Scientific Research Publishing

Advances in Infectious Diseases, 2024, 14, 810-821
https://www.scirp.org/journal/aid
ISSN Online: 2164-2656
ISSN Print: 2164-2648

Evaluation of the Diagnostic Capacity of Malaria by XN-31 Automate at Cotonou in an Endemic Area of Benin, West Africa

Tatchémè Filémon Tokponnon[1,2], Razaki Ossè[1,3], Yves Eric Denon[4], Idayath Joachelle Gounou Yerima[1,2], Festus Houessinon[2], Brunelle Agassounon[1,2], Thibaud Legba[1,4], Augustin Kpemasse[5], Mireille Fleure Dagbadji[6], Germain Gil Padonou[1], Martin Akogbeto[1]

[1]Centre de Recherche Entomologique de Cotonou (CREC), Cotonou, Benin
[2]Ecole Polytechnique d'Abomey-Calavi, Université d'Abomey-Calavi, Abomey-Calavi, Bénin
[3]Ecole de Gestion et d'Exploitation des Systèmes d'Elevage, Université Nationale d'Agriculture, Kétou, Benin
[4]Ministry of Health, Cotonou, Benin
[5]Programme National de Lutte contre le Paludisme, Cotonou, Bénin
[6]Clinic Boni, Cotonou, Bénin
Email: filemont@yahoo.fr

How to cite this paper: Tokponnon, T.F., Ossè, R., Denon, Y.E., Gounou Yerima, I.J., Houessinon, F., Agassounon, B., Legba, T., Kpemasse, A., Dagbadji, M.F., Padonou, G. G. and Akogbeto, M. (2024) Evaluation of the Diagnostic Capacity of Malaria by XN-31 Automate at Cotonou in an Endemic Area of Benin, West Africa. *Advances in Infectious Diseases*, **14**, 810-821.
https://doi.org/10.4236/aid.2024.144060

Received: September 31, 2024
Accepted: December 13, 2024
Published: December 16, 2024

Copyright © 2024 by author(s) and Scientific Research Publishing Inc.
This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).
http://creativecommons.org/licenses/by/4.0/

Open Access

Abstract

Reliable and rapid pre-force diagnosis of malaria is necessary in the fight against malaria. Due to the limitations of the Giemsa-stained thick and thin blood smears technique, new techniques are established for malaria diagnosis; this is the case with the XN-31 automated system. This study evaluates the performance of the XN-31 automated malaria diagnostic system compared to the Giemsa-stained thick and thin blood smears technique. 260 blood samples from plasma parasite positive individuals were collected between July 2021 and February 2022 at the Boni Clinic, along with the clinical information for each of these samples. These samples were run on the XN-31 automated system, which uses fluorescence flow cytometry to detect and quantify red blood cells parasitized by *Plasmodium* parasites and provides a complete blood count and visual image scattergram of the parasitized red blood cells for diagnosis of the species involved. The results from the automated system are compared with those obtained by reading the Giemsa-stained thick and thin blood smear slides, which are considered the reference method. Apart from one case of *P. vivax* only detected by the XN-31, both techniques mentioned the presence of *P. falciparum* (majority species), *P. malariae* and *P. ovale* in approximate proportions. In this study, the detection limit of the automaton is 40 parasites per ul of blood. A few cases of false positive, false negative and indeterminate were recorded by the machine. There was a strong correlation (r =

0.8019) between the Giemsa-stained thick and thin blood smears technique and the XN-31 machine in the estimation of parasite densities. The XN-31 can be used for clinical diagnosis of malaria in endemic areas. In case of diagnostic difficulties (undetermined, absence of scattergram) or abnormalities of red blood cells, it is necessary to dilute the sample to 1/7 or to use blood smears for the diagnosis of the species and the parasite load.

Keywords

Malaria Diagnostic, Microscopy, XN-31 Automated System, *Plasmodium* Parasites, Benin, West Africa

1. Introduction

Malaria is a deadly disease caused by the *Plasmodium* parasite and transmitted by an infected *Anopheles* bite. Reports indicate a high number of malaria cases, estimated at 229 million in 2019 worldwide. As in previous years, the African region bore more than 90% of the total disease burden. Benin has not remained on the sidelines of these affected African regions and cites malaria as the leading cause of hospitalization and healthcare seeking. According to the Annuaire des Statistiques Sanitaires (ASS) generated by the Système National d'Information et de Gestion Sanitaires (SNIGS) of the Ministry of Health for the year 2020, the human consequences of malaria amount to 2440 cases of death; malaria, in Benin, represents respectively 44.2% and 49.5% of the reasons for consultation within the general population and among children under five years of age. Five species of the genus Plasmodium cause human infection: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae* and the zoonotic parasite *Plasmodium knowlesi*. Over 90% of malaria cases (the most serious and potentially fatal disease) are caused by *P. falciparum* [1].

