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Comparison of the Novel Partec Rapid Malaria Test to the Conventional Giemsa Stain and the Gold Standard Real-Time PCR[▽]

Bernard Nkrumah,[1,2]* Alex Agyekum,[1,2] Samuel E. K. Acquah,[1,2] Jürgen May,[3] Egbert Tannich,[3] Norbert Brattig,[3] Samuel Blay Nguah,[4] Heidrun von Thien,[3] Yaw Adu-Sarkodie,[1] and Frank Huenger[2,5]

Department of Clinical Microbiology, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana[1]; Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR), Kumasi, Ghana[2]; Bernhard Nocht Institute for Tropical Medicine (BNITM), Hamburg, Germany[3]; Child Health Department, Komfo Anokye Teaching Hospital, Kumasi, Ghana[4]; and Institute for Transfusion Medicine, Laboratory of Medicine and Medical Microbiology, Dortmund, Germany[5]

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Malaria remains the single most frequent cause of death in Africa, killing one child every 30 s, but treatment decisions are often made only on clinical diagnosis, as laboratory techniques to confirm the clinical suspicion are labor intensive and costly. In this study, we evaluated the recently developed Partec rapid malaria test (PM) for the detection of *Plasmodium* spp. in human blood from patients in an area where malaria is endemic and compared the results with those of thick blood film Giemsa stain (GS) in terms of its performance and operational characteristics, using real-time (RT) PCR as the gold standard. The sensitivities of the PM and the GS were 62.2% (95% CI, 56.3 to 67.8) and 61.8% (95% CI, 55.9 to 67.4), respectively, while the specificities were 96.0% (95% CI, 92.3 to 98.3) and 98% (95% CI, 95.0 to 99.5), respectively. There was an excellent agreement between the results for the PM and those of the GS (k [level of agreement] = 0.96; $P < 0.001$). The results for the PM were obtained more quickly and at less cost than those for the GS. The performance characteristics of the PM were almost equal to those of the GS, but the operational characteristics were better, and the PM can therefore be considered as an alternative method for GS.

Malaria is the most common single diagnosis made in most countries in Africa (24). However, in many countries where malaria is endemic, clinical diagnosis is the only method used to decide on treatment, even though its accuracy is limited by the low specificities of the signs and symptoms of malaria (4, 5, 19). Presumptive antimalarial treatment for any fever with no obvious alternative cause is widely practiced, and studies suggest that this leads to significant overuse of antimalarial drugs throughout Africa (1, 2, 19). The overdiagnosis of malaria coexists with underdiagnosis, due to the lack of diagnostic laboratories, with the result that antimalarial drugs are given to people who do not need them and are not given to some of those who do (18, 23). The reliable diagnosis of malaria requires laboratories at which rapid, sensitive, and specific tests are available at affordable cost. However, in many countries of malaria endemicity, laboratory techniques to confirm the clinical suspicion of malaria are considered to be too labor intensive (3) and unreliable, due to a lack of skilled microscopists, limited supplies, inadequate maintenance of microscopes and reagents, and inadequate or absent quality control systems (7). In general, the screening of Giemsa stains (GS) by light microscopy is still considered the gold standard (11). This method is cheap and simple but labor intensive and time consuming and requires well-trained personnel (21), particularly for the detection of low levels of parasites (10). In resource-poor areas, microscopic diagnosis has been shown to be insensitive and nonspecific, especially when parasitemias are low or mixed infections are present (1, 7). The sensitivity of thick-film microscopy is 10 to 30 parasites/μl of blood (10). Parasitemias below this concentration may be difficult for even the best expert microscopist to detect. Having a simple and accurate test with high sensitivity and specificity that requires little or no training and is cost effective could greatly aid the diagnosis of malaria infection in remote areas where health facility coverage is low and the population is at high risk for contracting malaria (17, 25). This study evaluated the recently developed Partec rapid malaria test (PM) (Partec GmbH, Münster, Germany) for the detection of *Plasmodium* spp. in human blood (from patients in an area of malaria endemicity) and compared the results with those of GS in terms of performance (sensitivity, specificity, positive predictive value [PPV], negative predictive value [NPV], etc.) and operational characteristics (cost, time, etc.), using real-time (RT) PCR as the gold standard.

