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# Comparative laboratory diagnosis of malaria using rapid diagnostic test and Sysmex rapid malaria test with conventional light microscopy in villages around MAPÉ dam

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**Abstract**
The present study aimed at comparing the performance of Rapid Diagnostic Test (CareStart™ Malaria HRP2 Test), Sysmex/Partec Rapid Malaria test (FM) with reference to Light Microscopy (LM) for the diagnosis of malaria in rural area. 228 pupils, 6-15 years old were tested for malaria using the three methods. 61(26.75%) of the participants were positive for malaria. Light microscopy showed high sensitivity (96.23%) and specificity (95.43%) with positive predictive value and negative predictive value of 86.44% and 98.82% respectively, while Fluorescent Microscopy-Sysmex Rapid Malaria Test had a sensitivity and specificity of 92.45 and 98.86%, respectively, and positive predictive value and negative predictive value of 96.06% and 97.74%, respectively. The CareStart™ Malaria HRP2 and Fluorescent Microscopy-Sysmex Rapid Malaria tests demonstrated a good performance compared to conventional Light Microscopy. Both methods can be considered as alternative malaria diagnostic tools in rural areas. The setback being the non-quantification of parasites in blood.

**Keywords:** CareStart™ malaria HRP2, fluorescent microscopy, sensitivity, Specificity, malaria, magba dam

## 1. Introduction
Malaria, a parasitic disease, is an overwhelming problem in developing countries in tropical regions. About 3.2 billion people are at risk of contacting malaria, especially in Sub-Saharan Africa, South East Asia and Latin America regions. In 2015, there were about 214 million new cases of malaria with 438,000 deaths [1]. In 2016, up to an estimated 3.2 billion people in 91 countries and territories were at risk of infection with *Plasmodium*. In 2018, an estimated 445,000 deaths due to, malaria was recorded with African Region accounting for 91% of all malaria deaths [2, 3]. However, India is amongst the worst affected countries where malaria is one of the most common parasitic infections [1].
Over the past fifteen years, morbidity and mortality due to malaria have globally decreased. However, sub-Saharan Africa, where 90% cases of malaria and 92% of deaths related to malaria have occurred in 2015, still bears the highest burden of the disease [4, 5]. This, despite the free distribution on long lasting insecticidal nets in many countries like Cameroon. It was found in some area that malaria infection was not significantly associated with long lasting insecticidal nets possession [6]. The majority of malaria cases are found in countries where cost-effectiveness is an important factor and ease of diagnostic test performance and training of personnel are also major considerations.
Clinical diagnosis of Malaria is the most widely used diagnostic method in rural areas and where laboratory facilities do not exist. Syndromic approach is unreliable because of the non-specific and overlapping symptoms with other febrile diseases resulting in over diagnosis of malaria, over prescription of anti-malarial drugs, under diagnosis and inappropriate treatment of non-malarial febrile illnesses. Thus, diagnosis of malaria infection based on clinical decision alone is unreliable and, if possible, should be supported and verified with a laboratory-based confirmatory test [7]. However, clinical diagnosis of malaria based on symptoms and signs alone is not recommended since it has low specificity and increases chances of the patient being misdiagnosed leading to misuse of drugs [8, 9].
A laboratory diagnosis of malaria is one possibility in the management of a patient presenting

with fever. To improve quality care of the patients, many diagnostic procedures have been developed with an aim to have accurate diagnosis, to reduce the time of preparation and training needed. However, Giemsa stain followed by microscopic examination remains a gold standard method for diagnosis of malaria in many developing countries [10, 11].

Laboratory methods that require more than an hour to provide a clear diagnosis of malaria are not considered rapid tests, though they are considered as reference procedures [10] and remain a gold standard for malaria diagnosis despite some disadvantages such as inability to detect parasites at low parasitaemia, time consuming, requires electricity, and elaborate training to produce results [10]. Rapid diagnostic tests (RDTs) which counter these disadvantages have thus been developed and they focus on the RDT; performance characteristics which include detection of malaria antigens or antibodies, malaria DNA or entire parasites in red blood cells [10]. Such tests provide results within 2 to 15 min.

In Cameroon, the three rapid diagnostic tests most frequently used are the CareStart TM Malaria HRP2 pf [12], the Fluorescent Microscopy-Sysmex Rapid Malaria test (Cyscope), and SD Bioline Malaria Pf/PAN Rapid test [13].