Accurate and timely diagnosis of malaria is essential for the control of malaria transmission. Delay and/or misdiagnosis can result in significant morbidity and mortality. According to the World Health Organization (WHO), it is recommended that malaria be diagnosed promptly by either microscopy (MI) or a Rapid Diagnostic Test (RDT) in all patients suspected of having malaria before treatment is administered [2]. Diagnosis of malaria is routinely performed by morphological identification of the parasite through microscopic examination of a blood smear, which is considered the gold standard test for diagnosis in the clinical setting. This inexpensive diagnostic method allows the quantification of parasitaemia, is useful in monitoring the disappearance of parasitaemia and allows the possible identification of other pathogens. However, microscopy involves the competence of the diagnostic personnel and has a detection limit depending on the operator [3]. For this reason, RDTs for malaria have been developed, and the immunochromatographic card tests (ICTs) that detect *Plasmodium* antigens in the host's blood are the most commonly used. These TICs are easy to perform,

use approximately 10 - 20 μL of blood, and results exposed in 10 - 15 minutes [4]. However, in low parasitaemia infections, false negative results can be obtained [5]. In addition, the sensitivity of the RDT test decreases for the detection of *P. falciparum* due to specific mutations in the *P. falciparum* hrp2 gene or its complete deletion [6].

The design of new automated hematology analyzers is also part of this landscape of improved diagnostics given the challenges identified from previous techniques. Automated hematology analyzers can offer rapid, sensitive and cost-effective evaluations of all suspected malaria infections, as they reduce analysis time and improve accuracy [3]. This is the case of the Sysmex XN-31 automaton that generates for 60 ul of each blood sample absorbed, the parasite density in number of parasitized red blood cells per microliters of blood; a view on all the stages present of the parasites through a scattergram; a blood count and some pathologies related to the blood for example of leukopenia and anisocytosis [7]. This study therefore evaluates the performance of XN-31 in terms of malaria diagnosis via screw of microscopy.

2. Materials and Methods

2.1. Study Area

This study was conducted in the commune of Cotonou between July 2021 and February 2022. The Boni clinic (6˚21'57.0"N 2˚27'09.0"E) was selected for the collection of data and samples needed for the study. These samples are then sent to the Centre de Recherche Entomologique de Cotonou (CREC: 6˚28'23.0"N 2˚20'41.0"E), Abomey-calavi annex, for further manipulation.

2.2. Collection of Samples

Two hundred and forty seven (247) EDTA whole blood samples positive for malaria parasites were collected, which were read under microscopy for the detection of the parasite through a thick drop (TD) and a thin smear (TS) of blood, stained with Giemsa as indicated in the WHO standard protocol for malaria diagnosis. These two examinations allowed for the quantification of parasitemia and the diagnosis of the plasmodial species involved.

Samples are collected in batches per week and stored at 4˚C before being sent to the XN-31 automated diagnostic system. Recording sheets are attached to each batch of samples, giving the sample number, sex, age and the results of the microscopic diagnosis.

Test with the XN-31. The collected samples are now processed in the XN-31, a device that uses fluorescence flow cytometry to detect red blood cells infected with malaria parasites. This instrument was used in low malaria (LM) mode which absorbs 60 microliters of blood; it automatically counts and classifies cells through a 405 nm laser beam while analyzing the results of forward scattered light (FSC) and side fluorescent light (SFL) [8]. It provides a complete blood count (CBC), a qualitative result (positive or negative for malaria-infected red blood cells), a

quantitative result (an absolute number of MI-RBC and the percentage of red blood cells infected with Plasmodium), and a result for the suspected *Plasmodium* species [9].

The XN-31 results were compared to the microscopy results for the collected samples to determine the malaria diagnostic capability of the XN-31 in terms of sample positivity or negativity; the plasmodial species(s) demonstrated. In order to evaluate the sensitivity and specificity of this device, an additional 494 microscopy-negative samples were collected, added to the 247 baseline positive samples.

