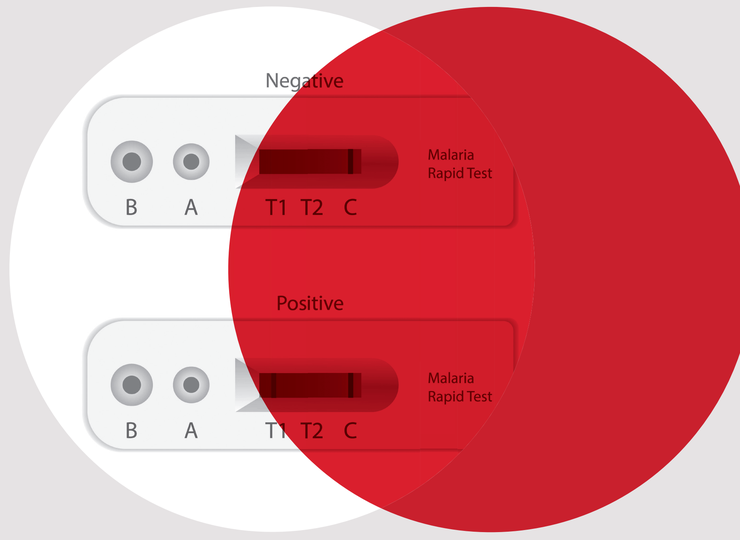
Malarial Parasite Rapid Diagnostic Testing – A Performance Review



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Introduction

Malaria is a parasitic infection with high morbidity and mortality. The gold standard for detecting and identifying Plasmodium species remains the visualisation of the parasite on light miscroscopy[1]. The Malaria Rapid Diagnostic Test (MRDT) is a supplementary immunochromatographic (IM) test for the detection, identification, and differentiation of malarial plasmodium species. The advantage of MRDT over conventional light microscopy is its application as a point of care (POC) device.

IM tests are based on the capture of the parasite antigens from the peripheral blood using either monoclonal (mAb) or polyclonal antibodies against the parasite antigen targets. The malaria antigens currently used as diagnostic targets are either specific to a *Plasmodium* species or are conserved across all four of the human malaria parasites. Falciparum specific monoclonals include histidine-rich protein-2 (c) and *P. falciparum* lactate dehydrogenase (pfLDH). Targets conserved across all human malaria have been identified on lactate dehydrogenase (pLDH) and aldolase enzymes[2,3].

RCPAQAP provides a proficiency program for MRDT designed to detect the presence or absence of PfHRP2 antigen. PfHRP2 was chosen as a target antigen since *P. falciparum* is the most clinically dangerous species; it replicates very quickly, and if treatment is delayed, outcomes of cerebral malaria and mortality can often result. The RCPAQAP program consists of 2 surveys per year and 2 case studies per survey. A Recombinant *P. falciparum* Histidine-Rich-Protein 2 (rPfHRP2) was commercially sourced. Participants were asked to test the samples using their MRDT kit and submit an interpretation of either negative or positive. An invalid option is also provided when kits do not comply with the manufacturer's internal controls.

Method

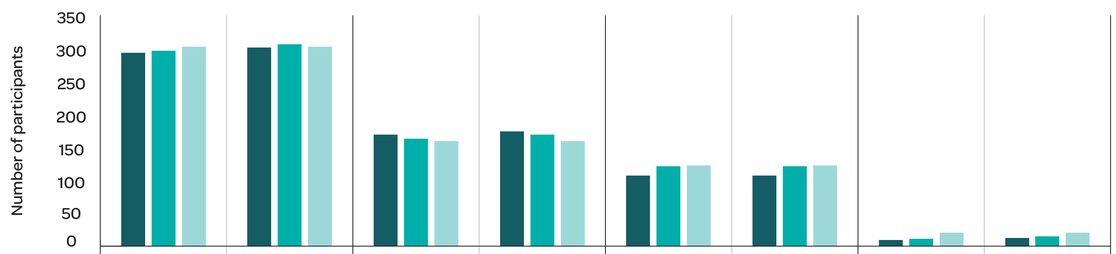
Six survey samples containing either 5 µg/mL of rPfHRP2 or phosphate buffer were reviewed from the 2018 and 2019 programs. The performance of MRDT kits in detecting the presence or absence of rPfHRP2 is presented. The percentage of false positive and false negative result returns were calculated based on the target response of positive (5 µg/mL of rPfHRP2) and negative (phosphate buffer). Only data from MRDT kits, whose user numbers were ≥ 10 is presented here.

