Curr Trop Med Rep (2015) 2:150–160
DOI 10.1007/s40475-015-0055-x

PROTOZOA (L BARTELT, SECTION EDITOR)

Advancing *Cryptosporidium* Diagnostics from Bench to Bedside

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Published online: 22 July 2015

Abstract *Cryptosporidium* is increasingly being recognized as an important cause of diarrhea worldwide. Although well known for its impact among HIV positive population, improved diagnostic tests have contributed to its emerging recognition one among the most prevalent causes of early childhood moderate to severe diarrhea, persistent diarrhea, and impaired neurocognitive development. The diagnosis of Cryptosporidiosis is generally carried out based on availability of skilled microscopist or advanced equipment for molecular- and immunologic-based assays. As an emerging enteric pathogen of medical importance, the need for point-of-care technology is deemed necessary for early identification of the pathogen and application of infection control measures for its potential risk of creating outbreaks. Current point-of-care technologies demonstrate varying sensitivities and specificities and may already address the present diagnostic need.

Keywords *Cryptosporidium* sp. · Diagnostics · Point-of-care

Introduction

The Need for Sensitive *Cryptosporidium* Diagnostics

The recent identification of cryptosporidiosis as an important cause of morbidity and mortality worldwide has been suggested to be due to gaps in diagnosis, treatment, and immunization [1•, 2, 3]. This article will focus on conventional and innovative technologies employed for sensitive and rapid *Cryptosporidium* diagnostics.

Cryptosporidium is a protozoan that is increasingly being recognized as an important cause of diarrhea worldwide. Of the more than 26 *Cryptosporidium* species, *Cryptosporidium*

This article is part of the Topical Collection on *Protozoa*

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parvum (humans and ruminants) and *Cryptosporidium hominis* (humans only) are the most important causes of human infections and are morphologically indistinguishable. All species of *Cryptosporidium* are obligate intracellular parasites that undergo development leading to the excretion of 4–6 μm sized oocysts in feces. Infected people can excrete oocysts for as long as 60 days after infection [4]. Transmission of *Cryptosporidium* is mainly through the oral-fecal route with an infectious dose as low as 10 oocysts [5]. The small size of *Cryptosporidium,* persistence of shedding, and low infective dose allow the parasite to easily spread in places with inadequate sanitation and hygiene. Even in resource abundant settings, the parasite can evade detection resulting in outbreaks, the largest of which was documented in Milwaukee, Wisconsin USA and accounted for more than 400,000 infections and 100 deaths [6].

Cryptosporidium sp. are ubiquitous and can be found in untreated drinking and recreational water, contaminated food, day care centers, and hospitals [7, 8]. Exposure to farm animals and children, initially infected with this parasitic organism can lead to the further propagation of the organism. Through flooding and increased rainfall, climate change is predicted to increase the burden of waterborne diseases including *Cryptosporidium* [9]. The observations of esophageal cryptosporidiosis, where parasites were found in squamous mucosa and submucosal lumen and border [10], extraintestinal manifestations of *Cryptosporidium* infection in severely immunocompromised patients [11•], and respiratory cryptosporidiosis concurrent with intestinal cryptosporidiosis in HIV-seronegative children [12] suggest the potential for more diverse routes of transmission. With the possible increase in the burden of C*ryptosporidium*, there is a need to develop more sensitive diagnostic techniques to detect the organism in clinical, biological, and environmental specimens [7].

Current Diagnostic Platforms

Serological and microscopic Diagnosis of *Cryptosporidium*

Studies focusing on comparisons of the effectiveness and reliability of various methods in diagnosing Cryptosporidiosis greatly increased since its identification as a gastrointestinal and respiratory tract parasitic disease among many species of animals, including mammals, in the 1970s To date, there is no single ideal test for the diagnosis of Cryptosporidiosis, and a significant percentage of *Cryptosporidium* infections can be missed if any of the available assays has been the sole method of diagnosis [13] (Table 1). Thus, current research mainly focuses on determining the most sensitive and cost-effective diagnostic method that can detect infection even at the earliest stages before the parasite has multiplied. Such research aims to draw benefit from knowing the most reliable tool in detecting the presence of *Cryptosporidium* parasite in vulnerable population groups and its environmental reservoir which may lead to enhanced diagnostic surveillance and clinical tools for effective eradication measures of the disease.