The Sysmex Rapid Malaria test is an innovative Fluorescence Microscopy method for detection of Plasmodium species DNA in human blood. It uses readily prepared and ready-to-use slides labelled with an unspecific DNA-binding fluorescent dye (4′-6-diamidino-2-phenylindole (DAPI); emission 365 nm) that detects plasmodial DNA. It is an ultra compact and robust microscope design which has connector for optionally available CCD camera upgrade for visualization of the slides on any PC Windows with USB connection [14, 15].

Thus this study was conducted to compare the performance of Rapid Diagnostic Test (CareStart™ Malaria HRP2) (RDT) and Cyscope-Florescence Microscopy (Sysmex Rapid Malaria test) (FM), in reference to Light Microscopy (Thick Blood Film-TBF) as conventional and gold-standard method.

**Note:** Sysmex Rapid Malaria test was formerly called Partec Rapid Malaria test. Partec, a German based company, was the manufacturer of the Cyscope-Fluorescent Microscope used for the purpose. Sysmex, a Japanese consortium, bought the German-Partec Company recently and the name Partec Rapid Malaria was changed to Sysmex Rapid Malaria test.

## 2. Materials and Methods

### 2.1. Study Area

The study area was located around the MAPÉ dam, built in July 1987, with a maximum water level of 715 m. It covers an area of about 550 km$^2$ from Adamaoua, Magba-West and North-West area in the Malantouen Health District. Magba is one of the sub-divisions in the Noun Division in the West Region of Cameroon located between Latitude 5°N and 6°N and Longitude11°E to 12°E. More than twenty ethnic groups are found including Bamoun, Kotoko, Tikar, Guiziga, Toupouri, Mudang, Junkums, Musgoum, Arabs, etc, with an estimated population of 40000 and a density of 30 inhabitants/km$^2$. The equatorial climate is made up of two seasons: a short dry season (November to March), with temperatures ranging from 30–35°C and a longer raining season (April to October), with temperatures of 24–28°C. The vegetation is dense savanna, often mouldy with agriculture and fishing being the very common activities and account for 60–70% of their economy and source of wealth [16, 17].

### 2.2. Study subject

Two hundred and twenty eight pupils (aged 6 to 15) in three villages (Matta Barrage, Matta Village and Cité) around the MAPÉ Dam participated in the study between September 2017 and March 2018 whereby consented children were screened for malaria infection using three different techniques.

### 2.3. Specimen collection and processing

Socio-demographic and clinical data of the pupils were collected using structured questionnaire. Three millilitres of venous blood were collected into EDTA tubes from each study subject [18] for smear preparation for light microscopy, Rapid Diagnostic Test and Fluorescent Microscopy-Sysmex Rapid Test. Rapid Diagnostic Test and test with fluorescent microscopy were processed on the site of collection while light microscopy was done in the Laboratory at the Bafoussam Regional Hospital, West Region Capital, Cameroon.

### 2.4. Rapid diagnostic test: CareStart™ *Malaria* HRP2

CareStart™ Malaria HRP2 Ag RDT Test, manufactured by Access Bio, Inc., was used during the study. The kit utilised, is an immunochromatographic test that contains a nitrocellulose membrane strip precoated with mouse monoclonal antibodies specific to Histidine rich Protein 2 (HRP2), antigen of *Plasmodium falciparum*. The antibodies were mixed with colloid gold which conjugate and react with the HRP2 antigen in the patients' sample. The HRP2 is highly abundant and heat stable; however, the HRP2 antigen remains in circulation for up to 4 weeks after the malaria parasites have been cleared [12].

Five micro-litres of whole blood were dispensed into the round sample well. Then, 4 drops of assay solvent were in turn dispensed into the square assay solvent well. 20 min later, the results were read (Figure 1). The procedure and interpretation of test results of the rapid tests were carried out according to manufacturer's literature guidelines [12].

