ORIGINAL ARTICLE

Diagnosis of malaria parasitemia in children using a rapid diagnostic test

TA Adesanmi, HU Okafor[1], AB Okoro[1], AG Mafe[2]

Emel Hospital Festac, Lagos. [1]Department of paediatrics University of Nigeria Teaching hospital, Enugu. [2]Departments of Parasitology and Histopathalogy, Nigerian Institute of Medical Research, Lagos, Nigeria

Abstract

Aim: This cross-sectional study was conducted to evaluate the diagnostic performance of a new rapid immunochromatographic test named "Paracheck *pf*" in the diagnosis of malaria in Nigerian children.

Materials and Methods: A total of 380 Nigerian children aged between 6 and 59 months who presented at the University of Nigeria Teaching Hospital (UNTH) Enugu with fever and no obvious focus of any other infection were consecutively recruited. Malaria parasitemia was determined using simple microscopy and "Paracheck *pf*".

Results: "Paracheck *pf*" has the following diagnostic performance characteristics: Sensitivity of 82%, specificity of 91.5%, negative predictive value of 91.5%, positive predictive value of 82%, a strong positive correlation between parasite density and test sensitivity, and a detection limit of 397 parasites/µl.

Conclusions: The test is, therefore, recommended for the use in Nigerian children aged between 6 and 59 months in Enugu for the diagnosis of malaria, but negative results should be cautiously interpreted in infants because symptomatic malaria may occur in these children at parasite densities as low as 100/µl.

Key words: Malaria, Diagnosis, Children, Rapid Test

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Introduction

About 300 million cases of malaria occur yearly, 90% of which occur in Africa.[1] Children suffer mostly from malaria and in absolute terms malaria kills 3000 children below 5 years old daily.[1] Malaria constitutes 25% of child mortality in Africa,[1] and 25-30% of below 5 mortality in Nigeria,[2-4] which means that at least 250,000 Nigerian children below 5 years die yearly from malaria.[3,4] In Nigeria, the prevalence of severe malaria in children varies between 25% and 34%.[5,6] The associated mortality varies between 11% and 30%.[5,7] Children aged 6 months to 5 years in areas holoendemic for malaria (for example, Nigeria) are most vulnerable to severe malaria because of their semi-immune status.[4,5-9] In the Children's Emergency Room at the University of Nigeria Teaching Hospital (UNTH) Enugu, Enugu State, South East Nigeria, severe malaria with severe anemia is the second commonest ailment seen, constitutes 18.4% of admissions and is the leading cause of death beyond the neonatal period, constituting 30% of mortality in this age group.[7] Severe malaria is also the commonest cause of severe anemia requiring blood transfusion in children aged less than 5 years at UNTH and other parts of Nigeria.[10-12]

Early diagnosis and prompt effective treatment of malaria will help to greatly reduce this malaria-related morbidity and mortality.[2,3,13,14] This is one of the technical strategies of the on-going Roll Back Malaria Programme.[13] *Simple microscopy* is the oldest laboratory method for the diagnosis of malaria. It has been in use for at least the past 120 years. In this method, thin and thick blood films are made and air-dried. The thin film is fixed in methanol, then both films are stained with either Field's stain, Leishman's

Address for correspondence:
Dr. TA Adesanmi,
Principal Investigator,FMCPAED, MWACP, Nigeria.
E-mail: Titiade26@yahoo.com

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stain, Giemsa stain or Wright's stain.[15,16] The Giemsa stain is preferred because it is suitable for both thick and thin films; therefore, both films made on one slide can be simultaneously stained. Field's stain is suitable only for thick films, while Leishman's is suitable only for thin films.[15] Wright's stain can be used for both films, but is less stable under storage conditions than Giemsa stain, especially in the tropics. The blood-stained films are then viewed under the microscope using oil immersion. When viewed by an expert using malaria microscopy, the following information can be obtained: Infecting species of *Plasmodium*, parasite stages present in the films and parasite density. Simple microscopy is therefore useful in making a diagnosis of malaria infection, assessing the degree of parasitemia and monitoring response to treatment. It is also useful in epidemiological surveys to assess parasite rates within a given population as well as the degree of transmission of malaria. It is the ability of this single test to provide so much information that gives it the pride of place in malaria diagnosis and control programmes. No other single test provides all these information. It is not surprising then that it has survived the test of time, and after 120 years it is still the "Gold Standard" in the diagnosis of malaria and validation of other diagnostic methods.[3,15,16]

