Potential application of the hematology analyzer XN-31 prototype for field malaria surveillance in Kenya

Wataru Kagaya (wataru.kagaya@gmail.com)
Osaka City University https://orcid.org/0000-0003-0998-5267
Ikki Takehara
Sysmex Corp: Sysmex Kabushiki Kaisha
Kyoko Kurihara
Sysmex Corp: Sysmex Kabushiki Kaisha
Gordon Okomo
Homa Bay County Government
Kongere James
Nagasaki University: Nagasaki Daigaku
Jesse Gitaka
Mount Kenya University
Akira Kaneko
Karolinska Institute: Karolinska Institutet

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Abstract

Early and accurate diagnosis is a key component in malaria control programs. Microscopy is the current gold standard, however it requires extensive training and the results largely rely on the skill of the microscopists. Malaria rapid diagnostic tests (RDT) can be performed with minimal training and offer timely diagnosis, but results are not quantitative. Moreover, some *Plasmodium falciparum* parasites have evolved to evade detection by RDT. Developed by the Sysmex Corporation, the XN-31 prototype (XN-31p) is an automated hematology analyzer capable of detecting *Plasmodium*-infected erythrocytes and providing species differentiation and stage specific parasite counts in venous blood samples in a minute without any sample preparation. Here we tested the performance of the XN-31p with capillary blood samples and evaluated the effect of sample storage time and temperature on the stability of results. Paired capillary and venous blood samples were collected from 169 outpatients with clinical malaria symptoms in Homa Bay County Referral Hospital, Kenya. Malaria infections were diagnosed with the XN-31p, microscopy, RDT, and PCR. Capillary blood samples were remeasured on the XN-31p after 24 hours of storage at either room (15 to 25°C) or chilled temperatures (2 to 8°C). Identical results in malaria diagnosis were observed between venous and capillary blood samples processed with the XN-31p. The sensitivity and specificity of XN-31p with capillary blood sample relative to PCR were 0.857 and 1.000 and those relative to microscopy and RDT were 1.000 and 0.986 to 1.000, respectively. Parasitemia and complete blood count (CBC) results were stable in capillary blood samples after 24 hours at room or chilled temperatures. These results showed that the XN-31p can be a useful tool to complement existing methods for routine malaria diagnosis in remote settings.

Introduction

Even though malaria is one of the oldest infectious diseases, it remains a major public health problem worldwide. In 2020, there was an estimated 241 million malaria cases and 627,000 malaria deaths globally [1]. After expanding the usage of novel tools such as rapid diagnostic test (RDT), insecticide treated bed net (ITN), and artemisinin-based combination therapy (ACT), these epidemiological indices have decreased remarkably since 2000. However, the reduction has slowed recently and especially the number of cases has slightly increased in the last five years and has been made worse by the COVID 19 pandemic [1]. This suggests a need for developing more effective diagnostics and medicines, improved and innovative vector control methods, and other tools such as vaccines [2].

In malaria diagnosis, microscopic examination of Giemsa-stained blood films relies on the visual detection of parasites, allows for estimate of parasite density, and remains the gold standard. However, training for competent microscopists takes time [3]. Also in elimination settings, malaria microscopy becomes more challenging because decreasing malaria prevalence presents fewer opportunities for microscopists to observe parasites and to maintain competence [4], and infections are more likely to have parasite density below the detection limits [5,6]. On the contrary, RDT requires less training, is more convenient, and can give results within fifteen minutes. Thus in many remote settings, RDT has

effectively replaced microscopy for routine diagnosis at health facilities as well as active case detection by community health workers. However, since RDT is not quantitative and does not differentiate the various parasite developmental stages, it has limited utility in determining the effect of treatment or monitoring the impact on transmission[7]. Furthermore, recent reports of RDT false negativity due to deletions of the gene encoding the target histidine rich protein 2/3 (HRP2/3) in *Plasmodium falciparum* have raised questions about diagnostic accuracy [8,9,10]. *Plasmodium* lactate dehydrogenase (pLDH)-detecting RDTs are also available, but they have lower sensitivity compared with HRP2-detecting RDT [11]. Polymerase chain reaction (PCR) has higher sensitivity and specificity relative to microscopy and RDT and is widely used for research purpose, but is not practical for clinical use since it requires more advanced equipment and skills and is more expensive. To complement existing methods, there exists a need for innovations in malaria diagnosis, especially those that are highly sensitive and easy to operate in malaria endemic settings.

