Ministry of Health

Document Title	Stool culture SOP
Document Type	Procedure
Directorate/Institution	Directorate General of Specialized Medical Care
Targeted Group	Medical laboratories
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Designation	Microbiology documents development team
Release Date	May 2023
Review Frequency	Three Years

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Acknowledgment

The diagnostic laboratories services at the Directorate General of Specialized Medical Care (DGSMC) at Ministry of Health (MOH) would like to thank and appreciate the great effort of the Microbiology documents development team. Participated and contributed personnel are:

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Acronyms:

BA	Blood agar
MAC	Mackonky plate
SMAC	Sorbitol mackonky plate
TCBS	Thiosulphate Citrate Bile Salt Sucrose Agar
XLD	Xylose Lysine Deoxycholate agar
APB	Alkaline Phosphate Broth
H&S	Health and Safety
ID	Identification
IQC	Internal Quality Control
SOP	Standard operating procedure
NICU	Neonatal intensive care unit
KIA	Kliger's Iron Agar

1. Purpose

This document describes the procedure for stool sample culture.

2. Scope

This document is applicable for all medical laboratories under MOH and other collaborative governmental and non-governmental health institutions.

3. Definitions

3.1 Diarrhea: defined as stools of a loose or liquid consistency, occurring more frequently than is normal for the individual and sually this is at least three or more instances in a 24-hour period.

4. Procedure

4.1. Clinical background:

Acute infectious diarrhea may be caused by bacteria, viruses or protozoa. The laboratory will routinely look for the most common causes of bacterial diarrhea. This includes *Campylobacter, Salmonella, Shigella, E.coli* O157:H7, and *Yersinia.* Less common causes such as *Aeromonas, Plesiomonas,* and *Vibrio* will be cultured only when clinically indicated or specifically requested.

There are three types of diarrhea:

1. Acute watery diarrhea: This is defined as diarrhoea not exceeding 14 days of duration and typically with frequency of 3 or more episodes a day. Organisms implicated include norovirus, rotavirus, adenovirus, Salmonella species, Campylobacter species and C. difficile. Other organisms that may be considered include the Vibrio species including Vibrio cholerae (the causative agent of cholera).
2. Acute bloody diarrhoea: This is a sudden onset of diarrhoea where frank blood is present. Dysentery is an acute infectious gastroenteritis characterized by loose stools with blood and mucus. Organisms implicated in acute bloody diarrhoea include Campylobacter species, Shigella species, Shiga toxin producing Escherichia coli (STEC) including serogroup O157, Salmonella species, Entamoeba histolytica.
3. Persistent and chronic diarrhoea: persistent diarrhoea is defined as diarrhoea of >14 days but fewer than 30 days in duration. It should be noted that viruses (eg norovirus) and bacteria (Salmonella, Shigella and Campylobacter species) can be the cause of

persistent diarrhoea in patients who are immunocompromised. Diarrhoea that lasts >30 days is referred to as “chronic”. Organisms implicated are predominantly parasites – Giardia species, Cryptosporidium species, Cyclospora cayetanensis and Microsporidia species. Chlamydia trachomatis infection including lymphogranuloma venereum (LGV) and Neisseria gonorrhoeae infection can present atypically as sexually transmitted infectious colitis in individuals who have unprotected anorectal sex.

4.2.Pre – analytical stage:

4.2.1. Sample:

- A single stool specimen for routine culture for enteric pathogens should be collected into a leak-proof container and transported to the laboratory as soon as possible after collection, Specimens should be processed upon arrival to the laboratory immediately, because the number of important pathogens such as *Shigella* species may not survive the pH changes that occur in feces specimens which are not promptly delivered to the laboratory even if refrigerated.
- Samples from hospitalized patients > 72 hours are not accepted (three day rule) and reported as hospital acquired diarrhea and released with comment: Diarrheal sample specimens from patients who have been admitted for more than 3 days are not routinely processed for stool culture, if clinically indicated please call microbiologist.

4.2.2. Material:

Reagents	**Supplies**	**Equipment**
Selenite F MAC, BA, XLD, Sorbitol MAC, CAMPY, TCBS 70% alcohol reagent. KIA TSI slants Distilled water. 6.5% Normal saline. Campy gas kit generator salmonella, sheigella, E.coli antisera	Disposable loops. wooden sticks, Racks. Plastic streaking loops. Universal plastic containers. Waste disposable, Sterility disposable bags.	43°C incubator O_2 incubator Fume hood Jars

- All the antisera has to be kept in fridge (2-8 C).

4.2.3. Safety precaution:

- All specimens need to be treated as potentially infectious. Standard procedures for handling of biohazard material must be followed at all times. Universal Precautions must be practiced at all stages of these procedures.
- Specimen processing in general requires containment level 2 for routine work. Containment level 3 if the following organisms are suspected from clinical information or laboratory findings: *Salmonella Typhi, Salmonella Paratyphi A, B, C, E.Coli 0157:H7, Shigella dysenteriae, Toxigenic V.cholerae.*

4.2.4. Quality control:

- Check the expiry dates of all media, reagents and stains before use.

- All media, reagents, kits, and stains MUST be quality controlled before use.
- Identification tests should be run with appropriate controls.
- Record the quality control results in the appropriate QC sheet.
- Monitor the 43 ° incubator temperature daily.

