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Evaluation of the Performances of Two Rapid Diagnostic Tests (Cyscope®mini and Paracheck-Pf®) in the Diagnosis of Malaria among Febrile Children in Southwest Nigeria

Olawunmi R. Rabiu[a, d] Ayokulehin M. Kosoko[b, d] Catherine O. Falade[c, d]
Olusegun G. Ademowo[c, d]

Departments of [a]Zoology, [b]Biochemistry, and [c]Pharmacology and Therapeutics, and [d]Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan, Ibadan, Nigeria

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Abstract
Objective: The aim of this study was to test the diagnostic performances of Cyscope®mini and Paracheck-Pf® for *Plasmodium falciparum* relative to microscopy. ***Subjects and Methods:*** 209 children aged 6 months to 12 years presenting with symptoms suggestive of malaria were enrolled at the University College Hospital, Ibadan, Nigeria, within a period of 6 months. Malaria parasites were identified in capillary blood samples using Cyscope®mini (parasite DNA-based fluorescence microscope) and Paracheck-Pf® (an HRP-II-based test) with microscopy of Giemsa-stained thick blood films as reference gold standard. The overall performances were calculated using OpenEpi version 2.3 statistical package. 209 samples were performed for Cyscope®mini and light microscopy while 140 samples were done by Paracheck-Pf®. ***Results:*** The prevalence of malaria parasitaemia by light microscopy was 22.0% (46/209), while those of Cyscope®mini and Paracheck-Pf® were 85.2% (178/209) and 32.1% (45/140), respectively. Parasite density ranged from 40 to 203,883/μl. Cyscope®mini and Paracheck-Pf® had sensitivities of 91.3 and 86.21%, respectively. The respective specificities were 16.56 and 81.98% for Cyscope®mini and Paracheck-Pf® with diagnostic accuracies of 33.01 and 82.86%. The diagnostic performances of the two rapid diagnostic tests were significantly different. ***Conclusion:*** Paracheck-Pf® performed better than Cyscope®mini for diagnosis of *falciparum* malaria and will be a good diagnostic tool for field studies.

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Introduction

Malaria is a widespread disease of major public health concern in sub-Saharan Africa. An estimated 655,000 deaths were attributed to malaria in 2010 of which 86% were children under 5 years of age [1]. In Nigeria, malaria is reported to be responsible for 25% of infant mortality and 30% of childhood mortality [2]. Until recently, presumptive diagnosis of malaria by healthcare professionals was the routine method of diagnosis of malaria [3]. Similarly, self-medication is a common practice among the general populace [4, 5]. However, the overlap of malaria symptoms with other febrile illnesses resulted in overdiagnosis and development of drug-resistant stag-

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Olusegun G. Ademowo
Institute for Advanced Medical Research and Training
College of Medicine, University of Ibadan
Ibadan 200212 (Nigeria)
E-Mail ademowo_g@yahoo.com

es of the parasite [6]. These underscore the need for accurate and prompt diagnosis of malaria before treatment to achieve better disease control. The World Health Organization (WHO) now recommends parasite-based diagnosis [7].

Microscopic examination of Giemsa-stained capillary blood for malaria parasites remains the gold standard [8]. However, microscopy is laborious, time-consuming and requires well-trained personnel, a good microscope, good quality reagents and clean slides [9–11]. These challenges necessitated the development of easier and faster diagnostic methods including rapid diagnostic tests (RDTs). Generally, RDTs are immunochromatographic tests targeting specific antigens of one or more *Plasmodium* species. They produce easily interpretable results within a short time, require minimal training and less expertise. The performance of Paracheck-Pf®, an HRP-II-based RDT, has been evaluated by many workers in sub-Saharan Africa [12–14], South America [15] and Asia [16]. However, there are only few reports evaluating Cyscope® – a fluorescent microscopic device for the rapid diagnosis of malaria [17–19]. Unlike most malaria RDTs which consist of lateral-flow immunochromatographic devices that detect parasite-specific antigens in the blood, Cyscope®mini RDT is a portable, battery-operated fluorescent microscopy manufactured by Partec, Germany. The principle is based on the detection of intraerythrocytic *Plasmodium* DNA [17] which results in a bright intracellular dot-shaped fluorescence if the red blood cells are infected with *Plasmodium* sp. The aim of this study was to test the diagnostic performances of Cyscope®mini (Partec, Germany) and Paracheck-Pf® (Orchid Biomedical Systems, Goa, India) for malaria parasite detection among febrile children in a malaria-endemic environment using light microscopy as reference gold standard.

