

CHAIN IDENTIFICATION OF *E coli*, *Salmonella* and *Shigella* ISOLATES FROM STOOL SAMPLES



(MASTER)

CHN56: IDENTIFICATION OF *E coli*, *Salmonella* and *Shigella* ISOLATES FROM STOOL SAMPLES

1.0 PURPOSE / INTRODUCTION:

1.1 Introduction:

Gastroenteritis is one of the most common diseases in humans with *Salmonella* and *Shigella* species being among the etiological agents. For diagnosis of these infections, stool samples are cultured on selective and differential media to aid isolation and identification of the causative agents.

1.2 Purpose:

To give guidance on correct identification of *Salmonella*, *Shigella* and *E. coli* isolates from stool/rectal swab samples. For diagnosis of these infections, stool samples are cultured on selective and differential media to aid isolation and identification of these enteric bacteria.

2.0 SCOPE / RESPONSIBILITY:

This SOP is applicable to all lab staff working in the CHAIN Study microbiology sections.

The Principal Investigator (through the study coordinator when applicable) retains the overall responsibility of implementation of these standard procedures.

The Study Laboratory Coordinator is responsible for answering questions you may have about the content of this SOP and any other relevant study documentation. Please contact that the Study Laboratory Coordinator through your site coordinator.

3.0 SAFETY/RISK ASSESSMENT:

Standard safety measures are paramount including wearing of appropriate personal protective gears

3.1. Always wear personal protective equipment (PPE) such as gloves, lab coats when handling samples (rectal or stool swabs) and treat all samples as potential hazards.

3.2. All samples should be handled under a BSC-2 cabinet.

3.3. All used materials e.g. gloves and waste papers should be disposed off in the right biohazard bin for future incineration.

4.0 DEFINITIONS:

4.1 O₂ - Oxygen/Aerobiasis.

4.2 °C - Degrees Celsius.

4.3 MAC -MacConkey agar.

4.4 XLD - Xylose Lysine Deoxycholate agar.

4.5 SS - Salmonella/Shigella agar.

4.6 SF - Selenite-F enrichment broth.

4.7 TSI - Triple Sugar Iron.

4.8 MIO - Motility-Indole-Ornithine decarboxylase.

4.9 SSP - Species.

4.8 TSB Tryptone soy broth.

4.9 **API 20E** Analytical profile index 20 Enterobacteraciae tests.

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5.0 SPECIMEN:

Growth of either lactose or non-lactose fermenting colonies obtained on either primary culture plates (MAC, XLD and SS) or SF subculture plates (XLD and SS).

6.0 EQUIPMENT / MATERIALS/ REAGENTS:

6.1 Equipments:

6.1.1 O₂ incubator.

6.2 Materials:

6.2.1 Four tube biochemical test media (TSI, MIO, Simmons Citrate and Urea).

6.2.2 Nichrome wire loop.

6.2.3 Bunsen burner.

6.2.4 Glass slides.

6.2.5 Normal saline/deionized water.

6.3 Reagents:

6.3.1 *Salmonella* antisera:

6.3.1.1 *Salmonella* Poly O (Group A-G).

6.3.1.2 *Salmonella* Poly-H phase 2.

6.3.1.3 *Salmonella* Vi (for serological identification of *Salmonella typhi*).

6.3.1.4 *Salmonella* 9-O (for serological identification of *Salmonella typhi*).

6.3.1.5 *Salmonella* d-H (for serological identification of *Salmonella typhi*).

6.3.2 *Shigella* antisera:

6.3.2.1 *S. sonnei* Phase I & II.

6.3.2.2 *S. boydii* 1(1-6), 2(7-11) 3(12-15).

6.3.2.3 *S. dysenteriae* polyvalent (1-10).

6.3.2.4 *S. flexneri* (1-6, X & Y).

7.0 METHODOLOGY:

PROCEDURE

7.1. Remove the MAC, XLD and SS media plates from the fridge and place them on the working bench to obtain room temperature.

7.2. Label the plates with the lab number.

7.3. Plating procedures

7.3.1. Pick rectal/stool swab using forceps, streak it directly onto MAC, XLD and SS media plates. Incubate the plates at 37°C for 18-24 hours.

7.3.2. Put rectal/stool swab on SF enrichment broth and incubate at 37°C for 18-24 hours.

7.3.3. After 18-24 hours incubation, streak the SF sub-cultured stool/rectal swab on MAC and XLD and SS.

7.3.4. Remove the direct media plates and subculture plates from the incubator after 18-24 hours and examine morphological characteristics of growth obtained.

