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HRP2 and PLDH Performance Comparison as Rapid Diagnostic Tools for Symptomatic Malaria Patients at Rugazi HC IV in Rubirizi District

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Abstract

Background: *Rapid and precise laboratory diagnosis is essential for effectively diagnosing and managing malaria patients. Malaria rapid diagnostic tests (mRDTs) play a critical role in malaria diagnosis in resource-constrained settings with limited access to labs and trained staff. The efficiency of two mRDTs (HRP2 and pLDH) was compared against microscopy, the gold standard for malaria diagnosis, in this study.*

Methods: *In a cross-sectional study, 308 participants were tested for malaria parasites using two mRDTs and blood smear microscopy as the gold standard. STATA software version 17 was used for data analysis.*

Results: *Out of the 308 participants, 82 tested positive for HRP2 mRDT (26.62% (95% CI: 21.97-31.86) positivity rate, 73.38% (95% CI: 68.14-78.83) negativity rate), and 99 tested positive for pLDH mRDT (32.14% (95% CI: 27.14-37.59) positivity rate, 67.86% (95% CI: 62.41-72.86) negativity rate). Using microscopy as the gold standard, the sensitivity, specificity, PPV, and NPV were determined. For HRP2 mRDT, sensitivity was 77.78% (95%CI: 73.13-82.42), specificity 97.61% (95%CI: 95.90 - 99.31), PPV 93.90% (95%CI: 91.23 – 96.56), and NPV 90.27% (95%CI: 86.96-93.58). For pLDH mRDT, the sensitivity was 94.95% (95% CI: 92.50-97.40), specificity was 97.61% (95% CI: 95.90-99.31), PPV was 94.95% (95% CI: 92.50 – 97.40), and NPV was 97.61% (95% CI: 95.90 - 99.31). The dominant malaria species was Plasmodium falciparum (74.7%), followed by Plasmodium vivax (12.1%) and Plasmodium ovale (1%). Mixed infections of plasmodium falciparum and plasmodium vivax (7.1%) and plasmodium falciparum and plasmodium ovale (5.1%) were also observed.*

Conclusion: *There was no significant difference in the positivity rate, negativity rate, specificity or PPV in both mRDTs. However, a significant difference in the NPV and sensitivity of HRP2 and pLDH mRDTs was observed where HRP2's sensitivity was below the WHO recommendation of ≥95% in this study population; thus, there is a need to improve the sensitivity of this mRDT kit. In our study, most malaria infections were caused by Plasmodium falciparum, followed by P. vivax and P. ovale.*

We therefore recommend the use of pLDH mRDTs over HRP2 mRDTs based on the results of our study.

Keywords: Malaria Rapid Diagnostic Tests, Malaria Microscopy, Symptomatic Infections.

Abbreviations and Acronyms.

DHO: District Health Officer.
HCIV: Health Centre Four.
HMIS: Health Management Information System.
HRP2: Histidine Rich Protein 2.
mRDTs: Malaria Rapid Diagnostic Tests.
MUST: Mbarara University of Science and Technology.
NPV: Negative Predictive Value
OPD: Out Patient Department.
P.f: Plasmodium falciparum.
PCR: Polymerase Chain Reaction.
pLDH: Plasmodium Lactate Dehydrogenase.
PPV: Positive Predictive Value
RBM: Roll Back Malaria
SOPs: Standard Operating Procedures.
WHO: World Health Organization.

1. Background

In Uganda, malaria is a serious public health issue with a high prevalence rate. Malaria is the primary cause of morbidity and mortality, according to the World Health Organization (WHO), accounting for 30% of all outpatient visits and 20% of all deaths [1]. Children under the age of five and pregnant women are extremely affected by the condition [2].

Malaria rapid diagnostic tests (mRDTs) are important tools for the diagnosis of malaria in environments where resources are limited and where laboratory facilities and trained staff are scarce [3,4]. Effective treatment and management of malaria patients depend on a quick and precise laboratory diagnosis. These mRDTs are inexpensive and simple to use, and they produce results quickly, making them ideal for use in remote or underserved areas [5]. Histidine-rich protein 2 (HRP2) and plasmodium lactate dehydrogenase (pLDH) tests are the two main common mRDT kits used for malaria diagnosis [6].

