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Prepared by QA Committee		
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## 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 sent to the Provincial Health Lab 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*, *Yersinia*, *Plesiomonas* and *Aeromonas*. 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*.

### II. Specimen Collection and Transport

See [Pre-analytical Procedure - Specimen Collection QPCMI02001](#)

### III. Reagents / Materials / Media

See [Analytical Process - Bacteriology Reagents\\_Materials\\_Media List QPCMI10001](#)

### IV. Procedure

#### A. Processing of Specimens:



See [Specimen Processing Procedure Manual](#)

- a) Direct Examination: Not routinely performed.  
Gram stain for faecal leukocytes if requested.

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b) Culture:

	Media	Incubation
MacConkey Agar (MAC)	O <sub>2</sub> ,	35°C x 18 - 24 hours
Hektoen Agar (HEK)	O <sub>2</sub> ,	35°C x 18 - 24 hours
MacConkey Sorbitol Agar (SMAC)	O <sub>2</sub> ,	35°C x 18 - 24 hours
Campylobacter Agar (CAMPY)	Campy Jar	42°C x 48 hours
Selenite Broth (SEL) <sup>1</sup>	O <sub>2</sub> ,	35°C 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 <sub>2</sub> ,	30°C x 48 hours
--	------------------	-----------------

If *Vibrio* is requested, **add:**

Thiosulphate Citrate Bile Salt Sucrose Agar (TCBS)	O <sub>2</sub> ,	35°C x 18 - 24 hours
Alkaline Peptone Water (APW) <sup>2</sup>	O <sub>2</sub> ,	35°C x 5 - 8 hours

If *Plesiomonas* is requested, **add:**

Blood Agar (BA)	O <sub>2</sub> ,	35°C x 24 hours
-----------------	------------------	-----------------

If *Aeromonas* is requested, **add:**

Blood Agar (BA)	O <sub>2</sub> ,	35°C x 24 hours
Cefsulodin Irgasan Novobiocin Agar (CIN)	O <sub>2</sub> ,	35°C x 24 hours

If *Neisseria gonorrhoeae* (GC) is requested (rectal swab only), **inoculate only:**

Martin-Lewis Agar (ML)	CO <sub>2</sub> ,	35°C x 72 hours
------------------------	-------------------	-----------------



If *C. difficile* toxin assay is requested set up Cepheid See

Specimen is also set up for Vancomycin Resistant Enterococcus (VRE) screen  
(on MSH clients only): See [Infection Control Manual](#)



Brilliance VRE agar	O <sub>2</sub>	35°C x 48 hours (In the dark)
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- Notes:**
1. Subculture Selenite broth following overnight incubation onto HEK. Incubate the sub-cultured HEK at 35°C in O<sub>2</sub> for 18 - 24 hours.
  2. Subculture APW 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°C in O<sub>2</sub> for 18 – 24 hours.

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## B. Interpretation of Cultures

Examine plates and perform Vitek-MS for suspected enteric pathogen colonies as per Table 1 below.



**Table 1: Suspect colonies for workup.**

Medium	Suspect colonies for Vitek-MS
MacConkey Agar (MAC)	Oxidase-negative, Non-Lactose Fermenter (NLF) (colourless or transparent)
Hektoen Agar (HEK)	Oxidase-negative, Green with or without H <sub>2</sub> S
MacConkey with Sorbitol (SMAC)	Oxidase-negative, Non-sorbitol Fermenter (NSF) (colourless)
Subbed Hektoen Agar (SFHEK) from Selenite broth	Oxidase-negative, Green with or without H <sub>2</sub> S
Campylobacter Agar (CAMPY)	Oxidase-positive, Grey, pinpoint, flat or mucoid colonies
Yersinia Agar (CIN) For <i>Yersinia</i> or <i>Aeromonas</i>	Oxidase-negative ( <i>Yersinia</i> ) Oxidase positive ( <i>Aeromonas</i> ) Small colony with a dark red centre surrounded by a transparent border (“bull’s eye”).
Subbed TCBS agar for <i>Vibrio</i> from Alkaline Peptone Water	Yellow or blue green colonies
Blood Agar (BA) For <i>Plesiomonas</i> or <i>Aeromonas</i>	Oxidase-positive colonies
Martin-Lewis (ML) For <i>Neisseria gonorrhoeae</i> (GC)	See <a href="#">Bacterial and Yeast work up Manual</a>

Any enteric pathogens identified by Vitek-MS require additional testing as per Vitek MS Manual [Organism Identification Acceptance criteria](#) for **NLF *E.coli*** and other enteric pathogens (*Salmonella spp*, NSF *E.coli*, *Campylobacter*, *Yersinea*, *Vibrio*, *Plesiomonas* or *Aeromonas*)

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If identification from MAC, HEK, SFHEK is unsuccessful from Vitek-MS, inoculate a urea slant and Trypticase Soy Broth (TSB). Incubate these for a minimum of 3 hours at 35°C in O<sub>2</sub>. Record Urea reactions and discard the tubes from urea positive isolates.