The sensitivity of a test is defined as "the probability that the test is positive if the pathology is present" and "the probability that the test is negative if the pathology is absent" [10].

Se = [*a*/(*a* + *c*)]*100

Sp = [*d*/(*b* + *d*)]*100

Cr: **=** [(*a* + *d*)/(*a* + *b* + *c* + *d*)]*100

Symbols used: *a* = True Positive (TD/TS positive samples), *b* = False Positive, c = False Negative, and *d* = True Negative (TD/TS negative samples). Se: sensitivity; Sp: specificity; Cr: concordance rate.

The plasmodial formula was evaluated with the results provided by Sysmex XN-31. This is according to the formula:

$$\text{Plasmodial formula} = \frac{\text{Number of the type of plasmodial species}}{\text{Number of samples tested positive}} \times 100$$

2.3. Statistical Analysis

The automaton generates a PDF file with information on each sample (sample number and results). An Excel file has been designed to summarize all the PDF data. The software R; GraphPad and Prism have been used to process the results.

3. Résults

3.1. Distribution of Samples According to Age, Sex and Parasite Density Classes

Of the 247 specimens collected, the age data for 12 specimens was not provided. Most of the patients collected were over 15 years old (**Figure 1**); there were more males than females, 159 males and 118 females (**Figure 2**). Parasite densities in most of the samples collected were between [10,000 - 99,999] parasitized red blood cells/blood unit (**Figure 3**).

3.2. Species Diagnosis According to Microspie and XN-31

The microscopic reading of the samples at the hospital showed the majority presence of *P. falciparum* (Pf); *P. ovale* (Po); *and P. malariae* (Pm), visualized on the scattergram (see **Figures 4-6**), which were alone or in combination in the blood samples (see **Figure 3**) while the XN-31 diagnosis revealed the presence of *P. vivax* alongside those encountered on microscopy in approximate proportions with 31 undetermined cases (see **Figure 6**).

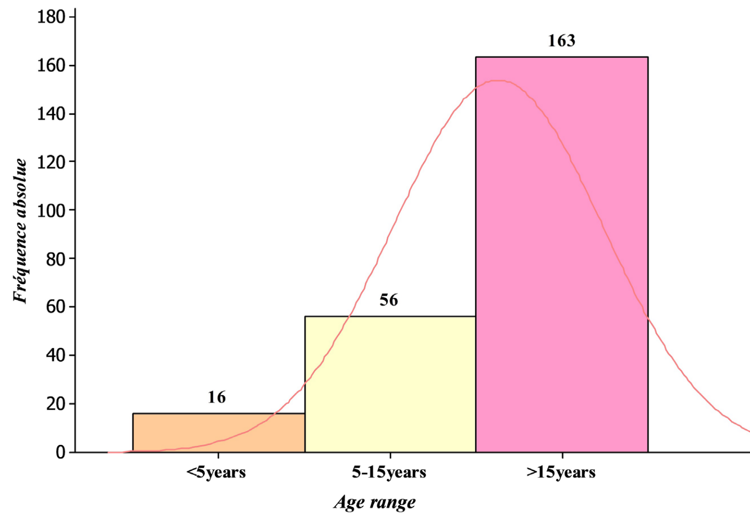

Figure 1. Distribution of subjects by age, <5 years 5 - 15 years >15 years.

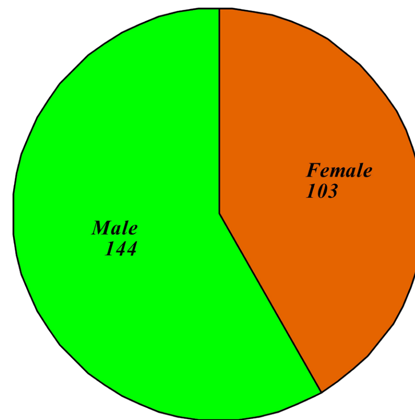

Figure 2. Distribution of subjects by gender.

