PURPOSE:

To culture stool specimens for the detection of major enteric pathogens that commonly cause diarrheal illness: *Aeromonas spp., Salmonella spp., Shigella spp., Campylobacter spp., Escherichia coli 0157* and *Yersinia enterocolitica*.

Specific examination for other potential causes of gastroenteritis, such as *Plesiomonas shigelloides* and *Vibrio spp.* can be performed if specifically requested by the ordering physician and a history of travel or consumption of seafood is provided or in the investigation of an outbreak where standard procedures have failed to find the cause.

SAMPLE INFORMATION:

Type	• Stool collected in enteric transport medium. • Stool in sterile container, if received within 2 hours of collection. • Rectal swab if feces cannot be obtained.
Source	Feces
Stability	Specimens should be received and processed with 24 hours of collection.
Storage Requirements	• Fresh stool, not in transport medium, needs to be processed within 2 hours after collection. • Fresh stool in transport medium may be refrigerated at 4°C and transported to the laboratory within 48 hours of collection.
Criteria for rejection and follow up action	1. Unlabeled/mislabeled specimen. 2. Not in enteric transport media and more than 2 hours old. 3. If specimen in transport medium is delayed for more than 48 hours at 4°C or is delayed more than 24 hours at 25°C. 4. Fecal cultures received from adults and pediatric patients (3 years of age or older) hospitalized for more than 3 days, unless patient is known to be HIV positive or there is a cluster epidemic within the institution. 5. Do not reject stool samples from infants and toddlers until after the fourth day of hospitalization, since it may take longer to collect a stool sample from pediatric patients admitted with gastroenteritis. 6. Stool with barium. 7. Specimens submitted in Ova and Parasite collection containers.

REAGENTS and/or MEDIA:

- Blood Agar (BAP), MacConkey Agar (MAC), Hektoen Agar (HEK), MacConkey Sorbitol Agar (MAC-S), Campylobacter Agar (CAMP), Yersinia Select Agar (CIN) and Selenite Broth
- Identification reagents: catalase, oxidase, Salmonella Vi and O antisera, E.coli 0157 serology, hippurate discs, TSI/Urea slants, etc.

SUPPLIES:

- Sterile pipettes and wooden sticks
- Glass test tubes
- Biosafety cabinet
- 35° O_2 incubator
- Vitek 2 Compact and supplies
- Campylobacter microaerophilic jar and pouch

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures.

- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used where there is a known or potential risk of exposure to splashes.
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
- The use of needles, syringes, and other sharp objects should be strictly limited.

All patient specimens are assumed to be potentially infectious. Universal precautions must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

QUALITY CONTROL:

- Refer to MIC60100 Non-Exempt Media Quality Control procedure
- Refer to Quality Control manual for reagent quality control procedures

PROCEDURE INSTRUCTIONS:

Step	Action
Processing Feces for Stool Culture	
1	In the biosafety cabinet, mix specimen and use cotton-tipped swab to inoculate each plate. Lightly inoculate BA, MAC and MAC-S. Use a heavier inoculum for the HEK, CAMP and CIN as they are more selective. Break off swab into Selenite Broth and recap tube loosely.
2	Streak for isolated growth using a disposable inoculation needle.  Streak out to cover the whole plate.
3	• Incubate BA, MAC, MAC-S and HEK in 35° O_2 incubator. • Incubate CAMP, in campy jar with microaerophilic pouch, in 42° incubator for 72 hours. • Incubate CIN plate at room temperature for 48 hours. • Incubate selenite broth in O_2 incubator before leaving at 20:00.
4	• Subculture selenite broth first thing the next morning. Saturate a cotton-tipped swab from the top of the selenite broth and inoculate the Hektoen plate reserved for selenite subculture. • Streak for isolated growth using a disposable inoculation needle and incubate in 35° O_2 incubator for 18-24 hours.

INTERPRETATION OF RESULTS:

Step	Action	
Examination of Blood Agar		
1	Predominate or pure growth *P.aeruginosa*, *S.aureus* or *Yeast species*: • Identify with GNI, WP, TC and RS as appropriate • Yeast species does not have to be identified	
2	Perform sweep oxidase to screen for *Aeromonas spp.*, which are oxidase positive and may be hemolytic. Note: If *Plesiomonas shigelloides* or *Vibrio species* was requested by ordering physician, these organisms are oxidase positive as well and will need to be investigated.	
3	**If:**	**Then:**
	Sweep oxidase negative	Discard plate, no *Aeromonas*, *Plesiomonas* or *Vibrio* isolated
	Sweep oxidase positive	Pick one representative colony of each morph type of oxidase positive colonies. Subculture each colony picked to a BA purity plate.

