I. GENERAL PRINCIPLE

CULTURE

The purpose of a stool culture (STC) is either to identify the causative agent of diarrhea, or to detect the bacterial carrier state in a patient. An order for "stool culture for pathogens" is interpreted as a routine stool culture, and in this laboratory, includes screening for the presence of Salmonella, Shigella, Arizona, Edwardsiella, Campylobacter, shiga toxin producing E. coli, and for a predominance of Staphylococcus aureus, Pseudomonas, or yeast. Screening for E. coli O157:H7 by culture methods has been replaced by testing for E. coli shiga-like toxin. Enterohemorrhagic E. coli produce a toxin capable of causing bloody diarrhea and can lead to hemolytic uremic syndrome as a result of toxin damage to the kidneys. An order for isolation of a single specific organism, such as Yersinia, Vibrio, Clostridium difficile or Neisseria gonorrhoeae must be requested separately, over and above an order for routine stool culture.

OVA / PARASITE EXAM

Diagnosis of intestinal parasitic infection is dependent on laboratory demonstration of the diagnostic stage of the parasite. Identification is by gross and/or microscopic exam of feces for eggs, larvae, or protozoa. An order for O & P (OP) will result in a screen of the specimen for most parasites. However, specific orders for Giardia antigen (GIAR), Cryptosporidium (CRYPTOSM), or pinworms (PIN), have special collection criteria and/or detection methods and so must be ordered as separate, distinct procedures.

OCCULT BLOOD

Also known as stool guaiac, the hemoccult test (OCCB) is a qualitative method for detection of blood in the stool. It is intended as:

- a diagnostic aid during routine physical exams,
- a monitor for bleeding patients with iron deficiency anemia or recuperating from surgery,
- peptic ulcer,
- ulcerative colitis,
- or other conditions;
- and as a screen for colorectal cancer.

LEUKOCYTES

Chronic ulcerative colitis and chronic bacillary dysentery can cause passage of pus with the stool. Examination of stool for fecal leukocytes (STLWBC) may also be requested as stool WBC, fecal leukocytes, or stool leukocytes.

CLOSTRIDIUM DIFFICILE TOXIN SCREEN

Inhibition of normal intestinal flora due to antibiotic treatment can lead to overgrowth of the bacteria, Clostridium difficile. The organism produces toxins that can cause tissue damage, and the result is antibiotic-associated pseudomembranous colitis. This laboratory performs a rapid screening test for the presence of C difficile antigen and toxin A. Also available from a reference lab, are screens for toxin B and/or Clostridium difficile culture.
ROTAVIRUS

Rotaviruses are the major cause of viral gastroenteritis in infants and children, worldwide. Rotavirus infection is self-limiting but the aim of treatment is to prevent severe dehydration and electrolyte imbalance. Diagnosis of rotavirus infection is best accomplished by antigen detection, not by culture.

II. STOOL CULTURE

A. COLLECTION

1. A single properly collected specimen is usually enough to identify the cause of acute bacterial diarrhea. To detect a carrier state, single specimens for three consecutive days are recommended. Only one specimen per patient per day will be accepted.

2. All stool specimens for culture should be submitted in a transport media. The transport media is designed to maintain pH levels because some pathogens are sensitive to the pH changes associated with normal bacterial metabolism. Currently, the transport vial for culture has an orange cap and contains a red-pink fluid. If the fluid is yellow, do not use the vial; or if the fluid turns yellow after the specimen has been added, it is not acceptable for culture and must be recollected.

3. Stool specimens should be collected in a clean, dry container. Stool specimens should not be contaminated with water, urine, barium, or mineral oil.

4. Transfer specimen from the collection container into a transport vial ASAP. Use the "spork" in the cap lid to sample the stool, particularly areas of blood or mucous. Pick up or pour a quantity of stool sufficient to bring the fluid volume up to the "fill-to-here" line. Do not overfill the transport vial.

5. Tighten the cap and shake well to mix.

6. Alternative collection methods:

   a. For children in diapers, if the quantity is sufficient to bring the transport fluid up to the fill-to-here line, scrape up the stool with the spork in the cap assembly of the transport vial, recap, shake well, and submit.

   b. For children with a watery diarrhea that soaks into the diaper, place a pedi urine collection bag over the child's anal area. Monitor closely, so the bag can be removed ASAP after a bowel movement to preserve as much specimen as possible in the bag. Watery specimens will tend to leak out of the pedi bag. Cut a corner from the bottom of the bag and pour contents into a transport vial.

   c. Rectal swabs are substituted for stool culture specimens only if absolutely necessary. Insert sterile culturette swab a short distance into the rectum. Hold in place for a minimum of 30 seconds to allow the swab to absorb specimen and withdraw. Remove cap and place swab in culturette sleeve. Squeeze sponge to moisten swab.

