**AASCIT Journal of Health**
2015; 2(4): 32-43
Published online July 30, 2015 (http://www.aascit.org/journal/health)




**Keywords**
Parasitic Diseases,
Microscopy,
Polymerase Chain Reaction,
Loop-Mediated Isothermal Amplification (Lamp),
Luminex xMAP Technology

Received: July 11, 2015
Revised: July 17, 2015
Accepted: July 18, 2015

# Emerging Molecular Methods for the Diagnosis and Epidemiological Study of Parasitic Infections

**Ukpai Agwu Eze[1, *], Ngozi Monica Eze[2]**

[1]Division of Molecular and Medical Microbiology, Parasitology and Infectious Diseases, Department of Medical Laboratory Sciences, Faculty of Health Sciences, Ebonyi State University, Abakaliki, Nigeria
[2]Directorate of Nursing Services, Ebonyi State Ministry of Health, Abakaliki, Ebonyi State, Nigeria

## Email address
ukpai.eze@ebsu-edu.net (U. A. Eze)

## Citation
Ukpai Agwu Eze, Ngozi Monica Eze. Emerging Molecular Methods for the Diagnosis and Epidemiological Study of Parasitic Infections. *AASCIT Journal of Health.* Vol. 2, No. 4, 2015, pp. 32-43.

## Abstract
Parasitic diseases constitute major public health challenges worldwide, especially in developing countries. It is estimated that more than one billion people worldwide are infected by parasites presenting with different disease conditions. The routine laboratory diagnosis of parasitic infections in most tropical countries involves conventional methods, such as optical microscopy, used for the morphological identification of parasites. Although the development and adaptation of new technologies for the genetic characterisation and identification of parasites continue to accelerate, providing an increasing number of epidemiological research and diagnostic tools in developed countries, little or none is available in most developing countries. This review examines the main current and new diagnostic techniques for confirmation of parasite infections, namely polymerase chain reaction (PCR), real-time polymerase chain reaction (RT-PCR), loop-mediated isothermal amplification (LAMP) and Luminex xMAP technology. Molecular assays have comprehensively assisted in the diagnosis, treatment and epidemiological studies of parasitic diseases that affect people worldwide, helping to control parasitic disease mortality.

## 1. Introduction

Parasitic diseases constitute major public health challenges worldwide. It is estimated that more than one billion people worldwide are infected by parasites presenting with different disease conditions (WHO, 2000). Parasitic diseases are closely related to geographic, social and economic factors driving the prevalence and incidence of these pathologies (WHO, 2010). Parasites are the causative agents of health disorders such as malaria, schistosomiasis and trypanosomiasis. In 2008, there were 247 million cases of malaria and nearly one million deaths from the disease, mostly among children living in Africa. In Africa, a child dies of malaria every 45 seconds; the disease accounts for 20% of all childhood deaths (WHO, 2010). Leishmaniasis threatens approximately 350 million men, women and children in 88 countries around the world. As many as 12 million people are believed to be currently infected by this disease, with approximately 1–2 million estimated new cases occurring every year. Additionally, an estimated of 10 million people are infected worldwide by Chagas disease (American trypanosomiasis), mostly in Latin America, where Chagas disease is endemic. More than 25 million people are at risk of acquiring this disease. It is estimated that in 2008, Chagas disease killed more than 10,000 people (Morrison, 2011). Similarly, Schistosomiasis is a chronic, parasitic disease caused

by blood flukes (trematode worms) of the genus *Schistosoma*. More than 207 million people are infected with these organisms worldwide, with an estimated 700 million people at risk in 74 endemic countries (Croft *et al*., 2003).

Lymphatic filariasis (caused by *W. bancrofti* and *Brugia malayi*) affects more than 1.3 million people in 81 countries. Approximately 65% of those infected live in Southeast Asia, 30% in Africa and the remainder in other tropical areas. Lymphatic filariasis afflicts over 25 million men with genital disease and over 15 million people with lymphoedema. Due to the prevalence and intensity of parasitic infection are linked to poverty, early diagnosis can result to elimination thereby contributing to achieving the United Nations Millennium Development Goals. Human African Trypanosomiasis (HAT) affects mostly poor populations living in remote rural areas of Africa. If untreated, it is usually fatal. Travellers also risk becoming infected if they venture through regions where the insect vector (tse tse flies) is common (WHO, 2010). It is estimated that 3 billion people worldwide are infected by intestinal parasites (WHO, 2008). Soil transmitted helminthes are responsible for 39.0 Million disability-adjusted life years (DALY) lost in sub-saharan Africa. In Nigeria, the prevalence of intestinal parasites ranges from 12-60%; rural areas having the highest prevalence. The high prevalence is as a result of several factors including lack of proper sanitation, indiscriminate defecation, polluted water, overcrowding and climate suitability for parasite development and transmission. Infestation causes a threat to the growth and development of the child. It causes imbalance in nutritional equilibrium, anaemia, growth retardation and impaired cognitive development (Chirdan *et al*., 2010).

In parasitology, the routine conventional diagnostic methods are microscopy (Figures 1, 2, 3, 4, 5 and 6) and serology. Microscopy remains the "gold standard" for diagnosis of parasites and indeed it is simple, can be rapid and does not involve the purchase and maintenance of expensive equipment. However, various problems are associated with microscopy as a diagnostic tool. For example, some parasites are morphologically similar or are very small and difficult to stain and detect. Consequently, results depend on the quality of staining and the microscope, the technique used for specimen film preparation, and the technical expertise of the microscopist is essential for accurate identification, and this holds true for many parasites. Furthermore, culture of parasites may be necessary as in the case of chronic *Trypanosoma cruzi* infections, where the numbers of blood trypomastigotes are low. Specialised media and laboratory facilities are required for culture and these are generally not available in countries where these infections are endemic. Besides, there is a relatively long period before results are obtained. Diagnosis by microscopy is also extremely labour intensive, especially when a large number of samples need to be screened in a relatively short time, such as during emergency cases and epidemiological studies.