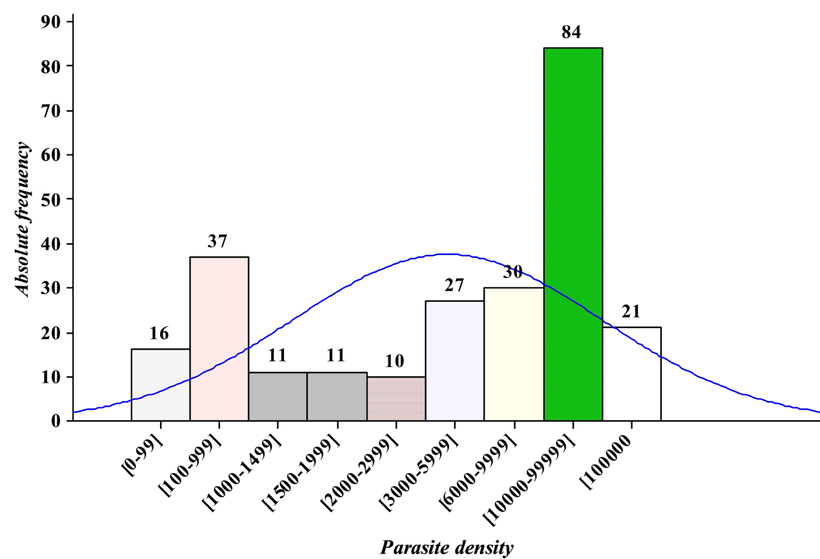

Figure 3. Distribution of subjects according to parasite densities obtained at XN-31.

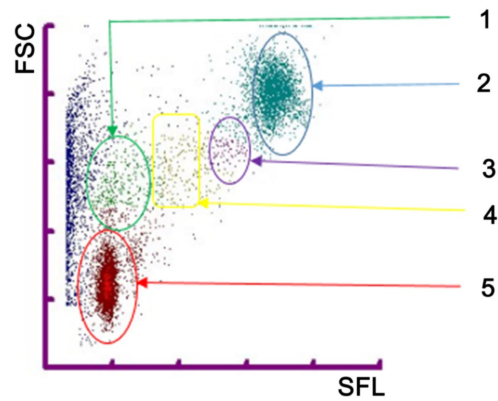

Figure 4. Scattergram showing all stages in a *P. falciparum* positive sample and "others". MIRCB: 4275 10^3 parasites/blood unit. 1: Gametocytes; 2: Lyse red blood cell debris 3: Schizontes; 4: Circulating Trophozoites; 5: Parasitized red blood cells.

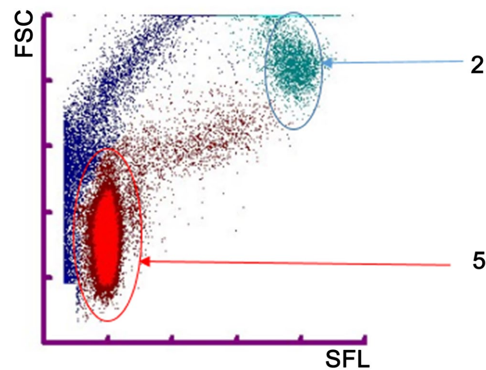

Figure 5. Scattergram of a *P. falciparum* positive sample. MIRCB: 22,508 10^3 parasites/blood unit.

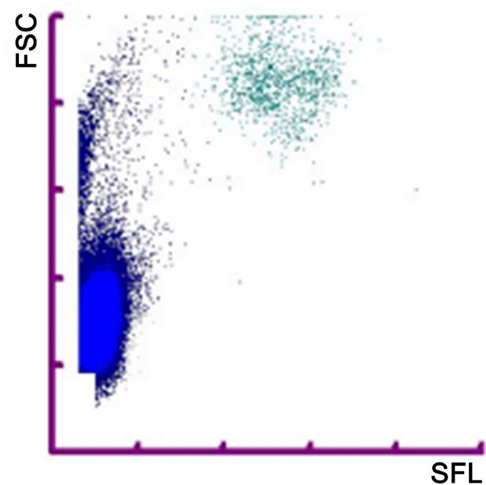

Figure 6. Scattergram of a sample with unknown species (Indeterminate). MIRCB: < Loq 10^3 parasites/blood unit.

Microscopy revealed that 211 participants carried only *Plasmodium falciparum* and one participant carried only *Plasmodium malariae*, while 35 participants were

mixed carriers of (Pf/Pm) for 20 people and (Pf/Po) for 15 people (Figure 7).