Histidine-rich protein 2 (HRP2) mRDTs detect the antigen histidine-rich protein 2, which is released by only the *P. falciparum* species that causes malaria [7]. HRP2 is released into blood as a byproduct of the parasite's life cycle, specifically during the asexual blood stage when the parasite replicates within red blood cells. This parasite species is the utmost cause of malaria in sub-Saharan Africa. Therefore, detection of this protein only confirms *Plasmodium falciparum* species [8].

On the other hand, pLDH mRDTs detect plasmodium lactate dehydrogenase (pLDH), which is an enzyme produced by all species of plasmodium. pLDH is released into the blood as a byproduct of parasite metabolism during the intraerythrocytic stage when the parasite replicates within red blood cells [9-11]. The presence of pLDH in blood is detected using mRDTs that look for pLDH antigen in the patient's blood. The pLDH antigen is released into the blood throughout the course of malaria infection, with increasing levels as the infection progresses, and can be detected in blood as early as a few days after infection, where levels of pLDH tend to peak around the time of symptom onset. The pLDH antigen levels gradually decline after treatment and can remain detectable in blood for a few days after successful treatment of infection; hence, this test may be more helpful for tracking patients' healing after treatment and preventing needless malaria treatment repetition [14-16].

However, microscopic examination and polymerase chain reaction (PCR) tests remain the gold standard methods by which malaria infection is diagnosed. The PCR test is a very sensitive replacement for microscopy; however, the method is not commonly used because it is very expensive to establish in a rural setting [17,18].

The pitfalls of most mRDTs are their low specificity and sensitivity in detecting malaria parasite antigens compared to gold standard tests. A recent study conducted in northern Uganda on asymptomatic patients showed that the sensitivity and specificity for HRP2 mRDTs were lower than those for *P. falciparum*/pLDH mRDTs (95.2% and 90%, respectively) [19]. Therefore, there is a need to study the sensitivity and specificity of mRDTs among symptomatic patients in a malaria-endemic area of southwestern Uganda, which will enable us to determine the most appropriate mRDT to be used for malaria diagnosis in Rugazi HC IV, Rubirizi District.

2. Methodology

2.1 Study Site

Rugazi Health Centre IV is located in Kasungu Village within the Parish of Kasarara Ward in the subcounty of Rubirizi Town Council, Rubirizi District western Uganda (0°15'43" south, 30°6'12" east), with a population of 144,100 according to 2020 estimates. The health center serves as a referral for the district and hence covers 90% of the population. The district's main physical features include natural rainforests, Kirinzu, Maramagambo and Kasyoha-Kitomi, a large concentration of crater lakes, over 30 in number. The main economic activities include fishing, brick

laying, and farming. All of these factors provide favorable habitats for mosquitoes that transmit the plasmodium species.

2.2 Study Design
We used a cross-sectional study design.

2.3 Study Population
All patients presented with malaria-like symptoms, such as fever, flu-like illness, shaking chills, headache, muscle aches, tiredness, nausea, malaise, vomiting, diarrhoea, joint pain, anaemia and jaundice (yellow colouring of the skin and eyes), at Rugazi Health Centre IV.

2.4 Sample Size Determination
This was calculated using the diagnostic accuracy test's formula for two proportions (Akoglu, 2022):

$$n = \frac{\left[U\sqrt{P_1(1-P_1)+P_2(1-P_2)} + V\sqrt{2P(1-P)}\right]^2}{(P_1-P_2)^2}$$

$$P = \frac{P_1+P_2}{2}$$

u- represents the desired power (typically 0.84 for 80% power).
v- Represents the desired level of statistical significance (typically 1.96 for 5% alpha).
P- Average proportion.
P_1 and P_2- Expected sensitivity or specificity of index tests 1 and 2. From a recent meta-analysis study regarding the precision of HRP2 and pLDH rapid diagnostic tests in diagnosing malaria, the combined sensitivity and specificity were 0.92 (95% CI 0.83-0.96) and 0.92 (0.86–0.95), the pLDH-based assays were 0.96 (0.85–0.98) and 0.93 (0.86–0.95), and the HRP2-based assays were 0.94 (0.84–0.98) and 0.86 (0.77–0.91), respectively (Hu et al., 2021).