One approach to detect *Cryptosporidium* infection is the serological detection of the specific antibody response. Following infection, there is usually the development of characteristic immunoglobulin G (IgG), IgA, and IgM antibody response against 2 sporozoite surface antigens with apparent molecular masses of approximately 27, 15, and 17 kDa [14•]. IgG responses to *Cryptosporidium* sporozoite antigens of low molecular size can persist for several months [1] in adults and have been shown to be consistent and of sufficient intensity to act as reliable markers of exposure [15, 16].

Prior studies suggested that serum *Cryptosporidium*-specific antibody levels remain elevated for weeks to months following infection [15]. Increases in the intensity of Western blot response for IgG antibody to two *Cryptosporidium* antigens (17 and 27 kDa) following infection were observed among highly exposed individuals. A 15 kDa protein isolated from the feces of infected calves by immunoaffinity adsorption using a monoclonal anti *C. parvum* antibody was recognized by IgA antibodies present in the saliva during the convalescent phase of infection. These results suggest that this coproantigen may be released from *C. parvum* sporozoites and may induce IgA antibody production in the mucosal immune system of infected calves [17]. The seroprevalence of IgG antibodies to the 27-kDa and 15/17-kDa *Cryptosporidium* antigen in sera was significantly greater (49–61 %) in settlements where the drinking water originated from surface water than in the control city where riverbank filtration was used (21–23 %). Hence, the elevated responses were most likely due to the use of contaminated water [18•].

It is necessary to evaluate the comparative diagnostic accuracy and feasibility of newly introduced test in comparison to the existing tests or the criterion standard to reveal how well this test discriminates between health and disease. The Center for Disease Control and Prevention Project #438 has developed a second generation ELISA that detected all laboratory confirmed cases. It showed improved sensitivity over the immunoblot test results [19]. There was also no cross-reactivity between *Cryptosporidium* and *Giardia* or *Toxoplasma*. Moreover, ELISA results from the same sample sets from two different laboratories showed excellent correlation. While these serological assays for *Cryptosporidium* are important for epidemiological studies because specific antibody responses develop after both symptomatic and asymptomatic infection, they cannot be used to diagnose active infection.

Cryptosporidium can only be cultivated for a short time in epithelial cell monolayers and does not grow in conventional laboratory media. Thus, microscopy is considered the conventional method of detecting active infection. Accuracy of diagnostic microscopy may be affected by several factors. The

Table 1 Comparison of *Cryptosporidium* diagnostic tests

Test	Advantages	Disadvantages	Clinical sensitivity	Clinical specificity	Diagnostic use	Product example
Microscopy [20, 23, 24, 25•]	Inexpensive, handy for field experiments	Tedious and time-consuming, prone to obtaining false-negative results	78–99 %	82–100 %	More complex techniques serve as tool for differentiating oocysts by species	No data
Western blot [14•, 15]	Fairly sensitive	Limited linear range, antibody levels are difficult to quantitate, cumbersome	71–87 %	89 %	Detect proteins specific to *Cryptosporidium*	No data
ELISA [13, 15]	Do not require an experienced and skilled morphologist, a good screening technique to test large numbers of specimens, can be automated	Tedious and time-consuming, remains cost-constraining in resource poor areas	92 %	100 %	Serologic detection of *Cryptosporidium* antibody	No data
PCR [29]	Sensitive, useful in epidemiologic studies	Inhibitory factors that may affect the quality of DNA, relatively expensive	83–100 %	100 %	Nucleic acid detection specific to *Cryptosporidium*	No data
LAMP [36, 41]	Rapid and simple, can detect organisms at relatively low concentration in environmental samples	Limited available sequences for primer design	No data	No data	Alternative tool and analytical tasks for *Cryptosporidium* detection	Loopamp® (Eiken, Chemical Co., Japan)
FISH/CISH [43, 45, 46, 52]	Can be designed to various degrees of specificity, powerful tool for the detection of microorganisms in a wide range of environmental samples	Limited to measuring the viability of C. *parvum* oocysts and not their infectivity, the extent to which FISH probes are useful for studies of oocyst viability is dependent on the rate at which SSU rRNA decays	80.6–100 %	57.5–100 %s	Provides an alternative tool for accurate identification of zoonotic *Cryptosporidium* which can be applied to both epidemiological and outbreak investigations	No data
Nanotechnology-based [56, 59, 60, 62]	Improves limit of detection, photostable, can be used to improve other diagnostic methods, rapid	Preparation of nanoparticles is costly	No data	No data	Alternative for organic dyes in microscopy and other diagnostic methods, point-of-care tests	No data
ICT assay [79, 83, 85, 89–91, 92•, 93•]	Rapid, simple, appropriate for limited-resource settings	Low sensitivity, qualitative test only, does not differentiate between genus	53.4–100 %	94.3–100 %	Point-of-care tests (dipsticks/casettes) in diagnosing *Cryptosporidium* infection	RIDA®QUICK (R-Biopharm) ImmunoCard STAT!® (Meridian)

