Appendix 1: Frequently asked questions

What is the etiology of infectious diarrhea?

Most acute diarrhea are caused by infections, most commonly viral followed by bacterial and infrequently, protozoal. Fecal-oral transmission is the predominant route of transmission. Exposures can occur through ingestion of contaminated food and water or contact with infected persons or contaminated surfaces. International travel to areas with inadequate sanitation is a known risk. High-risk areas for traveller's diarrhea include countries in Africa, Asia, the Middle East, and Latin America. Low-risk areas include North America, Central Europe, Australia, and Japan. Non-infectious causes include medications, gastrointestinal disease and endocrine disorders.[10]

Considering that the management of most infectious diarrhea is supportive, what is the rationale for performing diagnostic testing for diarrhea?

Infectious diarrhea is a common clinical presentation. Most infectious diarrhea episodes are self-limiting and resolve within 7 days, and these do not require further diagnostic testing as there will be marginal benefit to the patient. However, in selected cases, testing should be performed when the identification of the agent will benefit the patient in the management of the diarrheal episode.

For those with severe or prolonged (> 7 days) diarrhea, or immunocompromised individuals, syndromic diagnostic testing allows timely identification of the infectious agent where patient morbidity can be reduced through specific treatments such as antimicrobials and to provide a prognosis for the course of the illness. Potential outbreak situations may warrant appropriate diagnostic testing as directed by Public Health.[11–13]

The syndromic IDP test appears to oppose principles of test stewardship, where diagnostic tests should be judiciously selected based on the differential diagnosis. What was the rationale in adopting this approach?[14]

The use of IDP permits the detection of a broader range of pathogens, including enteric viruses and emerging bacterial pathogens. Several factors have come together to make this decision. Commercial multiplex NAAT assays for infectious diarrhea have become increasingly available and affordable compared with the traditional methods of culture and microscopy. These assays are less labour intensive compared with manual microscopy, and yet have faster turn-around time and sensitivity.

Although clinical symptoms and exposure history help narrow down the possible causative agent of diarrhea, there remains significant overlap of symptoms between the various diarrheal pathogens. This often results in the physician ordering multiple tests (e.g., Stool O&P, Stool Culture and *C. difficile*) for that diarrheal episode. This may also require multiple stool specimens, and hence multiple collections, multiple clinic visits and multiple laboratory drop-offs. The IDP simplifies this pre-laboratory stage and streamlines the workflow, all with a single stool specimen.

For those with chronic or severe diarrhea, the precision and higher sensitivity afforded by novel syndromic testing can aid with more timely specific treatment with antibiotics, such as for *Shigella* or *Giardia* infections. Even if a pathogen is identified where only supportive measures are available (e.g., Norovirus), clinicians receive a diagnosis that allows for the provision of a prognosis, advice about transmissibility to others and strategies for avoidance in the future. Public Health also receives a more accurate picture of the diarrheal disease burden in the community.

How was the list of pathogens in *Table 1* established?

This standardized list of pathogens was agreed upon by BC medical microbiologists, clinicians and public health, based on historical BC epidemiology, clinical significance and threats to the public. Each laboratory can choose or develop the particular multiplex kits and approach for IDP so long as it detects this list of "mandatory" pathogens. Note that prior to this, there were no agreed list of pathogens identified by stool culture or stool O&P. This new standardized list will be reviewed every several years and updated based on new clinical information and changes in available diagnostic assays. Other pathogens were not included because of the rarity of infection within BC, and/or these are not detectable by currently available assays. In selected

cases with the appropriate risk factors, specialized testing may be required if symptoms are ongoing, and the syndromic panel is non-diagnostic. Your local laboratory physician/medical microbiologist can be of assistance in determining what additional tests may be indicated.

What about *Blastocystis* hominis and *Dientamoeba fragilis*?

Certain organisms have been purposely excluded from being mandatory in the IDP, including, *Blastocystis hominis* and *Dientamoeba fragilis*. However, individual laboratories may choose to report these organisms.

