Proc# **2013.013**

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| Procedure | Stool culture |

**Principle**: To describe the proper procedure for the collection of the stool specimen, inoculation of media, and identification procedures to be used.

**Specimen Required:** (Special Requirement if any): Feces collected in an appropriate transport media.

**Reagents:** (Name & where located): Appropriate media – BAP, MacConkeys, HE, Campy plate, MacConkeys/sorbitol, & GN Broth. Also requires Campy bag for appropriate incubation temp & atmosphere.

**Instrumentation**: 42o C & 35-36o C incubators.

**Instructions**:

I. Collection

1. Stool specimens must be set up within an hour of collection or submitted in Cairy Blair Transport Medium. When submitting the specimen in Cairy Blair, do not fill the container past the red fill line. Rectal swabs may also be submitted in the Cairy Blair media. Specimens in Cairy Blair are good for 72 hours.

II. Direct Smear

1. A direct Smear is to be performed **only** when the physician requests one.
2. The smear may be made directly from the Cairy Blair media.
3. When examining the smear, look for fecal leukocytes and large numbers of yeast, Campylobacter-like organisms, or staph-like organisms.
4. If the smear appears normal, report out as “No fecal WBC noted, appears to be mixed fecal flora.” The code GMF in the WalkAway will print this out. If it is abnormal, quantitate the WBC/OIF and report as “few fecal WBC noted” or “Multiple fecal WBC noted,” as the case may be.

III. Inoculation & Incubation

1. All stool specimens will initially be inoculated on the following media: BAP, MacConkey, MacConkey/sorbitol, Hektoen, GN Broth & a Campy plate.
2. All media except the Campy plate are incubated at 36o C in aerobic conditions.
3. The Campy plate is incubated at 42o C under reduced oxygen. To achieve this, use a BBL Campy bag and incubate the packet in the 42o C incubator.
4. After overnight incubation, subculture the GN Broth to MacConkeys & HE.

IV. Examination of the Cultures

1. On the HE plate look for green or black colonies. These are nonlactose fermenting &/or H2S positive organisms which are properties of *Salmonella* &/or *Shigella.*
2. On MacConkey, look for clear colonies which are nonlactose fermenting organisms as opposed to the red lactose fermenting organisms.
3. Look for growth on the Campy plate. *Campylobacter* will appear 1-2 days after inoculation. Gram stain and look for gram negative curved rods.
4. On the BAP look for large amounts of staphylococci & yeast.

V. Identification

1. To identify *Salmonella* or *Shigella*: Pick all suspicious looking colonies from the HE or MacConkeys and streak for a pure culture if needed. Also take several identical colonies and set up an Aerobic ID panel. (See procedure for MicroScan panels.)
2. To identify *Campylobacter* perform the following tests for presumptive ID:
3. Oxidase – Should be positive ( may be weak)
4. Catalase – Positive
5. Motility – Do a hanging drop motility. Inoculate a broth tube with suspected Campylobacter and incubate at 42o C for 30 minutes or so. Observe for darting motility.
6. Gram Stain – Look for characteristic curved gram negative rods.
7. Growth at 25o C and 42o C– There should be no growth at 25o C & growth at 42o C. This step is optional.
8. Sensitivity – Optional step for qualitative ID. Put cephalothin & Erythromycin discs on a BAP streaked with a 0.5 McFarland Std solution of organism. Incubate overnight at 42°C. Erythromycin is sensitive and cephalothin is resistant. This sensitivity is for ID purposes only!
9. Identify *Staphylococcus* by catalase & coagulase
10. Identify yeast with germ tube or other yeast identification procedure if necessary.
11. *Escherichia coli* O157:H7 is screened for with the use of the MacConkey/sorbitol agar. These organisms are usually sorbitol negative & will show as clear or colorless colonies on the MacConkey/sorbitol agar. If plate shows clear colonies, run a latex test for *E. coli* O157 antigen. (Procedure 1008.161) If this test is negative, then the organism is not an O157:H7. If the test is positive, subculture the organism & refer to SDSHL or CLM for H7 typing. Phone the physician or physician on call the preliminary results.

**Calculations:** none

**Controls:** not applicable (see individual procedures)

**Normals:** not applicable

**Comments/Limitations:**

1. The most often encountered bacterial pathogens are *Salmonella, Shigella, Campylobacter, & E. coli* O157:H7. In large numbers, *S. aureus* & yeast are noted.
2. Stools are not cultured for *C. difficule*. They are tested for *C. diff* antigen & toxin A with the Triage kit & not performed on routine cultures. It is run only when specifically ordered.
3. For normal fecal cultures, report as follows: “No *Salmonella, Shigella, Campylobacter* isolated. *E.coli* O157:H7 screen negative.”
4. If Campylobacter is isolated, report as *Campylobacter jejuni*.
5. All *Salmonella & Shigella* isolates are sent to the State Health Lab for serotyping. Note this in the final report & add the State Health Lab results when available.
6. Stool cultures are held at least 48 hr before the final report is issued.
7. If rectal swab for GC is ordered – plate on MTM and hold in candle jar at 36o C for 72 hrs. Check all growth for oxidase positive gram negative diplococci.
8. The MacConkey/sorbitol agar is only a screening mechanism & will not detect all isolates. If questions arise concerning the procedure, refer to section tech, pathologist, or technical supervisor.

**References:**

1. Manual of Clinical Microbiology, 3rd Edition, ASM, Washington, DC
2. “Background & Culture Techniques for *Campylobacter fetus,”* Wilson, Nancy & Wang, Wen-lan, Campylobacter Lab, VA Hospital, Colorado, 18 Nov. 1979.
3. Clinical Microbiology Procedures Handbook, Isenberg, H, Editor, ASM, Washington, DC 1992.

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