Recent advances in nucleic acid-based methods for detection of helminth infections and the perspective of biosensors for future development

HANIF ULLAH[1], ABDUL QADEER[1], MUHAMMAD RASHID[2], MUHAMMAD IMRAN RASHID[3], GUOFENG CHENG[1]

[1]Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Key Laboratory of Animal Parasitology of Ministry of Agriculture and Rural Affairs, Shanghai, 200241, China;

[2]State Key Laboratory of Veterinary Etiological Biology, Key Laboratory of Veterinary Parasitology of Gansu Province, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, Gansu 730046, China.

[3]Department of Parasitology, University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan.

Short title: Nucleic acid-based methods for detecting helminth infections

*To whom correspondence may be addressed:

Guofeng Cheng, Ph.D.

Shanghai Veterinary Research Institute, 518 Ziyue Road, Shanghai, 200241, China.

Tel: 86-213-429-3659;

Fax: 86-215-408-1818;

E-mail: cheng_guofeng@yahoo.com.

This is an Accepted Manuscript for Parasitology. This version may be subject to change during the production process.
DOI: 10.1017/S0031182019001665

SUMMARY

Pathogenic helminth infections are responsible for severe health problems and economic losses worldwide. Timely and accurate diagnosis of helminth infections is critical for adopting suitable strategies for pathogen control. Here, we review recent advances in nucleic acid-based diagnostic methods, including polymerase chain reaction (PCR), quantitative qPCR (qPCR), loop-mediated isothermal amplification (LAMP), and recombinase polymerase amplification (RPA), and discuss their advantages and disadvantages for diagnosing helminth infections. In addition, we highlight recent advances in biosensors for the detection of nucleic acid biomarkers that can potentially be used for the diagnosis of helminth infection.

Keywords: Helminths, nucleic acids, biosensor, diagnosis, infection.

INTRODUCTION

Helminths are invertebrates morphologically characterized by elongated, flat or round bodies, and include trematodes, cestodes, and nematodes (Kaliappan *et al.*, 2013). Among them, soil-transmitted helminths (STHs) and schistosomes are responsible for the most prevalent parasitic infection of humans living in resource-limited areas of developing countries. Studies indicate that STHs, such as *Ascaris lumbricoides* and *Trichuris trichiura*, as well as hookworms, affect over 1.5 billion people worldwide (WHO, 2019a). Schistosomiasis caused by the genus *Schistosoma* affects at least 220.8 million people worldwide (WHO, 2019b). Currently, it is still prevalent in 78 countries where 700 million people are at the risk of contracting schistosomiasis. The World Health Organization has already developed a roadmap to control and eliminate the related neglected tropical diseases caused by these helminths. The major control strategy against these helminth infections is mass drug administration (Minetti *et al.*, 2016; Warren *et al.*, 2001). Consequently, it is critical to timely and accurately identify helminth infections to determine whether drug treatment is working efficiently and then deploy appropriate strategies for disease intervention. Currently, several “gold standard” techniques are used for diagnosing human infection including the identification of parasites in blood (lymphatic filariasis (LF)) or skin snip biopsy (onchocerciasis), eggs or larvae in stools or urine (STHs and schistosomiasis) by microscopy, and the detection of antibodies or pathogen antigens circulating in blood (onchocerciasis, LF and schistosomiasis) or urine (schistosomiasis) by immunological methods. However, these approaches usually have some limitations such as relatively low sensitivity (particularly for low and mid intensity infections), relatively low-throughput, increased time-consumption, skilled personnel being required for operation, and lack of onsite applicability. In addition,

serological methods for helminth diagnosis have limited ability to distinguish between ongoing and previous infections (Hinz *et al.*, 2017).

It is essential to develop rapid and sensitive techniques with high-throughput for diagnosing helminth infections. Immunodiagnostic techniques such as the detection of antibodies and antigens, and molecular diagnoses such as polymerase chain reaction (PCR), quantitative qPCR (qPCR), loop-mediated isothermal amplification (LAMP), and recombinase polymerase amplification (RPA) can fulfill these requirements. In addition, nucleic acid-based methods also offer the most rapid and sensitive detection with the potential to distinguish different species and to monitor potential genetic variation in pathogen populations owing to selection pressure caused by drug usage. Although the broad application of current nucleic acid-based detection methods for surveying, monitoring, and evaluating helminth control programs have so far been hindered by some obvious flaws such as lack of standards of sample preparation and nucleic acid isolation, lack of suitable internal control for target DNA/RNA amplification, potentially high background noise, potential amplification inhibition, and lack of accurate data interpretation for intensity of infection, these techniques may best guide decision making for controlling helminth diseases because of their higher sensitivity, rapid, and high-throughput (Corrado *et al.*, 2016). Hence, we mainly focused on nucleic acid-based diagnostic methods including PCR, qPCR, LAMP, and RPA, reviewed their recent advances and highlighted the potential usage of biosensors, a modern technique, for the diagnosis of helminth infection.

NUCLEIC ACID-BASED METHODS FOR DETECTION OF HELMINTH INFECTIONS

To date, several different nucleic acid-based methods are available for the detection of helminth infections such as PCR, multiplex PCR, qPCR, LAMP, and RPA. These methods offer considerable advantages such as precision, timeliness, and sensitivity of parasite detection. We have briefly reviewed recent advances in each method and then discussed their utility in the diagnosis of helminth infections in the following sections.

PCR

PCR is based on using the ability of DNA polymerase to synthesize a new strand of DNA complementary to the template strand. The technique has been successfully used for detecting schistosome DNA from host serum, urine, and feces as well as water containing cercaria and infected snails (Enk *et al.*, 2012; Sarhan *et al.*, 2015; Sengupta *et al.*, 2019). A new PCR-based approach has been shown to identify different *Schistosoma* species (*S. mansoni*, *S. japonicum*, *S. haematobium*, *S. intercalatum*, and *S. bovis*) and is applicable to human urine samples with high specificity and sensitivity (Sandoval *et al.*, 2006). Recently, a study documented that PCR assay targeting the 121-bp tandem repeat sequence sensitive for *S. mansoni* and the *Dra1* tandem repeat sequence specific for *S. haematobium* can distinguish *Schistosoma* species for diagnosis of early schistosomiasis (Bonnefond *et al.*, 2019). For filarial pathogens, the amplification of genomic DNA repeat sequences (188 bp DNA repeat sequence) *Ssp*I from *Wuchereria bancrofti* (Zhong *et al.*, 1996), *Hha* I repeat region from *Brugia malayi* (Fischer *et al.*, 2000), LLMF72 or a repeat region (15r3) from *Loa loa* (Fink *et al.*, 2011), and the O-150 repeat sequence from *Onchocerca volvulus* (Toé *et al.*, 1998) by PCR have been used to detect pathogen infection. In addition, PCR can also amplify *W. bancrofti* DNA from host sputum, serum, and urine with

high diagnostic accuracy (Kagai *et al.*, 2008; Ximenes *et al.*, 2014). Moreover, the detection of *Taenia solium* DNA from cerebrospinal fluid (CSF) samples by PCR has diagnostic potential for neurocysticercosis, which cannot be detected using radiology or serology (Chaya & Parija, 2014; Michelet *et al.*, 2011). Furthermore, PCR was used to amplify pathogen-specific DNA in host saliva and urine, which may become a new type of non-invasive biomarker for the diagnosis of schistosomiasis japonica (Weerakoon & McManus, 2016). In addition, this has been shown to have good potential for detecting early stage of schistosome infection by amplifying schistosome specific DNA from host sera using PCR (Kato-Hayashi *et al.*, 2010; Xia *et al.*, 2009).

Traditional PCR can be modified by incorporating additional primers and amplification to increase the sensitivity for detecting helminth infection. For example, a two-step semi-nested PCR for amplifying *Oesophagostomum bifurcum* egg DNA in human fecal samples was shown to achieve a specificity of 100% (Verweij *et al.*, 2000). In addition, the semi-nested PCR technique showed an 1,000,000-fold increase to the detection limit for *W. bancrofti,* compared with that of conventional PCR (Ximenes *et al.*, 2014).

By incorporating fluorescent dyes or fluorescently labeled oligonucleotide probes, the amplified DNA can be quantified. This is called real-time quantitative PCR (qPCR), which is now one of the most widely used tools in research and clinical diagnosis. qPCR has been used to detect schistosomes, soil-transmitted helminths (STHs) (e.g., *S. stercoralis, T. trichiura, A. duodenale, N. americanus, and A. lumbricoides*). and *O. volvulus* infection (Lier *et al.*, 2009; Morales-Hojas *et al.*, 2001; O'Connell & Nutman, 2016a; Prince-Guerra *et al.*, 2018). In schistosomes, qPCR was shown to identify considerably

more positive cases than the Kato-Katz method and could be adopted as a suitable strategy for schistosomiasis elimination (He *et al.*, 2018).

Recently, microRNAs (miRNAs) have been considered as a new class of biomarkers for diagnosing many diseases, particularly for circulating miRNAs (Cheng, 2015). For example, Cheng and coworkers (2013) identified five schistosome-specific miRNAs (*Bantam*, *miR-3479*, *sja-miR-8185*, *miR-10*, and *miR-3096*) in the plasma of rabbits infected with *S. japonicum*. Four out of these five miRNAs were shown to be significantly abundant in plasma of *S. japonicum*-infected mice, suggesting these miRNAs can serve as a potential biomarker for schistosomiasis diagnosis. More interestingly, several further studies demonstrated that parasite-derived miRNAs (*miR-277*, *miR-3479-3p* and *bantam*) could detect individuals infected with *S. mansoni* or *S. haematobium* from low and high infection intensity sites by real time qPCR (Hoy *et al.*, 2014; Meningher *et al.*, 2017).

