DETECTION OF *TRICHOMONAS VAGINALLIS*, *GIARDIA* and *CRYPTOSPORIDIUM* SPP. IN REMOTE INDIGENOUS COMMUNITIES IN CANADA USING A POINT-OF-CARE DEVICE

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Abstract

The Indigenous population in Canada disproportionately faces a large number of infectious diseases, such as *Trichomonas vaginalis* (Trich), *Giardia* and *Cryptosporidium* spp. For example, in Alberta, the highest prevalence of Trich in Indigenous women was found at 10.1%, which is concerning, considering that Indigenous people constitute 3.8% of Canada's population. Patient samples from remote Indigenous communities are currently sent to a central processing facility, which results in wait times of up to a month. This is worsened by a lack of access to full-time healthcare staff and clinics, along with the stigma of getting tested. The aim was to explore the current diagnosis and management strategies for Trich, *Giardia* and *Cryptosporidium* spp., along with issues faced in accessing primary healthcare in remote Indigenous communities. This was done to establish parameters for the development of a diagnostic device suitable for remote areas with resource constraints and cultural considerations. This investigation was done through a literature search and three structured interviews to obtain the perspectives of a healthcare worker, a researcher, and a member from Indigenous communities in Ontario. To meet the identified needs for a locally accessible, financially affordable, user-friendly, and rapid diagnostic device, a point-of-care device should be designed. This can ensure the diagnosis of various infectious diseases based on pathogen motility for patients of any sex, with or without symptoms. Understanding the current technologies available and gaps in access to healthcare can lead to the development of a suitable diagnostic device for a variety of resource-constrained communities.

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Table of Contents

List of Illustrations, Charts, Diagrams

Introduction

Infectious disease management in Indigenous communities

Based on socioeconomic status (SES), Canada is often ranked as one of the top five nations of the world[1]. Yet, when including the SES of the Indigenous population, Canada's ranking plummets[1]. The Indigenous population in Canada disproportionately face a large number of infectious diseases, such as pertussis, chlamydia, hepatitis A, Shigellosis, tuberculosis, and Human Immunodeficiency Virus / Acquired Immunodeficiency syndrome (HIV/AIDS)[2]. There are a variety of reasons as to why this may be the case, including government health coverage, poverty, education, nutrition, overcrowded housing, drinking water contamination, and health behaviour and practices[1,3,4].

First, within the government of Canada, there are different levels of government that provide healthcare to the Indigenous population. The federal government is responsible for the First Nations and Inuit populations, whereas the provincial government is responsible for the Métis populations, which differs in what constitutes the covered and non-insured portions of healthcare[1]. Second, with regards to poverty, about 52% of children in Indigenous communities in Canada are living in impoverished conditions, which is more than double the rate seen in the general population[3]. Third, regarding their drinking water contamination, from 1974 to 2001, there were 288 drinking water-related infectious disease outbreaks in Indigenous communities in Canada[4]. Accordingly, the number of gastrointestinal infections in these communities was 26 times larger than compared to the rest of Canada[4].

Given the aforementioned disparities, there is an identified need for collaboration in multi-jurisdictional health provisions. This includes involving the Indigenous communities when developing healthcare programs, providing more medical diagnostic and treatment resources, improving training of healthcare professionals, and creating comprehensive public health surveillance databases[1]. Thus, to provide affordable diagnostic tools that require minimal training and consider the needs of Indigenous communities, a point-of-care device is ideal for combating the infectious diseases experienced by these communities. For example, physicians and specialized nurses in First Nations communities in Saskatchewan are using portable and point-of-care fibroscans to assess liver fibrosis in patients with hepatitis B and C[5].

With these understandings, the focus of this paper will be on the use of an in-house point-of-care device (POCD) to detect *Trichomonas Vaginallis* (Trich) and its further application to detecting water-borne pathogens, such as Giardia and Cryptosporidium spp. as well.

Background on *Trichomonas Vaginallis* (Trich)

Why Trich diagnosis is required

Trichomonas vaginalis (Trich) is the most common, curable, non-viral sexually transmitted infection (STI) occurring in both men and women; however, it is often neglected with regards to its diagnosis[6,7]. In 2016, Trich was found to have made up almost half of all STI cases worldwide with 156 million cases[6]. Statistically, in Alberta, the highest prevalence of Trich was found in Indigenous women at 10.1% prevalence rate.

This data is concerning as the prevalence of STIs in the Indigenous population is higher than the 3.8% of their population in Canada[7]. Especially, remote and rural Indigenous communities in Canada face disproportionally high protozoal infection rates by Trich. The asymptomatic and non-specific symptoms that characterize these infections lead to a lack of diagnosis, and thus the accumulation of various health risks. An additional issue is that primary health care services provided in remote Indigenous communities are not equitable to that provided throughout Canada, and patients in these communities are deterred by the high cost and time required to travel to urban areas for diagnosis and treatment of diseases[8,9]. As such, to improve protozoal infection rates in these communities, it is vital to create an affordable POCD that can provide a timely and accurate diagnosis of *Trichomonas vaginalis*.

Symptoms and side effects of Trich

Around 70 to 85% of people infected with Trich are asymptomatic[6,7,10]. If they are symptomatic, they present non-specific inflammatory symptoms[6,7]. Trich can lead to negative consequences such as preterm birth, infertility, premature rupture of placental membranes, low-birth-weight infants, neonatal death, pelvic inflammatory disease, and a double to triple increased risk for HIV infection for both men and women[6,7,11].

For women specifically, symptoms of Trich include pruritus, odor, irritation, dysuria, frequency, urgency, pyuria, hematuria, and lower abdominal pain with urination as also seen with other STIs[10,16,17]. Symptoms are cyclic and more intense during menstruation[10]. Due to Trich, vaginal pH can increase from 4.5 to 5 and even reach as high as 7.5[10,17]. This increase leads to less lactobacillus acidophilus, healthy vaginal microbiota, which

still does not lead to a symptomatic condition[10]. A classical symptom for women is frothy and yellow or green vaginal discharge. The most specific symptoms are erythematous and edematous vagina and cervix, and the condition of “strawberry cervix”, which contains punctate hemorrhagic spots on the mucosa but is only diagnosed in 2-5% of women[10].

For quantitative diagnostics for women, a study by Khurana et al. in 2018, showed that 89% of cervical exams indicated that Trich infection was associated with cellular changes relating to inflammation[17]. Microscopy on infected urine sediment showed >20 red blood cells (RBC)/μL, >30 white blood cells (WBC)/μL, >30 epithelial cells/μL, bacteriuria[16,18]. In cases of mild pyuria and hematuria, there may be 20 to 22 WBCs/hpf, which is approximately 100 to 110 WBC/μL[17]. This is much higher than the expected range of 0 to 5 WBC/hpf for non-infected people. Higher levels of leukocyte esterase and protein can also be present[18].

For men, the infection can be diagnosed through urine cytology based on the following symptoms: lower genitourinary tract symptoms— including penile discharge and hematuria, nocturia, frequency, urgency, hesitancy, incontinence, increased serum PSA levels, urethral strictures, urethral discharge and residual urine sensation[12,13,19]. Other possible complications from Trich infection for men are urethral strictures, infertility and prostatitis, and symptomatic or asymptomatic nongonococcal urethritis[12,13,14,15].

Characteristics of Trich

Based on a case study by Khurana et al. in 2018, the morphology and motility pattern of Trich was similar to that of *B. coli*[17]. Trich has an oval-shaped body, around 60

µm in size, which is larger than WBCs and smaller than epithelial cells. It has pear-shaped flagellate with characteristic brisky and rotor motility, one to two food vacuoles, and a macronucleus. The body is covered with short and delicate cilia, with longer cilia lining at the mouth[17].

Trich flagellates do not survive outside the human body unless they are protected from drying and kept at a body-temperature environment[10,20]. Even after several hours of exposure to air and water, live Trich have been found in urine and in semen[10]. This explains why wet samples have higher sensitivity (100 Trich/mL) than the dry ones (10 Trich/mL)[21]. Trich will remain viable for 45 minutes on Bakelite and polished wooden seats, and for 30 minutes on an unpolished seat with an absorbent surface[22].

Diagnostic methods for Trich

Current diagnostic methods

Given the characteristics of Trich, there are ten well-known methods used to diagnose Trich, which are Wet mount test, Culture, Nucleic acid amplification test (NAAT), OSOM Trich rapid antigen test, polymerase chain reaction (PCR)-based tests, Affirm VP III, GeneXpert TV, Kalon TV agglutination, APTIMA *T. vaginalisis* assay, and Loop-mediated isothermal amplification (LAMP) assay. The advantages, limitations, sensitivity, specificity, and costs for each method are displayed in **Table 1**.

