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■ MINI-REVIEW

# Malaria Diagnosis: A Brief Review

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**Abstract:** Malaria is a major cause of death in tropical and sub-tropical countries, killing each year over 1 million people globally; 90% of fatalities occur in African children. Although effective ways to manage malaria now exist, the number of malaria cases is still increasing, due to several factors. In this emergency situation, prompt and effective diagnostic methods are essential for the management and control of malaria. Traditional methods for diagnosing malaria remain problematic; therefore, new technologies have been developed and introduced to overcome the limitations. This review details the currently available diagnostic methods for malaria.

**Key words:** *Plasmodium*, malaria, diagnosis, method

## INTRODUCTION

Malaria, sometimes called the "King of Diseases", is caused by protozoan parasites of the genus *Plasmodium*. The most serious and sometimes fatal type of malaria is caused by *Plasmodium falciparum*. The other human malaria species, *P. vivax, P. ovale, P. malariae,* and sometimes *P. knowlesi* can cause acute, severe illness but mortality rates are low. Malaria is the most important infectious disease in tropical and subtropical regions, and continues to be a major global health problem, with over 40% of the world's population exposed to varying degrees of malaria risk in some 100 countries. It is estimated that over 500 million people suffer from malaria infections annually, resulting in about 1-2 million deaths, of whom 90% are children in sub-Saharan Africa [1]. The number of malaria cases worldwide seems to be increasing, due to increasing transmission risk in areas where malaria control has declined, the increasing prevalence of drug-resistant strains of parasites, and in a relatively few cases, massive increases in international travel and migration [2]. The need for effective and practical diagnostics for global malaria control is increasing [3], since effective diagnosis reduces both complications and mortality from malaria. Differentiation of clinical diagnoses from other tropical infections, based on patients' signs and symptoms or physicians' findings, may be difficult. Therefore, confirmatory diagnoses using laboratory technologies are urgently needed. This review discusses on the currently available diagnostic methods for malaria in many settings, and assesses their feasibility in resource-rich and resource-poor settings.

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## DIAGNOSIS OF MALARIA

Prompt and accurate diagnosis is critical to the effective management of malaria. The global impact of malaria has spurred interest in developing effective diagnostic strategies not only for resource-limited areas where malaria is a substantial burden on society, but also in developed countries, where malaria diagnostic expertise is often lacking [4,5]. Malaria diagnosis involves identifying malaria parasites or antigens/products in patient blood. Although this may seem simple, the diagnostic efficacy is subject to many factors. The different forms of the 5 malaria species; the different stages of erythrocytic schizogony, the endemicity of different species, the interrelation between levels of transmission, population movement, parasitemia, immunity, and signs and symptoms; drug resistance, the problems of recurrent malaria, persisting viable or non-viable parasitemia, and sequestration of the parasites in the deeper tissues, and the use of chemoprophylaxis or even presumptive treatment on the basis of clinical diagnosis, can all influence the identification and interpretation of malaria parasitemia in a diagnostic test.

Malaria is a potential medical emergency and should be treated accordingly. Delays in diagnosis and treatment are leading causes of death in many countries [6]. Diagnosis can be difficult where malaria is no longer endemic for healthcare providers unfamiliar with the disease. Clinicians may forget to consider malaria among the potential diagnoses for some patients and not order

the necessary diagnostic tests. Technicians may be unfamiliar with, or lack experience with, malaria, and fail to detect parasites when examining blood smears under a microscope. In some areas, malaria transmission is so intense that a large proportion of the population is infected but remains asymptomatic, e.g., in Africa. Such carriers have developed sufficient immunity to protect them from malarial illness, but not infection. In such situations, finding malaria parasites in an ill person does not necessarily mean that the illness is caused by the parasites. In many malaria-endemic countries, the lack of resources is a major barrier to reliable and timely diagnosis. Health personnel are undertrained, underequipped, and underpaid. They often face excessive patient loads, and must divide their attention between malaria and other equally severe infectious diseases, such as tuberculosis or HIV/AIDS.

