OBJECTIVE
Alberta clinicians use a single initial stool test to improve the accuracy of stool testing for infectious diarrhea and tool collecting convenience for patients

TARGET POPULATION
Children and adults with suspected infectious diarrhea

EXCLUSIONS
Patients involved in a community or hospital outbreak
Food handlers to whom Public Health regulations apply
When an infectious etiology is not suspected

RECOMMENDATIONS

Stool testing may be required for patients with diarrhea. The following recommendations guide lab ordering for stool bacterial cultures (C & S), stool ova and 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 ordered initially and as indicated (see appendix A algorithm).
- X Stool C & S and/or stool O & P tests are generally NOT clinically indicated for patients with an onset of diarrhea four days after discharge from hospitalization (see Appendix A algorithm).
- ✓ Consultation with an appropriate specialist is recommended in circumstances where additional stool tests may be useful. Additional stool test(s) may be required if there is continued suspicion of enteric bacterial infection when the initial sample is negative and there has been recent travel to areas where E. histolytica, giardia lamlia or helminth infections are common.

BACKGROUND

DEFINITIONS

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

STOOL O & P

Stool examination to diagnose enteric parasitic colonization and infection requires 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.

C. DIFFICILE TOXIN

Stool testing to diagnose antibiotic-associated colitis requires 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 in their normal colonic flora.

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) under investigation. Although rapid antigen detection testing for Rotavirus infection in children is widely available, most other viral tests are only performed by the Provincial Public Health Laboratories in Calgary and Edmonton.

ENTERIC BACTERIAL INFECTION

Patients with bacterial enterocolitis typically present with an 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 patients with these infections will develop haematochezia. A stool C & S test must be performed to definitively diagnose suspected enteric bacterial infection.

Acute bacterial enterocolitis is acquired by ingesting the organism in food(s) and/or water contaminated by feces of an infected animal or person. Commonly implicated food sources include raw and undercooked eggs and egg products, raw milk and milk products, meat, salads, poultry and poultry 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 among travellers to underdeveloped countries where food handling practices and sanitary conditions may be poor. Epidemics may also occur in families, in groups eating in a specific restaurant, at a social function where a common contaminated food source is ingested, and 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, these are named in Table 1.

Enteric Bacterial Pathogens
Campylobacter jejuni
Salmonella spp.
Verotoxin producing E. coli (e.g., E. coli 0157:H7)
Shigella spp.
Aeromonas spp. (toxin producing strains)
Clostridium difficile
Yersinia enterocolitica
Pleisiomonas shigelloides

Table 1 Enteric Bacterial Pathogens

Table 1 also reflects the order of incidence of pathogens. Campylobactor infections are the most common enteric disease in Alberta. There is also no difference in the pattern or frequency of types of bacterial pathogens among patients admitted to the hospital with a primary diagnosis of acute enterocolitis compared with those attending out- patient facilities including the emergency department.[1,2,3] 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 by travelers to underdeveloped countries where sanitary conditions are poor (e.g., *Vibrio cholerae*), or those people eating contaminated shellfish (e.g., *Vibrio parahaemolyticus*).

In Alberta, enteric infections occur throughout the year but have a definite peak period in the summer months. This is likely due to improper food handling practices and is associated with outdoor activities. Infections occurring at other times of the year are more likely acquired while travelling in underdeveloped countries.

C. difficile is the primarily due to antibiotic associated diarrhea and pseudomembranous colitis. *C. difficile* has become one of the most commonly detected enteric pathogens, particularly in hospitalized patients and nursing home residents 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 a total colectomy, and although rare, death may occur if appropriate treatment is not provided. 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 occur among patients with no recent antibiotic treatment.

Physicians should conduct 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 and with protozoan parasites may present with persistent diarrhea (i.e., ≥ 5 days duration). Intestinal parasite infection may also cause abdominal bloating and flatulence, but symptoms alone will not distinguish enteric infection with protozoa versus bacteria. Some patients who have recently travelled to, or emigrated from under-developed countries, may have mixed infections. Microscopic examination of stool remains the primary 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 daycare centres, in institutionalized patients, and among homosexual men.