Wet mount test

The wet mount test is the most used Trich diagnostic method[10]. It is conducted through obtaining a sample of vaginal discharge and examining it under a microscope[10,23].

The patient tests positive for Trich if trichomonads are detected through observed motility and morphology[23]. The required sample for wet mount test is usually a vaginal swab but sometimes a urine sample may be used[11,24].

Culture

Cultures use specialized Diamond's or Trichosel medium or the newer InPouch device, which is just as sensitive as Diamond's[11,25,26]. The InPouch device is an oxygen-resistant, clear plastic bag of two chambers, where the specimen is placed in the upper chamber and the culture occurs in the lower chamber[11,25]. It can be stored at room temperature before use and remain at room temperature for a maximum of 48 hours after inoculation[25]. The Diamond's medium must be kept at 4˚C before use, warmed to room temperature before inoculation, and immediately incubated in anaerobic conditions[25]. Cultures should be inoculated less than an hour after sample collection, and are incubated at 37˚C and can be checked every other day for up to five to seven days by trained personnel[12,25,26,27]. Three negative readings lead to a negative result, and the detection of any live Trich leads to a positive result, confirmed by light microscopy[12,26]. Trich-positive cultures are often found three days after inoculation for women and can take five days or longer for men[25]. Culture is also used in cases of ongoing infection or potential treatment failure[25]. The sample requires vaginal fluid or vaginal swab for women, and urethral swab, urine, or semen for men[11,25,26,28,29]. For men, the urethral swab is less sensitive than the urine sample or the combination of the two for culture[25].

Nucleic acid amplification tests (NAATs)

NAATs are commercially available tests used on both symptomatic and asymptomatic subjects to diagnose Trich[11]. Two NAAT assays that have been FDA-cleared for use in Trich diagnose are the Aptima Trich vaginalis assay and BD Probetic Q^x assay[30,31]. All NAATs are FDA-cleared for men[13,15,19,32]. In the BD Probetic Q^x assay, DNA is extracted from the sample and then undergoes strand displacement amplification, which increases the detection of light emission of the specimen[31]. Endocervical or urinary samples, or urethral, vaginal, or rectal secretions may be used to perform the NAATs[10,11,28].

OSOM Trich rapid antigen test

OSOM Trich rapid antigen test is a type of non-amplified molecular test, specifically an immunochromatographic capillary-flow enzyme immunoassay dipstick test, that detects the Trich membrane protein[11,25,28]. It is an FDA-cleared point-of-care test (POCT) that uses specific antibodies to detect Trich protein antigens[25]. The sample used is a vaginal swab[29].

PCR-based tests

PCR-based tests are a form of NAATs used to diagnose Trich in laboratories[11,33]. To detect Trich using PCR, a primer is used to target a specific region of the genes that encodes for a protein that makes up much of the Trich flagella and cytoskeleton, such as TV-E650, and then binds with this target single strand portion of the DNA[12,34,35]. This process is repeated many times as the sample undergoes cycles of being heated and cooled, causing the DNA to amplify, which allows Trich to be easily detected by examining

the PCR product[34,35]. The test uses vaginal, urethral or endocervical swabs, or a urine sample[11,33,34]. One can use RT-PCR assay and mass spectroscopy to detect Trich 50-kDa metallopeptidase (TvMP50) that is only found in the sera of male Trich-infected patients[36]. This may be due to the presence of zinc in the male genitourinary tract at 4.5-7 mM, which allows for increased proteolytic activity of TvMP50, which can be inhibited by EDTA[36].

Affirm VP III

Affirm VP III is a non-amplified nucleic acid probe hybridization test that is cleared by the FDA[25,37]. It uses an oligonucleotide probe to detect nucleic acids from Trich, a heating block, and a processor instrument[25,]. The test can be completed in 45 minutes to an hour but is not used as a rapid test[25,28]. The sample used is a vaginal swab from symptomatic women or those suspected of having Trich exposure[25,37].

GeneXpert Trich assay

GeneXpert Trich assay is a type of NAAT with PCR-based methods[30,32]. The GeneXpert assay is completely integrated within a test cartridge[30]. In this test cartridge, fully-automated sample preparation, amplification and real-time detection of Trich all take place[30]. For female specimens, urine samples, self-collected vaginal swabs or endocervical swabs are required[30,32]. For males, urine samples are required[32]. GeneXpert TV assay is FDA-cleared for use with male urine, and the only rapid assay approved for men[30,32]. Sensitivity for male urine was 89.6-97.2% and specificity was 99.3-99.9%[30,32].

Kalon TV agglutination

Kalon TV agglutination is a type of non-amplified molecular test but is not an FDA-approved POCT. The test uses latex beads coated with an antibody to detect Trich protein antigens, which would cause cross-linking of the sensitized latex, and the beads would stick on a glass slide[23,25]. The kit includes sterile PBS and pH 7.2[23]. This test requires the use of vaginal swab and a culture medium[23,38].

APTIMA *Trichomonas vaginalis* (ATV) test

APTIMA *Trichomonas vaginalis* (ATV) test is a type of NAAT that uses the automated Tigris DTS instrumentation system to process the sample and detect Trich 16S rRNA[28,39,40]. The rRNA is extracted from the specimen, which is then transcription-mediated amplified so that the detection limitation is increased[31]. To achieve this, target capture specimen processing is used to purify target nucleic acid so that they can then undergo transcription-mediated amplification[10,39]. ATV also utilizes chemiluminescent probe hybridization for aid in identifying *Trichomonas vaginalis* rRNA[39]. For women, vaginal or endocervical swabs or urine specimens is required[10,28]. For men, urethral swabs, pharyngeal specimen, or urine is required[28,41].

Loop-mediated isothermal amplification (LAMP)

Loop-mediated isothermal amplification (LAMP) assay targets Trich adhesion protein 65 (AP65), which allows binding to host cells[42]. It amplifies a large number of target genes in isothermal conditions— 63 °C for 120 minutes during the incubation and 80 °C for 10 minutes during the termination in a heating block, and has been previously applied to virus, bacteria, and pathogen detection. It amplifies target DNA by a *Bacillus*

stearothermophilus (*Bst*) DNA polymerase using four to six primers[43]. The detecting limitation of LAMP was ten trophozoites of Trich and that of nested PCR was as many as 1000[42]. The sample is taken by a vaginal swab.

Table 1: Advantage, limitations, costs, and sensitivity (sens) and specificity (spec) of various methods used to diagnose *Trichomonas vaginalis* in the laboratory.

Method	**Advantages**	**Limitations**	**Sens & Spec, Cost approximation (USD, unless specified)**
Wet Mount Test	• Fast, simple and convenient[23,44] • Cheap compared to most other methods[23,44]	• A laboratory and a trained professional are needed[24,44] • Test must be conducted within a few minutes of sample collection[11] • Relatively low sensitivity[23,44]	• Sens: 38% - 82%[11,23,24,28] • Spec: 100%[23,24,44] • Cost: $13.57 CAD per test[45]
Culture	• Better sensitivity than microscopy[25] • Possible to conduct an antimicrobial susceptibility testing[25] • Works with a variety of sample collections types (fluids, swabs, urine) for both sexes[11,25,26,28,29]	• Requires an incubator, trained personnel, and temperature-controlled transport[11,25] • Takes 7 days to obtain test results[11,25] • Need a high load of 300-500 Trich/mL for detection[12,29]	• Sens: 40-96%[12,25,27,28,29] • Spec: up to 100%[25,27,28] • Cost: $216-350 for 3-6 L[46]
Nucleic Acid Amplification Test	• Can be used on both men and women[10,11] • Test can be conducted months	• High cost makes it infeasible to become the primary test for Trich[31,47]	• Sens: > 90%[31,33,48] • Spec: > 95%[31,33,48]

	after sample collection[27] • Can be completed in only 45 minutes[28]		• Cost: Almost $1000 per test[49]
OSOM Trich rapid antigen test	• Results in 10-30 mins[25] • Can be used with frozen samples, so no transport specificities[11,25] • Dipstick test that can be used as a self-test or a POCT[10,28,29] • No equipment needed, minimal training[25]	• Uses vaginal swabs only, not cleared for men or asymptomatic women[10,25]	• Sens: 77-98%[25] • Spec: 97-100%[10,29] • Cost: $858[50]
PCR-based tests	• Many laboratories already use similar techniques to diagnose STDs, so it would be easy to implement into laboratory workflow[35]	• Expensive to install[34] • More hands-on time required than culture and wet mount microscopy[51] • Requires trained professionals in microscopy to operate[34] • Takes 2-3 days to get results[35]	• Sens: 84% - 100%[51] • Spec: 94% - 100%[51] • Cost: $7,670.00 per thermal cycler[52]
Affirm VP III	• Results in 45-60 mins[25,28] • Delay in sample transport is not a hindrance[25]	• Requires the use of a complex equipment and technique[25,28] • Uses vaginal swabs only, not cleared for men or asymptomatic women[25,28]	• Sens: 40-95%[25, 28,37] • Spec: 92-100%[25,28,37] • Cost: $45-24,120[53]
GeneXpert TV	• Approved rapid assay for detection of Trich in men[30]	• More expensive than antigen detection rapid	• Sens: 96.4% - 100% [30,32]

