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Field Evaluation of SD Bioline Rapid Malaria Diagnostic Test among Asymptomatic Malaria Infected Children in Port Harcourt, Nigeria*

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Abstract: A rapid immunochromatographic test (SD Bioline malaria P.f/P.v) for the diagnosis of malaria was evaluated against thick blood smears in Port Harcourt, Nigeria, for the purpose of evaluating its efficacy for field work. Two hundred and forty children aged 1-8 years were surveyed in a cross sectional prospective study. For *P. falciparum*, the SD Bioline was 47% sensitive and 100% specific, with a positive predictive value (PPV) of 100% and a negative predictive value (NPV) of 83.2%. Efficiency of the test was 85.4%. The overall malaria prevalence in this study was 27.5%. *P. falciparum* accounted for 25.0%, while other species accounted for 2.75%. The cost of the SD Bioline test cassette (US \$ 1.98 (230 Naira) per test at the time of study) was quite affordable. We concluded that the sensitivity of SD Bioline is low but with a threshold of 1000 parasites/microlitre among asymptomatic malaria infected children and affordable cost, this RDT (SD Bioline) could serve as a useful epidemiological tool in *P. falciparum* malaria endemic areas in the developing countries.

Key words: *Plasmodium falciparum*, SD Bioline malaria P.f/P.v test, Niger Delta, Nigeria

INTRODUCTION

Malaria is the most important parasitic disease in the world and one of the most important causes of child mortality worldwide. *Plasmodium falciparum* kills an average of 1 million children in Africa annually. It is also estimated to cause about half a billion episodes of disease each year (Snow *et al.* 2005; Kwiatkowski, 2005).

Of an estimated 300,000-350,000 cases of *Plasmodium falciparum* in 2004, 70% occurred in Africa and 18% in the South East Asia region (Global, 2006). In Nigeria alone 60 million experience malaria attack at least twice a year with no less than 80% of the population exposed to the disease. Two hundred and twenty five children in Africa die every 2 and half hours while about 2173 children under the age of five die daily in the continent from malaria (Odotola, 2005).

One of the most pronounced problems in controlling the morbidity and mortality caused by malaria is the limited access to effective diagnosis and treatment in areas where malaria is endemic. The most widely used routine method of microscopy (the gold standard) needs laboratory infrastructure and expertise and is labor intensive. The urgent need for a simple and cost effective test to overcome the deficiencies of light microscopy has been recognized for a longtime (WHO, 1996). Rapid and specific diagnostic tests to identify individuals infected with malaria are very important for control of

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the disease. Also, in rural areas of our country with poor accessibility, which do not have these resources, many patients delay their treatment or receive their therapy based only on clinical criteria: this situation is not adequate and this increases costs, expose the patients to adverse reactions and contributes to the development and dissemination of drug resistance (Navitsky, 1997).

The commercially available methods using HRP- 2-antigen detection (Parasight TMF ICT, Opti MAL) have severally been evaluated to have a level of sensitivity comparable to or better than conventional microscopy (Singh *et al.*, 2000). However, a major limitation of antigen detection tests has been their inability to detect malaria caused by *Plasmodium vivax* with the exception of Optimal (Palmer *et al.*, 1998).

Recently, another rapid immunochromatographic test, the SD Bioline Malaria Pf/Pv was introduced into the Nigerian market for the rapid diagnosis of *P. falciparum* and *P. vivax*. To assess the usefulness of this test in the field, this kit was used in a cross-sectional survey of children aged 1-8 years in Port Harcourt, Nigeria.

MATERIALS AND METHODS

Study Area and Population

This prospective cross sectional study was conducted in the Rumueme Community in Port Harcourt, the capital city of Rivers State, Nigeria. Bayelsa, Imo, Abia and Akwa Ibom States bound Rivers State. The geographical location is latitude 4° 30'-5° 31' and Longitude 6° 30'- 7° 21'. Samples were collected randomly after obtaining a written or oral informed consent from the parents. Children residing in randomly selected households were recruited and the parents brought them to the research base in the Federal Housing Estate, Rumueme where demographic data and blood samples were collected from them. Selection of households for inclusion in this study was based on a random- cluster sampling of the entire household identified within the prescribed study area. Two millilitre of venous blood sample was collected into ethylenediaminetetraacetic acid (EDTA) bottle. Two hundred and forty children of both sexes aged 1-8 years participated in the study.

Laboratory Procedures

Staining of Thick and Thin Smears

Fresh working Giemsa stain was prepared by adding 1 mL of Giemsa stain stock into 39 mL of working Giemsa buffer and 2 drops of 5% Triton X-100 later added to the mixture. This mixture was poured into a standing 40 mL copling jar to fill. Well-made thick smears were placed in the working Giemsa stain (2.5%) for 45-60 min. Slides were removed at the end of the staining period, rinsed by dipping 3-4 times in the Giemsa buffer. The slides were further left in the buffer for 5 min after which they were dried upright in a rack. A positive smear was included with each new batch of working Giemsa stain for quality control. Thin smears were made, fixed in methanol before staining in the same concentration of Giemsa.

