Standard Operating Procedure for Identification of Salmonella by Biochemical testing

Purpose

To definitively identify *Salmonella* by biochemical testing of the isolated colonies in Laboratory

A. INTRODUCTION

Complete identification of *Salmonella* species requires both:

1. Biochemical characterization and,
2. Serological characterization

Before reporting an isolate as "*Salmonella* species", ensure that the isolate is identified as *Salmonella* species by tests / biochemicals (e.g., API 20E) **AND** serology.

This document contains instructions and various flowcharts outlining the minimal characteristics required to definitively identify *Salmonella* species - biochemically. For details around serological testing, refer to Procedure: Salmonella Serology in section B.

Salmonella taxonomy is described in the SUPPLEMENTARY INFORMATION section.

B. IDENTIFICATION

1. Tests

Refer to procedures in Section B for details regarding the following test procedures. Additional biochemical reactions are displayed in Procedure: "Shigella & Salmonella ID – TABLE 1" in section C.

b) **API 20E:** API 20E is used to finalize the identification of a salmonella-like organism based upon preliminary identification or screening tests. Refer to Microbiology Procedure: API 20E for instructions on how to perform this test.

c) **Colonial characteristics**: On blood agar: *Salmonellae* are grey/white, non-hemolytic, non-swarming colonies that range from 2 to 3 mm in diameter after 24 hours of incubation. On MacConkey: colourless colonies between 2 to 3 mm in diameter after 24 hours of incubation. On XLD: red-pink colonies from 2-3 mm in diameter at 24 hours; usually with

black centres. *Salmonella* that do not produce H_2S (e.g., most strains of S. paratyphi A) form red-pink colonies with no blackening.

e) **PYR**: Refer to Microbiology Procedure: "PYR" for instructions on how to perform the motility test.

- Use only fresh overnight growth **ONLY FROM NON-SELECTIVE MEDIA** (i.e., blood and chocolate plates). Do not test inoculum growing on MacConkey and XLD agars.

PYR-negative organisms require further confirmation as, in addition to *Salmonella*, organisms such as *Proteus*, *Hafnia* and *Morganella* have been found to be PYR-negative (Bennet).

f) **Serology:** Refer to Microbiology Procedure: "Salmonella Serology" for instructions on how to perform this testing. Serological characterization of *Salmonella* species is based upon the detection of 3 antigenic components:

i) Somatic or "O" antigens [heat stable polysaccharide on cell wall]
ii) Flagellar or "H" antigens [heat labile protein on flagella]
iii) Capsular or "Vi" antigen [capsular protein only found only in certain strains]

f) **TSI:** Refer to Microbiology Procedure: "Triple Sugar Iron (TSI)" for instructions on how to perform this test.

- Make one stab down the center of the medium to within 2 mm of the bottom; then streak the surface of the slant in a "zig zag" fashion.
- Read reactions no earlier or later than 18 to 24 hours.

