Received: 15 June 2021 | Revised: 6 September 2021 | Accepted: 7 September 2021

DOI: 10.1111/vox.13208

ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Evaluation of the Sysmex XN-31 automated analyser for blood donor malaria screening at Malawi Blood Transfusion Services

Bridon M'baya[1] | **Thom Mfune**[1] | **Aubrey Samon**[1] | **Talent Hwandih**[2] | **Marion Münster**[2]

[1]Malawi Blood Transfusion Service, Blantyre, Malawi

[2]Sysmex Europe, Norderstedt, Germany

Correspondence
Marion Münster, Sysmex South Africa, 5 Hunter Avenue, Ferndale, Randburg 2194, South Africa.
Email: munster.marion@sysmex.co.za

Funding information
Sysmex Europe funded the study and provided the XN-31 analyser and related reagents for the study duration

Abstract

Background and Objectives: Balancing blood supply safety and sufficiency is challenging in malaria-endemic countries where the risk of transfusion-transmitted malaria (TTM) is ever-present. In support of reducing this risk, our study aimed at evaluating the performance of the Sysmex XN-31 analyser in blood donor malaria screening, as compared with current practice in Malawi.

Materials and Methods: This prospective observational study was conducted on remnant venous donor blood samples collected at Malawi Blood Transfusion Service donation sites countrywide for routine blood-borne pathogen screening. XN-31 results were compared with routine thick smear malaria microscopy, using expert microscopy (phase 1 and 2) plus qualitative malaria polymerase chain reaction (PCR) (phase 2) to adjudicate discrepancies.

Results: XN-31 detected malaria in 614 (11.6%) of 5281 study samples compared with 341 (6.5%) for routine microscopy. Of the 273 discrepant samples, 60 smears (phase 1) could not be retrieved for expert microscopic review. Expert microscopy confirmed the XN-31 positivity in 78.8% (149/189) and 91.7% (22/24) of discrepant samples in phase 1 ($n = 4416$) and phase 2 ($n = 975$), respectively, with two cases requiring PCR testing, confirming one each as positive and negative, giving sensitivities of 100% and 75% and specificities of 99.9% and 100%, respectively, for XN-31 and routine microscopy.

Conclusion: The automated Sysmex XN-31 analyser's high sensitivity and specificity, ability to detect all *Plasmodium* species and high throughput with rapid turnaround-time, overcomes many of the limitations of currently available diagnostic tests, making it well-suited for malaria screening of donated blood in malaria-endemic countries in support of TTM risk reduction.

KEYWORDS
blood donation testing, blood safety, high throughput testing, malaria and protozoal infections, patient blood management, transfusion-transmissible infections

INTRODUCTION

Balancing blood supply safety and sufficiency is challenging in malaria-endemic countries with a lack of suitable diagnostics for pre-transfusion blood screening significantly contributing to transfusion-transmitted malaria (TTM) [1, 2]. Smear microscopy is laborious and subjective, with increasing workload compromising sensitivity. Rapid diagnostic tests, whilst simple to use, lack sensitivity and specificity and polymerase chain reaction (PCR) is cumbersome and costly. Pathogen inactivation is a tool aimed at the prevention of TTM [3, 4]. At a reported price of ~25 USD/unit, in Ghana, costs hinder its universal implementation. Prophylactic pre-transfusion antimalarials are advocated by World Health Organization (WHO), but this practice is not widely adopted because of cost and drug resistance concerns [5].

Malawi, a high malaria burden country with perennial transmission and the entire population at risk of infection, is one of three sub-Saharan African countries that screens donated blood for malaria [2]. In 2019, there were ~4 million confirmed malaria cases [6], accounting for 34% of hospital admissions and 40% of in-hospital deaths, impacting mostly pregnant women and young children [7]. In 2015, 68% of whole blood issued by the Malawi Blood Transfusion Service (MBTS) was for treating malaria, and 62% of recipients were children and pregnant women [8]. MBTS, contributing 77% of the national blood supply, utilizes thick smear microscopy for screening. MBTS donors with sub-microscopic parasitaemias would go undetected, and only 60% of hospital-based collections are screened for malaria (using rapid tests); however, there is no official data on the incidence of TTM in Malawi.

The Sysmex XN-31 analyser (XN-31) is a new higher diagnostic sensitivity automated malaria detection technology, which has shown great potential in research [9–11] and clinical settings [12–14]. This study was undertaken to establish the usefulness of XN-31 for malaria screening in a malaria-endemic setting. We compared the performance of XN-31 against routine MBTS practice of thick smear microscopy using expert microscopy (phase 1) for judgement of discrepant results, with the addition of qualitative malaria PCR in phase 2. The specific objectives were to determine the sensitivity and specificity of XN-31 for malaria screening compared with routine microscopy and to correlate malaria parasite quantification by XN-31 with microscopy grading.

