Eugene Y.H. Yeung, MD, MSc, BSc(Pharm), FRCPC, FCCM, D(ABMM), CTH

Prevalence of intestinal parasites identified by microscopy prior to implementation of infectious diarrhea panel nucleic-acid amplification testing (IDP-NAAT): What are we missing?

Many parasitic pathogens could be missed if only IDP-NAAT is used to diagnose intestinal parasitic infections.

ABSTRACT

Background: Since 2022, diagnostic laboratories in British Columbia have been advised to replace microscopy for intestinal ova and parasites with infectious diarrhea panel nucleic-acid amplification testing (IDP-NAAT). However, this multiplex assay captures only four common parasites: *Cyclospora cayetanensis*, *Cryptosporidium* spp., *Entamoeba histolytica*, and *Giardia* spp.

Methods: An audit was conducted from September 2022 to August 2023, 1 year prior to implementation of IDP-NAAT in LifeLabs BC, when microscopy was the method used to identify intestinal parasites.

Results: Pathogenic parasites were identified in 6149 of 52 221 stool specimens. The most common pathogens were *Blastocystis hominis* (78.47%), *Dientamoeba fragilis* (12.23%), *Giardia* spp. (4.93%), and *Cyclospora* spp. (1.07%). *Entamoeba histolytica/dispar*, *Strongyloides stercoralis*, *Cryptosporidium* spp., *Ascaris lumbricoides*, *Diphyllobothrium* spp., *Enterobius vermicularis*, *Hymenolepis nana*, *Schistosoma mansoni*, *Taenia* spp., *Trichuris trichiura*, and *Clonorchis sinensis* each accounted for less than 1% of the pathogenic parasites identified. *Enterobius vermicularis* was also identified in 46 of 1569 pinworm paddle specimens.

Conclusions: Several potentially pathogenic parasites could be missed if only IDP-NAAT is used to detect intestinal parasites. If indicated, microscopy orders would be needed to capture parasites not detected by IDP-NAAT.

Dr Yeung is a clinical assistant professor in the Faculty of Medicine and the Faculty of Pharmaceutical Sciences at the University of British Columbia and a staff medical microbiologist at LifeLabs BC.

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Background

Traditionally, stool culture and microscopy for ova and parasites are the diagnostic methods of choice to detect intestinal pathogens. In 2022, diagnostic laboratories in British Columbia were advised to replace this testing method with the infectious diarrhea panel nucleic-acid amplification test (IDP-NAAT).[1] It combines a multiple gene target (multiplex) that detects a minimum of 14 common viral, bacterial, and parasitic pathogens **[Box 1; Figure 1]**. The BC Guidelines state that the list of pathogens may be modified periodically, in line with changes in epidemiology and technology. Potential pathogens to be considered include parasites such as *Blastocystis hominis* and *Dientamoeba fragilis*, even though they may not be pathogenic in each case.[2] The minimum pathogen list in the BC Guidelines includes only four parasites, while the number of possible intestinal parasites can be countless.[2] Moreover, the BC Guidelines recommend that if either stool culture or microscopy for ova and parasites is ordered, the laboratories will automatically substitute with IDP-NAAT and, thus, potentially miss the correct diagnosis. The BC Guidelines state that stool microscopies may be warranted if patients have a history of recent travel or immigration from low- or middle-income countries or are severely immunocompromised.

LifeLabs BC implemented IDP-NAAT in September 2023. Our laboratory conducted a retrospective audit on all intestinal parasites detected in our regional microbiology laboratories from 1 September 2022 to 31 August 2023, 1 year prior to implementation of IDP-NAAT. Our regional

microbiology laboratories are connected with 129 collection centres in urban and rural communities in the province, and they provided the laboratory data on intestinal parasites, which indicated their local prevalence in community settings. The aim of this study was to identify intestinal parasites that would not be detected by IDP-NAAT and postulate plans to ensure the correct diagnosis is not missed.

Methods

Microscopies for ova and parasites in stool specimens

Microscopies for ova and parasites in stool specimens were ordered by clinicians. Patients or their caregivers were instructed to provide stool specimens in a clean vial with no liquid medium and a vial with sodium acetate–acetic acid–formalin fixative, which were then transported to the regional microbiology laboratories. Using a disposable plastic pipette, trained medical laboratory technologists placed a small amount of well-mixed sediment of the stool specimen on a plain glass microscope slide, which was then pressed by a coverslip. The technologists examined the entire area of the coverslip, first under 100× magnification and then under 400× magnification if suspicious features were seen. Iodine or carbol fuschin stain was used to enhance the detection of oocysts. When the presence of *Cryptosporidium* spp. or *Cyclospora* spp. was suspected, acid-fast stain was used. When testing for *Strongyloides* spp. or *Schistosoma* spp. was requested, a minimum of three slides were examined. The technologists were instructed to read each concentrate for an average of 9 minutes. The presence of ova, cysts, trophozoites, oocysts, larvae, adult worms of pathogenic parasites, and nonpathogenic parasites was reported.