## CLINICAL DIAGNOSIS OF MALARIA

A clinical diagnosis of malaria is traditional among medical doctors. This method is least expensive and most widely practiced. Clinical diagnosis is based on the patients' signs and symptoms, and on physical findings at examination. The earliest symptoms of malaria are very nonspecific and variable, and include fever, headache, weakness, myalgia, chills, dizziness, abdominal pain, diarrhea, nausea, vomiting, anorexia, and pruritus [7]. A clinical diagnosis of malaria is still challenging because of the non-specific nature of the signs and symptoms, which overlap considerably with other common, as well as potentially life-threatening diseases, e.g. common viral or bacterial infections, and other febrile illnesses. The overlapping of malaria symptoms with other tropical diseases impairs diagnostic specificity, which can promote the indiscriminate use of antimalarials and compromise the quality of care for patients with non-malarial fevers in endemic areas [8-10]. The Integrated Management of Children Illness (IMCI) has provided clinical algorithms for managing and diagnosing common childhood illnesses by minimally trained healthcare providers in the developing world having inappropriate equipment for laboratory diagnosis. A widely utilized clinical algorithm for malaria diagnosis, compared with a fully trained pediatrician with access to laboratory support, showed very low specificity (0-9%) but 100% sensitivity in African settings [11, 12]. This lack of specificity reveals the perils of distinguishing malaria from other causes of fever in children on clinical grounds alone. Recently, another study showed that use of the IMCI clinical algorithm resulted in 30% over-diagnosis of malaria [13]. Therefore, the accuracy of malaria diagnosis can be greatly enhanced by combining clinical-and parasite-based findings [14].

## LABORATORY DIAGNOSIS OF MALARIA

Rapid and effective malaria diagnosis not only alleviates suffering, but also decreases community transmission. The nonspecific nature of the clinical signs and symptoms of malaria may result in over-treatment of malaria or non-treatment of other diseases in malaria-endemic areas, and misdiagnosis in non-endemic areas [15]. In the laboratory, malaria is diagnosed using different techniques, e.g. conventional microscopic diagnosis by staining thin and thick peripheral blood smears [16], other concentration techniques, e.g. quantitative buffy coat (QBC) method [15], rapid diagnostic tests e.g., OptiMAL [17,18], ICT [19], Para-HIT-f [10], ParaScreen [20], SD Bioline [21], Paracheck [22], and molecular diagnostic methods, such as polymerase chain reaction (PCR) [23,24]. Some advantages and shortcomings of these methods have also been described, related to sensitivity, specificity, accuracy, precision, time consumed, cost-effectiveness, labor intensiveness, the need for skilled microscopists, and the problem of inexperienced technicians.

### Microscopic diagnosis using stained thin and thick peripheral blood smears (PBS)

Malaria is conventionally diagnosed by microscopic examination of stained blood films using Giemsa, Wright's, or Field's stains [25]. This method has changed very little since Laverran's original discovery of the malaria parasite, and improvements in staining techniques by Romanowsky in the late 1,800s. More than a century later, microscopic detection and identification of *Plasmodium* species in Giemsa-stained thick blood films (for screening the presenting malaria parasite), and thin blood films (for species' confirmation) remains the gold standard for laboratory diagnosis [26]. Malaria is diagnosed microscopically by staining thick and thin blood films on a glass slide, to visualize malaria parasites. Briefly, the patient's finger is cleaned with 70% ethyl alcohol, allowed to dry and then the side of fingertip is picked with a sharp sterile lancet and two drops of blood are placed on a glass slide. To prepare a thick blood film, a blood spot is stirred in a circular motion with the corner of the slide, taking care not make the preparation too thick, and allowed to dry without fixative. After drying, the spot is stained with diluted Giemsa (1 : 20, vol/vol) for 20 min, and washed by placing the film in buffered water for 3 min. The slide is allowed to air-dry in a vertical position and examination using a light micro-

scope. As they are unfixed, the red cells lyse when a water-based stain is applied. A thin blood film is prepared by immediately placing the smooth edge of a spreader slide in a drop of blood, adjusting the angle between slide and spreader to 45° and then smearing the blood with a swift and steady sweep along the surface. The film is then allowed to air-dry and is fixed with absolute methanol. After drying, the sample is stained with diluted Giemsa (1 : 20, vol/vol) for 20 min and washed by briefly dipping the slide in and out of a jar of buffered water (excessive washing will decolorize the film). The slide is then allowed to air-dry in a vertical position and examined under a light microscope [27]. The wide acceptance of this technique by laboratories all around the world can be attributed to its simplicity, low cost, its ability to identify the presence of parasites, the infecting species, and assess parasite density-all parameters useful for the management of malaria. Recently, a study showed that conventional malaria microscopic diagnosis at primary healthcare facilities in Tanzania could reduce the prescription of antimalarial drugs, and also appeared to improve the appropriate management of non-malarial fevers [16]. However, the staining and interpretation processes are labor intensive, time consuming, and require considerable expertise and trained healthcare workers, particularly for identifying species accurately at low parasitemia or in mixed malarial infections. The most important shortcoming of microscopic examination is its relatively low sensitivity, particularly at low parasite levels. Although the expert microscopist can detect up to 5 parasites/$\mu$l, the average microscopist detects only 50-100 parasites/$\mu$l [28]. This has probably resulted in underestimating malaria infection rates, especially cases with low parasitemia and asymptomatic malaria. The ability to maintain required levels of in malaria diagnostics expertise is problematic, especially in remote medical centers in countries where the disease is rarely seen [29]. Microscopy is laborious and ill-suited for high-throughput use, and species determination at low parasite density is still challenging. Therefore, in remote rural settings, e.g. peripheral medical clinics with no electricity and no health-facility resources, microscopy is often unavailable [30].