	• Provides results in as little as 40 minutes for positive specimens and 63 for negative specimens[30,32] • Easy to operate and can be used as a POCD[32]	tests and most other Trichs diagnose methods[30,39]	• Spec: 98.9% - 99.9%[30,32] • Cost: ?
Kalon TV agglutination	• Results in <10 mins[25] • No equipment and minimum training[25] • Delays in sample transport are not a hindrance[25]	• Uses vaginal swabs only, not cleared for men or asymptomatic women[25]	• Sens: 55-100%[23,25,38] • Spec: 81-100%[23,25,38] • Cost: $640[54]
APTIMA T. vaginalis assay	• Can be used for both men and women[10,28]	• Sensitivity dramatically decreases for men[28] • Expensive[49]	• Sens: 95.3% - 100%[10,28] • Spec: 95.2% - 100%[10,28] • Cost: ?
Loop-mediated isothermal amplification assay	• Results in 130 minutes[42] • Low cost[42]	• Need for equipment for incubation and detection[43]	• Sens: 100%[43] • Spec: 97%[43] • Cost: ?

Potential point-of-care tests (POCT)

Out of the techniques mentioned above, there are three rapid tests that have potential as a POCT: OSOM, Kalon TV, and GeneXpert TV.

OSOM has the highest potential as a POCT. It is FDA-cleared, does not need equipment, uses minimal training, provides quick results, can be developed into a self-test, and has relatively superior sensitivity and specificity. The main problem is the high cost and lack of clearance for use in men and asymptomatic women.

Kalon TV has advantages with regards to quick test results and lack of equipment and training required. It has similar problems with the high cost and lack of clearance for use in men and asymptomatic women. Further considerations are the wide range of sensitivity and specificity, along with the lack of FDA-clearance.

GeneXpert TV also has potential as a POCT and has advantages of quick test results, minimal training required because the assay is fully automated, very high sensitivity and specificity, and it has been approved for use in men. Its biggest issues are that the GeneXpert TV has yet to be approved as a POCT, and it is also very expensive when relatively compared to the OSOM and Kalon TV test costs.

Self-testing at home

Due to the lack of resources at clinics and stigma of STD testing, at-home tests may be considered for testing the presence of Trich. Home-collection is convenient and potentially cost-saving[19,55]. One method is to provide free kits to test for Trich infection. In a study by Gaydos et al. in 2013, male subjects mailed self-collected penile swabs back to the laboratory[19], and in a study by Gaydos et al. in 2011, females mailed self-collected vaginal samples[55]. Men were 60% more likely to complete screening if it is home-based[19], and favoured that method whether for urine or penile-meatal swab. Self-collect penile-meatal swabs showed 80.4-82% sensitivity, similar to the sensitivity of clinician-collected swabs at 85%[56]. However, urine samples only had 39.3-40% sensitivity[56]. Similarly, 75.7% of women preferred receiving home-test than visiting a clinic or their doctor[55]. The return rate of the collected samples was approximately 43% for women in 2011, and 50.2% for men in 2012, which improved from 15.3% in 2006[19,55].

With regards to attitudes towards self-testing, 71% were willing to perform a self-test if available over the counter, 49% preferred clinical examination or sending a self-collected sample to the clinician, and 23% preferred self-collection and home-testing[57]. There was an increase in trust and confidence after discussion with a clinician and by having a positive attitude and experience using other tests and products (i.e., pregnancy test, tampons). Specifically for the OSOM TV trichomonas rapid test, it was found that 99% women performed the test correctly and only 4% of results were discordant between the patients and the clinician[57].

However, there are several drawbacks to self-testing. Although it may be more comfortable to collect tests by oneself at home, patients would still have to wait for test results from centralized labs. When self-collected samples reach the central lab, the testing is often done through NAAT: Aptima TV, Analyte Specific Reagent (ASR), or Gen-Probe[19,21,55]. Furthermore, the result of the test would depend on whether the sample used is wet or dry. Home-collected dry urogenital swabs (held at 55˚C for five days before testing) containing more than 100 TV/mL tested positive[21]. On the other hand, home-collected wet urogenital swabs containing more than 10 TV/mL tested positive[21]. Thus, the testing of dry samples are not as sensitive as wet samples. Finally, with regards to accessibility, self-testing is still expensive. Women in Brazil said they would pay up to $4 for an STI self-test kit, but current home-test kits are $40-$250[57]. Given that a POCD is reusable, unlike home-test kits, it may be more economically feasible to use a POCD in a clinical setting.

Use of lensless microscopes as a POCD

Although the current POCDs still have room for improvement, POCDs are ideal for diagnosing diseases in areas with low medical resources as they have high sensitivity and specificity, are of reasonable cost, are easy to use, rapid, robust, have a long shelf life at ambient temperatures, and have a compact and portable design[58]. There are a couple of examples that highlight the suitability of using lensless microscopes as a POCD to detect a variety of pathogens, including Trich.

In general, lensless imaging platforms are of reasonable cost, due to their inexpensive light sources and imaging sensors, specifically light emitting diode (LED) and a complementary metal-oxide semiconductor (CMOS) imaging sensor[59,60].

In 2010, Mudanyali et al. used a portable lensless holographic microscope to image and automatically detect *Giardia Lamblia* and *Cryptosporidium Parvum* in contaminated drinking water samples. The device detected the parasites at a low concentration of approximately 380 parasites/mL with a mean error of less than 10% without any pre-concentration steps[59]. This renders the device easy to use, as laboratory technicians would not necessarily be needed. The microscope only weighs 46 grams, lacks lenses and bulky optical appendages, and can provide wireless transmission of the holographic images from a cellphone to a central computing station, without loss of resolution[59]. The above shows that the device is portable, compact, and allows for rapid attainment of results. This device is also cost-effective and can be further applied to diagnose malaria, HIV, and tuberculosis[61]. Lensless holographic imaging microscopes devices can also be used to detect the Herpes Simplex Virus (HSV-1), with a sensitivity of ~5 viral copies/µL[60]. An automated program counted the number of viral particles by

digitally filtering out the particles with sizes outside the 150-200 nm size range of HSV-1[60]. This example shows that these microscopes can have a high sensitivity and can automatically and thus quickly provide results.

In 2019, Kun et al. created a lensless optofluidic imaging microscope, which will be referred to as the in-house POCD in this paper, that detects the presence of red and white blood cells and Trich by motility rather than just morphological features. The optofluidic imaging device has a field of view of 2.15 mm^2 and a maximum sensor to sample distance of 100 μm[62]. The device only costs a few hundred CAD rather than the typical few thousand, weighs a few hundred grams rather than a couple kilograms, does not require calibration or change of regent, has a disposable sample holder, has a running time of a few to 15 minutes, and can provide tests results directly to a smartphone.

Regardless of whether a lensless imaging platform is holographic or optofluidic in nature, these microscopes show suitability as a POCD for Trich. The microscopes described above are portable, provide fast results, have high sensitivity, low cost, low weight, and do not require calibration and training.

Suitability of the in-house POCD for remote Indigenous communities

The in-house POCD is a reusable, low-cost, and portable lensless optofluidics microscope. The user, which would be a health care professional, can observe Trich in the fluidic samples, such as urine. With customized algorithms, Trich can be detected and counted automatically, allowing for clinical diagnosis. If necessary, Trich analysis can be further processed. With regards to sensitivity, the POCD can detect a single Trich at a time. **Figure 1** shows a schematic of the in-house POCD, and its other advantages are explained below.

