

## Diagnostic Performance of Histidine Rich Protein-2 and Parasite Lactate Dehydrogenase Based Rapid Diagnostic Tests for Detection of Malaria Parasites

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**Abstract:** AIM: Malaria remains as a life-threatening parasitic disease in Indian scenario and poses a great diagnostic challenge. RDTs have emerged as an alternative diagnostic tool, providing a giant leap in malaria diagnosis curtailing the disadvantages of microscopy in rural endemic areas. This study aims to assess the performance of two commercially available bivalent RDTs based on HRP-2 and pLDH with microscopy of blood films as diagnostic tool for malaria detection.

**METHODS:** Peripheral blood was taken from a total of 5,456 patients, presenting with fever, chills and rigor with profuse sweating and the JSB stained smears were examined for malaria parasites. Simultaneously, venous blood was tested by two different commercially available RDTs, AlereTrueline Malaria Ag P.f/Pan and Advantage Malcard Malaria Ag P.f/Pan.

**RESULT:** A total of 52 samples were found to be positive for malaria parasites by smear microscopy. Advantage Malcard and AlereTrueline picked up all falciparum malaria cases. Advantage Malcard was unable to detect only one case of non-falciparum malaria while AlereTrueline missed five of them. The analysis of results revealed sensitivity for Advantage Mal card (98.08%) to be higher than AlereTrueline (90.38%).

**CONCLUSION:** RDTs would be a better alternative diagnostic tool for malaria detection, especially for remote areas where smear microscopy is hard to establish. An ideal RDT with good diagnostic performances will play a pivotal role in speedy identification of malaria, in all levels of health care in near future.

**Key Words:** Malaria, Smear microscopy, Rapid Diagnostic Tests

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### I. Introduction

Malaria remains as a life-threatening parasitic disease in Indian scenario and poses a great diagnostic challenge<sup>1</sup>. WHO recommends that every suspected case of malaria needs to be confirmed by conventional smear microscopy or Rapid Diagnostic Tests (RDTs) before treatment<sup>2</sup>. For decades, malaria is detected traditionally by light microscopy of thick and thin Giemsa stained peripheral blood smears, which is considered as gold standard<sup>3</sup>. This method detects even 0.001% parasitemia and also identifies the species in 98%, if observed by competent persons<sup>4</sup>. It is inexpensive, relatively sensitive and easy to establish in limited resource settings but is very laborious and needs considerable expertise for correct interpretation<sup>5</sup>.

The proportion of malaria suspected cases confirmed by parasite-based test has shown a steady increase with the introduction of RDTs and has accounted for 74% among various methods for diagnostic testing<sup>2</sup>. RDTs have emerged as an alternative diagnostic tool, providing a giant leap in malaria diagnosis curtailing the disadvantages of microscopy in rural endemic areas. These immunochromatographic based tests detect *Plasmodium* antigen like Histidine Rich Protein-2 (HRP-2) or enzymes like parasite lactate dehydrogenase (pLDH) and aldolase<sup>4</sup>. HRP-2 is a surface-exposed protein complex and is produced by the asexual stages and young gametocytes of *P.falciparum*<sup>6</sup> and the enzyme of glycolytic pathway pLDH, by both sexual and asexual stages of all malaria parasites<sup>7</sup>.

RDTs are quick to perform; interpretation is easy; carried out by minimal technical expertise and stable under operational conditions<sup>8, 9</sup>. They are of relatively high cost and some of these tests are unable to differentiate individual *Plasmodium* species<sup>10</sup>. Significant variation in the performances of these tests due to several factors like different field conditions, parasite species, disease prevalence, and low parasite density has led to erroneous results and confusion<sup>11, 12</sup>. This study aims to assess the performance of two commercially available bivalent RDTs based on HRP-2 and pLDH with microscopy of blood films as malaria diagnostic tool

in a tertiary care teaching hospital of Kanyakumari district, Tamil Nadu where both *P.vivax* and *P.falciparum* are co-endemic.

## II. Materials and Methods

This Cross-sectional study was conducted at Department of Microbiology, Kanyakumari Government Medical College Hospital, from February 2017 to January 2018. A total of 5,456 patients, presenting with fever, chills and rigor with profuse sweating and other symptoms suggestive of malaria except pregnant women, referred by various clinicians were included in the study after obtaining informed written consent. A filled in proforma was obtained from all patients and the study was approved by the Institutional Ethical Committee.

**JSB staining:** The peripheral blood taken from all patients was prepared for thin and thick blood smears and staining done by JSB method as per standard protocol. Blood films were examined independently by two expert microscopists each blinded to the results of the other. In case of any discrepancies, opinion of a third expert microscopist was taken. Thick and thin smears were declared negative if no parasites were observed in 100 and 200 oil immersion fields respectively.

**RDTs:** About 1ml of venous blood with EDTA was taken and tested simultaneously by two different commercially available bivalent RDTs. The two RDTs used in this study were AlereTrueline Malaria Ag P.f/Pan (Alere Medical Pvt. Ltd., Gurgaon, India) and Advantage Malcard Malaria Ag P.f/Pan (J. Mitra & Co. Pvt. Ltd., New Delhi, India). The tests were performed and results interpreted as per manufacturer's instructions. These two RDTs differentiated falciparum and non-falciparum malaria.

**Falciparum malaria:** This was based on the presence of 2 bands (C and F) or by 3 bands (C, F and P) and was interpreted as falciparum malaria (including mixed infection by falciparum and other species).