Microscopies for pinworm paddle specimens

Microscopies for pinworm paddle specimens were ordered by clinicians. Patients or their caregivers were instructed to press the sticky surface of the pinworm paddle against the anal area early in the morning before arising

BOX 1. The minimum 14 pathogens in the infectious diarrhea panel nucleic-acid amplification test used in each diagnostic laboratory in BC.

- Viral pathogen: adenovirus 40/41, norovirus GI/GII, rotavirus.
- Bacterial pathogen: *Campylobacter* spp., *Clostridioides difficile*, Shiga toxin-producing *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica*, *Vibrio* spp.
- Parasitic pathogens: *Cyclospora cayetanensis*, *Cryptosporidium* spp., *Entamoeba histolytica*, *Giardia* spp.

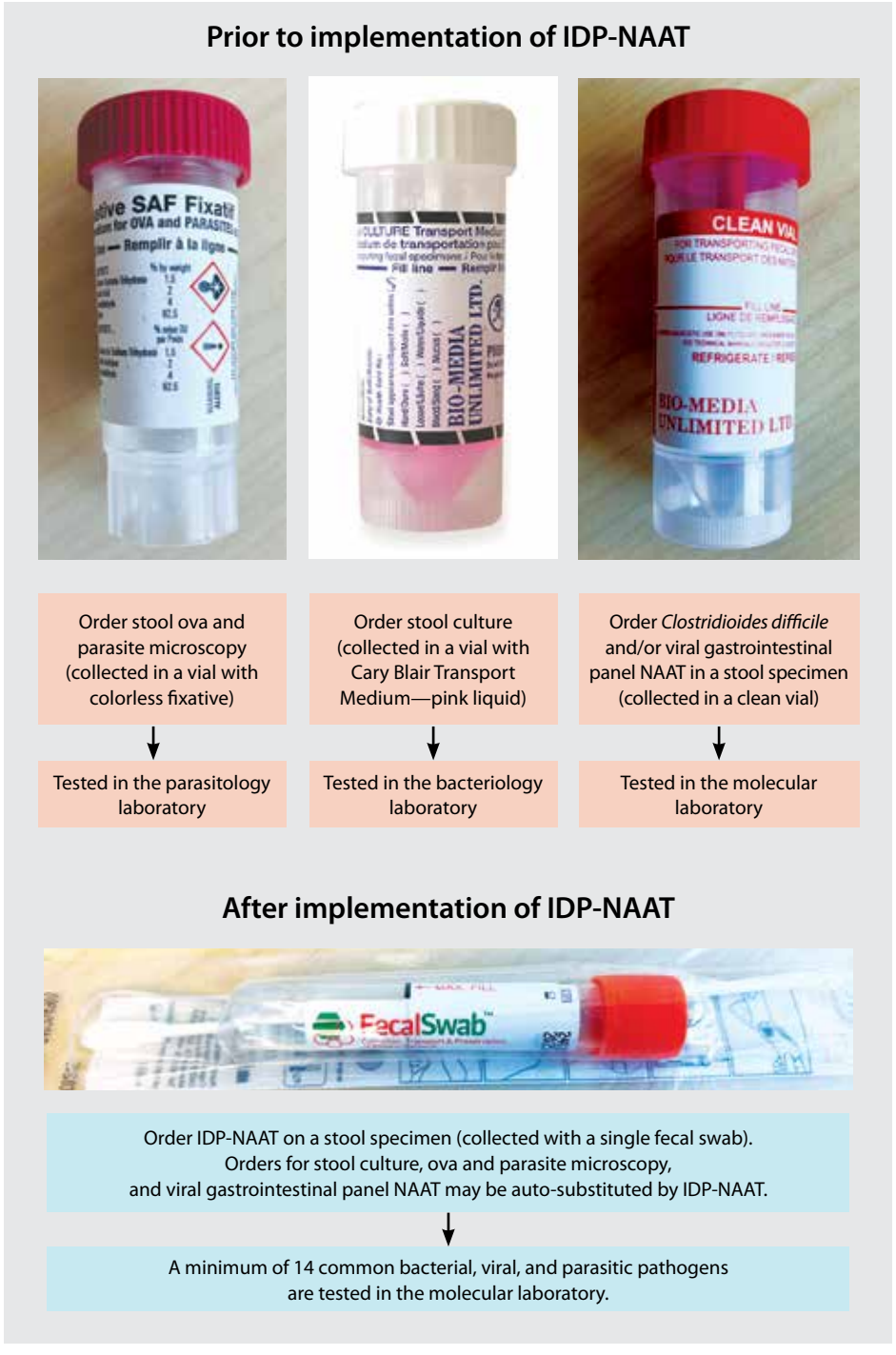


FIGURE 1. Flow chart explaining the change in stool microbiology test ordering in British Columbia prior to and after implementation of the infectious diarrhea panel nucleic-acid amplification test (IDP-NAAT).

or before bowel movement. The paddles were put in a vial and then transported to the regional microbiology laboratories. Each specimen was placed sticky side up on a glass microscope slide. Under 100× magnification, the technologists systematically examined the entire area of the paddle. The presence of pinworm (*Enterobius vermicularis*) ova and parasites was reported.

Data collection and analysis

Microscopies for ova and parasites and pinworm paddle orders were conducted from 1 September 2022 to 31 August 2023. The Microbiology Electronic Worksheet System software (version 5.00.267; LifeLabs, Toronto, ON) was used to generate data from all the microscopies. An entire year of data from patients of all ages and sexes was collected to reduce bias due to seasonality, differences in clinical practices, and other potential confounders. GraphPad Prism software (version 6.0c; GraphPad Software Incorporated, Boston, MA) was used to perform statistical analysis when needed.

Results

Microscopies for ova and parasites in stool specimens

Pathogenic and nonpathogenic parasites were identified in 6149 and 1016 of the 52 221 stool specimens, respectively. The most common pathogens were *Blastocystis hominis* (78.47%), *Dientamoeba fragilis* (12.23%), *Giardia lamblia* (4.93%), and *Cyclospora* spp. (1.07%). *Entamoeba histolytica/dispar*, *Cryptosporidium* spp., *Enterobius vermicularis*, *Hymenolepis nana*, *Strongyloides stercoralis*, *Diphyllobothrium* spp., *Clonorchis sinensis*, *Taenia* spp., *Ascaris lumbricoides*, *Schistosoma mansoni*, and *Trichuris trichiura* each accounted for less than 1% of pathogenic parasites identified **[Figure 2]**.

