Research Square

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Field evaluation of the Automated Hematology Analyzer XN-31 using blood samples from asymptomatic residents in a malaria-endemic area of Thailand: importance of sample transport and temperature control

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Abstract

Background

The Automated Hematology Analyzer XN-31 can readily quantify the number of malaria parasite–infected red blood cells (MI-RBCs) within approximately one minute using flow cytometry technology, and it has already demonstrated clinical utility for malaria patients in both endemic and non-endemic settings. Recently, asymptomatic carriers with low-density parasitemia in endemic regions have been identified as significant reservoirs contributing to ongoing malaria transmission. Therefore, field-based malaria surveys in endemic areas, including epidemiological investigations of asymptomatic infections, are important for malaria control.

Methods

To evaluate the feasibility and limitations of the XN-31 use under real-world field conditions, we collected a total of 1,799 blood samples from asymptomatic residents in a malaria-endemic area along the Thailand–Myanmar border during five field trips conducted between January 2021 and May 2022. Samples were transported within the same day to a central laboratory in Bangkok. XN-31 measurements were performed after transport, together with microscopy and rapid diagnostic tests (RDTs). Dried blood spots were also sent to Japan for PCR analysis.

Results

Microscopy detected one *P. vivax*–positive case. In contrast, PCR identified 2 *P. falciparum* and 4 *P. vivax* infections, whereas all RDT results were negative. The XN-31 detected 5 MI-RBC–positive samples, of which only one was confirmed as *P. vivax*–positive by both microscopy and PCR. Among the XN-31 measurements, 176 samples were flagged as “MI-RBC Abn Scattergram,” indicating the presence of unidentified particles in the scattergram that prevented proper interpretation. Following protocol adjustments for the transport of whole blood samples to Bangkok, the MI-RBC Abn Scattergram rate in XN-31 decreased from 23.8% (first trip) to 4.6% (fifth trip). Lower frequencies of MI-RBC Abn Scattergram results were observed following modifications to collection-to-analysis time, transport procedures, cooling methods, and transport temperature management.

Conclusions

In conclusion, although several operational challenges were encountered during field application, the XN-31 may be applicable to malaria surveys in endemic areas when appropriate sample handling and transport conditions are ensured.

1. Background

The Automated Hematology Analyzer XN-31, developed by Sysmex Corporation, can measure complete blood count (CBC) parameters and detect the number and proportion of malaria-infected red blood cells (MI-RBC#/%) using flow cytometry technology, with results available in approximately one minute. The XN-31 and the research-use-only model XN-30 have demonstrated the ability to efficiently and accurately detect malaria parasites in both endemic (1–3) and non-endemic settings (4–6), supporting their clinical utility. Particularly in non-endemic countries, malaria diagnosis typically targets symptomatic patients who have recently returned from endemic areas. In such cases, parasitemia levels tend to be relatively high, as these individuals are not likely to have acquired immunity to malaria, and the diagnostic goal is often a clear binary distinction between parasite-positive and parasite-negative cases. In contrast, many residents in endemic regions possess partial immunity due to repeated exposure to malaria parasites (7), which may result in a higher proportion of individuals with low parasitemia, often below the detection threshold of microscopy. Indeed, asymptomatic carriers with low-density parasitemia in endemic regions have recently been identified as significant reservoirs sustaining ongoing malaria transmission. However, field surveys in endemic regions often require transportation of blood samples from remote collection sites to centralized laboratories. Thus, the feasibility and limitations of the XN-31 use under real-world field conditions have not been fully evaluated. In this study, blood samples were collected from asymptomatic individuals along the Thailand–Myanmar border and transported to a reference hospital in Bangkok for analysis. We also assessed the effects of sample transportation conditions on analyzer performance, particularly in relation to MI-RBC Abn Scattergram findings (i.e., abnormal scattergram patterns indicating the presence of unidentified particles that can compromise result interpretation) on the XN-31.