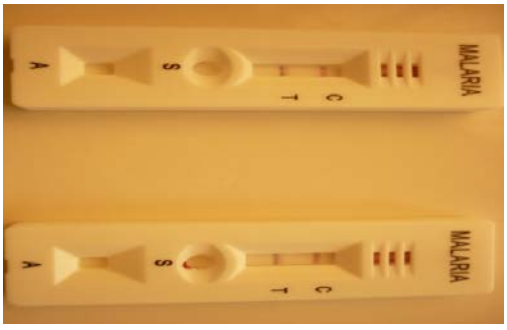


**Fig 1:** Rapid Diagnostic Positive device

### 2.5. Fluorescent microscopy-cyscope/sysmex rapid malaria test

Recently a portable fluorescence microscope- Sysmex Rapid Malaria Test was developed in Germany ("Cyscope", Sysmex Partec GmbH, Görlitz, Germany). Fluorescence microscopy is increasingly accepted and used in Cameroon [13, 12, 19]. The microscope is capable of both fluorescent and transmitted light operation, and incorporates powerful high-efficiency light-emitting diodes (LED) as light sources. The microscope is battery-powered and portable and can be used independently of mains power for many hours (Figure 2, B).

A built-in camera interface enables images of the slides to be snapped for further investigation by image analysis software if desired. It uses readily prepared and ready to-use slides labeled with an unspecific DNA-binding fluorescent dye (4'-6-Diamidino-2- phenylindole (DAPI); emission 443 nm) that detects Plasmodium DNA and the test was performed following manufacturer's instructions [14]. After mixing the sample and 10 µL of blood placed on the dye labeled area of a slide with the participant's number, the slide was cover-slipped, incubated at room temperature for a minute and observed under the 40X objective under UV light (365 nm). The presence of bright shiny intracellular tiny dots against a dark background observed under the UV light indicated the presence of malaria parasites in the erythrocytes [14].

### 2.6. Giemsa stained light microscopy (Thick and Thin Blood Film)

After collecting blood samples, thick and thin blood smears were prepared on the same slide (Figure 2, C). The thin blood film was fixed with methanol and stained with 10% Giemsa working solution for 10 min [18]. Blood films were observed under 100X objectives (Figure 2, A) for detection of malaria parasites, counted against 200 leucocytes and converted (using the formula below) to number of parasites per volume having obtained the total white cells with semi-automated blood counting machine in order to calculate parasite densities; the result was reported as positive if malaria parasites were seen or negative if malaria parasites were not seen after observing 100 fields of the thick smear.

$$\text{No. of parasites}/\mu L = \frac{\text{No. of Parasites counted}}{\text{No. of WBCs counted}} \times (\text{Total WBCs counted})$$

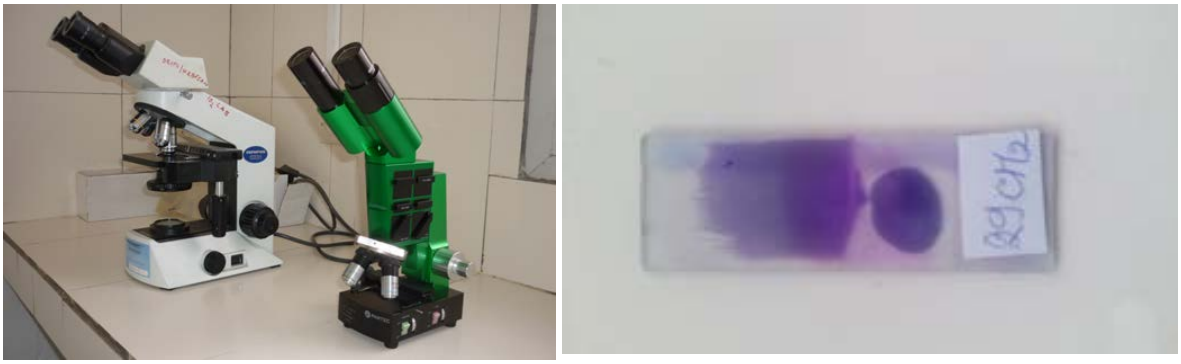

**Fig 2:** Microscopes used in the study and a stained slide. A: Light microscope; B: Cyscope Fluorescent microscope; C: Stained thick and thin blood film on the same slide.

### 2.7. Statistical analysis

Sensitivity, specificity, positive predictive value, and negative predictive value were calculated and used to compare methods. Data were compared using Chi square wherever possible. The data was analyzed using the Statistical Package for Social Sciences (SPSS) version 16.0 software, and the graphs were drawn using Excel 7.0 software.