The automated hematology analyzer XN-31 prototype (XN-31p) (Sysmex Corporation, Kobe, Japan) is a novel malaria diagnostic system based on the principle of fluorescence flow cytometry. The analyzer can perform a complete blood count (CBC), detect malaria infected red blood cells (iRBC), and count parasitemia with a limit of quantification of 20 iRBC/μL [12]. The instrument offers three measurement modes with different sample volumes and different limit of quantification, and takes only one minute for the measurement with simple operation. Previous studies from different malaria endemic settings showed that the XN-31p had comparable performance to microscopy and RDT in malaria diagnosis and good concordance with microscopy and quantitative PCR (qPCR) in parasite counts. However, venous blood samples were used in all previous evaluations except one, and venipuncture is generally not the blood collection method for routine malaria diagnosis in health facilities in endemic areas. Capillary blood samples were previously used to evaluate the performance of the XN-31p in pre-dilute (PD) mode, which requires a sample dilution step that can potentially introduce handling errors and result in lower accuracy. In this study, we evaluated the performance of the XN-31p using capillary blood samples in low malaria (LM) mode, which does not require any sample processing prior to measurement. The operation of the XN-31p is limited to clinical laboratories with stable electricity supply and as off-grid power system increases in most of low-middle-income countries, the equipment can be placed widely. To test the utility of the XN-31p for malaria diagnosis in remote locations in a hub and spoke design, we compared the results of capillary blood samples immediately after sample collection with those kept at either room or chilled temperatures for up to 24 hours. The results provided here expand the possibility of using the XN-31p in a wider range of settings.

Results

1. Participant characteristics

A total of 171 subjects were enrolled in this study, and 169 provided both venous and capillary blood samples. The demographic and clinical characteristics of the participants are described in Table 1. The

number of participants with fever at enrollment was 33 (19.8%). The median white blood cell (WBC) count was 5.93 × 10^3/μL, and the prevalence of anemia, defined as hemoglobin (Hb) of < 11 g/dL, was 20.1%.

Table 1
Participant demographic and clinical characteristics at enrollment

	Total enrolled (n=169)
Age; median (IQR)	23 (5-36)
Male; n (%)	75 (44)
Febrile subjects (≧37.5); n (%)	33 (19.8)
Duration of fever (days); median (IQR)	3 (2-3)
Having chronic conditions; n (%)	13 (7.7)
Took antimalarials in the last one month; n (%)	13 (7.7)
Traveled in the last 3 months; n (%)	13 (7.7)
Slept under a bed net in previous night; n (%)	155 (91.7)
Covered by IRS program for the last one year; n (%)	119 (70.4)
Enrolled in RTS,S vaccine trial; n (%)	3 (1.9)
Pregnancy; n (% of total female)	4 (2.4)
Hematological parameters*	
White blood cell (10^3/μL); median (IQR)	5.93 (4.82-8.93)
Red blood cell (10^6/μL); median (IQR)	5.04 (4.67-5.46)
Hemoglobin (g/dL); median (IQR)	13.1 (11.5-14.2)
Anemia (Hb < 11 g/dL); n (%)	34 (20.1)
Hematocrit (%); median (IQR)	39.8 (34.6-43.1)
Platelets (10^3/μL); median (IQR)	256 (197-364)
Thrombocytopemia (< 150 × 10^3/μL); n (%)	14 (8.3)
*Measured with venous blood in LM mode on the XN-31p.	

2. Diagnostic performance of XN-31p

Table 2 shows the number of malaria cases detected by each method. The XN-31p, microscopy, RDT, and PCR detected 18, 16, 18, and 23 *Plasmodium* infections, respectively. One *P. malariae* infection was

detected by microscopy while three *P. ovale* and one mixed infection of *P. falciparum* and *P. malariae* were detected by PCR. On the XN-31p, *Plasmodium* infections were detected in the same set of 18 samples using both capillary and venous blood; all were identified as *P. falciparum* except in one capillary blood sample where the species could not be specified. Inconclusive results (MI-RBC Abn Scattergram) were reported in five venous and eleven capillary blood samples by the XN-31p. The median parasite densities determined by the XN-31p using venous blood, capillary blood, and microscopy were 21,774, 21,357, and 2,356 parasites/μL, respectively.