Examination of Thick/thin Smears

The entire smear was first screened at a low magnification (10 or 40 x objective lens) to detect large parasites such as microfilaria. The smears were then examined using 100x oil immersion. A well-stained area, free of precipitate and well populated with white blood cells (10-20 WBCs/ field) was selected. No Parasite Found (NPF) was reported after 100 fields, each containing approximately 20 WBCs. These smears were examined in this study mainly for specie identification of parasites and not for the purpose of comparison. Examination was done using 100 x oil immersion objective.

Estimation of Parasite Density

Parasite densities were recorded as a ratio of parasites to white blood cells (WBCs) from thick smears. To quantify malaria parasites against WBCs on the thick smear, the parasites were tallied against WBCs until 500 WBCs were counted. Densities (parasite per microlitre of whole blood) then were calculated as follows:

Parasites/microlitre blood = parasites/WBC×WBC count per microlitre.

For the subjects in this study, parasite densities were calculated as follows:

= Parasites/ 500×WBC count of individual children.

Immunochromatographic Method

A rapid one step malaria anti-*Plasmodium falciparum* and *Plasmodium vivax* test (SD Bioline, Korea (LOT: 18032) was used alongside the gold standard (Gemsa stained film). The SD Bioline malaria Pf/Pv test is an immunochromatographic (rapid) test for the qualitative detection of the antibodies of all isotypes (IgG, IgM and IgA) specific to *P. falciparum* and *P. vivax* simultaneously in human serum, plasma or whole blood. The test cassette contains a membrane strip, which is precoated with recombinant malaria Pf capture antigen on test b and 1 region and with recombinant *P. v* capture antigen on test b and 2 region. Procedures were followed strictly as contained in the manufacturer's standard operating manual inserted in the kit.

Data Analysis

Sample sizes were not calculated as this study provided a preliminary estimate of the SD Bioline test sensitivity and specificity for *P. vivax* and *P. falciparum* measured against the standard of Giemsa thick blood films. To calculate sensitivity and specificity, the SD Bioline interpretation was compared with field microscopy. The figures of specificity, sensitivity, predictive values and efficiency were calculated as suggested by Tjitra *et al.* (1999). Performance indices were calculated for malaria as a whole (diagnosis of either species), *P. falciparum* malaria (including mixed infection and *P. vivax* malaria). The variables measured were number of true positives (TP), number of true negatives (TN), number of false positives (FP) and number of false negatives (FN). Sensitivity was calculated as $TP/(TP+FN) \times 100$ while specificity was calculated as $TN/(TN+FP) \times 100$. The positive predictive value (PPV) was calculated as $TP/(TP+FP) \times 100$ and negative predictive value (NPV) was calculated as $TN/(FN+TN) \times 100$. Test efficiency, the proportion of all tests that gave a correct result, was defined as $TP+TN/TP+FP+FN+TN \times 100$. Results were considered false positive if *P. falciparum* or *P. vivax* were detected in SD BIOLINE Pf/Pv and could not be detected on thick smear and vice versa.

RESULTS

Two hundred and forty children aged 1-8 years of both sexes were studied. 66 (25.7%) were found to be infected. 60 (25.0%) with *P. falciparum*, 6 (2.75%) with other species. Table 1 shows the breakdown of malaria cases in age groups.

Table 1: Malaria prevalence among 240 children in Port Harcourt

Children age groups (years)	Blood film examined	Malaria positive	Pf	Other species rate (%)	Slide positivity rate (%)	Slide falciparum rate (%)	<i>P. falciparum</i> (%)
1-4	99	36	36	0	36.36	36.36	100
5-8	141	30	24	6	21.27	17.0	80.0
Total	240	66	60	6	37.5	27.5	90.9

Pf- *Plasmodium falciparum*

Table 2: Results of SD Bioline and thick blood films of the 240 participants

Data	Positive thick blood film	Negative thick blood film	Total
Positive SD Bioline	31	0	31
Negative SD Bioline	35	174	209
Total	66	174	240

Sensitivity = $31/66 \times 100 = 47\%$, Specificity = $174/174 \times 100 = 100\%$, PPV = $31/31 \times 100 = 100\%$, NPV = $174/209 \times 100 = 83.2\%$, Efficiency = $31+174/240 \times 100 = 85.4\%$

Table 3: Sensitivity of SD Bioline related to parasitaemia levels

Parasitaemia (Parasites μL^{-1})	No. of subjects	Sensitivity (%)
≤ 500	18	55
501-1000	21	86
1,001-5,000	36	100
5,001-10,000	0	0
$\geq 10,000$	0	0

($p < 0.047$, Fisher's Exact test)

The test was sensitive (47%) and specific (100%) for the diagnosis of falciparum malaria with a PPV and an NPV of 100 and 83.2%, respectively. Efficiency was 85.4%. The corresponding values for *P. vivax* were not calculated because they were not encountered (Table 2).

