

Laboratory Guideline for

Ordering Stool Test for Investigation of Suspected Infectious Diarrhea

This guideline has been developed by an Alberta Clinical Practice Guidelines Program working group and is based on current scientific evidence. The opinions of laboratory physicians, family physicians, pediatricians, internists, gastroenterologists, public health and infectious disease physicians were used in the preparation of this guideline. The recommendations in this guideline reflect geographic and seasonal considerations in Alberta related to infectious diarrhea. This guideline was last reviewed in 2002.

GOALS

These guidelines are intended to assist practitioners with the following:

- ◆ To improve the accuracy of stool testing for infectious diarrhea.
- ◆ To improve convenience for patients and parents.
- ◆ To implement the practice of a single initial stool test sample.

EXCLUSIONS

These guidelines may not apply to the following:

- ◆ Patients involved in a community or hospital outbreak.
- ◆ Food handlers to whom Public Health regulations apply.
- ◆ Where an infectious etiology is not a consideration.

RECOMMENDATIONS

Stool testing may be required for patients with diarrhea. The following recommendations should guide the ordering of stool bacterial cultures (C & S), stool ova & parasite (O & P) tests and *C. difficile* toxin tests:

- ◆ A CLINICAL HISTORY should be provided to the laboratory, including the type and duration of symptoms, underlying medical conditions, recent travel and recent or current antibiotic therapy.
- ◆ A SINGLE stool test should be initially ordered where indicated (see algorithm.)
- ◆ Stool C & S and/or stool O & P tests are usually not clinically indicated for patients with onset of diarrhea - 4 days after hospitalization (see algorithm.)
- ◆ Consultation with an appropriate specialist is recommended in circumstances where additional stool tests may be useful. Additional stool test(s) may be done if there is continued suspicion of enteric bacterial infection when an initial sample is negative, and recent travel to areas where *E. histolytica*, *Giardia lamblia* or *helminth* infections are common.

The above recommendations are systematically developed statements to assist practitioner and patient decisions about testing that may be important for clinical management of specific clinical circumstances. They should be used as an adjunct to sound clinical decision making.

BACKGROUND

DEFINITIONS

1. Stool C & S

Stool culture for the isolation and identification of enteric bacterial pathogens requires collection of an adequate stool sample, planting of the specimen onto a number of selective and differential media, incubation of the plates one or more times, selection of appropriate colonies from incubated plates for identification, and in a limited number of cases, the provision of an antimicrobial susceptibility result.

2. Stool O & P

Stool examination for the diagnosis of enteric parasitic colonization and infection involves submitting a stool specimen in fixative to the laboratory, preparation of the stool for staining and examination (i.e., concentration and/or filtration), and staining with a standard stain such as iron haematoxylin.

3. *C. difficile* Toxin

Stool testing for diagnosis of antibiotic associated colitis involves submitting a stool specimen in a sterile container to the laboratory, for detection of *C. difficile* toxin(s) using a variety of different assay methods. There is controversy about the optimal method of toxin(s) detection. Currently, the three major assay methods include enzyme immunoassay for toxins A and B, latex agglutination assays for toxins A and B, and cell culture cytotoxicity assays with specific neutralization of toxin B. Culture of *C. difficile* from stool is not considered diagnostic since a significant number of children and adults carry this organism as part of their normal colonic flora.

4. Enteric Viral Infections

Enteric viral infections are diagnosed by submitting a stool sample in a sterile container using a variety of different methods depending upon the type of virus(es) being sought. Although rapid antigen detection testing for Rotavirus infection in children is widely available, most other viral tests are only done by the Provincial Public Health Laboratories in Calgary and Edmonton.

ENTERIC BACTERIAL INFECTIONS

Patients with bacterial enterocolitis typically present with the acute onset of diarrhea and may have associated fever, crampy lower abdominal pain and tenesmus. Although bloody diarrhea is more common when enterocolitis is caused by enteroinvasive pathogens such as *Shigella spp.*, not all such patients with these infections will develop haematochezia. A stool C & S test must be done to diagnose suspected enteric bacterial infection definitively.