Microscopies for pinworm paddle specimens

Enterobius vermicularis was identified in 46 of 1569 pinworm paddle specimens.

Discussion

The results of this study suggest that several potentially pathogenic parasites could be missed if only IDP-NAAT is used to detect intestinal parasites. The most common intestinal parasitic pathogens identified were *Blastocystis hominis* (78.47%) and *Dientamoeba fragilis* (12.23%); however, it remained controversial whether they were true pathogens in each clinical case. In the absence of other diagnoses, it may be important to report when patients' symptoms clinically correlate with the presence of these parasites, which can be roughly quantified (e.g., rare, few, many) in order to help clinicians determine the significance of their symptoms.[2] The current IDP-NAAT would not be able to report the presence of these parasites or quantify the amount of any parasite in a test sample.

Most of the parasites detected in this study accounted for less than 1% of all those found. Although it can be argued that the incidences of parasites missed by IDP-NAAT were statistically insignificant, statistics do not always apply in incidents of patient safety: one severe, highly nonconforming event, regardless of probability, would be considered significant.[3] If stool microscopy orders were automatically replaced with IDP-NAAT, as the BC Guidelines suggest, many intestinal parasite diagnoses could be missed. Clinicians do not always order intestinal parasite testing simply to determine the cause of diarrhea. They may look for the following parasites in other clinical situations:

- *Ascaris lumbricoides*—associated with eosinophilia, intestinal blockage, and impaired growth.[4,5]
- *Clonorchis sinensis*—associated with eosinophilia, gallbladder obstruction, jaundice, and hepatomegaly.[6]
- *Diphyllobothrium* spp.—associated with weight loss, vitamin B12 deficiency, pernicious anemia, intestinal obstruction, and gallbladder disease.[7]
- *Enterobius vermicularis*—associated with perianal itching.[8]
- Hookworm—associated with anemia and chronic protein deficiency, especially in children.[4]
- *Schistosoma* spp.—associated with fever and hematochezia (even though serologic testing may be preferred).[9,10]
- *Strongyloides stercoralis*—associated with hyperinfection syndrome, characterized by abdominal pain, diffuse pulmonary infiltrates, and septicemia or meningitis (even though serologic testing may be preferred).[11,12]
- *Taenia solium*—associated with cysticercosis (even though neuroimaging and serologic testing may be preferred).[13]
- *Trichuris trichiura*—associated with anemia and rectal prolapse.[4]