4.3.Analytical stage:

4.3.1 Direct exam: refer to stool microscopy SOP.

4.3.2 Culture set up:

1. Take the received fecal sample to the fume hood.
2. Prepare the appropriate plates according to the following table:

Media	Incubation
MacConkey Agar (MAC)	O_2, 35^{o}C x 18 – 24 hours
XLD	O_2, 35^{o}C x 18 - 24 hours
MacConkey Sorbitol Agar (SMAC)	O_2, 35^{o}C x 18 - 24 hours
Campylobacter Agar (CAMPY)	Campy Jar, 42^{o}C x 48 hours
Selenite F Broth (SEL)	O_2, 35^{o}C x 12-18 hours

- If *Yersinia* is requested or patient is >1 month–12 years old (except for NICU) OR If history of mesenteric adenitis, terminal ileitis add: Yersinia agar (if available) O_2,30^{o}C x 48 hours OR incubate MAC for 48hrs at room temperature
- If *Vibrio* is requested, or "if there is a history of shellfish ingestion or if there is a history of travel outside to areas where cholera/Vibrio associated diarrhoea is endemic", add: TCBS O_2, 35^{o}C x 18 - 24 hours APB O_2, 35^{o}C x 5 - 8 hours
- If *Plesiomonas* or *Aeromonas* is requested , or "if there is a history of sea food ingestion or if there is a history of travel outside to areas add: Blood Agar (BA) O_2, 35^{o}C x 24 hours

4.3.3 Isolation and Identification:

1. After 24 hrs of incubation: Identify any growth of *Salmonella*, *Shigella*, *E. coli* O157, or *Yersinia* by examining the MAC, XLD, and SMAC at 24 hr.
2. After overnight incubation, subculture Selenite broth onto XLD which is incubated in O_2 at 35°C x 24 hr. Workup as indicated above.
3. Examine MAC again at 48hr for *(*if *Yersinia* was requested or indicated by age group (>1 month–12 years old)
4. Examine Campy plates at 48 hr and identify any growth of *Campylobacter*.
5. See Identification schemes /flow charts for details. These, however, are meant as guides only.
6. Identify other pathogens only if specifically requested.

4.3.4 Screening for Salmonella and Shigella:

1. Examine MAC and DCA/XLD for suspected colonies:
2. Colourless or transparent NLF or very pale pink like LLF(*Shigella*) on MAC and oxidase negative .
    - Pink to red with or without black centres on XLD.
    - Colourless with or without black centres on DCA.
3. Pick one colony of each suspect morphotype and inoculate a KIA, peptone water. Incubate each according to protocol at 35ºC in O2 and add BA as purity plate.
4. Determine if the organism is a potential enteric pathogen according to results of Table 1.
5. Suggestive screening results require serologic confirmation and SET UP for available automated or manual identification system.
6. Set up of ID and serologic confirmation can be done directly from growth on the blood agar plate.
7. If biochemical tests indicate salmonella, send the isolate directly for susceptibility.

Table 1. Characteristic reactions of potential enteric pathogens and follow-up

KIA/gas/H_2S	**Urea**	**Indole**	**Motility**	**Follow-up**
K/A, + gas, + H_2S (degree of gas and H_2S may vary)[1''2]	-	-	+	Salmonella Anti-sera , API20E, AST and Send to PHL
K/A, no gas H_2S neg.[3]	-	- / +	-	Shigella Anti-sera , API20E, AST and Send to PHL
A/A, +/- gas	+/-	+	+/-	No further testing
K/K	+/-	Not applicable	+/-	No further testing
K/A or A/A No gas, no H_2S or "rainbow"	+	+	Positive at room temp. Negative at 35ºC	Pinpoint on MAC Yersinia API20E, AST and Send to PHL

Note: [1]*S. Typhi* may only produce small amounts of gas and/or H_2S.

[2]*S. Paratyphi* A may occasionally produce H_2S weakly.

[3]*Shigella Flexner* (type 6) may produce a small amount of gas.

Table 2. Characteristic reactions of potential enteric pathogens

Bacteria	Urea	Slant	Butt	H2s	Gas	Motility	Indole	Oxidase
E. coli	-	A	A	-	+	(+)	d	-
E. coli - Alk. Despair	-	K	A	-	-	-	+	-
Klebsiella	+	A	A	-	+	-	d	-
Enterobacter	-	A	A	-	+	+	-	-
Citrobacter	dw	d	A	d	+	+	-	-
Salmonella	-	K	A	dw	d	+	-	-
S. typhi	-	K	A	+	-	+	-	-
S. paratyphi A	-	K	A	-	+	+	-	-
S. arizonae	-	d	A	+	+	+	-	-
Shigelladysenteriae	-	K	A	-	-	-	d	-
S. flexneri	-	K	A	-	-*	-	d	-
S. boydii	-	K	A	-	-**	-	d	-
S. sonnei	-	K	A	-	-	-	-	-
Proteus vulgaris	+	K	A	+	d	+	+	-
P. marabilis	+	K	A	+	+	+	-	-
P. morganii	+	K	A	-	d	d	-	-
P. rettgeri	+	K	A	-	d	+	+	-
Providenciaalcalifaciens	-	K	A	-	d	+	+	-

P. stuartii	-	K	A	-	-	+	+	-
Yersinia enterolitica	+	K	A	-	-	V	d	
Aeromonas	-	K	A	-	+	+	+	+
Plesiomonas	-	K	A	-	-	+	+	+
Vibrio	-	K	A	-	-	+	+	+
Alkaligenes	-	K	N/K	-	-	+	-	+
Edwardsiellae	-	K	A	+	+	+	+	-
Hafnia	-	d	A	-	+	+	-	-

Symbols:
K = alkaline (red) reaction
A = acid (yellow) reaction
N = neutral reaction
+ = positive reaction
w = weak or delayed reaction
V = variable reaction (Y. enterolitica is motile at 25^{o} C but non-motile at 37^{o}C.
d = 10-85% positive
* Some S. flexneri serotype 6 gas (+)
** Serotype 13 and 14 gas (+).

