H2S)	+	Trace amount	-
TSI (gas)	+	No gas	+
LIA	+	+	-
MIO (Motility)	+	+	+
MIO (Ornithine)	+	+	+
MIO (Indole)	-	-	-
Urea	-	-	-

Appendix E[8]

Typical Results of Biochemicals for *Salmonella* Species and Subspecies

	Salmonella enterica						*Salmonella bongori* (formerly ssp. V)
Substrate	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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