Subjects and Methods

During a larger study evaluating the comparative efficacy of artemether-lumefantrine and dihydroartemisinin-piperaquine, the performances of Cyscope®mini and Paracheck-Pf® malaria RDTs were evaluated over a 6-month period starting in September 2010 at the General Out-Patients Department of the University College Hospital, Ibadan, Nigeria. 209 children between the ages of 6 months and 12 years presenting with symptoms suggestive of malaria were enrolled. Children with signs and symptoms of severe malaria were excluded from the study. A detailed clinical history and physical examination were carried out for each enrollee. Questionnaires were administered for relevant details such as age, sex, height, weight, axillary temperature, presenting features and treatment history after obtaining informed consent from the parents or guardians. Capillary blood was obtained through finger prick from each enrollee for haematocrit determination, thick and thin films, as well as for diagnosis by Cyscope®mini and Paracheck-Pf®. Ethical approval for the study was obtained from the University of Ibadan/University College Hospital Ethical Review Committee, Ibadan, Nigeria.

Thick and thin blood films were prepared from a finger-prick blood sample. The thin smears were fixed in absolute methanol and stored away after drying. The thick blood smears were processed as soon as the blood smears were dried. Thick smears were stained with 10% fresh Giemsa stain for 15 min, rinsed under gentle running water, allowed to air dry and then viewed at ×1,000 magnification under a light microscope for presence or absence of malaria parasites. Thick smears were considered negative if no parasites were seen in at least 100 high-power fields. Asexual parasites were counted until a total of about 200 white blood cells had been counted. Parasite density was estimated assuming a total white blood cell count of 8,000/μl blood [20]. 114 of the slides were re-screened by another microscopist for higher accuracy. The microscopists (B.A. and O.A.) were blinded to results of the RDTs.

A drop of fresh blood obtained from the same finger prick used for preparing blood smears was also placed on a Partec precoated test slide and covered with a coverslip. Each test slide had been labelled with an unspecific DNA-binding fluorescent dye (4′,6-diamidino-2-phenylindole – DAPI) that detects intraerythrocytic *Plasmodium* DNA [17] resulting in a bright intracellular dot-shaped fluorescence if the red blood cells are infected with malaria parasites. This was then viewed under the fluorescence Cyscope®mini with a ×40 objective lens after being allowed to incubate for 1 min. Small blue dots confirmed the presence of malaria parasites.

For malaria diagnosis using the Paracheck-Pf® device, 5 μl of fresh blood obtained from finger prick was placed directly on the test cassettes and six drops of the clearing buffer solution was added following the manufacturer's instructions. The presence of parasites was confirmed after 15 min when two red bands (control and test) were observed. Negative results had only the control band. Results were reported as invalidated when no control band was seen.

In order to make a fair comparison of the performances of the two RDTs, data with both results for Cyscope®mini and Paracheck-Pf® were selected. Hence, a total of 140 were used for the comparative analysis.

Blood from finger prick was likewise withdrawn into heparinised capillary tubes and spun at 3,000 rpm for 10 min using microhaematocrit centrifuge (Hawksley Ltd, Lancing, UK). The result was obtained using a Hawksley haematocrit reader.

Data were entered and analysed using SPSS version 16.0 statistical software (Chicago, Ill., USA). Frequencies were calculated and compared with McNemar test. The overall diagnostic performances including sensitivity and specificity were calculated using OpenEpi version 2.3 [21]. The likelihood ratio tests as performance indices were also included.

Table 1. Analysis of results obtained from RDTs compared to microscopy

RDT	True positives grouped by parasite densities per μl				False positives	True negatives	False negatives	Total
	<1,000	1,001–10,000	10,001–100,000	>100,000				
Cyscope®mini	9	10	18	5	136	27	4	209
Paracheck-Pf®	4	6	10	5	20	91	4	140

Table 2. Comparison of Cyscope®mini and Paracheck-Pf® results relative to parasite densities

	Parasite densities per μl			
	<1,000	1,001–10,000	10,001–100,000	>100,000
Microscopy	5	6	13	5
Cyscope®mini	5	6	13	5
Paracheck-Pf®	4	6	10	5

Table 3. Summary of comparative diagnostic performances of Cyscope®mini and Paracheck-Pf® in patients screened with both RDTs

	Cyscope®mini	Paracheck-Pf®
Sensitivity	100%	86.21%
Specificity	13.51%	81.98%
Positive predictive value	23.2%	55.56%
Negative predictive value	100%	95.79%
Diagnostic accuracy	31.43%	82.86%
Likelihood ratio of a positive test	1.156	4.784
Likelihood ratio of a negative test	0.0	0.1682
Cohen's κ (unweighted)	0.0608	0.5665

Results

A total of 209 children were enrolled. 50.7% (106/209) enrollees were males. The average age of the children was 40 ± 30.38 months (3 years 4 months) with a mean weight of 13.7 ± 6.1 kg. The mean temperature was 37.4 ± 1.1°C and the mean haematocrit was 32 ± 5.5%. A history of fever or fever at presentation was reported among 189 (90.4%) of the enrollees. However, only 95 (45.5%) were febrile (axillary temperature ≥37.5°C) at the time of presentation. The next four most frequent complaints among the enrollees were cough 107 (51.2%), loss of appetite 103 (49.3%), catarrh 79 (37.8%) and headache 79 (37.8%). Investigation into the antimalarial treatment history showed that 77 (36.8%) of the enrollees had used one or more antimalarial drugs (artemether-lumefantrine, chloroquine, artesunate, amodiaquine, sulphadoxine-pyrimethamine, artesunate-amodiaquine and quinine) within 2 weeks prior to presentation. Some of the enrollees (47, 22.5%) also used antibacterial agents (penicillins, septrin, flagyl, erythromycin, rocefin and ciprotab) before presentation.

