Contents list available at: https://journals.ekb.eg/
Sohag Journal of junior Scientific Researchers
journal homepage: https://sjyr.journals.ekb.eg/
ISSN 2735-5543

Review article

Conventional and recent approaches for diagnosis of malaria

Manal Reyad*

Medical Parasitology Department, Faculty of Medicine, Sohag University, Sohag 82524, Egypt
*Corresponding author: manalreyad@yahoo.com

Article info.

Citation: Reyad M. (2023). Conventional and recent approaches for diagnosis of malaria. ***Sohag Journal of Junior Scientific Researchers***, Vol. **3** (*1*). 18-24.

https://doi.org/10.21608/sjyr.2023.310791

Received: 18/01/2023
Accepted: 23/03/2023
Published: 01/12/2023

Abstract

Malaria is a disease caused by *Plasmodium* species infections. This disease causes significant health threats and death for inhabitants of endemic zones. Malaria is the most important infectious disease in tropical and subtropical regions and continues to be a major global health problem. Plasmodium is transmitted by the bite of an anopheles mosquito. Four species of the pathogen namely: *Plasmodium vivax* (*P. vivax*), *Plasmodium falciparum* (*P. falciparum*), *Plasmodium malariae (p. malariae)*, and *Plasmodium ovale* (*P. ovale*) are highly distributed among malaria-affected regions in the world. In addition, recently discovered *Plasmodium knowlesi*-associated zoonotic form of human malaria long-tailed and pig-tailed macaques. Among the species, *P. falciparum* accounts for almost all of the malaria-associated mortality. A rapid and precise diagnosis of the parasite is very important for the eradication of malaria. Healthcare workers in both endemic and non-endemic settings should be familiar with the latest evidence for the diagnosis. The quality of malaria diagnosis is important in all settings, as misdiagnosis can result in significant morbidity and mortality. Diagnostic methods play essential roles in dealing with the current global malaria situation and decreasing malaria incidence. The aim of this study is to spotlight conventional and recent approaches to the diagnosis of malaria.

Keywords: Approaches, Diagnosis, Malaria.

1. Introduction

Malaria is one of the leading causes of death worldwide. The female Anopheles mosquito serves as a competent vector to transmit the Plasmodium parasite to human hosts with each blood meal. a disease that seems to prevail over strategies used to combat it. It is becoming more challenging through the emergence of antimalarial drug resistance (Mbanefo and Kumar, 2020). Malaria is a febrile illness and clinical symptoms of uncomplicated malaria include fatigue. headaches, muscle aches, malaise, abdominal discomfort, fever, nausea, and vomiting White (2014).

in severe cases severe anemia, respiratory distress in relation to metabolic acidosis, or cerebral malaria (WHO, 2016).

2. Conventional methods in diagnosis of malaria

2.1. Clinical Diagnosis

Clinical Diagnosis is the most practiced method of diagnosing malaria as it is very cheap and rapid (Tangpukdee, 2009). The clinical diagnosis however possesses many problems due to the overlap of malaria symptoms with those of other common infections found in endemic regions such as bacterial infections. This overlap of symptoms with other diseases greatly reduces the specificity of clinical diagnosis (Oladosu and Oyibo, 2013). Clinical diagnosis is most useful in areas where there is no laboratory support, which is often the case in many areas in sub-Saharan Africa (Chandramohan *et al.* 2002). The combination of clinical diagnosis with other parasite-based methods greatly increases the accuracy of malaria diagnosis (Onile-ere *et al.* 2016).

2.2. Microscopy

2.2.1. light microscopy

Conventional microscopic examination of peripheral thick and thin blood smears remains the gold standard for malaria diagnosis (Noppadon *et al.* 2009). It has the advantage of being both qualitative(species, stage identification) and quantitative hence it's preferred use in the monitoring of patient response to treatment. Microscopy, however, falls short in that it requires skilled laboratory personnel for accurate results, maintenance costs, need for a constant supply of electricity. Sampling preparation also greatly affects the sensitivity of this test as the quality of the film, duration of staining, and quality of stain affect the ability to visualize the different stages of the parasite (Tangpukdee *et al.* 2009).