### QBC technique

The QBC technique was designed to enhance microscopic detection of parasites and simplify malaria diagnosis [31]. This method involves staining parasite deoxyribonucleic acid (DNA) in micro-hematocrit tubes with fluorescent dyes, e.g. acridine orange, and its subsequent detection by epi-fluorescent microscopy. Briefly, finger-prick blood is collected in a hematocrit tube containing acridine orange and anticoagulant. The tube is centrifuged at 12,000 g for 5 min and immediately examined using an epi-fluorescent microscope [27]. Parasite nuclei fluoresces bright green, while cytoplasm appears yellow-orange. The QBC technique has been shown to be a rapid and sensitive test for diagnosing malaria in numerous laboratories settings [15,32-35]. While it enhances sensitivity for *P. falciparum*, it reduces sensitivity for non-falciparum species and decreases specificity due to staining of leukocyte DNA [36]. Recently, it has been shown that acridine orange is the preferred diagnostic method (over light microscopy and immunochromatographic tests) in the context of epidemiologic studies in asymptomatic populations in endemic areas, probably because of increased sensitivity at low parasitemia [37]. Nowadays, portable fluorescent microscopes using light emitting diode (LED) technology, and pre-prepared glass slides with fluorescent reagent to label parasites, are available commercially [38]. Although the QBC technique is simple, reliable, and user-friendly, it requires specialized instrumentation, is more costly than conventional light microscopy, and is poor at determining species and numbers of parasites.

### Rapid diagnostic tests (RDTs)

Since the World Health Organization (WHO) recognized the urgent need for new, simple, quick, accurate, and cost-effective diagnostic tests for determining the presence of malaria parasites, to overcome the deficiencies of light microscopy, numerous new malaria-diagnostic techniques have been developed [39]. This, in turn, has led to an increase in the use of RDTs for malaria, which are fast and easy to perform, and do not require electricity or specific equipment [40]. Currently, 86 malaria RDTs are available from 28 different manufacturers [41]. Unlike conventional microscopic diagnosis by staining thin and thick peripheral blood smears, and QBC technique, RDTs are all based on the same principle and detect malaria antigen in blood flowing along a membrane containing specific anti-malaria antibodies; they do not require laboratory equipment. Most products target a *P. falciparum*-specific protein, e.g. histidine-rich protein II (HRP-II) or lactate dehydrogenase (LDH). Some tests detect *P. falciparum* specific and pan-specific antigens (aldolase or pan-malaria pLDH), and distinguish non-*P. falciparum* infections from mixed malaria infections. Although most RDT products are suitable for *P. falciparum* malaria diagnosis, some also claim that they can effectively and rapidly diagnose *P. vivax* malaria [21,42,43]. Recently, a new RDT method has been developed for detecting *P. knowlesi* [44]. RDTs provide an opportunity to

extend the benefits of parasite-based diagnosis of malaria beyond the confines of light microscopy, with potentially significant advantages in the management of febrile illnesses in remote malaria-endemic areas. RDT performance for diagnosis of malaria has been reported as excellent [14,19,20,22,45-47]; however, some reports from remote malaria-endemic areas have shown wide variations in sensitivity [36,40,48]. Murray and co-authors recently discussed the reliability of RDTs in an "update on rapid diagnostic testing for malaria" in their excellent paper [49]. Overall, RDTs appears a highly valuable, rapid malaria-diagnostic tool for healthcare workers; however it must currently be used in conjunction with other methods to confirm the results, characterize infection, and monitor treatment. In malaria-endemic areas where no light microscopy facility exists that may benefit from RDTs, improvements are required for ease of use, sensitivity for non-falciparum infection, stability, and affordability. The WHO is now developing guidelines to ensure lot-to-lot quality control, which is essential for the community's confidence in this new diagnostic tool [41]. Because the simplicity and reliability of RDTs have been improved for use in rural endemic areas, RDT diagnosis in non-endemic regions is becoming more feasible, which may reduce time-to-treatment for cases of imported malaria [30].