Table 2
Diagnosis of malaria by different methods

	XN-31p (venous blood)	XN-31p (capillary blood)	Microscopy	RDT	PCR
Plasmodium positive; n (%)	18 (10.7)	18 (10.7)	16 (9.47)	18 (10.7)	23 (13.6)
P. falciparum	18	17	15	18	19
Others*	0	1	-	-	-
P. malariae	-	-	1	-	0
P. ovale	-	-	0	-	3
P. falciparum + *P. malariae*	-	-	0	-	1
MI-RBC Abn Scattergram	5	11	-	-	-
Negative	146	140	153	151	146
Parasitemia (parasite/μL); median (IQR)	21,774 (9,490 -101,973)	21,357 (9,262 -104,193)	2,356 (1,004 -18,910)	-	-
Parasitemia (%iRBC); median (IQR)	0.44 (0.20 - 1.8)	0.45 (0.19 - 1.8)	0.27 (0.15 - 0.92)	-	-

*Except *P. falciparum*

Comparisons of the performance of the XN-31p with other diagnostic methods are described in Table 3. Samples showing MI-RBC Abn Scattergram were excluded in these comparisons. Performance measures of the XN-31p against microscopy, RDT, and PCR, were similar, with high sensitivity (0.818-1.000), specificity (0.986-1.000), positive predictive value (PPV; 0.889-1.000) and negative predictive value (NPV; 0.973-1.000). Against microscopy and RDT, all performance measures of the XN-31p were identical between capillary and venous blood. Against PCR, slight differences in sensitivity and NPV were found between capillary and venous blood. Detailed comparisons including samples with MI-RBC Abn Scattergram are shown in Supplemental Table 1.

Table 3
Assessment of the diagnostic performance of XN-31p using microscopy, RDT, and PCR as standards

		Microscopy		RDT		PCR	
		Pos	Neg	Pos	Neg	Pos	Neg
XN-31p (venous blood)	Pos	16	2	18	0	18	0
	Neg	0	146	0	146	4	142
		Value	95%CI	Value	95%CI	Value	95%CI
	Sen	1.000	0.713 - 1.000	1.000	0.740 - 1.000	0.818	0.597 - 0.948
	Spe	0.986	0.952 - 0.998	1.000	0.963 - 1.000	1.000	0.962 - 1.000
	PPV	0.889	0.653 - 0.986	1.000	0.740 - 1.000	1.000	0.740 - 1.000
	NPV	1.000	0.963 - 1.000	1.000	0.963 - 1.000	0.973	0.931 - 0.992
XN-31p (capillary blood)	Pos	16	2	18	0	18	0
	Neg	0	140	0	140	3	137
		Value	95%CI	Value	95%CI	Value	95%CI
	Sen	1.000	0.713 - 1.000	1.000	0.740 - 1.000	0.857	0.637 - 0.970
	Spe	0.986	0.950 - 0.998	1.000	0.961 - 1.000	1.000	0.960 - 1.000
	PPV	0.889	0.653 - 0.986	1.000	0.740 - 1.000	1.000	0.740 - 1.000
	NPV	1.000	0.961 - 1.000	1.000	0.961 - 1.000	0.979	0.939 - 0.996

3. Concordance in parasitemia and CBC between capillary blood and venous blood

The concordance in parasitemia between capillary and venous blood was evaluated by Bland-Altman analysis (Figure 1). The limits of agreement were between -2.622 and 3.750×10^3 for iRBC count per μL, and between -0.036 and 0.029% for %iRBC. There were both fixed and proportional biases in the iRBC count per μL ($p < 0.05$ with Wilcoxon matched pairs signed rank test, rho = 0.515, $p < 0.05$ with Spearman's rank correlation test), but not in iRBC%.