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

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

Table 2 Pathogenic Enteric Protozoa

A causal relationship between *Biastocystic hominis* and diarrhea has never been established. Diarrhea may be pathogenic among patients where no other cause is found. Other enteric protozoa not listed above may frequently colonize the bowel, but have not been documented to cause diarrhea or other gastrointestinal symptoms even among 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, Strongyloidia- sis, 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., two or three sequential samples) may be required. Three investigations may also be required if *E. his- tolytica*, or *Giardia lamblia* infection is suspected.

ENTERIC VIRAL INFECTION

Viral gastroenteritis occurs primarily in two distinct clinical forms. The first pattern of disease is typified by Rotavirus causing severe, acute watery diarrhea in infants and young children each year during the winter months, although sporadic cases can occur at other time of the year. Rotavirus infection may also be transmitted among 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 daycare. 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 those having contact with infected individuals. 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.

Enteric Viral Infections	
Rotavirus	Astroviruses
Adenovirus 40/41	Norwalk virus
Caliciviruses	Norwalk-like viruses

Table 3 Enteric Viral Infections

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

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 but not stool cultures for the organism.

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 daycare 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.

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 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, three sequential stool samples (i.e., different bowel movements) for stool C & S and stool O & P testing were ordered to diagnose 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 on studies that found 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. Furthermore there is little 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 demonstrated that there is high sensitivity using 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 four children with Giardiasis, four children with *Dientamoeba fragilis*, two children with *Blastocystis hom- inis* and two children with Cryptosporidiosis. However, due to the retrospective design of the study, the clinical significance of these cases in terms of patient symptoms or 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 is well documented that there is a very low yield of finding enteric infections (i.e., bacterial or protozoal) with onset ≥four 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 travel to an underdeveloped country has occurred or when additional stool examination is required for follow up of a previously diagnosed infection.

Most cases of diarrhea occurring in hospitalized patients is due to *C. difficile* toxin-mediated colitis when antibiotics have been recently administered (i.e., symptoms may occur up to eight weeks after antimicrobial(s) were discontinued). 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 supportive of the initial single sample rule, 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.

PROVIDING ADVICE TO PATIENTS

Physicians and other health care personnel must clearly communicate to the patient and/or parents to ensure they understand and can complete the test for diarrhea pathogens. It is important to explain the nature of their infection, the expected duration of diarrhea and to outline the plan to manage their diarrhea. The proper stool sample collection technique for different types of tests (i.e., stool C & S, stool O & P, *C. difficile*, Rotavirus) should be explained to ensure samples will be acceptable for laboratory analysis (see patient collection instruction sheet).

An important component of managing infectious diarrhea is to explain to patients the importance of keeping well-hydrated throughout their illness. For patients with confirmed bacterial enterocolitis, the reasons for not treating the illness with antibiotics should be explained. The potential side effects of antimicrobial therapy must be provided to patients (parents of children) with severe bacterial enterocolitis or pathogenic enteric protozoal infections and treated with antibiotics. Patients should also be informed that Public Health staff may be in contact with them to investigate foods and fluids recently consumed to try to identify the source of a notifiable infection.

REFERENCES

1. Montessori GA, Bishcoff L. Searching for parasites in stool: once is unually enough. CMAJ 1987;137:702.

2. Senay H, MacPherson D. Parasitology: diagnositc yield of stool examinations. CMAJ 1989;140:1327-31.

3. Asnis DS, Bresciani A, Ryan M, McArdle P, Mollura JL, Ilardi CF. Cost-effective approach to evaluation of diarrheal illness in hospitals. J CLIN MICROBIOL 1993;31:1675.

4. Kabani A, Cadrain G, Trevenen CL, Jadavji TJ, Church DL. Practice guidelines for ordering stool ova and parasite testing in a pediatric population. AM J CLIN PATHOL 1995;104:272-8.

5. Church DL, Cadrain G, Kabani A, Jadavji T, Trevenen C. Practice guidelines for ordering stool cultures in a pediatric population. AM J CLIN PATHOL 1995;103:149-53

6. Siegel DL, Edelstein PH, Nachamkin I. Inapproprite testing for diarrheal diseases in the hospital. JAMA 1990; 263:979-82.