METHODS

Study design and samples

This prospective observational study used venous blood from 5 ml sample tubes collected from donors at MBTS donation sites country-wide for routine blood-borne pathogen screening. Blood tubes were transported to the central laboratory in Blantyre within 24–72 h of collection and tested immediately upon receipt. K_2EDTA blood left-over after blood typing and malaria thick smear microscopy from consecutive blood donor samples received on low workload days was used for XN-31 testing. The study aimed to collect 4000 samples, using expert microscopy for the final judgement of discrepant malaria screening results (XN-31 vs. routine microscopy consensus result). The sample size was estimated using Buderer's methodology [15], incorporating the current MBTS donor malaria prevalence estimate of 1% and a confidence interval (CI) of 95%.

As several samples remained discordant after expert microscopy, a further 975 consecutive blood donors were included (phase 2), where qualitative PCR was used for the final judgement of samples unresolved by expert microscopy.

Ethical considerations

Study approval (protocol number #19/09/2396) was granted by the National Health Sciences Research Committee of the Malawi Ministry of Health. Donor informed consent was waived, as there was no additional blood draw and consent to donate includes consent to use donated blood samples and biodata for research use to improve blood safety and/or in studies of public health importance. Donors can opt-out at any time. In addition, as this study would not influence the standard of care, consent from recipients of donated blood included in this study was not required.

Routine testing

Blood typing, serology testing and malaria thick smear microscopy were performed according to MBTS standard procedures. The decision to release, quarantine or discard blood units was based exclusively on routine test results.

Routine malaria testing

Each study sample had a unique barcode. Each slide was examined by two microscopists who scanned 100 high-power fields before assigning a negative judgement. A semi-quantitative parasite density estimate (1+ to 4+) [16] was provided for positive samples. A blinded third MBTS microscopist served as an adjudicator for positive–negative discrepancies. Three microscopists shared the study workload, each reviewing up to 150 smears/day (over a period of 4–6 h).

Sysmex XN-31 analyser automated malaria screening

The measurement principle of XN-31, an analyser essentially identical to its research-use-only predecessor, the XN-30, has been detailed elsewhere [12]. Briefly, a reagent partially permeabilizes the red blood cell (RBC) membrane allowing entry of a fluorescent reagent that stains parasite nucleic acids. The RBC count is measured using a sheath flow direct current detection method, and the malaria-infected RBCs (MI-RBC) are measured by fluorescence flow cytometry.

MI-RBC are quantified and a qualitative judgement of malaria present–absent provided based on the analytical limit of quantification (LOQ) of 20 parasites/μl (p/μl). Where particle counts exceed 20 p/μl, but no distinct cluster is observed in the malaria gating area, an 'MI-RBC abnormal scattergram' flag is generated, and the result is deemed indeterminate. XN-31 can detect all *Plasmodium* species causing human malaria. Differentiation between *Plasmodium falciparum* and non-*falciparum* infections is available as a research-use-only suspect flag. Each measurement takes 1 min with a cost of ~3 USD/test (including analyser and related costs, assuming 20,000 tests/annum over 5 years).

Establishment of an XN-31 equivalent grading for parasite densities

The 'plus grading system' [16] and estimated thick smear blood volumes [17] were combined to calculate expected parasite density ranges.

Adjudication of discrepant malaria screening results

XN-31 and microscopy results were cross-checked daily to identify discrepancies.

Phase 1: Discordant samples were referred to an external WHO-certified microscopist for expert review using the same method as MBTS but without time pressure.

Phase 2: After preliminary data analysis, 975 additional samples were collected, and two dry blood spots were made on those with discrepant results. Qualitative PCR confirmatory testing, using *P. falciparum*-specific primers, was undertaken by an external molecular laboratory, in addition to expert microscopy, for final judgement. No non-*falciparum* malaria cases were reported for Malawi in 2019 [6].

Known positive and negative samples were included for expert microscopy and PCR for quality control purposes.

Study period

Donor blood samples included in phase 1 were collected from December 2019 to April 2020 and from November to December 2020 for phase 2.

