Policy: The Scoop on Poop - Number and Timing of Stool Specimen Collection

In order to clarify the appropriate means of stool testing and to provide a clinically relevant, cost-effective approach to the evaluation of diarrheal illness, the Elliot laboratory has developed a policy for the number and timing of stool specimen collections submitted for routine bacterial and parasitology testing.

Routine bacterial culture recommendations for diagnostic testing

A routine stool culture will detect the following: Salmonella species, Shigella species, Campylobacter species, Shiga-toxin producing *Escherichia coli*, Aeromonas species, and *Plesiomonas shigelloides*. In addition, by special request, the Laboratory will perform stool culture for the detection of Yersinia species and Vibrio species.

1. For stool culture, the Laboratory will accept no more than two specimens per patient, collected on consecutive days (preferably at least 24 hours apart). Multiple stool specimens are rarely indicated for detection of bacterial stool pathogens causing enterocolitis. In studies of adult and pediatric patients who submitted more than 1 specimen, the enteric pathogen was detected in the first sample 87-94% of the time, with the second specimen bringing the positive rate up to 98%. If the initial stool culture is negative, then an additional stool sample may be submitted for testing. Multiple stool samples from the same patient submitted on the same day will not be cultured. Also, the Lab will reject more than three stools from the same patient in a 3-week period.

2. The Laboratory will not accept stool specimens submitted for culture from inpatients after the third hospital day without prior consultation from the Microbiology Laboratory or an Infectious Disease physician. The Lab will reject stool cultures received from adults and older (≥16 yo) pediatric patients hospitalized for >3 days, unless the patient is known to be HIV positive or in a case of a cluster epidemic within the institution. Diarrheal stool samples from infants and toddlers should not be rejected after the fourth day of hospitalization since studies have shown that it may take longer to collect stool samples from pediatric patients admitted with gastroenteritis that are placed on bowel rest and are not eating a normal diet. For adults, data has shown that entero-pathogenic bacteria other than *Clostridium difficile* are grown from 0.5% of cultures obtained more than 3 days after hospital admission compared with a 10-fold higher yield of tests for *Clostridium difficile* toxin. Thus, testing for *C.difficile* infection and consideration of non-infectious causes such as antibiotic-associated or osmotic diarrhea should be the first steps in the evaluation of nosocomial diarrhea.

3. At least 1 gram of stool should be collected and submitted for bacterial culture testing on each of 2 consecutive days. More stool specimen will be required for multiple test requests on the same sample. Stool specimens should be collected in a clean, wide mouthed plastic container with a tight-fitting lid. The specimens should not be contaminated with water or urine. It is not appropriate for the Laboratory to process hard, solid, formed stools for bacterial culture or *C.difficile* toxin assay testing by PCR. The specimen of choice to diagnose diarrheal illness is diarrheal stool, not a formed stool or a swab.

4. Stool culture should not be performed for patients being treated with broad-spectrum antimicrobial agents, because it is likely that the antimicrobial therapy is responsible for the diarrhea. There may be overgrowth with other bacteria, including *Pseudomonas aeruginosa*, and Candida species, the role of which in disease production is not clear. Their presence will be reported along with a statement indicating that the organism was the predominant organism recovered and that expected enteric organisms were not present, which suggests an antimicrobial inhibition.

5. For *Clostridium difficile* toxin assay (by PCR) testing, a single loose, watery, unformed diarrheal stool specimen is recommended as sufficient for *C. difficile* testing. The specimen can be held for up to 24 hours at room temperature but must be tested within this time frame. Alternately, store the collected specimen at 2-8°C (refrigerated) for up to 5 days. Repeat testing of patients negative by the *C.difficile* toxin assay PCR test should not be performed for 7-14 days. The *C.difficile* PCR nucleic acid amplification test performed at the Elliot Hospital is not FDA approved for use on formed stools, stools from ostomy contents, or on patients less than 2 years of age. Only when a physician notes that the patient has ileus, a formed stool specimen submitted for *C. difficile* PCR testing will be sent to the reference laboratory for testing. Also, bear in mind, positive test results for *Clostridium difficile* do not correlate well with disease in young children. For the testing of pediatric patients under age two, stool specimens will be sent to the reference laboratory for testing. Please refer to Policy Manager for the Infection Prevention *C.difficile* testing policy for testing criteria.

6. The Lab will not accept rectal swabs for bacterial stool culture. It is appropriate, however, to collect swabs from rectal/anal folds for a VRE screen.

7. It is appropriate to submit stool specimens during the acute stage of infection (usually 5-7 days) because pathogens decrease in number with time.