Using the specificities of the two tests, P_1 and P_2 are 0.93 and 0.86, respectively:

$$P = \frac{0.93+0.86}{2} = 0.895.$$

$$n = \frac{\left[0.84\sqrt{0.93(1-0.93)+0.86(1-0.86)} + 1.96\sqrt{2\times 0.895(1-0.895)}\right]^2}{(0.93-0.86)^2}$$

n = 299.5412997
∴ n = 300 participants.

Therefore, 300 participants were recruited for the study, and data collection was conducted from June to July 2023.

2.5 Sampling Technique
It was a nonprobability sampling where all patients who presented with malaria-like symptoms at the outpatient department of Rugazi Health Centre IV were conveniently selected.

2.6 Study Selection Criteria
2.6.1 Inclusion Criteria
All patients presented with malaria-like symptoms, including fever, flu-like illness, shaking chills, headache, muscle aches, tiredness, nausea, malaise, vomiting, diarrhoea, joint pain, anaemia and jaundice (yellow colouring of the skin and eyes).

2.6.2 Exclusion Criteria
Patients who were on anti-malarial drugs and those who had just finished their dosage of antimalarials.

3. Data Collection and Laboratory Processing
The data collection tools were the patients' clinical notes, and tests were used. Upon consenting or assenting to the study, samples were collected from the patients by performing a venipuncture on the ideal and best locations of the body.

Each patient was tested for malaria using both HRP2 and pLDH mRDTs. The standard procedure per the manufacturer's instructions was followed, and the results were read and confirmed by a second person.

4.1 Malaria RDTs
These are lateral flow immuno-chromatographic antigen-detection tests, which rely on the capture of dye-labelled antibodies to produce a visible band on a strip of nitrocellulose, often encased in plastic housing, referred to as cassettes.

Generally, after labelling the mRDT with the patient's unique identification number, a drop of blood was added to the mRDT through the sample well, followed by four drops of buffer through a buffer well. Blood was carried by buffer along the length of the mRDT. With malaria RDTs, the dye-labelled antibody first bound to a parasite antigen, and the resultant complex was captured on the strip by a band of bound antibody, forming a visible line (T - test line) in the results window for positive patients. The control line (C- control line) provided information on the integrity of the antibody-dye conjugate but did not confirm the ability to detect parasite antigen.

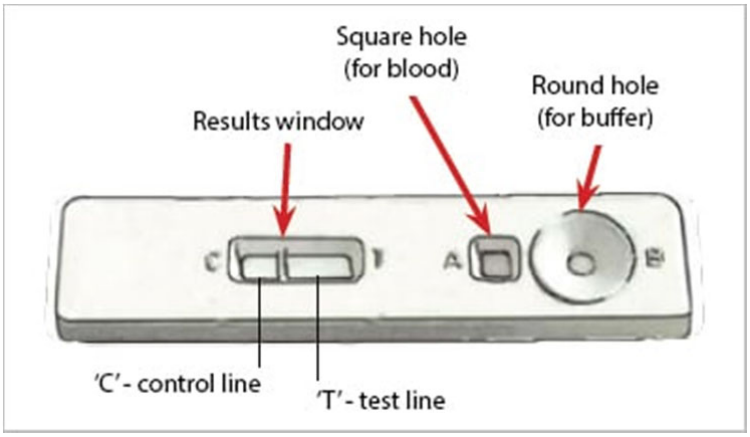

Figure 1: An mRDT Cassette

4.2 Malaria Microscopy

Microscopic examination was used as the "gold standard" for laboratory confirmation of malaria. A blood specimen collected from the patient was spread on a slide labelled with a unique laboratory number for a thick and thin blood smear. Then, the cells were stained with a Romanowsky stain (Giemsa was used) and examined with a 100X oil immersion objective. Visual criteria were used to detect malaria parasites and to differentiate the various species.

Examination of the slides was performed independently by two researchers and an experienced laboratory technologist/technician, and for varying results, another experienced laboratory technologist/technician re-examined the slides, and the majority decision was taken to be the final result. For each positive, the corresponding thin film was examined for species identification.

4.3 Quality Assurance

The SOPs in place at the Rugazi Health Centre IV OPD laboratory were strictly followed for each step of the testing procedure for different batches of mRDTs.

We had a one-day training on malaria microscopy before the start of the data collection.

Records including patient information, test results, quality control data and training records were kept safely.

We also tracked and monitored the expiration dates of the mRDT kits to ensure that they did not expire and were within their shelf life.