majority of positive *Cryptosporidium* samples are not usually found in watery stools, rather in loose mushy stools which implies that microscopy might not efficiently work for highly turbid samples as solid particles might cover the target organisms. Multiple staining and washing steps of the fecal sample on the slide could also lessen the detectable quantity of oocysts since oocysts can be washed away in the process [20]. The significant impact of the skills and experience in processing slides and analyzing stool samples of the microscopist is one limiting factor affecting accuracy of results. The generally low sensitivity results for microscopic diagnosis are usually augmented by performing repeated microscopic examination to confirm a microscopic finding. This basically implies that aside from being a less effective diagnostic tool compared to other serological techniques, conventional microscopy is also time-consuming and tedious, as it generally requires an average rate of 10 min per slide before oocysts can be detected and its diagnostic accuracy is largely dependent on the experience and skills of the microscopist [21•].

Several preparation and staining techniques were developed to improve detection of parasites in stool samples. Among the four existing commonly used preparation techniques for microscopic analysis of stool samples: Sheather's flotation (SF), normal saline sedimentation staining (NSSS), direct fecal smear staining (DFSS), and Sheather's flotation sedimentation staining (SFSS), the latter is considerably more sensitive and specific compared with the other three, having sensitivity and specificity values of 82.6 and 98.8 %, respectively [22]. Most laboratories use a modified acid-fast or Safranin stain to detect oocysts. Two other microscopic methods namely auramine phenol microscopy and immunofluorescence microscopy have improved sensitivity, 92.1 and 97.4 %, respectively, though it still has the potential to produce false-negative results [23]. Despite its downsides, the use of microscopy as a diagnostic tool for *Cryptosporidium* remains to be the most common technique to detect presence of active infection and is the only technique that can distinguish the presence of oocysts. However, when there is high index of suspicion, it is highly advisable to do confirmatory tests, such as PCR for microscopy-negative samples. While highly sensitive, molecular-based tests, such as polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), and some immunochromatographic tests such as the use of ImmunoCard STAT® [23, 24, 25•] are not routinely available in most laboratories.

In the clinical setting, there is a need for rapid, sensitive, and specific diagnostic methods that can guide appropriate control and therapy for cryptosporidiosis [23]. Antigen-based detection using ELISA and Western blot methods has been widely used. ELISA, however, has its limitations which involves the relative decrease specificity due to occurrence of cross reactions with closely related parasitic antigens. In addition, some comparative evaluation studies revealed the lack of sensitivity of ELISA compared with genotypic assays [26].

Polymerase Chain Reaction/Multiplex PCR

PCR is now gaining acceptability as the method of choice in the detection of *Cryptosporidium* because of higher sensitivity compared to other methods. Aside from this, it also has the capacity to detect co-enteropathogens (multiplex), to quantify the amount of parasites present in the sample when utilizing quantitative PCR (qPCR), and discriminate between infecting strains [27]. Also, qPCR is sensitive enough to detect exceptionally low copy number of the organism [28]. The simultaneous detection in a single assay provides substantial savings in cost and time in identifying the specific infectious agent and does not require visual determination or antibody binding, thus permitting early and appropriate therapy initiation in a timely and effective manner.

Detection of *C. parvum* by PCR was first reported in 1991. Since then, several techniques have been developed to detect and differentiate *Cryptosporidium* at the species and subtype level. Nucleic acid-based methods to screen or detect the presence of *Cryptosporidium* involves isolating its DNA, combining the isolate with PCR mixture that contains primers and/or probes targeting a region of its nucleic acid sequences, amplifying the target sequence, and detecting the product via gel or fluorescent dyes. Prior to DNA extraction, it is necessary to break open the oocysts. Various methods have been reported such as freeze-thawing, boiling, or use of beads [29]. Amplified products can be further analyzed by restriction enzyme digest or by sequence analysis. This approach has significant advantages due to the flexibility of the primer design. The development of the droplet digital PCR now provides absolute quantification without the need for calibration curves or normalization to reference genes. A recent article reported less affection by inhibitors, lower template copy numbers, but higher costs, when compared with qPCR [30] .