Statistical methods

Data analysis was done using MedCalc® Statistical Software version 19.8 (MedCalc Software Ltd, Ostend, Belgium). Continuous data were expressed as medians and interquartile ranges (IQR). Chi-squared test was used for the comparison of two independent proportions. *p* Values below 0.05 (two-tailed) were considered statistically significant. The sensitivity and specificity (with 95% CIs) of XN-31 and routine microscopy (consensus of two microscopists) were compared to that of the reference. For phase 1, the reference method was expert microscopy, and for phase 2, it was expert microscopy and PCR (for residual discrepancies post-expert microscopy).

RESULTS

Malaria screening

Malaria was observed in 341 (6.5%) of 5281 samples screened by routine microscopy with 76.3% (260/341) judged to have low parasitaemia (1+). The routine microscopists differed in parasite density grading in 21 cases and qualitative malaria assessment in 13 cases, necessitating the third microscopist. XN-31 judged 11.6% (614/5281) as malaria positive (MI-RBC present), with a median of 164 p/μl (IQR: 63–448) ranging from 20 to 13,514 p/μl. The detection rates of the screening methods differed significantly ($p < 0.0001$).

Diagnostic performance of XN-31 against routine microscopy

Phase 1: Expert microscopy adjudication of discordant results

After eliminating 190 samples (3.6%) with MI-RBC abnormal scattergram flags, (all routine microscopy negative), 4116 result pairs were available for analysis and revealed 249 (6%) samples with mismatched malaria screening results. For all, the XN-31 result was positive and routine microscopy negative. The expert microscopist detected malaria in 78.8% (149/189) of smears available for review. The diagnostic performance of routine microscopy and XN-31, using expert microscopy as the reference method, is shown in Table 1.

Routine microscopy detected 22% of samples with MI-RBC <100 p/μl, compared with 75% for that ≥100 p/μl. The median MI-

TABLE 1 Diagnostic performance of routine microscopy and XN-31 for blood donor malaria screening against expert microscopy (phase 1)

	Routine microscopy	XN-31
Sensitivity	64.6% (59.8%–69.2%) [272/421]	100% (99.1%–100%) [421/421]
Specificity	100% (99.9%–100%) [3635/3635]	98.9% (98.5%–99.2%) [3595/3635]

Note: Values are represented as follows: (95% confidence interval) [sample numbers].

RBC for samples positive by both methods ($n = 341$) was 227 p/μl (IQR 80–553), with 23 p/μl the lowest count. Malaria-infected donors missed by routine microscopy had low parasitaemias (median 101 p/μl, IQR 55–153), with one exception (6224 p/μl), which was reported 2+ positive by one microscopist but negative by three others, including the expert. The XN-31 scattergram of this case showed a distinctive *P. falciparum* pattern (Figure 1), which together with the 2+ grading of one microscopist suggests a sample mix-up during the smear review process.

After the review of discordant samples, 21.2% (40/189) remained discrepant as no malaria parasites were observed by expert microscopy. The lowest equivalent MI-RBC value detected by the expert was 20 p/μl.

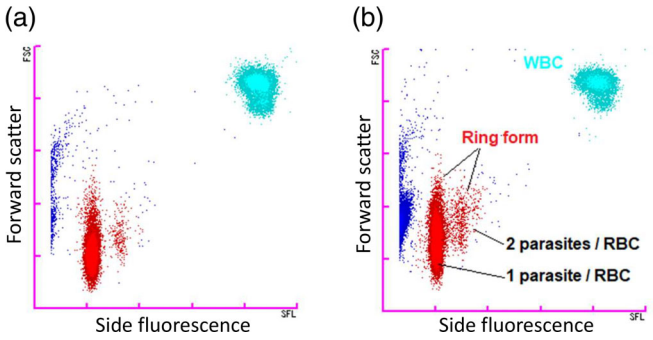

FIGURE 1 XN-31 scattergram of the sample adjudicated as 'false positive' by expert microscopy. (a) Study sample; (b) typical scattergram of confirmed *Plasmodium falciparum* infection. RBC, red blood cells

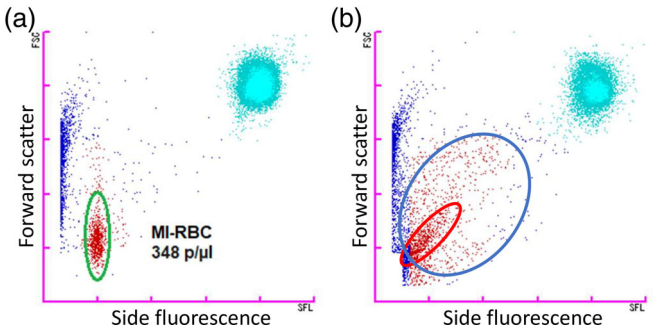

FIGURE 2 XN-31 scattergrams of the two samples that required polymerase chain reaction (PCR). (a) True positive (PCR confirmed), showing a vertically aligned cluster (green ellipse), non-continuous with debris and non-infected red blood cell (RBC) shown in blue along the *y* axis, typical of malaria-infected RBC. (b) False positive (PCR negative). The cluster of events is concentrated at a 45° angle (red ellipse), continuous with the blue events and associated with generalized scatter (blue oval), typically observed in cases of interference. MI-RBC, malaria-infected red blood cells