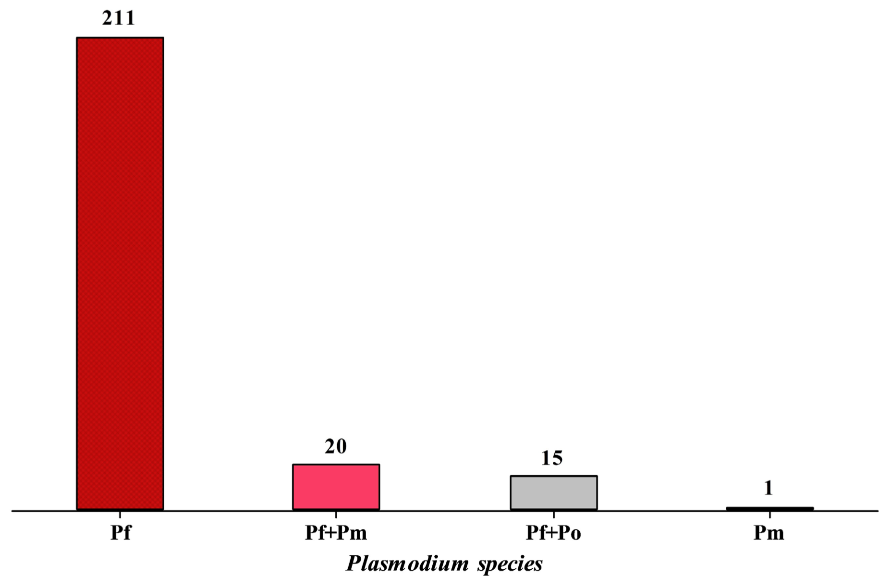

Figure 7. Distribution of subjects by plasmodial species (Giemsa-stained thick and thin blood smears).

Figure 8 shows that 185 participants carried Pf only, 5 carried Po only, 3 carried Pm and one carried Pv only. 31 participants were indeterminate, 19 carried both (Pf/Po) and 3 carried (Pf/Pm).

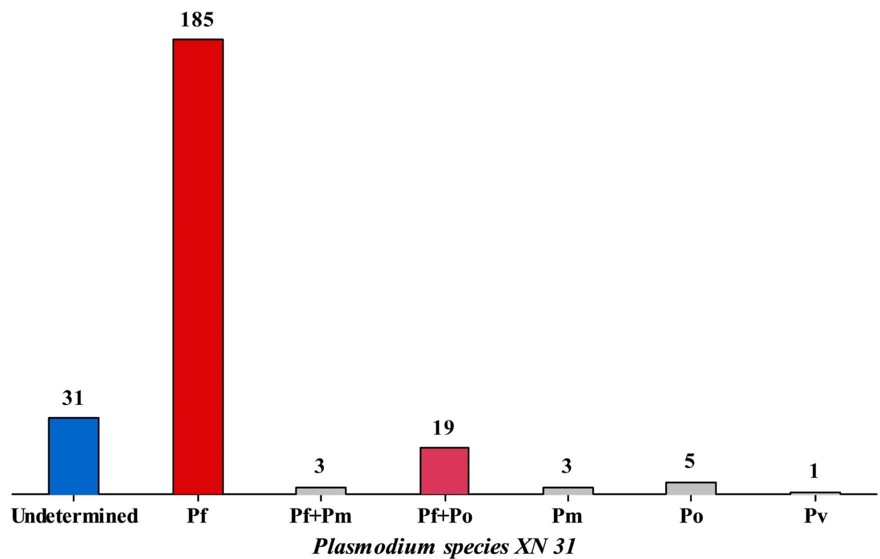

Figure 8. Distribution of subjects by plasmodial species (XN-31).

3.3. Calculation of the Plasmodial Formula

The plasmodial formula of each species is as follows in Table 1.

The plasmodial formula for each species is as follows: *Plasmodium falciparum* is in the majority with 91%, followed by *Plasmodium ovale* (around 7%), *Plas-*

modium malariae (2%) and *Plasmodium vivax* (0.46%). However, these species were present alone or in mixtures.

Table 1. *Plasmodium* formulas of the different species in the XN-31 automaton.

Plasmodium species (XN-31)	Numbers	Plasmodial formulas (%)
Pf	192	90.87
Po	24	6.62
Pm	2	2.05
Pv	1	0.46
Total	219	100

Pf: *P. falciparum;* Po: *P. ovale*; Pm: *P. malariae;* Pv: *P. vivax*.