MATERIALS AND METHODS

Study site. The Agogo Presbyterian Hospital is located in the Asante Akyem North District of the Ashanti Region of Ghana, West Africa, and is the principal hospital of the district. The district is located in the eastern part of the Ashanti region and covers a land area of 1,160 km^2, with an estimated population of about 150,000 (a projection from the 2000 population census). Over 40% of the population is under 15 years of age, and over 50% are under 20 years. The hospital runs an under-five clinic that attended to 13,060 patients in 2007 and 17,716 in 2008. The proportions of these children with malaria parasitemia were 42.5% in 2007 and 26.5% in 2008 (unpublished data of the Biostatistics Department of Agogo Presbyterian Hospital).

* Corresponding author. Mailing address: Kumasi Centre for Collaborative Research in Tropical Medicine, Asuogya Road, UPO, KNUST, Kumasi, Ghana. Phone: 233244788911. Fax: 233322062017. E-mail: nkrumah@kccr.de.

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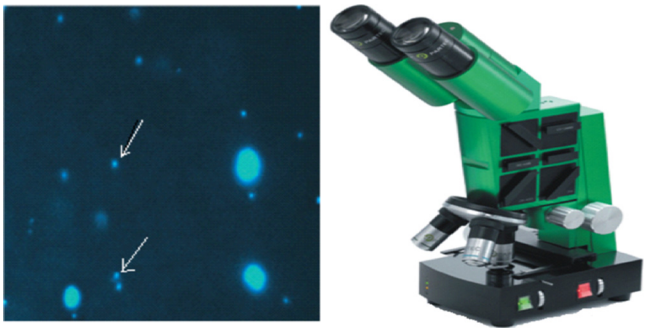

FIG. 1. The Partec CyScope and malaria parasites (arrows) as they appear under the CyScope.

Study population. Between May and December 2008 (i.e., from the beginning of the rainy season to the early dry season), 489 samples were collected from children attending the under-five clinic for the first time with fever or a history suggestive of malaria, who were enrolled in the study.

Sampling. A maximum of 1.5 ml of capillary blood was taken from each child in an EDTA tube labeled with the patient's barcode and pathology number. These samples were examined immediately by two microscopic methods, PM and GS. The two tests were done and examined by well-trained and expert laboratory staff independently and without knowledge of the health status of the child each sample was from. In cases where the two microscopic results were discordant, a third expert reader was used. The results of the third expert reader were considered the final result. Written results were communicated to the clinicians as soon as possible.

Ethical approval. Ethical approval for the study was obtained from the Committee on Human Research, Publication and Ethics (CHRPE) of the School of Medical Sciences, KNUST-Kumasi. The legal guardians of all participants consented before any study-related procedures were done. All procedures were done in accordance with internationally accepted good clinical and good laboratory practices.

Statistical analysis. Data were double entered into a predesigned electronic database using Epi-info version 6.04dfr (Centers for Disease Control and Prevention, Atlanta, GA) and were cleaned on a regular basis. The data were exported to R statistical software (R Development Core Team, 2008) for analysis. Sensitivities, specificities, and positive and negative predictive values were determined for the various tests and compared with one another. A P value of less than 0.05 was considered significant in all comparisons using a type I error of 0.95 and a power of 0.80.

Giemsa-stained blood film. Thick and thin blood films were prepared using standardized blood volumes of 10 μl and 2 μl, respectively, and air dried. The thin film was fixed in absolute methanol and heat fixed, and both thick and thin films were stained with a 10% Giemsa working solution for 12 min. A malaria blood film was considered negative if 100 high-power fields (HPF) were scanned and no parasite was observed. If parasites were observed, they were counted against 200 white blood cells (WBCs), but all parasites in a final HPF were counted even if a count of 200 WBCs had been exceeded. If counts exceeded 100 parasites/field in a thick film, the thin film was used for the count. Upon the observation of malaria parasites, the microscopist counted the parasitized red blood cells (RBCs) against 1,000 RBCs. The counts were done across the width of the thick and thin films, using the battlement method. The presence of other blood parasites was reported. Known positive and negative samples were used as controls for freshly prepared Giemsa working solution each day.