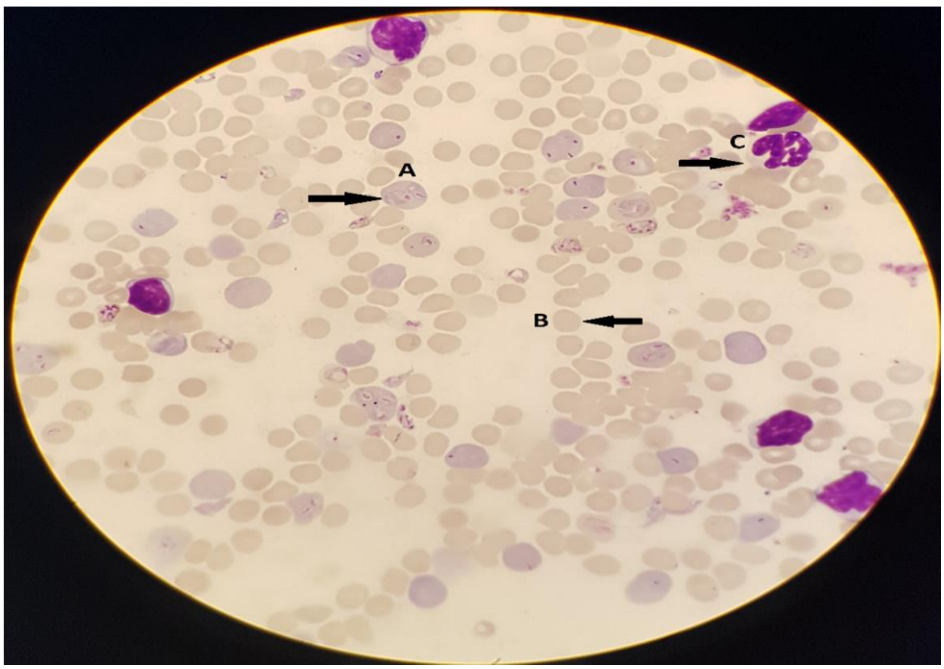

Figure 1. Light Microscopic examination of a thin smear of *P. falciparum*-infected erythrocytes stained with Giemsa. (A) A red blood cell infected with two malaria parasites in the "ring" stage at 100x oil immersion. (B) A normal uninfected red blood cell. (C) A normal leukocyte. Mbanefo and Kumar (2020).

2.3. Fluorescent microscopy

Three techniques using the fluorescence of the parasite for the diagnosis of malaria have been described. The quantitative buffy coat by Baird *et al.* (1995), the Kawamoto acridine orange process by Lowe *et al.* (1996), and the use of benzothiocarboxypurine (BCP) by Cooke *et al.* (1992). Both the QBC and Kawamoto methods use acridine orange (AO) as fluorochrome to stain the nucleic acids of any malaria parasites in the sample. BCP is another fluorochrome that stains nucleic acids. Although AO is a very intense fluorescent stain, it is non-specific and stains nucleic

acids from all cell types. Consequently, the microscopist using AO has to learn to distinguish fluorescent-stained parasites from other cells Delacollett and VanderStuyft (1994).