Apart from diagnosis, PCR and qPCR can also be used to distinguish between species of parasites. For example, a copro-PCR method, targeting a segment of the internal transcribed spacer (ITS) region and *COX1* gene was shown to distinguish between *O. viverrini* and *C. sinensis*; results suggested that *C. sinensis* was present in an area in central Thailand where it had not been previously detected (Cai *et al.*, 2014; Traub *et al.*, 2009). In addition, PCR and qPCR for diagnosis of soil-transmitted helminth infections and for monitoring genetic variation of helminths under mass drug administration have been elegantly reviewed (Corrado *et al.*, 2016; O'Connell & Nutman, 2016b; Papaiakovou *et al.*, 2019). qPCR was able to detect and distinguish filarial infection in domestic cats among *B. malayi, D. immitis,* and *D. repens* as well as *A. (Dipetalonema) reconditum*, showing more sensitivity than microfilariae detection

(Wongkamchai *et al.*, 2014).

Unlike conventional qPCR, Droplet Digital™ PCR (ddPCR) can perform absolute quantification of PCR products without the need for generating standard curves (Elmahalawy *et al.*, 2018). In principle, ddPCR can fractionate a loading sample into thousands to millions of droplets, and PCR amplification of the template molecules occurs in each individual droplet, followed by a real time or end point detection of the amplification. The distribution of target sequences into droplets is described by the Poisson distribution, thus allowing accurate and absolute quantification of the target without using reference materials with known target concentrations (Gutierrez-Aguirre *et al.*, 2015). ddPCR has been successfully used to detect *W. bancrofti* DNA (targeting the long DNA repeat (LDR) element) and *B. malayi* DNA (targeting the Hha I element) in host blood samples and mosquito vectors with high sensitivity and specificity (Jongthawin *et al.*, 2016). In gastrointestinal (GI) nematode parasitic infection, ddPCR can detect and distinguish major gastrointestinal nematodes by targeting the internal transcribed spacer region 2 (ITS2) of the ribosomal RNA gene in ovine parasites such as *Haemonchus contortus, Teladorsagia circumcincta* and *Trichostrongylus species* (Elmahalawy *et al.*, 2018) In addition, the ddPCR assay exhibited a high level of diagnostic performance compared with Kato Katz and antibody-based ELISA for diagnosis of *Schistosoma* infection although it is expensive and requires specialized equipment (Cai *et al.*, 2019; Weerakoon *et al.*, 2017a; Weerakoon *et al.*, 2017b).

Multiplex PCR

Multiplex PCR can amplify several different DNA sequences simultaneously using multiple primers (Hawkins & Guest, 2017; Touron *et al.*, 2005). Using selected target sequences for amplification, this

PCR approach can not only detect the pathogen's genotype, but also distinguish among species. For example, *S. mansoni* and *S. haematobium* from a set of duplicate stool samples can be distinguished using primers for species-specific amplification of *cytochrome C oxidase* genes by multiplex PCR, and the results showed a significant correlation with microscopic egg counts for *S. mansoni* in stool and *S. haematobium* in urine (ten Hove *et al*., 2008). In addition, multiplex PCR was also shown to distinguish between differential infections by *T. solium* and *T. asiatica* (Jeon & Eom, 2013; Wicht *et al.*, 2010). Moreover, multiplex PCR has been shown to discriminate *N. americanus, A lumbricoides,* and *T. trichiura* infections from fecal samples (Phuphisut *et al.*, 2014). In the Philippines, a study indicated that multiplex PCR could simultaneously detect two cestode parasites *T. saginata* and *T. solium* alongside *A. lumbricoides* and hookworms in stool samples (Gordon *et al.*, 2015). Moreover, a recent study indicated that a multiplex single-tube PCR assay can specifically discriminate trematode, cestode, and nematode species by targeting the 28S rRNA gene (trematode), *COX1* gene (cestode) and *COX1* gene (nematode) (Wong *et al.*, 2019).

By incorporating Luminex beads or fluorescent dye, multiplex PCR can be modified as qPCR to improve its sensitivity and specificity. For example, several helminths including *A. lumbricoides, A. duodenale, N. americanus* and *S. stercoralis* can be quantitatively co-detected using Luminex beads-based multiplex qPCR, showing high sensitivity (90–100%) and specificity (95–100%) from 319 stool samples (Taniuchi *et al.*, 2011). In addition, a multiplex qPCR for detecting *N. americanus* and *A. duodenale* also showed 100% specificity and 98.5% to 100% sensitivity (Verweij *et al.*, 2007). Moreover, a pentaplex qPCR was not only able to simultaneously detect *A. duodenale*, *N. americanus*, *A. lumbricoides*, and *S. stercoralis* but also to show significantly increased sensitivity of detection (up to 62.3% compared with the detection

of microscopic examination of 7.8%) for one or more helminth species (Basuni *et al.*, 2011). Recent studies also indicated that a multiplex TaqMan qPCR assay can specifically identify encapsulated *Trichinella* species in North America (Almeida *et al.*, 2018), and a multiplex qPCR can detect and quantify DNA from *F. hepatica* and the intermediate snail host, *A. tomentosa*, in water samples (Rathinasamy *et al.*, 2018).

Loop-mediated isothermal amplification (LAMP)

Although PCR and multiplex PCR offer significant advantages for detecting helminth infections such as more sensitivity and specificity, high-throughput, and cost-effectiveness, both methods are heavily dependent on a particular instrument or elaborate detection methods. Alternatively, LAMP can amplify target DNA by DNA polymerase based on a set of specific primers under a constant temperature of 60–65 °C without a thermocycler and accumulated 10^9 copies of target DNA in less than an hour, with a detection limit of a few copies. More importantly, the amplification endpoint could be observed using agarose gel electrophoresis for visual observation of DNA under UV light and using turbidity determination that is more suitable for field research. As a pyrophosphate ion is released once a nucleotide is added to the DNA strands, a visible white precipitate of magnesium pyrophosphate is formed and used to determine whether the target nucleic acid was amplified or not.

The use of LAMP for helminth detection has been recently summarized and reviewed (Amoah *et al.*, 2017; Deng *et al.*, 2019). Here, we highlight only the studies related to schistosomes. LAMP has been successfully used to amplify the *SjR2* gene of *S. japonicum* from the blood samples of infected rabbits at 3rd day post-infection, indicating the potential of early detection of *S. japonicum* infection (Xu *et al.*,

2015). In addition, by targeting *S. haematobium* ribosomal intergenic spacer (IGS) DNA, LAMP was shown to detect *S. haematobium* DNA in heated pellets from patients´ urine samples without requiring a complicated procedure for DNA extraction with 100% sensitivity and 86.67% specificity (Gandasegui *et al.*, 2015). Moreover, LAMP can detect miracidium DNA at the first day of snail exposure (intermediate hosts) to *Schistosoma* (Abbasi *et al.*, 2010) and also detect infection in pooled samples of field-collected snails (Tong *et al.*, 2015), Furthermore, LAMP was shown to be an effective means to detect low intensity infection of schistosomiasis (Lodh *et al.*, 2017). Overall, these studies suggested that LAMP might become a new surveillance and response tool for schistosomiasis control.

Compared to PCR, LAMP has better sensitivity that could enhance the effectiveness of surveillance and mass drug administration of control programs for helminth diseases. However, primer design is a critical part of LAMP assay, because the use of multiple primers for LAMP assay may increase the risk of primer-primer hybridization, resulting in template-free amplification that need to further evaluate false-positive outcomes (Hsieh *et al.*, 2014). Similar to PCR, LAMP cannot be used for detecting unknown or unsequenced targets of pathogens since specific DNA sequences are required for primer design (Drapala & Kordalewska, 2013).

Recombinase polymerase amplification (RPA)

RPA is another fast and sensitive isothermal amplification-based method for amplifying target DNA or RNA using a bacteriophage derived recombinase in combination with a polymerase (Euler *et al.*, 2012; Piepenburg *et al.*, 2006). RPA comprises three core enzymes including a recombinase, a single-stranded DNA-binding protein (SSB), and a strand-displacing polymerase. Recombinases pair

oligonucleotide primers with a homologous sequence, and the SSB binds to displaced strands to prevent primer displacement (Piepenburg *et al.*, 2006). Finally, the strand displacing polymerase begins DNA synthesis where the primer has bound to the target DNA (Euler *et al.*, 2012).

Studies have demonstrated that RPA can detect small amounts of viral, bacterial, and parasitic DNA with high sensitivity and specificity (Shahin *et al.*, 2018). In helminths, most of these studies focused on schistosomes. For example, RPA was used to amplify the *SjR2* gene of *S. japonicum,* and the result indicated that this assay could detect 0.9 fg *S. japonicum* DNA within 15 min and distinguish *S. japonicum* from other worm species (Xing *et al.*, 2017). In addition, RPA combined with oligochromatographic lateral flow strips was shown to detect low levels of *S. haematobium* DNA (Dra1) from crude urine (Rosser *et al.*, 2015). Similarly, an RPA based lateral flow was also developed in *S. mansoni* by amplifying 28S rDNA region and the result indicated that the lower limit of detection for this assay was 10 pg DNA with the lower test parameters permitting sufficient amplification being 6 min and 25 °C (Poulton & Webster, 2018).