However, simple microscopy has its limitations. These include the need for technical expertise, elaborate equipment (e.g., microscope, staining troughs/racks, drying rack, various reagents, and buffered water), and electricity. It is also time consuming, requiring on the average 60 min to perform. Of particular interest is the need for technical expertise because this depends on the quality of slides prepared; the quality of staining, the ability to distinguish artifacts from malaria parasite, species identification, and parasite detection/estimation at very low parasite density.[15-21] Kilian *et al*.[18] demonstrated that 74.6% of discordant slides occurred at parasite densities <100/μl, but interrater agreement at parasite densities >100/μl was excellent (κ coefficient = 0.94). McKenzie *et al*.[19] showed a slide discordance rate of 37.5% between routine microscopists and research microscopists; and among slides concordant for the presence of malaria parasitemia, species identification differed in 13.7%. The degree of slide discordance varied with parasite density. Barat *et al*.[20] evaluated the diagnostic performance of laboratory technicians in detecting malaria parasitemia, using expert microscopists as the reference standard. They found 88% sensitivity, 91% specificity, 76% positive predictive value, and 96% negative predictive value. In the same study, despite high specificity and negative predictive value, 35% of persons with negative slides still had antimalarials prescribed. This was because their clinical features as assessed by attending physicians strongly suggested a diagnosis of malaria. It seems therefore that there are cases of clinical malaria with undetectable malaria parasitemia on microscopy. For example, Gilles[15] reported that almost 50% of African children with clinical malaria did not have microscopically detectable parasitemia, even in expert hands. Two main mechanisms have been suggested for this, namely very low parasite density usually <100/μl; and the sequestration of parasitised red blood cells in deep vascular beds of vital organs such as the brain, placenta, spleen, and liver.[15,16,18,21]

The polymerase chain reaction (PCR) would help to identify parasites and infecting species in such cases of very low parasite density.[3,15,19] However, it does not distinguish between trophozoites and gametocytes; between living or dead parasites; nor can it detect sequestered parasites mentioned above[.3,15,16] It also requires high technology equipment and skills. Given the grave potential consequences of sequestered malaria parasites such as cerebral malaria, congenital malaria, early parasite detection is imperative. The ability of antigen detection methods upon which rapid immunochromatographic tests (RICTs) are based to detect such sequestered parasites; the simplicity and rapidity of RICTs, as well as their need for limited instrumentation poses the question: Can RICTs replace simple microscopy in the diagnosis of malaria in routine clinical practice?

Furthermore, given the low specificity of clinical case definitions of malaria[22,23-38] especially in an endemic area, and the contribution of presumptive malaria treatment to the constant evolution of antimalarial drug resistance,[39,40] there is a compelling need to make parasite detection more widely available.[41] RICTs were therefore developed to facilitate this.[13,42,43] "Paracheck *pf*" (Orchid Biomedical Systems, Verna, Goa, India) is an example of a malaria Histidine rich protein II (HRP2) detecting RICT.

The diagnostic performance of RICTs has been shown to vary with a location of study to a statistically significant degree.[44,45] This provides the impetus for local comparative studies of RICTs and microscopy. There is a heavy malaria burden in Enugu, and it is necessary to document the diagnostic performance of malaria RICTs in Enugu if their use is to be advocated. Furthermore, in Nigeria as a whole most studies evaluating HRP2-based RICT evaluated the diagnostic performance of the Parasight *f* test and ICT malaria *pf* but not Paracheck *pf*, which is reported remarkably cheaper than all the other RICTs.[46,48]

This study therefore sets out to determine the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of Paracheck *pf* in the diagnosis of malaria parasitemia as well as the relationship between parasite density and test positivity.