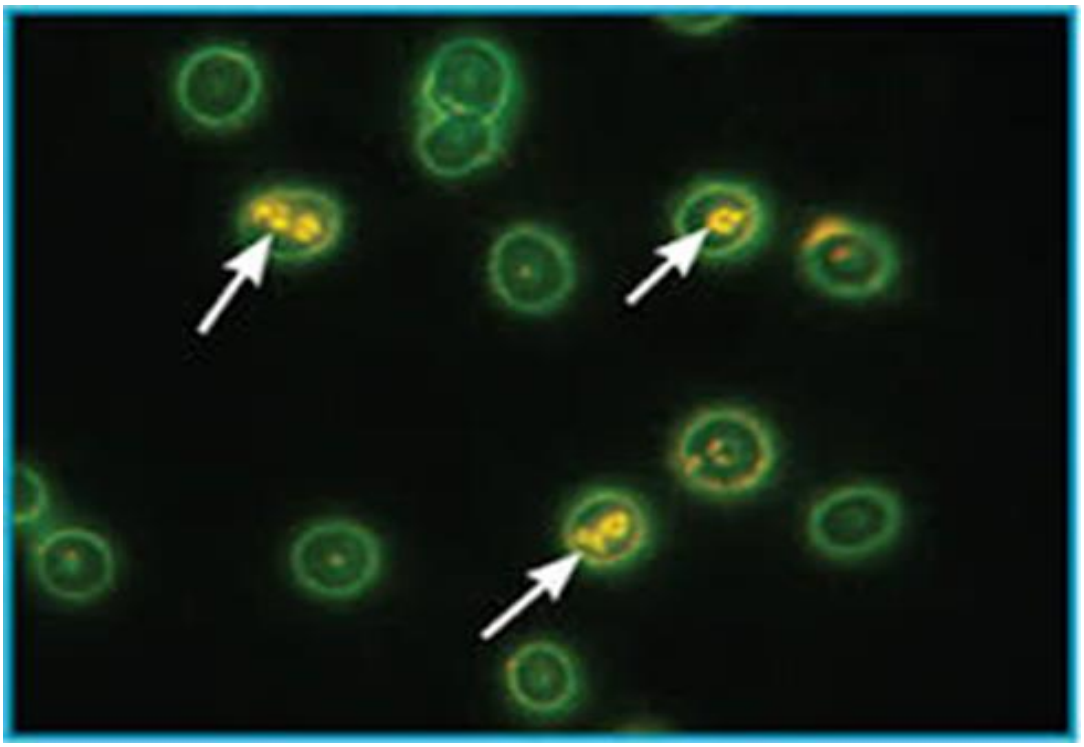

Figure 2. Orange or gold parasites stand out inside green blood cells Thin Film(1000x) Moody (2002).

2.4. Serological test

2.4.1. Antibody detection-Indirect Fluorescence Antibody (IFA).

2.4.2. Antigen detection-Immunochromatographic (ICT) Immunoassays.

2.5. Uses

2.5.1. Screening blood donors in case of transfusion-induced malaria where the donor's parasitemia may be below the detectable level of blood film.

2.5.2. Patient with fever suspected of malaria whose repeated blood smears are negative

2.5.3. Testing patients treated for malaria recently but whose diagnosis is doubtful Igbinosa *et al.* (2010).

2.6. Recent approaches in the diagnosis of malaria

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 World Health Organization (1996).

2.6.1. Rapid diagnostic tests (RDTs)

The field of rapid diagnostic test kits for malaria is almost completely dominated by antigen-binding strips or cassettes. These assays work on the same principles as standard pregnancy test kits Moody (2002). The Rapid Diagnostic Test (RDT) is a very effective tool in malaria diagnosis and forms the mainstay of diagnosis in many resource-poor areas where there is no access to a laboratory. It is especially useful as it requires no electricity, infrastructure, minimal sample preparation, and technical expertise, and interpretation of results is relatively easy. The RDT detects malaria antigens in 5-15 µL of blood using monoclonal antibodies, impregnated on a test strip, specific for the targeted antigens, in an immunochromatographic assay. Test results are interpreted by the absence or presence of a colored line on the strip and can be obtained in 5-20mins.

RDTs detect three plasmodial antigens; *P.falciparum* histidine-rich protein II (PfHRPII), Plasmodium lactate dehydrogenase (pLDH), and aldolase (Nyunt *et al.* 2013)

