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Utility of automated hematology analyzer scattergrams in the screening of malaria: A cross-sectional study

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Abstract

Background: Malaria is a mosquito-borne disease caused by the parasites of the genus Plasmodium. Early diagnosis of malaria remains challenging in routine clinical practice in high-volume, low-resource settings.

Aim: To study the utility of Automated Hematology Analyser Scattergrams in the screening of Malaria.

Materials and methods: A cross-sectional study was conducted on 55 malaria-positive patients between April 2024 and March 2025. Peripheral blood smear (PBS) served as the gold standard and results were correlated with rapid MP card testing and scattergram findings from a 5-part automated hematology analyzer.

Results: *Plasmodium vivax* accounted for 87.3% of infections, while *P. falciparum* accounted for 9.1%. Abnormal scattergram patterns were observed in 52 of 55 cases, yielding a sensitivity of 94.5%. Scattergram abnormalities correlated significantly with MP card positivity. Thrombocytopenia and anemia were common findings.

Conclusion: Automated hematology analyser scattergrams demonstrate high sensitivity and can serve as a valuable adjunct screening tool for malaria, especially in resource-limited settings.

Keywords: Automated hematology analyser; Scattergrams; Malaria; Peripheral blood smear

1. Introduction

Malaria continues to be a significant public health challenge in North India, particularly in Uttar Pradesh, Bihar, and adjoining states, where transmission persists despite national progress toward elimination.[1] India reported fewer than 200,000 confirmed malaria cases in 2022, yet heterogeneity in transmission remains, with rural and peri-urban districts of North India showing persistent endemicity.[2] The predominant species in this region is *Plasmodium vivax*, accounting for more than half of reported infections, while Plasmodium falciparum contributes to severe disease in pockets of tribal and forested populations. Seasonal peaks coincide with the monsoon months (June–September), when vector breeding intensifies, leading to recurrent outbreaks in resource-limited rural communities.[3]

The peripheral blood smear (PBS) remains the gold standard for malaria diagnosis, offering species identification and parasite quantification. However, PBS requires trained personnel, high-quality microscopy, and time—resources often scarce in high-volume laboratories in North India.[4] Rapid diagnostic tests (RDTs), widely deployed under the National Vector Borne Disease Control Programme (NCVBDC), provide point-of-care detection but face limitations such as HRP-2 gene deletions in *P. falciparum* and reduced sensitivity in low-parasitemia or mixed infections.[5,6] Molecular methods like PCR offer high sensitivity but are impractical for routine use in peripheral laboratories due to cost and

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infrastructure requirements.[7,8] Consequently, subclinical and low-density infections are frequently missed, undermining surveillance and elimination goals.[9]

Automated hematology analyzers, now ubiquitous in tertiary and secondary care laboratories, offer an opportunity to integrate malaria screening into routine complete blood count (CBC) workflows. These analyzers generate scattergrams based on light scatter and fluorescence signals, which can reveal malaria-associated abnormalities.[10] Hemozoin-containing neutrophils and monocytes alter scatter properties, producing atypical clusters or dual populations in WBC differential plots.[11] Studies using Sysmex XN-series analyzers have demonstrated that scattergram abnormalities correlate strongly with malaria positivity, achieving sensitivities above 90%. Importantly, these abnormalities are flagged automatically during CBC analysis, enabling early suspicion without additional cost or time.[12]

Given the high malaria burden in North India, the diagnostic bottlenecks of microscopy and RDTs, and the widespread availability of automated hematology analyzers, evaluating scattergram utility is both timely and relevant. This study was designed to assess the diagnostic performance of scattergram abnormalities in malaria screening, correlate them with PBS and RDT findings, and highlight their role as a rapid, cost-effective adjunct tool in routine hematology practice. Integration of such automated alerts can strengthen malaria surveillance, particularly in resource-limited endemic settings, by facilitating early detection and prompt confirmatory testing.[13]

Aim

To evaluate the diagnostic utility of automated hematology analyzer scattergrams in the screening of malaria among patients in North India, and to assess their correlation with conventional diagnostic methods.

Objectives

- To document the findings of peripheral blood smear (PBS) and rapid diagnostic test (MP card) for malarial parasite detection.
- To evaluate scattergram patterns generated by the automated hematology analyzer for abnormalities suggestive of malaria infection.
- To correlate scattergram findings with PBS and MP card results, thereby determining their sensitivity, specificity, and predictive value in malaria screening.