3.4. Accuracy of PD and Calculation of Sensitivity, Specificity and Concordance Rates of XN-31 Compared to Microscopy

The particle densities obtained by the two diagnostic methods were projected for each sample giving a correlation of 0.8019 of the XN-31 automaton to the microscopic reading (**Figure 9**). The sensitivity and specificity rates of XN-31 are respectively: 92% and 93%. It correlates 93% with the Giemsa-stained thick and thin blood smears results (**Table 2**).

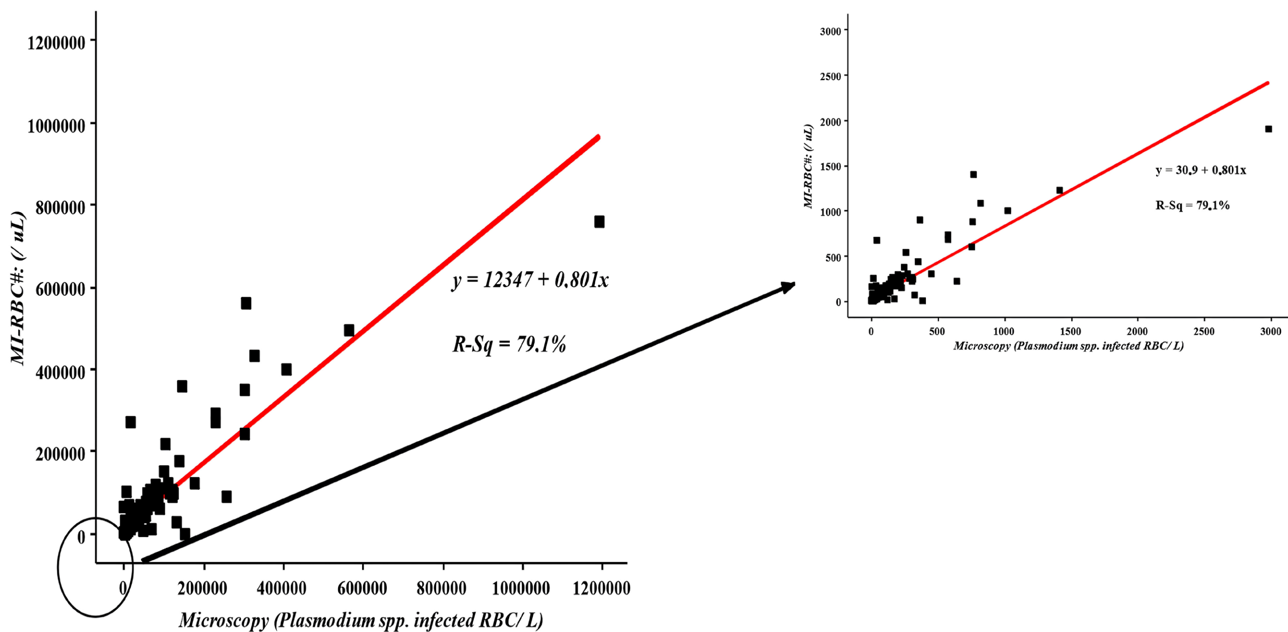

Figure 9. Accuracy of parasitaemia determination by XN-31 compared to parasitaemia determined by microscopy.

Table 2. Sensitivity, specificity and concordance rates of XN-31 compared to microscopy.

Parameters	Values
Sensitivity	92%
Specificity	93%
Concordance rate	93%

4. Discussion

The aim of this study was to evaluate the diagnostic performance of XN-31 in comparison to TD/TS in the diagnosis of malaria. For this purpose, 247 microscopy-positive and 494 microscopy-negative samples, regardless of age or sex, were collected and processed in XN-31. Microscopic reading revealed the presence of *P. falciparum* (majority species), *P. ovale and P. malariae* which were present alone or in combination in the collected blood samples. One case of *P. vivax was* detected by the XN-31 machine in addition to those found in the EW/Smear. The lower limit of detection of Plasmodia by the automaton during this work is 20 parasites per ul of blood. There is a strong correlation (R = 0.8019) between the two methods in the estimation of parasitaemia. The diagnosis with the XN-31 automaton has a specificity and a sensitivity of: 92% and 93%. Furthermore, the concordance rate between TD/TS compared to XN-31 results was 93%. Cases of false positive, false negative and indeterminate density and parasite species were noted by the automaton.