8.1 Macroscopic examination:

8.1.1 Examine either the primary or SF subculture plates for typical lactose and non- lactose fermenting colonies. Such colonies are identified by morphology;

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- MAC - *Salmonella* spp. (colourless, 2-3 mm diameter with entire edges).
- *Shigella* spp. (colourless, ~ 3 mm diameter with serrated edges).
- *E. coli* spp. (pink, 2-3 mm diameter with entire edges).
- XLD - *Salmonella* spp. (Colourless edges with or without black centre).
- *Shigella* spp. (Red, 2-3mm diameter).
- *E. coli* spp. (yellow, 2-3mm in diameter with entire edges).
- SS - *Salmonella* spp. (Colourless, 2-3mm diameter with entire edges with or without black centres).
- *Shigella* spp. (opaque + translucent & colourless).
- *E. coli* spp. (yellow, 2-3mm in diameter with entire edges).

8.2 Biochemical testing:

8.2.1 Using a pre-flamed straight wire, pick a suspected colony and inoculate TSI agar slant, MIO medium, Simmons Citrate agar, and Urea agar slope as follows:

8.2.2 Inoculate the TSI agar by stabbing the bottom of the tube with a single down and up motion. After stabbing, immediately streak the slant portion of the agar and loosely cap the tube.

8.2.3 Inoculate the MIO tube by stabbing in a single down and up motion in the centre of the agar going three-fourths of the way down the tube, keeping the wire as vertical as possible and loosely cap the tube.

8.2.4 Inoculate Simmons citrate tube by stabbing the bottom of the tube with a single down and up motion. After stabbing, immediately streak the slant portion of the agar and loosely cap the tube.

8.2.5 Inoculate urea agar tube by stabbing 2-3 times into the agar and loosely cap the tube.

8.2.6 Place the tubes in a rack and incubate at 37°C overnight in the O₂ incubator. After overnight incubation, examine the tubes for typical biochemical reactions. If the reactions suggests of either species (*Salmonella* or *Shigella*), perform slide agglutination tests with the respective antisera.

8.2.7 If the reaction suggests *E. coli* species, purity plate on MacConkey agar for stocking in TSB and record the results.

NB: API 20E biochemical test strip can also be used for confirmation of the isolates which are having inconclusive results by using the bio-typing method

8.3 Slide agglutination test:

8.3.1 On a glass microscope slide place a drop of saline.

8.3.2 Using a pre-flamed wire loop, pick the respective test organism from the TSI agar slant.

8.3.3 Emulsify the test organism in the saline on the slide and place a drop of the respective antiserum.

8.3.4 Rotate the slide for 30 seconds and record the presence of visible agglutination.

N/B:

The presence of agglutination with specific anti-sera denotes a positive reaction. No agglutination should be observed in the saline portion.

8.3.5 Perform antibiotic susceptibility testing for the identified isolate (Ref. SOP No :).

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8.3.6 Report the results on the study request form and log into the study site computer Data base.

8.3.7 Freeze the isolate in Tryptone Soy broth with 15% glycerol at -70°C freezer

9.0 APPENDICES:

9.1 Biochemical reactions of the genus *E coli*, *Salmonella* and *Shigella*:

SPECIES	TSI			GAS	MIO			CIT	UREA
	SLOP E	BUTT	H2S		MOT	IND	ORN		
<i>Salmonella typhi</i>	R	Y	+/ weak	-	+	-	+	-	-
<i>Salmonella paratyphi A</i>	R	Y	-	+	+	-	+	-	-
Other <i>Salmonella</i>	R	Y	+1	-d	+	-	+	d	-
<i>Shigella</i> spp	R	Y	-	-2	-	d	-	-	-
<i>E coli</i>	y	y	-	+	+	+	+	-	-

Key: R=Red-pink (Alkaline reaction), Y=Yellow (Acid reaction), H2S= Hydrogen sulphide (blackening), Mot=Motility, Ind=Indole, Orn=Ornithine decarboxylation, Cit=Citrate, d=different strains give different results.