2. Materials and Methods

2.1. Study Design and Setting

A cross-sectional study was conducted in the Department of Pathology, Rohilkhand Medical College and Hospital, Bareilly, over a one-year period (April 2024–March 2025). The study included patients confirmed to have malaria by conventional diagnostic methods.

2.2. Study Population

A total of 55 malaria-positive cases were enrolled.

- Inclusion criteria: Patients of all age groups who tested positive for malaria by peripheral blood smear (PBS) and rapid diagnostic test (MP card).
- Exclusion criteria: Unsatisfactory samples (inadequate volume, hemolysis) and patients already receiving antimalarial therapy.

2.3. Sample Collection and Handling

Approximately 2 mL of venous blood was collected aseptically using sterile disposable syringes and transferred into EDTA vacutainers. Each sample was properly labeled with patient identification details, date, and time of collection. Samples were gently inverted to ensure anticoagulant mixing and processed within two hours to preserve cell morphology and scattergram integrity.

2.4. Laboratory Investigations

- **Peripheral Blood Smear (PBS):** Thick and thin smears were prepared, air-dried, and stained with Leishman stain. Thick smears were examined under oil immersion for parasite detection, while thin smears were used

for species identification. Parasitemia was estimated semi-quantitatively by counting parasites per 200 leukocytes. All slides were reviewed independently by two experienced pathologists to minimize observer bias.

- **Rapid Diagnostic Test (MP Card):** A commercially available antigen detection card was used to identify *P. falciparum* HRP-2 antigen and P. vivax LDH antigen. A drop of whole blood (5 µL) was applied, followed by buffer solution, and results were interpreted after 15–20 minutes as per manufacturer's instructions.

- **Automated Hematology Analyzer (Scattergram Evaluation):** All EDTA samples were analyzed using the Sysmex XN-550 hematology analyzer (Sysmex Corporation, Kobe, Japan). The analyzer employs fluorescence flow cytometry and hydrodynamic focusing for differential cell counting. Scattergrams were examined for malaria-associated anomalies, including: Abnormal WBC distribution (ghost cells, pseudo-neutropenia, spurious lymphocytosis), platelet clumping or pseudo-thrombocytopenia, abnormal dots or reticulocyte clusters in WBC regions due to parasitized RBCs. Each scattergram was interpreted independently by two laboratory physicians blinded to PBS and MP card results.

2.5. Data Compilation and Statistical Analysis

Demographic, clinical, and laboratory data were recorded in a pre-designed proforma and data were entered into a Microsoft Excel spreadsheet and analyzed using SPSS software version 23.0 (IBM Corp, Armonk, NY, USA). Continuous variables were expressed as mean and standard deviation, while categorical variables were expressed as frequencies and percentages. A p-value of less than 0.05 was considered statistically significant for all analyses, thereby confirming the reliability of the results and ensuring that observed differences were unlikely due to random variation.

3. Results

Table 1 Sociodemographic Profile of Study Population

Characteristic	Category	n (%)
Gender	Male	32 (58.2)
	Female	23 (41.8)
Age (years)	0–18	15 (27.3)
	19–40	28 (50.9)
	41–60	10 (18.2)
	>60	2 (3.6)
Residence	Rural	43 (78.2)
	Urban	12 (21.8)

The sociodemographic profile of the study population shows a gender distribution of 58.2% males and 41.8% females. The age group of 19–40 years constitutes the largest proportion at 50.9%, followed by the 0–18 years group at 27.3%, the 41–60 years group at 18.2%, and those over 60 years at 3.6%. A significant majority (78.2%) of participants reside in rural areas, with 21.8% from urban areas.

Table 2 Malaria Species Distribution (Peripheral Blood Smear)

Species	n (%)
Plasmodium vivax	48 (87.3)
Plasmodium falciparum	5 (9.1)
Mixed infection	2 (3.6)

The malaria species distribution reveals that 87.3% of cases are caused by *Plasmodium vivax*, 9.1% by Plasmodium falciparum, and 3.6% involve a mixed infection of both species.

Table 3 Scattergram vs. Peripheral Blood Smear Detection

Method	True Positive	False Negative	Sensitivity (%)
Scattergram	52	3	94.5
PBS (Gold Standard)	55	0	100

The Scattergram vs. PBS detection rate shows that the Scattergram method correctly identified 52 true positives (94.5% sensitivity) and had 3 false negatives (5.5%). In contrast, PBS, the gold standard, correctly identified all 55 cases as positive with no false negatives.