4.3.5 Susceptibility testing:

After primary identification, follow the national antimicrobial susceptibility guidelines for all isolated enteropathogens.

4.4.Post – analytical stage:

4.4.1. Reporting :

- Negative Report: release the negative (non-significant) stool culture results as: *Salmonella, Shigella, Campylobacter* are NOT isolated. If specially requested for *Yersinia or E.coli O157,* include them pathogens to the comment.
- Positive Report: release the "Name of pathogen" ISOLATED.
- There is no need for quantification as any amount is significant.

- There is no need to comment on the absence of the other pathogens normally screened for.
- In case of any campy QC failure, release the stool culture report with the comment “"campylobacter was not tested, if patient is suspected to have campylobacter infection please submit another fresh stool sample".
- Follow the procedure for reporting of communicable enteric pathogens.
- Notify microbiologist/ senior of all *Shigella, E. coli O157, Salmonella Typhi or Paratyphi, or Vibrio cholera.*
- Refer to critical results notification S.O.P.

5. Responsibilities

5.1.Responsible staff:

- To ensure the adherence to critical result communication procedure
- To facilitate the alternative channels once needed

5.2.Quality manager /officer

- To follow up the implementation of the procedure
- To monitor regularly communication of critical results and raise non-conformance with corrective action once needed.

5.3.All lab staff:

- To adhere to the procedure.
- To document record and release results as recommended
- To report test failures or incident

6. Document History and Version Control

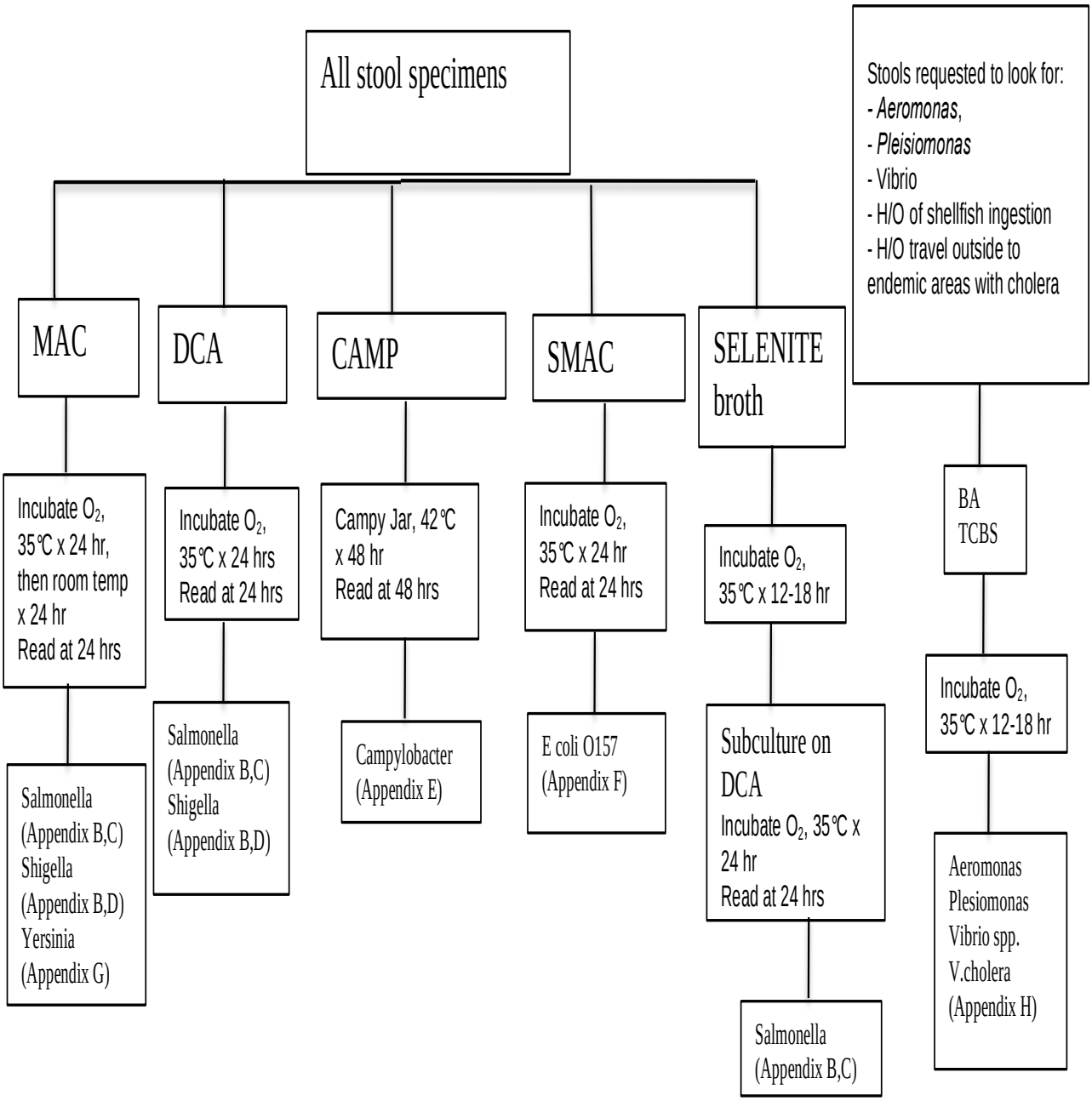
Version	Description	Review Date
1	Initial Release	May 2026