4.4 Quality Control

Internal quality control of mRDTs involved the visualization of the control line in the result window of the test card. This indicated that the active ingredients of the strip were functional and that migration was successful but was not an assurance that the specimen had been applied properly; it was not a positive specimen control.

Both positive and negative control samples were included with each batch of the mRDTs to ensure accuracy, and the controls were tested along with the patients' samples to verify that the test results were correct and reliable.

Prepared reagents or stains were quality controlled with known positive and negative samples from a confirmed symptomatic patient with malaria, and one without malaria was pretested with the reagents to check and confirm their quality.

Positive and negative control slides were used to validate the performance of the microscopist.
We compared the identified plasmodium species with those on the bench aid-colored charts and stained microscopic glass slides.

Positive samples were cross-checked by a certified malaria microscopist.

We took corrective actions for some quality control deviations, and this included replacing expired reagents.

5. Data Quality Control and Management

Data were collected based on the patients' requested clinical notes and tests. The results were recorded and checked for completeness and consistency, and the data were coded, double entered into an Excel spreadsheet to ensure the accuracy of the data and then cleaned before analysis to ensure quality. Raw data were later stored in the box files for retrieval.

5.1 Data Analysis

The raw data were double entered into a Microsoft Excel spreadsheet and then transferred to STATA software version 17 for analysis. The sociodemographic characteristics were compared between people who had malaria and those without malaria. Gender was summarized using frequencies and proportions that were expressed as percentages. A chi-square test was used to

compare the distribution of gender by malaria status. A P value less than 0.05 was considered to be significant. Age was not normally distributed according to the Shapiro–Wilk normality test (p value<0.001) and therefore was summarized using the median and interquartile range. The age distribution between those with malaria and those without malaria was compared using Student's t test, which compared the mean age in the two categories of malaria status.

The positivity or negativity rates of each of the mRDTs together with their 95% confidence intervals were expressed as a proportion by dividing the total number of patients who tested positive or negative for that particular mRDT by the total number of patients tested and then multiplying by 100%.

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) with their 95% confidence intervals were estimated using their respective formulas:

$$sensitivity = \frac{true\ positive}{true\ positive + false\ negative} \times 100\%$$

$$specificity = \frac{true\ negative}{true\ negative + fale\ positive} \times 100\%$$

$$PPV = \frac{true\ positive}{true\ positive + false\ positive} \times 100\%$$

$$NPV = \frac{true\ negative}{true\ negative + false\ negative} \times 100\%$$

The most common plasmodium species was calculated by dividing the total number of a specific plasmodium species by the total number of plasmodium species counted by microscopy. This is also a proportion, and was expressed as a percentage.

5.2 Ethical Considerations

Ethical authorization was acquired from the Faculty of Medicine Research Ethics Committee (*Appendix VI*) before conducting the study. Administrative approval (*Appendix VII*) from the DHO of Rubirizi district and the medical superintendent of Rugazi Health Centre IV was sought. The study sought informed consent and ascent (*Appendix IV and Appendix V*) from the study participants, and patient confidentiality was ensured by giving unique identification numbers to patients. All study data were kept securely and accessible only by the supervisor and research team.

6. Results

6.1 Sociodemographic Characteristics of the Participants

Out of the 308 participants, 189 (61.36%) were females and 119 (36.64%) were males. The median age was 15 years, with an interquartile range of 8 to 27 years. Ninety-nine of the 308 study participants were positive for malaria, giving an overall proportion of 32.14% (95% CI: 27.14-37.59%). There was no significant difference in the distribution of sex between people who had malaria and people who did not have malaria. There was also no significant difference between the mean age of study participants positive for malaria and those negative for malaria, as indicated in ***Table 1.***

Variable		Total	Malaria Status		P- Value
		308(100%)	Present	Absent	
			99(32.14%)	209(67.86%)	
Gender:	Male	119(38.64%)	45(45.45%)	74(35.41%)	0.091
	Female	189(61.36%)	54(54.55%)	135(64.59%)	
Age: (Median)(IQR) (Mean)(SD)		15(8-27)	17.60(16.67)	21.55(18.35)	0.070

Table 1: Sociodemographic Characteristics of the Study Participants Stratified by Malaria Status

6.2 Positivity and Negativity Rates of HRP2 and pLDH mRDTs.

Out of the 308 participants, 82 participants were reactive for HRP2 mRDT, giving an overall positivity rate of **26.62% (95% CI: 21.97-31.86).** However, 99 participants were reactive for pLDH mRDT, giving an overall positivity rate of **32.14% (95% CI: 27.14-37.59).** As indicated in ***Table 2*** and ***Figure 1*** below.