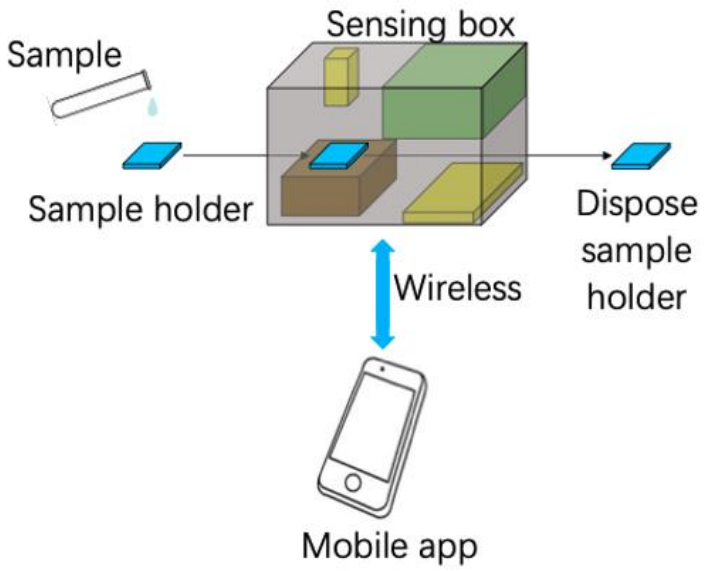

Figure 1: The in-house POCD is a low-cost and portable microscope that can be used by minimally trained professionals. The urine sample, which is being tested for the presence of Trich, is poured onto a sample holder. This sample holder is placed in the microscope sensing box, in which the fluid is being analyzed for traces of Trich as it flows through the channel. Within a few to 15 minutes, the results of whether Trich is present or not present in the fluid sample is sent to a health care professional's mobile app through wireless means. Upon receiving the results, the sample holder can be disposed. Image credits to the McMaster Biophotonics Research team in the Department of Engineering Physics at McMaster University.

Similar to the rapid tests — OSOM, Kalon TV, and GeneXpert TV — health care professionals, such as those at nurse-based clinics, require no to minimal training because the microscope is automated to analyze urine[63]. There is a fast turnover time because the automatic urine analyzer provides rapid and accurate screening, in a timeframe similar to that of Kalon TV[63]. This saves healthcare workers' time so that the lack of workers in remote Indigenous communities will not be a problem, and they can spend their time with patients. Because this test is a portable POCD, there is no need for transportation between these remote communities to a larger facility or laboratory, thus saving both time and financial resources[64].

Unlike the three rapid tests, the in-house POCD is inexpensive, as the most expensive component is the image sensor, which is typically only a few tens of dollars[64]. It also covers the weaknesses of the three rapid tests mentioned above. A weakness of OSOM and Kalon TV tests are that neither of them can be used on asymptomatic patients[25], but this POCD can[65]. This is a significant difference between these tests due to Trich's asymptomatic characteristic[10]. OSOM has not yet been approved for use on male specimens[10], while the lab POCD can be used on both men and women because the sample specimen is urine. This POCD is much cheaper than OSOM and the price per kit/test for Kalon, as the POCD price is just the installation price and has a low operating cost[66].

Overall, the in-house POCD is a viable choice for diagnosing Trich within remote Indigenous communities because it is rapid, portable, inexpensive, adaptable, and it does not require a trained professional or a laboratory. This device is more feasible than Kalon and OSOM because it can be used on asymptomatic people of any sex, and it tends to be cheaper. Considering that this POCD has the same benefits as the rapid tests, while also combatting the issue of cost and adaptability, it is ideal for implementation in all low-resource community clinics, such as remote Indigenous communities in Canada.

Treatment of Trich

The recommended treatment

Once Trich is detected, the standard drugs used in treating Trich are the 5-nitroimidazole class drugs, which are systemic[11,67,68]. Many compounds derived from the

5-nitroimidazole class could be used in curing Trich with a reported success rate of approximately 95%, if the partner of the infected individual is also treated[69,70]. The recommended 5-nitroimidazole derivative is metronidazole (MTZ) or tinidazole (TNZ)[11,67,71]. Metronidazole is the most used drug in the treatment of Trich; it is moderately cheap and effective, well-tolerated, usually only causes minimal and mild side effects, and is easy to administer through the oral, topical, intravaginal or intravenous routes[11,67,69,72].

The mechanism of the trichomonacidal activity of 5-nitroimidazoles is due to the ferredoxin-mediated reduction of their nitro group[73]. The creation of reactive metabolites interact with the Trich's DNA, leading to the inhibition of nucleic acid and protein synthesis. The reduced metabolites of nitroimidazoles may also covalently bind to tissue macromolecules and cause thiol depletion, causing further toxic effects[73]. In vitro studies show that the effects of the drugs can be seen in two to three days, by looking for remaining motile Trich[74,75,76,77]. The effects of an antibiotic can be seen 48 hours after the administration of the treatment, and by collecting a second sample from the patient.

To treat Trich, the World Health Organization (WHO) recommends first giving the patient a single two-gram dose of MTZ[67,69,72]. If this fails, the patient should either be administered a single two-gram dose of TNZ or be given a seven-day treatment regimen of 400-500 milligrams of MTZ taken twice a day[72,78]. If this also fails, then two-grams of either MTZ or TNZ should be administered daily for five days[11,72,78]. The approved treatment by the Government of Canada and the treatment recommended by the Centers for Disease Control and Prevention is similar to that of the WHO, except for the inclusion of TNZ as an option[70,79,80]. TNZ is only an option for MTZ-resistant patients in Canada,

and can only be obtained through Health Canada's Special Access to Drugs and Health Products Program[70,79].

It should be noted that through a set of randomized trials, it was determined that a single-dose MTZ and multi-dose MTZ treatment have similar cure rates, with the single-dose cure rate being slightly lower[72]. Additionally, higher doses of MTZ are known to cause more frequent and severe side effects[11,69,72]. This can lead to patient discomfort and possible treatment failure, and is a limiting factor in treating Trich, because the amount of MTZ that can be prescribed to a patient is dependent upon the severity of each patient's side effects[11].

Drug resistance to MTZ is a possible cause for repeat infections and occurs in about 2.2-9.6 % of patients[72,78]. Since TNZ has a similar chemical structure to MTZ, patients resistant to MTZ may also be incurable using the standard TNZ treatments[68]. However, most of these patients can be cured by receiving standard MTZ treatment again either at the same dosage for a prolonged time or at higher dosages[68,70,72].

In addition, previous research has shown that TNZ showed better treatment results compared to MTZ. A meta-analysis of eight randomized trials comparing MTZ and TNZ showed that patients treated with MTZ showed higher parasitological and clinical failure rates of 10.9% and 14.8%, respectively, than those treated with TNZ, with parasitological and clinical failure rates of 3.3% and 3.7%, respectively[78]. 47.3% of patients treated with MTZ reported adverse side effects from treatment, compared to only 28.9% of patients receiving TNZ[78]. Overall, a single two-gram dose of TNZ seems to be either as effective

or more effective than MTZ[71]. The cost of MTZ and TNZ are similar at $7.50-$9.69 per 500 milligram tablet compared to $6.71 per 500 milligram tablet, respectively[81].

Alternative treatment options

Although 5-nitroimidazole drugs are the most effective and only approved treatment for Trich, alternative treatments may be required in cases of resistance to MTZ, and allergies or hypersensitivities to 5-nitroimidazoles[11,72,78]. Hypersensitivity can occur in persistent cases of Trich as an adverse reaction, including anaphylactic shock[72]. As those reactions occur from receiving multiple doses of MTZ, hypersensitivity can be cured through desensitization, but only with a 42% success rate[72]. As such, alternative 5-nitroimidazole drugs consist of disulfiram and nithiamide, but have yet to be approved by the FDA[11]. Other potential alternatives include albendazole, mebendazole, and nitazoxanide, which have only shown *in vitro* effects so far[11].

When the patients cannot be desensitized or are allergic to nitroimidazole, they experience severe side effects. When systemic treatment options are limited, alternative treatment drugs should be administered vaginally because they either cannot be ingested, or they cannot be properly absorbed by the intestinal tract [11,68]. The following are drugs being studied as potential intravaginal treatment options: Albendazole, Mebendazole, Acetarsol, Boric acid, Furazolidone, Pentamycin, N-chlorotaurine, Drug-free chitosan, Paromomycin and Nitrazoxanide[11,68,71,72]. Unfortunately, intravaginal treatments are not as efficient as systemic treatments, such as nitroimidazole drugs. This is seen by the low success rate of 29.4% for intravaginal treatment compared to 95% for

MTZ[68,73,78]. Intravaginal metronidazole gel is not an option for treatment in Canada, due to its ineffectiveness[79].

Special considerations in the treatment of Trich

The findings of meta-analyses suggest that MTZ and intravaginal treatments are safe to use at any stage of pregnancy; however, the WHO recommends for women to not seek treatment in their first trimester[11,69,72]. Pregnant women can obtain a single two-grams dose of MTZ, or alternatively 100 milligrams of clotrimazole taken once a day at bedtime intravaginally for 14 days. The use of TNZ in pregnant women has not yet been studied[72].