Oxidase Interpretation	
Oxidase	**Next Steps:**
Negative	Discard purity plate. Go back to original BA plate and isolate oxidase positive colony again.
Positive	Vitek GNI to rule out Aeromonas, Plesiomonas shigelloides or Vibrio species • Perform GNS as per ASTM guidelines

Step	Action	
Examination of MacConkey Agar		
1	Examine plate for Non-lactose fermenting colonies. Record observations in LIS. If no Non-lactose fermenting colonies present, record "All Lactose Fermenters" in LIS.	
2	**If:**	**Then:**
	Lactose fermenters	Discard plate, no enteric pathogens isolated.
	Non-lactose fermenters	Pick one representative colony of each morph type of colorless colonies. Subculture each colony picked to a TSI, urea slant and BA purity plate.

TSI/Urea Interpretation for MacConkey Growth			
TSI	**Urea**	**Other Tests**	**Next steps:**
A/A Gas or H_2S+	Positive		Discard
A/A Gas and H_2S -	Positive		Vitek GNI to rule out *Yersinia enterocolitica* Perform GNS as per ASTM guidelines
A/A Gas and H_2S -	Negative	Oxidase -	Discard
A/A Gas and H_2S -	Negative	Oxidase +	Vitek GNI to rule out *Vibrio species* Perform GNS as per ASTM guidelines
A/A Gas or H_2S+	Negative		Discard
K/K Gas or H_2S +/-	Positive or Negative		Discard
K/A Gas or H_2S +/-	Positive		Discard
K/A Gas and H_2S -	Negative	Oxidase +	Vitek GNI to rule out *Aeromonas* and *Plesiomonas shigelloides* Perform GNS as per ASTM guidelines
K/A Gas and H_2S -	Negative	Oxidase -	Vitek GN to rule out *Salmonella* and *Shigella* • If identification is Salmonella species, perform Salmonella latex serology testing from BA purity plate Perform GNS as per ASTM guidelines
K/A Gas or H_2S +	Negative		Vitek GN to rule out *Salmonella* • If identification is Salmonella species, perform Salmonella latex serology testing from BA purity plate Perform GNS as per ASTM guidelines

Step	Action	
Examination of Hektoen Agar		
1	Examine plate for green (with or without black centers) or blue colonies. Record observations in LIS. If no green (with or without black centers) or blue colonies present, record "All yellow-orange or salmon pink" in LIS.	
2	**If:**	**Then:**
	Yellow-orange or salmon pink	Discard plate, no enteric pathogens isolated.
	Blue or green colonies with or without black centers	Pick one representative colony of each morph type of suspicious colonies. Subculture each colony picked to a TSI, urea slant and BA purity plate.

TSI/Urea Interpretation for Hektoen Growth				
TSI	**Urea**	**Next steps:**		
Any reaction	Positive	Discard		
K/K Gas or H2S +/-	Negative	Discard		
A/A Gas or H2S +/-	Negative	Discard		
K/A Gas and H2S -	Negative	Vitek GNI	*Shigella:* • Vitek 2 AST-N213	• Refer to ASTM for susceptibility reporting
K/A Gas or H2S +	Negative	Vitek GNI	*Salmonella*: • Perform Salmonella latex serology testing from BA. • Vitek 2 AST-N213	

Step	Action	
Examination of MacConkey Sorbitol Agar		
1	Examine plate for Non-sorbitol fermenters. Record observations in LIS. If no non-sorbitol fermenters present, record “All Sorbitol Fermenters” in LIS.	
2	**If:**	**Then:**
	Sorbitol fermenters	Discard plate, no enteric pathogens isolated.
	Non-sorbitol fermenters	Pick one representative colony of each morph type of colorless colonies. Subculture colony to a BA purity plate From BA purity plate, perform indole and oxidase.

