Malaria: A Comprehensive Review of Laboratory Diagnosis

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Abstract:

Malaria represents a worldwide health concern, contributing significantly to morbidity and mortality rates in both developing and developed countries, as well as underdeveloped ones. Insufficient resources in certain countries result in subpar diagnostic procedures. Therefore, advancements in malaria diagnostics should make it easier to identify people who have malarial parasite infections and to treat such patients with the appropriate medications. This review focuses on malaria diagnosis techniques, both conventional and modern. Due to a scarce of qualified technologists to read and interpret the slides, traditional diagnosis, which is based on the inspection of Leishman-Giemsa stained thick and thin blood smears under a microscope, is inappropriate in many areas. There are currently more sophisticated and reasonably effective diagnostic methods for malaria that are based on fluorescence microscopy or nucleic acid detection, such as PCR. In addition, many malaria-endemic nations lack the necessary equipment and skills for both fluorescent microscopy and nucleic acid detection procedures. This issue is resolved by recently developed technique and approach based on immuno-assays, which are simple to conduct and interpret and don't require complicated equipment or expert assistance. In addition, they are affordable, rapid (10 min/test), and just as sensitive as conventional microscopy.

Keywords: Fluorescent microscopy, Immuno-assay, Leishman-Giemsa stain, Malaria, Mortality, PCR.

Introduction:

Malaria, which comes from the Italian term "malaria," which means "unhealthy air," continues to be a global health issue. *Plasmodium falciparum* (Pf), *Plasmodium vivax* (Pv), *Plasmodium malariae* (Pm), *Plasmodium ovale* (Po), and *Plasmodium knowlesi* (Pk) are the five parasite species that can infect humans. [Ashley E. A et al.2018, Imwong Mallika et al.,2019]

A potentially lethal parasite disease spread by mosquitoes, malaria is responsible for an extensive amount of pain and fatalities worldwide due to its clinical manifestation. P. vivax, which accounts for 75% of infections and is the most prevalent species according to the World Health Organization (WHO), is followed by P. falciparum, which causes more than 90% of malaria deaths worldwide and continues to pose a serious threat to public health. [Imwong Mallika et al.,2019]

There is a global decrease in morbidity and mortality as a result of numerous efforts to eradicate malaria. [Newby Gretchen et al.2016] However, malaria continues to be a serious worldwide health issue, particularly in tropical areas. There has been a rise in imported malaria cases in non-endemic areas including the US and Europe.[Whitty Christopher J M et al.2006] The lack of analytical sensing instruments that enable early and precise detection in asymptomatic persons with low parasitemia levels in peripheral blood has contributed to the persistence of malaria.[Schellenberg J R et al.1994] The World Health Organization (WHO) currently advises that case treatment be directed by looking for Plasmodium antigens or parasites in the peripheral blood of patients who are feverish or carriers who are not exhibiting any symptoms. Nevertheless, the sensitivity of current methods, such as microscopy and rapid diagnostic tests (RDTs), is insufficient to detect parasitemia. Other approaches that perform better but have lower throughput and are more expensive make them unsuitable for everyday use. As a result, reliable diagnostic techniques must be developed for field use, as diagnostic knowledge of malaria is frequently deficient. [Reyburn Hugh et al.2007, Bell David R et

al.2005] Point-of-care assays that offer improvements in all test parameters are the focus of new technologies; these could support laboratory diagnosis in malaria endemic areas with limited resources.

The primary focus of this review is to enhance or create novel diagnostic methods for malaria detection and diagnosis. These include nucleic acid detection methods, biomarker identification strategies, high throughput immunochemical tests, and biosensing methodologies. Rather than just confirming the pathogen's existence, these methods enable the identification of biomarkers unique to a given disease.

Conventional Clinical Diagnosis Techniques

Traditional clinical diagnosis of malaria is the most common, least expensive, most extensively used method. Clinical diagnosis is made on the basis of physical examination findings as well as the signs and symptoms of the patient. The initial signs and symptoms of malaria are highly erratic and ambiguous; they include chills, fever, headache, weakness, myalgia, disorientation, diarrhea, nausea, vomiting, anorexia, and pruritus. Due to the ambiguous character of the signs and symptoms, which greatly overlap with other common as well as potentially fatal diseases, such as common viral or bacterial infections and other febrile illnesses, a clinical diagnosis of malaria is still difficult. The diagnostic specificity of malaria is restricted by its overlapping symptoms with other tropical diseases. This can lead to the indiscriminate prescription of antimalarial medications and lower the standard of care for patients in endemic areas who have fevers other than malaria. Clinical strategies for managing and diagnosing common pediatric illnesses by undertrained healthcare professionals in underdeveloped countries with inadequate equipment for laboratory diagnosis have been made available by the Integrated Management of Pediatric Illness (IMCI). Therefore, integrating clinical and parasite-based data can significantly improve the reliability of malaria diagnosis.