Acute bacterial enterocolitis is acquired by ingestion of the organism in food(s) and/or water contaminated by feces of an infected animal or person. Commonly implicated food sources include raw and under-cooked eggs and egg products, raw milk and milk products, meat, salads and poultry and their products. Direct contact with an infected animal (e.g., pet turtles or chicks) may also transmit Salmonellosis. The incidence of enteric bacterial infections is highest in travellers to underdeveloped countries where food handling practices and sanitary conditions may be poor. Epidemics may also occur in families, in groups attending a restaurant or social function where a common contaminated food source is ingested, or in institutions such as daycare centres and nursing homes.

In developed areas of the world, a limited number of bacterial pathogens are responsible for acute bacterial enterocolitis, as outlined in Table 1.

Table 1. Enteric Bacterial Pathogens

- *Campylobacter jejuni*
- *Salmonella spp.*
- *Verotoxin producing E. coli* (e.g., *E. coli* O157:H7)
- *Shigella spp.*
- *Aeromonas spp. (toxin producing strains)*
- *Clostridium difficile*
- *Yersinia enterocolitica*
- *Pleisiomonas shigelloides*

It remains controversial whether all or only toxin producing strains of *Aeromonas spp.* from stool samples are pathogenic.

Vibrio spp. infections are uncommon but are usually reported in travellers to underdeveloped countries where sanitary conditions are poor (e.g., *Vibrio cholerae*), or in people eating contaminated shellfish (e.g., *Vibrio parahaemolyticus*).

In Alberta, enteric infections occur year-round but have a definite peak in the summer months. This likely happens because of improper food handling practices which are associated with outdoor activities. Infections which occur at other times of the year are more likely to have been acquired while travelling in underdeveloped countries.

Table 1 reflects the order of incidence of pathogens. *Campylobacter* infections are the most common enteric disease in Alberta. There is also no difference in the pattern or frequency of isolation of the types of bacterial pathogens isolated from patients admitted to the hospital with a primary diagnosis of acute enterocolitis, versus those attending outpatient areas including the emergency room.^{1,2,3}

C. difficile is the main cause of antibiotic associated diarrhea and pseudomembranous colitis. *C. difficile* has become one of the most commonly detected enteric pathogens, particularly in hospitalized and nursing home patients where infection may be nosocomially transmitted. Symptoms range from mild diarrhea to the most severe form of the disease, toxic megacolon, where bowel perforation may require total colectomy. Rarely, death may occur if appropriate treatment is not given. Although microbial agents of all classes may cause this problem, the most commonly implicated agents include ampicillin, clindamycin and cephalosporins. *C. difficile* toxin mediated colitis may also rarely occur in patients who have not recently received antibiotic treatment.

Physicians should take a clinical history before ordering stool cultures. History should include questions about recent travel, other risk factors for acquiring bacterial infections and exposure to other people with similar symptoms.

ENTERIC PARASITE INFECTION

Patients with enteric infection with protozoan parasites may present with persistent diarrhea (i.e., ≥ 5 days duration). Intestinal parasite in-

fection may also cause abdominal bloating and flatulence, but symptoms alone cannot distinguish between enteric infection with protozoa versus bacteria. Some patients who have recently travelled to or immigrated from under-developed countries may have mixed infections. Microscopic examination of stool remains the main technique for confirming the presence of enteric parasitic infection, although antigen detection techniques may assume a greater role in the future.

Colonization and/or infection with protozoa is therefore most common in people who have travelled to an underdeveloped country where sanitary conditions may be poor, in children attending day care centres, in institutionalized patients, and in homosexual men.

Although all enteric protozoa encountered in a given stool specimen may be reported, not all may require specific antimicrobial treatment (Table 2).