Detection of Malaria Parasite by Microscopy. The diagnosis of malaria using the light microscope showed a *P. falciparum* prevalence of 22.0% (46/209). The parasite density ranged from 40 to 203,883/μl. The parasite density was ≤1,000/μl in 21.7% (10/46) of the malaria-positive enrollees. 31 (67.4%) of the children positive for malaria had parasite densities within the range of 1,001 to 100,000/μl.

Malaria Parasite Detection by Cyscope®mini Relative to Microscopy. Cyscope®mini had a prevalence rate of 85.2% (178/209). It was observed that the higher the intensity of fluorescing bodies observed under the microscope, the greater the parasite density from microscopy result. Cyscope®mini detected parasites in 19 (86.4%) out of 22 positive by microscopy at parasite densities ≤10,000/μl. At parasite densities >10,000/μl, the detection rate was 95.8% (23/24) (table 1). Although the sensitivity of Cyscope®mini was 91.3%, the large number of false positives (table 1) affected the specificity (16.56%).

Malaria Parasite Detection by Paracheck-Pf® Relative to Microscopy. Paracheck-Pf® had a prevalence rate of 32.1% (45/140). Of the 142 children screened with Paracheck-Pf®, two test kits gave invalid results (no control band seen). The sensitivity and specificity was 86.21 and 81.98%, respectively. Paracheck-Pf® was able to detect parasites in 10 (90.9%) out of the 11 positive by microscopy at a parasite density of ≤10,000/μl. At parasite densities >10,000/μl, the detection rate was 83.3% (15/18) (table 1). Furthermore, it was observed that the false negative results (table 1) obtained from Paracheck-Pf® had parasite densities ≤100,000/μl when viewed microscopically.

Comparative Performance of Cyscope®mini and Paracheck-Pf® Relative to Microscopy. Of the 140 samples screened with both methods, 29 were positive by microscopy. Cyscope®mini detected parasites at all densities but there were a few variations in the result from Paracheck-Pf®. It did not detect all positive samples at <1,000 and 10,000–100,000 μl (table 2). Cyscope®mini had 89.3% positive and 10.7% negative results relative to 32.1% positives and 67.9% negatives obtained from Paracheck-Pf®. There was a highly significant difference in the diagnostic performance of the two RDTs ($p < 0.05$). A summary of the overall comparative diagnostic performances of the RDTs among the 140 patients screened with both methods is given in table 3.

Discussion

The sensitivities of the Cyscope®mini (91.3%) and Paracheck-Pf® (86.21%) were quite impressive relative to the Giemsa-stained thick film slides screened microscopically used as gold standard. A similar finding of a high sensitivity of the Cyscope® in the diagnosis of malaria was recently reported [11, 19] among Ghanaian children (100%) and pregnant women in Sudan (97.6%), respectively. However, a high number of false positives were obtained in this study which culminated in a poor specificity outcome (16.56%) contrary to the 97.4% specificity reported by Nkrumah et al. [11] and 89.1% reported by Hassan et al. [19].

Low specificity of 16.56% of Cyscope®mini is similar to that observed in Uganda with specificities of 38.8 and 28.6% reported among adults and children, respectively [18]. The large number of false positives has been attributed to the presence of other unknown fluorescing bodies that could be mistaken as malaria parasites [18]. It was however interesting to note that the higher the parasite count obtained from light microscopy, the more the number of fluorescing bodies observed under the Cyscope®mini. Sousa-Figueiredo et al. [18] also reported that the Cyscope® was likely to record a false negative result at low parasite densities ≤400 parasites/μl. The limitation of this study was that the parasite quantification was not done using the Cyscope®mini. Likewise, some of the false-positive results of Cyscope®mini may be false-negative results of microscopy due to low parasitaemia. The use of PCR method would have been a better comparative tool. The sensitivity of Paracheck-Pf® in this study was 86.21%, but according to WHO standards a RDT is expected to have a sensitivity of 95% at a parasite density of 100 parasites/μl [21–23]. However, Paracheck-Pf® has markedly higher specificity than Cyscope®mini which is in agreement with a previous report [18].

Conclusion

Paracheck-Pf® performed better than Cyscope®mini and will be a good diagnostic tool in field studies for diagnosis of malaria in endemic areas.

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