Various Diagnostics Tools:

Currently available diagnostic tools for Plasmodium species identification include fluorescence and light microscopy, serology, Quantitative Buffy-Coat (QBC) concentration, Rapid Detection Techniques (such as immunochromatographic lateral flow assays) [W Chansuda et al. 2007], and nucleic acid amplification methods (such as polymerase chain reaction, or PCR), and isothermal amplification, which have been reviewed in detail elsewhere.[B J Wendi et al. 2013] Using an RDT to identify the causal Plasmodium species by Giemsa-stained microscopy or its antigens is the algorithm for laboratory diagnosis. The choice of treatment can only be made based on clinical diagnosis in children residing in high-transmission areas. The majority of people in this scenario are continuously parasite-positive; malaria may be a coexisting sickness, but it is not the causative agent of the fever. [C Daniel et al.2002]

The gold standard for diagnosing malaria remains microscopy, even with the advancements in diagnostic tools during the last two decades. The WHO states that rapid diagnostic tests (RDTs) or microscopic analysis of Giemsa-stained thin and thick blood smears for Plasmodium sp. identification and parasitemia count must be used in all clinical settings to diagnose malaria. [Malaria. CDC Yellow Book 2024] Within ≤24 hours of the patient's presentation, microscopy should be carried out immediately, and results should be obtained as soon as possible and no later than 2 hours after sample.[Malaria Microscopy manual,2023]

While thin blood films are helpful for speciation—that is, identifying the Plasmodium sp. and the circulating stages of the parasite's life cycle inside the patient's blood—thick blood films are primarily employed to detect the existence of malaria parasites and determine the degree of parasitemia. [Crutcher J.M et al. 2023]

As a proportion of infected red blood cells (RBC), the parasite count can also be determined by microscopically evaluating a well-stained thin blood film in places where the disease is not endemic and where incidents of malaria occur with low parasitemia. [Malaria Microscopy manual, 2023]
Light microscopy has the following benefits: (a) cost effective (b) high sensitivity and results in two hours (c)

identification of Plasmodium species and stage differentiation; and (d) parasitemia count. (e) monitoring drug-induced morphological changes (f) checking for parasites to determine if the plasmodia have been cleared; (g) immediately screening for other blood abnormalities and blood parasites (such as Babesia, Trypanosoma, and Filaria). [Huber J H et al. 2022]

However, microscopy has a number of difficulties. The morphology of all the stages between P. knowlesi and P. malariae cannot be distinguished, and the morphology of the early ring trophozoites between P. knowlesi and P. falciparum cannot be distinguished either.

Sometimes the staining process may miss a lot of parasites, which would diminish the method's sensitivity and result in an inaccurate parasite density count. The method's analytical sensitivity is also limited. [Singh B et al. 2004]

Based on stage identification, microscopy is the only diagnostic method that can show the existence of an active infection; in fact, given the life cycle of human Plasmodia parasites, it can even show "live" parasites. For the laboratory diagnosis of malaria, microscopy is still regarded as the gold standard method.

In order to achieve the fundamental goals of controlling malaria, which range from eradicating mortality and minimizing morbidity to lowering prevalence, all countries at risk of malaria must have access to reliable laboratory diagnostic services, accurate epidemiologic data, and security for potential epidemics. Due to its cost effectivity and ability to distinguish between different malaria species, and capacity of quantification parasites, Giemsa microscopy is considered the best diagnostic tool for controlling malaria. High-quality light-emitting diode (LED) lighting has made microscopy more practical in remote locations. On the other hand, microscopy necessitates skilled, qualified technologists as well as different types of functional infrastructure maintenance in addition to efficient quality control (QC) and quality assurance (QA).

