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ARTIFICIAL INTELLIGENCE IN HEMATOLOGY, FLAG, COUNT, CONFIRM: AUTOMATED CBC METRICS FOR HIGH PERFORMANCE MALARIA SCREENING

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ABSTRACT

Background: Automated hematology analyzers have shown promise as adjuncts for malaria detection through generation of parasite-related flags, service parameters and abnormal scattergrams. This study assessed the flagging capacity of the Sysmex XN-1000i, evaluated prbc–WDF# and prbc–WNR# service parameters, and described characteristic scattergram abnormalities in malaria-suspected samples.

Objective: To evaluate the diagnostic performance of the Sysmex XN-1000i in detecting malaria using the prbc flag, PRBC–WDF#, PRBC–WNR#, and scattergram abnormalities, with PCR as the reference standard.

Methods: A descriptive cross-sectional study was conducted from May to October 2020 at a tertiary hospital in Pakistan. Febrile patients suspected of malaria (n=87) underwent complete blood counts on the Sysmex XN-1000i. The presence of a prbc flag, PRBC WDF#, PRBC–WNR#, and abnormal WDF scattergrams were recorded. Real-time PCR served as the gold standard. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated.

Results: PRBC–WDF# and PRBC–WNR# both achieved 100% sensitivity, specificity, PPV, NPV, and accuracy, correctly identifying all PCR-confirmed cases and negatives. The prbc flag and scattergram abnormalities each demonstrated 97.6% sensitivity, 100% specificity, 100% PPV, and 33.3% NPV, missing two PCR-positive cases each.

Conclusion: The Sysmex XN-1000i's PRBC–WDF# and PRBC–WNR# parameters provide highly reliable malaria detection, even at low parasite levels. Combining these parameters with prbc flagging and scattergram review can enhance early case identification in routine CBC workflows, offering a practical screening tool in endemic and resource-limited settings.

Keywords: Malaria, Sysmex XN-1000i, parasitized red blood cells, PRBC WDF, PRBC WNR, scattergram abnormalities, PCR, automated hematology analyzer.

INTRODUCTION

Malaria continues to exert a considerable impact on global health, particularly in South Asia. The 2023 World Malaria Report estimated 249 million global cases in 2022, marking an increase over recent years (1). In endemic regions such as Pakistan facing persistent and, in some areas, increasing transmission. One of the sharpest rises, with cases increasing from 0.5 million in 2021 to over 2.6 million in 2022 (2). Surveillance in 2022 recorded more than 3.4 million suspected malaria cases nationally, up from 2.6 million the year before. This rise has been associated with climate related flooding, weakened public health monitoring, and interruptions to prevention and treatment services (3). In Khyber Pakhtunkhwa province alone, projections for 2023 estimated approximately 99,301 confirmed cases, highlighting ongoing transmission in remote and economically disadvantaged communities (4). A nationwide review further indicated that roughly 217 million people in Pakistan live in zones of moderate transmission risk, with an additional 63 million exposed to high-risk conditions (5).

The cornerstone of malaria control is reliable and early detection. Microscopic examination of blood films remains the reference method due to its ability to determine species and measure parasite density. However, it is labor intensive and depends on trained personnel and well equipped laboratories (6,7). Rapid diagnostic tests (RDTs) are simpler to deploy but can produce inconsistent results, particularly when parasite counts are low. Molecular methods such as polymerase chain reaction (PCR) have the highest analytical sensitivity and specificity but are hindered by their cost, technical demands, and longer turnaround times in routine clinical settings (8).

In recent years, automated hematology analyzers have attracted interest as a supplementary approach to detection of malaria, as they can identify hematological changes suggestive of infection during routine complete blood counts (CBSs). The Sysmex XN-1000i model, for instance, has the capacity to flag suspected cases through parameters like parasitized red blood cell counts (prbc–WDF#, prbc–WNR#) and atypical scattergram patterns. In a diagnostic study, Rehan et al. Reported that abnormal WDF scattergrams on this platform yielded a sensitivity of 80 %, specificity of 93.3 %, a positive predictive value of 95.8 %, and a negative predictive value of 99.9 % for identifying malaria cases (9). Similarly, work by Ningombam et al. Demonstrated that scattergram anomalies observed in both WDF and WNR channels showed a strong positive correlation with parasite density (Spearman $\rho = 0.77$; $p < 0.01$) (10).

Although these results are promising, detailed evaluations of the Sysmex XN-1000i—especially its performance in detecting malaria via prbc–WDF#, prbc–WNR#, and scattergram irregularities are limited particularly from high-burden countries such as Pakistan. This study was therefore designed to evaluate the flagging capacity of the Sysmex XN-1000i, assess the diagnostic utility of prbc–WDF# and prbc–WNR#, and describe scattergram abnormalities in malaria-positive samples.

MATERIALS AND METHODS

Study design and setting: Descriptive cross-sectional study at the Department of Pathology, Liaquat University of Medical and Health Sciences, Jamshoro, Pakistan (May–Oct 2020).