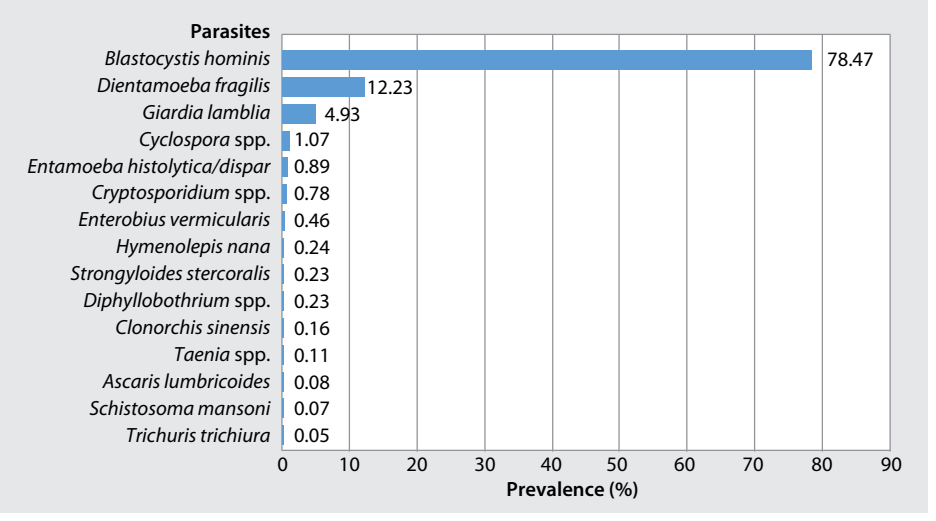
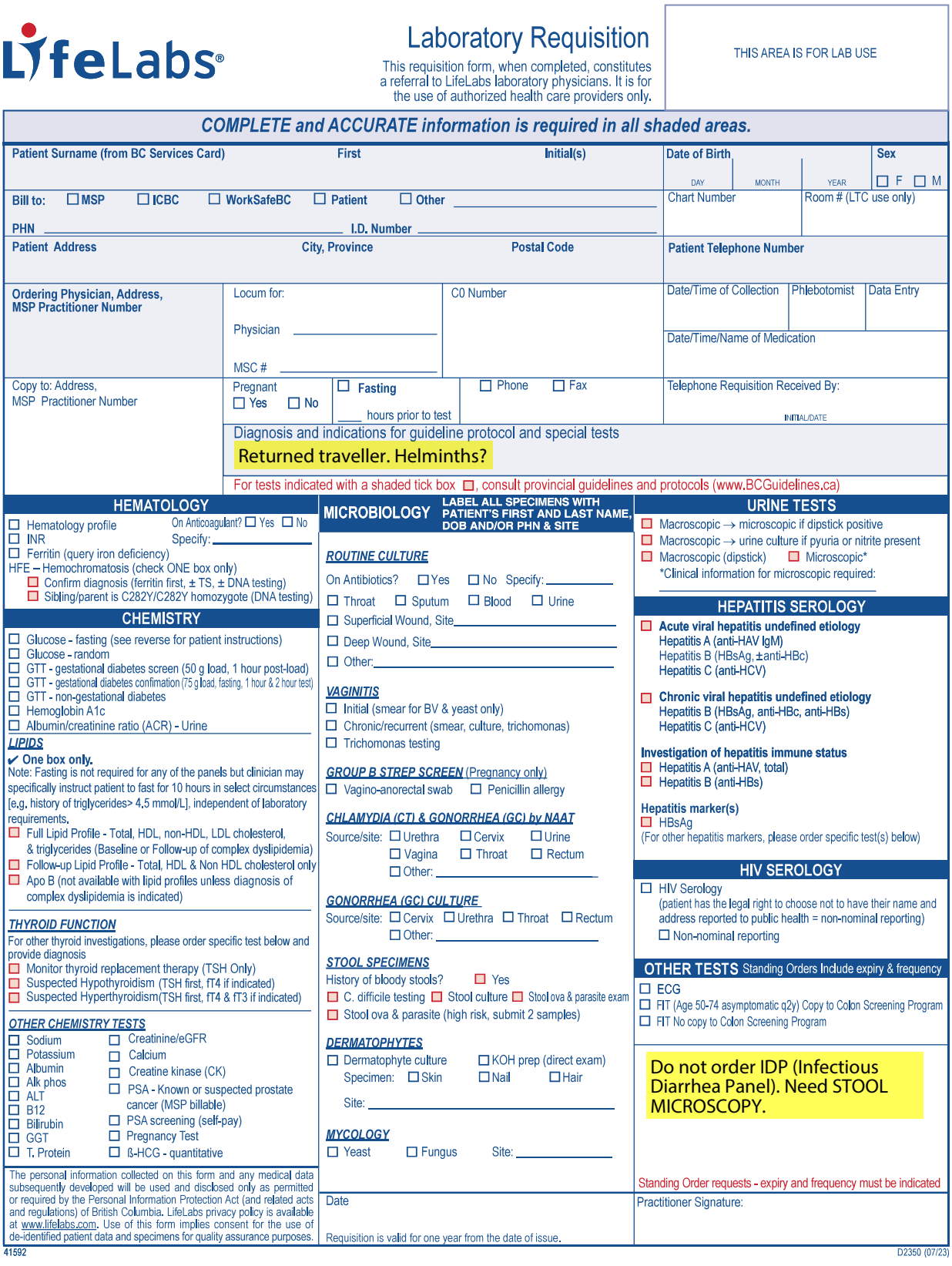