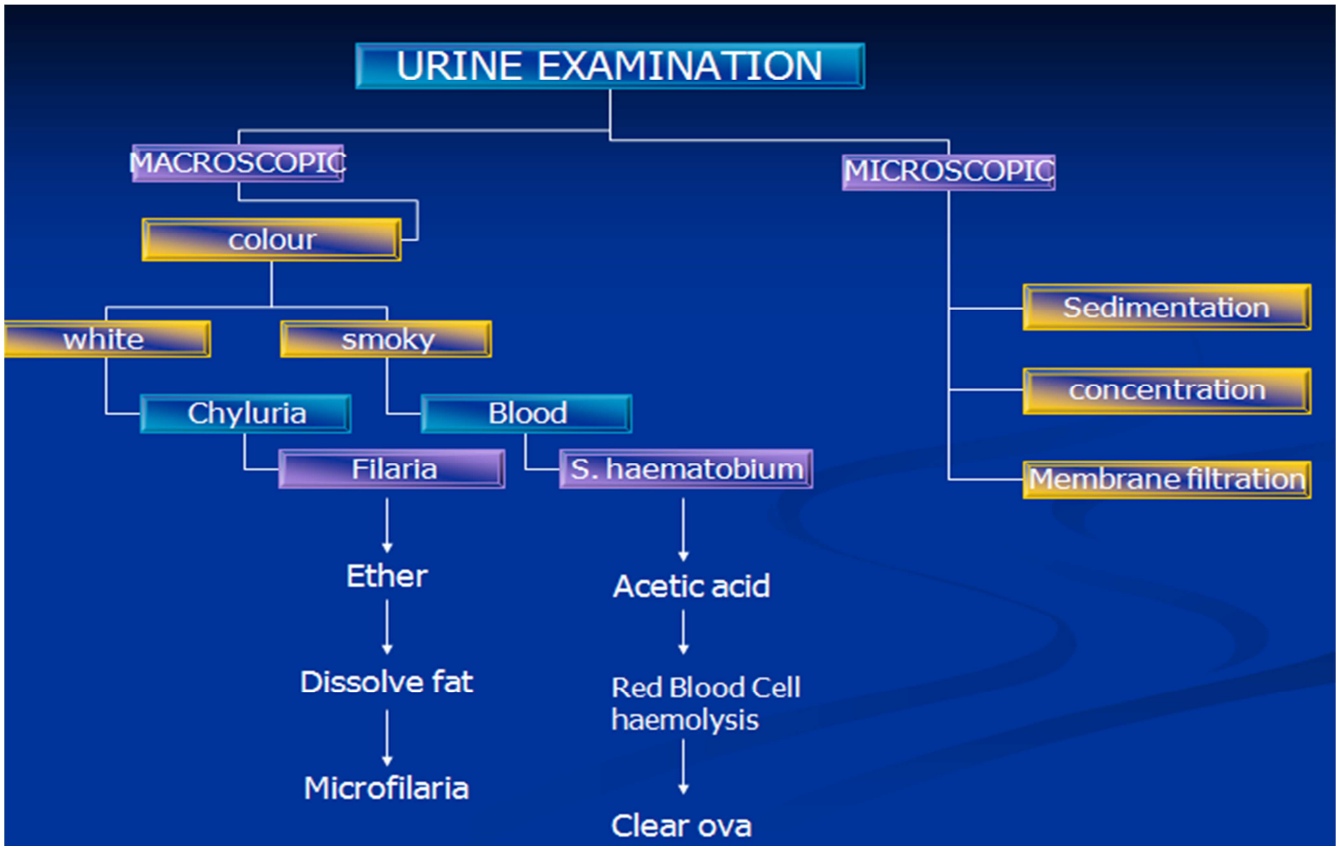


***Figure 1.*** *Flowchart for urine examination for parasite using conventional methods.*

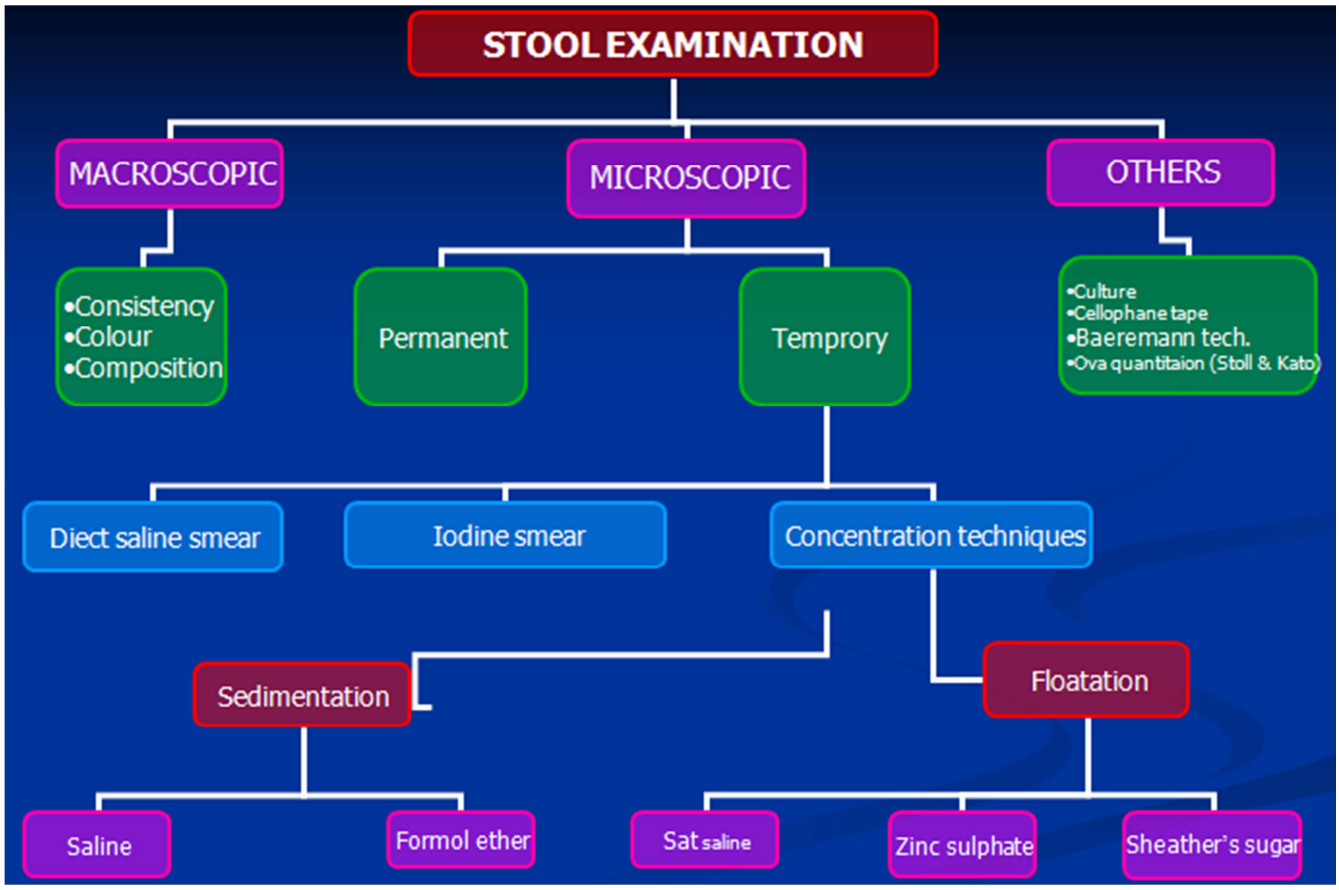


***Figure 2.*** *Flowchart for stool examination for parasites using conventional methods.*

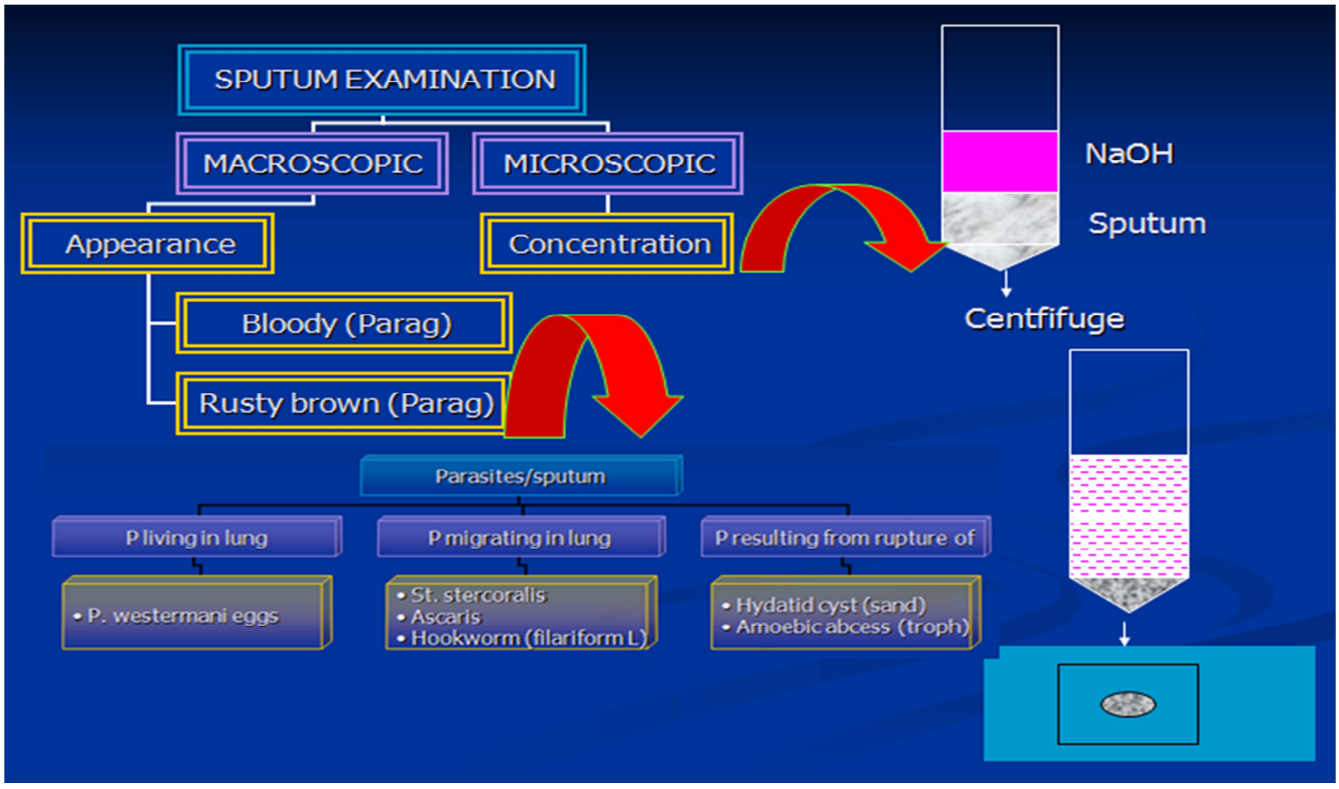


***Figure 3.*** *Flowchart for sputum examination for parasites using conventional methods.*

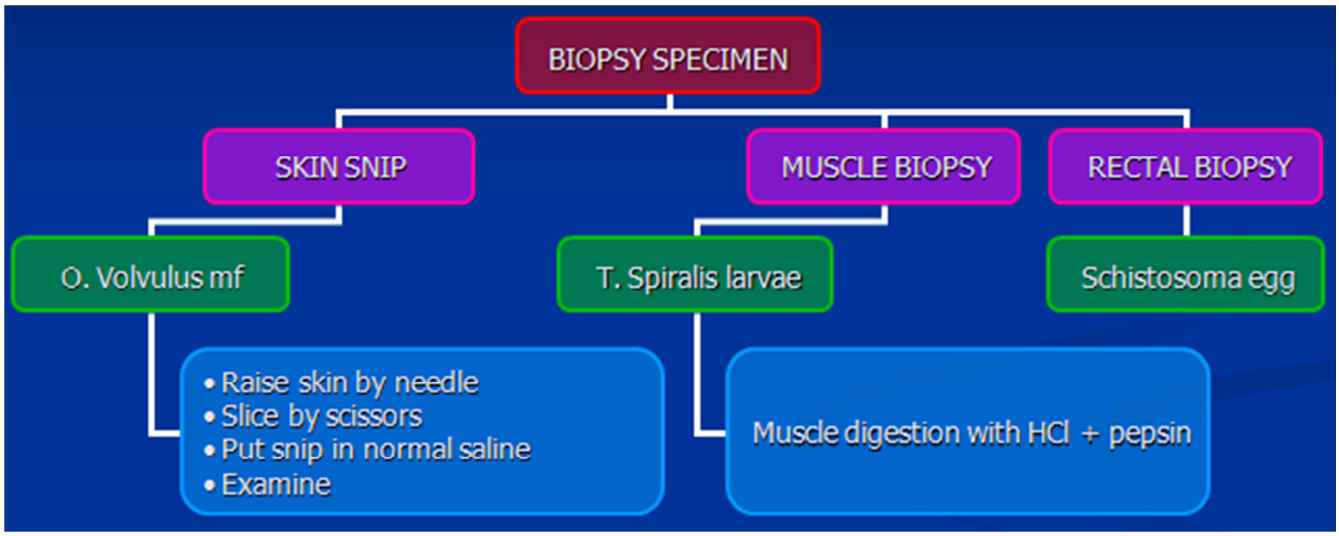


***Figure 4.*** *Flowchart for biopsy specimens examination for parasites using conventional methods.*

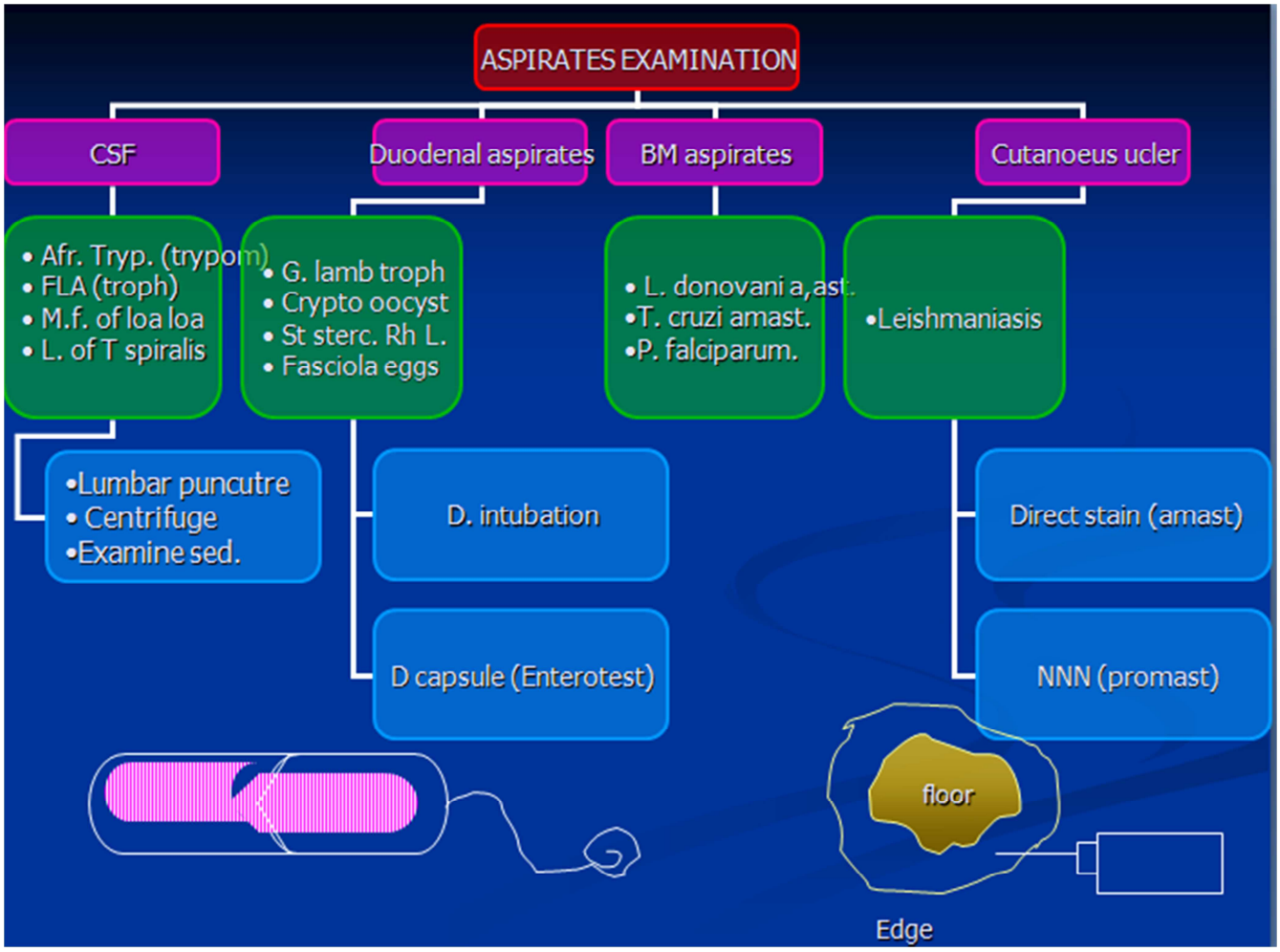


***Figure 5.*** *Flowchart for aspirates examination for parasites using conventional methods.*

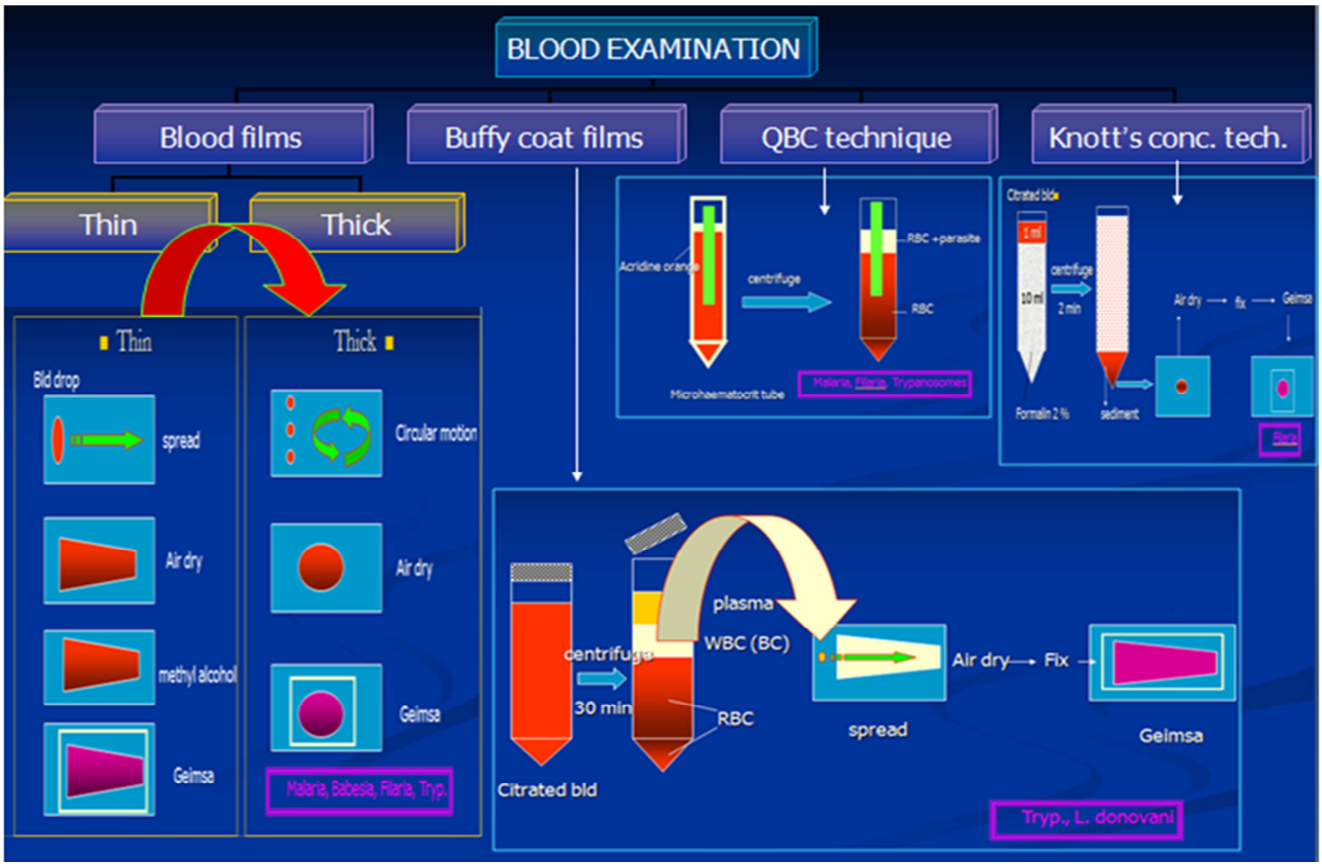


***Figure 6.*** *Flowchart for aspirates examination for parasites using conventional methods.*

In order to overcome some of the difficulties encountered using microscopy for parasite diagnosis, serological diagnostic methods have been developed. However, these methods have problems of their own. For example, it is difficult to differentiate between a current and previous parasite infection, and serological tests are of limited value when examining individuals from endemic areas with high circulating antibodies. In addition, the cost of producing specific purified antigens is generally very high and consequently crude antigen preparations are often used, resulting in reduced specificity and sensitivity.