The gene encoding the 18S rRNA region is being used more frequently as the target [31] because of more studies reporting a higher sensitivity in relation to its copy number, although other genes have also been reported, such as *Cryptosporidium* oocyst wall protein [32], 60-kDa glycoprotein, heat shock protein 70, Laxer locus, and microsatellite loci [33, 34]. Discrimination of species utilizes sequencing because of the very close similarity.

Loop-Mediated Isothermal Amplification

Loop-mediated isothermal amplification (LAMP) is an emerging technology and recognized as a useful diagnostic tool in *Cryptosporidium* detection [35]. It has major advantages over other diagnostic methods for detection of

Cryptosporidium especially at relatively low concentration in environmental samples [36]. Comparative detection of LAMP, PCR, and IFA has been documented in several epidemiological studies. In a study in Iran, LAMP showed higher sensitivity than PCR and IFA in the detection of *Cryptosporidium* oocyst in water samples [37•]. Similarly, a sensitivity assay performed used in a study in Turkey determined that LAMP can detect *C. parvum* DNA at 1.8 fg concentration, as compared to 100 fg by nested PCR [38•]. Development in LAMP assays has improved detection limits for *Cryptosporidium* in water and fecal samples. LAMP in combination of ARAD filter for the detection of *Cryptosporidium* oocysts in drinking water detected at least one oocyst in 10 l of drinking water [39]. Findings from a study using RT-LAMP targeting 18S sRNA had a detection limit of as low as 6×10^{-3} oocysts/test tube using water samples [40]. LAMP has been shown to be highly specific and sensitive for the detection of different *Cryptosporidium* species using different target genes. In particular, primer sets targeting gp60 was used to detect *C. parvum* [36], heat shock protein (HSP)-70 for *Cryptosporidium andersoni* [41], and S-adenosyl-methionine synthetase (SAM) gene for *C. parvum*, *C. hominis*, and *Cryptosporidium meleagridis* in fecal and water samples [38•, 41]. A new approach of an inclusion of a restriction site to generate clonable LAMP products was used to validate findings by sequencing [41]. A commercially available LAMP kit (Loopamp®, Eiken Chemical Co, Japan) is now being used for the detection of *Cryptosporidium* in environmental samples. Although LAMP is rapid and effective method of diagnostic method, one current limitation is the limited range of available sequences of *Cryptosporidium* species for primer design. Further development of different LAMP methods would lead to better decisions concerning treatment, contamination, and public health risks. Due to its sensitivity and simplicity, this method may prove to be a useful diagnostic tool in epidemiologic studies of *Cryptosporidium* detection.

Fluorescent In Situ Hybridization

The epidemiological importance of human cryptosporidiosis resulted in the development of innovative techniques that would identify oocysts in both clinical and environmental samples. Direct identification of zoonotic *Cryptosporidium* species without the use of PCR-based methods is of great importance during outbreak investigations. Simpler tools that provide species-specific information would enable water utilities and public health authorities to better assess the potential human health risks associated with *Cryptosporidium* positive environmental samples. One of these techniques is the fluorescence in situ hybridization (FISH). This method utilizes fluorescently labeled complementary DNA oligonucleotide probes that target-specific sequences of cellular rRNA for direct identification of microorganisms. Ribosomal RNA (rRNA) targeted oligonucleotide probes provide an advantage because they can be designed to various degrees of specificity, reaching from genus to species and even subspecies level. Fluorescently labeled rRNA targeted probes applied in FISH have emerged as a powerful tool for the detection of microorganisms in a wide range of environmental samples. The rRNA is used because it is a naturally amplified target for hybridization probes due to its high copy number. The procedure includes the following steps: (i) fixation of the specimen; (ii) preparation of the sample, including specific pre-treatment steps; (iii) hybridization with the respective probes for detecting respective targets; (iv) washing steps to remove unbound probes; (v) mounting, visualization, and documentation of results [42–44].