2. Methods

2.1. *Sample collection*

Blood samples were collected at the Mahidol University Rajanagarindra Tropical Disease International Centre (RTIC), located in the Thailand–Myanmar border area, during five visits from January 2021 to May 2022. A total of 1,799 blood samples were obtained from asymptomatic residents. Participants were aged 2 years or older and resided in the selected malaria-endemic study areas. Subject selection followed Thai national guidelines. Individuals living in the study area who voluntarily agreed to participate and provided informed consent were enrolled. Peripheral blood was collected by trained study nurses. The blood samples were then transferred into EDTA tubes and onto filter papers as dried blood spots (DBS).

2.2. *Transportation and XN-31 measurement*

The collected blood samples were transported on the day of collection to the laboratory at Mahidol University in Bangkok by car. During transport, the samples were maintained under either ice-cold conditions using polystyrene foam boxes or refrigerated conditions using BioBox PLUS (SUGIYAMA-GEN

Co., Ltd., Tokyo, Japan) with ice packs. In some cases, temperature monitoring during transport was conducted using a temperature logger system (SUGIYAMA-GEN). During the first and second trips, samples collected throughout the day were transported together to Bangkok. From the third trip onward, samples collected in the morning were transported in the early afternoon, whereas samples collected in the afternoon were transported in the evening, to shorten the interval between blood collection and XN-31 measurement. Upon arrival, the samples were examined using the XN-31 together with microscopy and rapid diagnostic tests (RDTs).

2.3. *Automated Hematology Analyzer XN-31*

The XN-31 (Sysmex, Kobe, Japan) is an automated hematology analyzer that utilizes flow cytometry for the rapid detection and quantification of malaria-infected red blood cells (MI-RBCs), along with standard hematological parameters. It employs a violet semiconductor laser (405 nm) and uses dedicated reagents—Fluorocell M (nucleic acid staining solution) and Lysercell M (lysis solution)—to differentiate infected from uninfected cells. The analyzer measures forward-scattered light (FSC), indicating cell size, and side fluorescent light (SFL), reflecting nucleic acid content, to generate an M scattergram that plots individual red blood cells based on these optical properties. From the M scattergram, the XN-31 can determine the presence and estimated species of *Plasmodium*, providing one of three interpretive flags: “Malaria?(P.f)” for *P. falciparum*, “Malaria?(others)” for non-falciparum species, and “Malaria?(UNC)” for unclassified malaria species. Representative M scattergram patterns and their interpretations for malaria-negative samples, malaria-positive samples (e.g., *P. falciparum* and *P. vivax*), and samples showing “MI-RBC Abn Scattergram” results are shown in Fig. 1. In this study, we used the Low Malaria (LM) mode of the XN-31, which is optimized for detecting low-density parasitemia. The limit of quantitation (LoQ) in LM mode is reported to be 20 MI-RBC/μL, and each measurement was performed using 60 μL of whole blood.

2.4. *Microscopy*

In Thailand, thick film microscopy is routinely used for parasite detection and species identification in clinical settings; the same approach was applied in this study to follow the standard diagnostic workflow at the Hospital for Tropical Diseases. For thick film examination, a negative result was defined as the absence of parasites after examination of 100 fields under 100× magnification. In thick films, asexual and sexual parasites were counted against 200 white blood cells (WBCs) when the parasite density was ≥ 100 parasites/μL or against 500 WBCs when the parasite density was < 100 parasites/μL, as previously described. Percent parasitaemia was calculated as the proportion of infected RBCs among 10,000 RBCs counted on a thin film, in accordance with WHO guidelines (11). All slides were examined by microscopists who had achieved Level 1 in the External Competence Assessment of Malaria Microscopists conducted by the WHO and the Ministry of Public Health (MOPH), Thailand. According to the institutional standard operating procedure (SOP), two microscopists independently read each thick film, and when the parasite density differed by more than 20%, a third reader was consulted.