Materials and Methods

A total of 380 children who presented at the outpatient pediatric clinic at UNTH and met the inclusion criteria (presence or history of fever, age between 6 and 59 months, and absence of obvious focus of infection) were consecutively recruited. Blood samples were collected as soon as patients were recruited and the rapid diagnostic test (RDT) was performed immediately.

The RDT used in this study is Paracheck *pf* It was obtained directly from the manufacturer's representative in Enugu. The test was performed by the investigator according to the manufacturer's instructions and was demonstrated to the parents/guardians. The Paracheck *pf* test procedure is briefly as follows: Four drops of clearing buffer was put into a test tube. A drop of blood obtained by the pinprick method was allowed to drop directly into the sampled area of the test strip. The test strip was then immediately put into the clearing buffer up to the arrow on the dipstick. The color change on the strip was read after 15 min. If only one pink band appears on the test strip the test is negative, but if two pink bands appear, the test is positive for *Plasmodium falciparum*. If no band appears, the test is invalid. Quality assurance was done by keeping all test kits at room temperature (26-28° C) throughout the study. This falls within the temperature range of 4-30°C specified by the manufacturer. In addition each sachet containing a test strip was immediately used when opened. This was to prevent excessive strip exposure to moisture which could affect the quality of the strip.

Thick and thin blood films were also prepared, stained, and examined according to World Health Organization (WHO) standards.[49] Diluted Giemsa stain (3% stock solution of pH 7.2) was used. Total parasite counts were obtained by counting the number of malaria parasites (trophozoite stage) seen after counting 200 white blood cells (WBC) in the thick film and applying the formula below using the total WBC previously estimated for the patient.

$$\frac{\text{Number of parasites}}{200} \times \frac{\text{Patient's total WBC}}{1}$$

This result is expressed per microliter (µl) of blood. This is parasite density. Data were analysed using SPSS computer software (version 11.5). Diagnostic performance was calculated using the standard WHO format[44,50] as follows:

1. Sensitivity (%)
$$= \frac{\text{Number of true positive (TP)}}{\text{Number of TP + false negatives (FN)}} \times 100$$
2. Specificity (%)
$$= \frac{\text{Number of true negatives (TN)} \times 100}{\text{TN + false positives (FP)}}$$
3. Positive predictive value (PPV) $= \frac{TP}{TP + FP} \times 100$
4. Negative predictive value (NPV)

$$\frac{\text{Number of parasites}}{200} \times \frac{\text{Patient's total WBC}}{1}$$

5. Relationship between parasite density and true positivity of the Paracheck strip (this is the diagnostic power of Paracheck *pf*).

Key:

(a) TP = sample is positive by both microscopy and Paracheck *pf*.
(b) TN = sample is negative by both microscopy and Paracheck *pf*.
(c) FP = sample in positive by Paracheck *pf* but negative by microscopy.
(d) FN = sample is negative by Paracheck *pf* but positive by microscopy.

Results

This RICT detected 100 true positives (TP), 22 false positives (FP), 236 true negatives (TN), and 22 false negatives (FN) [Table 1]. The ten cases of *P. malariae* only were all negative when tested with Paracheck *pf*. They were regarded as true negatives. Paracheck was positive in the two cases of mixed infection, did not detect any parasites below 397/µl [Table 2] and was negative in all slides containing only *P. falciparum* gametocytes [Table 1]. The diagnostic performance of Paracheck *pf* strips was as follows:

Sensitivity = 82%
Specificity = 91.5%
Positive predictive value (PPV) = 82%
Negative predictive value (NPV) = 91.5%

When stratified according to parasite density [Tables 3 and 4], there was a remarkable reduction in both test sensitivity and specificity from 100% and 100% at PD ≥ 500/µl to 12% and 91.2% at PD < 500/µl [Figures 1 and 2]. This difference is statistically significant ($P = 0.001$). The positive predictive value also dropped to 12% at PD < 500/µl, but the negative predictive value remained high (91%). Using Pearson's correlation test, there was a positive correlation between parasite density and true positivity rate of Paracheck *pf* when data were stratified according to PD, $R = 0.676$ [Figure 1]. True positivity rate also means test sensitivity.