For capillary blood samples, parasitemia determined by microscopy and XN-31p showed significant correlation in both iRBC count per μL (rho = 0.538, $p < 0.05$), and %iRBC (rho = 0.643, $p < 0.05$). For iRBC

count per µL, Bland-Altman analysis showed poor concordance between microscopy and XN-31p, with the limits of agreement between -90 and 210 × 10^3 iRBC/µL and fixed and proportional biases ($p < 0.001$ with Wilcoxon matched-pairs signed rank test, rho = 0.9412, $p < 0.001$ with Spearman's rank correlation test) (Figure 2a). On the other hand, for %iRBC microscopy and XN-31p showed good concordance without fixed and proportional bias. The limit of agreement was between -5.09 and 5.13%iRBC (Figure 2b).

Bland-Altman analyses of the concordance in CBCs between venous and capillary blood samples are shown in Figure 3. Fixed biases (all $p < 0.001$) were observed in WBC, hematocrit (HCT) and platelet while proportional bias was found only in platelet (rho = 0.344, $p < 0.001$).

4. Stability of iRBC and CBCs by time and temperature

Capillary blood samples were processed with the XN-31p again after 24 hours at either room (15 to 25°C) or chilled temperature (2 to 8°C). Bland-Altman analyses indicated good concordance in both iRBC count per µL and %iRBC after 24 hours, although results were more consistent for samples stored at chilled temperatures and samples with lower parasitemia (Figure 4).

The stability of CBCs is shown in Figure 5. Samples stored for 24 hours at 2 to 8°C had better concordance with the original measurements at 0 hour than those stored at room temperature. The biases detected in each measurement are summarized in Supplemental Table 2.

Discussion

Early diagnosis and treatment are key to malaria control. Currently malaria diagnosis in the field largely relies on microscopy and RDT, however both methods have limitations. Furthermore, malaria parasites are evolving to evade diagnosis and treatment, as demonstrated by the deletions of the *pfhrp2* and *pfhrp3* genes that allow parasites to escape detection by RDT [13]. Thus innovative tools are needed to overcome existing challenges toward global malaria elimination. In this study, we compared a novel malaria diagnostic system, the Sysmex XN-31p with conventional diagnostic methods in the Lake Victoria region, Kenya, and evaluated its applicability for use in the field. Several studies have shown the capability of the XN-31p to diagnose *Plasmodium* infections in different settings [12,14,15], but this is the first report showing the concordance of results between venous and capillary blood samples without any pretreatment or sample dilution prior to analysis. Also we showed that parasitemia results obtained from capillary blood were stable even after 24 hours, especially if the samples can be kept at chilled conditions. These positive findings unlock the potential use of the XN-31p as a diagnostic system for both clinical use and mass blood surveys in remote settings.

The diagnostic performance of XN-31p is quite comparable to that of existing field diagnostics, namely microscopy and RDT. We observed very high sensitivity (1.000) and specificity (0.986 to 1.000) against these conventional methods. These results are in a good agreement with previous reports [12,14,15]. When

compared to PCR, the sensitivity was lower (0.818 to 0.857) but the specificity remained very high (1.000). More importantly the performance was reproducible even with capillary blood samples.

The XN-31p has three measurement modes. Whole blood (WB) and LM modes do not require any pre-treatment prior to the measurement, while PD mode needs sample dilution before the measurement. PD mode requires only 20 µL of blood and thus is useful when the amount of available sample is very limited. The applicability of capillary blood in PD mode was previously tested, however our own preliminary tests indicated low consistency in parasite counts and CBCs due to handling errors during sample dilution. LM mode uses only 130 µL of blood, which can be readily obtained through finger prick. Our comparison showed excellent concordance between venous and capillary blood samples for malaria diagnosis and species differentiation when samples were measured in LM mode. Good concordance was also observed for %iRBC, with the limits of agreement between -0.036 and 0.029%, which are well accepted in the clinical context. However, the parasite count per µL had fixed and proportional biases and the ranges for the limits of agreement were quite wide. Since there was no significant difference in RBC counts between capillary and venous blood samples (as shown in Figure 3), the discrepancy must have derived from actual differences in iRBC count. The cause of differences in iRBC count is under investigation.