7. References

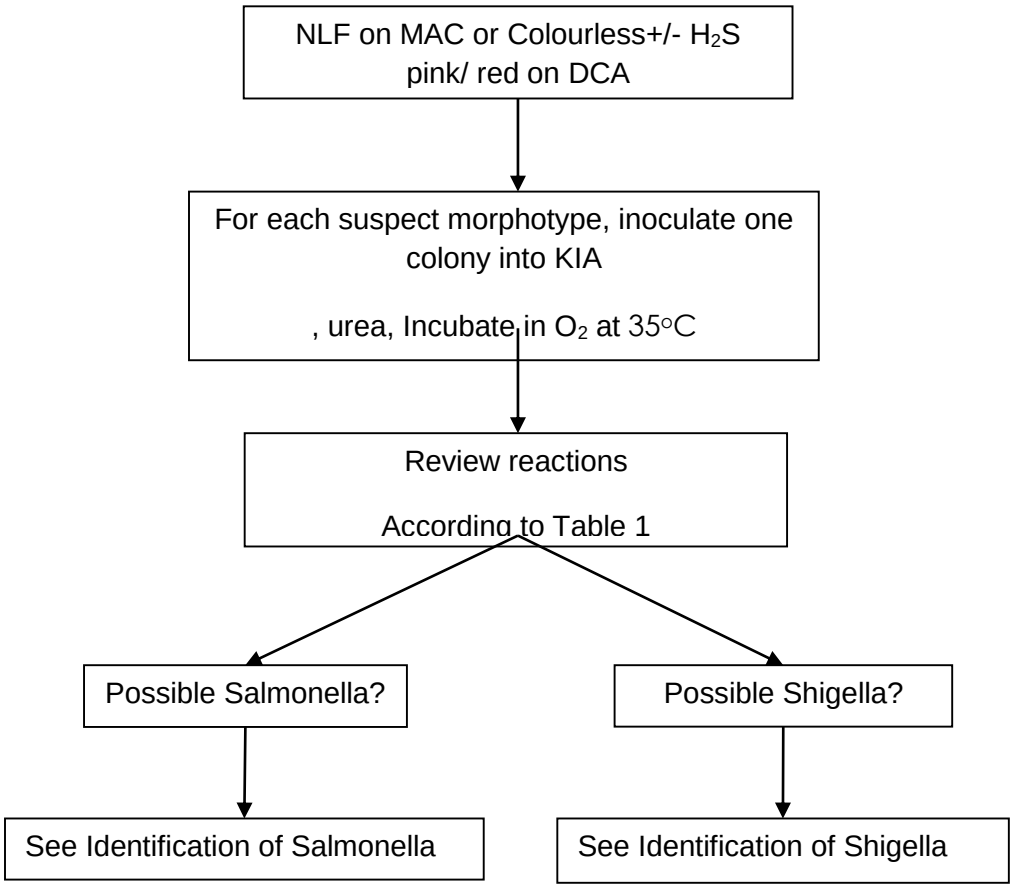
Title of book/ journal/ articles/ Website	Author	Year of publication	Page
1. Church et al. Am J ClinPathol Siegel 103		1995;	149-53.
2. Al. JAMA; 263		1990	979-82.
3. Brazier JS. JAC; 41, Suppl.		1998	C: 29-40.
4. UK SMI S 7		2020	

