National Department of Health

Title: Faeces Culture

ID: G_90_SOP_10_A

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1. Purpose and Scope

Acute infectious diarrhoea may be caused by a number of different agents including bacteria, viruses and protozoa.

This procedure applies to the clinical microbiology laboratory environment within the CPHL, and Human health Fleming Fund Country Grant partner laboratories.

2. Principle/Clinical application

Faeces culture is performed to detect pathogenic bacterial organisms that may be the cause of the infection. Bacterial pathogens include Salmonella, Shigella, Aeromonas, Plesiomonas shigelloides, Vibrio, Campylobacter and Yersinia enterocolitica. Toxigenic Clostridium difficile is an important cause of post antibiotic colitis in hospitalized and community patients.

Culture for faecal pathogens is only indicated for patients with loose stools who have been hospitalised for less than three days.

When faeces culture is requested, the specimen will be examined routinely for Salmonella and Shigella only. Culture for Vibrio cholerae is done on request, when there is a known outbreak occurring or when the sample looks like a rice water stool.

Culture for Aeromonas, Plesiomonas, Campylobacter and Yersinia are not currently performed in this laboratory. Detection of C. difficile also not performed.

3. Responsibilities

Role	**Responsibility**
Lab bench scientist	Setup, reading and data entry Identify and document AMR phenotypes that require storage/ referral
Reporting senior scientist	Validation (checking and report issue) of culture results Addition of necessary interpretative comments on the LIMS prior to issue of the report Second check that AMR phenotypes that require storage/ referral have been identified and actioned. Supervision and sign off of SO competency and media/reagent QC compliance/results
Scientist with this assigned duty	Media QC as per SOP Reagent QC as per JobAid

4. Specimen

Fresh faeces collected into clean sterile yellow urine or brown faeces container, promptly transported to the laboratory is the preferred sample**.**

Culture to be performed only on unformed /loose or watery stools.

If stool specimens are not readily available, the next best alternative is a rectal swab. Rectal swabs are of less value than fresh faeces since they yield a smaller number of enteric pathogens.

5. Equipment/Materials

- XLD agar (Xylose, lactose, Deoxycholate)
- TCBS agar (Thiosulfate-Citrate-Bile Salts-Sucrose)
- Blood agar
- Selenite broth
- Alkaline peptone water
- Sterile saline
- Urea and TSI slopes
- Indole
- Salmonella polyvalent O, H and Vi antisera
- Shigella antisera if available
- Vibrio antisera if available
- Mueller Hinton agar (CLEAR)
- Glass slides and coverslip
- Wooden applicator sticks
- Gram stain reagents

6. Procedure

6.1 Check patient name, MRN and DOB/age matches with specimen and request form.

6.2 Label matching accession number to the faeces sample container and request form

6.3 Check on LIMS for as to whether a faeces sample has been processed within 7 days.

Use this comment for rejection:

"Only one faeces specimen collected within a 7 day period is accepted; repeat testing provides no additional diagnostic benefit."

Description	**Appearance**
Hard lumps, or sausage-shaped with lumps or cracks, rattles in container	Formed
Soft blobs with defined shape	Soft
Fluffy pieces with ragged edges, mushy, takes shape of container	Unformed
Liquid with no solid pieces, watery, specimen can be poured	Watery/Fluid
Presence of blood or clots	Bloodstained

6.3 Describe the macroscopic appearance as above and additionally indicate if it contains blood

Soft or formed stools – add this rejection comment:

"The laboratory does not perform culture formed or soft stools."

6.4 Perform a wet mount preparation (Saline and Iodine):

a) Place sufficient drop of saline and Iodine on each end of a clean glass slide.

b) Use a wooden applicator stick, emulsify in the sample areas that appear bloody, mucoid or watery.

c) Add coverslips and examine under X10 and X40 for pus cells, red blood cells and fat globules.

d) Report pus cells and red cells as + (1-5/field), ++ (5-30) or +++ (>30)

e) Check for the presence of any ova, cysts or parasitic forms especially trophozoites of amoeba and protozoa.

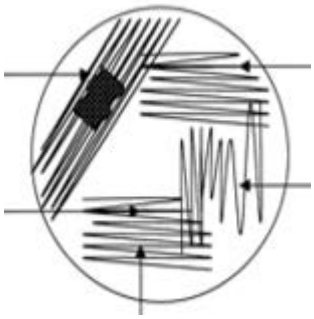
6.5 Bring XLD media and selenite broth to room temperature and TCBS if indicated. (rice water stools or specific request)

6.6 Label plates with patient name, date and accession number.

6.7 Choose area of faeces with blood or pus and inoculate a small amount of faeces onto XLD and selenite broth with a sterile swab.

