FAECES / RECTAL SWABS

I. Introduction

Acute infectious diarrhea may be caused by a number of different agents including bacteria, viruses and protozoa. The laboratory routinely searches for those bacteria that are most likely to cause diarrhea. Requests for viruses or protozoa will be processed in the Virology section or Parasitology section, respectively.

When stool C&S is requested, the specimens will be examined routinely for *Salmonella*, *Shigella*, Campylobacter, and *E. coli* 0157:H7. Upon special request, and if clinically indicated, the laboratory will also culture for the following: *Vibrio* and *Yersinia*. For children between one month and 12 years of age (except those in the neonatal intensive care unit), cultures will be routinely set up for *Yersinia*.

All reagents, kits and media **MUST** be quality controlled before use. All tests must include appropriate controls. (Refer to Quality Control Manual).

II. Specimen Collection and Transport

A single stool specimen should be collected and transported to the laboratory in Cary-Blair transport medium. When faeces cannot be obtained, a rectal swab is acceptable except for *Clostridium difficile* toxin assay. The specimen is collected with a sterile swab inserted approximately one inch beyond the anal sphincter and placed in Amies transport medium.

If Campylobacter other than *C. jejuni/coli* is requested, forward the specimen to PHL.

Stool specimens for *C. difficile* toxin assay should be collected in a clean, sterile container (Refer to Virology Manual).

Rectal swabs for GC must be sent in Amies transport medium.

Specimens for *Chlamydia* detection or virus isolation should be sent in appropriate transport media (Refer to Virology Manual).

Specimens for ova and parasites (O&P) must be collected in SAF (Refer to Parasitology Manual).

III. Specimen Rejection Criteria

Rejection Criteria	Report Comment
All formed stools except when *S. typhi* requested	Formed stool received. Test cancelled.
Patient hospitalized for 3 days or more	This specimen was not cultured for community acquired enteric pathogens because the patient has been hospitalised for 3 or more days. Discuss with the Medical Microbiologist if necessary.
Multiple specimens collected from the same in-patient the same day (only one specimen per patient per test per day is to be processed).	This specimen has not been processed as a specimen submitted from the same day has already been processed.
Stools from outpatients often arrive in batches and are usually a series taken from separate days. Accession and test the most recent specimen only.	Multiple specimens received. Only the most recently collected specimen has been processed.
Stool sample in Cary-Blair transport medium with yellow indicator indicating failure of the buffering system to maintain a neutral pH.	This specimen was not processed as the transport medium failed to stabilize the specimen and maintain a neutral pH.

Phone ward / physician and document on report.

IV. Reagents / Materials / Media

Refer to Appendix I.

V. Procedure

A. Processing of Specimens:

a) Direct Examination: Not routinely performed.
Upon special request, a Gram stain for faecal leukocytes may be performed.

b) Culture:

Media	**Incubation**	
MacConkey Agar (MAC)	O_2,	35^0C x 18 - 24 hours
Hektoen Agar (HEK)	O_2,	35^0C x 18 - 24 hours
MacConkey Sorbitol Agar (MAC-S)	O_2,	35^0C x 18 - 24 hours
Campylobacter Agar (CAMPY)	Campy Jar	42^0C x 48 hours
Selenite Broth (SEL)[1]	O_2,	35^0C x 12-18 hours
If *Yersinia* is requested or patient is >1 month–12 years old (except for NICU) add:		
Cefsulodin Irgasan Novobiocin Agar (CIN)	O_2,	30^0C x 24 hours
If *Vibrio* is requested, add:		
Thiosulphate Citrate Bile Salt Sucrose Agar (TCBS)	O_2,	35^0C x 18 - 24 hours
Alkaline Phosphate Broth (APB)[2]	O_2,	35^0C x 5 - 8 hours
If *Neisseria gonorrhoeae* (GC) is requested (rectal swab only), inoculate only:		
Martin-Lewis Agar (ML)	CO_2,	35^0C x 72 hours
If *C. difficile* toxin assay is requested and appropriate specimen received, forward the specimen to the Virology section (TML) or *C. difficile* bench (MSH) for testing. Specimen is also set up for Vancomycin Resistant Enterococcus (VRE) screen.		
Enterococcus Agar (6 μg/ml Vancomycin)	O_2	36^0C x 48 hours

Notes: 1. Selenite broth is subcultured following overnight incubation onto HEK which is incubated at 35°C in O_2 for 18 - 24 hours.