Subculture isolates with a negative urea test from the TSB into TSI, ONPG-PAM and MAC (half plate for purity). Read results after overnight incubation at 35°C in O<sub>2</sub> ([BACTERIA and YEAST WORKUP](#)- Enteric Pathogens).

#### C. Susceptibility Testing:



Refer to Susceptibility Testing Manual

### V. Reporting Results

Telephone all positive reports to ward or physician and infection control as per [Isolate Notification and Freezing Table QPCMI16003](#). These must be reported to the Medical Officer of Health and is flagged in the LIS as “[Communicable Disease \(CD\)](#)”.

#### **Direct Smear:**

Gram Stain: For faecal leukocytes, if requested:  
 “No pus cells seen”  
 “Pus cells seen”

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**Culture Report:**

**Negative Report:**

Routine: “No *Salmonella*, *Shigella*, *Campylobacter* or *E. coli* 0157:H7 isolated.”

If *Yersinia* culture is performed: “No *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia* or *E. coli* 0157:H7 isolated.”

If *Vibrio* is requested: “No *Salmonella*, *Shigella*, *Campylobacter* or *E. coli* 0157:H7 or *Vibrio* isolated.”

If *Pleisiomonas* is requested: “No *Salmonella*, *Shigella*, *Campylobacter* or *E. coli* 0157:H7 or *Pleisiomonas* isolated.”

If *Aeromonas* is requested: “No *Salmonella*, *Shigella*, *Campylobacter* or *E. coli* 0157:H7 or *Aeromonas* isolated.”

If *Neisseria gonorrhoeae* is requested: “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:**



***E. coli* O157, *Campylobacter* spp., and *Yersinia* spp.** - DO NOT report susceptibility result. Report with ISOLATE comment "In vitro susceptibility testing for this organism is not routinely performed and/or is unreliable. If advice on antimicrobial therapy is required, please contact the Medical Microbiologist".

*Salmonella* species

Preliminary report: *Salmonella* \_\_\_\_\_ isolated.  
\*Refer to [VITEK-MS-V2-speciesList Created-Translated list](#) for isolate comments specific to *Salmonella* spp. identified.

Final report: “*Salmonella* \_\_\_\_\_ isolated. as reported by Public Health Laboratory..... Report No. \_\_\_\_\_”.

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Shigella species

Preliminary report: “*Shigella* \_\_\_\_\_ isolated, confirmation from Public Health Laboratory to follow.

NOTE: Occasionally, some *E. coli* may identify as presumptive species using our current in-lab methodology; confirmation by Health Lab reference methodology to follow.”

Final report: “*Shigella* \_\_\_\_\_, “serotype (if given) \_\_\_\_\_ isolated, as reported by Public Health Laboratory..... Report No. \_\_\_\_\_”.

Campylobacter species

Final report: “*Campylobacter* \_\_\_\_\_ isolated.”

*E. coli* 0157:H7

Preliminary report: “*E. coli* 0157 isolated, confirmation from Public Health Laboratory to follow”.

Final report: “*E. coli* 0157:H\_\_\_\_\_” “isolated as reported by Public Health Laboratory..... Report No. \_\_\_\_\_”.

*Yersinia* species



Preliminary report: “*Yersinia enterocolitica* isolated, confirmation from Public Health Laboratory to follow”.

Final report: “*Yersinia enterocolitica*” “serotype \_\_\_\_\_ isolated. As reported by Public Health Laboratory..... Report No. \_\_\_\_\_”.

*Vibrio/Aeromonas/ Pleisiomonas* species

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Preliminary positive report: “*Vibrio or Aeromonas or Pleisiomonas* species isolated, confirmation from Public Health Laboratory to follow”.