RPA can amplify as low as 1–10 DNA/RNA target copies with a rapid turnaround time (less than 20 min) and simple set-up at a single low temperature (37–42 º C). Consequently, it provides significant advantages compared with other isothermal methods such as LAMP. In addition, RPA has been shown to amplify diverse targets, including RNA, miRNA, ssDNA, and dsDNA from a wide variety of organisms and samples (Euler *et al.,* 2012). It may be necessary to develop an RPA assay to detect pathogen-specific small RNAs such as miRNAs in future for diagnosing helminth infections. Moreover, RPA has good potential for field application and the use of this method can reduce diagnostic expenses in

resource-limited countries (Ajibola *et al.*, 2018). Although PCR, LAMP, and RPA methods can also be "sensed" by incorporating a fluorescent dye or probe and the amplified signal can be detected and presented by an electrical device, these methods usually carry disadvantages such as requiring a multi-step set up, being time-consuming, requiring a trained operator for use, and being difficult to apply in the field.

Biosensors for nucleic acid biomarker detection

With the development of modern electrical and chemical techniques, biosensors, a powerful and innovative analytical device, shows excellent characterizations, being highly sensitive, rapid, simple, low-cost, and field-applicable that can be widely used for drug discovery, diagnosis, biomedicine, food safety, processing, and environmental monitoring.

The first biosensor invented by Clark and Lyons (1962) for measuring glucose levels in biological samples was based on electrochemical detection of oxygen or hydrogen peroxide (Fracchiolla *et al.*, 2013). Since then, incredible progress has been made in terms of both the technology and applications of biosensors. Generally, biosensors consist of at least three elements, namely, a biological capture molecule (bioreceptor), a method of converting capture molecule-target interactions into a signal (transducer), and a data output system. A bioreceptor is an immobilized sensitive biological element (antibody, DNA probe, or enzyme) recognizing the analytic (antigen, complementary DNA, or enzyme substrate). A transducer is used to convert the (bio) chemical signal resulting from the interaction of the analytic with the bioreceptor into an electronic signal. The intensity of signals generated corresponds to the analyte concentration.

Currently, most reported biosensors have been designed to detect nucleic acid biomarkers and protein biomarkers related to pathogen infection. Nucleic acid biomarker-based biosensors show good potential for the detection and diagnosis of various diseases such as cancer, diabetes, cardiovascular diseases, tuberculosis, hepatitis, dengue, and food-borne diseases such as diarrhea, cholera, and salmonellosis (Bora *et al.*, 2013). Based on transducer methods, these biosensors can be divided into electrochemical biosensors and optical biosensors.

Electrochemical detection techniques

Various electrochemical biosensors have been reported to detect nucleic acid biomarkers during parasite infection, particularly protozoan infections. For example, Mohan and coworkers used a kind of Sol-gel synthesized nickel oxide (NiO) film deposited onto an indium tin oxide (ITO) coated glass plate to immobilize a 23-mer DNA sequence (oligonucleotide) from 18S rRNA gene sequences from *L. donovani* for the development of a DNA biosensor. Response studies of the ss-DNA/NiO/ITO bioelectrode in the presence of methylene blue redox dye as a redox mediator indicated a linear response in the wide concentration range of 2 pg/mL to 2 µg/mL of complementary target genomic DNA (parasite DNA), suggesting the potential of this biosensor for diagnosis of Visceral Leishmaniasis (Mohan *et al.*, 2011). In addition, a gold nanoleaf-based biosensor was constructed by immobilizing *Leishmania major*-specific DNA for detecting parasite infection. Further analysis indicated that the biosensor could detect a synthetic DNA target in the range of 1.0×10^{-10} to 1.0×10^{-19} molL^{-1} with a detection limit of 1.8×10^{-20} molL^{-1}, and genomic DNA in the range of 0.5 - 20 ng µL^{-1} with a detection limit of 0.07 ng µL^{-1}. More importantly, the biosensor could not only detect *L. major* in patient samples but also distinguish *L. major*

from a non-complementary-sequence oligonucleotide and *L. tropica* with high selectivity (Moradi *et al.*, 2016).

Recently, several electrochemical biosensors were also developed for detecting nucleic acid biomarkers for helminth infection. For example, Santos et al. used AuNPs that are the most commonly used nanomaterial in biosensor development through biomolecules conjugation and then obtained compelling sol-gel derivative films to immobilize AuNPs using the organosilane, 3-mercaptopropyltrimethoxysilane. Further analyses indicated that the biosensor could recognize the *S. mansoni* genome sequence at different concentrations in samples of urine, CSF, and serum with the limit detection of 0.6 pg μL^{-1} (Santos *et al.,* 2019). The authors also reported the development of another biosensor by using a self-assembled monolayer of mercaptobenzoic acid (MBA) immobilizing nanostructures composed of gold nanoparticles (AuNPs) and magnetite nanoparticles (Fe_3O_4_NPs) to recognize specific nucleotide sequence of *S. mansoni* present in CSF and serum samples, and this biosensor showed a DNA detection limit of 0.781 and 0.685 pg μL^{-1} (Santos *et al.*, 2017).

Apart from the detection of nuclear acid biomarkers, some biosensors were also developed to detect specific antibodies/antigens related to helminth infection. For example, a piezoelectric immunosensor assay was developed with immobilizing immunoglobulin G (IgG) as a probe to detect *S. japonicum* circulating antigens. By immobilizing purified IgG from infected rabbit's sera with *S. japonicum*, the developed biosensor was shown to specifically detect *S. japonicum* circulating antigens (Cheng *et al.*, 2008). In addition, a superhydrophobic surface-based magnetic electrochemical immunoassay for detection of *S. japonicum* antibodies was also developed and the results indicated that the biosensor is

quantitatively sensitive to SjAb concentrations ranging from 2 ng mL^{-1} to 15 µg mL^{-1}, with a detection limit of ~1.3 ng mL^{-1} (Nie *et al.*, 2012). Moreover, an electrochemical immunosensor was also developed by immobilizing *W. bancrofti* epitope antigens (Wb/ALT2-A5) on a screen-printed carbon electrode (SPCE) by their amine groups via chitosan film by coupling with glutaraldehyde as the crosslinker. The detection limitation of this biosensor was 0.002 µg mL^{-1} for human serum with a sensitivity of 1.86 µA (Prado *et al.*, 2018).

Optical detection techniques

Optical detection techniques are those that can sense phenomena related to the interaction of target compounds with the analytes. Usually, these biosensors can measure luminescence, fluorescence, or color changes. Several biosensors have used optical detection techniques to detect nucleic acid biomarkers for parasite infection, particularly for malaria. For example, Ittarat and coworkers described a genosensor based on quartz crystal microbalance (QCM) to detect malaria infection. In this method, the QCM surface was immobilized with a malaria biotinylated probe. Specific DNA fragments of malaria-infected blood were amplified. Hybridization between the amplified products and the immobilized probe resulted in quartz frequency shifts that were measured by an in-house frequency counter. Further analysis indicated that this biosensor can differentially diagnose blood infected with *P. falciparum* from that infected with *P. vivax* and no cross reaction with human DNA (Ittarat et al., 2013). In helminths, a diffraction-based optical biosensor was developed although it detects a protein biomarker (a 31-kDa recombinant antigen called NIE derived from *S. stercoralis*) to diagnose strongyloidiasis. The biosensor showed excellent agreement (κ = 0.98) with results obtained by an NIE-based ELISA method (Pak *et al.*, 2014).

In addition, as stated above, miRNAs are recognized a novel kind of biomarker for disease diagnosis, particularly for cancer, for example in early diagnostics, predicting responses to therapy, monitoring relapse, and molecular classification of tumors. a recent study documented that circulating miRNAs associated with cancer derived from serum can be detected with a biosensor using DNA concatemer amplification (Hong *et al.*, 2013). If sensitive and effective nucleic acid biomarkers can be identified during helminth infection, and suitable and effective probe layers can be immobilized on a transducer surface, we believe that electrochemical biosensors can become a next generation technique for diagnosis of helminth infections.

All the nucleic acid-based methods discussed and the related targets for detecting helminth infections are summarized and listed in Table 1.

ADVANTAGES AND LIMITATIONS OF NUCLEIC ACID-BASED METHODS FOR DETECTION OF HELMINTH INFECTIONS

Each nucleic acid-based method has its own advantages and limitations. We have summarized them as follows. PCR is one of the most promising approaches to detect parasite DNA or RNA in human, animal, or vector samples owing to its higher diagnostic sensitivity and specificity compared to microscopic examination or immunodiagnostics. However, it has limitations, such as the high cost of the instrumentation required for thermal cycling and signal detection, and the need for well-equipped laboratories (Verweij & Stensvold, 2014). Both qPCR and ddPCR methods can quantitatively determine helminth infection as they are amenable for automation and have relatively high-throughput, whereas

ddPCR can increase the accuracy of quantification at low target concentrations. Unlike PCR, LAMP offers sensitivity, high efficiency, rapidity, and simplicity for identifying certain infectious diseases, although the specificity is yet to be validated. The main shortcoming of LAMP is the false-positive production caused by non-specific amplification resulting from primers, which could be improved by optimization of the reaction conditions, primer design, detection approaches, etc. RPA offers several advantages such as operation at a low and constant temperature, without the need for an initial denaturation step or the use of multiple primers. Biosensor-based methods have the potential to deliver rapid, accurate, and affordable diagnosis of parasite infection. In addition, biosensors can improve diagnosis and follow-up treatment in terms of rapidity, real-time performance, and expense compared to current detection technologies such as PCR and ELISA. Furthermore, the use of biosensors offers significant advantages such as small fluid volume manipulation and high integration capability that facilitates the development of portable devices and ease of use, allowing use by non-specialized personnel in non-centralized laboratories (Sin *et al.*, 2014). The time-consuming preparative steps of gene probe evaluation make it hard to consider these as the basis of biosensors for *in situ* detection of pathogenic microorganisms. Although extensive efforts have been expended to develop biosensors, a relatively small number of analytics, particularly toxic materials, can be detected using commercially available devices (Alhamoud *et al.*, 2019). Although recent advancements in nanotechnology are facilitating the development of smaller, less-expensive, multiplex, easy-to-use, and fully integrated biosensors for diagnostics and food and water safety (Bouguelia *et al.*, 2013; Chung *et al.*, 2015; Verbarg *et al.*, 2013), future research must also focus on simplifying their fabrication techniques, miniaturizing devices, and developing the biocompatibility and environmental friendliness of the nanomaterials. Unfortunately, preparation and manipulation of nanomaterials in a reproducible and

economic fashion is still premature. More importantly, the development of Point of Care (POC) devices is the ultimate goal, particularly for human patients, which still faces a significant challenge. Consequently, techniques that employ and combine with chemistry, electrics, optics, material science, and mechanical passivation approaches could speed up the development of biosensors for commercial requirements. The advantages and limitations of each nucleic acid-based method are summarized in Table 2.