### 2.8. Ethical considerations

Ethical clearance was obtained from the National Ethical Committee and permission was obtained from the Malentouen District Health Officer to conduct the study. After informing about the objective of the study, written consent was taken from all study participants or parents/guardians. Participants who were positive for malaria parasite with either RDT or Fluorescent microscopy were taken to the nearest Health Centre for appropriate treatment and follow-up.

## 3. Results

Out of the 228 examined, a total of 61 cases were found to be positive using either of the three methods (Giemsa stain, FM test, or RDT). Thus, the prevalence of malaria among studied population was 26.75% (Table 1).

In the present study out of total 228 cases tested, 53 (86.9%), 51(83.6%) and 59 (96.7%) were positive by Giemsa stain, Sysmex Rapid Malaria Test or RDT respectively.

The overall prevalence of malaria in the studied area was 26.75%; without significant variation in infection neither by sex ($\chi^2$=0.050; p=0.879) nor by age range ($\chi^2$=4.398; p=0.210) (Table 1).

The village, Matta barrage had the highest prevalence of the disease (34.92%) while Cité (15.58%) and Matta village (15.94%) had lower prevalences (Figure 3).

**Table 1**: Prevalence of malaria in relation to sex and age range

| | N | Malaria positive | | Chi square (p) |
|---|---|---|---|---|
| | | n | Percentage | |
| Sex | | | | |
| Male | 132 | 35 | 26.52 | 0.050 (0.879) |
| Female | 96 | 26 | 27.10 | |
| Total | 228 | 61 | 26.75 | |
| Age range | | | | |
| 6-8 | 2 | 0 | 0.00 | 4.398 (0.210) |
| 9-11 | 81 | 26 | 32.10 | |
| 12-13 | 118 | 26 | 22.03 | |
| 14 and above | 27 | 9 | 33.33 | |
| Total | 228 | 61 | 26.75 | |

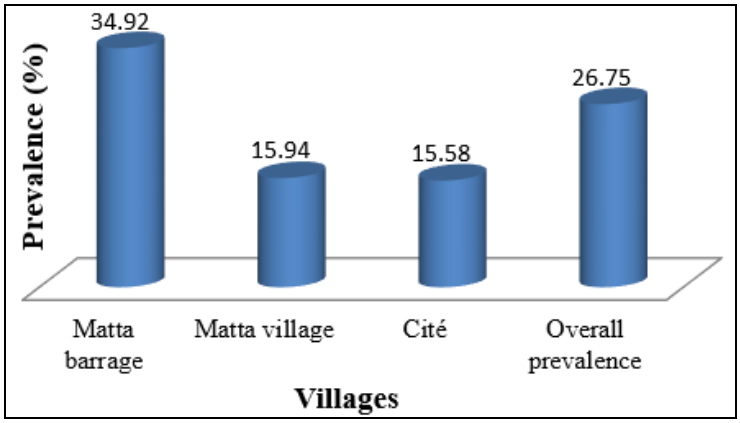


**Fig 3:** Distribution of Malaria in the three villages where samples were collected

Malaria was negatively correlated to distance from the village to the dam (r=-0.872). The prevalence of 34.92% pupils from Matta barrage showed they were more infected than those from the other two villages (Table 2).

The Rapid Diagnostic Test had higher sensitivity of detection (96.23) while Fluorescent microscopy- Sysmex Rapid Malaria test showed high specificity (98.86) (Table 3).

**Table 2:** Presence of parasites in studied population (villages) in relation to closeness to the dam and lake.

| Village | Distance to Dam (m) | Distance to Lake (m) | N | Number of Positive Plasmodium (%) | Correlation coefficient |
|---|---|---|---|---|---|
| Matta barrage | 2530 | 0 | 126 | 44(34.92) | -0.872 |
| Matta village | 3270 | 1200 | 69 | 11(15.94) | |
| Cité | 2500 | 2910 | 33 | 6(15.58) | |
| Total | | | 228 | 61(26.75) | |

**Table 3:** Performance of rapid diagnostic tests and fluorescent microscopy **(**Cyscope) using light microscopy as a standard.

| | TP | FP | TN | FN | Se | Sp | PPV | NPV |
|---|---|---|---|---|---|---|---|---|
| FM | 49 | 2 | 173 | 4 | 92.45 | 98.86 | 96.08 | 97.74 |
| RDT | 51 | 8 | 167 | 2 | 96.23 | 95.43 | 86.44 | 98.82 |
| LM | 53 | 0 | 175 | 0 | | | | |

TP: True positive; FP: False positive; TN: True negative; FN: False negative; Se: Sensitivity; Sp: Specificity; PPV: Positive predictive value; NPV: Negative predictive value.