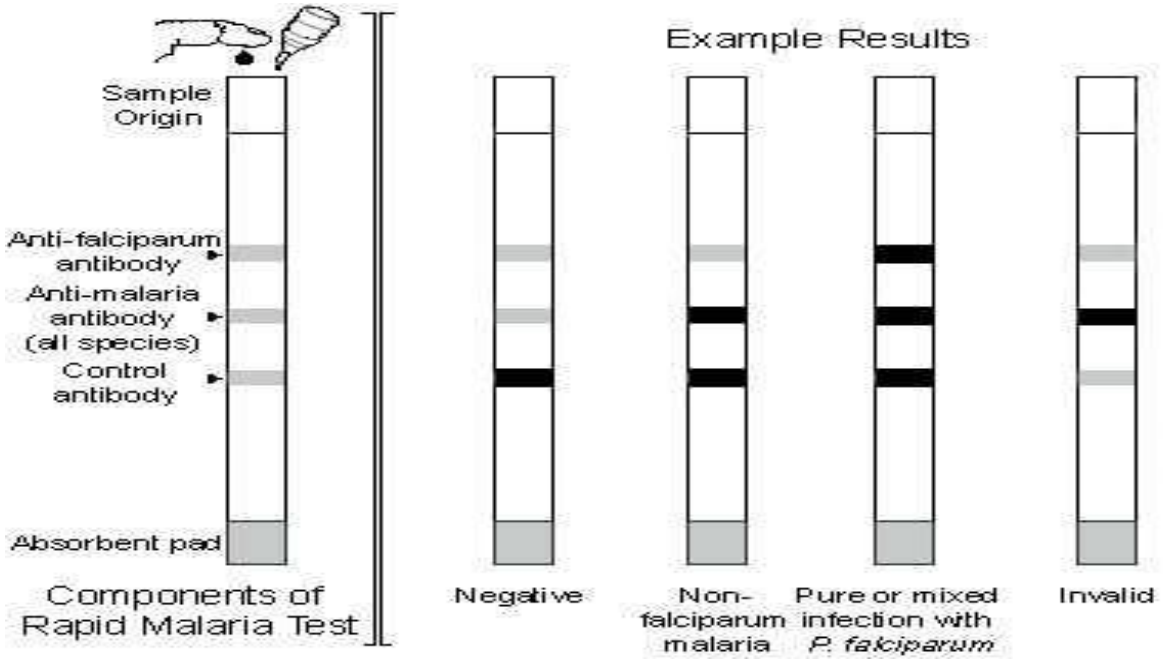

Figure 3. Sample test line configurations of commercial RDTs and their result interpretations Wongsrichanalai *et al.* (2007).

2.7. Molecular methods

2.7.1.1. Polymerase Chain Reaction (PCR)

Highly sensitive, specific, used for confirming uncertain species, detection of drug-resistant markers, and genotyping of *Plasmodium* strains. PCR can detect as few as 1-5 parasites/ul of blood compared with around 50-100 parasites/ul of blood by microscopy (Raghuveer and Mangala, 2012). Using PCR for the diagnosis of malaria is based on the detection of nucleic acid sequences specific to *Plasmodium*. Several PCR assays have been developed for the diagnosis of malaria. The18S rRNA gene has been used as a target for the differentiation of *Plasmodium* species by nested PCR and reverse transcription-PCR (Snounou *et al.* 1993).

A. Loop-mediated isothermal amplification (LAMP) technique

Detects 18S ribosome RNA of *Plasmodium falciparum*. It is easy, sensitive, quick, and cheaper than PCR. However, reagents require cold storage (Han *et al.* 2007). Despite its simplicity of use, the LAMP techniques with other molecular-based diagnostic methods are susceptible to DNA contamination, and sterile precautions should be taken to avoid false-positive results Char (pentier *et al.* 2020).

B. Mass spectrophotometry

Is based on the principle of detecting heme from hemozoin which is a parasite-specific biomarker and has the sensitivity of detecting up to 10 parasites /ul of blood. It comprises a protocol for the cleanup of whole blood samples, followed by direct ultraviolet laser desorption mass spectrometry (LDMS). It requires <1 minute, but not suitable for rural areas where electricity is a problem (Tangpukdee *et al.* 2009).

C. Sysmex XN-31 analyzer

The XN-31 is a fast and reliable screening method in the detection and quantification of *Plasmodium* species in patients However, if an abnormal red blood cell morphology is present, the results of the XN-31 should be interpreted with caution as false positive results can be caused by interfering abnormal erythrocytes (Khartabil *et al.* 2022).

The Sysmex XN-31 hemocytometer is an automated analyzer launched in September 2019 to support malaria diagnosis in whole blood samples in the clinical diagnostic laboratory. Using fluorescence flow cytometry (FFC) technology and a violet semiconductor laser with a 405 nm wavelength, this hemocytometer can detect, specify and quantitate malaria-infected red blood cells (MI-RBC) within a specific area of the scattergram known as the M-gating area. Previous studies on the XN-31 and its predecessor the XN-30 reported mainly data on *P. falciparum*-infected patients in endemic countries (Post *et al.* 2019).