Indole/Oxidase Interpretation for Sorbitol MacConkey Growth			
Indole	**Oxidase**	**Next steps:**	
Negative	Any reaction	Discard	
Positive	Positive	Discard	
Positive	Negative	Perform E.coli 0157 latex agglutination test	
		If Negative: • Discard	If Positive: • Perform GNI for identification to confirm E.coli • Susceptibility testing is not routinely performed on stool isolates of *E.coli 0157:H7*

Step	Action	
Examination of Yersinia Agar		
1	Examine plate for Bull's Eye colonies. Record observations in LIS. If no bull's eye colonies present, record "No Bull's Eye Colonies at 48hrs" or if there is no growth present, record "No Growth at 48h" in LIS.	
2	**IF**	**THEN**
	No growth or no bulls 'eye colonies at 48hrs	Discard plate, *Yersinia* not isolated.
	Bull's eye colonies	Pick one representative colony of each morph type of bull's eye colonies. Subculture each colony picked to a TSI, urea and BA purity plate.

TSI/Urea Interpretation for CIN Growth		
TSI	**UREA**	**Next steps:**
K/K Gas or H2S+/-	Any reaction	Discard
K/A Gas or H2S+/-	Any reaction	Discard
A/A Gas or H2S+/-	Negative	Discard
A/A Gas or H2S+	Positive	Discard
A/A Gas and H2S-	Positive	• Vitek GNI to rule out *Yersinia enterocolitica* • Perform GNS as per ASTM guidelines

Step	Action	
Examination of Campylobacter Agar		
1	Examine plate for grey, flat, irregular, spreading, sometimes mucoid colonies. Record observations in LIS. If no suspicious colonies present, record “No Growth at 72h” or “No Colonies Typical of Campy at 72h” in the LIS.	
2	**If:**	**Then:**
	No growth or no grey colonies	Discard plate, *Campylobacter* not isolated.
	Grey colonies	Pick one representative colony of each morph type of grey colonies colonies. Perform gram stain, catalase, oxidase and hippurate.

Interpretation for CAMP Growth				
Gram	**Catalase**	**Oxidase**	**Hippurate**	**Next steps:**
Small, curved Gram-negative bacilli	Positive	Positive	Positive	Report as *C.jejuni*
Small, curved Gram-negative bacilli	Positive	Positive	Negative	Send to Prov.Lab for further ID
Any other gram stain, catalase and oxidase reactions are not *Campylobacter* and can be discarded.				

REPORTING RESULTS:

Results	Action
No growth after 3 days	• Report "Reduced commensal flora present. No *Salmonella*, *Shigella*, *Campylobacter*, *E.coli 0157*, *Yersinia* or *Aeromonas species* isolated" • Report "No *Plesiomonas shigelloides* or *Vibrio species* isolated" if requested.
Normal enteric flora isolated	• Report "No *Salmonella*, *Shigella*, *Campylobacter*, *E.coli 0157*, *Yersinia* or *Aeromonas species* isolated" • Report "No *Plesiomonas shigelloides* or *Vibrio species* isolated" if requested.
Overgrowth of S.aureus, P.aeruginosa or Yeast	• Report "No *Salmonella*, *Shigella*, *Campylobacter*, *E.coli 0157*, *Yersinia* or *Aeromonas species* isolated" • Report "Predominant or pure growth of *Staphylococcus aureus*" or "Predominant or pure growth *of Pseudomonas aeruginosa*" or "Predominant or pure growth of Yeast species"
Aeromonas isolated	• Report "No *Salmonella, Shigella, Campylobacter, E.coli 0157, Yersinia* isolated" • Report **"*Aeromonas species*"** • List quantitation as **"Isolated"** • Report AST as per ASTM.
Plesiomonas shigelloides or Vibrio species isolated	• If organisms are found incidentally, only report it if no other pathogens were isolated. • Report "Report "No *Salmonella, Shigella, Campylobacter, E.coli 0157, Yersinia* or *Aeromonas species*" • Report **"*Plesiomonas shigelloides* or *Vibrio species*"** • List quantitation as **"Isolated"** • Report AST as per ASTM.