There was a high correlation but low concordance between the parasitemia determined by the XN-31p and microscopy. By microscopy, parasite density was determined by counting the number of iRBC against 200 WBCs in the thick blood smear and assuming 8,000 WBCs per µL of blood. In this study, WBC counts were quite variable among participants (Figure 3A), which might have contributed to the discordance. However, %iRBC was obtained without any assumption, thus the gap observed here may be purely due to the difference of two measurements. Similar trends in the concordance of parasitemia between the XN-31p and microscopy were reported previously [15]. Although microscopy is defined as the gold standard in malaria diagnosis, accurate determination of parasitemia depends on a number of factors including the skills of microscopists and the quality of the stained blood smears, thus other quantitative methods such as qPCR may give insights into the accuracy of parasite count with XN-31p.

Malaria-positive samples are flagged by the XN-31p as “Malaria?(P.f.)”, “Malaria?(Others)” or “Malaria?(UNC)” based on the pattern of scattergram. Unfortunately, few non-*P. falciparum* malaria infections were included in this study, making it difficult to evaluate the ability of XN-31p to differentiate *Plasmodium* species. All *P. ovale* infections in this study were detected by PCR only and thus were likely to have low parasitemia. It is reported that *P. ovale* infections tend to have lower parasitemia because of the species preference to invade reticulocytes [16]. Our PCR detected one case of *P. falciparum-P. malariae* co-infection, which was not detected by other methods likely due to low parasitemia. It would be interesting to test the diagnostic capability of the XN-31p in mixed-species infections since the scattergram patterns of RBC infected by different *Plasmodium* species may overlap with one another. No *P. vivax* was observed in this study in consistent with previous reports[17].

A small number of samples in our study had inconclusive results, reported as MI-RBC Abn Scattergram by the XN-31p. In the case of capillary blood, a previous study attributed this undesirable flag report to the presence of Howell-Jolly body or crystalized Sickle hemoglobin (HbS) [12]. However, in our study, only 3 samples out of 11 samples reported MI-RBC Abn Scattergram together with Sickle cells and one sample reported the presence of Howell-Jolly body did not report MI=RBC Abn Scattergram. Our previous preliminary tests revealed a relatively high number of results reporting MI-RBC Abn Scattergram, which we believed were caused by the finger prick method to obtain capillary blood. Repeated application of pressure on the finger can cause mechanical damage or deformation to blood cells, and damaged cells form aggregates with platelet and fibrin. The observation that inconclusive results were reported in more than twice as many capillary blood (11) as venous blood (5) samples is consistent with our hypothesis. To reduce the appearance of MI-RBC Abn Scattergram in our study, we retrained all laboratory technicians to follow the blood sampling procedure recommended by the Clinical and Laboratory Standards Institute (CLSI). Interestingly most of the MI-RBC Abn Scattergram (80% for venous blood samples and 81.8% for capillary blood samples) were observed in PCR negative samples in our study. Further tests with a larger sample size may give us a clearer answer to the causes of MI-RBC Abn Scattergram.

Operations of the XN-31p require stable electricity supply that are often unavailable in health facilities providing services in malaria endemic areas. To extend the utility of XN-31p as a malaria diagnostic tool in remote communities, we remeasured capillary blood samples after 24 hours at either room or chilled temperatures to simulate the time and condition of blood sample transport from distant villages to Homa Bay County Referral Hospital. Generally, samples stored at 2 to 8°C had better concordance in parasitemia with their 0-hour results than samples stored at room temperature, with narrower limits of agreement. The concordance would be higher if we excluded one outlier, though the numbers of *Plasmodium* positive samples available for analyses were small. CBC results also showed better concordance in samples stored at chilled temperatures. Samples were kept cold in an inexpensive, food-grade insulated cooler box with ice packs that can be readily maintained in basic health facilities. Some studies showed the correlation between the distance from health facility and malaria prevalence [18,19,20,21], thus in a hub and spoke design the potential addition of the XN-31p for malaria diagnosis in remote populations can advance universal access to malaria diagnosis and treatment.

One of the unique features of the XN-31p is the simultaneous measurement of CBCs, which may be useful in malaria eliminating areas. In such settings, lower malaria prevalence means fewer chances for microscopists to examine blood films and maintain competence in malaria diagnosis. Moreover, differential diagnosis of febrile diseases becomes more important in eliminating settings where malaria is no longer the main cause of fever [22,23,24,25], thus the ability to measure CBCs with malaria diagnosis can be useful in fever case management by differentiating bacterial and viral infections from malaria, when it is combined with the differential counts of WBCs, being able to help clinical practitioners in rural health facilities to decide an appropriate drug of choice.