2. Subculture APB to TCBS Agar after 5-8 hours incubation. Planter has to notify the technologist on the Enteric bench at the time of processing. Incubate the TCBS Agar at 35^0C in O_2 for 18 – 24 hours.

B. Interpretation of Cultures

MACCONKEY/HEKTOEN AGARS

Medium	**Suspect colonies**
MacConkey Agar (MAC)	Non-Lactose Fermenter (NLF) (colourless or transparent)
Hektoen Agar (HEK)	Green with or without H_2S

Pick one colony of each suspect morphotype and inoculate a urea slant and Trypticase Soy Broth (TSB). Incubate these for a minimum of 3 hours at 35^0C in O_2. Urea reactions are recorded and the tubes from urea positive isolates are discarded.

Isolates with a negative urea test are subcultured from the TSB into TSI, ONPG-PAM and MAC (half plate for purity). Results are read after overnight incubation at 35^0C in O_2 (See table below).

Table 1. Characteristic reactions of potential stool pathogens

Organism	**TSI**	**ONPG**	**PPA**	**Motility**	**Indole**
S. typhi	-/+[1]	-	-	+	-
S. arizonae	d/+ H_2S	+	-	+	-
S. paratyphi A	-/+[2]	-	-	+	-
Other Salmonella	-/+ H_2S	-	-	+	-
S. sonnei	-/+	+	-	-	-
S. dysenteriae	-/+	d	-	-	d
S. flexneri (1-5)	-/+	-	-	-	d
S. flexneri (type 6)	-/+[4]	-	-	-	d
S. boydii	-/+	-	-	-	d
Y. enterocolitica	d/+	+	-	-[3]	d

[1]may produce small amounts of gas and /or H_2S
[2]occasionally produces H_2S weakly
[3]non-motile at 35^0C; motile at room temperature
[4]may produce a small amount of gas
"d" indicates variable results

If results of these tests suggest:

Salmonella	Perform serotyping (Refer to Appendix II) and Vitek GNI+ If *S. typhi*, also set up a Vitek GNS-GA.
Shigella	Perform serotyping (Refer to Appendix II), Vitek GNI+ and GNS-GA.
Yersinia	Set up Vitek GNI+ and API 20E

Notes: 1. *Salmonella* and *Shigella* agglutination tests must be performed from a non-selective medium such as TSI. Agglutinations should not be performed from the MAC or HEK plates.
Send all *Salmonella* and *Shigella* isolates to the Public Health Laboratory (PHL) for further typing and / or identification.

MACCONKEY WITH SORBITOL AGAR

This plate is to be examined for the presence of *E. coli* 0157:H7. These organisms do not ferment sorbitol and will appear as non-fermenting (colourless) colonies on this medium.

Each non-fermenting morphotype is sub-cultured onto a BA and incubated at 35^0C in O_2 until sufficient growth to perform serologic testing (usually 4 to 24 hours). Using the 0157 latex agglutination test perform serologic testing on suspect colonies growing on the BA plate (Appendix II). Serology positive isolates should then be inoculated to a Vitek GNI+ card. (Check the Vitek card to confirm that the isolate is an *E. coli* and sorbitol negative. Discard sorbitol-positive isolates). Send the isolate to PHL for confirmation and H typing.

CAMPY AGAR

Examine after 48 hours incubation. Colonies of Campylobacter are grey or colourless, pinpoint flat or mucoid to convex to spreading across the plate.

Perform an oxidase test (strip method) on all suspect morphotypes. If the colonies are oxidase positive perform a Gram stain. Campylobacter will have a typical spiral or gull-winged shaped appearance on Gram stain.