8. Annexes

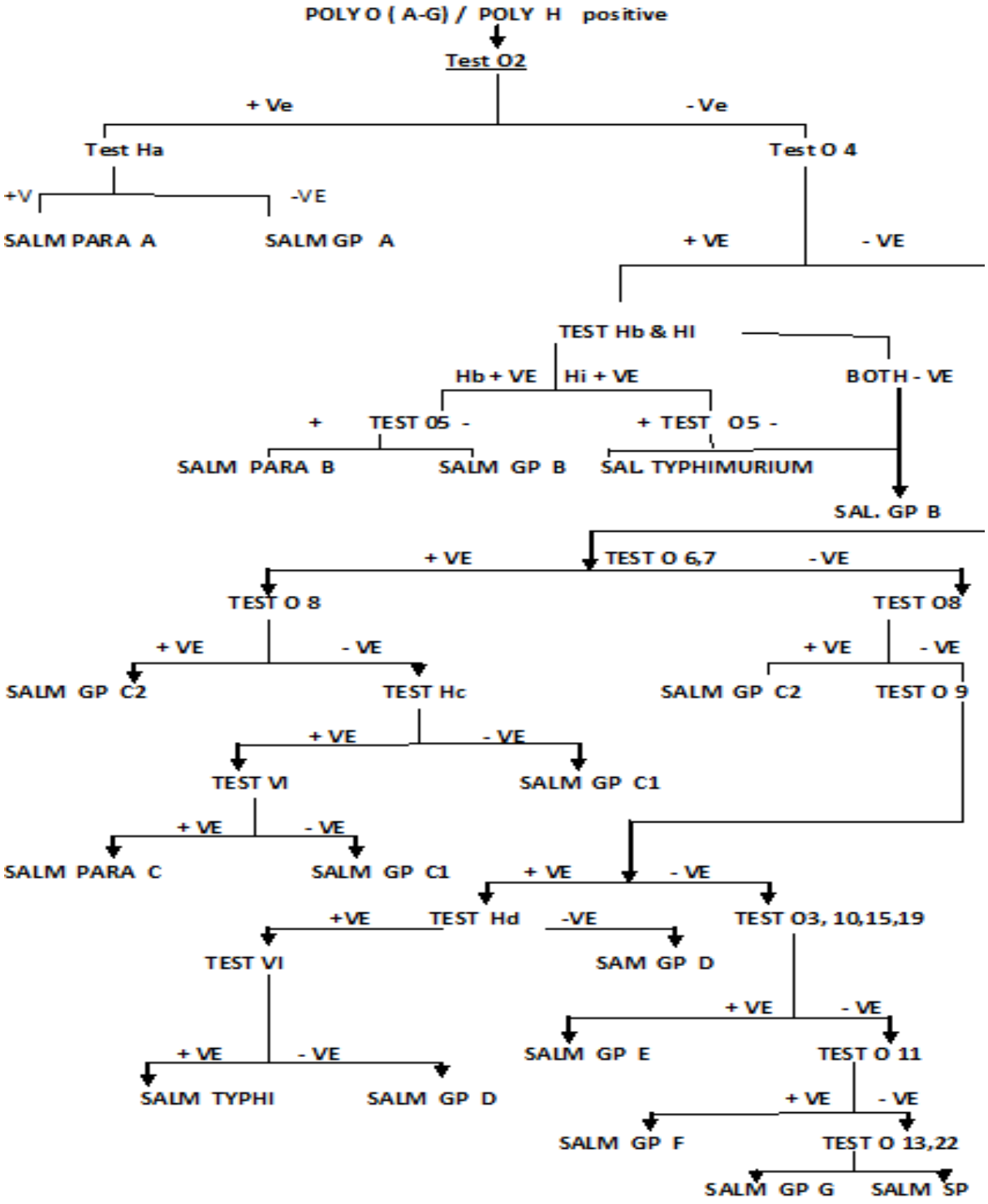
A. Stool culture algorithm



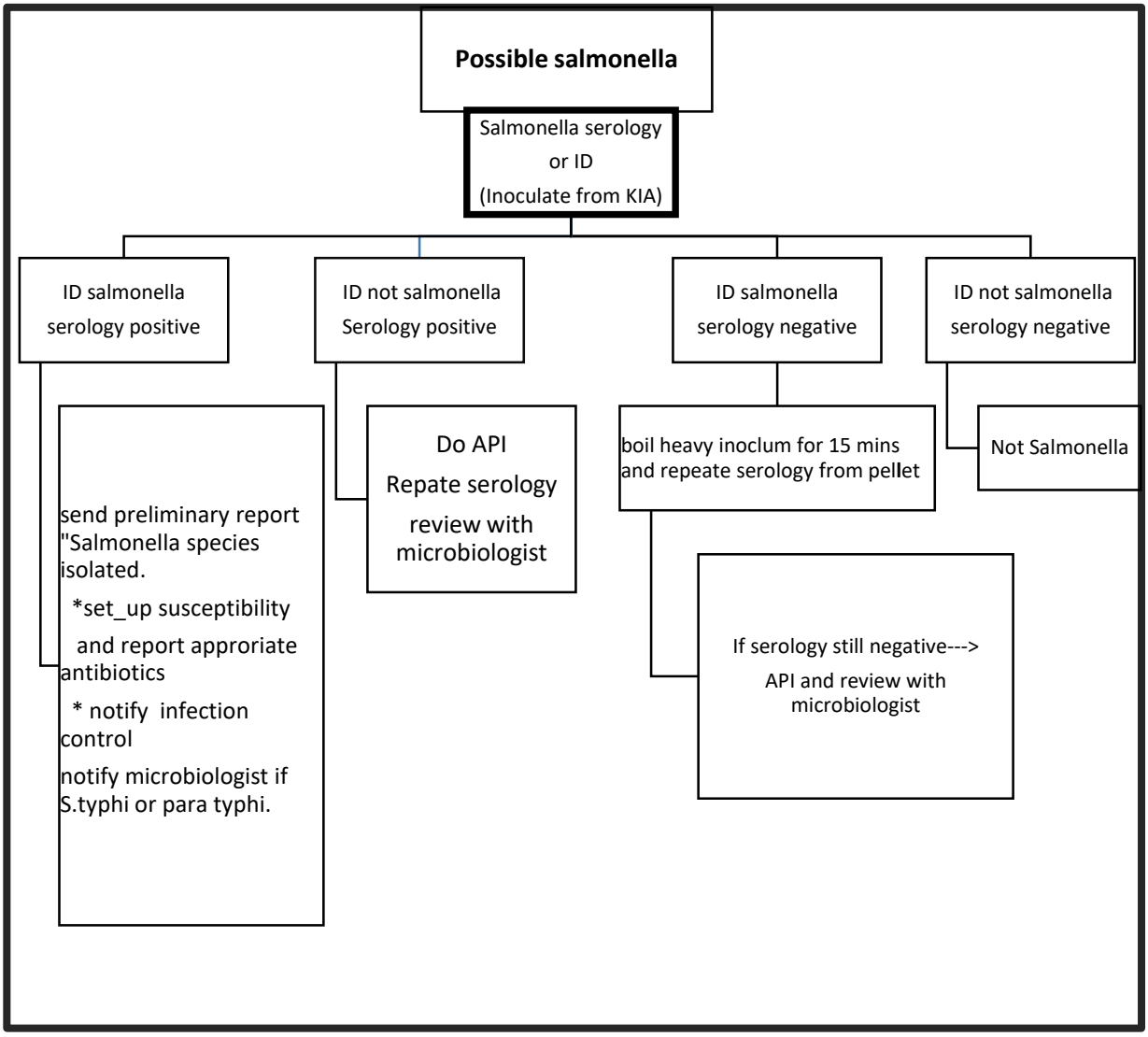
B. Screening for salmonella and shigella



