PURPOSE: To determine the presence or absence of bacterial pathogens in stool specimens, including: *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	If the sample is received in the laboratory and processed greater than 24 hours from collection: • Add specimen quality comment: "Delayed transport may adversely affect pathogen recovery"
Storage Requirements	Refrigerated
Criteria for rejection and follow up action	1. Unlabeled/mislabeled specimen. 2. Specimen container label does not match patient identification on requisition. 3. Specimen leaking. 4. Duplicate specimen within 24 hours. 5. Specimen received greater than 72 hours after collection. 6. Not in enteric transport media and more than 2 hours old. 7. Stool on patients hospitalized > 3 days. 8. Stool with barium. 9. Specimens submitted in Ova and Parasite collection containers.

REAGENTS and/or MEDIA:

- Blood agar (BA), MacConkey agar (MAC), Hektoen agar (HEK), MacConkey Sorbitol agar (SOR), Campylobacter agar (CAM), Yersinia Selective agar (CIN) and Selenite broth (SEL)
- Identification reagents: catalase, oxidase, Salmonella Vi and O antisera, E.coli 0157 serology, hippurate discs, TSI/Urea slants, etc.

SUPPLIES:

- Disposable inoculation needles
- Biosafety cabinet
- Campylobacter microaerophilic jar and pouch
- 35° ambient air incubator
- Wooden sticks
- Sterile pipettes
- Vitek 2 and supplies

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 MIC60040 – Culture Media Quality Control procedure.
- Refer to Test 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 SOR. • Use a heavier inoculum for the HEK, CAM 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, SOR and HEK in 35° O_2 incubator. • Incubate CAM, 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	During the morning startup, after 18-24 hours incubation, subculture the selenite broth to HEK agar: • Saturate a sterile swab in the broth. • Swab the first quadrant of the agar. • Streak for isolated growth using a disposable inoculation needle. • Streak out to cover the whole plate.

INTERPRETATION OF RESULTS:

NOTE: Record all observations in the LIS.

Step	Action	
Examination of Blood agar		
1	Predominate or pure growth *P.aeruginosa*, *S.aureus* or *Yeast* spp.: • Identify with GNI, WP, TC and RS as appropriate • Yeast species does not have to be identified to the species level	
2	Perform sweep oxidase to screen for *Aeromonas* spp., which are oxidase positive and may be hemolytic. **NOTE:** If *Plesiomonas shigelloides* or *Vibrio* spp. 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 morphotype 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* spp., *Plesiomonas shigelloides* or *Vibrio* spp. • Perform susceptibility testing as per ASTM.

Step	Action	
Examination of MacConkey agar		
1	Examine plate for non-lactose fermenting colonies.	
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:**
Acid/Acid Gas or H_2S+	Positive		Discard
Acid/Acid Gas and H_2S -	Positive		Vitek GNI to rule out *Yersinia* spp. Perform susceptibility testing as per ASTM
Acid/acid Gas and H_2S -	Negative	Oxidase -	Discard
Acid/acid Gas and H_2S -	Negative	Oxidase +	Vitek GNI to rule out *Vibrio* spp. Perform susceptibility testing as per ASTM
Acid/acid Gas or H_2S+	Negative		Discard
Alkaline/alkaline Gas or H_2S +/-	Positive or Negative		Discard
Alkaline/acid Gas or H_2S +/-	Positive		Discard
Alkaline/acid Gas and H_2S -	Negative	Oxidase +	Vitek GNI to rule out *Aeromonas* spp. and *Plesiomonas shigelloides* Perform susceptibility testing as per ASTM
Alkaline/acid 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 susceptibility testing as per ASTM
Alkaline/acid 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 susceptibility testing as per ASTM

Step	Action	
Examination of Hektoen agar		
1	Examine plate for green (with or without black centers) or blue colonies.	
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		
Alkaline/alkaline Gas or H2S +/-	Negative	Discard		
Acid/acid Gas or H2S +/-	Negative	Discard		
Alkaline/acid Gas and H2S -	Negative	Vitek GNI	*Shigella:* • Vitek 2 AST-N213	• Perform susceptibility testing as per ASTM
Alkaline/acid 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.	
2	**IF**	**THEN**
	Sorbitol fermenters	Discard plate, no enteric pathogens isolated.
	Non-sorbitol fermenters	• Pick one representative colony of each morphotype 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 • Perform susceptibility testing as per ASTM