Partec rapid malaria test. The Partec CyScope is a microscope that uses fluorescent light detection through incident UV light in addition to a bright field option. The microscope runs with integrated rechargeable batteries. The battery pack consists of nickel-metal hydride (NiMH) batteries with a built-in temperature probe that ensures an optimal recharging process. The lifetime of the batteries, however, depends on the usage; they can usually be recharged up to 1,000 times. The battery pack can be easily exchanged and is readily available (Partec GmbH, Münster, Germany). It uses readily prepared and ready-to-use test slides labeled with an unspecific fluorescent dye (4′,6-diamidino-2-phenylindole [DAPI]) that detects intraerythrocytic *Plasmodium* DNA. Tests were performed according to the manufacturer's instructions. Briefly, 5 μl of well-mixed capillary blood was placed on the dye-labeled portion of a slide with the patient's pathology number, cover slipped, incubated at room temperature for 1 min, and observed at a 100× objective under UV light. The presence of distinct bright, shiny, tiny dots observed under the UV light indicated the presence of malaria parasites (Fig. 1). To prevent the slides from drying out, they were kept in a wet chamber. Parasite counts were done as described for the GS method above. For counts that exceeded 100 parasites/field in a thick film, an approximate count was done by counting a quarter of the field and multiplying by four to get an approximate parasite count per field. This value was used to calculate the parasite density/μl. The presence of other blood parasites was reported. Positive and negative controls from known samples were done on each batch of ready-to-use test slides.

Real-time PCR. DNA extraction was done using a FlexiGene DNA kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions (20). DNA samples were stored at −20°C. *Plasmodium* DNA detection was performed by using a Corbett Rotor-Gene 6000 instrument (Corbett Life Science, Australia). The 18S rRNA gene was chosen as the target, since it contains both highly conserved and variable regions, and at least five copies of the gene are dispersed on separate chromosomes of the *Plasmodium* genome (9, 15). Consensus primers used for the study had gene sequences for each of the four *Plasmodium* spp. (PL1473F18 [5′-TAA CGA ACG AGA TCT TAA-3′] and PL1679R18 [5′-GTT CCT CTA AGA AGC TTT-3′]) (18). Each 20-μl reaction mixture contained 2 μl of sample DNA, 2 μl of FastStart DNA SYBR green reagent (Roche), 4.4 μl of 25 mM $MgCl_2$, 1 μl (10 pmol/μl) of each primer, and 9.6 μl of H_2O. The PCR conditions consisted of initial denaturation at 95°C for 10 min, amplification for 40 cycles of 10 s at 95°C, 5 s at 60°C, and 20 s at 72°C, and a touchdown of the first 11 steps by 0.5 s/cycle, with fluorescence acquisition at the end of each extension step. Amplification was immediately followed by a melt program consisting of 2 min at 95°C, 2 min at 68°C, a stepwise temperature increase of 0.6°C/cycle, a wait of 5 s for each step afterwards until 90°C was reached, with fluorescence acquisition at each temperature transition, and then cooling at 40°C for 20 s (14). Melting-curve analysis was used to determine the species-specific mean melting temperature (T_m) based on values determined for the positive controls. Each batch of sample run consisted of four positive-control DNAs from blood samples positive for *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium malariae*, and *Plasmodium vivax*, respectively, and DNA-free PCR grade water as a negative control. This assay for *Plasmodium* spp. differentiation required a run time of 1 h 20 min to complete (14).