Results and Discussion

Program enrolments grew from 242 to 302, from 2016 to 2019, with a peak of 311 in 2018. BinaxNow, CareStart, and SD Bioline were the most common MRDT kits used by our participants. A steady increase in the use of BinaxNow (159–174) and SD Bioline (12–20) kits was observed over the 2 year review period. Similarly, there was a significant increase in the number of CareStart users from 107 to 122.

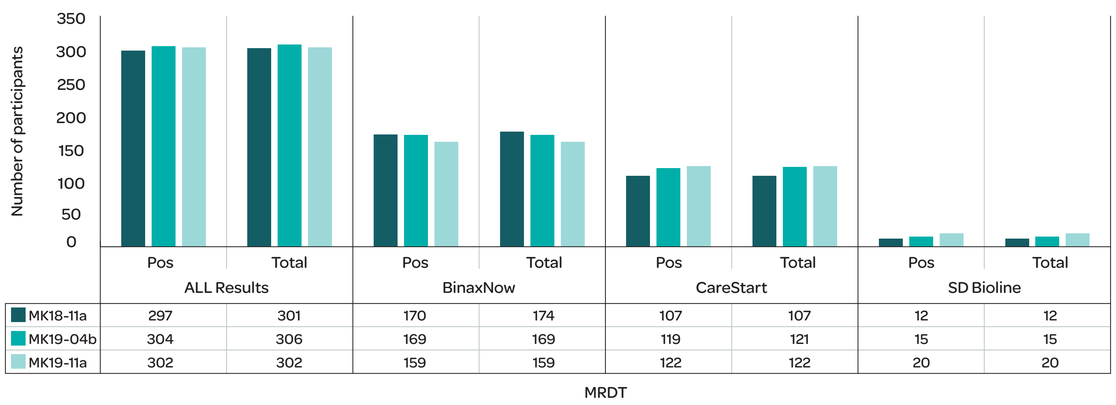
Results from the three samples containing 5 µg/mL of rPfHRP2 and Phosphate buffer (negative control) are illustrated in Figures 1a and 1b, respectively. Of the 3 samples containing 5 µg/mL of rPfHRP2, CareStart returned a 100% positive interpretation for all the three samples (no false negatives). SD Bioline returned negative results for all three samples with no rPfHRP2 (no false positives).

Figure 1a. Results from three surveys containing 5 µg/mL of rPfHRP2.



	ALL Results Pos	ALL Results Total	BinaxNow Pos	BinaxNow Total	CareStart Pos	CareStart Total	SD Bioline Pos	SD Bioline Total
MK18-11b	293	301	169	174	107	107	9	12
MK19-04a	296	306	163	169	121	121	11	15
MK19-11b	302	302	159	159	122	122	20	20

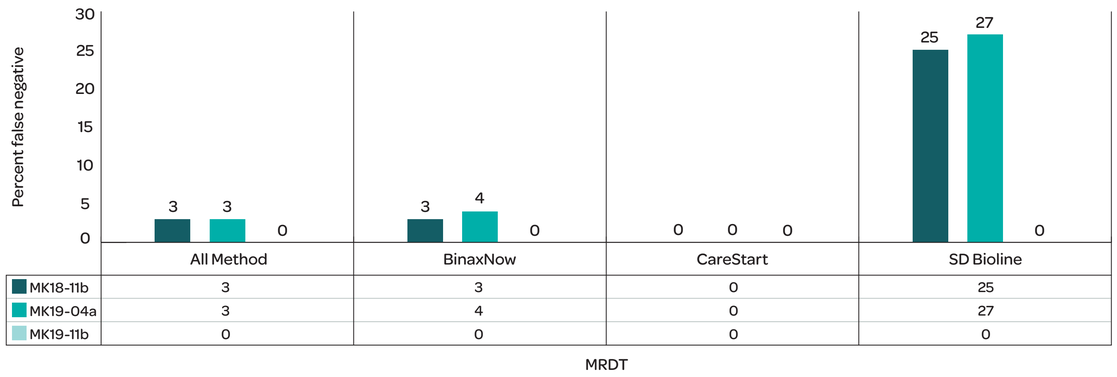
Figure 1b. Results from three surveys containing no rPfHRP2 (Phosphate buffer).