3. Conclusion

Early and accurate diagnosis of malaria is fundamental for successful and timely treatment of the disease, as delay and/or misdiagnosis can result in morbidity and mortality. There are several methods to find out the existence of parasites within the blood. The oldest one is by microscopy, which is still a gold standard Despite conventional techniques still being used in the field, the exploration and field implementation of advanced techniques for the diagnosis of malaria is still being developed rapidly.

References

Baird, J. K., Purnomo, and Jones, T. R. (1995). Diagnosis of malaria in the field by fluorescence microscopy of QBC capillary tubes. Trans R Soc Trop Med Hyg, 86(1):3-5. doi: 10.1016/0035- 9203(92)90412-6. PMID: 1566296.

Chandramohan, D., Jaffar, S. and Greenwood, B. (2002). Use of clinical algorithms for diagnosing malaria. Trop. Med. Int. Heal, 7(1), 45–52.

Charpentier, E., Benichou, E., Pagès A., Chauvin, P., Fillaux, J., Valentin, A., *et al.* (2020). Performance evaluation of different strategies based on microscopy techniques, rapid diagnostic tests, and molecular loop-mediated isothermal amplification assay for the diagnosis of imported malaria. Clin Microbiol Infect,26(1):115-121. doi: 10.1016/j.cmi.2019.05. 010.. PMID: 31158521.

Cooke, A. H., Morris-Jones, S., Horton, J., Greenwood, BM., Moody, AH. and Chiodini, PL. (1993). Evaluation of benzothiocarboxypurine for malaria diagnosis in an endemic area. Trans R Soc *Trop Med Hyg, 87*(5):549. doi: 10.1016/0035-9203(93)90082-2. PMID: 8266405.

Delacollette, C. and Van der Stuyft, P. (1994). Direct acridine orange staining is not a 'miracle' solution to the problem of malaria diagnosis in the field. Transactions of the Royal Society of *Tropical Medicine and Hygiene*, 88,187-188.

Han, E. T., Watanabe, R., Sattabongkot, J., Khuntirat, B., Sirichaisinthop, J., Iriko, H., *et al.* (2007). Detection of four *Plasmodium* species by genus and species-specific loop mediated-isothermal amplification for clinical diagnosis. J Clin Microbiol, 45,2521-2528.

Igbinosa, O., Osaser,e A. and Chenyi, J. (2010). A sequential review on the accuracy of detecting malaria parasitemia in developing countries with restriction on resources. J. Med. Med. Sci,1(9)385-390.

Khartabil, T. A., de Rijke, Y. B., Koelewijn, R., Jaap, J. van Helmond, and Henk Russcher (2022). Fast detection and quantification of *Plasmodium* species infected erythrocytes in a non-endemic region by using the Sysmex XN-31 analyzer. *Malar J.* , 21, 119 https://doi.org/10.1186/s12936-022-04147-0.

Lowe, B. S., Jeffa, N. K., New, L., Pedersen, C., Engbaek, K. and Marsh, K. (1996). Acridine orange fluorescence techniques as alternatives to traditional Giemsa staining for the diagnosis of malaria in developing countries. Trans R Soc Trop Med Hyg., 90 (1):34-36. doi: 10.1016/s0035-9203(96)90470-8.

Mbanefo, A. and Kumar, N. (2020). Evaluation of Malaria Diagnostic Methods as a Key for Successful Control and Elimination Programs. Tropical medicine and infectious disease, 5 (2), 102. https://doi.org/10.3390/tropicalmed5020102. PMID: 32575405; PMCID: PMC7344938.

Moody, A. (2002). Rapid diagnostic tests for malaria parasites. *Clin Microbiol Rev*, 15: 66-78.

Nyunt, M. H., Kyaw, M. P., Win, K. K., Myint, K. M. and Nyunt, K. M. (2013). Field evaluation of HRP2 and pan pLDH-based immunochromatographic assay in therapeutic monitoring of uncomplicated falciparum malaria in Myanmar. *Malaria Journal*, 12, 123. DOI: 10.1186/1475-2875-12-123. PMID: 23577630; PMCID: PMC3636062.

Oladipo, O. and Wellington, A. (2013). Overdiagnosis and Overtreatment of Malaria in Children That Presented with Fever in Lagos, Nigeria," ISRN *Infect. Dis,* 1–6.