Currently, molecular methods such as polymerase chain reaction (PCR), real-time polymerase chain reaction (RT-PCR), loop-mediated isothermal amplification (LAMP), Luminex xMAP, random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and restriction fragment length polymorphism (RFLP) are applied in the diagnosis of parasitic infections in developed countries while they are not easily available in developing countries. Molecular or DNA-based methods are developed for parasite detection to address some of the problems encountered using conventional methods such as microscopy for parasite diagnosis. Performance and rapid turn-around time are important aspects of any test, and clinicians and patients require a test that can produce a result rapidly and molecular diagnostic methods provides this platform. This paper is aimed at presenting, in simple terms, current molecular methods which have been developed for diagnosis and epidemiological studies of parasitic infections.

## 2. Molecular Methods for Diagnosis of Parasitic Infections

Several molecular tests to detect parasites have been developed in the last decade (Table 1). Their specificity and sensitivity have gradually increased, and parasites which were previously difficult to diagnose using traditional methods began to be identified by molecular techniques. Molecular assays have comprehensively assisted in the diagnosis, treatment and epidemiological studies of parasitic diseases that affect people worldwide, helping to control parasitic disease mortality. They have also been employed in the study of parasites in animal models, drug efficacy monitoring and vectorial capacity (Talmi-Frank et al., 2010).

### 2.1. Conventional Polymerase Chain Reaction

Conventional PCR involves the amplification of a specific target DNA sequence using a primer pair (forward and reverse) and the DNA product amplified is called the amplicon (Gordon *et al*., 2011). Due to the presence of multiple copies within each cell, mitochondrial DNA sequences can be targeted for the PCR diagnosis of schistosome eggs in stool samples; these sequences are sufficiently sensitive to detect the presence of a single egg (Gobert *et al*., 2005). Several studies have shown that conventional PCR is a highly

sensitive method for the detection of the blood flukes *Schistosoma japonicum* and *Schistosoma mansoni* in faeces (ALdV *et al*., 2006; Pontes *et al*., 2002; Pontes *et al*., 2003; Gobert *et al*., 2005; Lier *et al*., 2006; Lier *et al*., 2008) and serum (Pontes *et al*., 2002; Suzuki *et al*., 2006). Lier *et al*., (2008) in their studies compared a PCR based assay with a combined filtration and sedimentation method, and demonstrated a superior sensitivity of PCR for the detection of *S. japonicum* eggs. The ability of PCR to detect low amounts of parasite DNA was also assessed by Oliveira *et al*., (2010) who examined human faeces for the presence of *S. mansoni* eggs in humans with low intensities of infection. Three samples, with an intensity of less than 10 eggs per gram, were PCR-negative; however, 59% (13/22) of patients that were sero-positive for circulating antibodies and test-negative by microscopy, were test positive by PCR.

Conventional PCR has also been used to investigate the geographical range of *Opisthorchis viverrini* and *Clonorchis sinensis* in Southeast Asia (Traub *et al*., 2009). The eggs and metacercariae of these two species are morphologically similar to other fish-borne trematodes which do not infect humans, and the correct identification is both clinically and epidemiologically important. A conventional PCR targeting a segment of the internal transcribed spacer (ITS) region was able to differentiate between *O. viverrini* and *C. sinensis* and it demonstrated *C. sinensis* in an area in central Thailand where it previously had not been found, thereby extending the known endemic range of this liver fluke (Traub *et al*., 2009). *Echinococcus granulosus* and *Echinococcus multilocularis* have also been differentiated with conventional PCR (Shahnazi *et al*., 2011). Identifying and distinguishing between eggs of Echinococcus in dog (*E. granulosus*) and fox (*E. multilocularis*) as well as differentiating among different genotypes of these species and other related tapeworm is important for determining disease transmission routes to humans. This PCR approach does require the actual presence of eggs, and furthermore, the heterogeneous mixing of schistosome eggs in stool, the day-to-day fluctuations in egg output and the reduction in eggs released during a chronic infection, can present additional limitations for successful PCR-based detection, resulting in false negative results. Interestingly, PCR assays have also been described for the detection of *P. westermani* in infected freshwater crabs or crayfishes and have significantly increased the detection of *P. westermani* infection (Devi *et al*., 2010).

Malaria is a major challenge in tropical countries and PCR has a high throughput in the identification of mixed infections which may be underestimated using the traditional microscopic techniques (Tavares et al., 2011). However, the method is time-consuming and do not provide quantitative data (Lin et al., 2000).

## 2.2. Multiplex Polymerase Chain Reaction

Multiplex PCR allows the amplification of more than one target of interest in a PCR by using multiple primer pairs and producing amplicons of different sizes (Gordon *et al*., 2011). The design and selection of the multiple primer pairs can make the reaction specific for the target organism. This method was first employed to detect deletions in the Duchenne Muscular Dystrophy gene (Chamberlain *et al*., 1988). It has since been used in many applications, including the detection of multiple parasite species in individual samples from stool, serum, saliva or other environmental sources (Gordon *et al*., 2011). Multiplex PCR has been employed for microsatellite analysis and the 'genotyping' of viruses, bacteria and parasites. Multiplex PCR assays have been developed for the differential diagnosis of *Diphyllobothrium* species (Wicht *et al*., 2010),*T. asiatica*, *T. saginata*, and *T. solium* (Gottstein et al., 1991) and for distinguishing *T. saginata* and *T. solium* and *E. multilocularis* from other taeniids (Gordon *et al*., 2011). In addition, Le *et al*., (2006) developed a similar mPCR assay, targeting mitochondrial DNA sequences, that could effectively discriminate between *O. viverrini* and *C. sinensis*.

## 2.3. Real Time PCR (qPCR)

Real-time PCR (qPCR) allows the quantification of a PCR product (amplicon) by measuring fluorescence during the reaction as it occurs, that is "in real time" (Gordon *et al*., 2011). The fluorescence is achieved using Sybergreen, Taqman probes, fluorescence resonance energy transfer (FRET), and Scorpion primers (Muldrew, 2009). The qPCR output represents a graph, showing cycle numbers plotted against an increasing fluorescent signal, reducing the time- and labour needed to run an amplified product on a gel following PCR. Samples containing a higher amount of DNA show an increased fluorescence earlier in PCR than those with a smaller amount (Gordon *et al*., 2011). In addition to being able to quantify results, qPCR has a number of other advantages over conventional PCR. It is an automated process and thus reduces labour and time; results are saved to a computer hard-drive and can be viewed at any time, with no need to run an electrophoretic gel which often takes at least 40 min. Furthermore, the qPCR allows infection intensity to be estimated, thus potentially making it an efficient replacement for microscopy (Gomes et al., 2006).

Using the real-time PCR assay, Seung-Young *et al*., (2011) correctly diagnosed malaria in 32 clinical samples that had been determined to be negative by Gimesa-stained blood smear microscopy. Of 112 clinically suspected cases of malaria, only 80 (71.4%) were diagnosed with malaria based on microscopy, failing to detect 32 malaria infections. By contrast, qPCR diagnosed all of the 112 samples as malaria. Therefore, qPCR assay may have potential applications in detecting malaria parasites in asymptomatic infections; evaluating candidate malaria vaccines; screening blood donors, especially in endemic areas, and monitoring malaria treatment. Similarly, Calderaro *et al*., (2010) evaluated real time polymerase reaction in detecting *Giardia intestinalis* infection in comparison to conventional methods (microscopy and antigen detection assay) and reported that real-time PCR assay detected *Giardia intestinalis* DNA in 195 samples (106 patients), including 26 samples (21 patients) negative by the conventional assays.

Multiplex qPCR assays have also been developed for the

differentiation of genotypes of *E. granulosus* using different fluorophores (ten Hove *et al*., 2009), and for the detection and quantification of *S. mansoni* and *S. haematobium* infection in human stool samples (ten Hove *et al*., 2008). A multiplex qPCR has also been developed for the differential diagnosis of *Ancylostoma duodenale*, *Necator americanus* and *Oesophagostomum bifurcum* infections in humans (Verweij *et al*., 2007). Multiplexed RT-PCR was able to identify 4 species of *Plasmodium* (*P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*) that cause infections in humans in a single reaction and interestingly, even slightly infected samples were also identified (Shokoples et al., 2009). This method reduced the cost per test, increased the sensitivity for the detection of simple infections and also provided a rapid platform as results were generated in 3 hours comparable to traditional methods. Recently, Pilotte *et al*., (2013) described a multiplex, TaqMan-based, real-time PCR assay capable of simultaneously detecting *W. bancrofti* and *Brugia malayi* DNA extracted from human bloodspots or vector mosquito pools by amplifying Long DNA Repeat (LDR) element and HhaI Repeat element respectively. This is useful in programs tasked with monitoring infection levels, or conducting surveillance in locations co-endemic for Bancroftian and Brugian filariasis. Similarly, quantitative real-time PCR (Q-PCR) is reported as a rapid and accurate method for diagnosis of *Toxoplasma gondii* (Kompalic-Cristo *et al*., 2007), *Trypanosoma cruzi* (Piron *et al*., 2007), *Plasmodium spp*. (Perandin *et al*., 2004), and *Leishmania spp*. (Aoun *et al*., 2009; Kumar *et al*., 2009; Mary *et al*., 2004; Verma *et al*.,2010).