There was no significant difference between the positivity rate of HRP2 mRDT and the positivity rate of pLDH mRDT (**p value=0.1327,** proportion test).

Rapid Diagnostic Test	Results	Frequency	Percentage(95% CI)
HRP2 mRDT	Negative	226	73.38%(68.14 78.83)
	Positive	82	26.62%(21.97-31.86)
pLDH mRDT	Negative	209	67.86%(62.41-72.86)
	Positive	99	32.14%(27.14-37.59)

Table 2: Proportion of Study Participants Positive for HRP2 mRDT and pLDH mRDT Tests

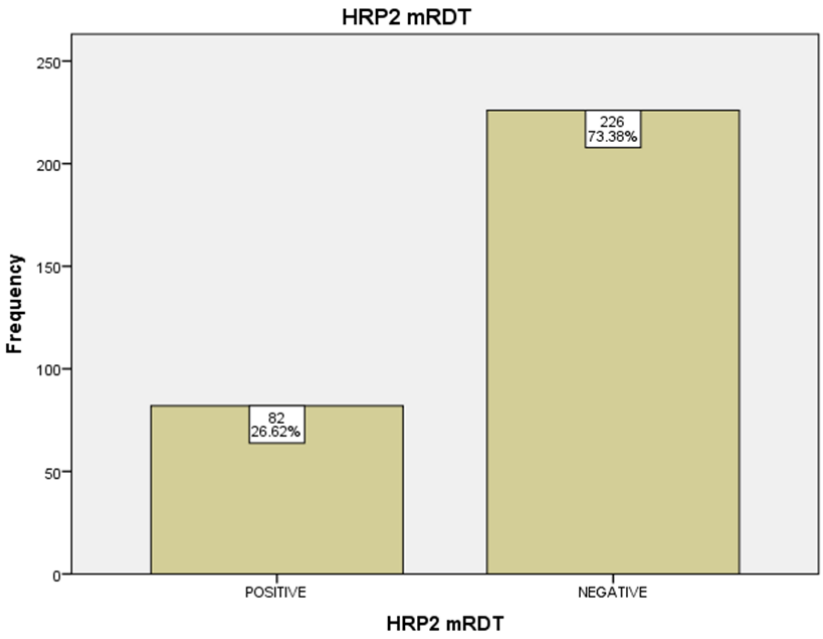

Figure 2: The Positivity and Negativity Rates of HRP2 mRDT

Figure 3 Positivity and Negativity Rates of pLDH mRDT

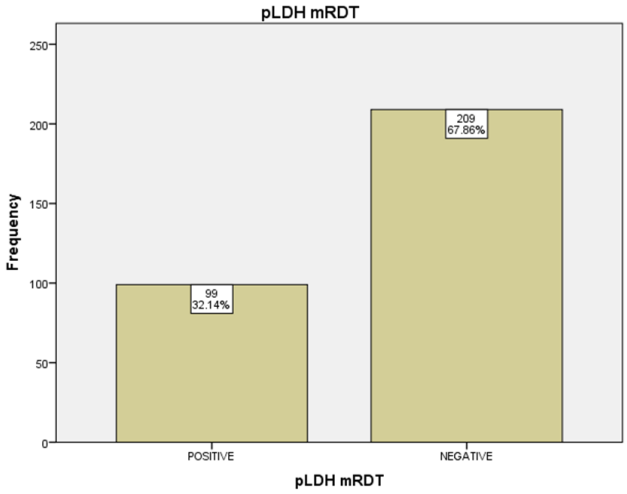

Figure 3: Positivity and Negativity Rates of pLDH mRDT

6.3 The Sensitivity, Specificity, and Positive and Negative Predictive Values of HRP2 and pLDH mRDTs

The sensitivity of pLDH mRDT [94.95% (95% CI: 92.50-97.40)] was observed to be significantly higher in this study than the sensitivity of HRP2 mRDT [77.78% (95% CI: 73.13-82.42)]. However, no significant difference was observed between the specificity of the pLDH mRDT (97.61%, 95% CI: 95.90-99.31) and the specificity of the HRP2 mRDT [97.61% (95% CI: 95.9-99.31)].