FIGURE 2. Pathogenic intestinal parasites (*n* = 6149), from the most to the least prevalent, collected from stool specimens in the community from 1 September 2022 to 31 August 2023.



LifeLabs®

Laboratory Requisition

This requisition form, when completed, constitutes a referral to LifeLabs laboratory physicians. It is for the use of authorized health care providers only.

THIS AREA IS FOR LAB USE

COMPLETE and ACCURATE information is required in all shaded areas.

Patient Surname (from BC Services Card) | First | Initial(s) | Date of Birth (DAY / MONTH / YEAR) | Sex ☐ F ☐ M

Bill to: ☐ MSP ☐ ICBC ☐ WorkSafeBC ☐ Patient ☐ Other _____ | Chart Number | Room # (LTC use only)

PHN _____ I.D. Number _____

Patient Address | City, Province | Postal Code | Patient Telephone Number

Ordering Physician, Address, MSP Practitioner Number | Locum for: Physician _____ MSC # _____ | C0 Number | Date/Time of Collection | Phlebotomist | Data Entry | Date/Time/Name of Medication

Copy to: Address, MSP Practitioner Number | Pregnant ☐ Yes ☐ No | ☐ Fasting ___ hours prior to test | ☐ Phone ☐ Fax | Telephone Requisition Received By: INITIAL/DATE

Diagnosis and indications for guideline protocol and special tests

Returned traveller. Helminths?

For tests indicated with a shaded tick box ☐, consult provincial guidelines and protocols (www.BCGuidelines.ca)

HEMATOLOGY

- ☐ Hematology profile — On Anticoagulant? ☐ Yes ☐ No
- ☐ INR — Specify: _____
- ☐ Ferritin (query iron deficiency)

HFE – Hemochromatosis (check ONE box only)

- ☐ Confirm diagnosis (ferritin first, ± TS, ± DNA testing)
- ☐ Sibling/parent is C282Y/C282Y homozygote (DNA testing)

CHEMISTRY

- ☐ Glucose - fasting (see reverse for patient instructions)
- ☐ Glucose - random
- ☐ GTT - gestational diabetes screen (50 g load, 1 hour post-load)
- ☐ GTT - gestational diabetes confirmation (75 g load, fasting, 1 hour & 2 hour test)
- ☐ GTT - non-gestational diabetes
- ☐ Hemoglobin A1c
- ☐ Albumin/creatinine ratio (ACR) - Urine

LIPIDS

✔ One box only.

Note: Fasting is not required for any of the panels but clinician may specifically instruct patient to fast for 10 hours in select circumstances [e.g. history of triglycerides> 4.5 mmol/L], independent of laboratory requirements.