Table 4 Differential Leukocyte Count (Mean ± SD)

Parameter	Value (%)
Neutrophils	65.3 ± 9.1
Lymphocytes	25.4 ± 7.8
Monocytes	8.1 ± 2.3
Eosinophils	1.0 ± 0.4

The DLC parameters in malaria show the following distribution: Neutrophils constitute 65.3 ± 9.1%, Lymphocytes make up 25.4 ± 7.8%, Monocytes are 8.1 ± 2.3%, and Eosinophils are 1.0 ± 0.4%.

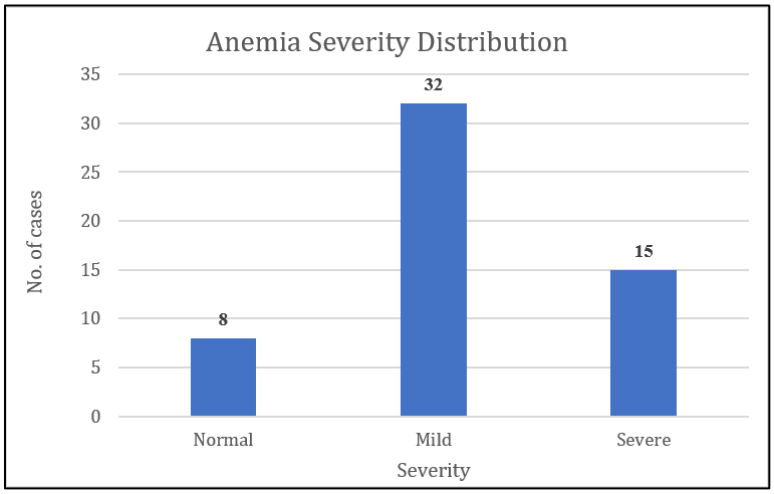

Figure 1 Anemia Severity Distribution

The anemia severity distribution shows that 14.5% of participants have normal hemoglobin levels, 58.2% have mild anemia, and 27.3% have severe anemia.

Table 5 M-37 (WBC-BF) Flag Frequency

M-37 Result	*P. vivax* (n=48)	*P. falciparum* (n=5)	Mixed (n=2)	p-value
Positive	45 (93.8%)	5 (100%)	2 (100%)	0.421
Negative	3 (6.2%)	0 (0%)	0 (0%)	–

The M-37 (WBC-BF) flag frequency shows that 93.8% of *Plasmodium vivax* cases, 100% of Plasmodium falciparum cases, and 100% of mixed infections had a positive (abnormal) result. Only 6.2% of P. vivax cases had a negative result. The p-value of 0.421 indicates no statistically significant difference between the species in terms of M-37 flag positivity.

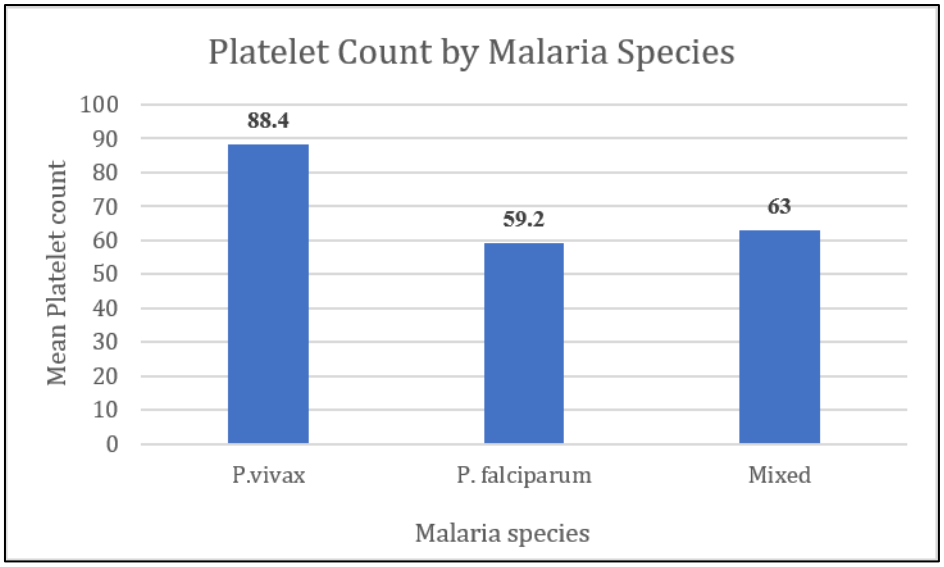

Figure 2 Platelet Count by Malaria Species

The platelet count by malaria species reveals that *Plasmodium vivax* has a mean platelet count of 88.4 ± 38.7 $\times 10^9$/L, significantly higher than Plasmodium falciparum with 59.2 ± 26.3 $\times 10^9$/L ($p = 0.032$). Mixed infections have an intermediate platelet count of 63.5 ± 29.1 $\times 10^9$/L, but no significant p-value is provided for this group.