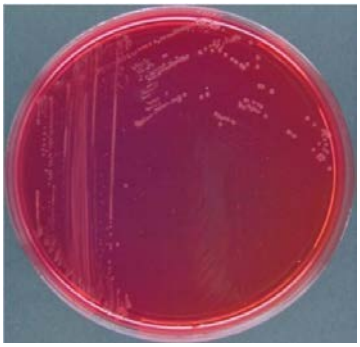
If indicated, add TCBS and alkaline peptone water. Incubate alkaline peptone water for 6-8hrs at 35°C in O_2 incubator.

6.8 Streak plates for single colonies and incubate for 24hrs @35°C in O_2 incubator.

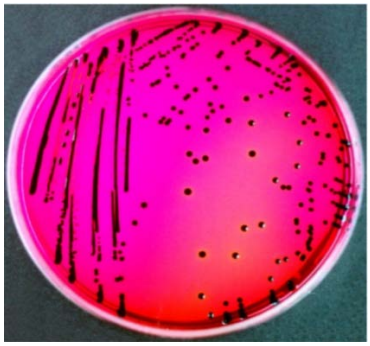


6.9 Collect all XLD, TCBS and selenite broth from incubator at day 2. Examine the plates for suspicious colonies that need further investigation.

a) XLD agar – Look for lactose/xylose negative colonies, which appear as red colonies on the XLD plate (Shigella) and for black H2S producing colonies. (Salmonella)



Shigella on XLD agar



Salmonella on XLD agar

b) TCBS agar- Look for yellow colonies that are sucrose fermenting and non-sucrose fermenting green colonies with blue green centres .

Vibrio on TCBS agar

c) Sub selenite broth onto XLD agar- incubate for 24 hrs @35C in O2 incubator.

d) Sub alkaline peptone water after 6-8hrs incubation onto TCBS. Perform a wet prep and gram stain after 6 hours. Look for motile organisms in a school of fish upstream movement.

e) Day 3 examine XLD for Salmonella and Shigella as per 6.9 (a) and examine TCBS for Vibrio as per 6.9 (b)

6.10 Identification of Salmonella and Shigella

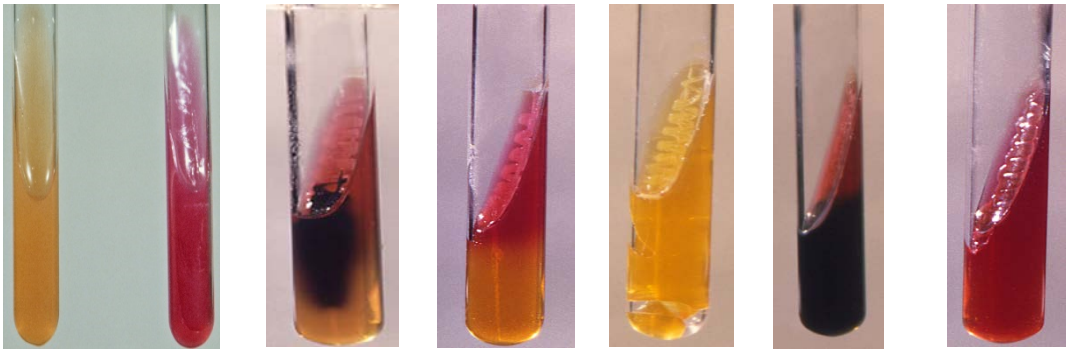
a) Identify presumptive *Salmonella* spp. or Shigella spp. from XLD agar by inoculation of a urea slope, Triple Sugar Iron agar slope (TSI) and Blood agar plate for purity.

b) Incubate at 35°C in O_2 incubator for 18-24 hrs.

Table 1.

Organism	Urea	TSI Slant	Butt	Gas	H2S
Shigella, Serratia	Neg clear	Alkaline (K) red	Acid (A) yellow	Neg	Neg
Salmonella	Neg clear	Alkaline (K) red	Acid (A) yellow	Pos	Pos Black
E coli, Klebsiella, Enterobacter	Neg clear	Acid (A) yellow	Acid (A) yellow	Pos	Neg
Pseudomonas	Neg clear	Alkaline (K) red	Alkaline (K) red	Neg	Neg
Proteus	Pos pink	Alkaline (K) red	Acid (A) red	Pos	Pos Black

Urea Neg Urea Pos **Salmonella** **Shigella** **E coli** **Proteus** **Pseudomonas**



Note: Salmonella Typhi may produce No or minimal H2S.

d) Perform susceptibility testing on Mueller Hinton agar (clear) at 35° in O_2 incubator for 18+/- 2hrs

(Refer to G_90_SOP_6_A Antibiotic disc susceptibility testing Eucast 11 – SSV antibiotic panel):

Ampicillin (AMP10)
Ceftriaxone (CRO30)
Sulph/trimethoprim (SXT25)
Chloramphenicol (C30)
Pefloxacin (PEF5)
Azithromycin (15ug)

e) Perform Salmonella serology testing using Poly O, Poly H and Typhi Vi antisera (see G_90_SOP_28_A. API20E NOT required.

f) Shigella: after presumptive pos with TSI and urea , set up an API 20E or Microbact, if no MALDI/Phoenix on site. Alternatively refer the isolate to CPHL/PMGH for MALDI identification.