Within a randomized clinical trial, a single two-gram dose of MTZ was found to be less effective than two doses per day of 500 milligrams of MTZ for seven days in patients infected with HIV[78]. Comparing the multi-dose versus single-dose treatments, only 8.5% versus 16.8% of the patients showed repeat Trich infection within days of receiving treatment, and only 11% versus 24.1% of patients showed repeat Trich infection within three months after treatment[78]. Therefore, the seven-day treatment option should be used to treat patients infected with HIV[72,78]. It should be noted that the antiretroviral therapy used to treat HIV may interfere with the administration of MTZ[72].

Accessibility to primary healthcare and the need for POCDs in remote Indigenous communities in Canada

Healthcare providers and patients in remote Indigenous communities often understand that there would be a lack of equal access to resources in remote

communities compared to the rest of Canada[8]. However, the same healthcare providers and patients highlighted the need for primary healthcare that was timely, close to home, and without risks and barriers to resources that are available[8]. By developing an effective and accessible POCD for diagnosis, timely and local diagnosis can be provided with ease by nurses.

Lack of availabilities of different health care professionals

With regards to non-urgent care, such as the diagnosis of protozoal diseases, the main issue identified was related to confidentiality[8]. This was more prevalent in smaller Indigenous communities that had a community healthcare worker (CHW) rather than a resident nurse. Patients reported feeling unsafe in sharing their health issues with CHWs for the fear that their information would be shared with other community members, leading to social exclusion[8,9]. Thus, patients would wait for nurses from outside the community to arrive, even if that would be weeks away[8]. Moreover, in many remote Indigenous communities, nurses and physicians are only present part-time[82]. For example, some communities in Northern Canada only have a nurse come in for two weeks or less and a physician for three days every six weeks[82]. Given that patients would rather delay the seeking of diagnosis and treatment, it is possible that they would have to wait an additional few weeks for diagnostic test results to be processed and reported from regional health centers. Hence, a POCD is essential to allow for rapid attainment of test results for infections such as Trich and avoid delays past waiting for the arrival of HCPs from outside the community or those that work part-time.

Lack of training for nurses

The primary healthcare system in Northern remote Indigenous communities is nurse-based, which means most duties that only a trained physician is qualified for are being completed by nurses[82]. The majority of healthcare workers within remote Indigenous communities are underqualified for the work they are completing and are responsible for jobs that far surpass the scope of their training[82,83,84]. As such, nurses identified a need for additional education and training, but that was often only available outside of the community[85]. Without such training, nurses have to consult physicians, which takes them on average 35 minutes, but it could take up to 140 minutes. To make matters worse, there is a chance that referrals can be denied or delayed[85]. From this, it can be deduced that any Trich diagnose methods that are appropriate for use within these remote Indigenous communities should by simple enough for a nurse to implement without advanced training. Accordingly, POCDs are ideal as they require none to minimal training, allowing nurses to easily use them without the need of regional training or the presence of a physician.

Additionally, patients preferred health services when HCPs were able to provide close monitoring and make the patients feel relaxed or calm by being confident rather than uncertain during consultations[9]. Thus, the delivery of processed results from a POCD to a smartphone can allow for both prompt monitoring and confidence, regardless of a nurse's level of training, as the HCP is not responsible for interpreting results. This is especially important, considering that the rates of infectious diseases, such as Trich, within remote Indigenous communities are also dramatically increased compared to other Canadian communities[83].

Nurses are understaffed and underequipped

Due to a lack of staffing resources and heavy workloads, nurses noted that they had limited time with patients[85]. For instance, some communities only have part-time nurses and those with full-time nurses only have one to three HCPs in a clinic[82]. This leads to patients being dissatisfied when HCPs are not able to attend to them or provide diagnostic results on a timely basis[9]. As such, diagnostic devices that are suitable for use in remote Indigenous communities are required, such as POCDs. POCDs can physically allow for rapid and close patient monitoring, especially in the limited time the nurses have available to spend with each patient.

Furthermore, there is a frequent lack of necessary medical equipment, leaving nursing stations extremely under-equipped[82,83]. A Webequie man from Thunder Bay named Norman Shewaybick discussed how First Nations communities often lack basic medical devices and diagnostic equipment, or if they do possess these resources, they are often in poor and unusable conditions[84]. Due to the lack of basic medical equipment in so many of these remote Indigenous communities, the only viable methods to diagnose Trich are those that do not rely on the presence of additional equipment in clinics. Hence, using a portable POCD can avoid the reliance on such equipment.

The lack of staffing and medical resources, can also cause the transportation of many patients to larger hospitals[82,83]. However, the priority for many patients is to receive primary healthcare without leaving the community, as this saves on monetary expenses and time from having to travel to urban areas for healthcare services[8,9]. In a study conducted on 49 North-Western Ontario First Nations communities, an approximate

24,000 out of roughly 49,000 people live in communities that do not have permanent road access, meaning the only way to travel to larger and more equipped health facilities is through medical air travel, which is expensive and should be avoided when unnecessary[82,83]. Thus, a POCD can allow patients' samples to be tested and for patients to receive results without having to travel outside of the community themselves. With a greater sense of convenience, more people may be willing to get tested for protozoal infections in their communities, preventing underdiagnosis.

Apart from expenses, patients did not prefer receiving consultation from HCPs in urban settings nor through telehealth, as there is an inability to build a trusting relationship with the provider[9]. This leads to insecurity and a lack of use of healthcare services[9]. It would be best for the POCDs to be used by the same nurses, whether from within or outside of the community, to develop a trusting relationship with the patient through repetitive exposure.

Variability in transportation accessibility

Inconveniently, the accessibility of traveling for care is correlated with the size of the Indigenous community in the North-West Territories. Residents in Wekweèti and Gamèti, with 140 and 250 residents respectively, can only travel by air, except for a period in the winter when an ice road is built over the snow[82]. The ice road is only open in the winter months, ranging from February to April, for 40 days in a year in Wekweèti and 56 days in a year in Gamèti[86]. In Tuktoyaktuk, a larger town with 850 residents, an all-weather road is currently under construction, and apart from this, all travel is done by air– similar to the smaller communities[82]. Larger communities such as Inuvik and Yellowknife,

with 3,460 and 19,230 residents respectively, have year-round road accessibility, providing access during all weather conditions[82]. As a general trend, the smaller the community, the less likely it is for residents to have access to roads for travel. This can restrict access to clinics, and thus highlights the need for a POCD to obtain diagnosis for infections such as Trich.

However, regardless of the large populations of remote First Nations communities in northwestern and northern Ontario, there is a lack of road accessibility and medical resources. The Nishnawbe Aski Nation (NAN) in northwestern Ontario consists of 49 First Nations communities and approximately 49,000 people who reside off and on the reservations[83]. Approximately half of the people who make up NAN do not have permanent road access and either only have access to seasonal ice roads or no roads at all[83]. These communities also lack in the number of hospitals and have nursing stations that are often under-equipped. This portion of the NAN population only have access to federal nursing stations or clinics instead of larger and more-equipped hospitals[83]. Similarly, the Sioux Lookout region in northern Ontario houses 33 First Nations communities and consists of approximately 30,000 people[84]. Approximately 80% of Sioux Lookout is accessible only by air, as there is a lack of road access. Additionally, this region lacks in basic medical devices and diagnostic equipment, along with clean water and housing[84]. The standard of care is also much lower here compared to other parts of Canada, much like other First Nations communities. Thus, the lack of access to medical resources is worsened by the lack of road access, preventing the reception of these medical resources from even neighbouring towns. As such, it seems essential to have a

POCD that can test samples and provide diagnosis results within the same community, without needing to travel.

Limiting infrastructure

In 2001, telephone providers began investing in a program that works towards ensuring telephone service access in all rural and remote regions within Canada that is up to par with those in other areas of Canada[87]. Since then, all regions of Canada have had telephone access, including Indigenous communities within Northern Canada[87].

Most First Nations within northern Ontario are not currently connected to Ontario's power grid and instead use diesel generation to produce electricity[88]. The Wataynikaneyap Power Project is made up of 22 First Nation communities and is working toward connecting 16 of the diesel dependent First Nations communities to Ontario's power grid, which provides energy that is cleaner, safer, more reliable, and cheaper[89]. Ontario's power grid utilizes hydroelectric plants, nuclear plants, gas-fired plants, wind plants, solar plants, bio-energy, and combined heat and power in order to generate electricity instead of just fossil fuels[90].

However, even with efforts to provide telephone services and electricity production, the question is whether these services are at par and whether they are consistent and reliable. Using a POCD would avoid the need of telehealth nor require large amounts of electricity, and thus, the uncertainties of basic telephone and electricity provisions may not be large hindrances in the provision of healthcare.