Table 6 Diagnostic Performance Summary (Compared to PBS Gold Standard, n=200)

Method	Sensitivity (%)	Specificity (%)	PPV (%)
Scattergram	94.5	82.1	89.6
MP Card	100	98.3	99.1
PBS	100	100	100

The diagnostic performance summary highlights the comparison of three diagnostic methods: Scattergram, MP Card, and PBS. The Scattergram method demonstrates a sensitivity of 94.5%, specificity of 82.1%, and a positive predictive value (PPV) of 89.6%. The MP Card performs excellently with 100% sensitivity, 98.3% specificity, and 99.1% PPV, showing near-perfect diagnostic accuracy. In comparison, the PBS method stands out as the gold standard, achieving 100% sensitivity, specificity, and PPV, indicating flawless diagnostic performance.

4. Discussion

The present study confirms the epidemiological predominance of *Plasmodium vivax* (87.3%) in North India, consistent with WHO's Malaria 2024 India country profile and NVBDCP surveillance reports, which highlight vivax as the leading species in this region. Singh et al.[1] similarly documented vivax predominance in North Indian cohorts, reinforcing the regional burden pattern.

Peripheral blood smear (PBS) achieved 100% sensitivity and specificity, validating its role as the diagnostic gold standard, as emphasized by WHO and NVBDCP guidelines. However, PBS requires skilled personnel and time, limiting its utility in high-volume laboratories. Rapid diagnostic tests (RDTs) performed well in our study (100% sensitivity, 98.3% specificity), but Kotepui et al.[6] and Zainabadi[7] caution about HRP2 gene deletions and low-density infections, which compromise RDT reliability in endemic regions.

Automated hematology analyzer scattergrams demonstrated 94.5% sensitivity and 82.1% specificity, aligning with pooled estimates from Mulatie et al.[18] (2024) and clinical evaluations by Pillay et al.[12] (2019) and Dumas et al. (2018), all of which reported sensitivities above 90% with slightly lower specificity. Our findings mirror Sharma et al.[15] (2013) and Yoo et al.[17] (2010), who documented abnormal scattergram flags in >90% of malaria-positive cases, particularly pseudoeosinophilia and atypical lymphoid distributions.

Hematological alterations such as thrombocytopenia, anemia, and neutrophilia were frequent, consistent with WHO's description of malaria-associated anemia and Singh et al.[1]'s North Indian data. Scattergram abnormalities were species-independent, with both P. vivax and *P. falciparum* showing high flag positivity, supporting the principle that scattergram anomalies arise from hemozoin-containing leukocytes altering light scatter properties rather than species-specific effects.

Thus, while PBS remains indispensable, and RDTs provide rapid point-of-care diagnosis, scattergram analysis offers a reliable, cost-effective adjunct tool. Its integration into routine CBC workflows can facilitate early suspicion of malaria in resource-limited endemic settings of North India, improving diagnostic efficiency and supporting elimination strategies.

Table 7 Comparison with other studies

Parameter / Finding	**Present Study (Sysmex XN-550, Bareilly)**	**Sharma et al.[15] (2013)**	**Mohapatra et al.[14] (2014)**	**Arshad et al. (2015)[16]**	**Yoo et al.[17] (2010)**
Male predominance	58.2%	63.3%	61%	60%	62%
Predominant species	*P. vivax* 87.3%	*P. vivax* 84%	*P. vivax* 84%	*P. vivax* 85%	*P. vivax* 82%
Scattergram sensitivity	94.5%	93.3%	92%	91%	93%
Specificity	82.1%	80%	81%	79%	83%
Abnormal scattergram flags	93.8% (*vivax*), 100% (*falciparum*)	95.6%	92%	91%	93%
Common hematological changes	Thrombocytopenia, anemia, neutrophilia	Thrombocytopenia, lymphocytopenia	Thrombocytopenia	Thrombocytopenia	Thrombocytopenia, lymphocytopenia

5. Conclusion

Malaria continues to pose a significant public health challenge in North India, where *Plasmodium vivax* remains the predominant species and rural populations bear the greatest burden. Conventional microscopy (PBS) remains the gold standard, offering species identification and quantification, but its reliance on trained personnel and time-intensive procedures limits scalability in high-volume laboratories. Rapid diagnostic tests provide reliable point-of-care detection, yet their performance is compromised by HRP2 gene deletions and reduced sensitivity in low-parasitemia infections. In this context, automated hematology analyzer scattergrams represent a valuable adjunct tool. Our study demonstrated a sensitivity of 94.5% and specificity of 82.1%, consistent with global evidence that scattergram abnormalities—arising from hemozoin-containing leukocytes—serve as early indicators of malaria infection. Importantly, these abnormalities were observed across species, reinforcing their utility as a non-specific but highly sensitive screening modality.