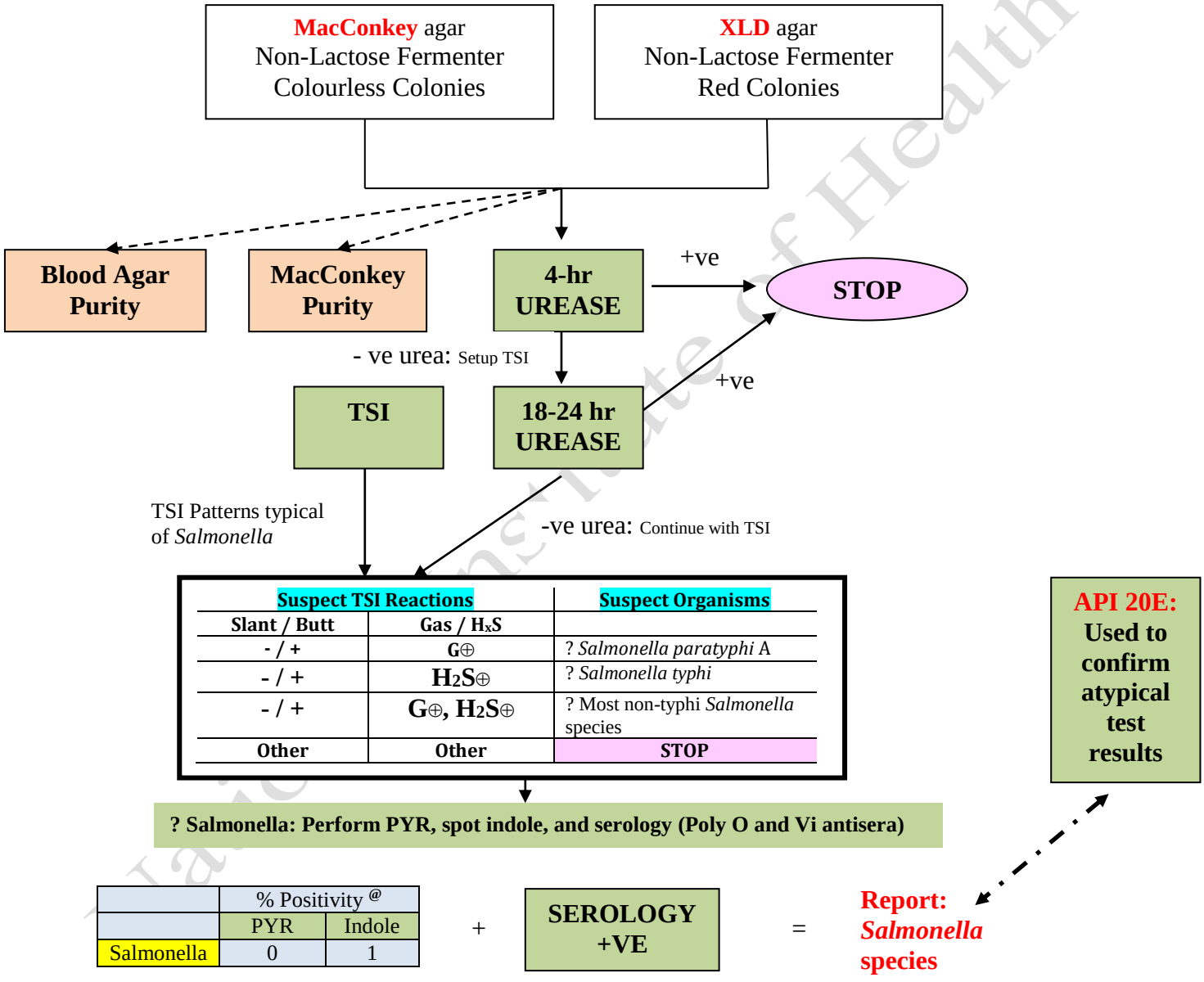
f) **Urease:** Refer to Microbiology Procedure: "Urease" for instructions on how to perform this test. Inoculate heavily the surface of the slant with growth from an 18 to 24-hour pure culture; streak in a "zig-zag" manner. Do not stab the butt.

B. IDENTIFICATION (continued)

2. Algorithms / Keys:

The following keys provide support for the definitive identification of *Salmonella*.

Figure 1. Use of screen tests to identify Salmonella-like organisms; leading to definitive identification.