The sensitivity and specificity of HRP2 and pLDH mRDTs are shown in table 3 and table 4, respectively:

		Microscopy (gold standard)		
		MPs seen	No MPs seen	Total
HRP2 mRDT	Positive	77	5	82
	Negative	22	204	226
	Total	99	209	308

Table 3: Sensitivity, Specificity, and Positive and Negative Predictive Values of HRP2 mRDT.

Sensitivity = 77.78% (95% CI: 73.13 - 82.42)
Specificity = 97.61% (95% CI: 95.90 - 99.31)
Positive predictive value = 93.90% (95% CI: 91.23 – 96.56)
Negative predictive value = 90.27% (95% CI: 86.96-93.58)

		Microscopy (gold standard)		
		MPs seen	No MPs seen	Total
pLDH mRDT	Positive	94	5	99
	Negative	5	204	209
	Total	99	209	308

Table 4: Sensitivity, Specificity, and Positive and Negative Predictive Values of the pLDH mRDT.

Sensitivity 94.95% (95% CI: 92.50 - 97.40)
Specificity 97.61% (95% CI: 95.90 - 99.31)
Positive predictive value = 94.95% (95% CI: 92.50 – 97.40)
Negative predictive value = 97.61% (95% CI: 95.90 - 99.31)

6.4 The Most Common Plasmodium Species

Plasmodium falciparum (74, 74.75%) was the most common plasmodium species among the study participants, followed by plasmodium vivax (12, 12.1%) and plasmodium ovale (1, 1.0%). Mixed infection of plasmodium falciparum and plasmodium vivax 7 (7.1%) was also observed to be greater than mixed infection of plasmodium falciparum and plasmodium ovale 5 (5.1%), as shown in ***Table 5*** below.

Plasmodium species	Frequency	Percentage (%)
Plasmodium falciparum	74	74.70
Plasmodium vivax	12	12.10
Plasmodium ovale	1	1.00
P.falciparum and *P.vivax*	7	7.10
P.falciparum and *P.ovale*	5	5.10
Total	**99**	**100.00**