RESULTS

Of 489 patients enrolled in the study, 182 (37.2%; 95% CI, 32.9 to 41.7), 187 (38.2%; 95% CI, 33.9 to 42.7), and 288 (58.9%; 95% CI, 54.4 to 63.3) were positive according to GS, PM, and RT PCR, respectively. There were 9 noncorresponding results between the GS and PM methods (Table 1). Of these, GS detected 2 positive results that were also positive by RT PCR, with a late cycle time (C_T) of 27.6, but were negative by PM. The PM detected 7 positive samples, 3 of which were positive by RT PCR (C_T = 28.1) and GS. RT PCR had a

TABLE 1. Summary of all tests, showing both concordant and discordant results

Test and result	No. of samples testing positive or negative by Giemsa stain[a]		No. of samples testing positive or negative by Partec rapid test[a]		No. of samples testing positive or negative by RT PCR[a]	
	Pos	Neg	Pos	Neg	Pos	Neg
Giemsa stain						
Pos	182		180	2	178	4
Neg		307	7	300	110	197
Partec rapid test						
Pos			187		179	8
Neg				300	109	193

[a] Pos, positive; Neg, negative.

TABLE 2. Performance characteristics of PM and GS at a 95% CI, using RT PCR as the reference standard

Test	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Partec rapid test	62.2 (56.3–67.8)	96 (92.3–98.3)	95.7 (91.7–98.1)	63.9 (58.2–69.3)
Giemsa stain	61.8 (55.9–67.4)	98 (95.0–99.5)	97.8 (94.5–99.4)	64.2 (58.5–69.5)

higher plasmodium-positive detection rate than the PM and GS; 109 (22.3%) and 110 (22.5%) of the total samples were positive by RT PCR but not by PM and GS, respectively. No other blood parasites were found by both PM and GS. Compared to that for RT PCR, the sensitivities of the PM and GS were 62.2% (95% CI, 56.3 to 67.8) and 61.8% (95% CI, 55.9 to 67.4), and the specificities were 96% (95% CI, 92.3 to 98.3) and 98% (95% CI, 95.0 to 99.5), respectively (Table 2). There was an excellent agreement between the two test methods (PM and GS), with concordant results for more than 98% of samples (k [level of agreement] = 0.96; $P < 0.001$). Table 3 shows the operational characteristics observed in this study. Compared to GS, the PM is less labor intensive, costs less per test performed on each patient, and requires less blood per sample, and results were obtained more quickly. On the other hand, the microscope needed for GS costs more than that for the PM.

DISCUSSION

In areas of Ghana where malaria is endemic, the predominant *Plasmodium* species is *P. falciparum*, thus making it an important cause of severe malaria. Early treatment is a key measure in the management of severe malaria, but to ensure correct treatment, early and definitive diagnosis must be available (3). Giemsa-stained thick blood film microscopy has historically been the mainstay of laboratory diagnosis and continues to be the gold standard despite its disadvantages (10, 21). As a result of these limitations, alternative techniques for the diagnosis of malaria that are easy and quick to perform as well as cheaper need to be evaluated.

The study results show comparable performance characteristics between the PM and GS. The PM detected 4 more positive malaria cases than GS. The PM uses a sensitive fluorescent dye, 4′,6-diamidino-2-phenylindole (DAPI), which strongly binds to intracellular double-stranded DNA (dsDNA) of the malaria parasite if it is present in the erythrocytes.

TABLE 3. Comparison of the operational characteristics of PM and GS

Parameter	Giemsa stain	Partec rapid test
No. of steps involved in performing test	6 steps	3 steps
Average time/test	25 min[a]	5 min[b]
Average cost/test	$1.00[c]	$0.50[b]
Average cost/microscope	$3,000[d]	$2,500[b]
Blood quantity/test	10 μl[e]	5 μl[b]
Can use electric current provided by a standby battery	No	Yes[b]

[a] Time observation made in this study.
[b] Data obtained from the manufacturer.
[c] Figure obtained from study site.
[d] Microscope cost from manufacturer.
[e] Blood quantity used in this study.