Final report: “*Vibrio or Aeromonas or Pleisiomonas* \_\_\_\_\_”  
isolated as reported by Public Health Laboratory Report No. \_\_\_\_\_”.

*Neisseria gonorrhoeae*



Final Report: “*Neisseria gonorrhoeae*” “isolated” (do not quantitate)

## VI. References:

Hardy Diagnostics. 2016. HDQA 2207F Rev. 012816hh  
[https://catalog.hardydiagnostics.com/cp\\_prod/Content/hugo/CINAgar.htm](https://catalog.hardydiagnostics.com/cp_prod/Content/hugo/CINAgar.htm)

P.R. Murray, E.J. Baron, M.A. Pfaller, R.H. Tenover, R.M. Tenover. 2003. Manual of Clinical Microbiology, 8<sup>th</sup> ed. ASM Press, Washington, D.C.

H.D. Izenberg. 2003. Fecal Culture for Aerobic Pathogens of Gastroenteritis, p.3.8.1.1-3.4.8.6 in Clinical Microbiology Procedures Handbook, 2<sup>nd</sup> ed. Vol.1 ASM Press, Washington, D.C.

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## DUODENAL OR SMALL BOWEL ASPIRATE / SWAB / BIOPSY

### I. Introduction

Duodenal and small bowel aspirates and biopsy specimens are processed for O&P only. Swabs are processed for aerobic and anaerobic culture only. Aspirates and biopsy specimens for O & P should be sent to PHL for processing. If the aspirate is not already in SAF, transfer to SAF and then forward for processing.

### II. Specimen Collection and Transport

See [Pre-analytical Procedure - Specimen Collection QPCMI02001](#)

### III. Reagents / Materials / Media

See [Analytical Process - Bacteriology Reagents Materials Media List QPCMI10001](#)

### IV. Procedure

#### A. Processing of Specimens



See [Specimen Processing Procedure Manual](#)

a) Direct Examination: Gram stain not performed.

b) Culture:

#### i) Duodenal or Small Bowel Aspirates

Duodenal and SB aspirates are processed for O&P only. If the specimen is not received in SAF, transfer the specimen to SAF and send to PHL. These specimens will not routinely be processed for bacterial culture.

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ii) Duodenal or Small Bowel Swab

<b>Media</b>	<b>Incubation</b>
Blood Agar (BA)	O <sub>2</sub> , 35 <sup>0</sup> C 18 x 48 hours
MacConkey Agar (MAC)	O <sub>2</sub> , 35 <sup>0</sup> C 18 x 24 hours
Fastidious Anaerobic Agar (BRUC)	AnO <sub>2</sub> , 35 <sup>0</sup> C x 48 hours
Kanamycin / Vancomycin Agar (KV)	AnO <sub>2</sub> , 35 <sup>0</sup> C x 48 hours

B. Interpretation of cultures



Refer to Miscellaneous / Wound Manual.

C. Susceptibility Testing

Refer to Susceptibility Testing Manual.

V. **Reporting Results**

Refer to Miscellaneous / Wound Manual.

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## RECTAL / LARGE BOWEL (COLON) BIOPSIES

### I. Introduction

Rectal and Large Bowel (Colon) biopsies are usually collected for investigation of patients with bloody diarrhoea. Cytomegalovirus is the most common viral agent associated with this syndrome. Although bacterial agents such as *Salmonella*, *Shigella*, *E. coli* 0157:H7 and others may cause bloody diarrhoea, the preferred specimen for detection of these organisms is a stool specimen. However, if requested, bacterial culture will be performed and the specimen will be processed as a stool specimen.

A portion of the specimen received in the Microbiology Laboratory should be forwarded to the Virology section for processing.

### II. Specimen Collection and Transport

See [Pre-analytical Procedure - Specimen Collection QPCMI02001](#)

### III. Reagents / Materials / Media

See [Analytical Process - Bacteriology Reagents\\_Materials\\_Media List QPCMI10001](#)

### IV. Procedure

#### A. Processing of Specimens



See [Specimen Processing Procedure Manual](#)

- a) Direct Examination: Gram stain not indicated.
- b) Culture:

Media	Incubation
MacConkey Agar (MAC)	O <sub>2</sub> 35°C x 18 – 24 hours
Hekton Agar (HEK)	O <sub>2</sub> 35°C x 18 – 24 hours
MacConkey Sorbitol Agar (SMAC)	O <sub>2</sub> 35°C x 18 – 24 hours
Camyplobacter Agar (CAMPY)	Campy Jar 42°C x 48 hours
Selenite Broth (SEL)	O <sub>2</sub> 35°C x 18 – 24 hours