Study population: Febrile patients suspected of malaria; exclusion known dengue.

Sample size: Sample size (n=87) was determined using openepi, with a malaria prevalence of 6.6%, a 95% confidence interval, and a 5% margin of error.

Procedures: Venous blood (6ml) collected in EDTA tubes. CBC performed on Sysmex XN-1000i; presence/absence of the manufacturer prbc flag recorded; prbc–WDF# and prbc–WNR# values recorded. WDF scattergrams were visually inspected for the characteristic patterns. RT-qpcr (Geno amp real-time qpcr) served as internal quality control (reference standard) in this analysis.

Ethical Considerations: Written informed consent was obtained from all participants or guardians. The study protocol was approved by the institutional ethical review committee.

Data analysis: Numerical variables reported as mean ± SD; categorical variables as counts and percentages. Contingency tables against PCR (positive n=85, negative n=2) were constructed to calculate sensitivity, specificity, PPV, NPV and accuracy.

RESULTS: Sysmex XN-1000i analysis revealed that the parasite related red blood cell (prbc) flag was present in 83 of 87 malaria-positive cases, yielding a detection rate of **95.4%**. This indicates that most infected samples were automatically flagged by the instrument without the need for manual review at the initial CBC stage. The prbc–WDF# parameter was measurable in 85 samples (97.7%) with a mean ± SD of 1,420 ± 2,290 and a median of 582 (range: 0–12,667). The prbc–WNR# was likewise measurable in 85 samples (97.7%), showing a mean ± SD of 1,257 ± 1,726 and a median of 582 (range: 0–8,141). These values were higher than the prbc flagging rate, indicating that even when the prbc flag was absent, these service parameters often still indicated an abnormal result suggestive of infection. Characteristic WDF scattergram abnormalities, defined as a distinctive purple cluster in the WDF plot, were observed in 83 cases (95.4%).

Table 1. Performance metrics of Sysmex XN-1000i parameters (reference: RT-qpcr, PCR pos n=85, neg n=2)

Parameter	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Prbc Flag	97.6	100	100	33.3	97.7
PRBC – WDF#	100	100	100	100	100
PRBC – WNR#	100	100	100	100	100
Scattergram Abnormalities	97.6	100	100	33.3	97.7

Tab 1: As shown in table above, against PCR as the reference, **PRBC–WDF#** and **PRBC–WNR#** achieved perfect agreement, with 100% sensitivity, specificity, PPV, NPV, and accuracy. This indicates they reliably detected all malaria cases and excluded all non-infected samples, even at low parasite levels, making them the most dependable Sysmex parameters in this study.

The pRBC flag and scattergram abnormalities each missed two PCR-positive cases, giving 97.65% sensitivity and 97.70% accuracy, likely due to very low parasitemia. Both maintained 100% specificity, preventing false positives. PPVs were 100% for all parameters, but NPVs were lower for prbc flag and scattergrams (33.33%), meaning a negative result from these alone cannot rule out malaria.

Overall, combining PRBC–WDF# and PRBC–WNR# with prbc flag and scattergram review can strengthen early malaria detection in routine CBC workflows, especially in resource-limited, endemic settings.

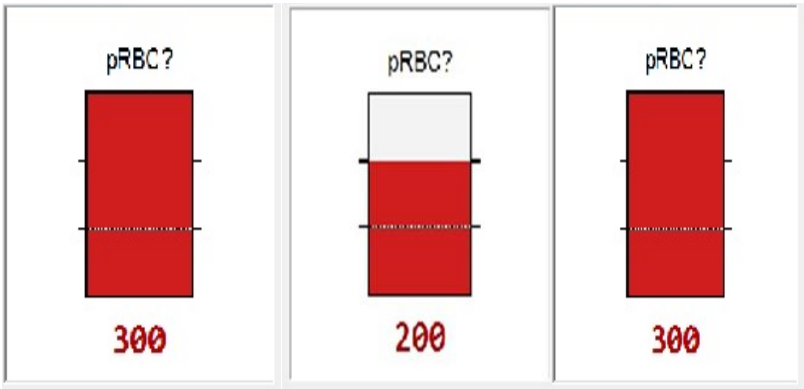

Fig 1: Plasmodium detection through PRBC flagging.

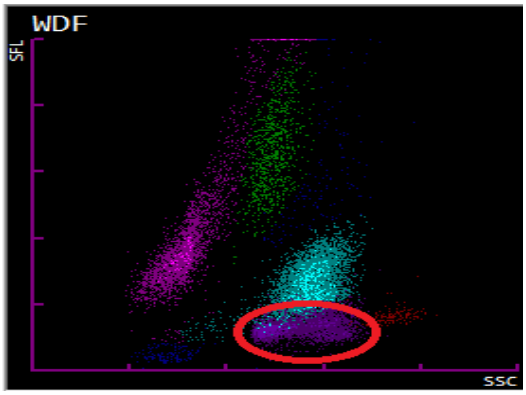

Fig 2: WDF Scattergram shows abnormal population of cells alongside PRBC flag indicating presence of malaria.