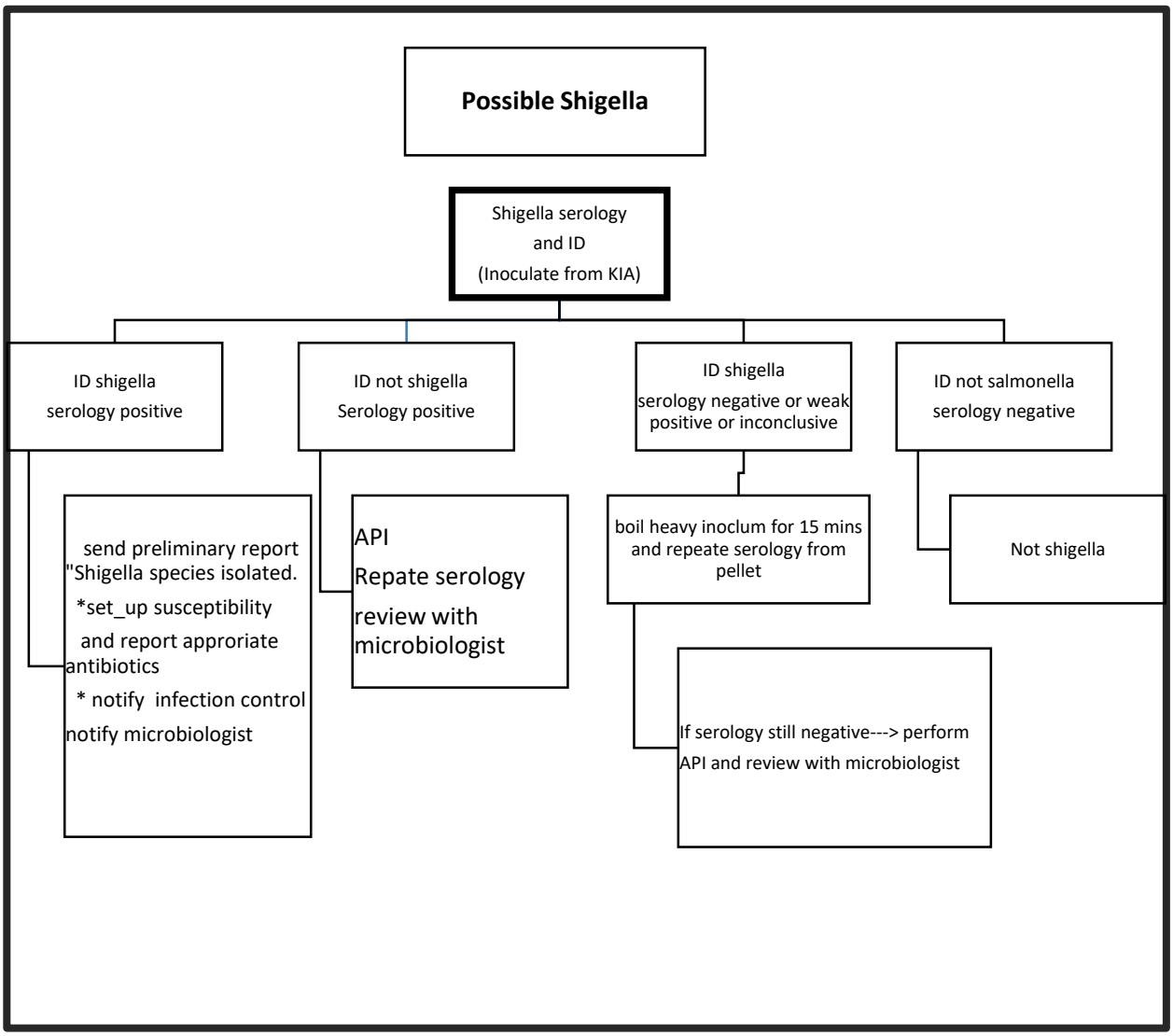
C. Identification of salmonella



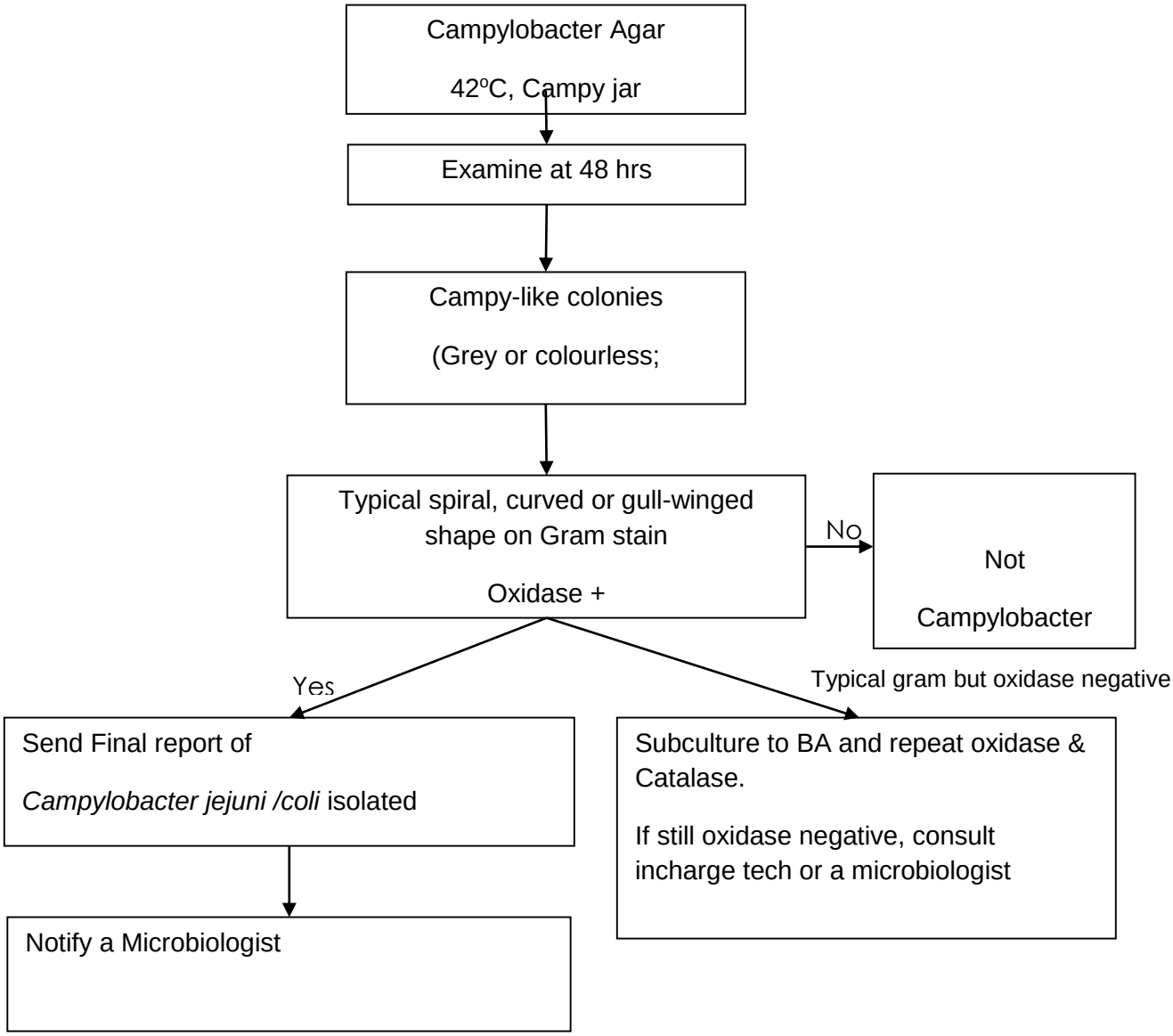
C. identification of salmonella continued