@Each number gives the % positivity after 2 days of incubation at 36ºC (Manual of Clinical Microbiology, 8th Edition)

B. IDENTIFICATION(continued)

2. Algorithms / Keys:

Figure 1. Screen tests and expected results for Salmonella-like organisms (Taken from: **Cheesbrough, page 7.11**)

				KIA Medium Reactions			
	Motility	Indole	LDC	Slope	Butt	Black (H_2S)	Cracks (Gas)
SHIGELLAE							
Shigella dysenteriae	–	d	–	R	Y	–	–
Shigella flexneri	–	d	–	R	Y	–	–[1]
Shigella boydii	–	d	–	R	Y	–	–[2]
Shigella sonnei	–	–	–	R	Y	–	–
SALMONELLAE							
Salmonella Paratyphi A	+	–	–	R	Y	–[3]	+
Salmonella Paratyphi B	+	–	+	R	Y	+	+
Salmonella Paratyphi C	+	–	+	R	Y	+[4]	+
Salmonella Typhi	+	–	+	R	Y	+ Weak	–
Other *Salmonella* serovars	+[5]	–	+	R	Y	+[6]	d

Key: KIA = Kligler iron agar, LDC = Lysine decarboxylase, d = different strains give different results, R = Red-pink (alkaline reaction), Y = Yellow (acid reaction).

Notes
1 Some strains of serotype 6 produce gas. 2 Serotypes 13 and 14 produce gas. 3 About 12% of strains produce H_2S weakly. 4 A minority of strains do not produce H_2S. 5 *Salmonella* Pullorum and *Salmonella* Gallinarum are non-motile. 6 A minority of strains do not produce H_2S.

The reactions of salmonellae and shigellae compared with other enterobacteria are summarized in Chart 7.10 in subunit 7.18.15.

B. IDENTIFICATION(continued)

2. Algorithms / Keys:

Figure 1. Use of screen tests to identify Salmonella-like organisms
(Taken from: Clinical Microbiology Procedures Handbook, 3rd Edition)