- ☐ Full Lipid Profile - Total, HDL, non-HDL, LDL cholesterol, & triglycerides (Baseline or Follow-up of complex dyslipidemia)
- ☐ Follow-up Lipid Profile - Total, HDL & Non HDL cholesterol only
- ☐ Apo B (not available with lipid profiles unless diagnosis of complex dyslipidemia is indicated)

THYROID FUNCTION

For other thyroid investigations, please order specific test below and provide diagnosis

- ☐ Monitor thyroid replacement therapy (TSH Only)
- ☐ Suspected Hypothyroidism (TSH first, fT4 if indicated)
- ☐ Suspected Hyperthyroidism(TSH first, fT4 & fT3 if indicated)

OTHER CHEMISTRY TESTS

- ☐ Sodium
- ☐ Potassium
- ☐ Albumin
- ☐ Alk phos
- ☐ ALT
- ☐ B12
- ☐ Bilirubin
- ☐ GGT
- ☐ T. Protein
- ☐ Creatinine/eGFR
- ☐ Calcium
- ☐ Creatine kinase (CK)
- ☐ PSA - Known or suspected prostate cancer (MSP billable)
- ☐ PSA screening (self-pay)
- ☐ Pregnancy Test
- ☐ ß-HCG - quantitative

The personal information collected on this form and any medical data subsequently developed will be used and disclosed only as permitted or required by the Personal Information Protection Act (and related acts and regulations) of British Columbia. LifeLabs privacy policy is available at www.lifelabs.com. Use of this form implies consent for the use of de-identified patient data and specimens for quality assurance purposes.

MICROBIOLOGY

LABEL ALL SPECIMENS WITH PATIENT'S FIRST AND LAST NAME, DOB AND/OR PHN & SITE

ROUTINE CULTURE

On Antibiotics? ☐ Yes ☐ No Specify: _____

- ☐ Throat ☐ Sputum ☐ Blood ☐ Urine
- ☐ Superficial Wound, Site _____
- ☐ Deep Wound, Site _____
- ☐ Other: _____

VAGINITIS

- ☐ Initial (smear for BV & yeast only)
- ☐ Chronic/recurrent (smear, culture, trichomonas)
- ☐ Trichomonas testing

GROUP B STREP SCREEN (Pregnancy only)

☐ Vagino-anorectal swab ☐ Penicillin allergy

CHLAMYDIA (CT) & GONORRHEA (GC) by NAAT

Source/site: ☐ Urethra ☐ Cervix ☐ Urine ☐ Vagina ☐ Throat ☐ Rectum ☐ Other: _____

GONORRHEA (GC) CULTURE

Source/site: ☐ Cervix ☐ Urethra ☐ Throat ☐ Rectum ☐ Other: _____

STOOL SPECIMENS

History of bloody stools? ☐ Yes

- ☐ C. difficile testing ☐ Stool culture ☐ Stool ova & parasite exam
- ☐ Stool ova & parasite (high risk, submit 2 samples)

DERMATOPHYTES

☐ Dermatophyte culture ☐ KOH prep (direct exam)

Specimen: ☐ Skin ☐ Nail ☐ Hair

Site: _____

MYCOLOGY

☐ Yeast ☐ Fungus Site: _____

Date

Requisition is valid for one year from the date of issue.

URINE TESTS

- ☐ Macroscopic → microscopic if dipstick positive
- ☐ Macroscopic → urine culture if pyuria or nitrite present
- ☐ Macroscopic (dipstick) ☐ Microscopic*

*Clinical information for microscopic required: _____

HEPATITIS SEROLOGY

☐ **Acute viral hepatitis undefined etiology**
Hepatitis A (anti-HAV IgM)
Hepatitis B (HBsAg, ±anti-HBc)
Hepatitis C (anti-HCV)

☐ **Chronic viral hepatitis undefined etiology**
Hepatitis B (HBsAg, anti-HBc, anti-HBs)
Hepatitis C (anti-HCV)

Investigation of hepatitis immune status

- ☐ Hepatitis A (anti-HAV, total)
- ☐ Hepatitis B (anti-HBs)

Hepatitis marker(s)

- ☐ HBsAg

(For other hepatitis markers, please order specific test(s) below)

HIV SEROLOGY

☐ HIV Serology
(patient has the legal right to choose not to have their name and address reported to public health = non-nominal reporting)
☐ Non-nominal reporting

OTHER TESTS Standing Orders Include expiry & frequency

- ☐ ECG
- ☐ FIT (Age 50-74 asymptomatic q2y) Copy to Colon Screening Program
- ☐ FIT No copy to Colon Screening Program

Do not order IDP (Infectious Diarrhea Panel). Need STOOL MICROSCOPY.