The benefit of having a period of latency between the collection and analysis of blood samples from suspected malaria cases is that "presumptive treatment" at the time of specimen collection is still effective in countries with well-managed Malaria Eradication programs. On the other hand, drug resistance spread to the economies where Malaria Eradication failed, and most of them were unprepared to face the new reality brought on by P. falciparum resistance.

Problem in Microscopy:

At relatively low parasite density, a skilled and knowledgeable microscopist or technologist should be able to accurately identify the Plasmodium species on thick blood films. It may sometimes be needed to examine the thin film for morphologic, differential-diagnostic details, including schizonts and gametes, as well as erythrocyte size, shape, and characteristic dots. The majority of recorded species errors most likely involve identifying sporadic human infections with simian plasmodia like P. knowlesi or distinguishing between P. vivax and P. ovale [Milne L M et al. 1994]. However, underreporting occurs when standard microscopy fails to distinguish P. falciparum from P. vivax, the two most common species [J Stephanie P et al.2006] Infections involving mixed species are frequently underreported. [Bell, David et al.2006]

Diagnosis By PCR:

According to CDC report the nested PCR was more sensitive than microscopy, Plasmodium could be found in instances with low parasitemia and mixed malaria infections. All cases involved the collection of PCR-positive and microscopy-negative material from symptomatic patients who had previously visited malaria endemic areas. Additionally, one mixed infection containing P. falciparum and P. ovale that was overlooked by microscopy was found by nested PCR, which also found three from submitted specimens from cases of telediagnosis. P. vivax and P. ovale could also be differentiated more clearly using PCR. According to the study, 2.2% of samples had their P. vivax or P. ovale identities misidentified using microscopy. [J Stephanie P et al. 2006]

Rapid Diagnostic Test:

In countries with endemic disease, most patients may benefit from rapid diagnostic tests (RDTs) for malaria, particularly in remote areas where there is a lack of trained employees. Nevertheless, there is not enough of data to inform decision-makers about the sensitivity and specificity of these RDTs, particularly from regions where malaria is endemic.

A rapid diagnostic test uses an immunochromatographic technique with monoclonal antibodies that are targeted against the target parasite antigen and impregnated on a test strip to detect malaria antigen in a small volume of blood, typically 5–15 μL. In 5-20 minutes, the result—typically a colourful test band—is developed. RDTs are straightforward to use, easy to read, and don't require any electricity or major expenditure. Compared to the earlier assays, the current RDT test formats—which are either plastic cassettes or cardboard attachments—promote ease of use and safety. RDT usage has increased significantly in recent years, particularly in emerging nations. The majority of RDTs are only able to identify P. falciparum; however, there are RDTs known as MPDA (malaria parasite dual antigen kit) that can differentiate P. falciparum from the three non-falciparum species.

Different target antigen combinations are used in commercial tests to accommodate regional variations in malaria epidemiology. [Bell, D et al.2001] The most often targeted malaria antigen, Histidine-Rich Protein 2 (HRP-2), is unique to P. falciparum. Another significant category of targeted antigens are enzymes known as parasite lactate dehydrogenase (pLDH). Commercially available monoclonal antibodies against pLDH are used to identify P. falciparum, P. vivax, and other Plasmodium species.

Thus, the purpose of this test method was to evaluate the two approaches of microscopy and RDTs for malaria diagnosis.

Materials And Methods:

The materials included syringes, light microscopes with proper visual acuity of 40x and 100x objectives lens, RDT kits, microscopic slides, Leishman and Giemsa stains, and ethylene diamine tetra-acetic acid (EDTA) vials.

Within ten minutes of collection, EDTA samples were processed into thick and thin films. Field's and Giemsa's techniques were used to stain thick films, while Leishman's and diluted Giemsa's techniques were used to stain thin films.

A whole blood sample was used for the antigen-based RDT. Using the RDTs-antibody detection approach, sera were analysed in duplicate to detect antibodies against malaria parasites.

Results:

Numerous studies revealed that it may be understood that the serum approach didn't seem to be a very accurate way to diagnose malaria. Since microscopy became the gold standard, as many samples that yielded negative results from the antigen technique continued to yield negative results from microscopy.

Discussion:

The diagnosis of malaria can be made using four main techniques such as Symptomatic, microscopy, antigen testing, and molecular techniques. In underdeveloped nations, the most common method of diagnosis is symptomatic diagnosis, wherein malaria is first diagnosed only based on symptoms, then using one of the alternative techniques.