The first use of 6-carboxyfluorescein phosphoramidite as a label for fluorescent in situ hybridization for specific detection of *C. parvum* was developed in 1997 [45]. The probe set consisted of two synthetic oligonucleotides each tagged with a fluorescent reporter molecule. Each probe strand detects ribosomal RNA from a range of isolates of this species, and the combination was designed to allow detection of all isolates. However, the sets were not tested for utility in actual water samples.

A fluorescent in situ hybridization (FISH) technique developed by Vesey et al. (1998) shows considerable promise as an indicator of *C. parvum* oocyst viability [46]. In these assays, a fluorescent DNA probe is targeted to the 18S rRNA of *C. parvum*. The 18S rRNA is usually present in viable organisms and is degraded by cellular RNases in dead or dying cells. Hybridization with the designed Cry1 probe resulted in fluorescence of sporozoites within oocysts that were capable of excystation, while oocysts that were dead prior to fixation did not fluoresce. Results also showed that the fluorescence of FISH-stained oocysts was not bright enough to enable detection of oocysts in environmental water concentrates containing auto-fluorescent algae and mineral particles. However, in combination with immunofluorescence staining, FISH enabled species-specific detection and viability determination of *C. parvum* oocysts in water samples. The 18S rRNA can be used successfully for species-specific design of probes for FISH detection of *Cryptosporidium* spp. FISH using a *C. parvum*-specific probe provided an alternative tool for accurate identification of zoonotic *Cryptosporidium* which can be applied to both epidemiological and outbreak investigations [43, 46, 47]. Since its development, several studies have utilized FISH in recovering *C. parvum* in flies which causes mechanical transmission of the pathogen [48], direct and rapid detection of *C. parvum* on polytetrafluoroethylene (PTFE) membrane [49], testing of recreational beach water samples [50], and visualizing *C. parvum* life-cycle stages in cell-free culture [51].

Although most studies focused on detection of *C. parvum* only, a two-color FISH assay, based on species-specific probes

for *C. parvum* and *C. hominis*, can distinguish between the two major species involved in human infections. The potential to detect and identify pathogenic *Cryptosporidium* species in clinical, water, and environmental samples within a 3-h time frame demonstrates that FISH presents an alternative to PCR-based assays [52].

The major drawback for FISH technique, however, is that it is limited to measuring the viability of *C. parvum* oocysts and not their infectivity. The extent to which FISH probes are useful for studies of oocyst viability is dependent on the rate at which SSU rRNA decays. Quantitative assessment of rapid decay upon cell death for SSU rRNA has not really been done, and it is likely that the rate of degradation will vary depending on different environmental conditions (i.e., temperature, pH, salinity, and/or RNase contamination present) [53].

Recent Advances

Nanotechnology-Based Platforms

The potential of nanotechnology-based materials has been utilized to improve specificity and sensitivity of other detection methods such as ELISA, PCR, lateral flow assays, and immunofluorescent-antibody microscopy [1•, 54•, 56]. One of the main problems encountered in IFA microscopy is the presence in water samples of inert particles or algal cells which have strong auto-fluorescence that can compete with the signal of labeled cells. Although fluorescent dyes have been suggested to effectively label oocysts in water samples, they have high susceptibility to photodegradation and have broad excitation and emission spectra. The use of semiconductor quantum dots for immunofluorescent labeling of *C. parvum* oocysts in water samples has shown to provide excellent and more consistent results [57–59]. Labeling with quantum dots (QD) can detect up to 4495 oocysts. The difference in detected oocysts using quantum dots and FITC does not have any significant difference even at various *C. parvum* oocyst concentrations [58]. In terms of photostability, QD-labeled oocysts exhibit better photostability after being exposed to continuous UV excitation for 5 min while the intensity of FITC-labeled oocysts can decrease to 19.5 % after the same period [57, 59]. Quantum dot labels also have approximately 50 % lower interference in concentrated water samples compared to organic fluorophore labels [59]. Another application of nanotechnology-based materials for *Cryptosporidium* diagnosis is the use of oligonucleotide gold nanoparticles for molecular detection without the need for amplification of nucleic acids and proteins [60]. Examples of assays that have been developed using gold nanoparticles are the electrochemical-based sandwich enzyme-linked immunosensor [61], rapid immunodot blot assay [62], and amplification-free detection systems [60] by using a dual-labeled gold nanoparticles (alkaline phosphatase (ALP) and anti-oocyst monoclonal antibody) functionalized indium tin oxide electrode. With this approach, the sensitivity of the electrochemical immunosensor increased with a limit of detection of 3 oocysts/ml in a minimal processing period [61].