2.5. *RDTs*

RDTs were performed using BinaxNOW® Malaria (Abbott Laboratories, Abbott Park, IL, USA) or Bioline™ Malaria Ag P.f/Pan (Abbott Laboratories). RDTs were performed for all 1,799 blood samples. BinaxNOW® Malaria was used for 741 samples during the first and second trips, whereas Bioline™ Malaria Ag P.f/Pan was used for 1,058 samples during the third, fourth, and fifth trips.

2.6. *DNA extraction*

The dried blood spot (DBS) samples were transported to the laboratory of the National Center for Global Health and Medicine in Japan. To prepare templates for PCR reactions, DNA was extracted from a 5-mm-diameter punched DBS using the Maxwell RSC DNA FFPE Kit (Promega Corporation, Madison, WI, USA) together with the automated Maxwell RSC Instrument (Promega Corporation). The DNA was eluted in 100 µL of elution buffer.

2.7. *PCR reaction*

In the Greater Mekong region, which includes Thailand, the majority of malaria cases are caused by either *P. falciparum* or *P. vivax* infections (12, 13). Consequently, we conducted real-time PCR to detect only *P. falciparum* and *P. vivax* DNA. Specific primers targeting the cytb gene of both *P. falciparum* and *P. vivax* were employed. A 20-µL PCR reaction mixture was prepared using SsoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad Laboratories, Hercules, CA, USA), containing 0.125 µM each of forward and reverse primers and 2 µL of template DNA. For the detection of *P. falciparum* DNA, primers PFF1 and PFR1 were used under the following conditions: an initial denaturation step at 95°C for 3 minutes, followed by 38 cycles of 95°C for 5 seconds and 60°C for 20 seconds. For the detection of *P. vivax* DNA, primers PVF11 and PVR7 were used under the following conditions: an initial denaturation step at 95°C for 3 minutes, followed by 38 cycles of 95°C for 5 seconds and 62°C for 20 seconds. Detailed primer sequences are provided in a previous report (14). PCR amplification and detection were performed using the CFX96 Real-time PCR Detection System (Bio-Rad Laboratories).

3. Results

3.1. *Overview of the results*

The overall results are summarized in the flowchart shown in Fig. 2. Among the XN-31 measurements of 1,799 blood samples, 1 sample yielded a measurement error, and 176 samples were classified as “MI-RBC Abn Scattergram” (Abn), indicating the presence of unidentifiable particles that may have interfered with the analysis. One sample yielded a measurement error and was excluded from further XN-31 evaluation because a valid XN-31 result could not be obtained. This sample was negative by microscopy, RDT, and PCR. Among the 176 samples classified as MI-RBC Abn Scattergram, all samples were negative by microscopy and RDT, whereas two samples were positive for *P. vivax* by PCR. After excluding the measurement error and the 176 Abn samples, 1,622 samples were available for further analysis. All samples were negative by RDTs; however, microscopy detected 1 sample as *P. vivax*-positive, which was also positive for *P. vivax* by PCR and flagged as “Malaria?(UNC)” by the XN-31.

Among the 1,621 microscopy-negative samples, PCR detected 2 *P. falciparum*-positive and 3 *P. vivax*-positive samples. However, all 5 PCR-positive samples were negative by the XN-31. Among the 1,616 PCR-negative samples, 1,612 were also negative by the XN-31, whereas 1 sample was flagged as both “Malaria?(P.f)” and “Malaria?(others),” and 3 samples were flagged as “Malaria?(UNC).”

3.2. *Conditions of each transportation and the incidence of Abn*

Because a high incidence of Abn results could hinder accurate interpretation, we took steps to reduce their frequency by improving transportation conditions (Table 1). During the first trip, the Abn rate was 23.8%, representing a substantial barrier to reliable analysis. At that time, XN-31 measurements were performed more than 24 hours after blood collection. From the second trip onward, all samples were analyzed within 24 hours of collection. In addition, based on previous observations suggesting that overcooling (below 2°C) increases the incidence of Abn results, temperature loggers were introduced during the second trip and continuously used thereafter. The recorded data confirmed a tendency toward overcooling. Beginning with the third trip, cold packs were introduced as an alternative to ice cooling to reduce the risk of overcooling. As a result, by the fifth trip, the Abn rate had decreased to 4.6%. For comparison, in a clinical study conducted at Mahidol University, Thailand, where patient samples were measured immediately after collection using the XN-31, the incidence of Abn results was 2.9% (15). These findings suggest that lower Abn rates were observed after modifications to transport procedures, and that the Abn rate observed during the fifth trip was comparable to that reported for bedside testing. Representative M scattergram patterns obtained by XN-31 measurements are shown in Fig. 3. In these scattergrams, unidentified particles were observed to be distributed in the central region.