*Toxoplasma gondii*, *Trypanosoma cruzi* and *Plasmodium spp*. are the major parasites associated with congenital infections in humans. These infections can have severe outcomes by compromising fetal/neonatal growth. Congenital infections with *T. gondii* and *T. cruzi* parasites can also lead to serious chronic infections later in adult life. In contrast, the sequestration of *Plasmodium falciparum*-infected erythrocytes in the intervillous spaces of the placenta together with localized inflammatory responses, sequestered parasites induce an impairment of placental transport resulting in stillbirths, perinatal mortality, low birth weight, and premature delivery (Carlier *et al*., 2012). Report have shown that PCR can detect 64–100% of fetal/neonatal toxoplasmosis cases using amniotic fluids and placental biopsies (Wallon *et al*., 2010) while Sterkers *et al*., (2011) in their study in France used PCR to establish the diagnosis of 5 (83%) of the 6 cases of congenital Toxoplasmosis using neonatal peripheral blood, thereby allowing for early treatment of such infection and reducing the adverse effects associated with parasite.

RT-PCR is very useful in quantifying parasitic nucleic acids from environmental samples or tissues as well as in estimating the intensity of infection and/or viability of parasites (Gasser, 2006) and can be employed in monitoring antimalarial therapy (Rougemont et al., 2004). The rapid results turnover and high throughput of this method warrant its use as a tool for the diagnosis of parasitic diseases. However, the high cost of RT-PCR makes this technique difficult to be applied routinely in resource poor settings.

***Table 1.*** *PCR-based and other DNA amplification techniques applied for diagnosis of human parasitic infection (■ means the method can be applied in diagnosis). Source: Adapted from Gordon et al., 2011).*

| Species | Copro -PCR | Tissue PCR | Blood/ Serum PCR | Conventional PCR | Nested PCR | Multiplex PCR | Real-time PCR | Multiplex real-time PCR | RFLP PCR | DNA probes | LAMP |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Trematoda | | | | | | | | | | | |
| *Schistosoma mansoni* | ■ | | ■ | ■ | ■ | ■ | ■ | ■ | | ■ | ■ |
| *Schistosoma japanicum* | ■ | | ■ | ■ | ■ | | ■ | ■ | ■ | ■ | ■ |
| *Schistosoma haematobium* | | ■ | | ■ | | | ■ | ■ | ■ | ■ | ■ |
| *Fasciola hepatica* | | ■ | | ■ | | ■ | ■ | | ■ | | ■ |
| *Fasciola gigantica* | | ■ | | ■ | | ■ | ■ | | ■ | | ■ |
| *Opisthorchis viverrini* | ■ | | | ■ | | ■ | ■ | | | | |
| *Clonorchis sinensis* | | | | ■ | ■ | ■ | ■ | | | | ■ |
| Cestoda | | | | | | | | | | | |
| *Taenia saginata* | ■ | | | ■ | | ■ | | | ■ | ■ | ■ |
| *Taenia solium* | ■ | | ■ | ■ | ■ | ■ | | | ■ | ■ | ■ |
| *Taenia asiatica* | ■ | | | ■ | | ■ | | ■ | | | ■ |
| *Echinococcus granulosus* | ■ | ■ | | ■ | ■ | ■ | ■ | | ■ | ■ | |
| *Echinococcus multicularis* | ■ | ■ | ■ | ■ | ■ | ■ | | | ■ | ■ | |
| Nematodes | | | | | | | | | | | |
| *Trichuris trichiura* | | | | ■ | | ■ | | | | | |
| *Ancylostoma caninum* | ■ | | | ■ | | | ■ | | ■ | ■ | |
| *Ancylostoma duodenale* | ■ | | | ■ | | ■ | ■ | ■ | | ■ | |
| *Necator americanus* | ■ | | | ■ | | ■ | ■ | ■ | | ■ | |
| *Onchocerca volvulus* | | | | ■ | ■ | | ■ | | | | |
| *Wuchereria bancrofti* | | | ■ | ■ | ■ | ■ | ■ | | ■ | | |
| *Brugia malayi* | | | | ■ | | | ■ | | ■ | | |
| *Toxocara canis* | ■ | | | ■ | | | | | | | ■ |
| *Toxocara cati* | ■ | | | ■ | | | | | | | ■ |
| *Ascaris lumbricoides* | ■ | | | ■ | | ■ | | ■ | | | |
| *Strongyloides stercoralis* | ■ | | | ■ | | ■ | ■ | ■ | | | |

## 2.4. Loop Mediated Isothermal Amplification (LAMP)

Loop mediated isothermal amplification (LAMP) is a novel nucleic acid amplification method developed by Notomi *et al*., (2000). This assay can amplify target deoxyribunucleic acid (DNA) to a quantity as high as $10^9$ copies in less than 1 h under isothermal conditions, and no thermocycler is needed (Notomi *et al*., 2000; Nagamine *et al*., 2002). In addition, the amplification product can be visually detected with the addition of fluorescent dyes such as SYBR Green I which allows a positive reaction to appear green or remain orange in a negative reaction (Poon *et al*., 2006). Four LAMP primers (B3, F3, BIP, and FIP) are designed to recognize six distinct regions on the target gene (B1, B2c, B3, F1c, F2, and F3) and this ensures the specific amplification of the target DNA (Chen *et al*., 2011). The method consists of incubating a mixture of a target gene, 4 different primers, *Bacillus stearothermophilus* (*Bst*) DNA polymerase, and substrates for 1 h at 60–65 °C, using basic equipment such as a heat block or water bath. Shorter reaction time with visual judgment of positivity without requiring sophisticated equipment makes it an attractive diagnostic method for field application. Furthermore, the sensitivity of LAMP is less affected by contamination with inhibitory components in DNA samples than that of conventional PCR (Kaneko *et al*., 2007). In addition, the *Bst* DNA polymerase acts at a relatively high temperature, which helps to reduce nonspecific priming. Moreover, this DNA polymerase is also more resistant to inhibitors than *Taq* DNA polymerase (Poon *et al*., 2006).

This method has been developed successfully for the diagnosis of parasitic infections such as schistosomiasis, malaria, trypanosomiasis, Paragonimiasis and clonorchiasis (Poon *et al*., 2006; Cai *et al*., 2010; Chen et al., 2011), *Toxoplasma gondii* (Sotiriadou and Karanis *et al*., 2008; Zhang *et al*., 2009; Lin *et al*., 2012), and *Cryptosporidium parvum* oocysts (Karanis *et al*., 2007; Bakheit *et al*., 2008). Schistosomiasis is a serious global public health problem affecting more than 200 million people. The WHO identified schistosomiasis as the second most important human parasitic disease in the world, after malaria (Croft *et al*., 2003). Various diagnostic techniques including parasitological and immunological methods have been established to diagnose schistosomiasis. Each of these methods has both advantages and disadvantages. Traditional parasitological methods, such as Kato–Katz assay, are inexpensive and simple, but lack sensitivity, and are not able to detect the infection until the parasite begins to lay eggs. On the other hand, immunological detection of schistosome infection also suffers from low sensitivity of the assays, as well as the fundamental problem of persistent antibodies after chemotherapy even though egg counts and circulating antigens (in the case of indirect assays) may have already decreased (Xu *et al*., 2010). Several PCR techniques have been developed for the diagnosis of *S. mansoni*, *S. haematobium* and *S. japonicum* infections. Although PCR-based assays provided reliable, sensitive and specific tools, these PCR techniques are not widely utilised due to economic and practical limitations. The dependence on expensive apparatus, low amplification efficiency, and long reaction time restricts their widespread application for clinical diagnosis. Recently, Xu *et al*., (2010) established the use of a loop-mediated isothermal amplification (LAMP) assay to detect *Schistosoma japonicum* DNA in faecal and serum samples of rabbits, and serum samples of humans infected with *S. japonicum*. The LAMP assay was based on the sequence of highly repetitive retrotransposon SjR2, and was able to detect 0.08 fg *S. japonicum* DNA, which is $10^4$ times more sensitive than conventional PCR. The LAMP assay was also highly specific for *S. japonicum* and able to detect *S. japonicum* DNA in rabbit sera at 1 week p.i. Following administration of praziquantel, detection of *S. japonicum* DNA in rabbit sera became negative at 12 weeks post-treatment. Their results demonstrated that LAMP was effective for early diagnosis of, and evaluation of therapy effectiveness for, *S. japonicum infection*. Comparing LAMP and conventional PCR, they also discovered that percentage sensitivity of LAMP was 96.7%, whereas that of PCR was only 60%, showing that LAMP was more sensitive than conventional PCR for clinical diagnosis of schistosomiasis cases in endemic areas. Similarly, a loop-mediated isothermal amplification (LAMP) assay has been developed and validated for the detection of *Paragonimus westermani* adults, metacercariae, and eggs in human, freshwater crabs and crayfish samples (Chen *et al*., 2011) and this is important for effective control of human paragonimiasis.