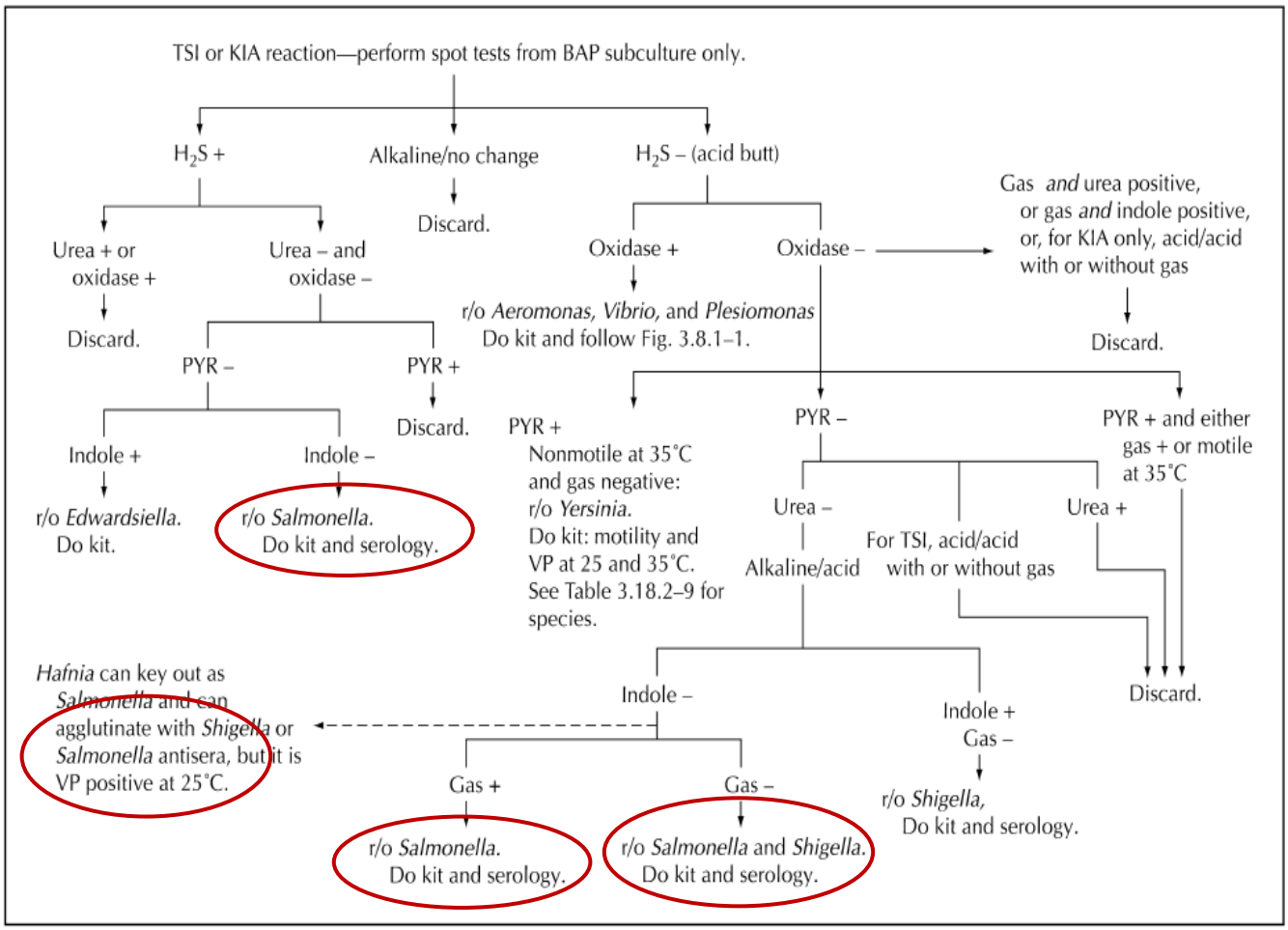
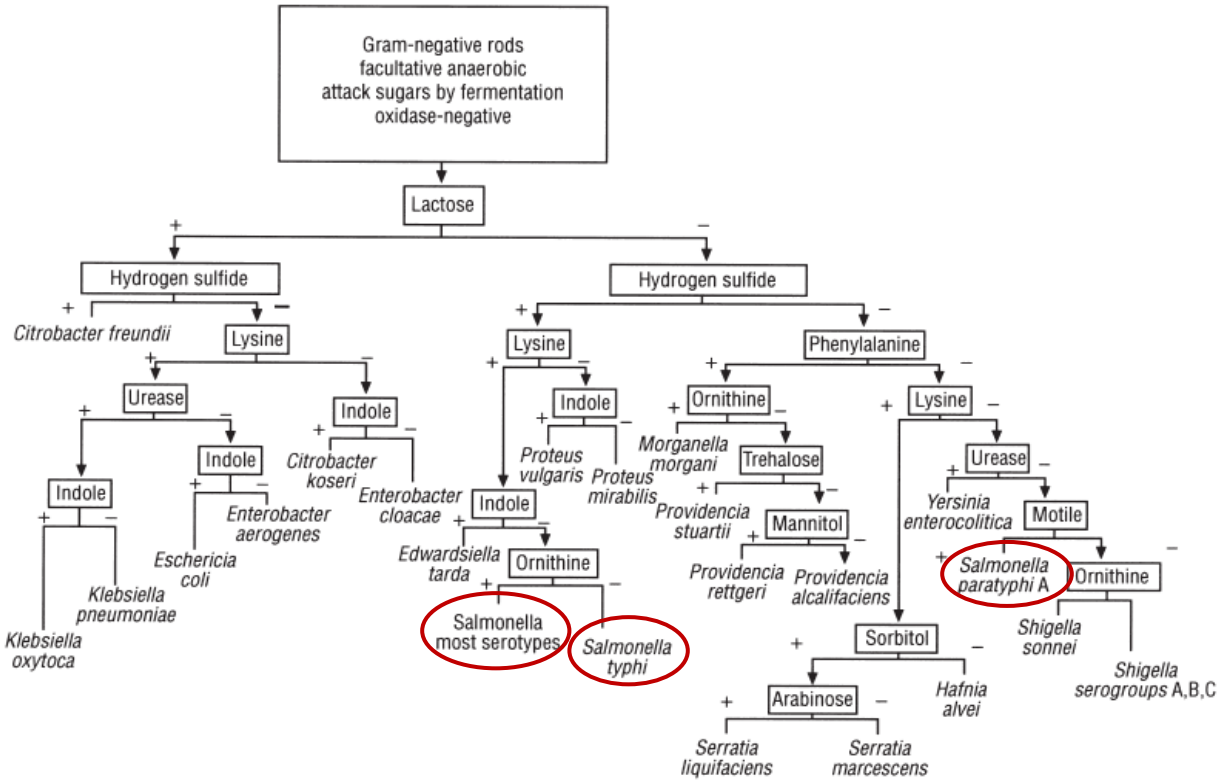