The other assay developed using gold nanoparticles is the immunodot blot assay. Compared to the conventional method of adding anti-oocyst monoclonal antibodies then ALP-conjugated secondary mouse antibodies to immobilized *C. parvum*, this enhanced method makes use of dual-labeled gold nanoparticles to detect the *C. parvum*. The sensitivity of the immunodot blot assay was improved by 500 times compared to the conventional method that it can detect as low as 10 oocysts/ml. The enhanced assay also had good coherence with the results of PCR detection of the organism in water samples [62].

Aside from detection using antibodies, RNA's coding for the 18S rRNA and HSP70 have also been targeted using oligonucleotide gold nanoparticles. The hybridization of nanoparticle probes to 18 s rRNA, which was done without amplification, was detected without complex technology was able to detect as few as 670 oocysts/ul in spiked stool samples. Cross-reactivity with either 18 s rRNA of other protozoan parasites or *Escherichia coli* was not observed [60]. Targeting the HSP70 gene, on the other hand, causes formation of oligonucleotide networks that are visually detected with simple colorimetric readout. Detection after inducing expression of HSP70 has sensitivity that can detect as low as 5000 oocysts [63]. Both of these methods have potential of being used for point-of-care assays for *C. parvum.*

There are other nanotechnology-based detection methods that have been less studied such as the background-free cytometry with rare earth nanoparticle bioprobes [64], bioimaging with silica-encapsulated europium particles [64], and the use of piezoelectric-excited millimeter-sized cantilever (PEMC) sensors [55, 65].

Towards Point-of-Care Technology

Lab-on-a-Chip

The concept of micro total analysis system (μTAS), later indicated as a lab-on-a-chip, was introduced by Manz and colleagues in the early 1990 [66, 67]. They introduced the concept of scaling down the size of analytical devices for improved performance and portability. An ideal μTAS requires only a small volume of sample and incorporates all necessary manipulations and analysis steps to deliver a qualitative or, in some cases, quantitative result in a single-in-answer-out manner [68]. The majority of μTAS platforms developed for detecting *Cryptosporidium* are principally for environmental analysis for cryptosporidial contamination from water

sources. The platforms used for detection range from size-filtering-based microfluidic devices [69, 70] to nucleic acid-based amplification systems [71], the protozoan's dielectrophoretic properties [72, 73], and immunological properties [74]. These μTAS technologies are promising platforms for future point-of-care (POC) platforms that may eventually be suitable not only for environmental surveillance but also as clinical diagnostic tools. One promising technology that has been tested for both epidemiological surveillance and now piloted for clinical diagnosis among HIV patients suspected of cryptosporidiosis is the use of microfluidic immunoassay system targeting the P23 antigen of the *Cryptosporidium* developed by Zhang and colleagues [75]. The detection process can be completed within 10 min using 2 μl of the sample volume. It generated a diagnostic sensitivity of 100 % and diagnostic specificity of 98.2 %. Although the technology remains "near" POC, the challenge of miniaturizing the digital conversion of fluorescence signal to a handheld device may well be expected in the near future.

Immunochromatographic Tests

The development of POC tests for the diagnosis of *Cryptosporidium* addresses the challenges posed by resource-limited settings and the patient's need for immediate intervention. The majority of POC test kits that have been commercially available over the past decade utilizes the principle of immunochromatographic assays (ICT) in the form of test strips [56, 76, 77, 78•, 79, 80, 81•, 82•]. Generally, the strips follow the lateral flow concept where *Cryptosporidium* antigen-specific antibodies are bound to a membrane and uses capillary flow to move the labeled antibody-antigen complex [83]. One of the considerations in gauging the performance of a POC test strip is having a turn-around time of 10 min—the assumed time a microscopist scans a slide prior to declaring negative results [82•, 84].

Despite its increasing commercial availability and simplicity, a few clinical laboratories in resource-replete regions resort to ICT rapid testing and still consider microscopy as the standard for diagnosis [85, 86•]. Aside from a relatively high testing cost, the available POC tests exhibit a wide range of sensitivity, from as low as 13.6 % [87•] to as high as 100 % [88•, 89], with a good specificity of 97–100 %, nonetheless [77, 80, 90, 91]. Due to the wide range of sensitivity, it is necessary to constantly evaluate the tests' diagnostic performance in varying settings with focus on the assessment of its effect on patient-centered outcomes [92•].