Cross tabulation of the RDT and FM showed that 59 samples were positive using RDT. Out of the 59, 51 were confirmed by light microscopy therefore, there were 8 false positive and 2 false negative using RDT. On the other hand, 51 samples were positive using Fluorescent microscopy, 49 confirmed by light microscopy, 2 results considered as false positive and 4 false negative were found (Table 4).

**Table 4:** Cross results of RDT and Fluorescent Microscopy- Sysmex Rapid Malaria compared to Light Microscopy (Standard method).

| Methods | Positive Microscopy | Negative Microscopy | Total |
|---|---|---|---|
| Positive RDT | 51 | 8 | 59 |
| Negative RDT | 2 | 167 | 169 |
| Total | 53 | 175 | 228 |
| Positive FM | 49 | 2 | 51 |
| Negative FM | 4 | 173 | 177 |
| Total | 53 | 175 | 228 |

RDT: Rapid Diagnostic Test; FM: Fluorescent microscopy.

It was observed that when parasitemia was at least 500 parasites/µL, sensitivity of the two RDTs was 100%. The sensitivity on the contrary was slightly reduced for parasitemia less than 500 parasites/µL (Table 5).

**Table 5:** Sensitivity of diagnostic methods according to the parasitemia in Thick Blood Film

| Parasitemia (LM) | PTBF | Sensitivity | |
|---|---|---|---|
| | | PFM (%) | PRDT (%) |
| 0 | 0 | 2 (-25.00) | 8 (-100) |
| 1-100 | 14 | 11 (78.57) | 12 (85.71) |
| 101-500 | 16 | 15 (93.75) | 16 (100) |
| 501-1000 | 16 | 16 (100) | 16 (100) |
| >1000 | 7 | 7 (100) | 7 (100) |

PTBF: positive tick blood film; PFM: positive fluorescent microscopy; PRDT: positive rapid diagnostic test; LM: light microscope

## 4. Discussion

Malaria is a parasitic infection of global importance. This study revealed that malaria is still a major public health problem despite intervention strategies that have been put in place in Cameroon over the years such as the free distribution of mosquito bed nets, sensitization on radio and television, and accounts for sizeable morbidity, mortality and economic loss. However, it is of prime importance to also tackle many other associated risk factors [6]. A key to proper control of malaria is accurate diagnosis and effective management.

The overall prevalence of malaria was 26.75%. Since 1982 with the work of Chauvet on the consequences of creating Magba dam on the epidemiology of malaria [16], no other data was recorded in the area reinforcing the insufficient consistent data for malaria in Cameroon [20]. The prevalence around the dam was close to the 27.7% earlier obtained in Mount Cameroon area [21] but not much different from the 29.6% observed in rural and semiurban communities in the South West Region of Cameroon [22]. On the contrary, higher prevalence of 46.7% and 36.9% in children less than 15 years had been reported in other areas in the Mount Cameroon area [23].

There was no significant difference ($p>0.05$) in prevalence of malaria in females and in males. This findings are contrary to earlier reports of Kimbi and collaborators (2012) who evaluated the performance characteristics of the Partec Cyscope [13]. We observed a negative correlation between the prevalence of malaria in each village and the distance between the village and the dam: the closer the village was to the dam, the higher the prevalence. This was surely due to the abundance of malaria vectors near the dam. Such findings were in accordance with result of Eliajah and collaborators (2014) while assessing risk factors associated with Malaria [24] and the work carried out in Ghana on environmental variables influencing malaria prevalence, where it was found that the incidence of malaria decreased as distance to the watercourses increased [25].

The present FM method used showed a sensitivity and specificity of 92.45% and 98.86% respectively, close to the 97.5 and 96.7 respective values obtained in Benin [26]. For the RDT, the sensitivity and specificity values of 96.23 and 95.43% obtained in detecting HRP2 antigen meet the acceptability limits recommended by the WHO for a RDT (sensitivity> 95%) [27]. On the other hand, sensitivity obtained during the study, met the acceptability limits recommended by the WHO for a RDT (sensitivity> 95%) when the parasitaemia was ≥100 parasites/µL of blood. This result is different from the findings of Teh and collaborators (2019), who observed that the RDT is most useful at parasitaemia ≥ 200 parasites /µL of blood and presentation with fever in malaria-endemic regions [21].