Interviews regarding Trich in remote Indigenous communities

To achieve the goal of creating a suitable POCD to provide clinical diagnoses of Trich in remote Indigenous communities, it is crucial to obtain the perspectives of healthcare professionals (HCPs), who are responsible for diagnosing and treating patients. It is also valuable to consult infectious disease specialists (IDSs), as they are responsible for obtaining test results and thus can provide input regarding diagnostic methods. Lastly, Indigenous community members should also be included, as they form the patient population and are most aware of accessibility to medical care and societal considerations regarding Trich infections in the community.

After emailing 28 various organizations and individuals who could provide direction in contacting relevant HCPs, IDSs, and community members, interviews were conducted with three members of Indigenous communities, specifically from the Anishinaabe and Six Nations. Doris Peltier is a community advocate for healthcare and is involved with research in sexually transmitted and blood-borne infections (STBBI). Maggie Powless-Lynes is a student at McMaster University, and Randy Jackson is a research and assistant professor at the same university, researching Aboriginal health and HIV. The interviews were conducted on Zoom and lasted approximately an hour. There was a 10-to-15-minute introduction to the study, including background information and motivation, followed by a 40-to-45-minute session of asking questions.

Appendix I consists of questions organized by those relevant to HCPs, IDSs, and community members. Peltier was able to answer most of the questions directed at HCPs,

while Powless-Lynes and Jackson answered questions directed at community members. Below are the responses obtained from the interviews.

Diagnostic methods of Trich

Peltier knows about the symptoms of Trich, as it has been around for a while, and noted that it is often confused with yeast infections, which are not STIs. Rates of STIs have been high for remote and Inuit communities in Nunavut. Also, Trich might go undiagnosed in Indigenous communities, allowing for spread if there are multiple partners. Trich is more common in younger people from 12 to 28 years old, in which the majority are females. There are also a lot of water advisories in First Nations communities, so environmental problems for accessing clean water could exasperate a person with diagnosis. Additional social factors add onto infection rates, such as alcoholism and some drug use. Furthermore, STBBIs can be gateway to HIV or more serious diseases. Powless-Lynes had only heard of the name Trich and Jackson had not known about the disease. Along with being underdiagnosed, Trich seems to be a topic that is relatively unknown or not discussed as much within communities.

Peltier explained that currently, test results are sent out of the community to a central lab, such as Northern communities sending samples to lab specialists at Health Canada in Winnipeg, and come in two weeks to a month later, which is too long of a wait. Powless-Lynes thinks that test results should be obtained within a week, but the sooner the better. Accordingly, all three interviewees think that a 15-minute POCT is ideal. There were varying opinions on whether a home test, POCT, or external sample processing would be the preferred method for diagnosis. One opinion that was consistent was that

none of the interviewees opted for the external sample processing, which is the current protocol.

Peltier and Jackson would prefer a POCT and Powless-Lynes would prefer a home-test. Peltier explained that the quick results and non-invasiveness of the in-house POCD would encourage more community members to get tested. As for home tests, she is not sure whether it would be covered by healthcare benefits and if Indigenous communities would have the funding to buy large quantities of such tests. Only a few communities are using non-central diagnostics, such as the recent acceptance of HIV-testing through blood spot testing, and more interest is building up now. Jackson agrees with using a POCT but for a different reason. He explains that care is a relational experience in Indigenous communities, so having a relationship with clinical staff is better. The fact that clinical help is involved, such as nurses who can easily use the POCT and deliver the results, can help patients. On the other hand, Powless-Lynes believes that a home test would be ideal, for privacy reasons. More people may want to do a test if they could pick it up at the pharmacy to do it at home, without worrying about booking an appointment or people knowing about it. Considering all the points of view, it seems most beneficial for nurses, but not CHWs, to use a POCD to diagnose Trich. The POCD can allow for financial practicality, and restricting the use to nurses can help community members build a relationship with a HCP and not worry about privacy being shared with community members such as CHWs, since sexual health is already a sensitive topic.

Treatment options for Trich

With regards to treatment options, Peltier believes that communities would have access to treatment based on the drugs covered by Health Canada, which varies by board. Treatment options are limited to what is covered within Indigenous communities, but antibiotics should be covered, and vaginal creams may be covered as well. Powless-Lynes thinks that antibiotics may be preferred, as they are easier to take and look more like a vitamin. Jackson mentioned that the access to cultural resources, specific to each community, may be meaningful when facilitating treatment.

All three interviewees agreed that it is more convenient for patients to have a 2 g single-dose medication than a 500 mg dose, twice a day, for seven days a week. According to Peltier, a single dose treatment would probably be better because often people stop taking their treatment after symptoms disappear, which is usually before the treatment regimen is completed. Jackson also mentioned that a single dose medication can help reduce the pill burden and encounters with medication, as the Indigenous population is already living with multiple chronic illness.

Peltier explains more about the administration of treatment in remote Northern communities. Doctors fly in for the clinics and do not live there. Nurses could prescribe treatment in remote communities and use telemedicine to check-in with a doctor, but CHWs cannot provide prescriptions. For a community of 2500 people, only four nurses are available. As such, test results can be received in person or through a phone call. For follow-up care regarding STBBI issues, a specific clinic is open once a week at nursing stations. However, by noticing who goes into the clinic on that day, rumours and

assumptions may be spread in the community, further deterring patients from seeking treatment.

As such, Powless-Lynes explains that the adherence to medication may be affected by stigma of taking medication. For instance, patients may be less likely to adhere if there are multiple follow-ups at the clinic, and more likely to adhere if they can take something like antibiotics at home. However, even at home, adherence to prescription may be affected by with whom the patient is living. If housemates or family members are judgmental about medications, that view could discourage patients from keeping up with treatment. Continuing this point, Jackson notes that multi-family dwellings are common in Indigenous communities due to poor housing, and patients may not want medications lying around. So, if a single treatment dose is administered by a nurse at the station, that may be better. Other factors affecting adherence is income and thus affordability of the treatment, and ease of access to the nursing station given that not many families own cars in Northern remote communities.

With regards to the likelihood of cross infection of patients with HIV also having Trich, Peltier notes that there is a lower incidence of STBBIs for people living with HIV. People not living with HIV are those who would have higher Trich incidences. That is because people living with HIV are more careful if engaging in sexual activities, as they do not want to transmit HIV. Moreover, one can be charged for aggravated sexual assault if one has sexual activities without disclosing oneself as HIV+. This leads to stigma.

Accessibility to healthcare

Accessibility to nursing stations is restricted to road access. Peltier explains that most Indigenous communities have good roads now and they are well-maintained. However, road access is an issue for remote northern communities. Powless-Lynes says that in the winter and flood seasons, communities are isolated as roads get flushed out. An exception is the availability of winter roads in Nunavut and North-West Territories all year-round.

In general, access to telephone, internet, and power services are not up to par in most communities. Powless-Lynes says that although the Six Nations reserve is close to Hamilton, finding reliable internet connection is hard. This is due to lower quality and service range. Jackson notes that officials in Nunavut recently demanded for better access to meet Canadian standards. Peltier says that although most First Nations communities have good power and hydro and only a few northern communities tend to be generator-based, sometimes the power does go out. Reliable access to the line, telephones, and internet is often restricted to health clinics and nursing stations.

Peltier explains that up North, you are more likely to have treatments available at the nursing station as these communities have less access to other communities. Powless-Lynes notes that larger communities, like Six Nations, are more likely to house health services on reserve, but smaller communities would not have walk-in-clinic or 24-hour health services.

With regards to pharmacies, Peltier notes that very few First Nations communities are big enough to have their own pharmacies. Thus, some patients have to leave their

communities to find a drug store in the closest non-First Nations town. The issue is with finding someone to drive one to the neighboring community, especially considering that most people live below the poverty line and so may not own cars. So far, only medicinal marijuana is sent by mail, but no other medicine. All three interviewees note that fly-in is an alternative to road travel, whether that is for patient or treatment movement, although probably a more expensive option.

With regards to access to HCPs, Powless-Lynes states that doctors are there temporally, so not much of a relationship is built with patients, thus risking the patients' concerns being brushed off. If doctors do not know their patients over a long-term, they may not know what their patients look like nor their concerns. Powless-Lynes indicates distrust towards temporary HCPs, but Jackson views the situation in a different light. He says that since doctors and some nurses are not part of the community, as CHWs are, it is easier for patients to trust that their information will not be shared with other community members. If turnover rate of HCPs is high, it would affect trust. However, if the medical team is on rotation and come back to serve the same community, the relationship between the medical team and patients is developed and maintained over time.