CONCLUSIONS

Traditional pathogen detection methods are usually time-consuming, and associated with low sensitivity, selectivity, or accuracy. Therefore, sensitive and specific methods for diagnosing parasitic diseases are urgently required. Recent advances in PCR, qPCR, ddPCR, LAMP, and RPA are proving to be highly sensitive and specific diagnostic approaches. By identifying effective biomarkers during helminth infection, these approaches could be further improved by increasing the sensitivity of detection and diagnosis. Current biosensors have the potential to provide rapid, accurate, and affordable diagnoses of parasite infection by offering significant advantages such as small fluid volume manipulation, high integration capability that facilitates the development of portable devices, and ease of use. Therefore, the combination of these outcomes with advanced technologies such as nucleic acid-based biosensors may change the current scenario of clinical diagnosis.

FINANCIAL SUPPORT

This study was, in part or in whole, supported by the National Key Research and Development Program of China (2017YFD0501306-3), National Natural Science Foundation of China (Grants No. 31472187

and 31672550) and the Science & Technology Innovation Program of the Chinese Academy of Agricultural Sciences.

ETHICAL STANDARDS

Not applicable.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest

REFERENCES

Abbasi, I., King, C. H., Muchiri, E. M. and Hamburger, J. (2010). Detection of *Schistosoma mansoni* and *Schistosoma haematobium* DNA by Loop-Mediated Isothermal Amplification: Identification of Infected Snails from Early Prepatency. *Am J Trop Med Hyg,* **83**, 427-432. doi: 10.4269/ajtmh.2010.09-0764.

Agarwal, M., Tomar, R. S. and Jyoti, A. (2014). Detection of water-borne pathogenic bacteria: where molecular methods rule. *International Journal of Multidisciplinary and Current Research,* **2**, 351-358.

Ajibola, O., Gulumbe, B., Eze, A. and Obishakin, E. (2018). Tools for Detection of Schistosomiasis in Resource Limited Settings. *Medical Sciences,* **6**, 39.

Alhamoud, Y., Yang, D., Kenston, S. S. F., Liu, G., Liu, L., Zhou, H., Ahmed, F. and Zhao, J. (2019). Advances in biosensors for the detection of ochratoxin A: Bio-receptors, nanomaterials, and their applications. *Biosensors and Bioelectronics*, 111418.

Almeida, M., Bishop, H., Nascimento, F. S., Mathison, B., Bradbury, R. S. and Silva, A. D. (2018). Multiplex TaqMan qPCR assay for specific identification of encapsulated Trichinella species prevalent in North America. *Mem Inst Oswaldo Cruz,* **113**, e180305. doi: 10.1590/0074-02760180305.

Amoah, I. D., Singh, G., Stenstrom, T. A. and Reddy, P. (2017). Detection and quantification of soil-transmitted helminths in environmental samples: A review of current state-of-the-art and future perspectives. *Acta Trop,* **169**, 187-201. doi: 10.1016/j.actatropica.2017.02.014.

Basuni, M., Muhi, J., Othman, N., Verweij, J. J., Ahmad, M., Miswan, N., Rahumatullah, A., Aziz, F. A., Zainudin, N. S. and Noordin, R. (2011). A pentaplex real-time polymerase chain reaction

assay for detection of four species of soil-transmitted helminths. *Am J Trop Med Hyg,* **84**, 338-343.

Bonnefond, S., Cnops, L., Duvignaud, A., Bottieau, E., Pistone, T., Clerinx, J. and Malvy, D. (2019). Early complicated schistosomiasis in a returning traveller: Key contribution of new molecular diagnostic methods. *Int J Infect Dis,* **79**, 72-74. doi: 10.1016/j.ijid.2018.11.018.

Bora, U., Sett, A. and Singh, D. (2013). Nucleic acid based biosensors for clinical applications. *Biosens J,* **1**, 104.

Bouguelia, S., Roupioz, Y., Slimani, S., Mondani, L., Casabona, M. G., Durmort, C., Vernet, T., Calemczuk, R. and Livache, T. (2013). On-chip microbial culture for the specific detection of very low levels of bacteria. *Lab Chip,* **13**, 4024-4032. doi: 10.1039/c3lc50473e.

Cai, P., Weerakoon, K. G., Mu, Y., Olveda, R. M., Ross, A. G., Olveda, D. U. and McManus, D. P. (2019). Comparison of Kato Katz, antibody-based ELISA and droplet digital PCR diagnosis of schistosomiasis japonica: Lessons learnt from a setting of low infection intensity. *PLoS Negl Trop Dis,* **13**, e0007228. doi: 10.1371/journal.pntd.0007228.

Cai, X.-Q., Yu, H.-Q., Li, R., Yue, Q.-Y., Liu, G.-H., Bai, J.-S., Deng, Y., Qiu, D.-Y. and Zhu, X.-Q. (2014). Rapid detection and differentiation of *Clonorchis sinensis* and *Opisthorchis viverrini* using real-time PCR and high resolution melting analysis. *The Scientific World Journal,* **2014**.

Chaya, D. and Parija, S. C. (2014). Performance of polymerase chain reaction for the diagnosis of cystic echinococcosis using serum, urine, and cyst fluid samples. *Tropical parasitology,* **4**, 43.

Chen, M., Ai, L., Zhang, R., Xia, J., Wang, K., Chen, S., Zhang, Y., Xu, M., Li, X. and Zhu, X. (2011). Sensitive and rapid detection of *Paragonimus westermani* infection in humans and animals by loop-mediated isothermal amplification (LAMP). *Parasitology research,* **108**, 1193-1198.

Cheng, C. W., Chen, C. K., Chen, Y. S. and Chen, L. Y. (2008). Determination of *Schistosoma japonicum* circulating antigens in dilution serum by piezoelectric immunosensor and S/N enhancement. *Biosens Bioelectron,* **24**, 136-140. doi: 10.1016/j.bios.2008.03.029.

Cheng, G. (2015). Circulating miRNAs: roles in cancer diagnosis, prognosis and therapy. *Adv Drug Deliv Rev,* **81**, 75-93. doi: 10.1016/j.addr.2014.09.001.

Chung, J., Kang, J. S., Jurng, J. S., Jung, J. H. and Kim, B. C. (2015). Fast and continuous microorganism detection using aptamer-conjugated fluorescent nanoparticles on an optofluidic platform. *Biosens Bioelectron,* **67**, 303-308. doi: 10.1016/j.bios.2014.08.039.

Corrado, M., E. James Lacourse, Lisa, R. and Stothard, J. R. (2016). Focusing nucleic acid-based molecular diagnostics and xenomonitoring approaches for human helminthiases amenable to preventive chemotherapy. *Parasitology Open,* **2**, 1-17.

Daher, R. K., Stewart, G., Boissinot, M. and Bergeron, M. G. (2016). Recombinase polymerase amplification for diagnostic applications. *Clinical chemistry,* **62**, 947-958.

Deng, M.-H., Zhong, L.-Y., Kamolnetr, O., Limpanont, Y. and Lv, Z.-Y. (2019). Detection of helminths by loop-mediated isothermal amplification assay: a review of updated technology and future outlook. *Infect Dis Poverty,* **8**, 20.

Diguta, C. F., Rousseaux, S., Weidmann, S., Bretin, N., Vincent, B., Guilloux-Benatier, M. and Alexandre, H. (2010). Development of a qPCR assay for specific quantification of *Botrytis cinerea* on grapes. *FEMS microbiology letters,* **313**, 81-87.

Drapala, D. and Kordalewska, M. (2013). Loop-mediated Isothermal Amplification (LAMP) as a diagnostic tool in detection of infectious diseases. *PhD Interdisciplanary Journal*, 19-23.

Elmahalawy, S. T., Halvarsson, P., Skarin, M. and Hoglund, J. (2018). Droplet digital polymerase

chain reaction (ddPCR) as a novel method for absolute quantification of major gastrointestinal nematodes in sheep. *Vet Parasitol,* **261**, 1-8. doi: 10.1016/j.vetpar.2018.07.008.

Enk, M. J., e Silva, G. O. and Rodrigues, N. B. (2012). Diagnostic accuracy and applicability of a PCR system for the detection of Schistosoma mansoni DNA in human urine samples from an endemic area. *PLoS One,* **7**, e38947.

Euler, M., Wang, Y., Nentwich, O., Piepenburg, O., Hufert, F. T. and Weidmann, M. (2012). Recombinase polymerase amplification assay for rapid detection of Rift Valley fever virus. *Journal of clinical virology,* **54**, 308-312.