False-positive PM results might be explained by the presence of fragmented nuclei from damaged reticulocytes or white blood cells. Also, the presence of bacterial or other microbial DNA as well as other nonspecific fluorescent artifacts might be misinterpreted as plasmodial dsDNA. Most of the possible nonplasmodial fluorescence signals may be identifiable. We observed that plasmodial DNA staining by DAPI produced brighter fluorescence than nonplasmodial DNA staining, and for confirmation of parasite localization within the erythrocyte, the PM microscope can be easily switched from fluorescence to bright field and back. The PM also generated two false-negative results. These could have been the result of the low level of parasitemia in blood films, as shown by the late RT PCR C_T values (28.1 and 27.6, respectively). Milne et al. (16) reported that most routine diagnostic laboratories generally achieve a lower level of sensitivity of detection (average, 0.01% RBCs infected; 500 parasites/μl) in the examination of blood films than that found by Gilles (10) under optimal conditions, for which the sensitivity of thick-film microscopy is 10 to 30 parasites/μl of blood (10); thus, lower parasite concentrations may not be detected by even the best expert microscopist. Semi-immune parasite carriers with low levels of parasitemia may also have had false-negative results. GS has been shown to be the less-sensitive test used as a reference method (6); nevertheless, it is cheap and can be used for both qualitative and quantitative purposes and is the only cheap method that can be used for species identification (6, 22). RT PCR has been reported to have 100% sensitivity and specificity (4) and an analytical sensitivity of 0.01 to 0.02 parasites/μl (14). RT PCR appears to be a useful method for detecting *Plasmodium* parasites in active malaria surveillance (6), even though DNA may persist for up to 8 days posttreatment (12). However, it is impossible to use it in rural areas of malaria endemicity due to its cost, sophistication, and labor intensiveness.

The PM has diagnostic abilities comparable to those of the GS but has various advantages. (i) Only a small amount of blood is needed, making it ideal for child patients. (ii) It is less labor intensive and faster to use and therefore has a better turnaround time than the GS and requires very little training and expertise. Furthermore, there is no need for reagent preparation, as reagents are already dried on the slides, which may also prolong the shelf life of the test reagents. (iii) The PM test is battery operated, making it ideal for field work and areas where there is no electricity. (iv) The PM is cheaper to use (Table 3). However, the test is not suited for species differentiation. In the study area, about 99% of malaria infections are due to *P. falciparum* (unpublished data of the Biostatistics Dept, Agogo Presbyterian Hospital). *P. falciparum* also predominates in most areas of West Africa where malaria is endemic (8); thus, distinction between species is of less concern in the study area. *P. malariae* and *P. ovale* were each detected in only one sample by GS and RT PCR. These two samples

were also positive by the PM, which shows that the PM is able to detect about 1% of non-*P. falciparum* malaria in the study area and areas where other species of malaria coexist at high frequencies. It may not be possible to store blood slides for an expanded period. However, sealing the ends of the coverslip with wax can enable the slides to be stored for a considerable period of time.

Conclusion. The PM represents an alternative method for GS in less-developed areas where malaria is endemic. More-sensitive tests such as RT PCR should be used at reference laboratories and institutions to further enhance diagnosis and reduce inappropriate treatment and eventual drug resistance.

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The findings and conclusions in this paper are those of the authors and do not represent the views of any of their affiliated research institutions.

Bernard Nkrumah designed the study protocol, analyzed the data, and headed the writing of the protocol. Frank Huenger, Jürgen May, Egbert Tannich and Yaw Adu-Sarkodie planned and initiated the study and contributed to the writing of the manuscript. Samuel E. K. Acquah and Alex Agyekum carried out the tests and also contributed to the writing of the manuscript. Samuel Blay Nguah contributed to the analysis of the results and the writing of the manuscript. Norbert Brattig organized the use of the Partec CyScope for the study and contributed to writing the manuscript. Heidrun von Thien contributed to the running of the PCR. All authors have read and approved the final version of the manuscript.

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