8. If fresh stool is submitted for culture that is not preserved in transport medium, the specimen must be transported to the Laboratory for processing within 2 hours of collection. The Lab is only able to accept stool to perform a Gram stain for leukocytes on an unpreserved specimen within two hours of collection.

9. If fresh stool is submitted for culture in transport medium, the preserved specimen may be held at room temperature and transported to the Lab within 24 hours for the best recovery of pathogens. Keep in mind, refrigeration of the specimen may adversely affect the ability of the Laboratory to recover Shigella species.

Routine parasitology recommendations for diagnostic testing

Stool antigen testing (Ova & Parasite Screen by EIA) performed at the Elliot Microbiology Lab is the optimal test method for determining the parasitic presence of Giardia cyst antigen and Cryptosporidium oocyst antigen.

Full Ova & Parasite stool testing by wet mount and trichrome stain performed at the reference laboratory will detect intestinal parasites. The reference laboratory requests submission of three separate stool specimens within a 10-day period with an individual order submitted for each specimen. The full O&P Alcor Parasep device provided by the laboratory for specimen transport must be used within ONE hour of specimen collection. Please note: full O&P exam performed at the reference laboratory does not specifically detect Cryptosporidiuim, Cyclospora, Cystoisospora, and Microsporidia. By special request, the presence of these organisms in stool can be identified by the reference laboratory. Also, note stool antigen testing for the presence of *Entamoeba histolytica antigen*, the optimal test method, can be performed at the reference laboratory.

1. Cost-effective testing includes the examination of a single diarrheal stool specimen. A second specimen for O&P EIA screen is suggested only when the first is negative for common Giardia and Cryptosporidium parasites and the patient remains symptomatic. A third specimen can be submitted only if the patient continues to be O&P negative and symptomatic. At this time it is wise to consider a full O&P examination. For O&P screen, the Laboratory will not accept more than three specimens per patient collected and submitted preferably on separate days. Many organisms, particularly the intestinal protozoa, do not appear in the stool in consistent numbers on a daily basis, and the series of three specimens collected every other day is considered adequate for

parasitic screening; otherwise the series of three specimens should be submitted within no more than 10 days. Multiple specimens from the same patient should not be submitted on the same day. One possible exception would be a patient who has severe, watery diarrhea, in whom any organisms present might be missed because of a tremendous dilution factor related to fluid loss. These specimens will be accepted only after consultation with the physician.

2. The Laboratory will not accept specimens for parasitic detection from inpatients after the fourth hospital day, without prior consultation from the Microbiology Laboratory or an Infectious Disease physician.

3. Stool specimens should be collected in a clean, wide mouthed plastic container with a tight-fitting lid. The specimens should not be contaminated with water or urine because water may contain free-living organisms that can be mistaken for human parasites and urine may destroy motile organisms. The presumptive diagnosis or relevant travel history information is helpful and should accompany the test request. If there are delays from the time of specimen passage until examination in the laboratory, the use of stool preservative should be considered. Specimens preserved in stool fixatives should be stored at room temperature. It is also important to use the correct ratio of stool and fixative to ensure proper fixation. Commercial vials are marked with a "fill-to" line for the addition of stool to the container. The stool preservative container should not be over-filled or under-filled.

4. Collection of stool for parasite detection should always be performed before barium is used for radiological exam. Intestinal protozoa may be undetectable for 5 to 10 days after barium is given to a patient. The following substances may also interfere with the detection of intestinal protozoa. These include: mineral oil, bismuth, antibiotics (metronidazole or tetracycline), antimalarial agents, and non-absorbable antidiarrheal preparations. After administration of any of these compounds, parasites may not be recovered for a week to several weeks. Therefore, specimen collection should be delayed for 5-10 days or at least 2 weeks after barium or antibiotics, respectively, are administered.

5. Three specimens have also been recommended for post-therapy examinations. However, a patient who has received treatment for a protozoan infection should be checked 3 to 4 weeks after therapy and those treated for Taenia infections should be checked 5 to 6 weeks after therapy. In many cases, post-therapy specimens are not collected, often as a cost containment measure. If the patient becomes symptomatic again, additional stool specimens can be submitted. The probability of detecting clinically relevant parasites in a single stool specimen may be as low as 50 to 60% but is >95% if three samples are examined.

6. To evaluate patients who are at risk for giardiasis, the negative predictive value of some of the immune-assays on a single stool specimen is not sufficiently high to exclude the possibility of a *Giardia lamblia* infection. In cases where the clinical suspicion for *G. lamblia* infection is moderate or high and the first assay yields a negative result, testing of a second specimen is recommended.

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