Feng, K., Li, W., Guo, Z., Duo, H., Fu, Y., Shen, X., Tie, C., XIAO, C., LUO, Y. and QI, G. (2017). Development of LAMP assays for the molecular detection of taeniid infection in canine in Tibetan rural area. *Journal of Veterinary Medical Science,* **79**, 1986-1993.

Fernández-Soto, P., Arahuetes, J. G., Hernández, A. S., Abán, J. L., Santiago, B. V. and Muro, A. (2014). A loop-mediated isothermal amplification (LAMP) assay for early detection of *Schistosoma mansoni* in stool samples: a diagnostic approach in a murine model. *PLoS Negl Trop Dis,* **8**, e3126.

Fink, D. L., Kamgno, J. and Nutman, T. B. (2011). Rapid molecular assays for specific detection and quantitation of Loa loa microfilaremia. *PLoS Negl Trop Dis,* **5**, e1299.

Fischer, P., Supali, T., Wibowo, H., Bonow, I. and Williams, S. A. (2000). Detection of DNA of nocturnally periodic Brugia malayi in night and day blood samples by a polymerase chain reaction-ELISA-based method using an internal control DNA. *Am J Trop Med Hyg,* **62**, 291-296.

Flores, M. D., Gonzalez, L. M., Hurtado, C., Motta, Y. M., Domínguez-Hidalgo, C., Merino, F. J., Perteguer, M. J. and Gárate, T. (2018). HDP2: a ribosomal DNA (NTS-ETS) sequence as a

target for species-specific molecular diagnosis of intestinal taeniasis in humans. *Parasit Vectors,* **11**, 117.

Fracchiolla, N. S., Artuso, S. and Cortelezzi, A. (2013). Biosensors in clinical practice: focus on oncohematology. *Sensors,* **13**, 6423-6447.

Fung, M. S., Xiao, N., Wang, S. and Carlton, E. J. (2012). Field evaluation of a PCR test for *Schistosoma japonicum* egg detection in low-prevalence regions of China. *Am J Trop Med Hyg,* **87**, 1053-1058.

Gandasegui, J., Fernández-Soto, P., Carranza-Rodríguez, C., Pérez-Arellano, J. L., Vicente, B., López-Abán, J. and Muro, A. (2015). The rapid-heat LAMPellet method: a potential diagnostic method for human urogenital schistosomiasis. *PLoS Negl Trop Dis,* **9**, e0003963.

Gobert, G. N., Chai, M., Duke, M. and McManus, D. P. (2005). Copro-PCR based detection of *Schistosoma* eggs using mitochondrial DNA markers. *Mol Cell Probes,* **19**, 250-254.

Gordon, C. A., Acosta, L. P., Gray, D. J., Olveda, R. M., Jarilla, B., Gobert, G. N., Ross, A. G. and McManus, D. P. (2012). High prevalence of *Schistosoma japonicum* infection in carabao from Samar province, the Philippines: implications for transmission and control. *PLoS Negl Trop Dis,* **6**, e1778.

Gordon, C. A., McManus, D. P., Acosta, L. P., Olveda, R. M., Williams, G. M., Ross, A. G., Gray, D. J. and Gobert, G. N. (2015). Multiplex real-time PCR monitoring of intestinal helminths in humans reveals widespread polyparasitism in Northern Samar, the Philippines. *International journal for parasitology,* **45**, 477-483.

Gutierrez-Aguirre, I., Racki, N., Dreo, T. and Ravnikar, M. (2015). Droplet digital PCR for absolute quantification of pathogens. *Methods Mol Biol,* **1302**, 331-347. doi: 10.1007/978-1-4939-2620-

6_24.

Hajia, M., Rahbar, M., Farzami, M. R., Dolatyar, A., Imani, M., Saburian, R. and Farzanehkhah, M. (2014). Efficacy of multiplex PCR procedure for Iranian *Streptococcus pneumoniae* isolates. *Caspian journal of internal medicine,* **5**, 109.

Hawkins, S. F. and Guest, P. C. (2017). Multiplex analyses using real-time quantitative PCR. In *Multiplex Biomarker Techniques* pp. 125-133. Springer.

He, P., Gordon, C. A., Williams, G. M., Li, Y., Wang, Y., Hu, J., Gray, D. J., Ross, A. G., Harn, D. and McManus, D. P. (2018). Real-time PCR diagnosis of *Schistosoma japonicum* in low transmission areas of China. *Infect Dis Poverty,* **7**, 8. doi: 10.1186/s40249-018-0390-y.

Hernández, M., Gonzalez, L., Fleury, A., Saenz, B., Parkhouse, R., Harrison, L., Garate, T. and Sciutto, E. (2008). Neurocysticercosis: detection of *Taenia solium* DNA in human cerebrospinal fluid using a semi-nested PCR based on HDP2. *Annals of Tropical Medicine & Parasitology,* **102**, 317-323.

Hinz, R., Schwarz, N. G., Hahn, A. and Frickmann, H. (2017). Serological approaches for the diagnosis of schistosomiasis - A review. *Mol Cell Probes,* **31**, 2-21. doi: 10.1016/j.mcp.2016.12.003.

Hong, C.-Y., Chen, X., Liu, T., Li, J., Yang, H.-H., Chen, J.-H. and Chen, G.-N. (2013). Ultrasensitive electrochemical detection of cancer-associated circulating microRNA in serum samples based on DNA concatamers. *Biosensors and Bioelectronics,* **50**, 132-136.

Hoy, A. M., Lundie, R. J., Ivens, A., Quintana, J. F., Nausch, N., Forster, T., Jones, F., Kabatereine, N. B., Dunne, D. W. and Mutapi, F. (2014). Parasite-derived microRNAs in host serum as novel biomarkers of helminth infection. *PLoS Negl Trop Dis,* **8**, e2701.

Hsieh, K., Mage, P. L., Csordas, A. T., Eisenstein, M. and Soh, H. T. (2014). Simultaneous elimination of carryover contamination and detection of DNA with uracil-DNA-glycosylase-supplemented loop-mediated isothermal amplification (UDG-LAMP). *Chemical communications,* **50**, 3747-3749.

Hussein, H. M., El-Tonsy, M. M., Tawfik, R. A. and Ahmed, S. A.-E.-G. (2012). Experimental study for early diagnosis of prepatent schistosomiasis mansoni by detection of free circulating DNA in serum. *Parasitology research,* **111**, 475-478.

Ibironke, O. A., Phillips, A. E., Garba, A., Lamine, S. M. and Shiff, C. (2011). Diagnosis of *Schistosoma haematobium* by detection of specific DNA fragments from filtered urine samples. *Am J Trop Med Hyg,* **84**, 998-1001.

Ittarat, W., Chomean, S., Sanchomphu, C., Wangmaung, N., Promptmas, C. and NgrenngarmLert, W. (2013). Biosensor as a molecular malaria differential diagnosis. *Clinica Chimica Acta,* **419**, 47-51.

Janissen, R., Sahoo, P. K., Santos, C. A., da Silva, A. M., von Zuben, A. A., Souto, D. E., Costa, A. D., Celedon, P., Zanchin, N. I. and Almeida, D. B. (2017). InP nanowire biosensor with tailored biofunctionalization: ultrasensitive and highly selective disease biomarker detection. *Nano letters,* **17**, 5938-5949.

Jeon, H.-K. and Eom, K. S. (2013). Molecular approaches to Taenia asiatica. *The Korean journal of parasitology,* **51**, 1.

Jeon, H.-K., Yong, T.-S., Sohn, W.-M., Chai, J.-Y., Hong, S.-J., Han, E.-T., Jeong, H.-G., Chhakda, T., Sinuon, M. and Socheat, D. (2011). Molecular identification of *Taenia* tapeworms by Cox1 gene in Koh Kong, Cambodia. *The Korean journal of parasitology,* **49**, 195.

Jongthawin, J., Intapan, P. M., Lulitanond, V., Sanpool, O., Thanchomnang, T., Sadaow, L. and

Maleewong, W. (2016). Detection and quantification of *Wuchereria bancrofti* and *Brugia malayi* DNA in blood samples and mosquitoes using duplex droplet digital polymerase chain reaction. *Parasitol Res,* **115**, 2967-2972. doi: 10.1007/s00436-016-5051-0.

Kagai, J., Mpoke, S., Muli, F., Hamburger, J. and Kenya, E. (2008). Molecular Technique Utilising Sputum For Detecting *Wuchereria bancroft i* Infections In Malindi, Kenya. *East African medical journal,* **85**, 118-122.

Kaliappan, S. P., George, S., Francis, M. R., Kattula, D., Sarkar, R., Minz, S., Mohan, V. R., George, K., Roy, S. and Ajjampur, S. S. R. (2013). Prevalence and clustering of soil‐transmitted helminth infections in a tribal area in southern India. *Tropical Medicine & International Health,* **18**, 1452-1462.

Kato-Hayashi, N., Kirinoki, M., Iwamura, Y., Kanazawa, T., Kitikoon, V., Matsuda, H. and Chigusa, Y. (2010). Identification and differentiation of human schistosomes by polymerase chain reaction. *Experimental parasitology,* **124**, 325-329.

Kato-Hayashi, N., Leonardo, L. R., Arevalo, N. L., Tagum, M. N. B., Apin, J., Agsolid, L. M., Chua, J. C., Villacorte, E. A., Kirinoki, M. and Kikuchi, M. (2015). Detection of active schistosome infection by cell-free circulating DNA of *Schistosoma japonicum* in highly endemic areas in Sorsogon Province, the Philippines. *Acta Trop,* **141**, 178-183.