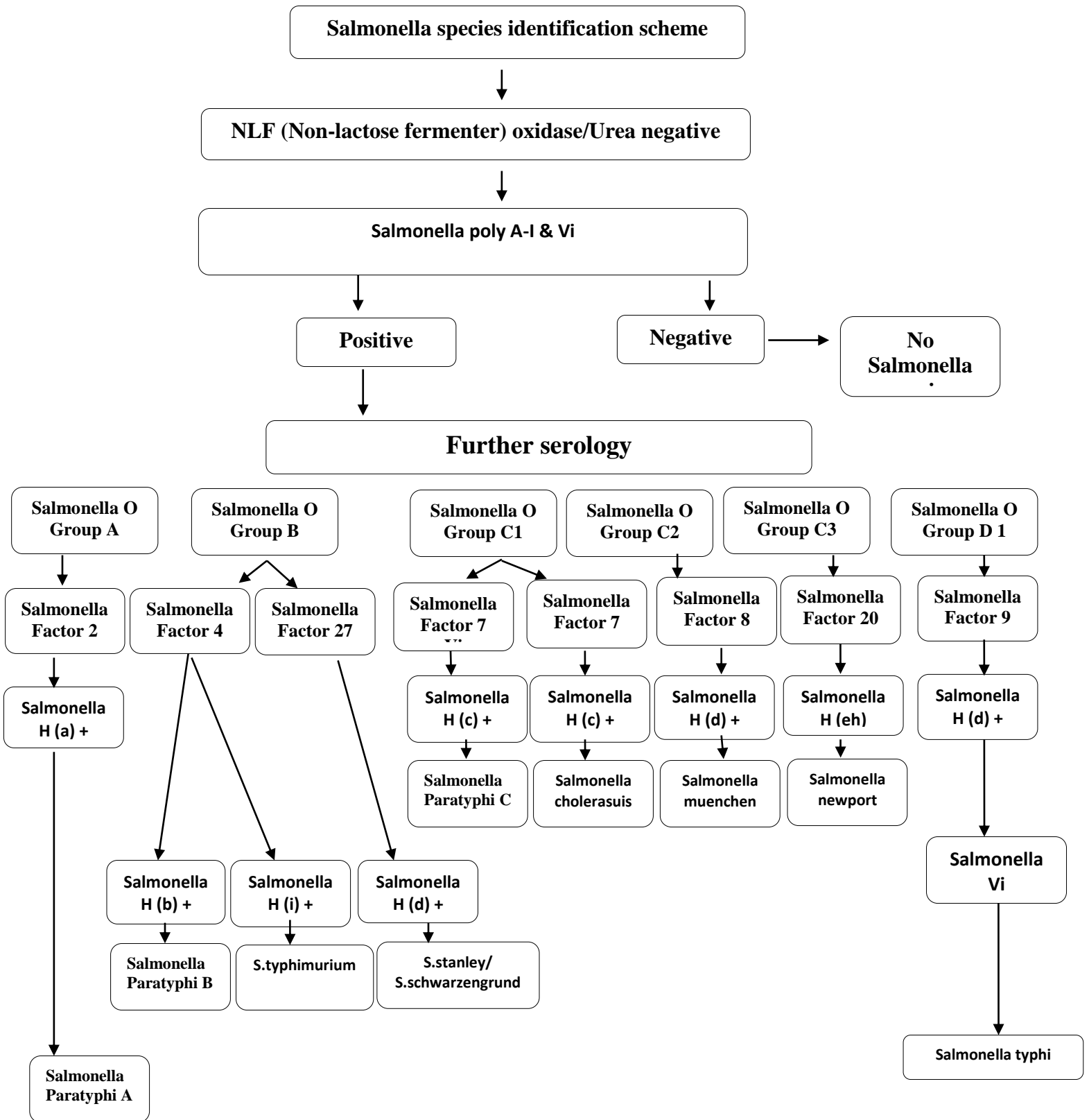
Notes:

1. A minority of strains give a negative results.
2. A minority of strains give a positive result.

9.3 *Salmonella* serotyping scheme.

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10.0 REFERENCES:

- 10.1 **De Mol, P. et al.** Enteropathogenic agents in children with diarrhoea in rural Zaire. Lancet, 1, pp 516-518, 1983.
- 10.2 **Mackie and MacCartney.** Practical medical microbiology, thirteenth edition.
- 10.3 **Edwards, P. R., and Ewing, W. H.** Identification of Enterobacteriaceae, 3rd Edition. Burgess Publishing Company, Minneapolis, 1972.
- 10.4 **Monica Cheesbrough.** Medical Laboratory Manual for Tropical Countries, Volume 11, 1984.

Document history

Version	Author	Approved by	Dated
1.01 (MASTER) IDENTIFICATION OF E coli, Salmonella and Shigella ISOLATES FROM STOOL SAMPLES .CHN	Edwin Machanja	Caroline Tigoi	10/10/2016
1.02			

Site training record

All sites are required to maintain a master copy of this SOP that documents the site staff that have been trained on this SOP.

Document History				
Version No.	Trained staff initials	Signature of trained staff	Date	Trainer's Initials
1.01	KDT	Example row	1 st Jan 2016	DM