Table 1
Sample transport conditions and incidence of Abn results during each trip

Trip (Date)	No. of samples	No. of Abn results	Abn rate	Transport conditions and comments
1st (28 Feb. 2021)	261	62	23.8%	All samples cooled with ice. High Abn rate observed. Measurements performed more than 24 hours after blood collection.
2nd (27 Jun. 2021)	480	63	13.1%	Measurements performed within 24 hours after collection. All samples cooled with ice. Temperature logger data revealed that samples were overcooled (0.6–2°C).
3rd (29 Jan. 2022)	260	11	4.2%	Cold packs with the BioBox PLUS system were introduced. The Abn rate was 4/100 (4.0%) with ice cooling and 7/160 (4.4%) with cold packs.
4th (7–8 May. 2022)	470	25	5.3%	To avoid overcooling with cold packs, the packs were left at room temperature for 1 hour after removal from the freezer. Room-temperature transport was also evaluated. The Abn rate was 3/50 (6.0%) with ice cooling, 13/320 (4.1%) with cold packs, and 9/100 (9.0%) at room temperature.
5th (14–15 May. 2022)	328	15	4.6%	All samples were successfully transported using cold packs while maintaining temperatures above 2°C.
Total	1799	176	9.8%	Lower Abn rates were observed after modifications to transport and cooling procedures.

3.3. *M scattergrams of XN-31 positive samples*

To further investigate the nature of the MI-RBC–positive results obtained by the XN-31, we examined the M scattergrams of all five positive samples. Figure 4 shows the M scattergram patterns of the five XN-31–positive samples. Only one of these samples (LF0206) was confirmed as *P. vivax*–positive by both microscopy and PCR. Under microscopic examination of a thick blood smear, the parasite density was estimated to be 51 gametocytes/μL of blood. By XN-31 analysis, the parasite density was calculated to be 32 parasites/μL. In the M scattergram generated by the XN-31, a small red cluster was observed. This red cluster represented a population identified by the XN-31 as ring-stage parasites. However, the cluster was located in a higher FSC region than the representative *P. vivax* ring-stage cluster shown in Fig. 1. Because FSC is associated with cell size, this finding may indicate the presence of a larger infected red blood cell population than that typically observed for ring-stage parasites. Since *P. vivax* gametocytes were observed by microscopy in this sample, the cluster may have represented gametocytes rather than typical *P. vivax* ring forms. In contrast, the other four samples (LF0012, LF0673, LF0839, and LF0997) were positive only by the XN-31, while all other diagnostic methods yielded negative results, suggesting that these were unconfirmed XN-31-positive samples. In fact, the M scattergrams of these samples did not show clearly defined red-dot clusters. Instead, together with blue dots (indicating debris), they

formed irregular structures aligned along the diagonal axis, consistent with artifact patterns rather than true parasite signals.

These unconfirmed XN-31-positive samples were observed only in samples obtained during the first, second, and third trips. Notably, no unconfirmed XN-31-positive samples were identified in samples collected during the fourth and fifth trips.

4. Discussion

In this study, the XN-31 successfully detected a low-density malaria infection in an asymptomatic individual—the only case that was also identified by microscopy. The microscopist involved in this study demonstrated a high level of diagnostic expertise, as the case showed a parasitemia as low as 51 *P. vivax* gametocytes/μL. In general, microscopy is considered to have a detection limit of approximately 50–100 parasites/μL (16). Therefore, this finding highlights the potential utility of the XN-31 for detecting low-density infections. Notably, five additional cases were identified as submicroscopic infections, being PCR-positive but microscopy-negative; however, all of these cases were also negative by the XN-31. Given the limited number of positive cases, further studies may be required to evaluate the utility of the XN-31 for detecting parasite carriers below the microscopic detection threshold.