### Serological tests

Diagnosis of malaria using serological methods is usually based on the detection of antibodies against asexual blood stage malaria parasites. Immunofluorescence antibody testing (IFA) has been a reliable serologic test for malaria in recent decades [50]. Although IFA is time-consuming and subjective, it is highly sensitive and specific [51]. The literature clearly illustrates the reliability of IFA, so that it was usually regarded as the gold standard for malarial serology testing [47]. IFA is useful in epidemiological surveys, for screening potential blood donors, and occasionally for providing evidence of recent infection in non-immunes. Until recently, it was a validated method for detecting *Plasmodium*-specific antibodies in various blood bank units, which was useful for screening prospective blood donors, so avoiding transfusion-transmitted malaria [52,53]. In France, for example, IFA is used as a part of a targeted screening strategy, combined with a donor questionnaire [54]. The principle of IFA is that, following infection with any *Plasmodium* species, specific antibodies are produced within 2 wk of initial infection, and persist for 3-6 months after parasite clearance. IFA uses specific antigen or crude antigen prepared on a slide, coated and kept at -30°C until used, and quantifies both IgG and IgM antibodies in patient serum samples. Titers > 1 : 20 are usually deemed positive, and < 1 : 20 unconfirmed. Titers > 1 : 200 can be classified as recent infections [27]. In conclusion, IFA is simple and sensitive, but time-consuming. It cannot be automated, which limits the number of sera that can be studied daily. It also requires fluorescence microscopy and trained technicians; readings can be influenced by the level of training of the technician, particularly for serum samples with low antibody titers. Moreover, the lack of IFA reagent standardization makes it impractical for routine use in blood-transfusion centers, and for harmonizing inter-laboratory results.

## MOLECULAR DIAGNOSTIC METHODS

As mentioned above, traditional malaria diagnostic methods remain problematic. New laboratory diagnostic techniques that display high sensitivity and high specificity, without subjective variation, are urgently needed in various laboratories. Recent developments in molecular biological technologies, e.g. PCR, loop-mediated isothermal amplification (LAMP), microarray, mass spectrometry (MS), and flow cytometric (FCM) assay techniques, have permitted extensive characterization of the malaria parasite and are generating new strategies for malaria diagnosis.

### PCR technique

PCR-based techniques are a recent development in the molecular diagnosis of malaria, and have proven to be one of the most specific and sensitive diagnostic methods, particularly for malaria cases with low parasitemia or mixed infection [55]. The PCR technique continues to be used extensively to confirm malaria infection, follow-up therapeutic response, and identify drug resistance [27]. It was found to be more sensitive than QBC and some RDTs [56,57]. Concerning with the gold standard method for malaria diagnosis, PCR has shown higher sensitivity and specificity than conventional microscopic examination of stained peripheral blood smears, and now seems the best method for malaria diagnosis [55]. PCR can detect as few as 1-5 parasites/$\mu$l of blood ($\leq$ 0.0001% of infected red blood cells) compared with around 50-100 parasites/$\mu$l of blood by microscopy or RDT. Moreover, PCR can help detect drug-resistant parasites, mixed infections, and may be automated to process large numbers of samples [58,59]. Some modified PCR methods are proving reliable, e.g., nested PCR, real-time PCR, and reverse transcription PCR, and appear to be useful second-line techniques when the

results of traditional diagnostic methods are unclear for patients presenting with signs and symptoms of malaria; they also allow accurate species determination [58,60-62]. Recently, the PCR method has become widely accepted for identifying *P. knowlesi* infections [63-65]. Although PCR appears to have overcome the two major problems of malaria diagnosis-sensitivity and specificity- the utility of PCR is limited by complex methodologies, high cost, and the need for specially trained technicians. PCR, therefore, is not routinely implemented in developing countries because of the complexity of the testing and the lack of resources to perform these tests adequately and routinely [66]. Quality control and equipment maintenance are also essential for the PCR technique, so that it may not be suitable for malaria diagnosis in remote rural areas or even in routine clinical diagnostic settings [67].