Societal considerations

The issue of stigma surrounding STIs is very prevalent in Indigenous communities. Peltier explains that people know there are treatment for STIs, but it is harder to want access to treatments due to stigma. There are also not enough private screening areas, so private information can more easily reach community members. Powless-Lynes agrees, saying that STIs are a taboo subject, so it is not discussed in many communities.

In fact, people would be discouraged from being aware about it and wanting to go get tested. Although there is more awareness in the younger generation, STIs are not talked about in school as much. Peltier says that currently, the aim is to run education, testing, and screening campaigns to normalize the discussion on sexual activities and promote wellness without judgement. She also said that the aim is to start teaching pre-pubescent students at school about sexual health. Powless-Lynes appreciates seeing more researchers and HCPs providing more focus on HIV and STI awareness.

Peltier explains more about the stigma, saying that it is socially uncomfortable or even triggering for some people to talk about sexual activities, based on norms or experience with abuse. If it is known that someone has HIV or HepB, they are often stigmatized with having STBBI and are known for having “the dirty disease”. The situation ranges from older generations experiencing inter-generational trauma and having experienced sexual abuse in residential school or within the family, to the younger generations being more open to talk about sexuality and more proactive in sharing information with peers. The difference in experience between the older and younger generation, however, can impact changes made by the younger generation. Powless-Lynes explains that there is an overall lack of awareness and stigma on STI testing and treatment, especially for youth or younger people trying to get treatment without parents or guardians knowing. Youth are afraid that parents do not understand that STIs are normal and is not necessarily about being unsafe. Jackson says that similar to outside Indigenous communities, the stigma of STIs within Indigenous communities negatively impacts diagnosis and access to treatment or care.

Discussion of primary healthcare access in Indigenous communities

From the research in the literature and results from the interviews, the following four major themes were found regarding issues in the general and STBBI-based provision of primary healthcare in Indigenous communities: 1) Lack of privacy and presence of social stigma, 2) Strain on the role of nurses, 3) Importance of community-centred care, 4) Lack of medical and infrastructure-related resources.

Lack of privacy and presence of social stigma

First, the lack of privacy and the presence of social stigma appears to hinder the seeking of medical support. In general, patients have a lack of trust in CHWs and they would rather wait for rotating HCPs to return to the community to discuss medical issues. STBBI clinics open on certain days, requiring many follow-ups or multiple dosages of treatment can all lead to stigma, as it is easier for community members to track patients' visits to these clinics. This may be a reason why Trich is underdiagnosed and underdiscussed. Unfortunately, this stigma is impacting the young female population the most, as they are the most heavily affected by Trich but are reluctant to seek testing and treatment. While the older generation may be traumatized by past abuses leading to STBBIs and thus prefer not discussing the issue, the younger generation is unable to be honest with elders for fear of misjudgement, thus diminishing both diagnosis and treatment. To tackle this issue, there is a greater need for educational awareness and more privatization of patient medical information and testing areas.

Strain on the role of nurses

Second, there is a huge strain on the role of nurses, whether external and rotating or internal and part-time. Often, due to the lack of training, nurses have to wait on physicians' orders. This is an important consideration as only nurses and fly-in doctors can prescribe treatments, not CHWs. This factor, along with the high patients-to-nurses ratio, leaves nurses with limited time to attend patients and causes a delay in medical diagnosis and treatment. However, patients prefer their nurses to be confident in their skills and to provide close monitoring of their treatment. Thus, a POCD may be useful as a quick, non-invasive, and cost-effective diagnosis device that can allow nurses to provide accurate diagnoses while providing a relational and trustworthy healthcare experience to patients.

Importance of community-centred care

Third, patients place grave importance on the provision of community-centred care. Specifically, patients prefer consults from within the community and would rather not leave the community, as it is a waste of their time and money. However, the feasibility of community-centered care depends on the affordability of treatment and access to nursing stations. One has better luck of finding the treatment one needs in bigger communities or those really isolated in northern Canada, where it would not be possible to rely on a neighbouring larger city. Only a few communities are big enough to have pharmacies, and these are usually the ones closer to cities. Otherwise, only medical marijuana can be mailed-in and other treatments needs to be picked up by road travel. Community-centered care is also dependent on the building of rapport with the healthcare

team. Rotating physicians may lack rapport with patients, but if the same one is seen consistently, perhaps trust can be established.

Lack of medical and infrastructure-related resources

Fourth, there is a serious lack of resources. These resources range from medical equipment, to road access, to telephone and electricity provisions. Usually, diagnostic tests and treatment options, such as a POCD and a 2 g single dose of metronidazole or tinidazole, is better suited for these communities that lack medical resources and are faced with the pill burden. Additionally, the access to roads usually correlates with how close a community is to cities. On the other hand, winter roads are only available in communities where it is winter all year-round, such as in northern Canada. Lastly, telephone, internet, and power services have poor quality and service range, even if these communities are near major cities. The infrastructure demands in northern Canada do not meet the Canadian standard and a few remote communities depend on a generator. If there is provision of telephone and electricity services, these are most reliable at health clinics and nursing stations.

Detecting *Giardia* and *Cryptosporidium* spp.

Giardia and *Cryptosporidium* spp. are waterborne pathogens that cause gastrointestinal illnesses. Contamination of drinking water sources by *Giardia* and *Cryptosporidium* spp. constitutes a serious concern, as these protozoa are resistant to chemical disinfection[91,92]. They are highly infectious and cause non-specific clinical symptoms, similar to Trich.

In Canada, the Indigenous population in rural and remote communities have a higher risk of exposure than other Canadians to canine fecal zoonoses, which contain protozoa such as *Giardia* and *Cryptosporidium*[92]. From a northern Saskatchewan Indigenous community, the prevalence of *Giardia* was 61% and that of *Cryptosporidium* was 3%. The prevalence of the former was much higher than the expected prevalence of 10%, but the prevalence of the latter was similar to that in other North American locations[92].

Characteristics of *Giardia* and *Cryptosporidium* spp.

The *Giardia lamblia* cysts outside the body are round to oval and 8-19 µm long[93]. On the other hand, trophozoites in the body look teardrop or pear-shaped and are 10-20 µm long, with motility that resembles a falling leaf. This pathogen resides in the duodenum. The stool specimens may be negative, thus requiring other sampling techniques such as aspiration, Entero-Test, and fecal immunoassays[93].

The *Cryptosporidium* spp. oocyst is spherical shape, 8-10 µm long, and non-motile[93]. This pathogen resides in the intestinal mucosa at the edge of brush border, the gallbladder, and the lungs. The oocysts are seen in the stool or sputum but may be negative if only a few are present. The pathogen can cause severe diarrhea but is ineffective once exposed to the outside environment[93].

Diagnostic methods

Common laboratory tests for *Giardia* and *Cryptosporidium* spp. include test stools, the *Giardia/Cryptosporidium* enzyme immunoassay, modified acid-fast stain, and

multiplex molecular panels. For test stools, if fecal immunoassays are performed, it is recommended to test two separate stools that are collected at least one day apart for *Giardia* but not necessarily for *Cryptosporidium* spp[93]. The *Giardia/Cryptosporidium* enzyme immunoassay is used when a patient has diarrhea, especially for a waterborne outbreak in a municipal water supply, and AIDS or another cause of immune deficiency. This is because *Cryptosporidium* affects both immunocompetent and immunocompromised individuals[93]. The sensitivity and specificity for this ELISA is 71.8% and 94.3%[94]. The modified acid-fast stain is used when *Cryptosporidium* is suspected due to diarrhea from a potential foodborne outbreak, and immunologic status. Lastly, multiplex molecular panels, such as PCR, are moderately or highly complex tests used in smaller laboratories, with a sensitivity and specificity of 100% and 0%[93,94].

There are three commercially available fecal-antigen assays classified as rapid diagnostic tests, that can detect *Cryptosporidium* spp. and *Giardia duodenalis* infections in stool samples. Compared to a PCR assay, these tests are less time-consuming, simpler to carry out, cost-effective and do not require specialised equipment[94,95]. These tests include QUIK-CHEK, RIDA-QUICK, and CRYPTO/GIARDIA DUO-Strip, and may be used as alternative diagnostic tools when microscopic examination and technical expertise are unavailable in remote locations[94,95]. A study by Bitilinyu-Bangoh et al. in 2019 tested these rapid diagnostic tests. The sensitivities of these tests were found to be moderate compared to PCR, ranging from 48.2% to 85.7% for *Giardia duodenalis* and from 42.9% to 76.9% for *Cryptosporidiosis*. In contrast, the specificity of the tests ranged from 88.4 to 100%[95]. While the specificity values were similar to those reported by the manufacturer of 95.2 to 100%, the sensitivities were much lower than the reported 86.7

to 100%. The difference could be due to the lack of detection of rare genetic variants or less common species. Also, the antibodies that are used to detect the parasites are species-specific and not genus-specific, thus limiting detection[95]. However, in a study by Kabir et al. in 2018, QUIK-CHEK was noted to display high sensitivity and specificity levels of 92.3% and 97.1%, respectively.