Table 5: Percentage Frequencies of the Different Plasmodium Species

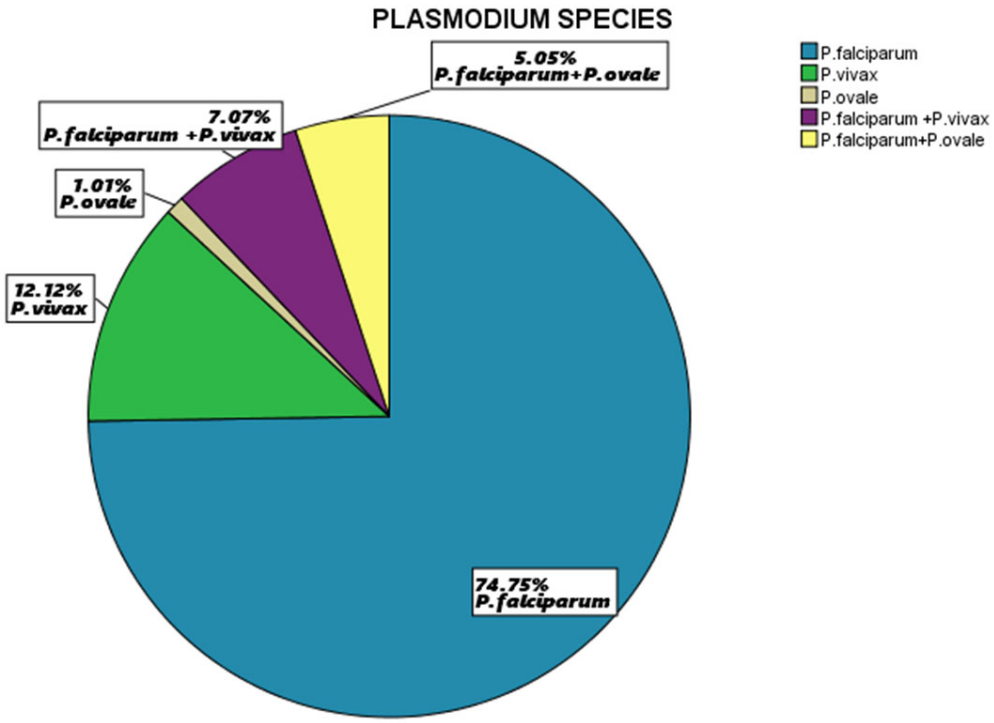

Figure 4: Percentage Distribution of the Plasmodium Species

7. Discussion

7.1 Positivity and Negativity Rates of HRP2 and pLDH mRDTs.

A similar study conducted in Kenya had positivity and negativity rates of 45.65%[95%CI(39.0-52.0)] and 54.35%[95%CI(48.0-61.0)], 46.09%[95%CI(40.0-53.0)] and 53.9%[95%CI(47.0-60.0)] for HRP2 and PLDH mRDTs, respectively [20]. These results are similar to those reported from Nigeria and Cameroon, which turned out to be significantly higher than what we found in our study [21,22]. This is because of the small sample sizes in their studies compared to our study, making the positivity rate more susceptible to specific positive or negative cases. In other words, even though the prevalence of the disease in the community as a whole is low, a small number of positive instances can result in a significantly higher positivity rate. As a result, the computed positive rate may be more variable when sample sizes are small. Another reason is the high endemicity of malaria in their study site compared to our study.

The results from some studies agree with our study results [19,23-25]. These studies also concentrated on using mRDTs to identify

malaria. There are various reasons for the parallelism in the positive and negative rates between these studies and ours. We all had comparable demographics, geographic areas, and malaria frequencies and were directed towards similar people. Consistent frequencies of positive cases discovered by both types of mRDTs result from the diagnostic sensitivity and specificity for both HRP2 and pLDH markers being similar across investigations. These studies all used the same testing procedures and protocols, and the mRDTs' similar test quality, production standards, and storage conditions also contributed to the consistency that was seen [26,27].

We performed a proportion test to assess whether there was a significant difference between the positivity rates of HRP2 and pLDH mRDTs. The p value obtained from the proportion test was 0.1327, which indicated that the difference in positivity rates between HRP2 and pLDH mRDTs was not statistically significant; thus, both tests are comparable in terms of their ability to detect malaria infections among the study population.

7.2 Sensitivity, Specificity, and Positive and Negative Predictive Values of HRP2 and pLDH mRDTs

Expert blood smear microscopy was utilized as the gold standard in this study to compare the performance of two distinct mRDTs. The pLDH mRDTs demonstrated a higher sensitivity of 94.95% (95% CI: 92.50-97.40) compared to HRP2 mRDTs, which gave 77.78% (95% CI: 73.13-82.42) in this study population, which is lower than the WHO recommendation of ≥95% and the manufacturer's sensitivity of 99.7% (95% CI: 98.5-100). The specificities were the same for both mRDTs [97.61% (95% CI: 95.90 - 99.31)] because they were able to detect the same number of true negatives [20].

A similar study in Kenya showed that HRP2 mRDTs had a higher sensitivity of 99.4% compared to our study, whereas the sensitivity of pLDH mRDTs (94.4%) was not significantly different from our study results. Their specificities (85.71% and 85.00%) for HRP2 and pLDH mRDTs, respectively, were lower in identifying patients without malaria compared to our study results [28]. Other studies in agreement with this study include [24,29,30]. This could have resulted from the difference in antigen expression brought about by the genetic diversity of malaria parasites, which impairs the test's capacity to detect the parasites accurately. Variations in sample collection, storage and laboratory techniques may also have an impact on test outcomes. Changes in the local prevalence of several malaria species may be important [31-34].

Additionally, the sensitivity of HRP2 (77.78%) is also compromised by the ability to identify the remaining HRP2 antigen after treatment and parasite clearance. HRP2 mRDTs are currently the most widely used mRDTs for malaria diagnosis in Uganda and other sub-Saharan African countries where *P. falciparum* is the main parasite species [35,36]. However, several factors can impair the usefulness of mRDTs as malaria diagnostic instruments, necessitating regular monitoring [37-39].

The high PPV and NPV of pLDH mRDTs are in agreement with a study conducted in Nanoro, Burkina Faso, which reported PPV and NPV values of 85.2 and 97.6, respectively, for pLDH, whereas the PPV and NPV of HRP2 mRDTs were lower [40]. Studies also agree with these findings [41-43]. This could be attributed to the higher specificity and lower susceptibility to false positives of pLDH mRDTs. HRP2 mRDTs are sensitive to *P. falciparum* but can produce false positives due to antigen persistence [22], whereas pLDH mRDTs are more specific and efficient in areas with mixed infections or nonfalciparum species [44].