### LAMP technique

The LAMP technique is claimed to be a simple and inexpensive molecular malaria-diagnostic test that detects the conserved 18S ribosome RNA gene of *P. falciparum* [68]. Other studies have shown high sensitivity and specificity, not only for *P. falciparum*, but also *P. vivax*, *P. ovale* and *P. malariae* [69,70]. These observations suggest that LAMP is more reliable and useful for routine screening for malaria parasites in regions where vector-borne diseases, such as malaria, are endemic. LAMP appears to be easy, sensitive, quick and lower in cost than PCR. However, reagents require cold storage, and further clinical trials are needed to validate the feasibility and clinical utility of LAMP [30].

### Microarrays

Publication of the *Plasmodium* genome offers many malaria-diagnostic opportunities [71,72]. Microarrays may play an important role in the future diagnosis of infectious diseases [73]. The principle of the microarrays technique parallels traditional Southern hybridization. Hybridization of labeled targets divided from nucleic acids in the test sample to probes on the array enables the probing of multiple gene targets in a single experiment. Ideally, this technique would be miniaturized and automated for point-of-care diagnostics [23]. A pan-microbial oligonucleotide microarray has been developed for infectious disease diagnosis and has identified *P. falciparum* accurately in clinical specimens [74]. This diagnostic technique, however, is still in the early stages of development [30].

### FCM assay

Flow cytometry has reportedly been used for malaria diagnosis [75-77]. Briefly, the principle of this technique is based on detection of hemozoin, which is produced when the intra-erythrocytic malaria parasites digest host hemoglobin and crystallize the released toxic heme into hemozoin in the acidic food vacuole. Hemozoin within phagocytotes can be detected by depolarization of laser light, as cells pass through a flow-cytometer channel. This method may provide a sensitivity of 49-98%, and a specificity of 82-97%, for malarial diagnosis [78,79], and is potentially useful for diagnosing clinically unsuspected malaria. The disadvantages are its labor intensiveness, the need for trained technicians, costly diagnostic equipment, and that false-positives may occur with other bacterial or viral infections. Therefore, this method should be considered a screening tool for malaria.

### Automated blood cell counters (ACC)

An ACC is a practical tool for malaria diagnosis [80], with 3 reported approaches. The first used a Cell-Dyn® 3500 apparatus to detect malaria pigment (hemozoin) in monocytes, and showed a sensitivity of 95% and specificity of 88%, compared with the gold-standard blood smear [81]. The second method also used a Cell-Dyn® 3500, and analyzed depolarized laser light (DLL) to detect malaria infection, with an overall sensitivity of 72% and specificity of 96% [82]. The third technique used a Beckman Coulter ACC to detect increases in activated monocytes by volume, conductivity, and scatter (VCS), with 98% sensitivity and 94% specificity [83]. Although promising, none of the 3 techniques is routinely available in the clinical laboratory; further studies are required to improve and validate the instrument and its software. The accuracy these methods promise, for detecting malaria parasites, mean ACC could become a valuable and routine malaria-diagnostic laboratory method.

### Mass spectrophotometry

A novel method for in vitro detection of malaria parasites, with a sensitivity of 10 parasites/$\mu$l of blood, has been reported recently. It comprises a protocol for cleanup of whole blood samples, followed by direct ultraviolet laser desorption mass spectrometry (LDMS). For malaria diagnosis, the principle of LDMS is to identify a specific biomarker in clinical samples. In malaria, heme from hemozoin is the parasite-specific biomarker of interest. LDMS is rapid, high throughput, and automated. Compared with

the microscopic method, which requires a skilled microscopist and up to 30-60 min to examine each peripheral blood smear, LDMS can analyze a sample in < 1 min [84]. However, the remote rural areas without electricity are inhospitable for existing high-