False positive results were obtained during the study; this observation could be attributed to the presence of the HRP2 antigen in the blood up to 28 days after treatment [28, 29]. Humar and collaborators (1997) detected circulating antigens in 68% of patients, seven days after the beginning of treatment and, in 27% cases, 28 days later had been detected while comparing two different techniques for the diagnosis of malaria. Also, some false positives might have been due to presence of the rheumatoid factor [26], or related to an inflammatory syndrome, phlebitis or viral hepatitis [30].

Some cases of false negatives were recorded. Such results could be due to either low parasitaemia or perhaps a genetic variety of HRP2 [31], or to the production of anti-HRP2 antibodies [32].

The RDT had a low PPV of 86.44 but high NPV of 98.82. Thus, a high NPV indicates a probability that a participant might not have had the disease with high certainty, meaning that RDT is a reliable test method in diagnosing malaria parasites.

The current study revealed a high sensitivity and specificity of Sysmex Rapid Malaria test (92.45% and 98.86% respectively); the sensitivity being lower than the requirements of the WHO. The high sensitivity and specificity are in agreement with earlier observations on pregnant women in Sudan [33]. However, the sensitivity was lower than the 100% reported in Zimbabwe while using Cyscope-Fluorescent Microscopy- Sysmex Rapid Malaria [34]. These differences might be due to observer variation or host factors. Sysmex Partec rapid malaria test had high PPV and NPV. Thus, as in the case of RDT observed earlier, a high NPV indicates that a person does not have the disease with high certainty while a high PPV indicates a person has the disease with high certainty, meaning that FM is a reliable test method in diagnosing malaria parasites. In our study, the performance of the portable fluorescence microscope was slightly higher as seen with the lower number of false positives compared to RDT. This is probably related to the detection threshold of the portable FM (30 to 40 parasites / µL) lower than that of HRP2 RDT (100 to 200 p / µL). During the study, a sensitivity of 78.57% was found in participants with parasite densities less than 100 parasites / µL of blood. These findings are in accordance with a review of malaria diagnostic tools that revealed a sensitivity of the portable fluorescence microscope of 78.9% in a population with parasite densities less than 100 parasites / µL [35].

Sysmex Rapid Malaria Test had detected two cases, which were negative by Light Microscopy. Unlike Giemsa stain light microscopy, Sysmex Rapid Malaria Test has sensitive fluorescent dye, 4, 6-diamidino- 2-phenylindole (DAPI), which can be detected in such low levels of parasites. In addition, the presence of artefacts such as nonspecific aggregated DAPI, immature erythrocytes, or bacterial cells might have been misinterpreted as *Plasmodium* DNA [15]. On the other hand, Sysmex Rapid Malaria Test produced four false negative results. This might be due to the fact that analyzed red blood cells may lie on each other or overlap with each other thereby preventing parasites in red blood cells that may be lying beneath other cells. The performance characteristics of the tests were very good as indicated by their sensitivities, specificities, PPV, and NPV. Compared to light microscopy again, determination of parasitemia with Sysmex Rapid Malaria Test is difficult, because the red blood cell may overlap each other and make the parasites invisible under the UV light. This finding corroborates results obtained in a study conducted in Ghana [15]. Sysmex Rapid Malaria Test has some advantages like reduction of the training time necessary for diagnosis of malaria, reduction of slide preparation time, simplicity of reading, functional autonomy of more than 24 h facilitating its usage in rural areas. It equally has some disadvantages as the impossibility of calculating parasite density and species identification; however it can be used as screening tools in malaria endemic areas.

## 5. Conclusion

The present study showed that the two rapid tests used for the study (CareStart™ Malaria HRP2 and Fluorescent

Microscopy- Sysmex Rapid Malaria Test) could be used as alternative diagnostic tools in malaria endemic areas in addition to or instead of Light microscopy since they are reliable , sensitive and specific in diagnosing malaria infection. Furthermore, they have the advantages that, the length of time required to report results is short, little training is required and they can be used in field conditions.

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