Phase 2: Expert microscopy and PCR adjudication of discordant results

Of the additional 975 samples that underwent malaria screening, 24 (2.5%) were discordant (XN-31 positive, routine microscopy negative). Expert microscopy confirmed malaria positivity in 22 cases. PCR testing of the two negative cases confirmed one positive (MI-RBC 348 p/μl) and one malaria negative. Upon review, the XN-31 scattergram of the PCR negative sample revealed a pattern typically observed in cases of interference (Figure 2). The diagnostic performance of routine microscopy and XN-31 using expert microscopy, and PCR where needed, as the reference method, is shown in Table 2.

Comparison of microscopy parasite density estimation with XN-31 MI-RBC quantification

Calculated p/μl values correlating to the 'plus' parasite density grading system are shown in Table 3. MI-RBC values for the semi-quantitative microscopy grades overlapped substantially (Figure 3). The agreement between microscopic grading and the corresponding absolute value ranges is shown in Table 4. Twenty percent (62/312) of only malaria-infected units (co-infections excluded) would have been discarded due to higher parasitaemia (≥2+), with the remaining

TABLE 3 Absolute parasite counts equivalent to the 'plus' microscopic parasite density grades

Microscopic grading	Parasites per HPF (100× oil immersion objective)	Parasites/μl of blood
1+	1–10 parasites/100 HPF	<67
2+	11–100 parasites/100 HPF	67–667
3+	1–10 parasites/1 HPF	668–6667
4+	>10 parasites/1 HPF	>6667

Abbreviation: HPF, high power field.

TABLE 2 Diagnostic performance of routine microscopy and XN-31 blood donor malaria-screening against expert microscopy and polymerase chain reaction (phase 2)

	Routine microscopy	XN-31
Sensitivity	75.0% (64.9%–83.4%) [69/92]	100% (96%–100%) [92/92]
Specificity	100% (99.6%–100%) [883/883]	99.9% (99.4%–99.997%) [882/883]

Note: Values are represented as follows: (95% confidence interval) [sample numbers].

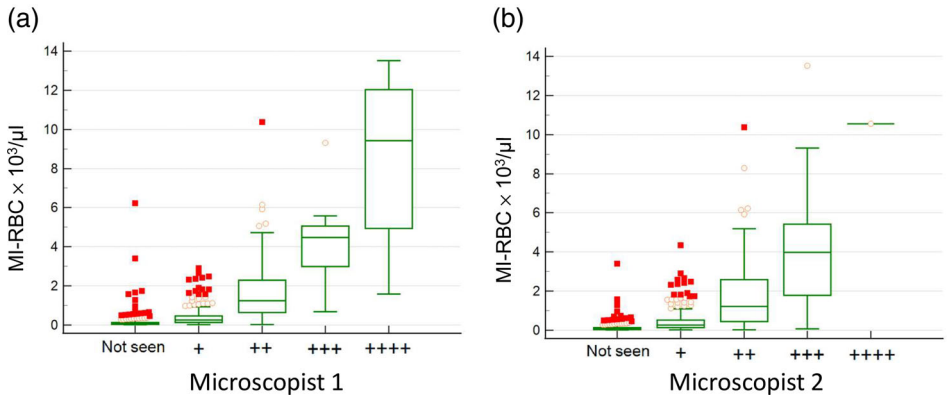

FIGURE 3 XN-31 malaria-infected red blood cells (MI-RBC) distribution for semi-quantitative microscopy grades. (a) Microscopist 1; (b) Microscopist 2. The boxplots highlight the respective MI-RBC medians, lower and upper quartiles and mild (orange dots) and extreme (red squares) outliers

TABLE 4 Comparison of semi-quantitative microscopy grades with expected parasites/µl values as measured by XN-31