Standing Order requests - expiry and frequency must be indicated

Practitioner Signature:

41592 D2350 (07/23)

FIGURE 3. An example of how to fill out a laboratory requisition form to prevent the auto-substitution of microscopy with infectious diarrhea panel (IDP) nucleic-acid amplification test orders. The highlighted area in the "Other tests" box (bottom-right corner of the page) indicates a stool microscopy rather than IDP testing is needed. The highlighted area in the "Diagnosis and indications" box (top third of the page) explains the rationale for the order.

Implications for clinicians

Although IDP-NAAT, in general, is more sensitive than microscopy and culture testing, a "multiplex" IDP-NAAT is not "omniplex." Now that BC diagnostic laboratories are transitioning to IDP-NAAT, which captures 14 common intestinal pathogens, it is expected that clinicians will need to be more familiar with the various pathogens beyond these 14 common ones to aid their differential diagnoses. If the multiplex IDP-NAAT does not include targets for the potential pathogens in their differentials, additional specific testing would be required, especially if the patient is still symptomatic and has no clear diagnosis.

Another potential change of practice is the timing to order test of cure for occupational clearance, especially for bacterial intestinal infections, depending on local occupational health and public health policies. IDP-NAAT is highly sensitive and could generate reactive results, even when patients are no longer infectious.[14] The BC Guidelines do not specify whether a test of cure is needed for each pathogen. Laboratories may have their own laboratory-developed assays and therefore no published guidance on when to order repeat testing, if indicated.

Furthermore, if laboratories are transitioning to automatic substitution of stool microscopy orders with IDP-NAAT, clinicians would have to clearly indicate on their order requisition forms that microscopy is needed and provide the rationale for it. An example of how to fill out a requisition form is provided in **Figure 3**. To further prevent errors, when handing requisition forms to patients, clinicians may want to remind them that they should anticipate receiving vials (for stool microscopy) rather than swabs (IDP-NAAT) from the laboratory patient service centres **[Figure 4]**. These tips are summarized in **Box 2**.

Although the BC Guidelines recommend stool microscopies if patients have a history of recent travel or immigration from low- or middle-income countries or are severely immunocompromised, these criteria may not capture all at-risk patients. For instance, *Diphyllobothrium* infections generally occur in the northern hemisphere, including Europe; newly independent states of the former Soviet Union; North America; and Asia.[7] Fish-borne parasitic infections, secondary to *Anisakis* spp. and *Diphyllobothrium* spp., are endemic in cosmopolitan regions in Japan.[15] Several intestinal parasites, including *Ascaris lumbricoides*, *Trichuris trichiura*, and *Taenia* spp., isolated from human stool specimens in Ontario were deemed to be endemic in Canada rather than imported.[16] Strongyloidiasis could be asymptomatic or cause minimal symptoms in its acute phase **[Figure 5]**. Thus, it is not apparent in recent travelers and immigrants, but clinical disease can be lifelong and turn into hyperinfection or disseminated disease when patients become immunocompromised.[11,12]

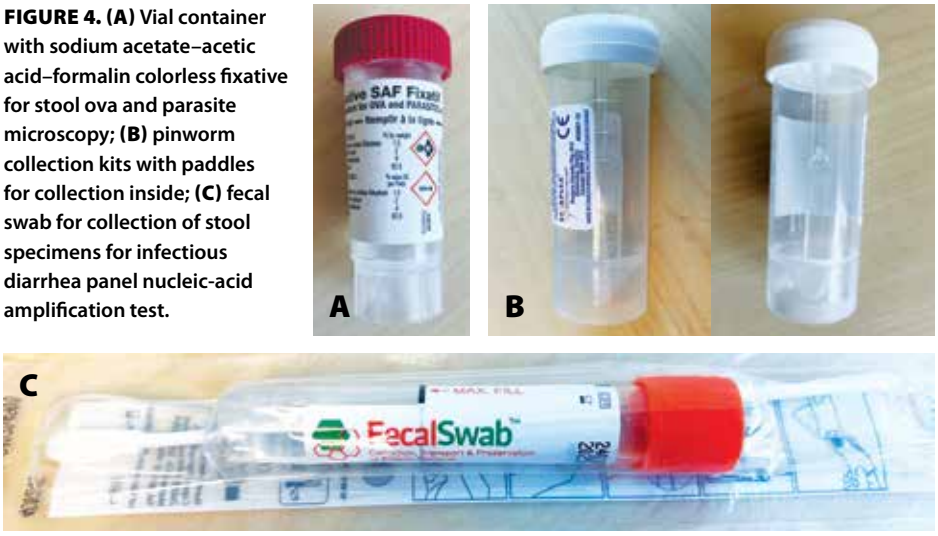


FIGURE 4. (A) Vial container with sodium acetate–acetic acid–formalin colorless fixative for stool ova and parasite microscopy; **(B)** pinworm collection kits with paddles for collection inside; **(C)** fecal swab for collection of stool specimens for infectious diarrhea panel nucleic-acid amplification test.