Furthermore, the current commercially available POC tests are limited to detecting the presence or absence of the parasite without the capacity to quantify the burden of infection and differentiate between genus [87•]. However, these limitations can be outweighed if POC tests will be used to accelerate treatment initiation, to test a relatively large number of at-risk patients or to screen individuals during epidemics. Some of the commercial tests comprise of a combination of antibodies specific for 2–3 antigens such as the RIDA® QUICK *Cryptosporidium*/*Giardia* Combi cassettes (R-Biopharm, Germany) [77], ImmunoCard STAT® Crypto/Giardia (Meridian Bioscience, Inc.) [91], and RIDA® QUICK *Cryptosporidium*/*Giardia*/*Entamoeba* Combi dipsticks (R-Biopharm, Germany) [93•]. These single panels are used to detect an enteric infection caused by either *Cryptosporidium* or *Giardia*, as in the case of RIDA® QUICK *Cryptosporidium*/*Giardia* Combi and ImmunoCard STAT® Crypto/*Giardia*, and *Cryptosporidium*/*Giardia*/*Entamoeba* for the RIDA® QUICK *Cryptosporidium*/*Giardia*/*Entamoeba* Combi, with comparable performance metrics (see Table 2) [25•, 81•, 87•, 88•, 93•]. Although this diagnostic platform is best coupled with standard/routine diagnostic

Table 2 List of common commercially available immunochromatographic tests and their corresponding performance metrics

ICT test	Sensitivity	Specificity	PPV	NPV
Savyon® CoproStrip™ [82•]	74	98	97	94
Coris Duo-Strip† [88•]	66.7	95.2	–	–
Dia.Pro CA-RT [81•]	86.7	100	100	95.2
Coris Crypto-Strip [77, 91]	61.1	99.3	80	98
Techlab Quik Chek [88•, 89]	100	100	100	100
Meridian ImmunoCard STAT!® CGE‡ [88•]	100	95.4	–	–
Meridian ImmunoCard STAT!® CG† [25•, 81•, 91]	87.3	99.6	95.6	98.6
ThermoFisher Remel™ Xpect™ [91]	68.8	100	–	–
R-Biopharm RIDA®QUICK *Cryptosporidium* [23, 77, 88•, 90, 93•]	83.1	98.6	89.4	97.8
R-Biopharm RIDA®QUICK CG Combi† [77, 87•, 93•]	53.4	98.9	91.7	82.6
R-Biopharm RIDA®QUICK CGE Combi‡ [93•]	71.1	94.3	65.2	95.6

† A single platform that simultaneously detects *Cryptosporidium* and *Giardia* infections.

‡ A single platform that simultaneously detects *Cryptosporidium*, *Giardia* and Entameoba infections.

procedures, it can be a primary option for the rapid qualitative diagnosis of a possible enteric parasite for providing immediate intervention or a basis for performing further laboratory testing.

Conclusion

While the challenge of therapeutic intervention for *Cryptosporidium* remains a huge task, the challenge of diagnostic technology for active case detection has moved at considerable speed. The major limiting factor of these existing technologies is access. The major driver for developing diagnostic point-of-care testing is to improve the turn-around-time of such testing, thereby allowing clinicians and patients to make a quick clinical decision. For point-of-care tests to have maximal impact in resource-limited settings, it is necessary for the cost of the technology to be affordable even to the marginalized sector of society. To optimize point-of-care testing in resource-limited settings, diagnostic tests need rigorous assessments focused on relevant clinical outcomes and operational costs, which differ from assessments of conventional diagnostic tests [92•]. The development of a fully integrated POC platform for the diagnosis of *Cryptosporidium* must include each aspect of test performance such as sample preparation, on-chip nucleic acid analysis and immunoassay, and system integration/automation. At this point in time, ICT platforms remain to be the closest to bedside; however, a challenge remains on the ability to identify and discriminate intensity disease burden. This can be augmented with strong clinical correlation and the knowledge on the burden and epidemiology of the disease in clinical setting.

Compliance with Ethics Guidelines

Conflict of Interest The authors declare that they have no competing interests.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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