This is the first test of the XN-31p in East Africa. The Lake Victoria basin has some of the highest malaria burden in Kenya, and our previous cross-sectional surveys in Homa Bay County showed malaria

prevalence of approximately 20% and 40% by microscopy and PCR, respectively [17,26]. Malaria prevalence in the study area has decreased substantially due partly to the roll out of the indoor residual spray (IRS) program. Also the onset of the COVID-19 pandemic coincided with the start of this study, greatly reducing the number of outpatients seeking treatment at the hospital and resulting in a study sample size much smaller than we had expected due to altered care seeking behaviors driven by social distancing measures such as lock downs and travel restrictions. However, the results from this study are in general agreement with those from previous reports and provide evidence to support further development and evaluation of the XN-31p as a malaria diagnostic tool.

In summary, this study showed that capillary blood can be used in lieu of venous blood for malaria diagnosis on the XN-31p hematology analyzer. Moreover, capillary blood can be stored at chilled temperature for up to 24 hours without adversely affecting malaria diagnostic and CBC results, which broaden the applicability of the XN-31p as a rapid and accurate malaria diagnostic method for mass surveys and case confirmation in remote locations. The ability of the XN-31p to detect asymptomatic and submicroscopic infections and mixed-species infections needs to be investigated.

Methods

1. Study design and study site

An observational cross-sectional study was conducted to compare the effects of blood sampling methods (venous vs. capillary) and sample storage temperature and length on the performance of the XN-31p in the detection of *Plasmodium* infections. The study was conducted in the Homa Bay County Referral Hospital in Kenya between March and April 2020. The hospital is the major referral hospital in Homa Bay County, accepting patients throughout county. As a normal clinical procedure, all outpatients were first examined by clinical officers. Once malaria was suspected, the patient was included in the study after obtaining informed consent.

Homa Bay County lies along the eastern shore of Lake Victoria and is among the regions with the highest malaria prevalence in Kenya [27]. Two rainy seasons are observed in the Lake Victoria basin: the long rainy season runs from March to June and the short rainy season from November to December. Malaria incidence peaks one to two months after the rainy seasons. The average *P. falciparum* parasite prevalence in 2 to 10 years old in the Lake Victoria region in Kenya is estimated to be more than 30% [27]. Long lasting insecticidal nets (LLINs) were distributed for free by the Ministry of Health in 2017, but the proportion of households with at least one LLIN for every two persons was only 50% in the Lake region [28]. Indoor residual spraying (IRS) has been conducted across Homa Bay County once in a year since 2018 [29], and the third round of IRS was implemented in February and March 2020, just prior to our study [30]. A pilot introduction of the RTS,S vaccine was started in September 2019 [31].

2. Procedures for the sample collection

Prior to blood sampling, a questionnaire was administered to study participants to obtain sociodemographic information and known malaria risk factors. Capillary blood (250 µL) was obtained by finger prick in a BD K2-EDTA Microtainer blood collection tube (BD, New Jersey, US). After inversions, 70 µL of blood was spotted on Whatman ET31 Chr filter paper (Whatman International, Maidstone, UK) and stored in a zipped plastic bag after drying at room temperature. Capillary blood was also used to prepare thick and thin blood smears for microscopic examination, and for RDT diagnosis using the SD Bioline Malaria Ag Pf/Pv RDT (Standard Diagnostics Inc., Gyeonggi-do, South Korea). A detailed protocol of capillary blood collection was shared with laboratory technicians (Supplemental Figure 1). Venous blood (500 µL) was collected with a hypodermic needle and syringe and transferred to a different BD K2-EDTA Microtainer blood collection tube.

3. Diagnostic methods: Measurement with XN-31p, microscopy, RDT, and PCR

All diagnostic methods except PCR were carried out at the Homa Bay County Referral Hospital laboratory. PCR was performed in Osaka City University, Japan.

Immediately after sampling, both capillary and venous blood samples were directly analyzed on the XN-31p (Sysmex Corporation, Kobe, Japan) using the low malaria (LM) mode [32]. In LM mode, approximately 130 µL of blood is needed, although only 60 µL of the sample is used for analysis. Detailed features of the XN-31p were described in a previous report [15].

