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Rapid Diagnostic Tests in Malaria Diagnosis and Discordance between RDT and Microscopy: A Review

Bhattacharyya S*, Dan A, Dey K and Sadhukhan G

Associate Professor, Microbiology, AIIH&PH, Kolkata, India

ABSTRACT

Malaria can be diagnosed with microscopy as well as by Rapid diagnostic tests or RDTs. RDTs may miss low level of parasitemia and in many cases, may also be false positive. RDTs can detect various antigens in different stages of the malaria parasite. Sometimes stained smear may be positive but RDT may appear to be negative, owing to low parasite count. The reverse is also true in many cases, where gene deletions or antigenic persistence occur. This is important from the viewpoint of applied immunology. All these interesting aspects have been reviewed here.

***Corresponding author**

Bhattacharyya S, Associate Professor, Microbiology, AIIH&PH, Kolkata, India.

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Introduction

Malaria remains a major global health burden, particularly in tropical and subtropical regions, from 40 N to 60 S latitudes. Globally in 2023, there were an estimated 263 million malaria cases and 597000 malaria deaths in 83 countries [1]. There are 5 known causative species of Plasmodium producing Malari ain man, namely P. vivax, P. falciparum, P. ovale, P. malariae and P. knowlesi [2].

It is a killer disease where microscopy as well as rapid antigen tests are mainstay for diagnosis. Microscopy and rapid diagnostic tests (RDTs) are two principal diagnostic methods widely used in endemic areas. Light microscopy is the diagnostic standard against which other diagnostic modalities have traditionally been compared [3]. While microscopy is the gold standard for malaria diagnosis, RDTs offer simplicity and speed. However, diagnostic discordance between these methods can occur, notably in cases where blood smear microscopy is positive and RDT is negative (Smear+/RDT−), or RDT is positive and smear is negative (RDT+/Smear−). This review explores the causes, implications, and management strategies for such discordance. Understanding these diagnostic inconsistencies is essential for improving malaria detection, guiding treatment, and preventing misdiagnosis.

Epidemiology

Malaria, caused by Plasmodium spp., affects over 240 million individuals annually and causes over 600,000 deaths annually, predominantly in sub-Saharan Africa. The cases and deaths are concentrated in Africa and in young children, but a large chunk of the world's population remain at risk of infection [4]. Prompt and accurate diagnosis is critical for effective treatment and control. The World Health Organization (WHO) recommends parasitological confirmation of malaria before treatment through either light microscopy or rapid diagnostic tests (RDTs).

Microscopy detects Plasmodium parasites in Giemsa or Leishman-stained blood films, providing species identification and parasite density quantification. However it needs trained eyes and a good binocular microscope, which can be costly. RDTs are lateral flow tests or Immunochromatographic tests which detect parasite antigens (like histidine-rich protein 2 [HRP2] or lactate dehydrogenase [pLDH]) and are ideal for resource-limited settings due to ease of use and quick results and even no need of electricity [5]. Some other tests are also there based on Microscopy, like quantitiative buffy coat (QBC) test and Acridine orange test that detect parasite DNA inside RBCs by fluorescence. Other antibody-based tests are also there to detect malaria, like ELISA using different malarial antigens. Figure 1: Below Shows an Image of Rapid Antigen Test.

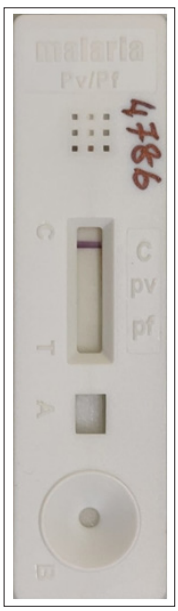

Figure 1: Dual Antigen Test for Malaria (Original Image of Authors).

However, diagnostic discordance challenges clinical decision-making. This review hence focuses on:

- Smear positive / RDT negative (Smear+/RDT−) malaria
- RDT positive / Smear negative (RDT+/Smear−) malaria

It is important to examine their causes, clinical implications, diagnostic pitfalls, and recommendations.

Type and Nature of Antibodies Against the Malaria Antigens

- 2. Overview of Diagnostic Modalities
- 2.1 Microscopy

Microscopy remains the gold standard in malaria diagnosis. Thick films improve chances of parasite detection, while thin films aid in accurate species identification.

Advantages of a well-made smear include:

- Detection of all Plasmodium species
- Quantification of parasitemia
- Evaluation of treatment efficacy

Limitations include the need for:

- a. Skilled technicians, Quality staining,
- b. Proper equipment like microscope and slide preparation

Rapid Diagnostic Tests (RDTs)

RDTs detect circulating parasite antigens like LDH, Aldolase and HRP-2 (Histidine rich protein). They use nitrocellulose strips that are tagged with monoclonal antibodies against these parasitic antigens. The monoclonal antibodies are also bound to colloidal gold. When they react with parasitic antigens, a visible band appears due to clustering of the gold nanoparticle. The control band should come positive always, since it has antibodies against human IgG. There is also a slot to put buffer. RDTs permit a more or less reliable detection of malaria infections particularly in remote areas with limited access to good quality microscopy services [6].

HRP2

This antigen is specific to P. falciparum and is found on the knobs developing in late trophozoite stages of the parasites.

pLDH and Aldolase

They are pan-Plasmodium markers or species-specific targets. LDH can even be P. vivax-specific and P. falciparum specific.