Perform catalase and set up susceptibility tests for nalidixic acid (30 μg) and cephalothin (30 μg) on BA (Appendix III). If there are any suspected Campylobacter isolates which do not fit the following reactions, send the organism(s) to PHL for further identification.

Test	***C. jejuni / coli***
Catalase	+
Oxidase	+
Cephalothin 30 μg	R
Nalidixic acid 30 μg	S

CIN AGAR

Yersinia enterocolitica appears as a small colony with a dark red centre surrounded by a transparent border ("bull's eye"). Any morphotype resembling *Y. enterocolitica* is set up for Vitek and/or API identification. If a *Yersinia* species is isolated, consult the microbiologist or charge technologist before reporting. Send the isolate to PHL for confirmation.

TCBS AGAR

After 18 – 24 hours incubation, subculture all yellow or blue green colonies to BA and incubate at 35^0C in O_2 x 18-24 hours. Perform an oxidase test and Gram stain on all morphotypes growing on BA (Do not perform the oxidase test directly on colonies from the TCBS Agar). Set up a Vitek GNI + card on all oxidase positive gram negative bacilli.

MARTIN-LEWIS AGAR

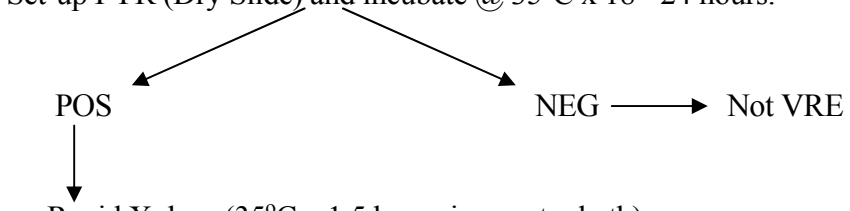
1. Examine the plate after 48 and 72 hours incubation.
2. Perform oxidase test and Gram stain on suspected GC.
3. If there is sufficient growth, perform a Gonogen GC coagglutination test from the primary plates (Refer to Appendix III Genital Manual).
4. Make two CHOC purity plates from suspect GC colonies and incubate in CO_2 at 35^0C for 18-24 hours.
5. After 18-24 hours incubation, do the following:
   a) Inoculate API NH Strip from the purity plate (Refer to Appendix IV, Genital Manual).
   b) Perform Gonogen GC coagglutination from the purity plate if unable to perform from the original culture. (Refer to Appendix III, Genital Manual).
6. If after 72 hours incubation, bacterial growth is not typical of GC, flood the plate with oxidase reagent and immediately subculture any oxidase positive colonies onto CHOC. Repeat step 5 on all suspect colonies.
7. GC is positively identified by the reactions set out in Appendix V, Genital Manual.

ENTEROCOCCUS AGAR

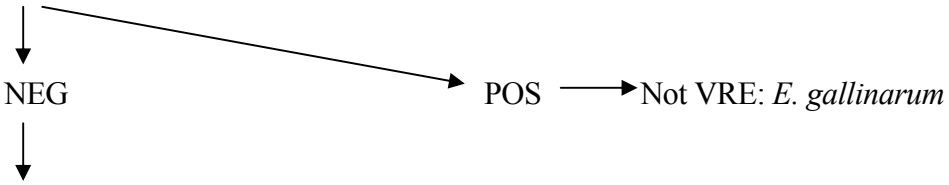
1. Examine VRE screen plates after 24, 48 and 72 hours incubation.
2. Gram stain any growth on the VRE plate. Subculture all Gram positive cocci to Blood Agar (BA) and incubate in O_2 at 35^0C x 18 - 24 hours prior to identification.
3. Identify *E. faecium* and *E. faecalis* as outlined below.

Figure 1. Work-up of Suspected Vancomycin Resistant Enterococci (VRE)

Step 1. Examine the BA plate for purity and colony pigment production (by swab)
Discard all yellow-pigmented colonies as "Not VRE"
Set-up PYR (Dry Slide) and incubate @ 35°C x 18 - 24 hours.