Malaria is one of the most important public health problems in the world. In sub-Saharan Africa alone, there are 400–900 million cases each year with an annual mortality of 1–2 million occurring mostly in children and pregnant women (Snow *et al*., 2005; Hay *et al*., 2008) and increasing resistance to affordable antimalarials has recently worsened these health and economic burdens (Trape, 2001). In a retrospective study using *P. falciparum*-specific primers for the 18S rRNA gene, LAMP exhibited 95% sensitivity and 99% specificity compared to PCR (Poon et al., 2006). Another study using four sets of species-specific primers found 98.5% sensitivity and 94.3% specificity compared to microscopy (Han *et al*., 2007). In another study, Jun-Hu et al., (2010) employed nested PCR to detect malaria parasites in patients with low level *P. vivax* parasitemia of <0.0001% (approximately 3 parasites/µl), and LAMP was found to detect malaria parasites at <0.001% (approximately 30 parasites/µl), which is 1.6-fold more sensitive than that of microscopic examinations. Compared to the results of microscopic examination, LAMP had a sensitivity of 98.3% and a specificity of 100%, which were similar to the results of nested PCR (99.0 and 100%, respectively). The use of molecular techniques has also enabled the identification of parasite gene polymorphisms associated with malarial drug resistance and this has allowed the use of molecular techniques for surveillance of antimalarial resistance. For instance, it has been shown that *P. falciparum* resistance to chloroquine both *in vitro* and *in vivo*

requires a key lysine-to-threonine mutation at codon 76 (K76T) of the *pfcrt* gene, and may be modulated by mutations in *pfmdr1*. Similarly, resistance to antifolates is associated with various combinations of mutations within the genes of drug targets, *dhfr* and *dhps*. Furthermore, Mefloquine resistance has been linked to *pfmdr1* copy number and atovaquone resistance to cytochrome B gene mutations, and artemisinin resistance has been associated with a mutation in *PfATPase6* (Wilson *et al*., 2005; Erdman *et al*., 2008).

In a more recent study, Verma *et al*., (2013) applied LAMP assay using SYBR Green for clear-cut naked eye detection *of Leishmania* (*Leishmania*) *donovani* in 200 clinical samples of visceral leishmaniasis (VL) and post-kala-azar dermal leishmaniasis (PKDL). The assay was positive in 53/55 VL blood samples (sensitivity, 96.4%), 15/15 VL bone marrow aspirate samples (sensitivity, 100%), 60/62 PKDL tissue biopsy samples (sensitivity, 96.8%), and 1/68 control samples (specificity, 98.5%). The assay was specific for *L*. (*L*.) *donovani*, the causative species for VL and negative for *L*. (*L*.) *infantum*, *L*. (*L*.) *tropica*, and *L*. (*L*.) *major* with a detection limit of 1fg DNA.

From the foregoing, it can be deduced that LAMP assay is a rapid, simple, and cost effective diagnostic tool as compared to PCR assay which requires a thermal cycler and more handling techniques for post-PCR manipulations. Therefore, LAMP assay is advocated as a low-technology diagnostic tool for resource-poor settings (Notomi *et al*., 2000).

## 2.5. Luminex xMAP Technology

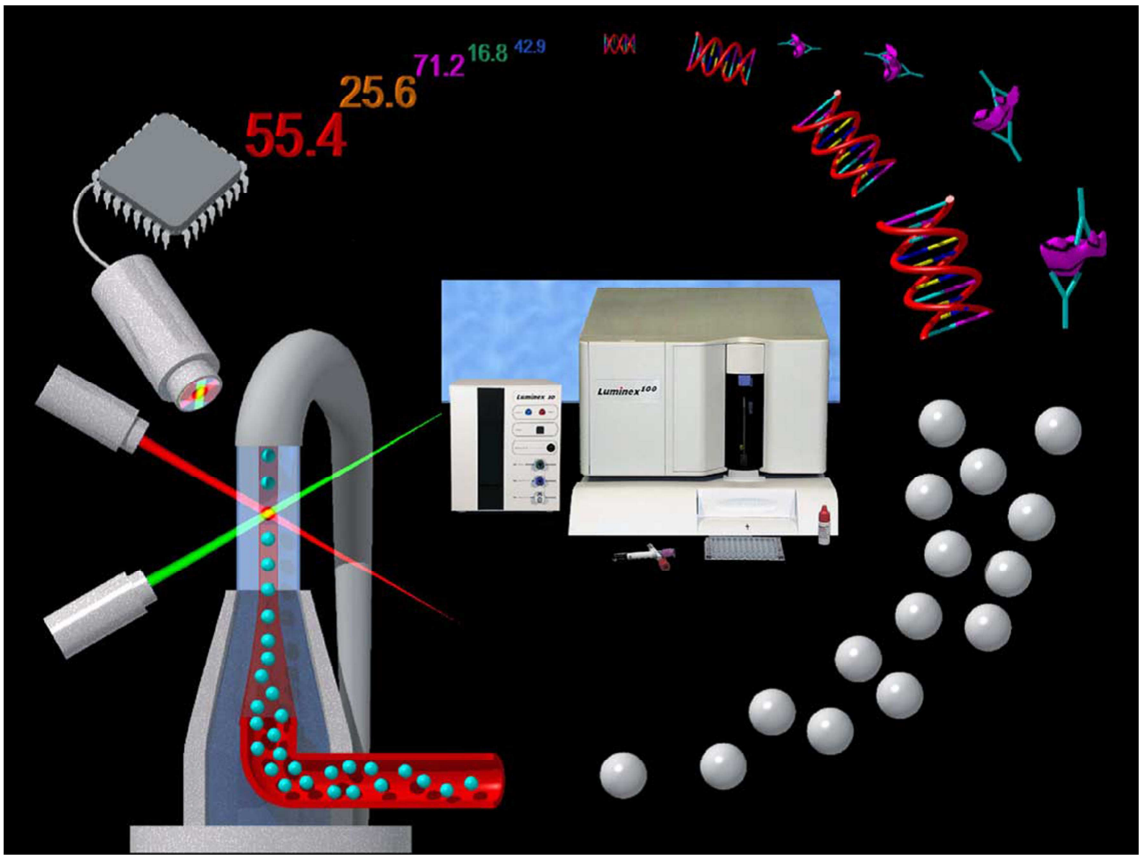


***Figure 7.*** *Luminex xMAP system and components. The four main components of the xMAP system are shown, clockwise from top-right: biomolecular reactants; fluorescently color-coded microspheres; fluidics and optics; and high-speed digital signal processing. The Luminex 100 analyzer with XY platform and sheath delivery system is pictured in the center. Source: Adapted from Dunbar, 2006.*

Luminex is a bead-based xMAP technology (multianalyte profiling), a system that combines flow cytometry, fluorescent microspheres (beads), lasers and digital signal processing (Figure 7), which has the capacity of simultaneously measuring up to 100 different analytes in a single sample (Tavares et al., 2011). Consequently, the capturing and detection of specific analytes from a given sample is allowed by the method. The microspheres can be covalently linked to antigens, antibodies or oligonucleotides, which serve as probes in the assay (Tavares et al., 2011). Several DNA tests developed in the Luminex platform over the years have been applied in the detection and genotyping of Escherichia coli, Mycobacterium, Trichosporon, Salmonella, Listeria monocytogenes, Campylobacter jejuni, and Candida spp. (Tavares et al., 2011). The ability of Luminex technology to identify multiple organisms or different genotypes of one particular organism during the same reaction using a very low volume may be useful in studies involving antigenic diversity, drug-resistant alleles and diagnosis of parasitic diseases.

Bandyopadhyay and his colleagues employed Luminex to differentiate the species of Cryptosporidium; C. hominis and C. parvum in 143 DNA extractions, using

oligonucleotide-specific probes for the ML-2 regions of each species, without the need for DNA sequencing (Bandyopadhyay et al., 2007). The species C. hominis and C. parvum differ genetically by a single nucleotide in the microsatellite region-2 (ML-2), making them indistinguishable using antigen detection or serology tests. The Luminex technology enabled the generation of results in about five hours, being faster and less expensive than PCR followed by DNA sequencing. Luminex assay was also proved to be 100% specific and more sensitive than direct immunofluorescence (DFA), a method routinely employed to identify species of Cryptosporidium and Giardia.

Li *et al*. (2010) allied techniques of nested PCR and Luminex for the diagnosis of *Cryptosporidium* spp., *C. parvum,* and *Giardia duodenalis*, seeking to increase sensitivity and specificity. This adapted approach proved to be 100% specific and accurate in tests of a total of 240 fecal samples. Similarly, Luminex technology has been employed to detect the four human *Plasmodium* species (*falciparum, vivax, malariae* and *ovale*) simultaneously in blood samples (McNamara *et al*., 2006). Therefore, Luminex can improve the speed, accuracy, and reliability of other PCR methods, in addition to costing less per test than other molecular techniques.