Throughout the study, the high frequency of Abn results posed a challenge in accurately evaluating the performance of the XN-31 using field-collected blood samples. As efforts were made to reduce the frequency of Abn results, the proportion of Abn samples decreased from 23.8% (first trip) to 4.6% (fifth trip). Lower Abn rates were observed when XN-31 measurements were performed within 24 hours of blood collection and when transport temperatures remained above 2°C. To date, several causes of Abn results have been reported (1, 2, 5), including anemia and abnormally shaped red blood cells (RBCs). Empirically, RBC breakdown caused by overcooling below 2°C has also been considered a potential cause of Abn results. The findings of this study suggest that temperature control during sample storage and transport may affect XN-31 result evaluability.

Notably, no unconfirmed XN-31-positive samples were observed during the fourth and fifth trips, suggesting that efforts to reduce Abn results also contributed to minimizing unconfirmed XN-31-positive results. In the M scattergram patterns of both Abn and unconfirmed XN-31-positive samples, particles were distributed within the central region and aligned along the diagonal axis. These findings suggest that RBC breakdown caused by overcooling may have produced irregular M scattergram patterns that were subsequently misclassified as positive by the XN-31 analysis program. These findings suggest the importance of performing XN-31 measurements within 24 hours of blood collection and maintaining appropriate temperature control during sample transport for field application of the XN-31 in malaria-endemic areas.

Unlike the previous Thailand evaluation conducted in a hospital setting, the present study provides important insights into XN-31 use among residents under real-world field conditions. Although the number of malaria-positive cases was limited, the study provides insight into operational factors that

may affect XN-31 performance, particularly sample transport and temperature management. These findings provide important guidance for establishing practical workflows in which blood samples collected in malaria-endemic areas are transported to central hospitals for diagnosis using the XN-31.

5. Conclusions

In conclusion, although several operational challenges were encountered during field application, this study describes the operational experience of using the XN-31 in a malaria-endemic setting. In particular, strict control of sample storage temperature and prompt measurement after blood collection were associated with lower rates of Abn and unconfirmed XN-31-positive results. Further large-scale studies are warranted to validate its performance under field conditions. Nevertheless, the present findings support the potential utility of the XN-31 for field application in malaria-endemic areas.

Abbreviations

Abn
MI-RBC Abn Scattergram
CBC
Complete blood count
DBS
Dried blood spot
EC
Ethics Committee
FSC
Forward-scattered light
LM
Low Malaria
LoQ
Limit of quantitation
MI-RBC
Malaria parasite-infected red blood cell
PCR
Polymerase chain reaction
Pf
Plasmodium falciparum
Pv
Plasmodium vivax
RBC
Red blood cell
RDT
Rapid diagnostic test

RTIC
Rajanagarindra Tropical Disease International Centre
SFL
Side fluorescent light
UNC
Unclassified
WHO
World Health Organization
XN-31
Automated Hematology Analyzer XN-31

Declarations

Ethics approval and consent to participate

The study protocol and all amendments were approved by the Ethics Committee (EC) of the Faculty of Tropical Medicine, Mahidol University (EC submission No.: TMEC 18–062; approval date: January 10, 2019) and the EC of the National Center for Global Health and Medicine (Reference No.: 3054; approval date: November 9, 2018).

Consent for publication

Not applicable.

Competing Interests

Miki Kawaguchi, Kenichi Fujimaki, and Aya Konishi are employees of Sysmex Corporation. The other authors declare no competing interests.

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Author Contribution

M.K., K.F., A.K., and Shigeyuki Kano conceived and designed the study and were involved in participant enrollment and sample collection. Srivicha Krudsood supervised malaria diagnosis at the field site. N.K. and M.H. supported the setup and implementation of the clinical study. M.N. performed the PCR

analyses. K.K.-Y. drafted the manuscript with input from all other authors. All authors read and approved of the final manuscript.