Although the above rapid tests may be valid to use in remote Indigenous communities, it would be more useful to use the in-house POCD as a multi-purpose diagnostic tool. This option can avoid the wide range in sensitivity and the limitations of species-specific antibodies. This option is also more considerate of the lack of financial and medical equipment resources in remote Indigenous communities, as previously discussed. Since the in-house POCD tracks motility of species, it can differentiate *Giardia* and *Cryptosporidium* spp., as the former has fluttering motility and the latter is non-motile[93].

Treatment

Regarding preventative treatment, *Giardia* and *Cryptosporidium* spp. have developed resistance to chemical treatments, such as chlorine, so new treatments are being studied. It has been found that low doses of UV can eliminate *Giardia*, whereas high doses of UV are required to eliminate *Cryptosporidium* spp.[96].

For individuals who do encounter infection with these pathogens, those who are immunocompetent can recover in about two weeks after experiencing diarrhea and gastroenteritis[97]. On the other hand, those who are immunocompromised, such as individuals with HIV/AIDS, the resulting diarrhea can be fatal[97]. Treatments such as

paromomycin, azithromycin, and nitazoxanide can be used to reduce symptoms from cryptosporidium infection[98]. For *Giardia*, nitroimidazole derivatives— such as metronidazole, tinidazole, nitrofuran, and benzimidazole drugs can be used. Similar to the treatment of Trich, these is resistance developing against metronidazole, so nitazoxanides may be used[98]. However, the efficacy of nitazoxanide is moderate for malnourished and immunocompetent individuals and lacking for immunocompromised people[97]. Although nitazoxanides may have up to 90% efficacy, psychiatric and dermatological side effects may occur[97].

A future treatment option may be using drugs initially used to treat rheumatoid arthritis, as they have shown effects against *Cryptosporidium* spp. *in vitro*[97]. These drugs include: the human 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitor, itavastatin, and auranofin (Ridaura®)[97].

Local anecdote from Dr. Paul Andrus

To obtain more information on the prevalence, diagnosis, and treatment of *Giardia* and *Cryptosporidium* spp., questions in **Appendix II** were emailed to Dr. Paul Andrus, a physician in a family medicine clinic in Ancaster, Ontario. He explained that *Giardia* and *Cryptosporidium* spp. are not common in urban areas unless there is an outbreak at a daycare. As a physician, he orders the ova and parasites stool test. Usually, antibiotics are prescribed to treat the individual infected and prevent dehydration, as well as to contain the outbreak.

Conclusion

To diagnose infectious diseases in remote Indigenous communities in Canada, there is a need for a locally accessible, financially affordable, user-friendly, and rapid diagnostic device. These requirements can be met by the in-house POCD. Compared to the current diagnosis and management strategies, the in-house POCD has the lowest cost, can test for both male and females, and can be used for asymptomatic patients. Also, the POCD relies on pathogen motility rather than genes and antibodies, which can help distinguish between pathogens such as Trich, *Giardia*, and *Cryptosporidium* spp., while avoiding false negatives. Furthermore, the in-house POCD considers the requirements by remote Indigenous communities in Canada by 1) providing immediate results to patients, 2) allowing nurses to provide quick and patient-centered care, 3) allowing for diagnosis from within the community, and 4) not depending on medical and other infrastructural-related resources. Overall, the use of the in-house POCD can be generalized to any community that lacks access to resources for the purposes of diagnosing pathogens with characteristic motility.

Understanding the current technologies available and gaps in access to healthcare can lead to the development of a suitable diagnostic device for a variety of resource-constrained communities to diagnose a variety of diseases. It is crucial to identify and then minimize the gaps in access to healthcare to ensure that patients receive the same quality of care, no matter where they live.

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Appendix I: Interview Questions for Trich

DIAGNOSITIC

Health Care Providers (HCPs)

D1. Do you know about Trich? How often do you diagnose Trich during patient visits?

D2. How long do you think the test needs to be? What is the tolerable time limit in obtaining a diagnostic result?

D3. How are people diagnosed for Trich in Indigenous communities? Do you use a point-of-care test (POCT) or are all samples sent to a central processing facility?

D4. What is your opinion on the practicality of diagnosing Trich using the following options: i) home-test, ii) POCT, iii) external sample processing?

D5. Are there different professionals responsible for different aspects of diagnosis (i.e. sample collection, sample processing, and result interpretation)?

Infectious Disease Specialists (IDSs)

D1. Do you know about Trich? How often do you come across a Trich diagnosis?

D6. Do diagnostic tests need to be FDA-cleared in order to be administered?

D7. Currently, there is an OSOM Trich rapid antigen test, which is a dipstick diagnostic test for Trich that comes in a kit of 25 individual tests. However, they all need to be used within an hour of opening the canister. Thus, is it practical for everyone to come in for testing on the same day within one hour? Are there enough resources and labour to conduct all these tests?

TREATMENT

HCPs

T1. How do you proceed with a Trich diagnosis? What is the decision-making process for choosing a treatment option in remote Indigenous communities?

T2. What is the preferred treatment for Trich: broad-spectrum antibiotics, specific oral antibiotics (metronidazole or tinidazole), or intravaginal creams/gels?

T3. If specific oral antibiotics are prescribed, would you prescribe metronidazole or tinidazole? For dosage, do you prescribe a 2 g single-dose or a 500 mg dose, twice a day, for 7 days a week?

T4. Can the community health workers (CHWs) administer the treatment themselves, or must they wait for the arrival of a physician?

T5. How long does it take to know whether the treatment worked? How is this determined? What if physicians are not on-site?

T6. Does the patient come back to get results on the progress of the treatment or is this done over a phone call?

T7. Are there any issues with adhering to medication prescription guidelines in Indigenous remote communities?

T8. How many treated for Trich are also HIV-infected in the remote Indigenous communities? Are these patients monitored more closely and/or given more treatment options?

ACCESSIBILITY

Community members

A1. How frequently do patients have access to roads? What are the alternatives?

A2. Is there a main powerline or a generator-based system? Are there reliable telephone, internet, and power services to provide/receive consultations and prescriptions?

A3. How frequently do patients have access to health care facilities and providers?

HCPs/IDSs

A4. Are there resources and technologies available to freeze Trich samples or incubated them at 37˚C? Or, must they be processed ASAP?

A5. Do pharmacies fill and store prescribed treatment in these communities, or is a mail-in service used instead?

A6. For antibiotics, do you have a variety of choices for when some do not work?

SOCIETY

Community members

S1. Are there any social/cultural expectations for STD/STI testing? Are there annual screenings that are mandatory or optional? What are the procedures?

S2. Are there any social stigmas or considerations when seeking treatment for Trich or Trich-like infections?

HCPs

S1. Are there any social/cultural expectations for STD/STI testing? Are there annual screenings that are mandatory or optional? What are the procedures?

S3. Is the diagnosis of Trich in remote Indigenous communities more prevalent in a certain demographic profile (i.e. age, sex)?

S4. Is there a correlation of Trich diagnosis and other diseases? Are there any social-environmental (i.e. water treatment and cleaning/sanitation facilities) reasons for Trich infection?

Appendix II: Interview Questions for Giardia and Cryptosporidium

Health Care Providers (HCPs) and Infectious Disease Specialists (IDSs)

1. Do you have experience diagnosing Giardia and Cryptosporidium spp.? If so, how prevalent are cases in urban centres vs. Indigenous or rural/remote communities?
2. With regards to diagnosis, is your goal to prevent outbreaks or to allow for source tracing? Does it depend upon the location (i.e. urban centre vs. Indigenous/rural/remote communities)?
3. For diagnosis, which of the following tests have you ordered (if applicable)?:
    - test stools
    - Giardia/Cryptosporidium enzyme immunoassay
    - modified acid-fast stain
    - multiplex molecular panels
    - QUIK-CHEK, RIDA-QUICK
    - CRYPTO/GIARDIA DUO-Strips
4. Do you have a preference for any of these? Is a specific diagnosis required to differentiate between Giardia and Cryptosporidium spp.?

HCPs only

5. What are the treatment options that you prescribe upon pathogen detection? Are they general antibiotics or medications specific to the pathogen?