7.3 The Most Common Plasmodium Species

Speciation of Plasmodium species is an important malaria epidemiological metric for assessing a country's parasite population, which eventually guides public health management actions for malaria. The study identified three plasmodium species among the study participants, with *Plasmodium falciparum* being the most common, followed by *Plasmodium vivax* and *Plasmodium ovale*, and there were also mixed infections of *Plasmodium falciparum* with Plasmodium vivax and *Plasmodium falciparum* with *Plasmodium ovale*.

A study conducted in four contagious villages in Mulanda subcounty in Tororo District, Eastern Uganda, showed that infection due to *Plasmodium falciparum* was 94.0%, which was also the most common species identified; *Plasmodium malariae* was 6.0%, and there were no mixed infections seen [45]. Similar studies conducted in selected health care facilities across Ghana and Kisoro Hospital in southwestern Uganda showed results that were not significantly different from our study, with the exception of *P. malariae*, which was not identified in our study [24,46]. Studies in the same region of Uganda show results that are not significantly different from our study results [47-49]. This is due to the geographical distribution. Plasmodium falciparum is the most predominant plasmodium species because it is well adapted to the female anopheles that are abundant in Sub-Saharan Africa, leading to higher transmission rates than the other plasmodium species, which have a lower level of compatibility with the dominant mosquito species in the region, leading to reduced transmission opportunities [50].

Plasmodium falciparum is the most dangerous pathogenic strain of malaria, causing severe malaria; hence, the implementation of its control measures should be revised [51]. Because the present HRP2 tests only detect *P. falciparum* monoinfection, the presence of other species has an impact on the type of mRDTs to be used in this situation [7]. In situations where *P. falciparum*-only mRDTs are utilized exclusively, non-*P. falciparum* can cause misleading negative mRDT results. Furthermore, the existence of mixed infections of *P. falciparum* + *P. vivax*, as well as *P. falciparum* + *P. ovale,* may have consequences for the training and development of laboratory employees in this environment to be able to report all species [52,53].

8. Conclusion and Recommendation

There was no significant difference in the positivity rate, negativity rate or specificity in both mRDTs. However, a significant difference in the sensitivity of HRP2 and pLDH mRDTs was observed where HRP2's sensitivity was below the WHO recommendation of ≥95% in this study population; thus, there is a need to improve the sensitivity of this mRDT kit. In our study, most malaria infections were caused by *Plasmodium falciparum*, followed by *P. vivax* and *P. ovale.* Mixed infections were also observed, which may have implications for the choice and deployment of diagnostic tools.

We therefore recommend the use of pLDH mRDTs over HRP2 mRDTs basing on the results of our study.

Limitations of the Study

The study had limited geographical coverage; therefore, generalizability may not be possible. The sample size of 308 participants may be relatively small, and the study might not have captured the full heterogeneity of malaria cases in the population [54].

Although blood smear was used as a gold standard as recommended by WHO, the use of molecular tools such as PCR could have been more precise in detecting and speciation of the plasmodium parasites.

Among the uncooperative patients, some failed to consent, and others withdrew from the study after realizing there was no reward for their participation in the study, whereas others withdrew because of a phobia for needles.

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Authors' contributions.

NA, MIA, LH, APR, AE, SF, KR, SPR, AB and BNC designed the study. NA, MIA, LH, APR and AE collected data. CNB and MIA performed data cleaning, data analysis and results interpretation. MIA drafted the manuscript. NA, MIA, LH, APR, AE, AB, SPR, OB, ME all reviewed the manuscript. All authors read and approved the final manuscript.

Declarations

Ethical Considerations

Ethical authorization was acquired from the Faculty of Medicine Research Ethics Committee before conducting the study. Administrative approval from the DHO of Rubirizi district and the medical superintendent of Rugazi Health Centre IV was sought. The study sought informed consent and ascent from the study participants and patient confidentiality was ensured by giving unique identification numbers to patients. All study data was kept securely and only accessible by the supervisor and research team.

Competing Interests

All authors declare that they have no competing interests.

Supplementary Information

The detailed datasets analysed are uploaded, follow the DOI: https://doi.org/10.6084/m9.figshare.24624768.v1

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