

## Comparative Evaluation of Available Immune-Chromatographic Tests Used for Detection of Plasmodium Species, A Tertiary Care Hospital Experience

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### ABSTRACT

**Objective:** To test the sensitivity of locally available strips for the detection of Plasmodium Vivax and Plasmodium Falciparum species at a tertiary care hospital.

**Study Design:** Cross-sectional study.

**Place and Duration of Study:** Armed Forces Institute of Transfusion, Rawalpindi Pakistan, done over one week during Nov 2021.

**Methodology:** Four different commercially available brands of Malaria Rapid Diagnostic Tests were taken. They were tested against venous blood sample taken from only one patient who had lab detected Malaria. Light microscopy showed trophozoites of both Plasmodium Vivax and Falciparum. Nine serial dilutions of different strengths were made from blood sample. These dilutions were tested against commercially available four different Rapid Diagnostic Tests strips.

**Results:** Malaria Rapid Diagnostic Tests kits from all the four brands which were commercially available showed positive results for Plasmodium vivax up to 1:512 dilutions. However, only one brand Rapid Diagnostic Tests kits showed positive results with Plasmodium falciparum in 1:2 dilutions, in addition to Plasmodium vivax.

**Conclusion:** The commercially available Rapid Diagnostic Tests Immunochromatographic Technique have high sensitivity in diagnosing Malaria, but are not resolute when it comes to speciation, particularly for Plasmodium falciparum.

**Keywords:** Immunochromatographic technique, Malaria rapid diagnostic tests, Plasmodium falciparum, Plasmodium vivax

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### INTRODUCTION

Malaria is a major health concern, with around 229 million cases reported worldwide.<sup>1</sup> About 409,000 deaths have been reported globally due to Malaria in 2019, majority of them were reported from sub-Saharan Africa.<sup>2</sup> Children and pregnant women are the most vulnerable group among those who have died.<sup>3</sup> Malaria can lead to hematological and cerebral complications, particularly with falciparum species.<sup>4,5</sup> The first step toward effective Malaria management is a prompt and accurate diagnosis. According to recent WHO recommendations, all cases with Malarial suspicion should be screened with laboratory based microscopic diagnosis as well as rapid diagnostic tests before starting the treatment.<sup>6</sup> According to World Malaria Report published in 2019, the percentage of Malaria suspected patients tested positive with RDT, or microscopy increased, from 36% in 2010 up to 84% in 2018. Various diagnostic procedures are used in labs for accurate Malaria detection, which include microscopic examination, hematological analyzers, immune-

florescence technique, immune-chromatographic testing (ICT) and PCR.<sup>7</sup> Gold standard approach for the diagnosis of Malaria has been microscopic examination of thick and thin blood films stained with Giemsa stain.<sup>8,9</sup> However, in the event of non-availability of reliable microscopy, a number of quick diagnostic techniques have recently been established. One of the established approaches for the quick diagnosis of Malaria is immunochromatographic technology (ICT), as an alternative to light microscopy.<sup>10</sup> Only P. falciparum produces the HRP2 protein, whereas other Plasmodium species generate LDH and aldolase. Commercially available dipstick format kits for detecting various Malaria antigens with good sensitivity and specificity are available. Different studies have been conducted to compare performance efficacy of RDTs and microscopy worldwide. However, no proper study has been done to compare the sensitivity of locally available ICTs used for detection of Plasmodium species in this particular region.

### METHODOLOGY

The cross-sectional study was conducted at Armed Forces Institute of Transfusion (AFIT), Rawalpindi Pakistan, lasting one week during

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November 2021. Permission of the hospital Ethical Committee (AFIT-ERC-21-038, dated 15th November 2021) was sought before commencing this study.

**Inclusion Criteria:** Venous blood sample from a patient with symptomatic diagnosis of Malaria based on the presence of intermittent fever (temperature >37.5°C) with rigors and chills was taken after proper consent.