7. Fan K, Morris AJ, Reller LB. Application of rejection criteria for stool cultures for bacterial pathogens. J CLIN MICROBIOL 1993;31:2233-5.

8. Alicna AD, Fadell AJ. Advantage of purgation in re- covery of intestinal parasites or their eggs. AM J CLIN PATHOL 1959;31:139-42.

9. Andrews J. The diagnosis of intestinal protozoa from purged and normally passed stools. J PARASITOL 1934;20:253-4.

10. Morris AJ, Wilson ML, Reller LB. Application of rejection criteria for stool ovum and parasite examinations. J CLIN MICROBIOL 1992;30:3213-16.

11. Koplan J, Benfari Ferraro MJ, Fineberg HV, Rosenberg ML. Value of stool cultures. LANCET 1980;23:413-16.
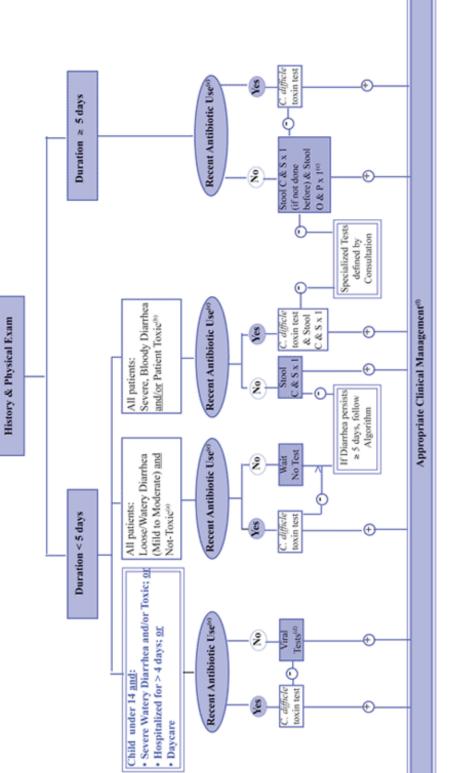
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For more information see www.topalbertadoctors.org

GUIDELINE COMMITTEE

The committee consisted of representatives of microbiology, general practice, gastroenterology, pathology, infectious disease, public health, and the public.

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APPENDIX A - ALGORITHM

Algorithm for Investigation of Suspected Infectious Diarrhea

History & Physical Exam

Duration < 5 days

Duration ≥ 5 days

Child under 14 and:
- Severe Watery Diarrhea and/or Toxic; or
- Hospitalized for > 4 days; or
- Daycare

All patients:
Loose/Watery Diarrhea
(Mild to Moderate) and
Not-Toxic(d)

All patients:
Severe, Bloody Diarrhea
and/or Patient Toxic(b)

Recent Antibiotic Use(c)

Yes / No

C. difficile toxin test

Viral Tests(d)

Wait No Test

If Diarrhea persists ≥ 5 days, follow Algorithm

Stool C & S x 1

C. difficile toxin test & Stool C & S x 1

Specialized Tests defined by Consultation

Stool C & S x 1 (if not done before) & Stool O & P x 1(e)

Appropriate Clinical Management(f)

Notes:

a) Some cases of watery diarrhea in these patients do not require investigation and are self limiting.

b) Bacterial enterocolitis is most often acquired in Alberta from late spring (May/June) to fall (Oct/Sept). Cases outside of these peak months occur most frequently in travellers/immigrants from developing countires.

c) Antibiotic associated colitis (AAC) due to C. difficile may occur up to eight weeks after antibiotics have been stopped. AAC is the most common cause of diarrhea in hospitalized patients.

d) Rotavirus infections occur predominantly in yung infants/children ≤ age 3 years0 and are epidemic in Alberta each year from early winter (Nov/Dec) to early spring (Apr/May). Rotavirus infections may also be nosocomially transmitted between hospitalized children. Adenovirus 40/41 and other enteric viral infections occur much less frequently throughout the year.

e) Multiple, sequential stool ova and parasite examinations may be necessary for patients who have recently travelled and/or immigrated (i.e., usually within the past six months) from an underdeveloped country in order to diagnose E. histolytica, G. lamblia and/or enteric helminth infections.

f) Rehydration is central to the management of patients with infectious diarrhea.