	ALL Results Pos	ALL Results Total	BinaxNow Pos	BinaxNow Total	CareStart Pos	CareStart Total	SD Bioline Pos	SD Bioline Total
MK18-11a	297	301	170	174	107	107	12	12
MK19-04b	304	306	169	169	119	121	15	15
MK19-11a	302	302	159	159	122	122	20	20

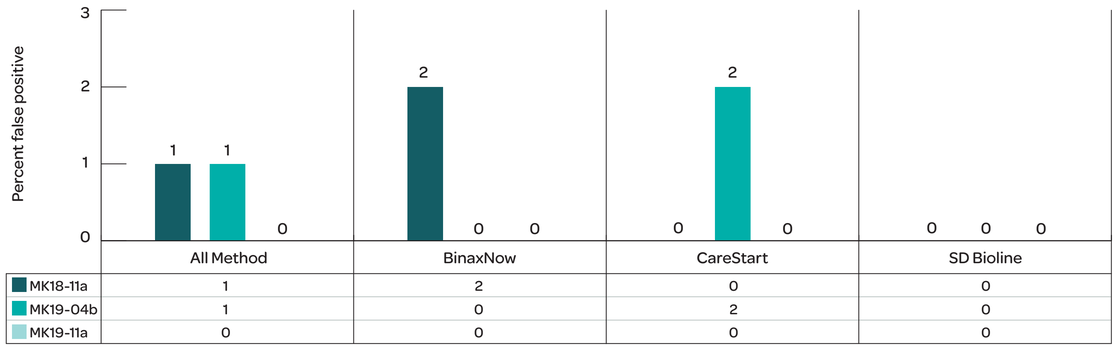
The percentage of false negatives (Figure 2a) and positives (Figure 2b) ranged from 0 to 27%. CareStart was the best performing MRDT kit, with no false negative results amongst the 122 participants. This finding is consistent with its published sensitivity and specificity of 98%. In contrast, BinaxNow returned 3 and 4% false positive results on samples MK18-11b and MK19-04a, respectively. These results also match the 97% sensitivity and 95% specificity manufacturer claim[4]. The rate of false negatives for the SD Bioline is 25 and 27% for MK18-11b and MK19-04a, respectively. The high false negative results for this kit may be associated with the total number of participants. The number of BinaxNow and CareStart users were more than 100. In contrast, there are < 25 participants for SDBioline. The reported sensitivity and specificity for SD Bioline was 99.7 and 95.5% respectively[4].

Figure 2a. Percent of false-negative results from samples containing 5 µg/mL of rPfHRP2



	All Method	BinaxNow	CareStart	SD Bioline
MK18-11b	3	3	0	25
MK19-04a	3	4	0	27
MK19-11b	0	0	0	0

Figure 2b. Percent of false-positive results from samples containing no rPfHRP2 (Phosphate Buffer).



	All Method	BinaxNow	CareStart	SD Bioline
MK18-11a	1	2	0	0
MK19-04b	1	0	2	0
MK19-11a	0	0	0	0

In a clinical setting, it is worth noting that other clinical conditions (i.e. Chronic hepatitis C, Toxoplasmosis, Human African Trypanosomiasis, Dengue fever, Leishmaniasis, Chagas disease, and Schistosomiasis) may contribute to a false-positive result. The presence of rheumatoid factor and heterophile antibodies may also be associated with a false-positive result[5].

False-negative tests have been observed even in severe malaria with parasitemias >40000 parasites/µg. The reason outlined includes the presence of blocking antibodies for PfHRP2 or immune-complex formation; or a prozone phenomenon seen with high parasite densities[2]. On a molecular level, the genetic heterogeneity of PfHRP2 expression and deletion of the HRP2 gene could also lead to a false-negative result. Additionally, technical errors due to users not adhering to manufacturer recommendations for processing of samples could give a false-negative result.

In human blood samples, the performance of the different MRDT kits is subject to the presence of interfering substances. However, the samples provided by the RCPAQAP are not of human origin but a purified recombinant protein to give a concentration of 5 µg/mL of fHRP2. Therefore, the false positive and negative results were more than likely due to 'technical errors.' For example, incorrect addition of sample or transport buffer, interpretation of test results beyond the specified period, labeling, and readability of processing instructions[6,7].

Conclusion

The steady increase in enrolments for the MRDT program reflects the uptake of the point of care test as a tool in the diagnosis and identification of malaria infection. The false negative rate for quality assurance samples that were expected to return a positive result confirms the ongoing need to monitor both kit performance and operator competency.

References

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