These TD/TS species results parallel those obtained by Damien *et al.* who had made the same finding in blood samples of asymptomatic children from 1 year to 5 years old in the health district of Ouidah, Kpomasse, Tori Bossito; in the South of Benin [11]. The single case of *P. vivax* suspected on the XN-31 joins the work of Poirier *et al.* who reported the presence of *P. vivax* in Benin, in blood bags from asymptomatic Beninese donors [12]. XN-31 has this ability to identify red blood cells parasitized by parasites of the genus *Plasmodium* as mentioned by Zuluaga-Idárraga *al.* in a context of endemicity of *P. falciparum* and *P. vivax* in Colombia [3]. However, the proportions of identification of *Plasmodium* species in XN-31 versus TD are (211/247) and (188/247) respectively for cases where *P. flaciparum* is only present. This difference would be due to the fact that the XN-31 suspects other species ("malaria? Pf Malaria? other") in addition to the identified *P. falciparum* while the microscopy only reports *P. falciparum*. The correlation obtained between TD/TS and XN-31 results in the estimation of malaria parasite densities (r = 0.801) is similar to that of Khartabil *et al.* [9] (r = 0.791) for samples that are not necessarily positive on microscopy that she had to collect in a non malaria endemic area. This finding suggests a possible use of the XN-31 in clinical diagnosis in *P. falciparum* endemic areas. The specificity and sensitivity rates of XN-31 compared to microscopy were 92% and 93% respectively, which are close to 100% and 99.9% obtained by M'baya *et al.* [13] for the same techniques in the context of malaria screening in 5,281 samples collected from blood donation sites in Malawi. This slight discrepancy could be explained by the nature of the collection of our samples, only the *Plasmodium* positive cases in the Boni clinic, which represent 1/3 of the samples that arrive in this clinic; that is 2/3 (negative samples) not taken into account whereas the estimation of sensitivity and specificity takes into account the number of negative samples.

On the other hand, cases of indeterminate, false positives or negatives have been observed in XN-31; they may be due to the presence of reticulocytes which are still

nucleated red blood cells; in fact, the device lists the nucleus of these red blood cells as *Plasmodium* resulting in false positives. This was noted in the work of Khartabil *et al.* [9] who concluded that in the case of abnormal red blood cell shapes, the results of the XN-31 should be considered with caution. Samples may be weakly positive (low MIRBC), and without scattergram for species diagnosis. In this case, a confession of a blood smear is necessary to give the species. They are often observed when the parasite density is extremely high; a repeat of the sample diluted at 1/7 allows us to obtain better results.

5. Conclusion

The XN-31 automaton is faster than the Giemsa-stained thick and thin blood smears in the diagnosis of malaria. It is reliable and can be recommended for clinical diagnosis in endemic areas. In case of abnormalities in red blood cells, an EKG/swab test is recommended to confirm or invalidate the diagnosis provided by the device.

Acknowledgements

We thank Professor Martin Akogbeto of the Centre de Recherche Entomologique de Cotonou for the contribution to this study. We also thank the management of the Centre de Recherche Entomologique de Cotonou and its staff for field and laboratory work. We thank the agents of Boni Clinic at Cotonou and all participants.

Authors' Contributions

TFT, RO, GGP and MA designed the research; TFT, MFD, TL, YED, IJGY, BA and FH conducted data collection; all authors conducted data analysis. TFT, BA, AK and YED coded the data; TFT, FH and IJGY led the drafting with substantive input from MA and TL in the results section; all authors revised the manuscript.

All authors read and approved the final manuscript.

Funding

The study was the fruit of local efforts of the personal contributions of researchers.

Ethics Approval

The study was conducted in accordance with the Declaration of Helsinki and approved by the institutional ethics committee of the Cotonou Entomological Research Center (N06/IECC of January 7, 2021).

Availability of Data and Materials

Data is contained within the article.

Conflicts of Interest

The authors declare that they have no competing interest.