Suspected Shigella	• Report "No *Salmonella, Campylobacter, E.coli 0157, Yersinia* or *Aeromonas* species isolated" • Report **"*Shigella group*"** with isolate comment **&SHIG** to state**: "Isolate has been referred to Alberta Provincial Laboratory for Public Health (Tel: 1 780 407 7121) for confirmation and typing"** • List quantitation as **"Possible"** • Report AST as per ASTM. • Freeze specimen and enter into specimen isolate log. • Go to Order Entry; copy report to Chief Medical Officer of Health (HPU) and Infection Control Nurse (SOHS) if in-patient. • Add test ?REFE. Send to Prov. Lab for confirmation and serotyping.
Salmonella	• Report "No *Shigella, Campylobacter, E.coli 0157, Yersinia* or *Aeromonas* species isolated" • Report **"*Salmonella spp*"** with isolate comment **&SALM** to state: **"Isolate has been referred to Alberta Provincial Laboratory for Public Health (Tel: 1 780 407 7121) for further identification and typing"** • List quantitation as **"Isolated"** • Report AST as per ASTM. • Freeze specimen and enter into specimen isolate log. • Phone results to ordering location and Chief Medical Officer of Health (HPU1) and document in call log. Results must be phoned to HPU1 within 24 hours of isolation. • Go to Order Entry; copy report to Chief Medical Officer of Health (HPU1) and Infection Control Nurse (SOHS) if in-patient. • Add test ?REFE. Send to Prov. Lab for further identification and serotyping.

E.coli 0157	• Report "No *Salmonella, Shigella, Campylobacter, Yersinia* or *Aeromonas* species isolated" • Report **"Escherichia coli 0157"** with isolate comment **&0157** to state: **"Isolate has been referred to Alberta Provincial Laboratory for Public Health (Tel: 1 780 407 7121) for confirmation and typing"** • List quantitation as **"Isolated"** • AST not performed on this organism. • Freeze specimen and enter into specimen isolate log. • Go to Order Entry; copy report to Chief Medical Officer of Health (HPU) and Infection Control Nurse (SOHS) if in-patient. • Add test ?REFE. Send to Prov. Lab for confirmation and serotyping.

Campylobacter	• Report "No *Salmonella, Shigella, E.coli 0157, Yersinia* or *Aeromonas* species isolated"
	• Report organism according to hippurate result.
	  ◦ Hippurate positive: Report **"*C.jejuni*"**
	  ◦ Hippurate negative: Report **"*Campylobacter spp*".** with isolate comment **&CAMP** to state: **"Isolate has been referred to Alberta Provincial Laboratory for Public Health (Tel: 1 780 407 7121) for further identification"**
	• List quantitation as **"Isolated"**
	• Report AST as per ASTM.
	• Freeze specimen and enter into specimen isolate log.
	• Go to Order Entry; copy report to Chief Medical Officer of Health (HPU) and Infection Control Nurse (SOHS) if in-patient.
	• Add test ?REFE. Send to Prov. Lab for further identification if hippurate result is negative.

Yersinia	• Report "No *Salmonella, Shigella, Campylobacter, E.coli 0157* or *Aeromonas* species isolated". • Report **"Yersinia spp"** with isolate comment **&YERS** to state: **"Isolate has been referred to Alberta Provincial Laboratory for Public Health (Tel: 1 780 407 7121) for confirmation and typing"** • List quantitation as **"Isolated"** • Report AST as per ASTM. • Freeze specimen and enter into specimen isolate log. • Go to Order Entry; copy report to Chief Medical Officer of Health (HPU) and Infection Control Nurse (SOHS) if in-patient. • Add test ?REFE. Send to Prov. Lab for confirmation and serotyping.

NOTES AND PRECAUTIONS:

All cultured organisms sent to referral lab must be sent according to current transportation of dangerous goods protocols.

PROCEDURE NOTES:

- Direct examination of fecal cultures is not indicated
- Fecal cultures should not be performed for patients being treated with broad-spectrum antimicrobial agents because it is likely that this therapy is responsible for the diarrhea. These cultures may show overgrowth of other organisms including Pseudomonas aeruginosa and Candida species – the role of which in disease production is not clear

REFERENCES:

Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016

Jorgensen J.H., Pfaller M.A., Carroll K.C., Funke G., Landry M.L., Richter S.S., Warnock D.W. 2015. Manual of Clinical Microbiology, 11th edition, ASM Press, Washington, D.C.

Northwest Territories Health and Social Services. NWT Reportable Diseases as per 2009 Public Health Act.
http://www.hss.gov.nt.ca/sites/default/files/nwt_communicable_disease_report_form.pdf

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	23 Jun 2017	Initial Release	L. Steven

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