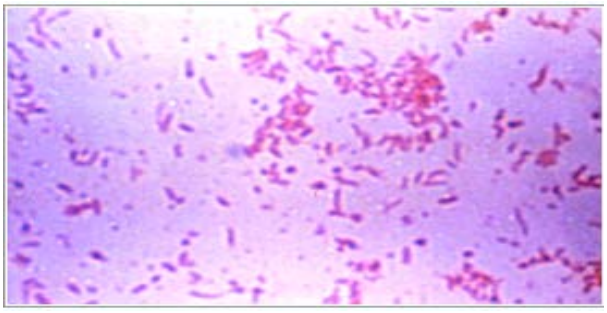
f) Store all Shigella or Salmonella isolates at -20 °C and refer monthly to NRL for confirmatory identification and AST.

6.11 Identification of Vibrio

a) Identify presumptive Vibrio spp. from TCBS agar by subbing to a blood agar plate.

b) Incubate at 35°C in O_2 incubator for 24 hrs.

c) Gram stain can be done on suspect colonies and look for typical comma shaped Gram negative rods.



d) Perform an oxidase test to confirm as TCBS medium interferes with oxidase testing and may give false negative results.

e) Perform indole and motility

Table 2

Organism	Oxidase	Indole	Motility
V. cholerae	Pos	Pos	Pos
V. parahaemolyticus	Pos	Pos	Pos

e) Set up an API 20E using saline unless MALDI available.

f) Submit isolates of confirmed V. cholerae to the NRL for serotyping.

g) Perform susceptibility testing on Mueller Hinton agar at 35° in O2 incubator for 18+/-2hrs

(Refer to G_90_SOP_6 Antibiotic disc susceptibility testing Eucast - SSV antibiotic panel)

Ampicillin (AMP10)
Ceftriaxone (CRO30)
Sulph/trimethoprim (SXT25)
Chloramphenicol (C30)
Pefloxacin (PEF5)
Azithromycin (15ug)

7. Results Recording

- Record all results onto paper worksheet with registered lab number and patient identification.
- Final results recorded into LIMS

8. Interpretation

8.1 Report all isolates of Salmonella sp., Shigella sp., and Vibrio sp. and dispatch results to wards. Vi positive isolates reported as S Typhi.

8.2 Add comment:
"Antibiotic therapy is indicated only for significant systemic symptoms or prolonged illness, particularly at the extremes of age."

8.3 For all negative cultures report as "No Salmonella or Shigella species isolated"

8.4 If Vibrio tested and not detected. Add "No Vibrio species isolated"

8.5 Ring any positive Salmonella or Shigella results to the Doctor looking after the patient. Record date, time and who was phoned on the patient worksheet.

8.6 Vibrio cholerae – inform one of the Pathologists as soon as you think you may have an isolate. Do NOT wait until it is confirmed. Record date, time and who was phoned on the patient worksheet. Notify local public health.

9. Safety

For safety aspects, please review this document G_10_Info_3 Laboratory Biosafety

10. Quality Control

10.1 Media QC is to be done and recorded for each batch of relevant media

10.2 AST QC is to be done weekly

10.3 Salmonella serology QC is to be performed and recorded each time a patient is tested

10.4 Gram stain, Indole and Oxidase test QC is to be performed and recorded daily on Bench Reagent QC Worksheet G _90_WS-1_A

11. Related documents

For access, refer to https://path-png.org/microbiology-sops-fleming-fund/

Disc diffusion quality control	G_90_SOP_3
Antibiotic disc susceptibility testing	G_90_SOP_6
Enteric parasite examination (pends)	G_90_SOP_20
Setup of disc antibiotic susceptibility tests	G_90_J_1
Gram Stain	G_90_T_1
Oxidase Test	G_90_T_8
Indole Test	G_90_T_9
Bench Reagent QC Worksheet	G_90_WS_1
Salmonella serological testing	G_90_SOP_28

12. References

- Stool Culture PMGH Lab SOP 18
- DMDP – 258- Stool Culture SOP
- RNS Microbiology – Faeces Manual Culture
- CDC Public health image Library https://phil.cdc.gov/Details.aspx?pid=5158