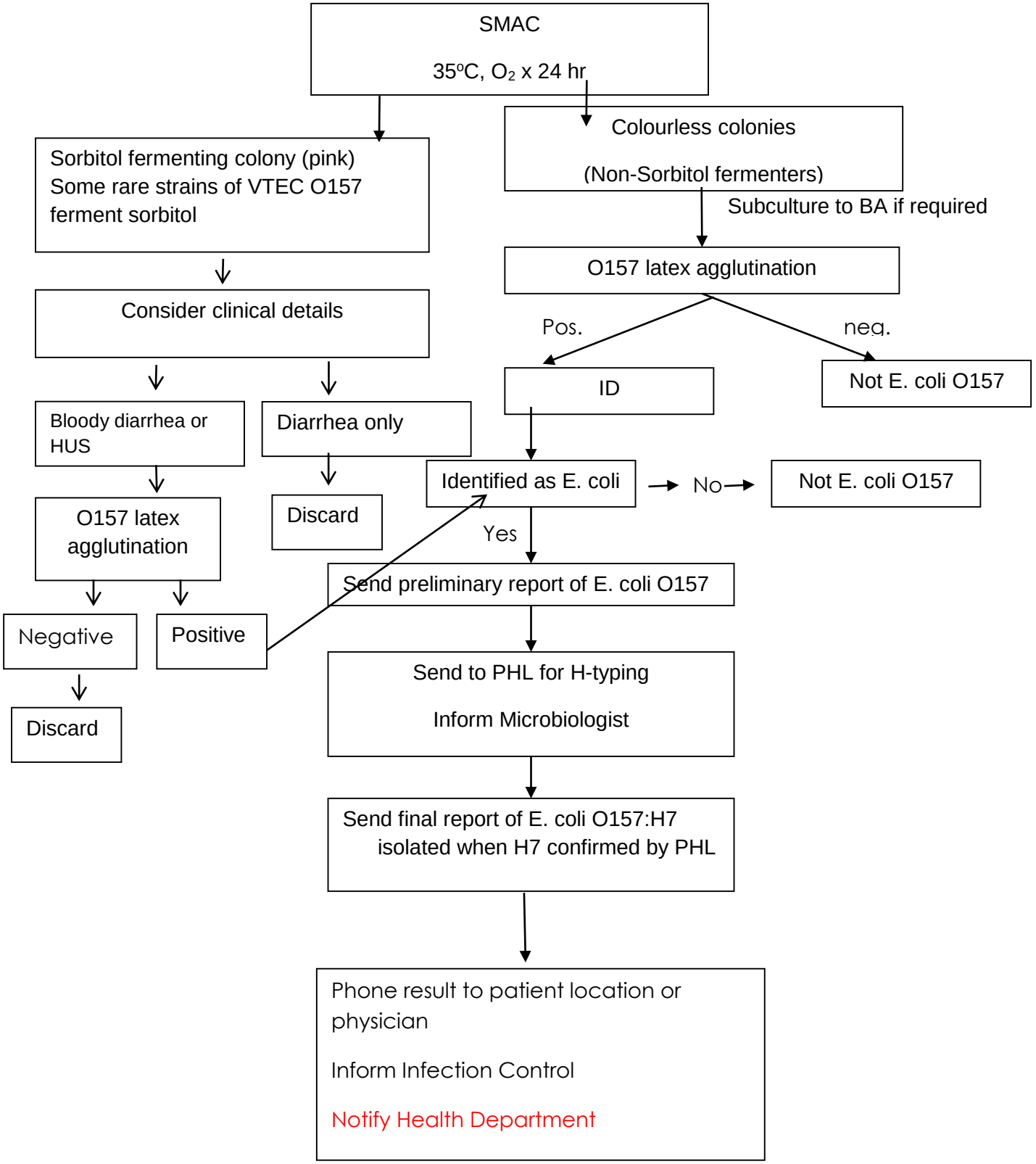
D Identification of shigella



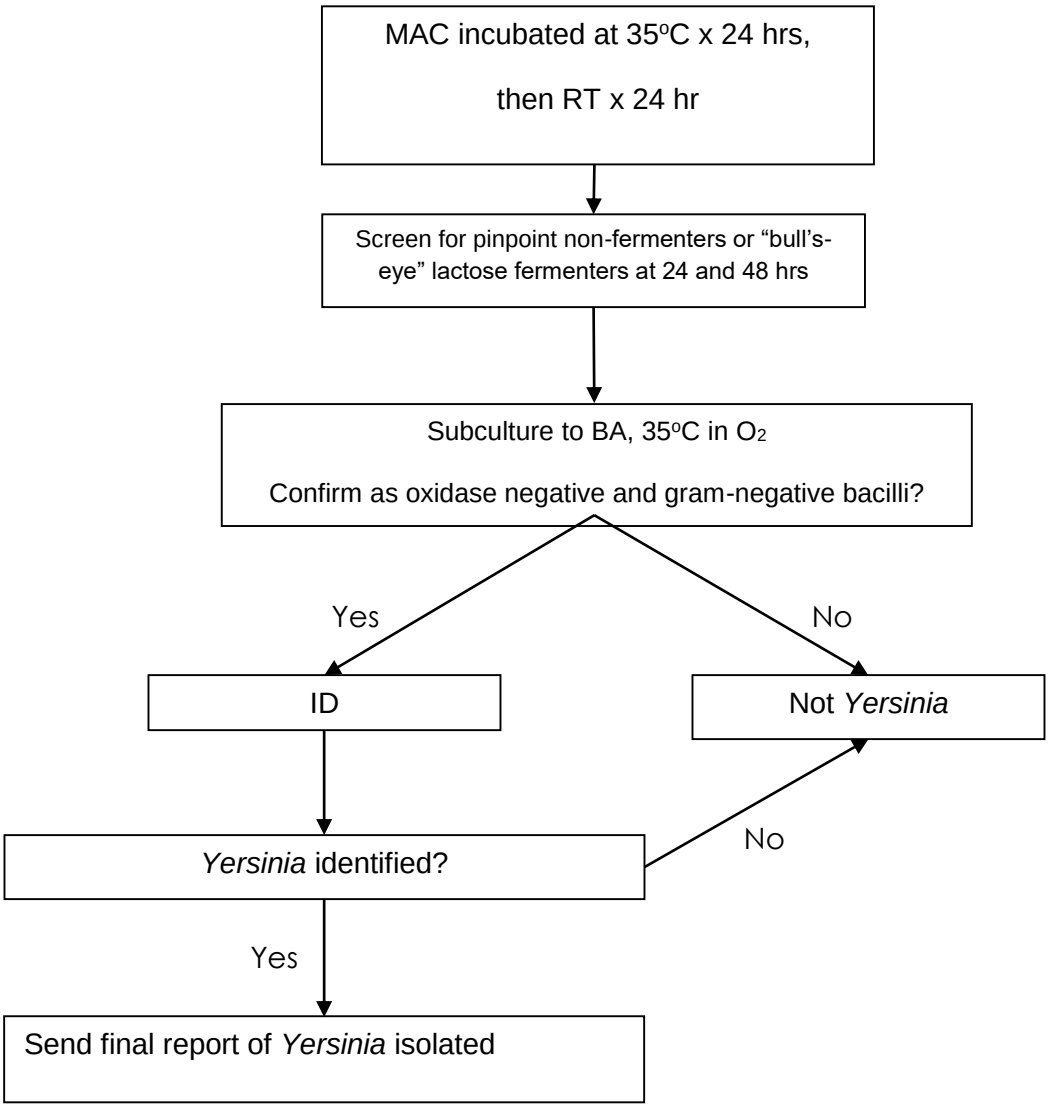
E. Identification of campylobacter



F. Identification of E.coli 0157:h7



G. **Screening primary MacConkey Agar for Yersinia**



H. Screening primary BA plate for Vibrio, Aeromonas& Plesiomonas