Step	Action	
Examination of Yersinia agar		
1	Examine plate for bull's eye colonies.	
2	**IF**	**THEN**
	No growth or no bulls eye colonies at 48 hours	Discard plate, *Yersinia* spp. 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:**
Alkaline/alkaline Gas or H2S+/-	Any reaction	Discard
Alkaline/acid Gas or H2S+/-	Any reaction	Discard
Acid/acid Gas or H2S+/-	Negative	Discard
Acid/acid Gas or H2S+	Positive	Discard
Acid/acid Gas and H2S-	Positive	• Vitek GNI to rule out *Yersinia* spp. • Perform susceptibility testing as per ASTM

Step	Action	
Examination of Campylobacter agar		
1	Examine plate for grey, flat, irregular, spreading, sometimes mucoid colonies.	
2	**IF**	**THEN**
	No growth or no grey colonies	Discard plate, *Campylobacter* spp. not isolated.
	Grey colonies	• Pick one representative colony of each morphotype of grey 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* spp. and can be discarded.				

REPORTING RESULTS:

IF	REPORT
No growth after 3 days	• Report: "Reduced commensal flora present" • Report: "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 or Yersinia isolated" • Report: **"Aeromonas species"** • List quantitation as **"Isolated"** • Report susceptibility as per ASTM.
Plesiomonas shigelloides or Vibrio species isolated	• If organisms are found incidentally, only report it if no other pathogens were isolated. • Report: "No Salmonella, Shigella, Campylobacter, E.coli 0157, Yersinia or Aeromonas species" • Report: **"Plesiomonas shigelloides"** or **"Vibrio species"** • List quantitation as **"Isolated"** • Report susceptibility 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 susceptibility as per ASTM. • Freeze specimen and enter into specimen isolate log. • In order entry, copy report to Chief Medical Officer of Health (HPU1) and if in-patient, Infection Control (SOHS). • Add test ?REFE and finalize with "." • Send to Provincial Laboratory for confirmation and serotyping. • Refer to MIC 10510 – Referral of Category B specimens to DynaLIFE and Provincial Laboratory.
Salmonella	• Report: "No Shigella, Campylobacter, E.coli 0157, Yersinia or Aeromonas species isolated" • Report: **"Salmonella species"** 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 susceptibility as per ASTM. • Freeze specimen and enter into specimen isolate log. • In order entry, copy report to Chief Medical Officer of Health (HPU1) and if in-patient, Infection Control (SOHS). • Add test ?REFE. and finalize with "." • Send to Provincial Laboratory for confirmation and serotyping. • Refer to MIC 10510 – Referral of Category B specimens to DynaLIFE and Provincial Laboratory.

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"** • Report susceptibility as per ASTM. • Freeze specimen and enter into specimen isolate log. • In order entry, copy report to Chief Medical Officer of Health (HPU1) and if in-patient, Infection Control (SOHS). • Add test ?REFE. and finalize with "." • Send to Provincial Laboratory for confirmation and serotyping. • Refer to MIC 10510 – Referral of Category B specimens to DynaLIFE and Provincial Laboratory.
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 susceptibility as per ASTM. • Freeze specimen and enter into specimen isolate log. • In order entry, copy report to Chief Medical Officer of Health (HPU1) and if in-patient, Infection Control (SOHS). • Add test ?REFE. and finalize with "." • Send to Provincial Laboratory for confirmation and serotyping. • Refer to MIC 10510 – Referral of Category B specimens to DynaLIFE and Provincial Laboratory.

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 susceptibility as per ASTM. • Freeze specimen and enter into specimen isolate log. • In order entry, copy report to Chief Medical Officer of Health (HPU1) and if in-patient, Infection Control (SOHS). • If hippurate result is negative, add test ?REFE. and finalize with "." • If hippurate result is negative, send to Provincial Laboratory for identification. • Refer to MIC 10510 – Referral of Category B specimens to DynaLIFE and Provincial Laboratory.

LIMITATIONS:

1. Direct examination of fecal cultures is not indicated
2. 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
2.0	30 Nov 2018	Updated to include new Vitek 2 instrument	L. Steven