7. Please note any mention of a specific organism on the request form and/or request specific culture type in the computer. See special requests section below.
B. TRANSPORT

1. Specimens are acceptable for culture as long as the transport fluid has not turned yellow.

2. Do not refrigerate.

3. Transport ASAP to the laboratory.

C. SPECIAL REQUESTS

1. Culture for single, specific organisms -- *Yersinia, Vibrio, Clostridium difficile*
   a. Place stool specimen in an orange cap preservative vial, as instructed above.
   b. Request as a specific culture type. **Do not issue request as a comment attached to a routine culture order.**
   c. A single orange cap vial is sufficient for both a routine stool culture and a request for isolation of a single organism.

2. Culture for *Neisseria gonorrhoeae*
   a. Notify laboratory before collection.
   b. Request as GC culture (GCC).
   c. Collect a rectal swab, as described above in alternative collection methods.
   d. Transport to the laboratory immediately.

III. OVA / PARASITE EXAM

A. COLLECTION

1. **Three specimens** collected on **three successive days** are recommended for optimum parasite detection. Collect specimen in a clean, dry container. **Stool swabs are unacceptable for O&P exams.**

2. Interfering substances - Certain substances and medications interfere with parasite detection. These are listed below:
   - Specimen should not contain water or urine.
   - Contamination with mineral oil, barium, bismuth, antibiotics, anti-malarials, or non-absorbable antidiarrheal agents can prevent parasite recovery for one to several weeks.
   - Purging or bowel prep agents must be cleared before the specimen can be submitted for O&P exam, as they are crystalline in nature and obscure any parasitic elements that might be present.
   - Specimen collection should take place a minimum of 7 days after administration of any of the interfering agents mentioned above.

3. **All specimens should be placed in fixative ASAP** after collection.
a. A two-vial system is currently in use for ova and parasite fixation and preservation. Both vials must be submitted for the complete OP exam.
   • The pink cap vial contains formalin and is used in an iodine concentration procedure.
   • The blue or gray cap vial contains PVA and is used to prepare a permanent stained smear.
b. Pour over or use the "spork" provided in the cap lid to sample the specimen, in particular areas of blood or mucous.
c. Add specimen to each vial until fluid level reaches the fill-to-here line. **Do not overfill the vials.**
d. Tighten caps and shake vigorously to mix.

4. **Note specimen consistency** on the outside of the vial and/or on the computer order.
   - **Liquid** = of pourable consistency
   - **Soft** = having no shape
   - **Formed** = having a definite shape

5. Include recent travel history of the patient, if known.

6. Unpreserved liquid specimens may be submitted to the lab in a clean, dry container, **immediately** after passage, if amoebiasis is suspected. A direct wet mount procedure can be performed to look for motile protozoans.

B. **TRANSPORT**

1. Request should include all pertinent clinical information, including suspected diagnosis and travel history. Note any specific parasite the physician mentions by name.

2. Transport to the laboratory **ASAP. Do not refrigerate.**

3. Fresh liquid stool must be transported immediately and rapidly to the lab and hand delivered to the technologist on duty with verbal instructions to look for amoebae.

C. **SPECIAL REQUEST**

1. **Giardia Antigen**
   a. Collect specimen as for O & P.
   b. Transfer stool to **only the pink cap vial** (formalin) of the O & P collection set.

2. **Cryptosporidium**
   a. Collect specimen as for O & P.
   b. Transfer stool to **only the pink cap vial** (formalin) of the O & P collection set.

3. **Pinworm**
   a. Contact the laboratory for commercial collection kit or **clear Scotch tape** and a glass slide. **Opaque tape is unacceptable.**
b. As the patient sleeps at night, the pinworm emerges from the rectum to deposit eggs on and around the anus. Collect the specimen first thing after patient awakens in the morning, before arising from bed. Timing is critical for detection of eggs.

c. Loop a strip of clear tape length-wise down a wooden tongue depresser, around the end and back up the other side of the stick, sticky side out. Hold both ends of the tape securely against the tongue depressor.

d. Have the patient lie face down and spread the buttocks.

e. Press the sticky side of the tape against several areas of the perianal region.

f. Place the tape, sticky side down on a glass slide and smooth with cotton or gauze.

g. Transport to laboratory ASAP.