Figure 3.8.1–2 Flowchart for identification of stool pathogens from routine stool cultures. Set up either TSI or KIA, BAP, and urea agar (or rapid urea tube) from all lactose-negative or H_2S-positive colonies on enteric selective agars. Reactions of the slant are listed with a slash before the butt reaction. Optionally for H_2S-negative colonies, Andrade's glucose tube with Durham tube for gas will eliminate most questionable production of gas and provide a broth for VP testing. *Perform spot tests (indole, oxidase, PYR) only from BAP.* r/o, rule out.

B. IDENTIFICATION (continued)

2. Algorithms / Keys:

Figure 2. Differentiation of *Salmonella* from common members of the *Enterobacteriaceae* family.



Taken from: WHO, Basic Laboratory Procedures in Clinical Bacteriology, 2nd Edition
http://www.who.int/medical_devices/publications/basic_lab_procedures_clinical_bact/en/

C. SUPPLEMENTARY INFORMATION

Salmonella nomenclature varies throughout the world. However, uniformity in *Salmonella* nomenclature is necessary for communication between scientists, health officials, and the public. Unfortunately, current usage often combines several nomenclatural systems that inconsistently divide the genus into species, subspecies, subgenera, groups, subgroups, and serotypes (serovars), and this causes confusion.

The Kauffmann-White Scheme for designation of *Salmonella* serotypes is maintained by the WHO Collaborating Centre for Reference and Research on *Salmonella* at the Institute Pasteur and is used by most of the world (JCM, Brenner). In this scheme, the **genus *Salmonella*** is divided into two species, *Salmonella enterica* and *Salmonella bongori.*

Salmonella enterica is further subdivided into 6 subspecies that are designated by names or Roman numerals. The Roman numerals are simpler and more commonly used. Subspecies IIIa and IIIb were historically considered a separate genus, ***Arizonae***, and are still sometimes referred to by this name.

***Salmonella enterica* subspecies**		
I	*enterica*	Warm-blooded animals
II	*salamae*	Cold-blooded animals and the environment
IIIa	*arizonae*	Cold-blooded animals and the environment
IIIb	*diarizonae*	Cold-blooded animals and the environment
IV	*houtenae*	Cold-blooded animals and the environment
VI	*indica*	Cold-blooded animals and the environment

http://jcm.asm.org/cgi/content/full/38/7/2465

Salmonella bongori was originally designated *S. enterica* **subspecies V**. It has since been determined to be a separate species of *Salmonella*. However, for simplicity and convenience, these strains are commonly referred to as "subspecies V" for the purpose of serotype designation.

There are over 4400 serovars of Salmonella. The vast majority of human isolates (>99.5%) are subspecies *S. enterica.* For the sake of simplicity, the CDC recommends that *Salmonella* species be referred to only by their genus and serovar, e.g., *Salmonella* Typhi instead of the more technically correct designation, *Salmonella enterica* subspecies *enterica* serovar Typhi. Refer to the 8th Edition of the Manual of Clinical Microbiology for a full discussion of taxonomic nomenclature.

References

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