AFRICAN JOURNAL OF CLINICAL & EXPERIMENTAL MICROBIOLOGY JAN. 2003 ISSN1595-689 Vol 4 No 1
AJCEM/200251/2302

INVESTIGATION OF THE EFFICACY OF TWO RAPID ASSESSMENT TECHNIQUES (OPTIMAL 1 AND SD-BIOLINE) FOR THE DIAGNOSIS OF MALARIA IN RURAL AREAS OF NIGERIA

P.U. AGOMO, V.N. ASIANYA, S.K. AKINDELE, C.O. AGOMO, M.O. AKINYELE, T.A. ADEWOLE, U.T. IGBASI, RC ANYANWU and K.N. EGBUNA. Division of Biochemistry and Nutrition, Nigerian Institute of Medical research, P.M.B. 2013, Yaba, Lagos.
Correspondence: Dr. P.U. AGOMO, Biochemistry Division, NIMR, PMB 2013, Yaba, Lagos

We had previously studied the efficacy of three new techniques-Para Sight ®F, (PSF), Immunochromatographic Test (ICT) and Quantitative Buffy Coat (QBC) – as possible replacements for the time-consuming microscopy in the diagnosis of malaria. Two more rapid assessment techniques (the Optimal 1 and SD-BIOLINE) were recently introduced into Nigeria and claimed to exhibit high sensitivity and specificity. Optimal 1 was particularly claimed to distinguish between *P falciparum, P. malariae, P ovale and P. vivax.* We have in this work evaluated the efficacy of both the Optimal 1 and SD-Bioline in 240 patients from Ibafo and Magboro Communities in Obafemi-Owode LGA of Ogun State, Nigeria. Results showed that with regard to the detection of *P. falciparum,* Optimal 1 gave a sensitivity, specificity, positive and negative predictive values of 63.95%, 92.20%, 82.1% and 82.1% respectively, while the SD-Bioline gave 54.84%, 42.9%, 68.0% and 68.0% respectively. In retrospect, the sensitivities shown by 3 other techniques (ICT, PSF and QBC) investigated by us were 88.63, 89.95 and 87.6% respectively. Their specificities on the other hand were 94.60, 91.17, 94.70% respectively. The main advantage of the rapid Optimal 1 technique is that it was able to detect *P. malariae* which microscopy also detected in three patients. The SD-BIOLINE gave the worst comparative result and could not be recommended for use in Nigeria. This work in conclusion has shown that Optimal 1 could be useful in the rapid diagnosis of the various species of *Plasmodium* in Nigeria provided the patients could afford the test.
Key Words: Malaria, Optimal 1, SD-Bioline, *P. falciparum.*

INTRODUCTION:

Since its introduction by Ross in 1903, the thick blood film microscopy (TFM) has been the primary method of malaria diagnosis throughout the world (1). Despite the simple technology, this technique is still expensive and requires adequate infrastructure to maintain, train health workers and ensure quality assurance of the service. Microscopy also requires electricity, which is not available in most Health centres in rural areas. As a result of these limitations, laboratory support which is needed due to problems of over-prescription of antimalarial drugs and drug-resistance is not available in Primary Health care centres where clinical diagnosis and presumptive treatment are the only method adopted. The urgent need for a rapid, simple, reliable and cost-effective diagnostic technique to overcome the deficiencies of light microscopy has been recognised for a long time. In 1993, rapid diagnosis of malaria was made a key feature of the world Health Organisation Global Malaria Control Strategy so that effective treatment could be administered quickly to reduce morbidity and mortality especially in children and pregnant women (2,3). Rapid diagnosis is also a key feature of one of the six elements of the Roll Back Malaria Programme (4).