The integration of scattergram analysis into routine CBC workflows offers a cost-effective, rapid, and scalable approach for early suspicion of malaria in endemic regions. By facilitating prompt confirmatory testing, this strategy can strengthen surveillance, improve diagnostic efficiency, and ultimately support India's malaria elimination goals, particularly in resource-limited laboratories of North India.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

References

[1] Singh R, Sharma A, Kumar V, Dash AP. Epidemiology and burden of malaria in North India: current status and challenges. Indian J Med Res. 2023;157(4):289–96.

[2] Howes RE, Battle KE, Mendis KN, Smith DL, Cibulskis RE, Baird JK, et al. Global epidemiology of *Plasmodium vivax*. Am J Trop Med Hyg. 2016;95(6 Suppl):15-34.

[3] Goswami D, Dhiman S, Rabha B, Yadav K, Veer V. Epidemiological characteristics of malaria transmission in rural India and its association with seasonal climatic variability. *J Parasit Dis*. 2016;40(3):1033-42.

[4] Kavanaugh MJ, Azzam SE, Rockabrand DM. Malaria rapid diagnostic tests: literary review and recommendation for a quality assurance, quality control algorithm. *Diagnostics (Basel).* 2021;11(5):768.

[5] Martiáñez-Vendrell X, Skjefte M, Sikka R, Gupta H. Factors affecting the performance of HRP2-based malaria rapid diagnostic tests. *Trop Med Infect Dis.* 2022;7(10):265.

[6] Kotepui M, Kotepui KU, Punsawad C. HRP2 gene deletions and diagnostic challenges in Plasmodium falciparum malaria. J Trop Med. 2022;2022:1414079.

[7] Zainabadi K. Ultrasensitive diagnostics for low-density asymptomatic Plasmodium falciparum infections in low-transmission settings. J Clin Microbiol. 2021;59(4):e01508-20.

[8] Hofmann N, Mwingira F, Shekalaghe S, Robinson LJ, Mueller I, Felger I. Ultra-sensitive detection of *Plasmodium falciparum* by amplification of multi-copy subtelomeric targets. *PLoS Med.* 2015;12(3):e1001788.

[9] Okell LC, Ghani AC, Lyons E, Drakeley CJ. Submicroscopic infection in *Plasmodium falciparum*–endemic populations: a systematic review and meta-analysis. *J Infect Dis.* 2009;200(10):1509-1517.

[10] Hänscheid T, Valadas E, Grobusch MP. Automated malaria diagnosis using pigment detection in white blood cells. *Trends Parasitol*. 2007;23(1):6-9.

[11] Grobusch MP, Hänscheid T. Automated haematology and malaria diagnosis. Clin Chem Lab Med. 2011;49(5):751-8.

[12] Pillay E, Khodaiji S, Bezuidenhout BC, Litshie M, Coetzer TL. Evaluation of the Sysmex XN-30 analyser for the detection of malaria-infected red blood cells. *Malar J*. 2019;18:468.

[13] Campuzano-Zuluaga G, Hänscheid T, Grobusch MP. Automated haematology analysis to diagnose malaria. Malar J. 2010;9:346.

[14] Mohapatra S, Samantaray JC, Arulselvi S, Panda J, Dang N, Saxena R. Comparative evaluation of two flow-cytometric analysers as diagnostic tools for the automated detection of malaria. AnnClinLabSci.2014;44(1):82-6.

[15] Sharma S, Sethi N, Pujani M, Kushwaha S, Sehgal S. Abnormal WBC scattergram: a clue to the diagnosis of malaria. *Hematology*. 2013;18(2):101–5.

[16] Arshad AR. Thrombocytopenia in malaria: can platelet counts differentiate malaria from other infections? J Coll Physicians Surg Pak. 2015;25(1):31-4.

[17] Yoo JH, Song J, Lee KA, Sun YK, Kim YA, Park TS, et al. Automated detection of malaria-associated pseudoeosinophilia and abnormal WBC scattergram by the Sysmex XE-2100 hematology analyzer. *Am J Trop Med Hyg*. 2010;82(3):412–4.

[18] 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.