**Exclusion Criteria:** None

A single patient's venous sample was tested against WHO approved four distinct brands of commercially available Malaria RDTs. (Bio Check AgPf/Pv (pLDH/pHRPII), Healgen AgPf/Pv (pLDH/pHRPII), Acu-check Ag Pf/Pan and Accurate Ag Pf/Pan). Microscopic examination of both thick and thin film by experienced hematologist confirmed trophozoites of both Plasmodium vivax and falciparum and few gametocytes of Plasmodium falciparum with parasite density of 1080 parasites/ul as shown in Figure-1.

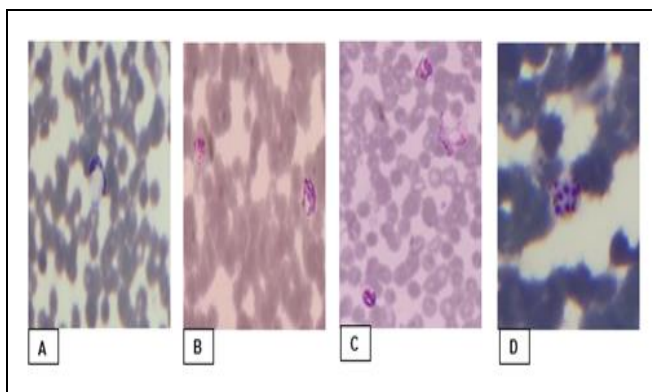


Figure-1: (A)Gametocyte of Plasmodium Falciparum, (B)(C)Trophozoites of Plasmodim vivax, (D)Schizonts of Plasmodium vivax.

Concurrently whole blood sample was centrifuged to obtain plasma as per manufacturer's instruction. Nine serial dilutions of 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512 strengths were made from sample plasma. These dilutions were tested against four different commercially available RDTs strips. (Bio Check Ag Pf/Pv (pLDH/pHRPII), Healgen Ag Pf/Pv (pLDH/pHRPII), Acu-check Ag Pf/Pan and Accurate Ag Pf/Pan).<sup>10</sup> µl from each of the prepared dilutions were added to the sample pad which were pre-coated with colloidal gold-labeled antibodies. Each RDT consisted of a membrane strip coated with target protein antibodies and was tested according to the

given instructions. The released HRP-2 and plasmodium LDH antigens get attached to the respective antibodies on the pad. Antigen antibody complexes migrated forward on the test strip, where they crossed two test lines and a control line. If the HRP2-specific line was evident, regardless of whether the LDH antigen line was visible, the test was interpreted as P. falciparum-positive. When all the three lines were visible, the results were interpreted as mixed infection with P. falciparum and vivax. If only the control and LDH antigen lines were visible, sample was considered positive for a Malaria parasite other than P. falciparum.

## RESULTS

RDTs kits from all the four brands (Bio Check Ag Pf/Pv (pLDH/pHRPII), Healgen Ag Pf/Pv (pLDH/pHRPII), Acu-check Ag Pf/Pan and Accurate Ag Pf/Pan) showed positive results for Plasmodium vivax up to 1:512 dilutions. However, only one brand RDT kits showed positive result with Plasmodium falciparum in 1:2 dilutions, in addition to Plasmodium vivax. The results were shown in Figure-2.

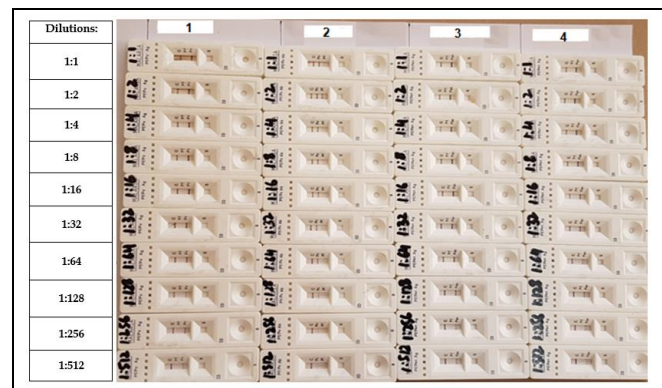


Figure-2: Commercially Available ICTs Showing Positive Malaria Results with Different Dilutions of Patient's Plasma.