		XN-31 MI-RBC count based parasite density grading (parasites/µl)			
		<67	67–667	668–6667	>6667
Microscopy grading	Samples (*n*)	1+	2+	3+	4+
1+	232 (80%)	31 (13.4%)	**165 (71.1%)**	**36 (15.5%)**	**0 (0%)**
2+	48 (16.5%)	*1 (2.1%)*	12 (25%)	**34 (70.8%)**	**1 (2.1%)**
3+	9 (3%)	0 (0%)	0 (0%)	8 (88.9%)	**1 (11.1%)**
4+	1 (0.5%)	0 (0%)	0 (0%)	0 (0%)	1 (75%)
Total	290	32 (11%)	179 (61%)	78 (27%)	3 (1%)

Note: Co-infected samples and those without grading consensus were excluded. 'Bold', 'italic' and 'regular' font represent underestimated, overestimated and correctly estimated microscopic parasite densities, respectively.

80% (250/312) kept in quarantine (for release under special conditions in the event of negative-screened blood stock-outs) in line with national guidelines [18]. Agreement between the microscopic estimates and XN-31 result was observed in only 17.9% (52/290) samples, with 81.7% (237/290) underestimation and 1 sample overestimated. Using XN-31, 143 and 414 malaria-infected units, after exclusion of co-infections, would have been graded as 1+ and ≥2+, respectively.

DISCUSSION

In this study, we evaluated the performance of the Sysmex XN-31 automated analyser for malaria screening at MBTS, comparing it with their routine of thick smear microscopy.

The malaria detection rate, in 5281 donor blood samples tested, was significantly higher ($p < 0.0001$) using XN-31 (11.6%) compared with routine microscopy (6.5%). All samples with discrepant results gave an XN-31 'MI-RBC present' result with 'no parasites observed' by routine microscopy. In phase 1, with a 6% (249/4116) discrepancy rate, a WHO certified expert microscopist observed malaria parasites in 78.8% (149/189) of cases, concurring with the XN-31 result. Compared with expert microscopy, the respective sensitivities and specificities for XN-31 and routine microscopy were 100% versus 64.6% and 98.9% versus 100%. This 'trade-off' in specificity is unsurprising when new automated technology such as XN-31, with a LOQ of 20 p/µl, achievable with high precision, is compared with an inherently subjective manual reference method, such as microscopy, even in the hands of experts, with achievable sensitivities ranging from 5 to 50 p/µl, and up to 500 p/µl under high workload conditions [19, 20]. The median parasite count of microscopically detected malaria-infected donors was 227 p/µl (IQR 80–553) whereas those missed had a median of 101 p/µl (IQR 55–153), in line with sensitivities expected for routine microscopy. Interestingly, although the lowest parasite counts detected by XN-31, routine and expert microscopy, were equivalent (20–23 p/µl), routine microscopy detected only 22% of samples <100 p/µl, compared with 75% of samples ≥100 p/µl, suggesting that accuracy of malaria microscopy is influenced more by the inconsistency of manual counting when parasite density is low, rather than the limit of detection. Our findings of differences between the routine microscopists in 10.0% of cases (34/341) in qualitative judgement of malaria or parasitaemia

grading, further highlight the inherent subjectivity of manual microscopy [21].

As 21.2% (40/189) XN-31 positive cases remained discrepant after expert microscopic review and were classified as 'false positives' under this study design, we extended the study (phase 2) to incorporate qualitative PCR, with undisputed superior analytical sensitivity, for final judgement. In phase 2, the agreement rate between routine microscopy and XN-31, and expert microscopy and XN-31, was higher than in phase 1, hence PCR was only required for 2/24 samples, confirming one positive and one negative result. Upon review of the XN-31 scattergram (Figure 2), the PCR positive sample showed a classical malaria pattern whereas the negative sample was clearly a false positive XN-31 result, which should have triggered an MI-RBC abnormal scattergram flag making the outcome indeterminate. The sample was no longer available at the time of data analysis, precluding further investigation.

Our study showed that XN-31 had superior sensitivity to routine microscopy (100% vs. 75%) for the detection of malaria-infected donors, whilst maintaining excellent specificity (99.9%). Deployment of XN-31 as a routine malaria screening tool within blood transfusion services in malaria-endemic countries such as Malawi would reduce the risk of TTM beyond what is currently achievable with microscopy.

Malaria-infected blood is not automatically discarded. At MBTS, low parasitaemia-infected blood (1+) is kept in quarantine as reserve in case of negative-screened blood stock depletion [18]. Based on current MBTS practices, 62 units would have been discarded exclusively because of malaria, compared with 414 had the XN-31 MI-RBC value of >67 p/μl (as equivalent to ≥2+) been applied, with the remaining 4.7% (250/5281) and 2.7% (143/5281) for microscopy and XN-31, respectively, of the donor pool being kept in quarantine. The significant overlap in measured parasite counts for the plus grades, and substantial microscopic parasite density underestimation (Table 4), highlight serious limitations of microscopic parasite density estimates to inform usage of malaria-infected blood products.