Onile-ere, O., John, O. and Grace, O. (2016). Malaria Diagnosis: Current Approaches and Future Prospects. 3rd International Conference on African Development Issues.ISSN:2449-075X.

Post, A., Kabore, B., Reuling, I. J., Bognini, J., van der Heijden, W., Diallo, S., *et al.* (2019); The XN-30 hematology analyzer for rapid sensitive detection of malaria: a diagnostic accuracy study. *BMC Med*, 17, 103.

Raghuveer, C. V. and Goneppanavar, M. (2012). Laboratory diagnosis of malaria, a review. *Journal of Evolution of Medical and Dental Sciences, 1*, 453-462.

Snounou, G., Viriyakosol, S., Jarra, W., Thaithong, S. and Brown, K. N. (1993). Identification of the four human malarial species in field samples by the polymerase chain reaction and detection of a high prevalence of mixed infections. *Molecular and Biochemical Parasitology*, 58, 283-292.

Tangpukdee, N., Duangdee, C., Wilairatana, P. and Krudsood, S. (2009) Malaria diagnosis: a brief review. Korean J Parasitol, 47 (2), 93-102. doi: 10.3347/kjp.2009.47.2.93. PMID: 19488414; PMCID: PMC2688806.

White, N. J., Pukrittayakamee, S., Hien, T. T., Faiz, MA., Mokuolu, O. A. and Dondorp, A. M. (2014). Malaria. Lancet, 22, 723-35. doi: 10.1016/S0140-6736(13)60024-0. PMID: 23953767.

World Health Organization (1996). information consultation on recent advances in diagnostic techniques and vaccines for malaria: a rapid dipstick antigen capture assay for the diagnosis of falciparum malaria. Bull World Health Organ, 74, 47-54.

World Health Organization (2009). Parasitological confirmation of malaria diagnosis. Geneva: World Health Organization.

World Health Organization (2016). "Malaria,". (Online). Available: http://www.who.int/media-centre/factsheets/fs094/en/. (Accessed: 25-Mar-2016).

Wongsrichanalai, C., Barcus, M. J., Muth, S., Sutamihardja, A., andWernsdorfer, W. H. (2007). A review of malaria diagnostic tools: microscopy and rapid diagnostic test (RDT). Am J Trop Med Hyg,77 (6) ,119-27. PMID: 18165483.

مقال مرجعي

الملخص العربي

الأساليب التقليدية والحديثة لتشخيص الملاريا

منال رياض*

قسم الطفيليات الطبيه، كلية الطب البشري، جامعة سوهاج، سوهاج ٨٢٥٢٤، مصر
المؤلف المختص: manalreyad@yahoo.com

ملخص البحث

الملاريا مرض تسببه أنواع العدوى المتصورة. يسبب هذا المرض تهديدات صحية كبيرة وموت سكان المناطق الموبوءة. الملاريا هي أهم الأمراض المعدية في المناطق الاستوائية وشبه الاستوائية ، ولا تزال مشكلة صحية عالمية كبرى. ينتقل المتصورة عن طريق لدغة بعوضة الأنوفيلة. أربعة أنواع من المرض : المتصورة النشيطة ، المتصورة المنجلية ، الملاريا المتصورة .المتصورة البيضوية منتشرة بشكل كبير بين المناطق المصابة بالملاريا في العالم. بالإضافة إلى ذلك ، اكتشف مؤخرًا شكل حيواني المنشأ المرتبط من الملاريا البشرية طويلة الذيل وذيل الخنازير. من بين الأنواع ، المتصورة المنجلية مسؤولة عن جميع الوفيات المرتبطة بالملاريا تقريبًا. إن التشخيص السريع والدقيق للطفيلي مهم للغاية للقضاء على الملاريا. يجب أن يكون العاملون في الرعاية الصحية في كل من البيئات المتوطنة وغير المتوطنة على دراية بأحدث الأدلة للتشخيص. تعد جودة تشخيص الملاريا مهمة في جميع الظروف ، حيث يمكن أن يؤدي خطأ التشخيص الخاطئ إلى انتشار المرض وحدوث وفيات كبيرة. تلعب طرق التشخيص أدوارًا أساسية في التعامل مع الوضع العالمي الحالي للملاريا وتقليل حدوث الملاريا.

الكلمات الرئيسية: الأساليب، التشخيص، الملاريا.