## 3. Conclusion

Parasitic diseases pose a major public health challenge, especially in developing countries. The early detection of parasitic infection in humans allows for early treatment of such infection and reduces the adverse effects associated with the parasite. Microscopy remains the "gold standard" for diagnosis of parasites and indeed it is simple, can be rapid and does not involve the purchase and maintenance of expensive equipment. However, results depend on the quality of staining and the microscope, the technique used for specimen film preparation, and the technical expertise of the microscopist is essential for accurate identification. Consequently, misdiagnosis may occur among infected individuals who are not sensitive to traditional diagnostic methods, causing the need for more sensitive and efficient diagnostic methods. Molecular or DNA-based methods have recently been developed for parasite detection to address some of the problems encountered using these traditional methods such as microscopy for parasite diagnosis. The molecular methods detects parasites based on their antigenic components or DNA segments and have paved way for early diagnosis of parasitic infection. The use of molecular techniques has also enabled the identification of parasite gene polymorphisms associated with drug resistance, especially in malaria infections and this has allowed the use of molecular techniques for surveillance of antimalarial resistance. The limiting factor for the universal application of molecular techniques in developing countries is mainly their expensive nature; however, these tests are increasingly being used in clinical diagnosis, treatment monitoring, and epidemiological studies of parasitic diseases affecting people worldwide. They have become indispensable tools for acquiring detailed knowledge on the morphology, genetic characteristics and behavior of parasitic disease in the affected populations.

## References

[1] Adams, E. R., Schoone, G. J., Ageed, A. F., Safi, S. E. and Schallig, H. D. (2010) Development of a reverse transcriptase loop-mediated isothermal amplification (LAMP) assay for the sensitive detection of Leishmania parasites in clinical samples. *Am J Trop Med Hyg*. 82:591–6.

[2] ALdV, G., Melo, F. L., Werkhauser, R. P. and Abath, F. G. C. (2006) Development of a real time polymerase chain reaction for quantitation of Schistosoma mansoni DNA. *Memorias Instituto Oswaldo Cruz*, 101:133-6.

[3] Bakheit, M. A., Dena, T., Lily, A. P., Oriel, M. M. T., Peter, A. M., Jerry, O. and Panagiotis, K. (2008) Sensitive and specific detection of *Cryptosporidium species* in PCR-negative samples by loop-mediated isothermal DNA amplification and confirmation of generated LAMP products by sequencing. *Veterinary Parasitology*, 158: 11–22.

[4] Bandyopadhyay, K., Kellar, K. L., Moura, I., Casaqui Carollo, M. C, Graczyk, T. K., Slemenda, S., Johnston, S.P. and da Silva AJ. (2007) Rapid Microsphere Assay for Identification of *Cryptosporidium hominis and Cryptosporidium parvum* in Stool and Environmental Samples. *Journal of Clinical Microbiology*, 45(9): 2835–2840.

[5] Cai, X. Q., Xu, M. J., Wang, Y. H., Qiu, D. Y., Liu, G. X., Lin, A., Tang, J.D., Zhang, R.L. and Zhu, X. Q. (2010) Sensitive and rapid detection of *Clonorchis sinensis* infection in fish by loop-mediated isothermal amplification (LAMP). *Parasitology Research*, 106:1379–1383.

[6] Calderaro, A., Chiara, G., Sara, M., Simona, P., Giovanna, P., Sabina, R., Franco, G., Nino, M., Giuseppe, D. and Carlo C. ( 2010) Evaluation of a real-time polymerase chain reaction assay for the laboratory diagnosis of giardiasis. *Diagnostic Microbiology and Infectious Disease*, 66: 261–267.

[7] Carlier, Y., Carine, T., Philippe, D. and Francois, P. (2012) Congenital parasitic infections: A review. *Acta Tropica*, 121: 55– 70.

[8] Catherine, A. G., Darren, J. G., Geoffrey, N. G. and Donald, P. M. (2011) DNA amplification approaches for the diagnosis of key parasitic helminth infections of humans. *Molecular and Cellular Probes*, 25: 143-152.

[9] Chamberlain, J. S., Gibbs, R. A., Ranier, J. E., Nguyen, P. N. and Caskey, C. T. (1988) Deletion screening of the Duchenne muscular dystrophy locuse via multiplex DNA amplification. *Nucleic Acids Research*, 16: 11141-11156.

[10] Chen, J. H., Lu, F., Lim, C. S., Kim, J. Y., Ahn, H. J., Suh, I. B., Takeo, S., Tsuboi, T., Sattabongkot, J. and Han, E. T. (2010) Detection of Plasmodium vivax infection in the Republic of Korea by loop-mediated isothermal amplificationn(LAMP). *Acta Tropica*, 113:61–5.

[11] Chen, M. X., Ai, L., Zhang, R. L., Xia, J. J., Wang, K., Chen, S. H., Zhang, Y. N., Xu, M. J., Li, X., Zhu, X. Q. and Chen, J. 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.

[12] Chirdan, O. O., Akosu, J. T. and Adah, S. O. (2010). Intestinal parasites in children attending day care centres in Jos, Central Nigeria. *Nigeria Journal of Medicine*, 19(2): 219-222.

[13] Croft, S. L., Vivas, L. and Brooker, S. (2003) Recent advances in research and control of malaria, leishmaniasis, trypanosomiasis and schistosomiasis. *East Mediterranean. Health Journal*, 9: 518–533.

[14] Devi, K. R., Narain, K., Agatsuma, T., Blair, D., Nagataki, M., Wickramasinghe, S., Yatawara, L. and Mahanta, J. (2010) Morphological and molecular characterization of Paragonimus westermani in northeastern India. *Acta Tropica*, 116: 31–38.

[15] Dunbar, S. A. (2006) Applications of LuminexR xMAPi technology for rapid, high-throughput multiplexed nucleic acid detection. *Clinica Chimica Acta*, 363: 71 – 82.

[16] Erdman, L. K. and Kevin, CK. (2008) Molecular diagnostic and surveillance tools for global malaria control. *Travel Medicine and Infectious Disease*, 6: 82–99.

[17] Gasser, R. B. (2006) Molecular tools – advances, opportunities and prospects. *Veterinary Parasitology*, 136(2): 69-89.

[18] Gobert, G. N., Chai, M., Duke, M. and McManus, D. P. (2005) Copro-PCR based detection of Schistosoma eggs using mitochondrial DNA markers. *Molecular and Cell Probes*, 19:250-4.

[19] Gordon, C.A., Gray, D. J., Gobert, G. N. and McManus, D. P. (2011) DNA amplification approaches for the diagnosis of key parasitic helminth infections of humans. *Molecular and Cellular Probes*, 25: 143-152.

[20] Gottstein, B., Deplazes, P., Tanner, I. and Skaggs, J. S. (1991) Diagnostic identification of *Taenia saginata* with the polymerase chain reaction. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 85: 248-249.

[21] Gottstein, B. and Mowatt, M. R. (1991) Sequencing and characterization of an *Echinococcus multilocularis* DNA probe and its use in the polymerase chain reaction. *Molecular and Biochemical Parasitology*, 44: 183-193.

[22] Han, E. T., Watanabe, R., Sattabongkot, J., Khuntirat, B., Sirichaisinthop, J., Iriko, H., Jin, L., Takeo, S. and Tsuboi, T. (2007). Detection of four *Plasmodium species* by genus- and species-specific loop-mediated isothermal amplification for clinical diagnosis. *Journal of Clinical Microbiology*, 45: 2521–2528.

[23] Hay, S. I., Smith, D. L. and Snow, R. W. (2008) Measuring malaria endemicity from intense to interrupted transmission. *Lancet Infectious Disease*, 8(6): 369–378.

[24] Jun-Hu, C., Feng, L., Chae, S. L., Jung-Yeon, K., Heui-June, A., In-Bum, S., Satoru, T., Takafumi, T., Jetsumon, S. and Eun-Taek, H. (2010) Detection of Plasmodium vivax infection in the Republic of Korea by loop-mediated isothermal amplification (LAMP). *Acta Tropica*, 113: 61–65.

[25] Karanis, P., Thekisoe, O., Kiouptsi, K., Ongerth, J., Igarashi, I. and Inoue, N. (2007) Development and preliminary evaluation of a loop-mediated isothermal amplification procedure for sensitive detection of *Cryptosporidium* oocysts in fecal and water samples. *Applied Environmental Microbiology*, 73: 5660–5662.

[26] Le, T. H., Van De, N., Blair, D., Sithithaworn, P. and McManus, D. P. (2006) *Clonorchis sinensis* and Opisthorchis viverrini: development of a mitochondrial-based multiplex PCR for their identification and discrimination. *Experimental Parasitology*, 112: 109-114.

[27] Li, W., Zhang, N., Gong, P., Cao, L., Li, J., Su L., Li, S., Diao, Y., Wu, K., Li, H. and Zhang, X. (2010) A novel multiplex PCR coupled with Luminex assay for the simultaneous detection of *Cryptosporidium* spp., *Cryptosporidium parvum* and *Giardia duodenalis*. *Veterinary Parasitology*, 173(1-2): 11-18.

[28] Lier, T., Johnasen, M. V., Hjelmevoll, S. O., Vennervald, B. J. and Simonsen, G. S. (2008) Real-time PCR for detection of low intensity *Schistosoma japonicum* infections in a pig model. *Acta Tropica*, 105: 74-80.

[29] Lier, T., Simonsen, G. S., Haaheim, H., Hjelmevoll, S. O., Vennervald, B. J. and Johansen, M. V. (2006) Novel real-time PCR for detection of *Schistosoma japonicum* in stool. *Southeast Asian Journal of Tropical Medicine and Public Health*, 37:257-64.

[30] Lin, M. H., Chen, T. C., Kuo, T. T., Tseng, C. C. and Tseng, C. P. (2000) Real-time PCR for quantitative detection of *Toxoplasma gondii*. *Journal of Clinical Microbiology*, 38(11): 4121-4125.