Maintaining an adequate safe blood supply is challenging in sub-Saharan Africa where severe blood shortages are widespread and parasitaemic blood donors are common. Malaria drives the demand for blood in most malaria-endemic countries where those most in need, namely young children, and pregnant women, are also at greatest risk of an adverse clinical outcome of TTM [22, 23].

Unsurprisingly, countries with the greatest malaria burden, and thus need for blood, also have the highest prevalence of parasitaemic blood donors. Here, identification of low-risk donors is virtually impossible and rejection of all malaria parasite-containing blood units untenable, as this would cripple the blood supply. Blood transfusion practice in malaria-endemic countries is highly complex, where compromise is unavoidable to achieve an acceptable balance between supply and demand, without undue loss of life. Withholding blood from someone in critical need carries a high certainty of death, whereas releasing a blood unit, even if malaria-infected may be life-saving despite the risk of TTM.

Our study has some limitations. Notably, expert microscopy was evoked only for the assessment of discrepancies, with PCR analysis reserved for selected samples. As there were no 'XN-31 negative-microscopy positive' mismatches, the extent of missed parasitaemias with <20 p/μl is unknown. An important caveat for determining the actual prevalence of malaria-infected donors, and thus risk of TTM, is to detect low-level parasitaemia [22]. This would require PCR for adjudication of all samples. This was not included in our study design as it was cost-prohibitive, which is also partly why, despite its excellent sensitivity, PCR is not routinely used for pre-transfusion malaria screening in malaria-endemic settings. Consequently, assuming a PCR: microscopy detection ratio of ~2:1 [24], we calculated a hypothetical PCR detection rate of 12.9% (682/5281), and thus that 1.29% (68/5281) of donors would theoretically have gone undetected as malaria-infected by XN-31 in this study.

Furthermore, we did not subject the indeterminate XN-31 samples to adjudication to confirm the routine microscopy finding of malaria absence, although other studies indicate that such samples are largely malaria negative [12–14]. Likewise, although we suspected sample ageing due to transport delays as the primary cause of indeterminate results (as these occurred in clusters), absence of individual sample collection times precluded further analysis. The most notable reported clinical cause of abnormal scattergrams is reticulocytosis [12]. Although we were unable to analyse this, we consider reticulocytosis an unlikely cause of indeterminate results in our subjectively healthy blood donor study participants based on a recent report of no increase in reticulocyte counts in asymptomatic malaria in subjects >15 years [25].

In conclusion, we showed that microscopy underestimates malaria prevalence in blood donors (6.5% vs. 11.6%) and is unreliable for parasite density estimation when compared with XN-31. Other advantages of XN-31 include high sensitivity (100%) and specificity (99.9%), ability to detect all *Plasmodium* species and automated nature providing high throughput and rapid result availability. This overcomes many of the limitations of currently routinely available diagnostic tests and would thus be well-suited for pre-transfusion malaria screening in malaria-endemic countries such as Malawi, where 100% of the population is considered to have been exposed to the parasite and at high risk of ongoing exposure [6, 7], to reduce the risk of TTM.

It has been reported that as few as 10 parasites can cause malaria [26] hence no diagnostic method, including PCR, can eliminate TTM risk and pathogen inactivation is not universally available nor affordable. Controlled human malaria infection studies suggest that a minimal infective dose is required for establishment of malaria [27] and rodent malaria experiments have shown that clinical severity correlates with parasite inoculation dose [28]. XN-31 will miss very low-level malaria-infected blood units, but we hypothesize that such units are less infectious and less likely to cause severe clinical disease than unscreened blood or malaria-positive blood, when viewed from a public health perspective in settings where the probability of prior exposure to malaria is high amongst recipients, compounded by ongoing risk of mosquito-acquired malaria [6]. In high malaria-burden countries, the greatest consumers of blood products are also those most vulnerable to TTM. Consequently, we believe that malaria screening with XN-31, complemented with selective malaria chemoprophylaxis

for high-risk recipients, could provide a balance between ensuring blood safety and supply. Blood that has tested malaria-negative, could preferentially be reserved for high-risk recipients, such as young children and pregnant women, with malaria-positive blood, selected based on the lowest parasitaemia available being issued to individuals, such as adults with semi-immunity and at low-risk of clinical malaria, if the malaria-negative blood stock is depleted, supplemented with prescription of antimalarials.