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B. Interpretation of Cultures

See [Faeces/Rectal Culture Section](#)

C. Susceptibility Testing

Refer to Susceptibility Testing Manual



V. **Reporting Results**

Refer to [Faecal/Rectal Culture Section](#)

VI. **References**

P.R. Murray, E.J. Baron, M.A. Pfaller, R.H. Tenover, R.M. Tenover. 2003. Manual of Clinical Microbiology, 8<sup>th</sup> ed. ASM Press, Washington, D.C.

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Annual Review	May 12, 2003	Dr. T. Mazzulli
Annual Review	May 26, 2004	Dr. T. Mazzulli
Annual Review	May 12, 2005	Dr. T. Mazzulli
Specimen collection procedure – see <a href="#">Pre-analytical Procedure - Specimen Collection QPCMI02001</a>	July 23, 2006	Dr. T. Mazzulli
Specimen processing procedure - See <a href="#">Specimen Processing Procedure QPCMI06003</a>	July 23, 2006	Dr. T. Mazzulli
Faces/Rectal Swab C&S – <i>Pleisiomonas</i> and <i>Aeromonas</i> added	July 23, 2006	Dr. T. Mazzulli
CIN agar for <i>Yersinia</i> – changed to 48 hour incubation	July 23, 2006	Dr. T. Mazzulli
Page 4 Volume of specimen to send to PHL	July 23, 2006	Dr. T. Mazzulli
Annual Review	July 23, 2006	Dr. T. Mazzulli
Annual Review	August 13, 2007	Dr. T. Mazzulli
Annual Review	August 15, 2008	Dr. T. Mazzulli
Annual Review	August 15, 2009	Dr. T. Mazzulli
Annual Review	August 15, 2010	Dr. T. Mazzulli
Annual Review	August 15, 2011	Dr. T. Mazzulli
Added Motility testing for identification of <i>Campylobacter</i>	March 01, 2012	Dr. T. Mazzulli
Annual Review	March 01, 2012	Dr. T. Mazzulli
Annual Review	May 31, 2013	Dr. T. Mazzulli
Updated with MS ID	February 05,2014	Dr. T. Mazzulli
Removed <i>Campylobacter</i> ID in appendix	February 05,2014	Dr. T. Mazzulli
Updated using Wellcolex for <i>Shigella</i> serological typing	February 05,2014	Dr. T. Mazzulli
Annual Review	February 05,2014	Dr. T. Mazzulli
<i>E. coli</i> may identify as presumptive <i>Shigella</i> species using our current in-lab methodology; confirmation by PHL	July 28, 2014	Dr. T. Mazzulli
Update stool pathogens canned messages	December 30, 2014	Dr. T. Mazzulli
Change <i>Campylobacter</i> canned message	March 9, 2015	Dr. T. Mazzulli
Rectal/Fecal swab manual revised.	March 9, 2015	Dr. T. Mazzulli
Removed appendix II <i>Salmonella</i> serology to technical	March 9, 2015	Dr. T. Mazzulli

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manual		
Annual Review	March 9, 2015	Dr. T. Mazzulli
Annual Review Add Remel <i>Shigella</i> serology for <i>Shigella</i> testing Changed Alkaline phosphate Broth to Alkaline peptone Water for vibrio Updated MSH logo in header	February 21, 2016	Dr. T. Mazzulli
<b>Section Faeces and Rectal swab:</b> Under Table 2 added foot note to <i>Yersinia /Vibrio /Aeromonas / Pleisiomonas</i> <sup>2</sup> If identified by routine specimen processing, proceed to report isolate despite the fact no request for isolation was received. Added <i>Vibrio/Aeromonas</i> with <i>Pleisiomonas</i> in reporting section.	March 26, 2016	Dr. T. Mazzulli
Annual Review CIN added to <i>Aeromonas</i> set up 35C for 24hrs	January 4, 2017	Dr. T. Mazzulli
Removed table 2 Workup of enteric pathogen ID from Vitek MS and replaced with a link to Vitek MS manual where table is now (organism acceptance table)	August 15, 2017	Dr. T. Mazzulli
Annual Review	January 4, 2018	Dr. T. Mazzulli
Minor format change	September 14, 2018	Dr. T. Mazzulli

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