It should be remembered, though, that the symptoms of many other illnesses can be strikingly similar to those of malaria, making a diagnosis based solely on symptoms potentially dangerous. Therefore, it is essential to use one of the other, more precise techniques to confirm the clinical diagnosis.

Diagnosing malaria through microscopic examination of blood is the most reliable way. As a result, the malaria parasite is checked for using a microscope on a sample of blood. While less invasive techniques can also be employed with other physiological fluids such as saliva or urine, blood is the preferred fluid due to its higher concentration of the parasite.

As of right now, the World Health Organization's provisional recommendation states that, with the probable exception of children residing in high-prevalence areas and specific other circumstances, parasite-based

identification should be employed in all suspected cases of malaria. [Nandwani, S et al.2005]
Comparing the effectiveness of microscopy with polymerase chain reaction (PCR) in malaria detection is one way to create a quick and precise malaria diagnosis approach. [Moody A.2002] The PCR procedure took approximately 10–11 hours to complete, while microscopy took in the range of 40–45 minutes, according to this study, despite being 96.8% sensitive. Malaria parasites are found in red blood cells by PCR. Because PCR requires electric power, which is quite expensive, it is not a practical option for usage in developing nations. It also takes a long time and fails to advance as quickly as we would want.
The fastest way to identify the presence of the malaria parasite seems to be immunochromatographic examination, which also requires little to no training to the technologist or stuff. [J Stephanie P et al.2006] The immunochromatographic technique depends on liquid moving across a nitrocellulose membrane's surface, based on the extraction of parasite antigen from peripheral blood using monoclonal antibodies which is prepared against malaria antigen target conjugated to gold particles in a mobile phase; alternatively, antigen can be incorporated into cellulose strip to capture the antibody present in the serum or plasma instead of using monoclonal antibodies to capture the antigen.

Currently, HRP-2, plasmodium aldolase, and parasite lactate dehydrogenase (pLDH) are the malaria antigens that RDTs target. According to a different study, these proteins are secreted by Plasmodium species, which explains why RDTs' sensitivity and specificity are evaluated using them. It has been demonstrated that P. falciparum secretes significantly more HRP-2 than HRP-1 and HRP-3, while other Plasmodium species have pLDH and PL-aldolase.
Since P. falciparum is not only the most deadly but also the most prevalent in this region of the world, it is reasonable that the RDTs approach was more sensitive to HRP-2. The gold standard of microscopy supports this viewpoint as well.

Conclusion:

Therefore, in this review it can be concluded that only the antigen based method kits be imported in worldwide mainly the parts of the tropics with malaria endemicity. As a result, it is concluded that the RDT for malaria diagnosis is as reliable as microscopy, but only the antigen based method is suitable in other parts of the tropics where malaria is endemic. Otherwise Microscopical identification by inspecting thick and thick smear is the most reliable but the only requirement is qualified professionals.

REFERENCES:

Ashley, E. A., Pyae Phyo, A., & Woodrow, C. J. (2018). Malaria. *Lancet (London, England)*, *391*(10130), 1608–1621.

Bell, D. R., Jorgensen, P., Christophel, E. M., & Palmer, K. L. (2005). Malaria risk: estimation of the malaria burden. *Nature*, *437*(7056), E3–E5. https://doi.org/10.1038/nature04179

Bell, D., Wongsrichanalai, C., & Barnwell, J. W. (2006). Ensuring quality and access for malaria diagnosis: how can it be achieved? *Nature reviews. Microbiology*, *4*(9), 682–695.

Bell, D., Go, R., Miguel, C., Walker, J., Cacal, L., & Saul, A. (2001). Diagnosis of malaria in a remote area of the Philippines: comparison of techniques and their acceptance by health workers and the community. *Bulletin of the World Health Organization*, *79*(10), 933–941.

