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| Section: Bacteriology Procedures | Subject Title: Enteric Culture Manual | |
| Prepared by QA Committee | | |
| Issued by: Laboratory Manager | Revision Date: 6/15/2023 | |
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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*. Should one of these organisms be isolated during workup, they shall be reported.

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 ₂ , | 35°C x 18 - 24 hours |
| Hektoen Agar (HEK) | O ₂ , | 35°C x 18 - 24 hours |
| MacConkey Sorbitol Agar (SMAC) | O ₂ , | 35°C x 18 - 24 hours |
| Campylobacter Agar (CAMPY) | Campy Jar | 42°C x 48 hours |
| Selenite Broth (SEL) ¹ | O ₂ , | 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 ₂ , | 30°C x 48 hours |
|--|------------------|-----------------|

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

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

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

| | | |
|-----------------|------------------|-----------------|
| Blood Agar (BA) | O ₂ , | 35°C x 24 hours |
|-----------------|------------------|-----------------|

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

| | | |
|--|------------------|-----------------|
| Blood Agar (BA) | O ₂ , | 35°C x 24 hours |
| Cefsulodin Irgasan Novobiocin Agar (CIN) | O ₂ , | 35°C x 24 hours |

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

| | | |
|------------------------|-------------------|-----------------|
| Martin-Lewis Agar (ML) | CO ₂ , | 35°C x 72 hours |
|------------------------|-------------------|-----------------|

If *C. difficile* toxin assay is requested set up Cepheid See C difficile PCR by
Luminex [Aries](#)



Notes: 1. Subculture Selenite broth following overnight incubation onto HEK. Incubate the sub-cultured HEK at 35°C in O₂ for 18 - 24 hours.

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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 O₂ 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 ₂ 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 ₂ 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 Bacterial and Yeast work up Manual |

Any enteric pathogens identified by Vitek-MS requires additional testing as per Vitek MS Manual [Organism Identification Acceptance criteria](#) (*NLF/NSF E.coli*, *Salmonella spp*, *Shigella spp*, *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 O₂. 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 O₂ ([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 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

P.R. Murray, E.J. Baron, M.A. Pfaller, R.H. Tenover, R.M. Tenover. 2003. Manual of Clinical Microbiology, 8th 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, 2nd 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

| Media | Incubation |
|----------------------------------|--|
| Blood Agar (BA) | O ₂ , 35 ⁰ C 18 x 48 hours |
| MacConkey Agar (MAC) | O ₂ , 35 ⁰ C 18 x 24 hours |
| Fastidious Anaerobic Agar (BRUC) | AnO ₂ , 35 ⁰ C x 48 hours |
| Kanamycin / Vancomycin Agar (KV) | AnO ₂ , 35 ⁰ 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 ₂ 35°C x 18 – 24 hours |
| Hekton Agar (HEK) | O ₂ 35°C x 18 – 24 hours |
| MacConkey Sorbitol Agar (SMAC) | O ₂ 35°C x 18 – 24 hours |
| Camyplobacter Agar (CAMPY) | Campy Jar 42°C x 48 hours |
| Selenite Broth (SEL) | O ₂ 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, 8th 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, 2nd ed. Vol.1 ASM Press, Washington, D.C.

VII. Related Documents

MI_SO Send-Out Manual

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Record of Edited Revisions

Manual Section Name: Enteric Bench Manual

| Page Number / Item | Date of Revision | Signature of Approval |
|--|-------------------|-----------------------|
| 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 Pre-analytical Procedure - Specimen Collection QPCMI02001 | July 23, 2006 | Dr. T. Mazzulli |
| Specimen processing procedure - See Specimen Processing Procedure QPCMI06003 | 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 09, 2015 | Dr. T. Mazzulli |
| Rectal/Fecal swab manual revised. | March 09, 2015 | Dr. T. Mazzulli |
| Removed appendix II <i>Salmonella</i> serology to technical | March 09, 2015 | Dr. T. Mazzulli |

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| manual | | |
| Annual Review | March 09, 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 |
| Section Faeces and Rectal swab: Under Table 2 added foot note to <i>Yersinia /Vibrio /Aeromonas / Pleisiomonas</i> ² 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 04, 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 04, 2018 | Dr. T. Mazzulli |
| Minor format change | September 14, 2018 | Dr. T. Mazzulli |
| Annual Review Minor updates to introduction and Table 1: Suspect colonies for workup notes under table. Paralinks fixed. | January 13, 2019 | Dr. T. Mazzulli |
| pg 13 added Related Documents MI_SO | September 06, 2019 | Dr. T. Mazzulli |
| Annual Review -Removed setting up Vancomycin Resistant Enterococcus (VRE) screen (on MSH clients only) for C.diff specimens - Changed C difficile PCR testing by Cepheid GeneXpert to Luminex Aries | June 15, 2020 | Dr. T. Mazzulli |

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Full document review included in all updates. Bi-annual review conducted when no revision had been made within 2 years.

| Page Number / Item | Date of Revision | Edited by: |
|---|------------------|----------------|
| Minor formatting change | April 11, 2021 | Jessica Bourke |
| Nomenclature update – removed clostridium difficile | April 19, 2021 | Wayne Chiu |
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