IV. OCCULT BLOOD

A. COLLECTION

1. There are no restrictions on the number of times an occult blood test may be ordered. Logically, if the first specimen is positive, the reason for testing should be evaluated before ordering additional tests. However, with an initial negative test, additional specimens may be required. Since gastrointestinal lesions may bleed intermittently, the recommendation is a specimen from three consecutive bowel movements.

2. **IF PATIENT PREPARATION GUIDELINES CANNOT BE MET, BE ADVISED THAT RESULTS MAY BE FALSELY POSITIVE OR FALSELY NEGATIVE**, and evaluate your patient accordingly.

   • For females, do not submit specimens during, or until three days after a menstrual period.

   • Do not submit specimens while the patient has bleeding hemorrhoids or blood in the urine.

   • For 7 days prior to and during the collection period, avoid aspirin or other non-steroidal anti-inflammatory drugs, anticoagulants, or any substance which could irritate the gastrointestinal tract, including alcohol.

   • For 72 hours prior to and during the collection period, avoid:

     Vitamin C, or iron supplements containing Vitamin C
     Red meat
     Artichokes, mushrooms, bean sprouts, apples, oranges, bananas, grapes.

   • If specimen is collected over a toilet bowl, remove toilet bowl cleaners from the tank and flush twice before proceeding.

3. Place stool specimen in a clean, dry container. The Hemoccult test requires only a small amount of specimen, but submit enough of a quantity so that the stool does not dry out before reaching the lab.
4. Alternative collection method:
   a. If Hemoccult cards are available, specimen may be applied to the card at the bedside.
   b. Flip open the cover of the card to expose the two areas of guaiac paper. Use a wooden applicator to sample the stool and apply a THIN SMEAR to box A. Use the same applicator to sample a second, different area of the stool specimen and repeat the THIN SMEAR for box B. Discard the applicator and close the cover flap.
   c. Label the card and return it to the laboratory. DEVELOPMENT OF THE TEST MUST TAKE PLACE IN THE LABORATORY OR BY A PHYSICIAN, IF PERFORMED IN A PATIENT AREA.

B. TRANSPORT
   1. Transport stool in containers to the laboratory as rapidly as necessary to prevent drying of the specimen.
   2. Hemoccult slides prepared at the bedside may be stored for up to 14 days at room temperature before development.

V. FECAL LEUKOCYTES

A. COLLECTION
   1. Only liquid stools or those containing recognizable mucous should be submitted for microscopic examination of stool leukocytes. Formed stool is an unacceptable specimen.
   2. Collect specimen prior to procedures requiring administration of barium.
   3. Specimen should be submitted in a clean, dry container.

B. TRANSPORT
   1. Transport specimen to the laboratory ASAP after collection. Significant deterioration occurs with prolonged storage.
   2. Refrigerate specimen if necessary due to delay in transport.
VI. **CLOSTRIDIUM DIFFICILE ANTIGEN / TOXIN SCREEN**

A. **COLLECTION**
   1. **Formed stools are not acceptable** for *C. difficile* toxin testing.
   2. A diarrheal type stool specimen should be collected in a clean, dry container from a patient with a history of previous or current antibiotic usage.
   3. An initial positive report should cause re-evaluation of additional test orders.

B. **TRANSPORT**
   1. Transport immediately to the laboratory.
   2. If transport is delayed, **refrigerate**. Stool may be stored for up to 3 days at 2-8° C. If this much delay has occurred, be sure to note on the request.

V. **ROTOVIRUS**

A. **COLLECTION**
   1. Submit stool or feces laden rectal swab in a clean dry container.
   2. Stool in preservative is unacceptable.

B. **TRANSPORT**
   1. Transport to laboratory ASAP on cold packs or ice.
   2. Refrigerate if transport is delayed.

**WRITTEN BY:** D’Ann Spinks, MT(ASCP)  **DATE:** 6-96

**APPROVED BY:** Martin F. Belli, M.D.  **DATE:** 6-96

**REVISED BY:** D’Ann Spinks, MT(ASCP)  **DATE:** 4-99, 5-2000, 11-2001, 4-2002, 2-2006, 11-2006, 3-2008, 2-2014

**APPROVED BY:** Martin F. Belli, M.D.  **DATE:** 4-99, 5-2000, 11-2001, 4-2002, 2-2006, 11-2006, 3-2008, 2-2014

*See original policy in the Laboratory for all documented biennial reviews.*
REFERENCES:

Clinical Microbiology Procedures Handbook; 1992; Isenberg; American Society for Microbiology.


A Guide to Specimen Management in Clinical Microbiology; 1999, 2nd edition; Miller; American Society for Microbiology


Parasites: A guide to laboratory procedures and identification; 1987; Ash and Orihel; ASCP Press.


Package inserts.