The role of these organisms in causing infectious diarrhea is controversial. *Dientamoeba fragilis* is often asymptomatically carried, particularly by children. In such populations, the detection of *D. fragilis* is an incidental finding and requires no treatment. *Blastocystis hominis* is similarly often carried asymptomatically although there is some evidence that different genotypes found around the world may be potentially pathogenic in terms of causing diarrheal symptoms. Treatment may be considered for *B. hominis* or *D. fragilis* if either is detected as the sole pathogen in a symptomatic patient, and other causes of diarrhea have been excluded.

Why is *C. difficile* included in IDP?

While *C. difficile* infection was previously associated mostly with recent health-care exposure (i.e., hospital admission, long-term care facility setting) and/or recent antibiotic use (within 3 months), it has now become an increasingly important cause of community acquired diarrhea with some patients having no clear risk factors. In BC, *C. difficile* is the most common pathogen identified in all stool specimens. On review of such cases, many of them were not symptomatic or had an alternative diagnosis. Instead, they are thought to be colonized with *C. difficile*.

Clinically, it can be hard to differentiate *C. difficile* from other causes of infectious diarrhea. Generally, those with moderate-severe and/or prolonged diarrhea should be tested for *C. difficile*. Timely diagnosis and treatment reduce morbidity. This is especially true for debilitated patients with multiple medical co-morbidities, including advanced age, as they have the highest risk of complications.

How do I discern *C. difficile* infection from colonization?

Colonization is defined as the absence of clinical symptoms of *C. difficile* infection (i.e., diarrhea, ileus, toxic megacolon) or the presence of an alternative explanation of these symptoms.[1] After colonization with the bacterium, patients may be asymptomatic *C. difficile* carriers. A disruption of the normal gut microbiota (i.e., antibiotic administration) may lead to *C. difficile* infection. Within BC, the estimated *C. difficile* colonization is 10-15% in those greater than 2 years old. Past epidemiologic studies have found that up to 71% of children are asymptomatically colonized with *C. difficile*.[15]

A positive test does not differentiate infection from colonization. Up until recently, most BC labs utilized the highly sensitive nucleic-acid amplification tests (NAAT) to detect *C. difficile*. This method often identified asymptomatic carriers, which led to unnecessary treatment and patient angst. To facilitate differentiation of *C. difficile* infection from colonization, a supplemental test to detect the *C. difficile* toxin by enzyme immunoassay (EIA) is performed in both the IDP and the standalone *C. difficile* tests. This combination of test has three possible results:

- Positive (Toxin protein detected by EIA) – High likelihood of *C. difficile* infection, although asymptomatic colonization is possible.
- Indeterminate (Toxin gene detect by NAAT, but toxin protein not detected by EIA) – Patient is colonized with *C. difficile*, although *C. difficile* infection is possible.
- Negative (Toxin gene not detected by NAAT) – *C. difficile* infection is excluded in >99% of patients.

Clinical correlation is always required to establish the diagnosis of *C. difficile* infection versus colonization. Furthermore, neither the *C. difficile* test nor the IDP should not be requested in patients without diarrhea – i.e., those with formed stools. Repeat testing during the same episode of *C. difficile* infection and test of cure are not useful for the management of patients.

What is the difference between Shiga toxin-producing *E. coli* (STEC) and *E. coli* O157:H7?

As its name implies, STEC produce Shiga toxins, which are part of a family of toxins first described in *Shigella dysenteriae*. STEC, also known as enterohemorrhagic *E. coli* (EHEC), includes multiple strains of *E. coli*. Each strain of *E. coli* is defined by its O- and H-antigens (e.g., O157:H7). Within STEC, the different strains have different pathogenicities, such as the amount and types of Shiga toxin produced. STEC O157:H7 is perhaps the highest risk strain, notably due to its ability to cause hemolytic uremic syndrome (HUS). Of note, the association of HUS with the toxin, also known as verotoxin, was established by Canadian researchers in 1985.[16]

Routine stool cultures can detect STEC O157:H7, but not the other strains. Occasionally, non-O157:H7 STEC were detected by verotoxin assay, but this assay was not frequently used and was limited to bloody stools only. IDP detects all STEC strains, and its introduction will result in more confirmed cases of non-O157:H7 STEC.