Step 2. Set-up Rapid Xylose (35°C x 1.5 hours in a water bath)



Step 3. 1. If ***E. faecium*** is suspected, set-up MIC panel. Interpret biochemical and susceptibility results after 24 hours incubation as outlined in Table 2.

2. If ***E. faecalis*** is suspected, set-up Arabinose fermtentation, MGP broth, Ampicillin KB, and BHI vancomycin 6 mg/L screen plate puls growth control plate.

For Mount Sinai Hospital patients only, notify Infection Control of negative Xylose test and species of suspected VRE.

Step 4. Read the BHI-6 vancomycin plate at 18, 24 and 48 hours for any growth. If vancomycin-resistant, identify enterococci as outlined in Table 1 below.

Step 5. **Notify Infection Control of *E. faecalis* growing on BHI vancomycin agar and set-up MIC panel. (For Mount Sinai Hospital: do not enter as VRE into LIS until MIC complete).**

Step 6. Confirmation of vancomycin-resistance and species identification of enterococci is performed from MIC panels after 24 hours incubation as outlined in Table 2.

Step 7. 1. If a VRE is identified from the MIC panel:

- **Notify Infection Control and the Ward**
- Set-up 10 mL Brain Heart Infusion broth for Pulse Field Gel Electrophoresis (MSH clients only)
- Freeze and enter into the Freezer program

Table 1. Identification of Vancomycin-Resistant Enterococci

Organism	Arabinose	MGP	Ampicillin
E. faecium	Positive	Negative	Resistant
E. faecalis	Negative	Negative	Susceptible
E. gallinarum/casseliflavus	Positive	Positive	Susceptible

Salmonella

1. If an isolate is suspected to be Salmonella based on biochemical reactions only, but does not react with any antisera, send the organism to PHL for further testing. Cross-reactions with the Salmonella antisera can occur with some *E. coli* and other Enterobacteriaceae.

Shigella

1. Check for autoagglutination and serotype using all available *Shigella antisera* (Appendix I).
2. If an isolate is suspected to be *Shigella* based on biochemical reactions but does not react with any antisera, send the organism to PHL for further testing.

VI. Reporting Results

Telephone all positive reports to ward / physician. Inform infection control of any positive cultures for enteric pathogens from all In-patients. These must be reported to the Medical Officer of Health and flagged in the LIS as "Communicable Disease (CD)".

Negative Report: "No *Salmonella, Shigella*, Campylobacter or *E. coli* 0157:H7 isolated."

Negative Report when Yersinia Culture Performed: "No *Salmonella, Shigella*, Campylobacter, *Yersinia* or *E. coli* 0157:H7 isolated."

Positive Report:

Salmonella species

Preliminary report: "*Salmonella* species. Further identification to follow".

Final report: "*Salmonella* _____. Public Health Laboratory Report No. _____".

Shigella species

Preliminary report: "*Shigella* _____. Serotyping to follow".

Final report: "*Shigella* _____, serotype _____. Public Health Laboratory Report No. _____".

Campylobacter species

Final report: *"Campylobacter jejuni/coli"*

E. coli 0157:H7

Preliminary report:	"*E. coli* 0157. Further identification to follow".
Final report:	"*E. coli* 0157:H___. Public Health Laboratory Report No. _____".

Yersinia species

Preliminary report:	"*Yersinia enterocolitica*. Serotyping to follow".
Final report:	"*Yersina enterocolitica*, serotype _____. Public Health Laboratory Report No. _____.

Vibrio species

Negative Report:	"*Vibrio* species not isolated".
Preliminary positive report:	"*Vibrio* species. Further identification to follow".
Final report:	"*Vibrio* _____. Public Health Laboratory Report No. _____".

Neisseria gonorrhoeae

Negative Report:	"No *Neisseria gonorrhoeae* isolated" If ML plate is overgrown by swarming Proteus or yeast report ONLY as "Unable to rule out *Neisseria gonorrhoeae* due to bacterial/yeast overgrowth."
Positive Report:	"*Neisseria gonorrhoeae* isolated (do not quantitate) beta-lactamase positive or negative" (enter beta-lactamase result under "breakpoint panel" in LIS isolate screen)