**Table 1.** Summary of modalities and issues for consideration in malaria diagnosis

| | Clinical diagnosis | PBS | QBC | RDTs | Serological tests | PCR | LAMP | Microarrays | FCM | ACC | MS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Principle of the method | Based on presenting malarial signs and symptoms | Visualization of morphological distinguishable stages of parasites under light microscope by thick and thin blood smear and staining | Blood staining with acridine orange and detection by epi-fluorescent microscope | Detection of parasite antigens or enzyme | Detection of antibodies against parasites | Specific amplification of malaria DNA | Detection of turbidity by a turbidity meter after amplifying DNA sequences | Hybridization of DNA isolate and quantified by fluorescence-based detection | Detection of hemozoin by flow cytometer | Detection of malarial pigment in activated monocyte | Identification of heme by LDMS |
| Sensitivity and specificity | Depends on malarial endemicity | Depends on good technique, good reagent and microscopist's skill | Sensitivity and specificity higher than PBS | Moderate if more than 100 parasite/$\mu$l | Relatively high but not correlate to clinical symptoms of patients | Excellent | Excellent | Relatively high | Variable sensitivity, high specificity | Variable in both sensitivity and specificity | Undetermined |
| Time consumed (min) | Depends on physician's skill | 30-60 | <15 | 10-15 | 30-60 | 45-360 depends on the methods | < 60 | < 60 | Automated, <1/sample | Automated, <1/sample | Automated, <1/sample |
| Detection limit (parasites/$\mu$l) | Undetermined | Expert ~5-10 Routinely > 50 | > 5 | 50-100 | Undetermined | ≥ 1 | > 5 | Undetermined | Poor correlation with parasitemia | 5-20 | 100 for whole blood |
| Expertise required | High: in non-endemic areas | High: in non-endemic areas | Moderate | Low | Moderate | High | High | High | High | High | High |
| Instrument cost | - | Low cost | Moderate | Moderate | Moderate | Expensive | Moderate | Expensive | Expensive | Expensive | Expensive |
| Other considerations | Easy to follow the diagnostic algorithm, Results in significant over-treatment of malaria, especially in highly endemic areas but lower estimation in hypoendemic areas; severe malaria is commonly caused by *P. falciparum*; mixed infection is still problematic | Gold standard method, Good for all human species (except *P. knowlesi*); need considerable expertise; mixed infection and low parasitemia may cause misdiagnosis | Simple and user-friendly; electricity is needed; limit for species identification and quantitative parasite; cannot store capillaries for later reference | 1st line diagnostic in all areas; suitable in field work; may not possible for differentiation between *P. vivax*, *P. ovale* and *P. malariae*; limited for quantifying parasites; low parasitemia may cause misdiagnosis | Results can be influenced by trained technicians; possible for all human species; useful for epidemiological surveys; not useful for treatment decision-making | 2nd line diagnosis in well-equipped laboratories; useful for identify the development of drug-resistance, species identification; and for quantifying parasite density at low parasitemia | Limit for quantifying parasites; possible for all human species; clinical trials are needed to validate feasibility and clinical utility | Still in the early stages of development for diagnosis of malaria | Useful for diagnosis of clinically unsuspected malaria; clinical trials are needed to validate feasibility and clinical utility | Clinical trials are needed to validate feasibility and clinical utility | Still in early stages of development for diagnosis of malaria |

PBS, peripheral blood smears; QBC, quantitative buffy coat; RDTs, rapid diagnostic tests; PCR, polymerase chain reaction; LAMP, loop-mediated isothermal amplification; FCM, flow cytometry; ACC, automated blood cell counter; MS, mass spectrometry; LDMS, laser desorption mass spectrometry.

tech mass spectrometers. Future improvements in equipment and techniques should make this method more practicable.

Recently, other reliable malaria-diagnostic tests have been developed and introduced, and some tests are commercially available, for example, enzyme linked immunosorbent assay (ELISA)/ enzyme immunoassay (EIA) [50,54,85], latex agglutination assay [86], and cultivation of live malaria parasites [87,88]. Post-mortem organ diagnoses, by investigating malaria parasites in tissue autopsy, e.g. liver and spleen [89], kidney [90] and brain [91], have also been described. However, parasite culture, molecular techniques, serology techniques and pathobiological diagnostic techniques, although sometimes useful in research laboratories, are not practical or appropriate for the routine clinical diagnosis of malaria. Table 1 summarizes of modalities and issues for consideration in malaria diagnosis.

## CONCLUSION

Conventional microscopic examination of peripheral thick and thin blood smears remains the gold standard for malaria diagnosis. Although this method requires a trained microscopist, and sensitivity and specificity vary compared with recent technical advances, it is inexpensive and reliable. Quick and convenient RDTs are currently implemented in many remote settings, but are costly and need improved quality control. Serological tests are useful for epidemiological surveys, but not suitable for the diagnosis of acute malaria. Molecular-biological techniques are appropriate for research laboratories; they can be used to identify the development of drug-resistance, are useful for species identification, and also for quantifying parasite density with low parasitemia. Finally, the level of malaria endemicity, the urgency of diagnosis, the experience of the physician, the effectiveness of healthcare workers, and budget resources, are all factors influencing the choice of malaria-diagnostic method.

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### Conflict of interest

The authors declare no conflict of interest.

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