Our knowledge of non-O157:H7 STEC is evolving. While many strains can cause a mild, self-limited disease, certain strains (particularly those that produce Shiga toxin 2) can present similarly as *E. coli* O157:H7 (i.e., fever, abdominal pain, bloody diarrhea and progression to HUS) and may be associated with outbreaks. Hence all individuals who have STEC detected in their stool require assessment of disease severity and, if there are ongoing symptoms, bloodwork (i.e., complete blood count, urea, creatinine, electrolytes and lactate dehydrogenase). In those at risk of HUS or severe disease, hospitalization for fluid management is recommended. HUS can occur in any age, but the risk is highest in children less than 10 years old. Antibiotics should be avoided or discontinued, as antibiotics can lyse the bacterium resulting in release of more toxin.

Why are helminths (worms) not included in IDP?

Most infections with gastrointestinal **worms do not cause diarrhea**. Only very heavy infections with some types of worms (e.g., *Schistosoma mansoni*, *Trichuris trichiura*), not endemic to Canada, may cause diarrhea. Instead, worm infections are often asymptomatic, or they may cause non-specific gastrointestinal symptoms such as vague abdominal pain. Rarely, they may result in nutritional deficiencies, eosinophilia or cause complications such as biliary colic by migrating within the body. Very few gastrointestinal worm infections are endemic to Canada. In BC, the detection of worm ova from stool O&P is <0.1%. Risk factors for these worm infections are migration from or prolonged travel to regions, outside of Canada, USA, western Europe, Australia or New Zealand or, occasionally, consumption of undercooked imported food products.

I'm still concerned about a worm infection. What do I order to make a diagnosis?

If there is suspicion for gastrointestinal worm infection, order **Stool Microscopy Exam for Worm-Ova**. Due to intermittent shedding of worm ova and the inherent limited sensitivity of microscopy, repeat testing on different days may be required to rule out an infection. Additional laboratory requisitions may be required for multiple samples. Generally, three stool samples collected on different days is > 95% sensitive and adequate to rule out a clinically significant gastrointestinal worm infection.

Diagnosis of infection with the worm *Strongyloides stercoralis* is an exception. This infection can affect anyone who has walked barefoot in a high-risk area, particularly in Southeast Asia and the Indian subcontinent.[17] Stool microscopy examinations for this parasite has low sensitivity (< 20%) and may be negative in chronically infected patients. The appropriate screening test is **Strongyloides serology**. This serum antibody test is > 95% sensitive for detecting chronic infection. *Strongyloides stercoralis* is transmitted from contaminated soil endemic to tropical and subtropical climates worldwide. Farmers, military personnel, or adventurers are at higher risk as they may have had direct skin penetration by the infectious form of larvae from contaminated soil. *Strongyloides* causes a chronic subclinical infection that may be relatively asymptomatic for decades or may manifest as intermittent eosinophilia. Immunosuppressed states, including advanced age, can allow a hyper-infection syndrome to develop which carries high mortality. It is important to identify chronically infected individuals, particularly prior to immunosuppression (e.g., high dose steroids, chemotherapy, transplant) so that they can be offered definitive anti-helminthic treatment and avoid developing this complication.

My patient passed something that is concerning for a worm. What do I with it?

If an object visible to the naked eye is passed in stool that is suspected to be a worm, submit it to the laboratory in a clean container separately from the stool sample and order **Worm ID**. Adult round worms (i.e., *Ascaris*) and tapeworm segments may be visualized with the naked eye without a microscope.

My patient is worried about worm infection after eating sushi. What is the prevalence and trends of such infections in BC?

The risk of worms from sushi consumed in North America is very low thanks to the implementation of food safety standards. Particularly for sushi consumed within North America, there are two worms of concern, *Anisakis* and *Diphyllobothrium*. Both of which can be killed by cooking or by freezing the fish.18,19 These do not cause diarrhea and hence do not usually require testing.