Advantages

- Fast, user-friendly, no need for electricity
- Useful in peripheral or field settings

Limitations

- Variability in sensitivity
- Low sensitivity if parasite count below 160 per microliter of blood.
- False negatives/positives
- Inability to quantify parasitemia

Causes of Smear Positive / RDT Negative (Smear+/RDT−) Malaria

HRP2 Gene Deletion

- *P. falciparum* parasites lacking the pfhrp2 gene do not express HRP2, leading to false-negative HRP2-based RDTs.

Low Antigen Levels

- In early infections, antigen levels may be below detection thresholds despite visible parasites.

Prozone Effect

Very high antigen concentrations can saturate antibodies in RDTs, blocking signal formation.

d.Non-HRP2 Species:

- RDTs targeting only HRP2 will miss non-falciparum species like P. ovale, P. malariae, or P. knowlesi.

RDT Storage/Degradation

- Heat or humidity can degrade test performance.

Antigen-Antibody Interference

- Immune complexes or antibodies may bind antigens, hindering their detection.

Clinical and Public Health Implications of RDT Negative Malaria

- **Underdiagnosis and delayed treatment:** Too much of reliance on RDT may miss many smear-positive cases and this delays timely treatment.

- **Misinterpretation of RDTs as superior to microscopy:** RDT is in no way superior to microscopy, and in many cases, both have to be done for better interpretation. In fact, it is advisable to confirm RDT positive cases by microscopy.

- **Risk of transmission due to untreated infections:** Missed cases or false negative cases may transmit infection to vulnerable population.

- **Underestimation of malaria prevalence in surveillance:** RDTs may miss low parasitemia cases and this may miss diagnosis. A falsely low prevalence may be reported.

Several RDTs are there in the market, like kit for detecting P. vivax and P. falciparum as separate bands, or kit for detecting P. falciparum only (using HRP-2 antigen). Microscopy is still regarded as the traditional diagnostic standard of care, since it provides the diagnostic capability to include speciation, quantitative assessment of parasitemia, and also generates evidence on treatment responsiveness [7]. Thus, the US Center for Disease Control stance on RDTs is that it "does not remove the requirement for microscopy in malaria diagnostics" [8]. Kits looking for dual antigens are also good, like the BinaxNOW kit. The BinaxNOW Malaria test is an *in vitro* immunochromatographic test for the qualitative detection of *Plasmodium antigens* circulating in human venous and capillary blood of individuals with signs and symptoms of malaria. The test targets the histidine-rich protein II (HRP-II) antigen specific to *Plasmodium falciparum* (P.f.) and a pan-malarial antigen which is common to all 4 common malaria species capable of infecting humans - *P. falciparum, P*. vivax (P.v.), *P. ovale* (P.o.), and *P. malariae* (P.m.) [9].

RDTs for Detecting P. Knowlesi

Although no specific P. knowlesi RDTs have been devised till date, cross-reactivity between certain pLDH epitopes for *P. falciparum* and *P. vivax* with a portion of *P. knowlesi* parasites which also express these epitopes allows their detection, with sensitivity associated with the degree of binding affinity. In addition, tests targeting non-specific Plasmodium species pLDH or aldolase enzyme are also able to detect *P. knowlesi* [10].

Case Studies

Several studies in Africa and Asia have documented Smear+/RDT− cases, particularly in areas with high prevalence of HRP2 deletions (like Eritrea, Ethiopia and India). In fact, studies have documented that Pfhrp-2 gene deletions have been seen in Odisha and Gujarat in India, which lead to false negative results in RDT [11]. Also, these antigens like HRP-2 are found in peripheral circulation of P. falciparum infected patients and detected by RDTs, which is tagged with nitrocellulose membrane where monoclonal anti-HPR2 antibody is coated. Although this antibody is supposed to detect mainly HRP2 antigen, structural motif similarity between HRP2 and HRP3 contribute to cross reactivity of the antibody with HRP3. This is also another factor to ponder upon [12].

- **Conditions where microscopy is positive but antigen test is negative:** In low parasitemia, microscopy may find some results but rapid antigen test will be negative.
- **Conditions where microscopy is negative but antigen test is positive:** In case of P. falciparum, the HRP-2 antigen may persist in blood till 6 months after parasitological cure. This may yield a false positive result in rapid antigen test.

Discussion

Malaria is an age-old disease that continues to take many lives every year. Early diagnosis and treatment are essential for malaria control. RDT for malaria detects antibodies to different parasitic antigens. RDT is advantageous in many ways, like ease to do and yield of quick results. However, it only serves as an indicator of malaria. In symptomatic individuals it may be regarded as confirmatory.IN most cases, smear is still the gold standard. However, RDT is a good and reliable immunological method for screening malaria in endemic areas, where microscopy may be difficult, especially in remote settings. Various types of RDTs are there, but their results should be dealt with caution. These aspects of immunological diagnosis of malaria are quite intriguing and interesting, and warrant more scientific attention and research.

Conclusion

Antibody-based tests or immunochromatographic tests are often very useful in diagnosis of malaria. They may suffer from lack of sensitivity and specificity. More and more advancements are taking place in rapid diagnostics of malaria. Microscopy needs to be combined with RDT to ensure a more accurate diagnosis of malaria. Clinicians, Microbiologists and Immunologists must be well-versed with these things.

RDT is advised in field settings while microscopy is usually carried out in referral centres. Health programs do not recommend combining microscopy and RDT, but RDT negative symptomatic cases may be confirmed by microscopy

Microscopy is done from Primary Health Centre upwards. NVBDCP does recommend that if one of the tests are positive then it can be treated as Malaria. However, in the present scenario many of the RDT negative cases are suffering from malaria. So, in this scenario we recommend a change in the programme directive. Symptomatic but RDT negative cases have to be tested by microscopy. This will prevent transmission of malaria.

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