Khoshakhlagh, P., Spotin, A., Mahami-Oskouei, M., Shahbazi, A. and Ozlati, M. (2017). Loop-mediated isothermal amplification as a reliable assay for *Toxocara canis* infection in pet dogs. *Parasitology research,* **116**, 2591-2597.

Lier, T., Simonsen, G. S., Wang, T., Lu, D., Haukland, H. H., Vennervald, B. J., Hegstad, J. and Johansen, M. V. (2009). Real-time polymerase chain reaction for detection of low-intensity

Schistosoma japonicum infections in China. *Am J Trop Med Hyg,* **81**, 428-432.

Liu, J., Gratz, J., Amour, C., Kibiki, G., Becker, S., Janaki, L., Verweij, J. J., Taniuchi, M., Sobuz, S. U. and Haque, R. (2013). A laboratory-developed TaqMan Array Card for simultaneous detection of 19 enteropathogens. *Journal of clinical microbiology,* **51**, 472-480.

Lodh, N., Mikita, K., Bosompem, K. M., Anyan, W. K., Quartey, J. K., Otchere, J. and Shiff, C. J. (2017). Point of care diagnosis of multiple schistosome parasites: Species-specific DNA detection in urine by loop-mediated isothermal amplification (LAMP). *Acta Trop,* **173**, 125-129. doi: 10.1016/j.actatropica.2017.06.015.

Lodh, N., Naples, J. M., Bosompem, K. M., Quartey, J. and Shiff, C. J. (2014). Detection of parasite-specific DNA in urine sediment obtained by filtration differentiates between single and mixed infections of *Schistosoma mansoni* and *S. haematobium* from endemic areas in Ghana. *PLoS One,* **9**, e91144.

Luz, J. G., Souto, D. E., Machado-Assis, G. F., de Lana, M., Luz, R. C., Martins-Filho, O. A., Damos, F. S. and Martins, H. R. (2016). Applicability of a novel immunoassay based on surface plasmon resonance for the diagnosis of Chagas disease. *Clinica Chimica Acta,* **454**, 39-45.

Mejia, R., Vicuña, Y., Broncano, N., Sandoval, C., Vaca, M., Chico, M., Cooper, P. J. and Nutman, T. B. (2013). A novel, multi-parallel, real-time polymerase chain reaction approach for eight gastrointestinal parasites provides improved diagnostic capabilities to resource-limited at-risk populations. *Am J Trop Med Hyg,* **88**, 1041-1047.

Meningher, T., Lerman, G., Regev-Rudzki, N., Gold, D., Ben-Dov, I. Z., Sidi, Y., Avni, D. and Schwartz, E. (2017). Schistosomal MicroRNAs Isolated From Extracellular Vesicles in Sera of Infected Patients: A New Tool for Diagnosis and Follow-up of Human Schistosomiasis. *J Infect*

Dis, **215**, 378-386. doi: 10.1093/infdis/jiw539.

Meurs, L., Brienen, E., Mbow, M., Ochola, E. A., Mboup, S., Karanja, D. M., Secor, W. E., Polman, K. and van Lieshout, L. (2015). Is PCR the next reference standard for the diagnosis of *Schistosoma* in stool? A comparison with microscopy in Senegal and Kenya. *PLoS Negl Trop Dis,* **9**, e0003959.

Michelet, L., Fleury, A., Sciutto, E., Kendjo, E., Fragoso, G., Paris, L. and Bouteille, B. (2011). Human neurocysticercosis: comparison of different diagnostic tests using cerebrospinal fluid. *Journal of clinical microbiology,* **49**, 195-200.

Minetti, C., LACOURSE, E. J., Reimer, L. and STOTHARD, J. R. (2016). Focusing nucleic acid-based molecular diagnostics and xenomonitoring approaches for human helminthiases amenable to preventive chemotherapy. *Parasitology Open,* **2**.

Mohan, S., Srivastava, P., Maheshwari, S., Sundar, S. and Prakash, R. (2011). Nano-structured nickel oxide based DNA biosensor for detection of visceral leishmaniasis (Kala-azar). *Analyst,* **136**, 2845-2851.

Moradi, M., Sattarahmady, N., Rahi, A., Hatam, G. R., Sorkhabadi, S. M. R. and Heli, H. (2016). A label-free, PCR-free and signal-on electrochemical DNA biosensor for *Leishmania major* based on gold nanoleaves. *Talanta,* **161**, 48-53. doi: 10.1016/j.talanta.2016.08.030.

Morales-Hojas, R., Post, R. J., Shelley, A. J., Maia-Herzog, M., Coscarón, S. and Cheke, R. A. (2001). Characterisation of nuclear ribosomal DNA sequences from *Onchocerca volvulus* and *Mansonella ozzardi* (Nematoda: Filarioidea) and development of a PCR-based method for their detection in skin biopsies. *International Journal for Parasitology,* **31**, 169-177.

Mugambi, R. M., Agola, E. L., Mwangi, I. N., Kinyua, J., Shiraho, E. A. and Mkoji, G. M. (2015).

Development and evaluation of a loop mediated isothermal amplification (LAMP) technique for the detection of hookworm (Necator americanus) infection in fecal samples. *Parasit Vectors,* **8**, 574.

Ngui, R., Lim, Y. A. and Chua, K. H. (2012). Rapid detection and identification of human hookworm infections through high resolution melting (HRM) analysis. *PLoS One,* **7**, e41996.

Nie, J., Zhang, Y., Wang, H., Wang, S. and Shen, G. (2012). Superhydrophobic surface-based magnetic electrochemical immunoassay for detection of *Schistosoma japonicum* antibodies. *Biosens Bioelectron,* **33**, 23-28. doi: 10.1016/j.bios.2011.11.053.

O'Connell, E. M. and Nutman, T. B. (2016a). Molecular diagnostics for soil-transmitted helminths. *Am J Trop Med Hyg,* **95**, 508-513.

O'Connell, E. M. and Nutman, T. B. (2016b). Molecular Diagnostics for Soil-Transmitted Helminths. *Am J Trop Med Hyg,* **95**, 508-513. doi: 10.4269/ajtmh.16-0266.

Papaiakovou, M., Gasser, R. B. and Littlewood, D. T. J. (2019). Quantitative PCR-Based Diagnosis of Soil-Transmitted Helminth Infections: Faecal or Fickle? *Trends Parasitol,* **35**, 491-500. doi: 10.1016/j.pt.2019.04.006.

Phuphisut, O., Yoonuan, T., Sanguankiat, S., Chaisiri, K., Maipanich, W., Pubampen, S., Komalamisra, C. and Adisakwattana, P. (2014). Triplex polymerase chain reaction assay for detection of major soil-transmitted helminths, *Ascaris lumbricoides*, *Trichuris trichiura*, *Necator americanus*, in fecal samples. *Southeast Asian Journal of Tropical Medicine and Public Health,* **45**, 267.

Piepenburg, O., Williams, C. H., Stemple, D. L. and Armes, N. A. (2006). DNA detection using recombination proteins. *PLoS biology,* **4**, e204.

Plichart, C. and Lemoine, A. (2013). Monitoring and evaluation of lymphatic filariasis interventions: an improved PCR-based pool screening method for high throughput *Wuchereria bancrofti* detection using dried blood spots. *Parasit Vectors,* **6**, 110.

Poole, C. B., Tanner, N. A., Zhang, Y., Evans Jr, T. C. and Carlow, C. K. (2012). Diagnosis of brugian filariasis by loop-mediated isothermal amplification. *PLoS Negl Trop Dis,* **6**, e1948.

Poulton, K. and Webster, B. (2018). Development of a lateral flow recombinase polymerase assay for the diagnosis of *Schistosoma mansoni* infections. *Anal Biochem,* **546**, 65-71. doi: 10.1016/j.ab.2018.01.031.

Prado, I. C., Mendes, V. G., Souza, A. L. A., Dutra, R. F. and De-Simone, S. G. (2018). Electrochemical immunosensor for differential diagnostic of *Wuchereria bancrofti* using a synthetic peptide. *Biosens Bioelectron,* **113**, 9-15. doi: 10.1016/j.bios.2018.04.045.

Prince-Guerra, J. L., Cama, V. A., Wilson, N., Thiele, E. A., Likwela, J., Ndakala, N., wa Muzinga, J. M., Ayebazibwe, N., Ndjakani, Y. D. and Pitchouna, N. A. (2018). Comparison of PCR methods for *Onchocerca volvulus* detection in skin snip biopsies from the Tshopo province, Democratic Republic of the Congo. *Am J Trop Med Hyg,* **98**, 1427-1434.

Rahman, S. M., Song, H. B., Jin, Y., Oh, J.-K., Lim, M. K., Hong, S.-T. and Choi, M.-H. (2017). Application of a loop-mediated isothermal amplification (LAMP) assay targeting cox1 gene for the detection of *Clonorchis sinensis* in human fecal samples. *PLoS Negl Trop Dis,* **11**, e0005995.

Rashwan, N., Diawara, A., Scott, M. E. and Prichard, R. K. (2017). Isothermal diagnostic assays for the detection of soil-transmitted helminths based on the SmartAmp2 method. *Parasit Vectors,* **10**, 496.

Rathinasamy, V., Hosking, C., Tran, L., Kelley, J., Williamson, G., Swan, J., Elliott, T., Rawlin, G.,

Beddoe, T. and Spithill, T. W. (2018). Development of a multiplex quantitative PCR assay for detection and quantification of DNA from *Fasciola hepatica* and the intermediate snail host, *Austropeplea tomentosa*, in water samples. *Vet Parasitol,* **259**, 17-24. doi: 10.1016/j.vetpar.2018.06.018.