Implications for epidemiologists

Changes in test methods can lead to pseudo-outbreak.[17] An increase in incidents of certain pathogens could be due to the superior sensitivity of IDP-NAAT compared with microscopy.[18] Epidemiologists may need to determine the implementation dates of IDP-NAAT in different laboratories and set new baseline surveillance rates. The prevalence of some intestinal pathogens, such as *Blastocystis hominis* and *Dientamoeba fragilis*, may seem to decline, but it may be that they are not being identified by IDP-NAAT.

Implications for laboratorians

Similar to epidemiologists, laboratorians should conduct their own surveillance studies of intestinal pathogens detected in their

BOX 2. Practice tips on how to prevent auto-substitution of microscopy with infectious diarrhea panel orders, if clinically indicated.

When and how to order microscopy rather than infectious diarrhea panel (IDP)—use the acronym PICH:

- Consider stool microscopy if **p**arasitic infections are in your differential diagnoses but are beyond the four parasites in the IDP (i.e., *Cyclospora cayetanensis*, *Cryptosporidium* spp., *Entamoeba histolytica*, *Giardia* spp.). Consider pinworm collection kit when **p**inworm (*Enterobius vermicularis*) is suspected.
- **I**ndicate the rationale for why stool microscopy is needed in the "Diagnosis and indications" section of the laboratory requisition form (e.g., recent travel or immigration from a low- or middle-income country, immunocompromised).
- **C**larify in the "Other tests" box of the laboratory requisition form that microscopy, not IDP, is needed.
- When **h**anding out the requisition form, remind the patient that a vial container, not a swab, should be given.

laboratories. If there has been a significant decrease in the prevalence of intestinal pathogens since the implementation of IDP-NAAT, it could be that they are not being identified by that test. Consideration can be given to incorporating those pathogens into the multiplex panel.

Comparison to other studies

In this study, the most common intestinal parasitic pathogens identified were *Blastocystis hominis* and *Dientamoeba fragilis*. They were also the most predominant intestinal parasites found in stool testing among refugees at a primary care clinic in Toronto, Ontario.[19] Similar to our study, that study found infrequent occurrences of parasitic helminths,[19] which are not among the 14 common intestinal pathogens listed in the BC Guidelines. The variety of parasites identified in our study was consistent with studies of parasitic diseases that were conducted in Canada in the 1970s and 2000s.[16,20] However, unlike those studies, our study did not detect *Toxoplasma* spp. or *Trichinella spiralis*, which are diagnosed mainly using serological testing.

Study limitations

A major limitation of this study was the exclusion of data from hospitals, whose clinicians may have different indications warranting the need for stool microscopies in addition to IDP-NAAT. Hospital laboratories are welcome to conduct their own audits to observe changes in pathogen prevalence due to implementation of IDP-NAAT. Another limitation of this study was that not all of the 53 790 orders (52 221 stool and 1569 pinworm paddle specimens) were evaluated to determine whether a diagnosis could be missed. This study was meant to hypothesize about diagnoses that could be missed if only IDP-NAAT was used for diagnosing intestinal parasitic infections. In addition, this study did not investigate bacterial pathogens that could be missed, because IDP-NAAT recommends testing only seven common bacterial pathogens but not some rare ones such as *Aeromonas* spp., *Plesiomonas* spp., *Edwardsiella* spp., and *Yersinia* spp., other than *Yersinia enterocolitica*.[1,21,22] Further studies may determine whether testing for these pathogens should be included in IDP-NAAT. Despite this study's limitations, its major strength was the inclusion of almost all community microbiology data; therefore, the results should be generalizable to the community population in BC.

FIGURE 5. ***Strongyloides stercoralis*** **larvae detected by stool microscopy at LifeLabs. This microorganism would not be detected if a fecal swab were submitted for IDP-NAAT, whose targets do not include *S. stercoralis*.**

Conclusions

This 1-year study demonstrated that many parasitic pathogens could be missed if only IDP-NAAT is used to diagnose intestinal parasitic infections. If indicated, microscopy orders would be needed to capture these additional parasites. ■

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Competing interests

Dr Yeung has been paid for working as a microbiologist, physician, and pharmacist. The views and opinions expressed are those of the author and do not necessarily reflect the views or positions of his employers.

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