[31] Lin, Z., Yanlei, Z., Houshuang, Z., Yongzhi, Z., Jie, C. and Jinlin, Z. (2012) Comparison of loop-mediated isothermal amplification (LAMP) and real-time PCR method targeting a 529-bp repeat element for diagnosis of toxoplasmosis. *Veterinary Parasitology*, 185: 296– 300.

[32] Maurelli, M., Rinaldi, L., Capuano, F., Perugini, A. and Cringoli, G. (2009) Development of a real-time PCR for the differentiation of the G1 and G2/G3 genotypes of *Echinococcus granulosus*. *Parasitology Research*, 105: 255-229.

[33] McNamara, D. T., Kasehagen, L. J., Grimberg, B. T., Cole-Tobian, J., Collins, W. E. and Zimmerman, P. A. (2006) Diagnosing infection levels of four human malaria parasite species by a polymerase chain reaction/ligase detection reaction fluorescent microsphere-based assay. *American Journal Tropical Medicine and Hygiene*, 74(3): 413-421.

[34] Morrison, L. J. (2011) Parasite-driven pathogenesis in *Trypanosoma brucei* infections. *Parasite Immunology*, 33:448-455.

[35] Muldrew, K.L. (2009). Molecular Diagnostics of Infectious Diseases. *Current Opinion in Pediatrics*, 21(1):102–111.

[36] Nagamine, K., Hase, T. and Notomi, T. (2002) Accelerated reaction by loop mediated isothermal amplification using loop primers. *Molecular and Cell Probes*, 16: 223–229.

[37] Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N. and Hase, T. (2000) Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research*, 28: E63.

[38] Oliveira, L. M. A., Santos, H. L. C., Gonçalves, M. M. L. , Barreto, M. G. M. and Peralta, J. M. (2010) Evaluation of polymerase chain reaction as an additional tool for the diagnosis of low-intensity *Schistosoma mansoni* infection. *Diagnostic Microbiology and Infectious Disease*, 68: 416-420.

[39] Pilotte, N., Torres Tomaino, M. F. R., Laneya, S. J. and Williams, S. A. (2013) A TaqMan-based multiplex real-time PCR assay for the simultaneous detection of *Wuchereria bancrofti* and *Brugia malayi*. *Molecular & Biochemical Parasitology*, 189: 33– 37.

[40] Pontes, L. A. and Dias-Neto, E. (2002) Detection by polymerase chain reaction of Schistosoma mansoni DNA in human serum and faeces. *American Journal of Tropical Medicine and Hygiene*, 66: 157-162.

[41] Pontes, L. A., Oliveira, M. C., Katz, N., Dias-Neto, E. and Rabello, A. (2003) Comparision of a polymerase chain reaction and the kato-katz technique for diagnosing infection with *Schistosoma japonicum*. *American Journal of Tropical Medicine and Hygiene*, 68: 652-656.

[42] Poon, L. L., Wong, B. W., Ma, E. H., Chan, K. H., Chow, L. M., Abeyewickreme, W., Tangpukdee, N., Yuen, K. Y., Guan, Y., Looareesuwan, S. and Peiris, J. S. (2006) Sensitive and inexpensive molecular test for falciparum malaria: detecting *Plasmodium falciparum* DNA directly from heat-treated blood by loop-mediated isothermal amplification. *Clinical Chemistry*, 52: 303–306.

[43] Rougemont, M., Van Saanen, M., Sahli, R., Hinrikson, H. P., Bille, J. and Jaton, K.(2004) Detection of four *Plasmodium* species in blood from humans by 18S rRNA gene subunit-based and species-specific real-time PCR assays. *Journal of Clinical Microbiology*, 42(12): 5636-5643.

[44] Seung-Young, H., So-Hee, K., Ga-Young, L., Vu Thi, T. H., Chi-Sook, M., Jeong, H. S., Wan-Lim, K., Seong-Youl, K., Hae-Joon, P., Han-Oh, P. and Weon-Gyu, K. (2011) A novel real-time PCR assay for the detection of *Plasmodium falciparum* and *Plasmodium vivax* malaria in low parasitized individuals. *Acta Tropica*, 120: 40– 45.

[45] Shahnazi, M., Hejazi, H., Salehi, M. and Andalib, A. R. (2011) Molecular characterization of human and animal *Echinococcus granulosus* isolates in Isfahan, Iran. *Acta Tropica*, 117:47-50.

[46] Shokoples, S. E., Ndao, M., Kowalewska-Grochowska, K. and Yanow, S. K. (2009) Multiplexed real-time PCR assay for discrimination of *Plasmodium* species with improved sensitivity for mixed infections. *Journal of Clinical Microbiology*, 47(4): 975-980.

[47] Snow, R. W., Guerra, C. A., Noor, A. M., Myint, H. Y. and Hay, S. I. (2005) The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Nature*, 434(7030): 214–217.

[48] Sotiriadou, I. and Panagiotis, K. (2008) Evaluation of loop-mediated isothermal amplification for detection of *Toxoplasma gondii* in water samples and comparative findings by polymerase chain reaction and immunofluorescence test (IFT). *Diagnostic Microbiology and Infectious Disease*, 62:357–365.

[49] Sterkers, Y., Jennifer, R., Sahar, A., Eric, I., Patrick, B. and Francine, P. (2011) Diagnosis of congenital toxoplasmosis by polymerase chain reaction on neonatal peripheral blood. *Diagnostic Microbiology and Infectious Disease*, 71: 174–176.

[50] Suzuki, T., Osada, Y., Kumagai, T., Hamada, A., Okuzawa, E. and Kanazawa, T. (2006) Early detection of *Schistosoma mansoni* infection by touchdown PCR in a mouse model. *Parasitology International*, 55: 213-8.

[51] Talmi-Frank, D., Nasereddin, A., Schnur, L. F, Schönian, G., Töz, S. O., Jaffe, C. L. and Baneth, G. (2010) Detection and identification of old world *Leishmania* by high resolution melt analysis. *PLoS Neglected Tropical Diseases*, 4(1): e581. doi:10.1371/journal.pntd.0000581

[52] Tavares, R. G., Staggemeier, R., Borges, A. L. P., Rodrigues, M. T., Castelan, L. A., Vasconcelos, J., Anschau, M. E. and Spalding, S. M. (2011) Molecular techniques for the study and diagnosis of parasite infection. The Journal of Venomous Animals and Toxins including Tropical Diseases, 7(3): 239-248.

[53] 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.

[54] Trape, J. F. (2001) The public health impact of chloroquine resistance in Africa. *American Journal of Tropical Medicine and Hygiene*, 64(1–2 Suppl): 12–17.

[55] Traub, R. J., Macaranas, J., Mungthin, M., Leelayoova, S., Cribb, T., Murrell, K. D. and Thompson R. C. A. (2009) A new PCR-based approach indicates the range of *Clonorchis sinensis* now extends to Central Thailand. *PLoS Neglected Tropical Disease*, 3 (1): 367. doi:10.1371/journal.pntd.0000367

[56] Verma, S., Kumar, A., Vanila, S., Narendra, S. N., Venkatesh, R. and Poonam, S. (2013) Application of loop-mediated isothermal amplification assay for the sensitive and rapid diagnosis of visceral leishmaniasis and post-kala-azar dermal leishmaniasis. *Diagnostic Microbiology and Infectious Disease*, 75: 390–395.

[57] Verweij, J. J., Brienen, E. A. T., Ziem, J. B., Polderman, A. M. and Van Lieshout, L. (2007) Simultaneous detection and quantificaton of *Ancylostoma duodenale*, *Necator americanus*, and *Oesophagostomum bifurcum* in fecal samples using multiplex realtime PCR. *American Journal of Tropical Medicine and Hygiene*, 77: 685-690.

[58] Wallon, M., Franck, J., Thulliez, P., Huissoud, C., Peyron, F., Garcia-Meric, P. and Kieffer, F. (2010) Accuracy of real-time polymerase chain reaction for *Toxoplasma gondii* in amniotic fluid. *Obstetrics and Gynecology*, 115: 727–733.

[59] WHO. (2008) Public Health Significance of intestinal parasitic infections. Bulletin of WHO, 65(5): 575-588.

[60] WHO. (2010) Millennium Development Goals: progress towards the health-related Millennium Development Goals .

[61] Wicht, B., Yanagida, T., Scholz, T., Ito, A., Jimenéz, 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.

[62] Wilson, P. E., Alisa, P. A. and Steven, R. M. (2005) Real-time PCR methods for monitoring antimalarial drug resistance. *TRENDS in Parasitology*, 21(6): 44-50.

[63] Xu, J., Rong, R., Zhang, H. Q., Shi, C. J., Zhu, X. Q., and Xia, C. M. (2010) Sensitive and rapid detection of *Schistosoma japonicum* DNA by loop-mediated isothermal amplification (LAMP). *International Journal for Parasitology*, 40: 327–331.

[64] Zhang, H., Thekisoe, O. M., Aboge, G. O., Kyan, H., Yamagishi, J., Inoue, N., Nishikawa, Y., Zakimi, S. and Xuan, X. (2009). *Toxoplasma gondii:* sensitive and rapid detection of infection by loop-mediated isothermal amplification (LAMP) method. *Experimental Parasitology*, 122: 47–50.