Table 2. Pathogenic Enteric Protozoa

- *Giardia lamblia (intestinalis)*
- *Cryptosporidium spp.*
- *Entamoeba histolytica*
- *Dientamoeba fragilis*
- *Microsporidium spp.*
- *Blastocystis hominis*
- *Cyclospora spp.*
- *Isospora belli*

Although the role of *Blastocystis hominis* in causing symptoms is controversial, it may be pathogenic in patients where no other cause is found for diarrhea. Other enteric protozoa not listed above may frequently colonize the bowel, but have not been documented to cause diarrhea or other gastrointestinal symptoms even in immunocompromised patients.

Although intestinal helminth infections rarely cause diarrhea, stool O & P tests are also used to diagnose these infections including roundworms (nematodes) such as hookworm, Strongyloidiasis, Ascariasis etc., tapeworms (cestodes) such as *Taenia spp.*, *Hymenolepsis spp.*, etc. and flukes (trematodes)

such as Schistoso-miasis, Clonorchiasis etc. Because the exact number of stool examinations needed to diagnose enteric helminth infections is not known, additional stool samples for examination (i.e., 2 or 3 sequential samples) may be required. Three investigations may also be required if *E. histolytica*, or *Giardia lamblia* infection is suspected.

Enteric Viral Infections

Viral gastroenteritis occurs predominately in two distinct clinical forms. The first pattern of disease is typified by Rotavirus which primarily causes severe, acute watery diarrhea in infants and young children each year during the winter months, although sporadic cases can occur at other times during the year. Rotavirus infection may also be transmitted amongst hospitalized children, and nosocomial Rotavirus outbreaks are well described. Enteric adenovirus 40/41 infections are a much less common cause of pediatric diarrhea but sporadic cases may be reported. Caliciviruses and astroviruses may also cause diarrhea in children, but in general, these viruses are associated with milder diarrheal symptoms. Calicivirus infection may be more prevalent in children in day care. Enterovirus infections most often present as aseptic meningitis and rarely cause diarrheal illness. The second pattern of illness is typified by Norwalk virus and is characterized by evolving community-wide outbreaks which affect one or more family members as well as their contacts. Most symptomatic viral gastroenteritis illness in adults occurs within this setting, and outbreaks in institutionalized elderly patients have been documented. Table 3 outlines the most common types of enteric viral infections diagnosed in Alberta in order of decreasing frequency from left to right.

Table 3. Enteric Viral Infections

- | | |
|--------------------|------------------------|
| • Rotavirus | • Astroviruses |
| • Adenovirus 40/41 | • Norwalk virus |
| • Caliciviruses | • Norwalk-like viruses |

CLINICAL HISTORY

A clinical history is essential for the efficient and appropriate work-up of stool C & S and stool O & P samples. The following information must be provided on the requisition:

- ◆ Clinical symptoms and duration

- ◆ Type of suspected infection/exposure
- ◆ Underlying disease(s) - immunosuppression
- ◆ Recent travel history (Dates/Location)
- ◆ Recent or current antibiotic therapy

1. Enteric Bacterial Infections

Microbiology laboratories routinely inoculate stool C & S specimens onto a variety of selective and differential medias and into broth which enhances the isolation of all major enteric bacterial pathogens of interest. Special planting media to recover *Vibrio spp.* will only be inoculated when symptomatic patients have recently travelled to an endemic area for *V. cholerae* infection, or have recently ingested shellfish (i.e., suspected *V. parahaemolyticus* infection). A history of recent or current antibiotic use should be provided when ordering *C. difficile* toxin testing. Most microbiology laboratories only perform toxin(s) testing and do not do stool cultures for the organism.

2. Enteric Parasitic Infections

Routine stool staining and examination will detect most enteric parasites of interest except for *Cryptosporidium spp.*, *Microsporidium spp.* and *Cyclospora spp.* Special stool concentration and staining methods are required to detect these infections. The microbiology laboratory may only perform these tests if an appropriate history is provided (i.e., child in a day care centre, HIV seropositive patient, recent travel to the tropics or farm exposure). In addition, recent or current antibiotic therapy may decrease the laboratory's ability to detect enteric parasite infection.