Rosser, A., Rollinson, D., Forrest, M. and Webster, B. (2015). Isothermal Recombinase Polymerase amplification (RPA) of *Schistosoma haematobium* DNA and oligochromatographic lateral flow detection. *Parasit Vectors,* **8**, 446.

Sandoval, N., Siles-Lucas, M., Perez-Arellano, J. L., Carranza, C., Puente, S., Lopez-Aban, J. and Muro, A. (2006). A new PCR-based approach for the specific amplification of DNA from different *Schistosoma* species applicable to human urine samples. *Parasitology,* **133**, 581-587. doi: 10.1017/s0031182006000898.

Santos, G. S., Andrade, C. A., Bruscky, I. S., Wanderley, L. B., Melo, F. L. and Oliveira, M. D. (2017). Impedimetric nanostructured genosensor for detection of schistosomiasis in cerebrospinal fluid and serum samples. *J Pharm Biomed Anal,* **137**, 163-169. doi: 10.1016/j.jpba.2017.01.031.

Sarhan, R. M., Kamel, H. H., Saad, G. A. and Ahmed, O. A. (2015). Evaluation of three extraction methods for molecular detection of *Schistosoma mansoni* infection in human urine and serum samples. *Journal of parasitic diseases,* **39**, 499-507.

Sengupta, M. E., Hellstrom, M., Kariuki, H. C., Olsen, A., Thomsen, P. F., Mejer, H., Willerslev, E., Mwanje, M. T., Madsen, H., Kristensen, T. K., Stensgaard, A. S. and Vennervald, B. J. (2019). Environmental DNA for improved detection and environmental surveillance of schistosomiasis. *Proc Natl Acad Sci U S A,* **116**, 8931-8940. doi: 10.1073/pnas.1815046116.

Shahin, K., Gustavo Ramirez-Paredes, J., Harold, G., Lopez-Jimena, B., Adams, A. and Weidmann,

M. (2018). Development of a recombinase polymerase amplification assay for rapid detection of *Francisella noatunensis* subsp. *orientalis*. *PLoS One,* **13**, e0192979. doi: 10.1371/journal.pone.0192979.

Sin, M. L., Mach, K. E., Wong, P. K. and Liao, J. C. (2014). Advances and challenges in biosensor-based diagnosis of infectious diseases. *Expert review of molecular diagnostics,* **14**, 225-244.

Taniuchi, M., Verweij, J. J., Noor, Z., Sobuz, S. U., Lieshout, L., Petri, W. A., Jr., Haque, R. and Houpt, E. R. (2011). High throughput multiplex PCR and probe-based detection with Luminex beads for seven intestinal parasites. *Am J Trop Med Hyg,* **84**, 332-337. doi: 10.4269/ajtmh.2011.10-0461.

ten Hove, R. J., Verweij, J. J., Vereecken, K., Polman, K., Dieye, L. and van Lieshout, L. (2008). Multiplex real-time PCR for the detection and quantification of *Schistosoma mansoni* and *S. haematobium* infection in stool samples collected in northern Senegal. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **102**, 179-185.

Toé, L., Boatin, B. A., Adjami, A., Back, C., Merriweather, A. and Unnasch, T. R. (1998). Detection of *Onchocerca volvulus* infection by O-150 polymerase chain reaction analysis of skin scratches. *Journal of Infectious Diseases,* **178**, 282-285.

Tomita, N., Mori, Y., Kanda, H. and Notomi, T. (2008). Loop-mediated isothermal amplification (LAMP) of gene sequences and simple visual detection of products. *Nature protocols,* **3**, 877.

Tong, Q. B., Chen, R., Zhang, Y., Yang, G. J., Kumagai, T., Furushima-Shimogawara, R., Lou, D., Yang, K., Wen, L. Y., Lu, S. H., Ohta, N. and Zhou, X. N. (2015). A new surveillance and response tool: risk map of infected *Oncomelania hupensis* detected by Loop-mediated isothermal amplification (LAMP) from pooled samples. *Acta Trop,* **141**, 170-177. doi:

10.1016/j.actatropica.2014.01.006.

Touron, A., Berthe, T., Pawlak, B. and Petit, F. (2005). Detection of Salmonella in environmental water and sediment by a nested-multiplex polymerase chain reaction assay. *Research in Microbiology,* **156**, 541-553.

Traub, R. J., Macaranas, J., Mungthin, M., Leelayoova, S., Cribb, T., Murrell, K. D. and Thompson, R. A. (2009). A new PCR-based approach indicates the range of *Clonorchis sinensis* now extends to Central Thailand. *PLoS Negl Trop Dis,* **3**, e367.

Verbarg, J., Plath, W. D., Shriver-Lake, L. C., Howell, P. B., Jr., Erickson, J. S., Golden, J. P. and Ligler, F. S. (2013). Catch and release: integrated system for multiplexed detection of bacteria. *Anal Chem,* **85**, 4944-4950. doi: 10.1021/ac303801v.

Verweij, J. J., Brienen, E. A., Ziem, J., Yelifari, L., Polderman, A. M. and Van Lieshout, L. (2007). Simultaneous detection and quantification of *Ancylostoma duodenale*, *Necator americanus*, and *Oesophagostomum bifurcum* in fecal samples using multiplex real-time PCR. *Am J Trop Med Hyg,* **77**, 685-690.

Verweij, J. J., Polderman, A. M., Wimmenhove, M. C. and Gasser, R. B. (2000). PCR assay for the specific amplification of *Oesophagostomum bifurcum* DNA from human faeces. *International journal for parasitology,* **30**, 137-142.

Verweij, J. J. and Stensvold, C. R. (2014). Molecular testing for clinical diagnosis and epidemiological investigations of intestinal parasitic infections. *Clinical microbiology reviews,* **27**, 371-418.

Vică, M. L., Matei, H. V. and Siserman, C. V. (2016). The Advantages of Using Multiplex PCR for the Simultaneous Detection of Six Sexually Transmitted Diseases. In *Polymerase Chain Reaction for Biomedical Applications* InTech.

Warren, K., Bundy, D. and Anderson, R. (2001). Helminth infection. Dalam: Jamison DT, Mosley WH, Measham AR, penyunting: Disease control priorities in developing countries. In *Disease Control Priorities in Developing Countries* (ed. Jamison DT, M. W., Measham AR, Bobadilla JL), pp. pp. 131–160. . New York: Oxford University Press for World Bank, 1993.

Watts, M. R., James, G., Sultana, Y., Ginn, A. N., Outhred, A. C., Kong, F., Verweij, J. J., Iredell, J. R., Chen, S. C. and Lee, R. (2014). A loop-mediated isothermal amplification (LAMP) assay for *Strongyloides stercoralis* in stool that uses a visual detection method with SYTO-82 fluorescent dye. *Am J Trop Med Hyg,* **90**, 306-311.

Weerakoon, K. G., Gordon, C. A., Cai, P., Gobert, G. N., Duke, M., Williams, G. M. and McManus, D. P. (2017a). A novel duplex ddPCR assay for the diagnosis of schistosomiasis japonica: proof of concept in an experimental mouse model. *Parasitology,* **144**, 1005-1015. doi: 10.1017/S003118201700021X.

Weerakoon, K. G., Gordon, C. A., Williams, G. M., Cai, P., Gobert, G. N., Olveda, R. M., Ross, A. G., Olveda, D. U. and McManus, D. P. (2017b). Droplet Digital PCR Diagnosis of Human Schistosomiasis: Parasite Cell-Free DNA Detection in Diverse Clinical Samples. *J Infect Dis,* **216**, 1611-1622. doi: 10.1093/infdis/jix521.

Weerakoon, K. G. and McManus, D. P. (2016). Cell-free DNA as a diagnostic tool for human parasitic infections. *Trends in parasitology,* **32**, 378-391.

WHO (2019a). World Health Organisation. Soil-transmitted helminth infections fact sheet. Avaibale from: https://www.who.int/en/news-room/fact-sheets/detail/soil-transmitted-helminth-infections.

WHO (2019b). World Health Organization. Schistossomiasis fact-sheets. Available from: http://www.who.int/news-room/fact-sheets/detail/schistosomiasis.

Wicht, B., Yanagida, T., Scholz, T., Ito, A., Jiménez, J. A. and Brabec, J. (2010). Multiplex PCR for differential identification of broad tapeworms (Cestoda: *Diphyllobothrium*) infecting humans. *Journal of clinical microbiology,* **48**, 3111-3116.

Wong, S. S. Y., Poon, R. W. S., To, K. K. W., Chan, J. F. W., Lu, G., Xing, F., Cheng, V. C. C. and Yuen, K. Y. (2019). Improving the specific diagnosis of trematode, cestode and nematode infections by a multiplex single-tube real-time PCR assay. *J Clin Pathol,* **72**, 487-492. doi: 10.1136/jclinpath-2018-205590.

Wongkamchai, S., Nochote, H., Foongladda, S., Dekumyoy, P., Thammapalo, S., Boitano, J. J. and Choochote, W. (2014). A high resolution melting real time PCR for mapping of filaria infection in domestic cats living in brugian filariosis-endemic areas. *Vet Parasitol,* **201**, 120-127. doi: 10.1016/j.vetpar.2013.12.011.

Xia, C.-M., Rong, R., Lu, Z.-X., Shi, C.-J., Xu, J., Zhang, H.-Q., Gong, W. and Luo, W. (2009). *Schistosoma japonicum*: a PCR assay for the early detection and evaluation of treatment in a rabbit model. *Experimental parasitology,* **121**, 175-179.