DISCUSSION

This study found that the Sysmex XN-1000i's **PRBC–WDF#** and **PRBC–WNR#** parameters matched PCR results exactly, producing 100% values for sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy. This perfect agreement indicates that these numerical outputs are highly dependable for identifying malaria, including cases with low parasite densities.

These findings are in line with a recent meta-analysis, which reported pooled sensitivity above 97% and specificities close to 99% for Sysmex platforms when compared with PCR as the gold standard (11). Such consistency suggests that incorporating WDF and WNR parameters into frontline laboratory workflows could enhance malaria screening, particularly where molecular tests are not readily accessible.

In contrast, both the pRBC flag and scattergram abnormalities failed to detect two PCR-positive cases each, resulting in sensitivities of 97.6% and overall accuracies of 97.7%. The missed detections were likely due to infections with very low parasitemia that did not trigger analyzer thresholds. Similar performance trends for the prbc flag have been reported, with sensitivities varying from the low 80s to full detection depending on parasite density, but specificity consistently remaining high (12). While scattergram interpretation offers valuable visual clues such as abnormal cell cluster patterns. It is best used alongside the numeric service parameters to avoid false negatives in low density infections (13).

CONCLUSION

The Sysmex XN-1000i demonstrated high diagnostic performance for malaria detection when evaluated against PCR, particularly through the **PRBC–WDF#** and **PRBC–WNR#** parameters, which achieved 100% sensitivity, specificity, PPV, NPV, and accuracy. While pRBC flag and scattergram abnormalities also showed excellent specificity and PPV, their slightly lower sensitivity indicates that they should be interpreted in combination with service parameters to avoid missed low parasitemia cases.

These findings support the integration of PRBC–WDF# and PRBC–WNR# into routine hematology workflows as a rapid, reagent free screening approach for malaria. In resource limited, endemic settings, this could enable earlier detection and timely initiation of treatment contributing to improved patient outcomes and strengthened malaria surveillance.

REFERENCES

1. World Health Organization. World Malaria Report 2023. Geneva: WHO; 2023.
2. Venkatesan P. Pakistan sees sharp rise in malaria cases after floods. Lancet Microbe. 2024;5(1):e4. Doi:10.1016/S2666-5247(22)00323-0.
3. Afzal M, et al. Clinical burden of Plasmodium infection and associated factors in Chakwal district, Punjab, Pakistan. Malar J. 2024;23:91. Doi:10.1186/s12936-024-00190-1.

4. Khan MI, et al. Dynamics of malaria incidence in Khyber Pakhtunkhwa: projected prevalence by December 2023. Parasitol Res. 2024;123:2145-2154. Doi:10.1007/s00436-024-07963-3.
5. Khan MI, et al. A systematic review of malaria risk and prevalence in Pakistan. Malar J. 2023;22:144. Doi:10.1186/s12936-023-04638-y.
6. Wongsrichanalai C, et al. A review of malaria diagnostic tools: microscopy and rapid diagnostic test (RDT). Am J Trop Med Hyg. 2007;77(6 Suppl):119–127.
7. Moody A. Rapid diagnostic tests for malaria parasites. Clin Microbiol Rev. 2002;15(1):66–78.
8. Adu-Bonsu, Gideon & Addy, Francis & Ankrah, Jacob & Kpangkpari, Sylvanus & Dongdem, Julius & Abugri, James & Helegbe, Gideon & Wezena, Cletus. (2024). Comparative analysis of Plasmodium falciparum-specific RDT and PCR for detecting malaria in northern Ghana. Journal of the Ghana Science Association. 22.
9. Rehan M, et al. White blood cell differential fluorescence abnormal scattergram: A useful indicator for early detection of malarial parasite. Pak J Med Sci. 2022;38(3 Pt 1):687-691. Doi:10.12669/pjms.38.3.5370.
10. Ningombam A, Sarkar A, Acharya S, Chopra A, Kumar K, Subramanian A. Application of Sysmex XN-Series automated haematology analyser in the rapid detection of malaria. Indian J Hematol Blood Transfus. 2020;36(3):512-518. Doi:10.1007/s12288-019-01240-4.
11. Mulatie Z, Kelem A, Chane E, Tarekegn AM, Teketelew BB, Yenesew A, et al. Diagnostic role of Sysmex hematology analyzer in the detection of malaria: A systematic review and meta-analysis. Plos One. 2024;19(9):e0296766.
12. Huh HJ, Chung JW, Park SY, Chae SL. Vivax malaria detection using a parasitic red blood cell flag generated by the Sysmex XN-9000 hematology analyzer. Int J Lab Hematol. 2021;43(6):1403-1407.
13. Dumas C, Tirard-Collet P, Mestrallet F, Girard S, Jallades L, Picot S, et al. Flagging performance of Sysmex XN-10 hematology analyzer for malaria detection. J Clin Pathol. 2020;73(10):676-677.