Table 3 shows the sensitivity of SD Bioline in relation to parasitaemia levels. Sensitivity was lower when subjects had parasitaemia levels of less than 1000 parasites, μL ($p = 0.047$, Fisher's exact tests) > 0.05).

DISCUSSION

One of the top priorities in WHO's action plan for malaria control (1995-2000) is strengthening national capabilities to provide early diagnosis and treatment both within and outside the health services (WHO, 1996). In view of this and in keeping with the objective of the millennium development goals (MDG), we tested one of the Rapid Diagnostic Test (RDT) kit the SD Bioline P.f/P.v that came into the Nigerian market on a population survey of children aged 1-8 years in the Niger Delta, Nigeria and compared the results with the traditional blood film (the gold standard).

The prevalence rate of *P. falciparum*, in this study was found to be 25.0%, While the overall malaria prevalence was 27.5%. This prevalence rate was found to be lower than an earlier report by Jeremiah and Nsa (2005) in a study conducted in Calabar where a prevalence rate of 40.98% was observed. Since the subjects were selected randomly from a survey and not from symptomatic children alone, it is possible that this could have influenced the prevalence rate of malaria infection in this study. This study observed that the under fives carried a higher Pf rate (36.4%) than the above fives (21.3%). The malaria prevalence rate in this study is however in consonance with previous studies conducted in Nigeria (Ejezie and Ezedinachi, 1992; Ekanem *et al.*, 1994).

Several studies have been done on some of the rapid strips like optimal (Jelinek *et al.*, 1999; Cooke *et al.*, 1999), SD Bioline (Cavanagh *et al.*, 1998), ICT (Singh *et al.*, 2000) and others. Irrespective of the type of rapid malaria screening strip used, it was found that the only measurement for evaluating the diagnostic value of the strip is to determine the sensitivity and specificity using the gold standard as a basis of comparison (Tarazon *et al.*, 2004; WHO, 1995). The sensitivity of most of the reagent strips were found to reduce with decrease in parasite density, thus suggesting that they are most useful in areas endemic for the disease (Tarazon *et al.*, 2004).

In this study, the SD Bioline P.f/P.v RDT sensitivity was found to be low (47%) while the specificity was high (100%) for the diagnosis of *P. falciparum*. This finding is similar to the observation of Agomo *et al.* (2003) in whose report, the SD Bioline's sensitivity was reported to be 54.84%. The PPV and NPV of 58.0 and 68.0% are however at variance with the PPV (100%), NPV (83.2%) obtained in this study. The specificity value of 42.9% in their report is also at variance with

the specificity of 100% obtained in this study. We found that the SD Bioline sensitivity was 100% when the parasitaemia was higher than 1,000 parasites μ L but decreased with lower parasitaemia levels. This phenomenon has been described by many authors, both for *P. vivax* and *P. falciparum* diagnosis with 100% sensitivity for parasitaemia higher than 1000 parasites μ L and 40% for values lower than 100 parasites (Palmer *et al.*, 1998; Tarazon, 2004). The explanation for this phenomenon could be that the quantity of P.f./P.v enzyme, the antigen detected by SD Bioline is in direct proportion to the number of parasites in the blood (Makler and Hinrichs, 1993). This problem must be considered when this test is applied for fieldwork. With the high specificity value, the SD Bioline may be most useful in areas of endemic infection with high parasitaemia levels.

The SD Bioline RDT however has an advantage that it differentiates between *P. falciparum* and *P. vivax* malaria. Despite the few limitations highlighted above, the test can be used as an epidemiological tool because in areas where *P. vivax* and *P. falciparum* are prevalent, it can be used to identify the plasmodium species infecting the patients in each village quickly and it allows public health workers to deliver the appropriate chemotherapy rather than just give blinded treatment to every one with malaria symptoms. Also, in addition to these advantages over microscopy and clinical diagnosis, the cost of the SD Bioline P.f/P.v (US \$ 1.98 (230 Naira) per test at the time of study was quite affordable and would favor its widespread use in malaria endemic areas of developing countries where many patients need a fever screen.

We concluded that, with a threshold of 1000 parasites/microlitre among asymptomatic malaria infection, this RDT (SD Bioline) could be a useful epidemiological tool in the diagnosis of Pf malaria diagnosis in highly endemic areas in the tropics.

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