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Data Availability

The datasets generated and/or analyzed during the current study are not publicly available because the informed consent obtained from participants and ethics approvals for this study do not permit secondary sharing of individual-level participant data.

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Figures

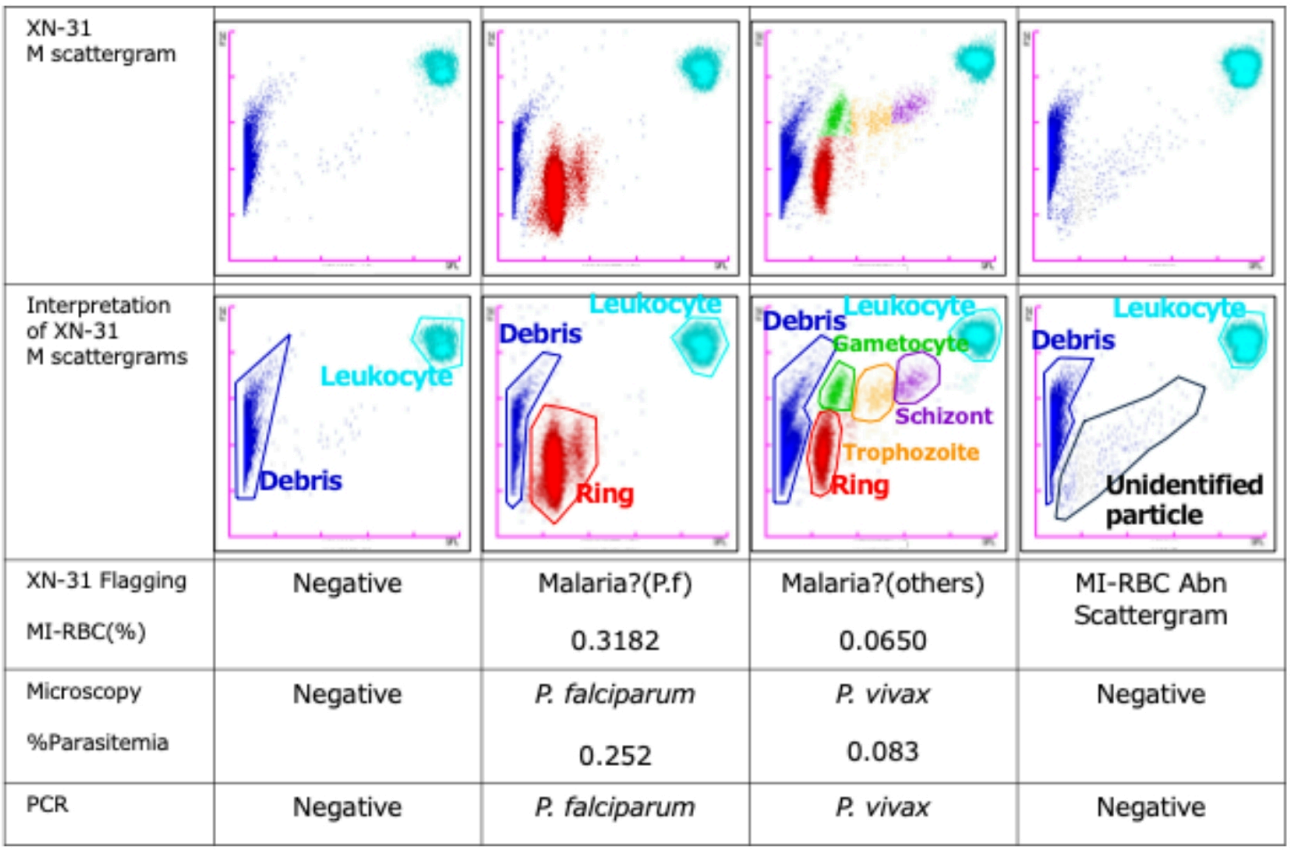


Figure 1

Representative M scattergram patterns generated by the XN-31 from malaria-negative samples, malaria-positive samples, and samples showing "MI-RBC Abn Scattergram" results. The lower panels illustrate the interpretation of each scattergram pattern. Reproduced from Komaki-Yasuda K, et al. Clinical performance testing of the Automated Hematology Analyzer XN-31 prototype using whole blood samples from patients with imported malaria in Japan, Malaria Journal, 2022, under the