3. Enteric Viral Infections

Laboratory testing for enteric viral infections is not usually necessary except in young children with acute, severe acute diarrheal illness during the late fall and winter months when Rotavirus infection becomes epidemic each year. Rapid antigen detection tests such as latex agglutination or enzyme immunoassay are widely available for Rotavirus infections. Although Adenovirus 40/41 infection occurs much less frequently, it can also be diagnosed using an enzyme immunoassay. Electron microscopy and stool viral cultures must be performed to diagnose other types of enteric viral infections.

RESEARCH FINDINGS

Basis of the Multiple Sample Rule

Historically, 3 sequential stool samples (i.e., different bowel movements) for stool C & S and stool O & P testing were ordered for diagnosis of enteric bacterial and/or parasitic infection respectively. The Centre for Disease Control (CDC) in the United States recommended the practice of performing multiple sequential stool O & P examinations based upon studies which showed that the rate of recovery of *E. histolytica* from asymptomatic patients increased from 50%, with only a single stool sample examination to 90%, after examination of 6 sequential stool samples.⁴ Several other studies have also reported that multiple stool specimens are required to achieve adequate sensitivity of recovery of parasites on examination of stool specimens.^{5,6} However, these studies were epidemiological in nature, aimed at primarily diagnosing asymptomatic *E. histolytica* excretion in patients in the underdeveloped world, and stool concentration methods were not always used. It has become clear that these guidelines may not be applicable or appropriate for patients in the developed world where there is not a high incidence of *E. histolytica* infection. There has also not been scientific evidence to support the routine ordering of multiple sequential stool C & S samples, and this practice was likely extrapolated from the recommendations for stool O & P testing.

Evidence for the Single Sample Rule

Studies have documented the high sensitivity of a single stool culture for enteric bacterial pathogens and stool examination for parasites in a large general hospital setting.⁷⁻¹¹ Work from the Alberta Children's Hospital has also demonstrated the high efficiency for diagnosis of enteric infections of a single initial stool culture and stool parasite examination in both hospitalized and ambulatory children.^{1,11} Most pediatric cases of enterocolitis (190 of 194, 98%) are confirmed from a single stool culture, and a second sample is seldom required.^{1,3} Most clinically relevant protozoal infections (102 of 112, 91%) were also detected in the first stool specimen examined.³ Infections which would have been missed on a single stool O & P specimen included

4 children with Giardiasis, 4 children with *Dientamoeba fragilis*, 2 children with *Blastocystis hominis* and 2 children with Cryptosporidiosis.

However, due to the retrospective design of the study, the clinical significance of any of these cases in terms of patient symptoms or institution of treatment could not be ascertained.

Evidence for Not Performing Stool C & S or Stool O & P Tests on Hospitalized Patients (i.e., ≥ 4 days in hospital)

It has been well documented that there is a very low yield of finding enteric infections (i.e., bacterial or protozoal) with onset ≥ 4 days after hospitalization regardless of the age of the patient or their immune status.^{1,7-11} Stool C & S and stool O & P tests are therefore not recommended in hospitalized patients except in rare situations where the patient has recently travelled to an underdeveloped area, or where an additional stool examination is required for follow up of a previously diagnosed infection.

Most diarrhea in hospitalized patients is due to *C. difficile* toxin-mediated colitis when antibiotics have been recently given, i.e., symptoms may occur up to 8 weeks after antimicrobial(s) were stopped. Viral gastroenteritis, particularly Rotavirus infection, is a common cause of diarrhea in children during the late fall and winter months and infection may be nosocomially transmitted in hospitalized children. Other causes of viral gastroenteritis such as Adenovirus 40/41 or other enteric viruses are much less common but should be considered when other types of infection have been ruled out.