Even where rejection of any malaria-infected blood is simply untenable, such as in Nigeria where prevalence rates reach ~55% [29], screening with a technology such as XN-31 provides an opportunity for informed matching of blood with recipient, and the initiation of prophylactic treatment for the recipient as needed, thereby enhancing overall transfusion safety, without unduly compromising supply.

Malawi, a high malaria burden country, currently only meets 72% (86,811/1,200,000) of its estimated annual blood requirements [30]. In the context of chronic blood shortages, and malaria being a treatable disease, the Malawian Ministry of Health, aimed at improving clinical outcomes, permits the issue of low parasitaemia blood when malaria-negative blood is depleted, upon special request with clinicians taking full responsibility for their decision to transfuse malaria-infected blood, in patients for whom transfusion is deemed to be a necessary life-saving intervention. In such cases, prophylactic antimalarials are always prescribed pre-transfusion, a TTM preventive measure endorsed by WHO [5]. This approach is supported by data showing that lack of timely access to blood is a major contributor to mortality in Africa [31], together with critical lack of evidence of the true prevalence and clinical impact of TTM in malaria-endemic countries [29]. In this regard, as the primary aim of blood donor/donation malaria screening is to improve clinical outcomes of recipients, we aim to conduct a retrospective follow-up of sub-microscopic XN-31 positive blood unit recipients identified during this study.

XN-31 also provides the added benefit of a full blood count (FBC) with each malaria analysis, which would be beneficial for donor health management and quality control testing of blood products, the latter currently being achieved using haematology analysers, which can only perform FBCs.

ACKNOWLEDGEMENTS

B.M., M.M. and T.H. designed and supervised the research; T.M. and A.S. performed the research; T.H. conducted statistical analysis; M.M. wrote the manuscript; all authors reviewed and approved the final version. We thank all the blood donors of Malawi for the gift of their blood in support of saving lives; MBTS staff for their contributions throughout the study; James Palapandu for expert microscopy; Siyanda Mngoma for training and technical support for the XN-31 analyser and Karl Seydel and Andrew Nyambalo of the Blantyre Malaria Project molecular laboratory for the polymerase chain reaction testing.

CONFLICT OF INTEREST

M.M. and T.H. are full-time Sysmex employees.

ORCID

Marion Münster https://orcid.org/0000-0002-5366-4842

REFERENCES

1. Owusu-Ofori AK, Betson M, Parry CM, Stothard JR, Bates I. Transfusion-transmitted malaria in Ghana. Clin Infect Dis. 2013;56:1735–41.
2. World Health Organization. The 2016 global status report on blood safety and availability. Geneva: WHO; 2017.
3. Drew VJ, Barro L, Seghatchian J, Burnouf T. Towards pathogen inactivation of red blood cells and whole blood targeting viral DNA/RNA: design, technologies, and future prospects for developing countries. Blood Transfus. 2017;15:512–21.
4. Allain JP, Owusu-Ofori AK, Assennato SM, Marschner S, Goodrich RP, Owusu-Ofori S. Effect of Plasmodium inactivation in whole blood on the incidence of blood transfusion-transmitted malaria in endemic regions: the African Investigation of the Mirasol System (AIMS) randomised controlled trial. Lancet. 2016;387:1753–61.
5. World Health Organization. Screening donated blood for transfusion transmissible infections–recommendations. Geneva: WHO; 2010.
6. World Health Organization. World malaria report 2020. Geneva: WHO; 2020.
7. Battle KE, Gumbo A, Hamuza G, Kwizombe C, Banda AT, Chipeta S, et al. Consultative meeting that examined alignment and discrepancies between health facility and household survey data in Malawi. Malar J. 2019;18:411.
8. Njolomole SE, M'baya B, Ndhlovu D, Mfune T, Yonamu F, Phiri P, et al. Post baseline situational analysis of blood safety in Malawi 2015. Results of the 2026 survey. Blantyre, Malawi: Malawi Blood Transfusion Services; 2017.
9. Tougan T, Suzuki Y, Itagaki S, Izuka M, Toya Y, Uchihashi K, et al. An automated haematology analyser XN-30 distinguishes developmental stages of falciparum malaria parasite cultured in vitro. Malar J. 2018;17:59.
10. Tougan T, Suzuki Y, Izuka M, Aono K, Okazaki T, Toya Y, et al. Application of the automated haematology analyser XN-30 in an experimental rodent model of malaria. Malar J. 2018;17:165.
11. Tougan T, Toya Y, Uchihashi K, Horii T. Application of the automated haematology analyzer XN-30 for discovery and development of antimalarial drugs. Malar J. 2019;18:8.
12. Pillay E, Khodaiji S, Bezuidenhout B, Litshie M, Coetzer TL. Evaluation of automayed malaria doagnosis using the Sysmex XN-30 analyser in a clinical setting. Malar J. 2019;18:15.
13. Post A, Kaboré B, Reuling IJ, Bognini J, van der Heiden W, Diallo S, et al. The XN-30 hematology analyser for rapid sensitive detection of malaria: a diagnostic accuracy study. BMC Med. 2019;17:103.
14. Zuluaga-Idárraga L, Rios A, Sierra-Cifuentes V, Garzón E, Tobón-Castaño A, Takehara I, et al. Performance of the hematology analyser XN-31 prototype in the detection of Plasmodium infections in an endemic region of Colombia. Sci Rep. 2021;11:5268.
15. Hajian-Tilaki K. Sample size estimation in diagnostic test studies of biomedical informatics. J Biomed Inform. 2014;48:193–204.
16. World Health Organization. Basic malaria microscopy. Part 1 Learner's guide. 2nd ed. Geneva: WHO; 2010.
17. World Health Organization. Microscopy for the detection, identification and quantification of malaria parasites on stained thick and thin blood films in research settings–methods manual. Geneva: WHO; 2015.
18. Ministry of Health, Lilongwe, Malawi. Guidelines for the clinical use of blood and blood products in Malawi. 1st ed. Lilongwe: MOH; 2012.
19. Moody A. Rapid diagnostic tests for malaria parasites. Clin Microbiol Rev. 2002;15:66–78.