Ximenes, C., Brandao, E., Oliveira, P., Rocha, A., Rego, T., Medeiros, R., Aguiar-Santos, A., Ferraz, J., Reis, C., Araujo, P., Carvalho, L. and Melo, F. L. (2014). Detection of *Wuchereria bancrofti* DNA in paired serum and urine samples using polymerase chain reaction-based systems. *Mem Inst Oswaldo Cruz,* **109**, 978-983. doi: 10.1590/0074-0276140155.

Xing, W., Yu, X., Feng, J., Sun, K., Fu, W., Wang, Y., Zou, M., Xia, W., Luo, Z., He, H., Li, Y. and Xu, D. (2017). Field evaluation of a recombinase polymerase amplification assay for the diagnosis of *Schistosoma japonicum* infection in Hunan province of China. *BMC Infect Dis,* **17**, 164. doi: 10.1186/s12879-017-2182-6.

Xu, J., Duan, Z. L., Guan, Z. X., Wang, Y. Y., Lin, C., Zhang, T. T., Zhang, H. Q., Qian, X. and Xia, C. M. (2017). Early detection of circulating DNA of *Schistosoma japonicum* in sentinel mice models. *Experimental parasitology,* **176**, 82-88.

Xu, J., Guan, Z. X., Zhao, B., Wang, Y. Y., Cao, Y., Zhang, H. Q., Zhu, X. Q., He, Y. K. and Xia, C. M. (2015). DNA detection of *Schistosoma japonicum*: diagnostic validity of a LAMP assay for low-intensity infection and effects of chemotherapy in humans. *PLoS Negl Trop Dis,* **9**, e0003668. doi: 10.1371/journal.pntd.0003668.

Xu, J., Rong, R., Zhang, H., Shi, C., Zhu, X. and Xia, C. (2010). Sensitive and rapid detection of *Schistosoma japonicum* DNA by loop-mediated isothermal amplification (LAMP). *International journal for parasitology,* **40**, 327-331.

Zhong, M., McCarthy, J., Bierwert, L., Lizotte-Waniewski, M., Chanteau, S., Nutman, T. B., Ottesen, E. A. and Williams, S. A. (1996). A polymerase chain reaction assay for detection of the parasite *Wuchereria bancrofti* in human blood samples. *Am J Trop Med Hyg,* **54**, 357-363. doi: 10.4269/ajtmh.1996.54.357.

Table 1 Nucleic acid-based methods for the detection of helminth infections

Pathogens	Target genes	Detection methods	Types of sample	References
S. japonicum	*SjR2*	PCR	Feces	(Fung *et al.*, 2012)
S. mansoni	121-bp tandem repeat sequence	PCR	Sera	(Hussein *et al.*, 2012)
S. mansoni *S. haematobium*	*28S rDNA*	PCR	Urine	(Sandoval *et al.*, 2006)
S. haematobium	*Dra* 1	PCR	Urine	(Ibironke *et al.*, 2011; Lodh *et al.*, 2014)
S. japonicum	*COX1*	PCR	Sera and urine	(Kato-Hayashi *et al.*, 2015)
S. japonicum	Repetitive retrotransposon SjCHGCS19	PCR	Serum	(Xu *et al.*, 2017)
T. saginata *T. solium*	*HDP2*	qPCR	Feces	(Flores *et al.*, 2018)
W. bancrofti	*LDR*	qPCR	Blood	(Plichart & Lemoine, 2013)
A. lumbricoides *A. duodenale* *N. americanus* *S. stercoralis* *T. trichiur*	*ITS1, ITS2*	qPCR	Feces	(Mejia *et al.*, 2013)
S. japonicum	*NAD6* and *NAD1*	PCR and qPCR	Feces	(Gordon *et al.*, 2012)
S. mansoni *S. haematobium*	*ITS2*	qPCR	Feces	(Meurs *et al.*, 2015)
N. americanus *A. duodenale* *A. ceylanicum* *A. caninum* *A. braziliense*	*ITS2*	qPCR	Feces	(Ngui *et al.*, 2012)
T. trichiura *A. lumbricoides*	*18S rDNA* and *ITS1*	qPCR	Feces	(Liu *et al.*, 2013)
A. lumbricoides *A. duodenale* *N. americanus* *S. stercoralis*	*ITS1, ITS2* and 18S rDNA	Multiplex qPCR	Feces	(Basuni *et al.*, 2011)
T. solium *T. saginata*	*COX1*	Multiplex PCR	Feces	(Jeon *et al.*, 2011)
S. japonicum *S. mansoni*	*NAD1, 2, 6* and *COX2* *NAD5, 6* and *COX2*	Multiplex PCR	Feces	(Gobert *et al.*, 2005)
F. hepatica *A. tomentosa*	*ITS-2*	Multiplex PCR	Water	(Rathinasamy *et al.*, 2018)
Trichinella Spp.	*ITS1* and *ITS2*	Multiplex qPCR	Meat	(Almeida et al., 2018)
W. bancrofti	Repeated DNA sequence	Semi Nested PCR	Urine and sera	(Ximenes *et al.*, 2014)

W. bancrofti	*Long direct repeat element (LDR)*	ddPCR	Blood, vectors	(Jongthawin *et al.*, 2016)
B. malayi	HhaI element	ddPCR	Blood, vectors	(Jongthawin *et al.*, 2016)
S. japonicum	*NAD 1* and *SjR2*	ddPCR	Urine, feces, salivary gland, and serum	(Cai *et al.*, 2019; Weerakoon *et al.*, 2017a; Weerakoon *et al.*, 2017b)
H. contortus, T. circumcincta and Trichostrongylus spp.	*Internal transcribed spacer region 2*	ddPCR	Parasite DNA	(Elmahalawy *et al.*, 2018)
T. solium	*HDP2*	Semi nested PCR	Cerebrospinal fluid	(Hernández *et al.*, 2008)
S. japonicum	*SjR2*	LAMP	Serum	(Xu *et al.*, 2010)
S. mansoni	Mitochondrial minisatellite DNA	LAMP	Feces	(Fernández-Soto *et al.*, 2014)
S. stercoralis	*28S rDNA*	LAMP	Feces	(Watts *et al.*, 2014)
C. sinensis	*COX1*	LAMP	Feces	(Rahman *et al.*, 2017)
T. trichiura N. americanus A. lumbricoides	*β-tubulin*	LAMP	Feces	(Rashwan *et al.*, 2017)
T. canis	*ITS2*	LAMP	Feces	(Khoshakhlagh *et al.*, 2017)
E. multilocularis	*COX1*	LAMP	Feces	(Feng *et al.*, 2017)
N. americanus	*ITS2*	LAMP	Feces	(Mugambi *et al.*, 2015)
B. malayi B. timori	*Hha* I	LAMP	Blood	(Poole *et al.*, 2012)
P. westermani	*ITS2*	LAMP	Sputum and pleural fluid	(Chen *et al.*, 2011)
S. haematobium	*Dra* 1	RPA	Urine	(Rosser *et al.*, 2015)
S. mansoni	*28S rDNA* and *ITS*	RPA	Genomic DNA	(Poulton & Webster, 2018)
S. japonicum	*Sj*Ab	Immunosensor	Sera	(Nie *et al.*, 2012)
S. mansoni	*Genomic DNA*	Genosensor	Urine, cerebrospinal fluid, and serum.	(Santos *et al.,* 2019)
S. mansoni	*Genomic DNA*	Genosensor	Cerebrospinal fluid and sera	(Santos *et al.*, 2017)
S. japonicum	*S. japonicum* circulating antigens	Immunosensor	Sera	(Cheng *et al.*, 2008)
W. bancrofti	*W. bancrofti epitope antigens*	Immunosensor	Sera	(Prado *et al.*, 2018)

Table 2 Summary of advantages and limitations of each nucleic acid-based method

Techniques	Advantages	Limitations	References
PCR	Relatively high specificity and sensitivity; can detect small amounts of target genes; lower turnaround time; relatively high throughput; high dynamic range of detection.	Relatively expensive instruments are required, risk of false negatives in pathogen detection.	(Agarwal *et al.*, 2014; Diguta *et al.*, 2010)
Multiplex PCR	High sensitivity and specificity; can detect multiple genes simultaneously with relatively high-throughput.	Primer design is critical; the primers may interfere with each other during amplification.	(Hajia *et al.*, 2014; Vică *et al.*, 2016)
ddPCR	High sensitivity and specificity; accurate and absolute quantification of the target without using reference materials with known target concentrations; low copy detection for target	Expensive instruments are required.	(Gutierrez-Aguirre *et al.*, 2015)
LAMP	Rapid, highly efficient, and cost-effective; denaturation step not required; more rapid than other conventional PCR-based methods; can directly visualize results; less equipment.	Primer design is critical for LAMP assay; risk of false-positive results.	(Drapala & Kordalewska, 2013; Hsieh *et al.*, 2014; Tomita *et al.*, 2008)
RPA	Cost-effective, highly sensitive and specific, short amplification time. Takes place at the ambient temperature without the need for an initial denaturation step or the use of multiple primers.	Risk of false-positive outcomes.	(Daher *et al.*, 2016; Shahin *et al.*, 2018)
Biosensors	High sensitivity and reliability; fast detection; reduction in reagent consumption; detection of small molecules, proteins, and nucleic acid.	Not commercially available for most pathogens, further research required for developing the system.	(Janissen *et al.*, 2017; Luz *et al.*, 2016; Sin *et al.*, 2014)