Discussion

This study established a high degree of concordance between Paracheck *pf* and microscopy in the diagnosis of malaria parasitemia. The overall sensitivity was reasonably

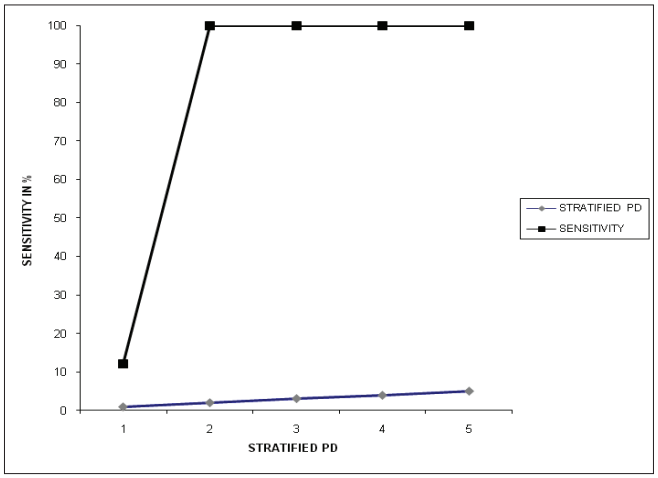

Figure 1: : Positive Correlation between Parasite Density and Sensitivity of Paracheck pf 1.= Parasite Density < 500/ul; 2. = Parasite Density 500 - < 1,000/ul; 3. = Parasite Density I,000 - < 5,000/ul; 4. = Parasite Density 5,000 - < 10,000/ul; 5. = Parasite Density > 10,000/ul

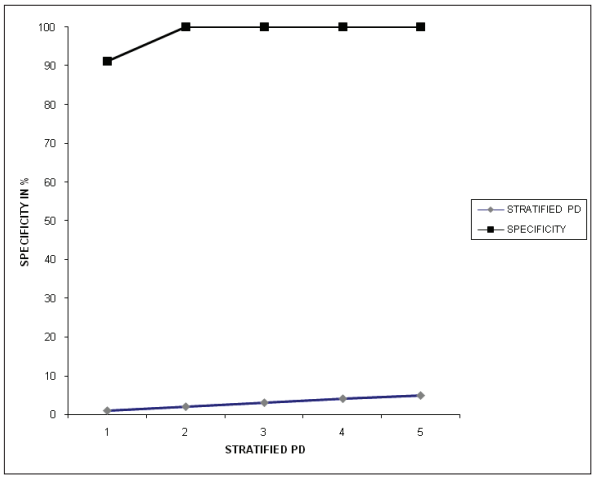

Figure 2: : Positive Correlation between Parasite Density and Specificity of Paracheck pf 1. = Parasite Density < 500/ul; 2. = Parasite Density 500 - < 1,000/ul; 3. = Parasite Density I,000 - < 5,000/ul; 4. = Parasite Density 5,000 - < 10,000/ul; 5. = Parasite Density > 10,000/ul

Table 1: Cross tabulation of results of microscopy and paracheck *pf*

	Microscopy	Paracheck *pf*
P. falciparum trophozoites	120	98
P. malariae trophozoites	10	0
Mixed parasitemia	2	2
P. falciparum gametocytes only	8	0
No malaria parasitemia	240	258
Total	380	356

Table 2: Stratified parasite density vs. Paracheck *pf* results

Microscopic parasite density	Performance of paracheck *pf*			
	TN	FP	FN	TP
<500	228	22	22	3
500-<1000	1	0	0	8
1000-<5000	4	0	0	50
5000-<10,000	1	0	0	21
>10,000	2	0	0	18
All	236	22	22	100

TN = True negative; FP = False positive; FN = False negative; and TP = True positive

Table 3: Stratified parasite density vs. true positivity rate of paracheck *pf*

Microscopic parasite density	Performance of paracheck *pf*		
	FN	TP	TPR (%)
<500	22	3	12
500-<1000	0	8	100
1000-<5000	0	50	100
5000-<10,000	0	21	100
>10,000	0	18	100
All	22	100	82

FN = False negative; TP = True positive; TPR = True positivity rate = Test sensitivity