20. Milne LM, Kyi MS, Chiodini PL, Warhurst DC. Accuracy of routine laboratory diagnosis of malaria in the United Kingdom. J Clin Pathol. 1994;47:740–2.
21. Sori G, Zewdie O, Tadele G, Samuel A. External quality assessment of malaria microscopy diagnosis in selected health facilities in Western Oromia, Ethiopia. Malar J. 2018;17:233.
22. Ahmadpour E, Foroutan-Rad M, Majidiani H, Moghaddam SM, Hatam-Nahavandi K, Hosseini S, et al. Transfusion-transmitted malaria: a systematic review and meta-analysis. Open Forum Infect Dis. 2019;6:ofz283.
23. Freimanis G, Sedegah M, Owusu-Ofori S, Kumar S, Allain JP. Investigating the prevalence of transfusion transmission of Plasmodium within a hyperendemic blood donation system. Transfusion. 2013; 53:1429–41.
24. Okell LC, Ghani AC, Lyons E, Drakely CJ. Submicroscopic infection in *Plasmodium falciparum*–endemic populations: a systematic review and meta-analysis. J Infect. 2009;200:1509–17.
25. Kaboré B, Post A, Berendsen MLT, Diallo S, Lompo P, Derra K, et al. Red blood cell homeostasis in children and adults with and without asymptomatic malaria infection in Burkina Faso. PLoS One. 2020;15: 0242507.
26. Seed CR, Kitchen A, Davis TME. The current status and potential role of laboratory testing to prevent transfusion-transmitted malaria. Transfus Med Rev. 2005;19:229–40.
27. Gómez-Pérez GP, Legarda A, Muñoz J, Sim KL, Ballester MR, Dobaño C, et al. Controlled human malaria infection by intramuscular and direct venous inoculation of cryopreserved *Plasmodium falciparum* sporozoites in malaria-naïve volunteers: effect of injection volume and dose on infectivity rates. Malar J. 2015;14:306.
28. Timms R, Colegrave N, Chan BHK, Read AF. The effect of parasite dose on disease severity in the rodent malaria *Plasmodium chabaudi*. Parasitology. 2001;123:1–11.
29. Owusu-Ofori AK, Parry C, Bates I. Transfusion-transmitted malaria in countries where malaria is endemic: a review of the literature from sub-Saharan Africa. Clin Infect Dis. 2010;51:1192–8.
30. Ministry of Health, Lilongwe. Malawi. HIV and AIDS integrated quarterly reports. Lilongwe: MOH; 2018.
31. World Health Organization. Department of essential health technologies: blood transfusion safety. Geneva: WHO; 2011.

How to cite this article: M'baya B, Mfune T, Samon A, Hwandih T, Münster M. Evaluation of the Sysmex XN-31 automated analyser for blood donor malaria screening at Malawi Blood Transfusion Services. Vox Sang. 2022;117: 346–53.