terms of the Creative Commons Attribution 4.0 International License (CC BY 4.0). The red, orange, purple, and green clusters in the scattergrams represent ring-stage, trophozoite-stage, schizont-stage, and gametocyte-stage parasites, respectively, as automatically classified by the analysis algorithm implemented in the XN-31. The blue cluster located on the left side of the scattergram represents debris, whereas the light blue cluster located in the upper right region corresponds to leukocytes. In samples with "MI-RBC Abn Scattergram" results, unidentified particles are distributed in the central region of the scattergram.

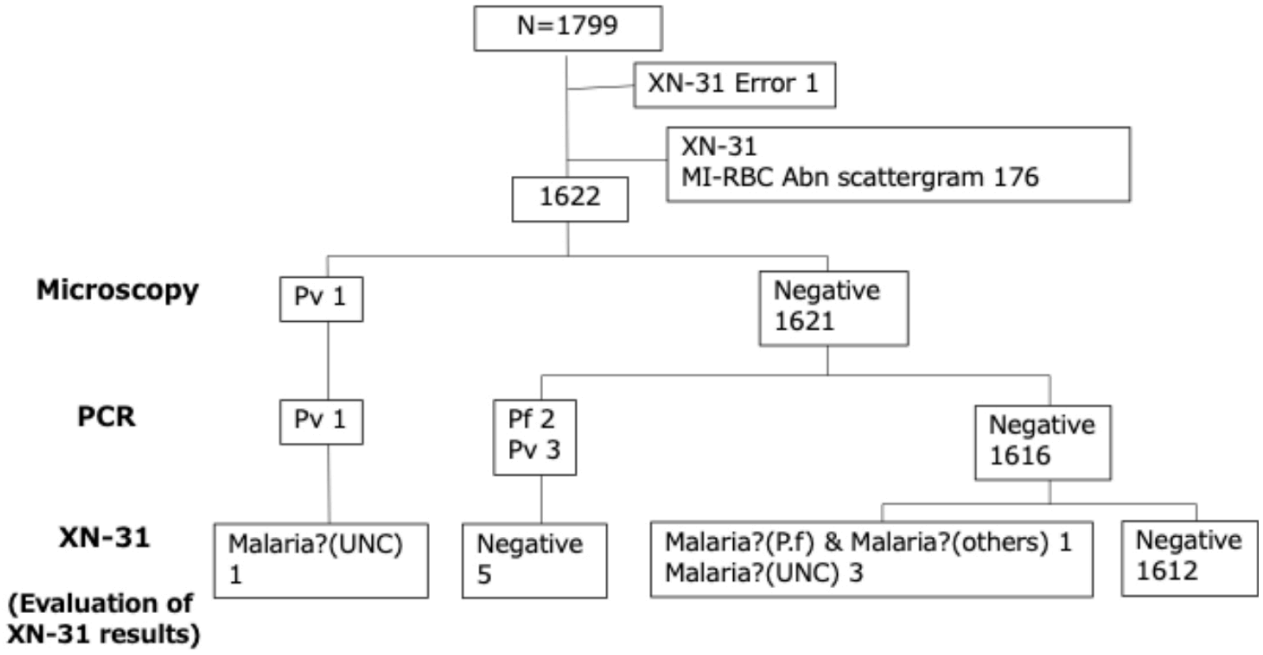


Figure 2

Flow diagram summarizing the diagnostic test results obtained in this study, including microscopy, PCR, and XN-31 findings. For microscopy and PCR results, “Pf” and “Pv” represent *Plasmodium falciparum* and *P. vivax*, respectively.