References

[1] Snow, R.W. (2015) Global Malaria Eradication and the Importance of *Plasmodium falciparum* Epidemiology in Africa. *BMC Medicine*, **13**, Article No. 23. https://doi.org/10.1186/s12916-014-0254-7

[2] World Health Organization (2017) Malaria Rapid Diagnostic Test Performance: Results of WHO Product Testing of Malaira RDTs: Round 7 (2016-2017). https://iris.who.int/handle/10665/255836

[3] Zuluaga-Idárraga, L., Rios, A., Sierra-Cifuentes, V., Garzón, E., Tobón-Castaño, A., Takehara, I., *et al.* (2021) Performance of the Hematology Analyzer XN-31 Prototype in the Detection of Plasmodium Infections in an Endemic Region of Colombia. *Scientific Reports*, **11**, Article No. 5268. https://doi.org/10.1038/s41598-021-84594-y

[4] Mishra, M. and Misra, R. (2007) Immunochromatographic Methods in Malaria Diagnosis. *Medical Journal Armed Forces India*, **63**, 127-129. https://doi.org/10.1016/s0377-1237(07)80054-2

[5] Portero, J., Rubio-Yuste, M., Descalzo, M.A., Raso, J., Lwanga, M., Obono, J., *et al.* (2010) Accuracy of an Immunochromatographic Diagnostic Test (ICT Malaria Combo Cassette Test) Compared to Microscopy among under Five-Year-Old Children When Diagnosing Malaria in Equatorial Guinea. *Malaria Research and Treatment*, **2010**, Article ID: 858427. https://doi.org/10.4061/2010/858427

[6] Cheng, Q., Gatton, M.L., Barnwell, J., Chiodini, P., McCarthy, J., Bell, D., *et al.* (2014) *Plasmodium Falciparum* Parasites Lacking Histidine-Rich Protein 2 and 3: A Review and Recommendations for Accurate Reporting. *Malaria Journal*, **13**, Article No. 283. https://doi.org/10.1186/1475-2875-13-283

[7] Pillay, E., Khodaiji, S., Bezuidenhout, B.C., Litshie, M. and Coetzer, T.L. (2019) Evaluation of Automated Malaria Diagnosis Using the Sysmex XN-30 Analyser in a Clinical Setting. *Malaria Journal*, **18**, Article No. 15. https://doi.org/10.1186/s12936-019-2655-8

[8] Komaki-Yasuda, K., Kutsuna, S., Kawaguchi, M., Kamei, M., Uchihashi, K., Nakamura, K., *et al.* (2022) Clinical Performance Testing of the Automated Haematology Analyzer XN-31 Prototype Using Whole Blood Samples from Patients with Imported Malaria in Japan. *Malaria Journal*, **21**, Article No. 229. https://doi.org/10.1186/s12936-022-04247-x

[9] Khartabil, T.A., de Rijke, Y.B., Koelewijn, R., van Hellemond, J.J. and Russcher, H. (2022) Fast Detection and Quantification of *Plasmodium* Species Infected Erythrocytes in a Non-Endemic Region by Using the Sysmex XN-31 Analyzer. *Malaria Journal*, **21**, Article No. 119. https://doi.org/10.1186/s12936-022-04147-0

[10] Loewenberg, S. (2007) The US President's Malaria Initiative: 2 Years On. *The Lancet*, **370**, 1893-1894. https://doi.org/10.1016/s0140-6736(07)61795-4

[11] Damien, G.B., Djènontin, A., Rogier, C., Corbel, V., Bangana, S.B., Chandre, F., *et al.* (2010) Malaria Infection and Disease in an Area with Pyrethroid-Resistant Vectors in Southern Benin. *Malaria Journal*, **9**, Article No. 380. https://doi.org/10.1186/1475-2875-9-380

[12] Poirier, P., Doderer-Lang, C., Atchade, P.S., Lemoine, J., de l'Isle, M.C., Abou-bacar, A., *et al.* (2016) The Hide and Seek of *Plasmodium vivax* in West Africa: Report from a Large-Scale Study in Beninese Asymptomatic Subjects. *Malaria Journal*, **15**, Article No. 570. https://doi.org/10.1186/s12936-016-1620-z

[13] M'baya, B., Mfune, T., Samon, A., Hwandih, T. and Münster, M. (2021) Evaluation

of the Sysmex XN-31 Automated Analyser for Blood Donor Malaria Screening at Malawi Blood Transfusion Services. *Vox Sanguinis*, **117**, 346-353.
https://doi.org/10.1111/vox.13208