Bailey, J. W., Williams, J., Bain, B. J., Parker-Williams, J., Chiodini, P. L., & General Haematology Task Force of the British Committee for Standards in Haematology (2013). Guideline: the laboratory diagnosis of malaria. General Haematology Task Force of the British Committee for Standards in Haematology. *British journal of haematology*, *163*(5), 573–580. https://doi.org/10.1111/bjh.12572

Chandramohan, D., Jaffar, S., & Greenwood, B. (2002). Use of clinical algorithms for diagnosing malaria. *Tropical medicine & international health : TM & IH*, *7*(1), 45–52. https://doi.org/10.1046/j.1365-3156.2002.00827.x

Crutcher J.M., Hoffman S.L. *Medical Microbiology*. 4th ed. University of Texas Medical Branch at Galveston; Galveston, TX, USA: 1996. [(accessed on 13 November 2023)].
Chapter 83. Malaria. Available online: https://www.ncbi.nlm.nih.gov/books/NBK8584/

Huber, J. H., Elliott, M., Koepfli, C., & Perkins, T. A. (2022). The Impact of Emerging Plasmodium knowlesi on Accurate Diagnosis by Light Microscopy: A Systematic Review and Modeling Analysis. *The American journal of tropical medicine and hygiene*, *108*(1), 61–68.

Imwong M, Madmanee W, Suwannasin K, Kunasol C, Peto TJ, Tripura R, von Seidlein L, Nguon C, Davoeung C, Day NPJ, Dondorp AM, White NJ. Asymptomatic Natural Human Infections With the Simian Malaria Parasites Plasmodium cynomolgi and Plasmodium knowlesi. J Infect Dis. 2019;219(5):695-702.

Johnston, S. P., Pieniazek, N. J., Xayavong, M. V., Slemenda, S. B., Wilkins, P. P., & da Silva, A. J. (2006). PCR as a confirmatory technique for laboratory diagnosis of malaria. *Journal of clinical microbiology*, *44*(3), 1087–1089. https://doi.org/10.1128/JCM.44.3.1087-1089.2006

Malaria Microscopy. Quality Assurance Manual. [(accessed on 13 November 2023)].
Available
online: https://www.who.int/docs/default-source/documents/publications/gmp/malaria-micros copy-quality-assurance-manual.pdf

Milne, L. M., Kyi, M. S., Chiodini, P. L., & Warhurst, D. C. (1994). Accuracy of routine laboratory diagnosis of malaria in the United Kingdom. *Journal of clinical pathology*, *47*(8), 740–742. https://doi.org/10.1136/jcp.47.8.740

Malaria. CDC Yellow Book 2024. [(accessed on 13 November 2023)]; Available online: https://wwwnc.cdc.gov/travel/yellowbook/2024/infections-diseases/malaria

Moody A. (2002). Rapid diagnostic tests for malaria parasites. *Clinical microbiology reviews*, *15*(1), 66–78.

Nandwani, S., Mathur, M., & Rawat, S. (2005). Evaluation of the polymerase chain reaction analysis for diagnosis of falciparum malaria in Delhi, India. *Indian journal of medical microbiology*, *23*(3), 176–178. https://doi.org/10.4103/0255-0857.16590

Newby G, Bennett A, Larson E, Cotter C, Shretta R, Phillips AA, Feachem RG. The path to eradication: a progress report on the malaria-eliminating countries. *Lancet*. 2016;387(10029):1775-1784.

Reyburn H, Mbakilwa H, Mwangi R, Mwerinde O, Olomi R, Drakeley C, Whitty CJ. Rapid diagnostic tests compared with malaria microscopy for guiding outpatient treatment of febrile illness in Tanzania: randomised trial. BMJ. 2007;334(7590):403.

Schellenberg JR, Smith T, Alonso PL, Hayes RJ. What is clinical malaria? Finding case definitions for field research in highly endemic areas. Parasitol Today. 1994; 10(11):439-42.

Singh, B., Kim Sung, L., Matusop, A., Radhakrishnan, A., Shamsul, S. S., Cox-Singh, J., Thomas, A., & Conway, D. J. (2004). A large focus of naturally acquired Plasmodium knowlesi infections in human beings. *Lancet (London, England)*, *363*(9414), 1017–1024.

Wongsrichanalai, C., Barcus, M. J., Muth, S., Sutamihardja, A., & Wernsdorfer, W. H. (2007). A review of malaria diagnostic tools: microscopy and rapid diagnostic test (RDT). *The American journal of tropical medicine and hygiene*, *77*(6 Suppl), 119–127.

Whitty CJ, Lalloo D, Ustianowski A. Malaria: an update on treatment of adults in non-endemic countries. BMJ. 2006;333(7561):241-5.