**Non-falciparum malaria:** This was based on the presence of 2 bands (C and P).

**Negative:** Presence of only C band was interpreted as negative.

**Invalid:** Presence of F and/or P bands with no C band made the result invalid and test was repeated again.

**Data analysis:** The results of smear microscopy and RDTs were matched by an independent expert who had been blinded and the result of smear microscopy was taken as the gold standard for comparing the performance of the two RDTs used in this study. All the statistical analysis was performed by SPSS version 21. All the calculated parameters were based on 95% Confidence Interval (CI).

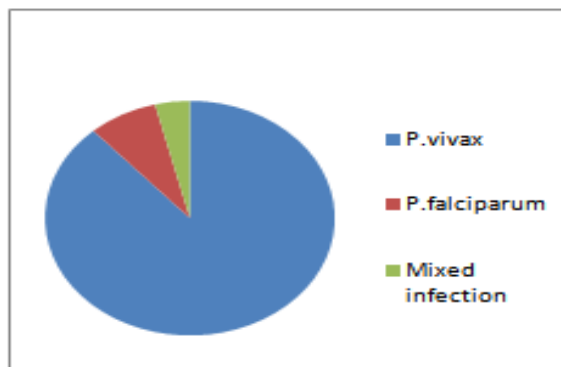
## III. Results

During the one year duration, 5,456 blood samples were screened for malaria parasites among JSB stained smears by light microscopy. A total of 52(0.95%) samples were found to be positive for malaria parasites while the remaining 5,404(99.05%) of them, were negative. Out of 52 blood smear positive samples, 46 (88 %) were infected with *P.vivax* (*P.v*), 4(8%) with *P.falciparum* (*P.f*) and 2 (4%) showed mixed infections for *P.f* and *P.v*(Figure.1).

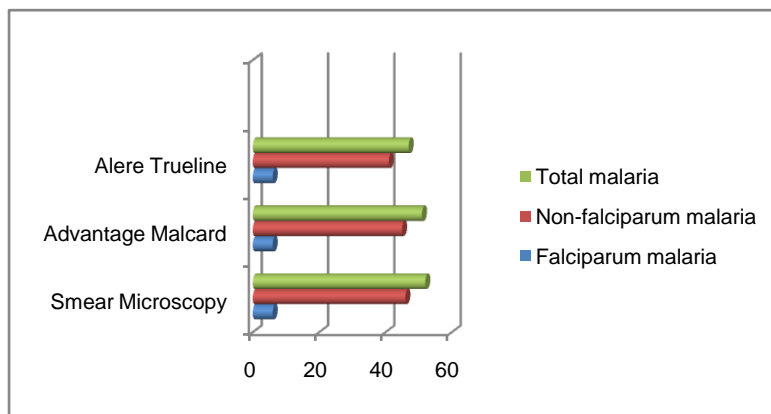
The RDTs, Advantage Malcard and AlereTrueline picked up all falciparum malaria cases. Advantage Malcard was unable to detect only one case of non-falciparum malaria while AlereTrueline missed five of them (Figure.2). Mixed infections (*P.f/P.v*) were identified as only falciparum malaria by above two RDTs.

The analysis of results revealed sensitivity for Advantage Mal card (98.08%) to be higher than AlereTrueline (90.38%) (Table.1).The specificity and Positive Predictive Value (PPV) approaches 100% and the Negative Predictive Value (NPV) nearly 99.9% (99.98% for Advantage Mal card and 99.91% for AlereTrueline) for both the RDTs.

**Figure.1**Total malaria positives by Smear microscopy



**Figure.2**Comparative evaluation of various tests for Malaria detection



**Table.1** Performance characteristics of RDTs

	Advantage Mal Card	AlereTrueline
Sensitivity (%)	98.08	90.38
(95% CI)	(89.74-99.95)	(78.97-96.80)
Specificity (%)	100.00	100.00
(95% CI)	(99.93-100.00)	(99.93-100.00)
PPV (%)	100.00	100.00
(95% CI)		
NPV (%)	99.98	99.91
(95% CI)	(99.87-100.00)	(99.79-99.96)

#### IV. Discussion

Since malaria is a potentially fatal disease, rapid and accurate methods are vital for its earlier identification. Now-a-days, though with more technical improvements, smear microscopy still remains the backbone for diagnosis of malaria. The present study had evaluated the performance of two different bivalent RDTs with smear microscopy for detection of malaria parasites from suspected patients. According to smear microscopy, non-falciparum were predominant than falciparum malaria. This study confirms the co-existence of both *P.vivax* and *P.falciparum* in the district<sup>13</sup>.

Some smear positives for non-falciparum malaria have been missed by both RDTs. The samples which are negative for non-falciparum may also be due to dead parasites in the blood since pLDH is produced by living malarial parasites only<sup>14</sup>. Missing of malaria positive cases by RDTs, may also be due early presentation of the patient to hospital, during this time sufficient level of enzymes wouldn't have been attained for a positive test and may be well below the detection limit of the kits employed<sup>15</sup>.

In the present study, the sensitivity and specificity of Advantage Malcard was 98.08% and 100%. This is in concurrence to the observation by Sushilet *al*<sup>16</sup>. They had a sensitivity of 97% for Advantage Malcard. But Jitendraet *al*<sup>17</sup> had a sensitivity and specificity of 91.67% and 85.42% respectively for this RDT in malaria detection.

The analysis of the results obtained showed a better performance of Advantage Malcard in detecting both falciparum and non-falciparum malaria. Advantage Malcard has detected non-falciparum cases approximately in close proximity to smear microscopy than AlereTrueline. Both the RDTs are effective in detecting falciparum malaria, as they pose more threat among healthcare personals.

#### V. Conclusion

RDTs would be a better alternative diagnostic tool for malaria detection, especially for remote areas where smear microscopy is hard to establish. An ideal RDT with good diagnostic performances will play a pivotal role in speedy identification of malaria, in all levels of health care in near future.

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