Based on the results of this initial analysis, samples were divided into two groups and stored at either room or chilled temperature for 24 hours. Samples in the room temperature group (8 positive and 39 negative samples) were kept in an air-conditioned room with temperature set at 22°C, while those in the chilled temperature group (8 positive and 43 negative samples) were kept in a cooler box with frozen ice packs (2 to 8°C). The ice packs were changed in the morning. After 24 hours all samples were analyzed again on the XN-31p using the LM mode.

Microscopic examination was performed as described by malaria microscopy standard operating procedure (MM-SOP-09) by World Health Organization [33]. Briefly, thin smears were fixed with methanol, and both thin and thick smears were stained with 3% Giemsa solution for 30 minutes. The positivity of the microscopy is determined by the thick smear. %iRBC and iRBC counts were calculated as described in Supplemental Figure 2. The slides were examined by two experienced microscopists and samples with discordant results were further examined by a third experienced microscopist who was blind to results from the first two readings. In addition to *Plasmodium* parasites, the presence of sickle cell, target cell, dacrocyte, Howell-Jolly body (if present, the count), hemolysis, morphological abnormality of WBC, fibrin clots, and platelet aggregation was noted. RDT results were obtained by following manufacturer's instructions. For PCR diagnosis, DNA was extracted from quartered blood spots (17.5 µL) using the QIAamp Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. PCR

amplification of the *Plasmodium* mitochondrial cytochrome c oxidase III (*cox3*) gene was performed as described previously [34].

4. Statistical analyses

All questionnaire data together with RDT, microscopy, and PCR results were entered into a Microsoft Excel spreadsheet and cross-checked for errors. The sensitivity, specificity, positive and negative predictive values of the XN-31p were calculated in reference to RDT, microscopy, and PCR using the R-based statistical software EZR [35]. Concordance in malaria parasitemia and CBC between capillary blood sample and venous blood sample, between XN-31p and microscopy, and between initial measurement and measurement after 24 hours with storing in room temperature or cool condition were evaluated by Bland-Altman plot method using GraphPad Prism 9.2.0 (GraphPad Software Inc, CA, USA). Wilcoxon matched pair signed rank test, and Spearman's rank correlation test were performed to examine the presence of proportional bias and fixed bias, respectively with STATA/MP 16.1 (StataCorp, TX, USA).

5. Ethics approval and consent to participate

The study was approved by the Kenyatta National Hospital/University of Nairobi-Ethics and Research Committee in Kenya (registration number: P609/10/2014), and Osaka City University Institutional Review Board (registration number: 3206) and performed in accordance with relevant guidelines and regulations. Community consent to study participation was sought through workshops and sensitization meetings with the island communities. Informed consent was obtained from all study participants at enrollment.

Declarations

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions

WK, GO, JG and AK conceived and designed the experiments, WK, IT, KK, and JK performed the experiments, WK, IT, and KK analyzed the data, WK wrote the paper. All authors reviewed the manuscript.

Additional information

Competing interests

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Figures

Figure 1

Bland-Altman analyses of concordance in (A) iRBC count per µL and (B) %iRBC between venous and capillary blood samples measured by XN-31p.

Figure 2

Bland-Altman analyses of concordance in (A) iRBC count per µL and (B) %iRBC in capillary blood between microscopy and XN-31p.

Figure 3

Bland-Altman analyses of concordance in (A) WBC, (B) RBC, (C) Hb, (D) hematocrit, and (E) platelet between venous and capillary blood samples measured by XN-31p.

Figure 4

Effects of storage time and temperature on parasitemia in capillary blood samples measured by XN-31p. Bland-Altman analyses of concordance in (A) iRBC count per μL after 24 hours at 2 to 8°C, (B) %iRBC after 24 hours at 2 to 8°C, (C) iRBC count per μL after 24 hours at room temperature, and (D) %iRBC after 24 hours at room temperature.

Figure 5

Effect of storage temperature on concordance in the complete blood counts (CBCs) measured by XN-31p. Capillary blood samples were kept for 24 hours at either chilled (2 to 8°C; A through E) or room temperature (15 to 25°C; F through J). WBC (A and F), RBC (B and G), Hb (C and H), hematocrit (D and I), and platelet (E and J) were measured.

Supplementary Files

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