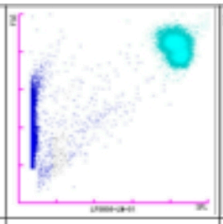
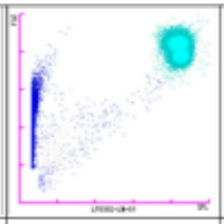
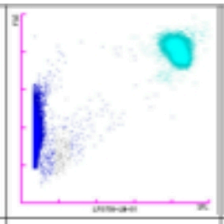
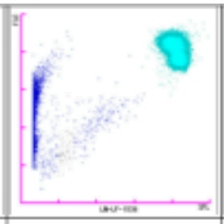
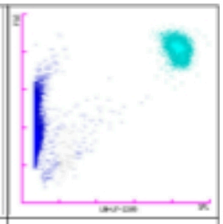
XN-31 M-scattergram					
ID	LF0006	LF0302	LF0759	LF1539	LF2260
Trip	1st	2nd	3rd	4th	5th
XN-31 Flagging	MI-RBC Abn Scattergram	MI-RBC Abn Scattergram	MI-RBC Abn Scattergram	MI-RBC Abn Scattergram	MI-RBC Abn Scattergram
Microscopy	Negative	Negative	Negative	Negative	Negative
PCR	Negative	Negative	Negative	Negative	Negative

Figure 3

Representative M scattergram patterns of samples flagged as “MI-RBC Abn Scattergram (Abn)” during each trip from the first to the fifth. In each scattergram, unidentified particles are distributed in the central region, in addition to the blue cluster on the left side representing debris and the light blue cluster in the upper right region corresponding to leukocytes. The samples shown were the first flagged cases observed during each respective trip.

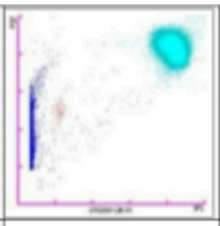
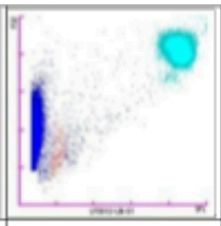
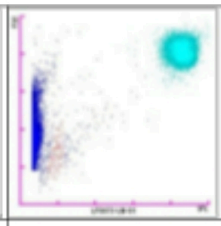
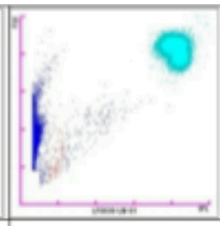
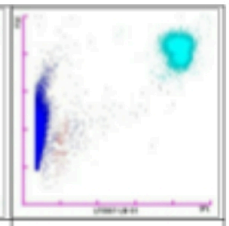
XN-31 M-scattergram					
ID	LF0206	LF0012	LF0673	LF0839	LF0997
Trip	1st	1st	2nd	3rd	3rd
XN-31 Flagging	Malaria?(UNC)	Malaria?(P.f) & Malaria?(others)	Malaria?(UNC)	Malaria?(UNC)	Malaria?(UNC)
Number of #MI-RBC	32 /μL	108 /μL	59 /μL	45 /μL	54 /μL
Microscopy	*P. vivax* (51 gametocytes /μL)	Negative	Negative	Negative	Negative
PCR	*P. vivax*	Negative	Negative	Negative	Negative

Figure 4

M scattergram patterns of samples positive for MI-RBCs by the XN-31. In each scattergram, red clusters represent cells classified as ring-stage parasites by the analysis algorithm implemented in the XN-31. Among these samples, LF0206 was confirmed as *P. vivax*–positive by both microscopy and PCR, whereas the remaining four samples were negative by all other diagnostic methods, suggesting that these were unconfirmed XN-31-positive samples. In LF0206, which was positive by XN-31, microscopy, and PCR, a relatively distinct red cluster was observed. In contrast, the unconfirmed XN-31-positive samples showed irregular particle distributions aligned along the diagonal axis without clearly defined red clusters.