Table 4: Stratified parasite density versus true negativity of paracheck *pf*

Microscopic parasite density	Performance of paracheck *pf*		
	FN	TP	TPR (%)
<500	228	22	91
500-<1000	1	8	100
1000-<5000	4	0	100
5000-<10,000	1	0	100
>10,000	2	0	100
All	236	22	91.5

TN = True negative; FP = False positive; TNR = True negativity rate = Test specificity

high at 82%. This Figure is comparable to that obtained in India[51] but less than results obtained elsewhere.[46-48,52] The direct implication is that the use of Paracheck *pf* in our study population missed 18% of patients with malaria parasitemia as against 3-8% in some other studies.[46-48,52] This difference may have been due to the predominance of adults in each of those studies. In holoendemic areas, adults are likely to have symptomatic malaria at much higher parasite densities than children, who are less immune to malaria. This possibility is strengthened by the fact that in this study, the sensitivity of Paracheck *pf* at parasite density ≥ 500/µl was 100% and in another study in Thailand[47] where a sensitivity of 92% was obtained, the range of parasite density was 1000-40,000/µl.

With respect to specificity, the high Figure of 91.5% obtained in this study falls within the range of 78.8% and 100% found in earlier studies.[46-48,51] Specificity essentially is the ability of a test to avoid false positives. Thus the current findings provide confidence that the risk of making judgmental errors arising from false positivity is less than 10%. However, there is another theoretically possible explanation. The false positives in this study were defined relative to microscopy. In other words, these were cases identified by Paracheck *pf*

but not by microscopy. The theoretical possibility is that the affected patients may have truly had malaria, but for some reason were missed by microscopy. The reasons include recent exposure to antimalarial drugs or technical problems with microscopic identification of parasites.

Closely related to specificity was the predictive value of a negative test. Indeed, the two results in this study were identical. The result assures the investigator that a negative test excluded the diagnosis of malaria in more than 90% of cases. Thus for the busy clinician, the implication is that an alternative explanation of the patient's symptoms must be sought.

On the other hand, the predictive value of a positive test of 82% obtained in this study assures the clinician that 82% of Paracheck *pf* positive results truly have malaria parasitemia. Thus, the clinician may be prepared to allow a reasonable time interval between commencing antimalarial treatment and deciding whether or not to investigate for an alternative cause of the illness.

FN obtained in this study all corresponded to parasite densities <397/µl thereby giving this as the detection limit of Paracheck *pf* in this study. The detection limit obtained in other studies on Paracheck *pf* was not clearly stated. The implication of a detection limit of 397 parasites per microliter obtained in this study needs to be evaluated against the threshold parasite density for symptomatic malaria. In areas holoendemic for malaria (e. g. West Africa), asymptomatic parasitemia is common and may exist concurrently with fever due to other illnesses. Therefore, it is necessary to define malaria as a febrile illness accompanied by malaria parasitemia above a certain threshold.[53] The value of this threshold varies with the level of acquired immunity. A study done in a neighbouring West African country, Ghana, obtained a threshold of ≥100/µl for infants and ≥3500/µl for children aged between >1 year and <5 years. Applying this threshold to this study group, failure of Paracheck *pf* to detect PD ≥ 100/µl < 397/µl would mean missing cases of symptomatic malaria in children <1 year old, which may be ultimately fatal.

Paracheck *pf* did not detect any none *falciparum* malaria, a finding also previously observed in another study.[54] This suggests that cross reactivity between *Plasmodium falciparum* HRP2 antigen and other species of *Plasmodium* is unlikely; however, further studies are required to verify this.

As at the time of this study, in monetary terms, Paracheck pf costs US$ 1.00 (1 US dollar), i. e, N135.00 (135 naira) per test. The market survey done at that time showed that this was cheaper than simple microscopy which costs between N150.00 and N250.00 per test. The cost of other commercially available RICTs varied between N380.00 and N660.00 (380 naira to 660 naira). Paracheck pf, like other RICTs, also requires less time to perform than simple microscopy. Test results are available by the end of 15 min unlike simple microscopy which requires at least 60 min for the test result to be ready.[42]

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