## DISCUSSION

Malaria is a parasitic infection spread by Anopheles mosquito bite prevalent in tropical and subtropical regions around the world. It has inflicted utmost health and social burden worldwide. With an estimated 1 million cases per year, Pakistan is among the highest burden-sharing countries in the battle against Malaria. According to the current World Health Organization report, Plasmodium vivax is about 84 %prevalent in Pakistan, while Plasmodium falciparum and mixed infections accounts for 14.9 % and 1.1 % of cases, respectively.<sup>11-13</sup> Monitoring the

presence of Malaria is essential for appropriate Malaria management in resource-limited settings. Despite the infrastructure and technical limitations, microscopic examination of blood film is still the gold standard method for diagnosis of Malaria with sensitivity of 5-20 parasites/ul.<sup>14</sup> However, the major shortcoming of microscopic examination is its intense inter-observer variability and discrepancy, mainly for samples affected with mixed Plasmodium species and with low parasitic index. Rapid diagnostic tests (RDTs) for quick Malarial identification have been recognized worldwide as an efficient tool for the diagnosis of Malarial parasite in many Malaria-endemic countries, owing to their easy availability, quick result output, and straightforward interpretation. The diagnostic accuracy of rapid diagnostic test kits, on the other hand, has been a source of debate with sensitivity of 100 parasites/ ul. Molecular testing based on polymerase chain reaction although considered as very precise Malarial diagnostic tool; it is difficult to apply in point-of-care settings. Therefore, for rapid diagnosis of Malaria in many endemic regions, the role of RDTs as rapid diagnostic aid is increasing worldwide.<sup>15</sup> Despite the easy availability and rapid interpretation of results by Malaria RDTs, reliable and appropriate reporting of Malaria cases is still a challenge for tracking Malaria trends in resource-constrained settings. Furthermore, RDTs do not outperform microscopic tools in terms of sensitivity, because their overall sensitivity is reduced when parasitemia levels are low.<sup>16</sup> According to WHO standards, efficient RDTs must have greater than 95% sensitivity.<sup>17</sup> In a recent international systematic review based on 30-year meta-analysis data comparing the performance of Malarial RDTs and light microscopy (gold standard), Kojom *et al.*<sup>18</sup> reported that pooled estimates of RDTs performances showed sensitivity of 97.0% and specificity of 96.0%, positive likelihood ratio (PLR) was 22.4, negative likelihood ratio (NLR) 0.02 and diagnostic odd ratio (DOR) of 1080. RDTs aiming for Plasmodium falciparum revealed highly sensitive and specific outcomes as compared to those targeting non-falciparum and mixed infections. Another study conducted in Pakistan assessed the effectiveness of two commercially manufactured rapid test devices, the ICT Malaria Combo and First Response Malaria, using microscopy as the gold standard. With a sensitivity of 91.52% (95% CI: 87.52–95.52), the First Response Malaria device was proven to be more effective. The sensitivity with ICT Malaria Combo, on the other hand, was shown to be

lower (90.83%; 95% CI: 86.83–94.83)<sup>5</sup>. Similarly in our study the four tested RDTs brands showed discrepancy in results due to inability to recognize mixed infection in microscopic, tested sample. The RDT kits tested in this study appeared to be relatively unreliable diagnostic devices for detecting Plasmodium vivax, Plasmodium falciparum and mixed infections.

## CONCLUSION

The commercially available strips of Malaria detection based on immunochromatographic Technique (ICT) have high sensitivity in diagnosing Malaria, but are not resolute when it comes to speciation, particularly for Plasmodium falciparum. In practical settings, Malaria RDTs can show wide variation in their performance. Moreover, false-negative results can be the consequences of numerous factors. A more accurate and sensitive method for Malaria diagnosis need to be used in resource constraint Malaria endemic settings.

## Conflict of Interest:

There are no conflicts of interest to disclose.

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## Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

NR & MAN: Data acquisition, data analysis, drafting the manuscript, critical review, approval of the final version to be published.

MK & MBA: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

ZF & SU: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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