Experience with Implementation

Since these recommendations were implemented at the Alberta Children's Hospital several years ago, there has been a sustained reduction in both stool C & S and stool O & P testing. Physicians have been very supportive of the initial rule of a single sample, and these types of stool tests are now rarely requested for patients with prolonged hospital stays. Nursing personnel and parents also appreciate not having to collect multiple stool samples.

ADVICE TO PATIENTS

Clear communication by physicians and other health care personnel to the patient and/or parents is integral to their understanding and compliance with the need to test for diarrhea pathogens. It is important to explain the nature of their infection, the expected duration of diarrhea and to outline the management plan. The proper stool sample collection methods for different types of tests (i.e., stool C & S, stool O & P, *C. difficile*, Rotavirus) should be outlined to ensure that the samples will be of acceptable quality to the laboratory (see patient collection instruction sheet).

An important part of managing patients with infectious diarrhea is to tell them how they can remain well hydrated throughout their illness. For patients with confirmed bacterial enterocolitis, the reasons for not routinely treating the illness with antibiotics should be explained. The potential side effects of antimicrobial therapy must be outlined to patients (parents of children) with severe bacterial enterocolitis or pathogenic enteric protozoal infections who receive antibiotics. Patients should also be informed that Public Health staff may contact them about what they have been eating or drinking to try to identify the source of a notifiable infection.

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Toward Optimized Practice (TOP) Program

Arising out of the 2003 Master Agreement, TOP succeeds the former Alberta Clinical Practice Guidelines program, and maintains and distributes Alberta CPGs. TOP is a health quality improvement initiative that fits within the broader health system focus on quality and complements other strategies such as Primary Care Initiative and the Physician Office System Program.

The TOP program supports physician practices, and the teams they work with, by fostering the use of evidence-based best practices and quality initiatives in medical care in Alberta. The program offers a variety of tools and outreach services to help physicians and their colleagues meet the challenge of keeping practices current in an environment of continually emerging evidence.

TO PROVIDE FEEDBACK

The Alberta CPG Working Group for Microbiology is a multi-disciplinary team composed of microbiologists, general practitioners, and a gastroenterologists, pathologist, university representative, member of the public and representative of Alberta Health and Wellness. The team encourages your feedback. If you need more information or if you have difficulty applying this guideline, please contact:

Toward Optimized Practice Program
12230 - 106 Avenue NW
EDMONTON, AB T5N 3Z1
T 780.482.0319
TF 1-866.505.3302
F 780.482.5445
E-mail: cpg@topalbertadoctors.org

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Laboratory Appendix for Ordering Stool Tests for Investigation of Suspected Infectious Diarrhea

This appendix complements the Guideline for Ordering Stool Tests for Investigation of Suspected Infectious Diarrhea prepared by the Microbiology Working Group of the Clinical Practice Guidelines Program.

RECOMMENDATIONS

- ◆ Use containers with enteric solution for stool culture and sensitivity specimens which require transportation of greater than two hours from the site of collection to the laboratory.
- ◆ Use containers with **SAF fixative** for parasite testing.
- ◆ Use **normal sterile containers** for:
 - stool culture and sensitivity tests where transport of the specimen for a period of greater than two hours from collection site to laboratory is not required;
 - testing for *C. difficile*; or
 - testing for Rotavirus and other enteric viruses.
- ◆ Relevant clinical history should be provided to properly process the specimen.
- ◆ In patients with AIDS, laboratories may choose to look routinely for Microsporidiosis, Cryptosporidiosis and *Mycobacterium avium* complex (MAC).
- ◆ Many of the infectious agents mentioned in the Guideline, “Ordering Stool Tests for Investigation of Suspected Infectious Diarrhea,” are classified as notifiable to Public Health.

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12230 - 106 Avenue NW
EDMONTON, AB T5N 3Z1
T 780.482.0319
TF 1-866.505.3302
F 780.482.5445
E-mail: cpg@topalbertadoctors.org