Some scientists have made attempts at developing alternative techniques (5). Some of the early attempts concentrated on the improvement of the sensitivity of light microscopy by concentrating the parasites in the blood sample, improving the visualization and detection, staining with fluorescent dyes or, a combination of these approaches such as the quantitative buffy coat technique (QBCT) (6) and the kawamoto fluorescent technique (7). Newer methods which have been claimed to be better than the TFM were based on the identification of parasite-specific antigens and molecules such as nucleic acids, the sensitivity in some cases being improved by the use of the polymerase chain reaction (PCR) technique (5). All these techniques utilize sophisticated equipment and electricity, and thereby suffer from similar logistic problems as the microscopic technique. A new direction was introduced when Howard et al described the secretion of a malarial histidine-rich protein-2 from

Plasmodium falciparum infected erythrocytes(8). A rapid technique, ParaSight®F was then developed by Becton Dickinson Tropical Diseases Diagnostics, Sparks, MD. This was based on the detection of *P. falciparum* histidine-rich protein-2 in human blood within 10 minutes and without the use of electricity. The usefulness of ParaSight®F was reported in Thailand(9) Tanzania(10) Brazil(11) Zimbabwe(12) and Kenya(13). In our laboratories, preliminary evaluation of ParaSight®F in 235 patients in Nigeria expressed some reservation considering the cost of the strips and the spill-over effect of antigenaemia long after parasitaemia had cleared(14). However, further work with a larger number of patients (501) presented the benefits of ParaSight®F in better light especially when the results were stratified according to the various health facilities and Local Governments (15).

A new technique, the Immunochromatographic Test (ICT) was introduced not long after the introduction of ParaSight®F. Although it is based on the same principle of detection of the histidine-rich protein-2 in *P. falciparum* infection, the manufacturers (ICT Diagnostics, Sydney Australia) and some investigators outside Nigeria claimed that it is an improvement on all other rapid assessment techniques (16,17,18,19). The ICT has been evaluated in our Laboratory (20) and its comparative assessment with ParaSight®F in two Local Government Areas showed the former to have an edge over the latter, although no statistical conclusions could be drawn(21).

Another addition to rapid antigen detection tests is the optimal 1 assay (Flow Inc) which has been claimed to detect all four species of malaria parasites, namely, *P. vivax, P. falciparum, P. malariae* and *P. ovale*(22). The optimal 1 technique is said to detect the metabolic enzyme, parasite lactate dehydrogenase (pLDH) produced by viable malaria parasites and also released from parasite-infected erythrocytes. Differentiation of the malaria species is said to be based on the antigenic differences between the pLDH isoforms. The presence of pLDH in the blood is also claimed to reflect the presence of viable malaria parasites. Optimal 1 is new in Nigeria and we are not aware of anyone who has undertaken the evaluation of the efficacy of this technique in Nigeria. There is however evidence of its review by Bailey(11) and its evaluation in Honduras(22). We therefore deemed it useful to investigate the performance of Optimal 1 inorder to compare its efficacy with those of the microscopic technique and other rapid assessment methods for possible utilization in rural areas.

Also new in Nigeria is another rapid assessment device, SD-BIOLINE, for the diagnosis of Plasmodium falciparum. The SD BIOLINE Plasmodium falciparum test is claimed to be rapid test for the qualitative detection of antibodies of all isotypes (IgG, IgM, IgA) specific for *Plasmodium falciparum* in human serum, plasma or whole blood. The SD BIOLINE *Plasmodium falciparum* test is said to contain a membrane strip which is pre-coated with recombinant *P. falciparum* capture antigens, merozoite surface protein (MSP) and Circumsporozoite protein, (CSP) on the test band region. The principle of this device has it that the recombinant malaria Plasmodum falciparum antigen (MSP & CSP) – colloid gold conjugate and blood/serum sample move along the membrane chromatographically to the test region and form a visible line as the antigen-antibody gold particle with a high degree of sensitivity and specificity. In a comparative analysis of the SD BIOLINE *P.falciparum* test versus microscopic examination of whole blood, the manufacturers recorded a sensitivity of 88.2%, a specificity of 98.5% and a total agreement of 93.8%. These claims

prompted us to investigate the performance of the two new techniques in Nigeria in comparison with other rapid assessment techniques.

MATERIALS AND METHODS

Study sites and Study subjects:
Ibafo-Magboro group of communities is the rural parts of Obafemi-Owode LGA, Ogun State lies 80 KM east of Lagos. Most of the 18 villages in the area have neither electricity nor pipe borne water. The only Police Station is in Ibafo town which is the only developed part of the group of Communities. Their LGA headquarters is in Owode near Abeokuta, about 50km away. Most of the villages are surrounded by thick forest although in recent times they have started selling the wooded lands to some organizations which are now cutting down some of the trees. A total of 240 study subjects (65 adults and 175 children, 133 females and 107 males) were randomly recruited for this work from some of these villages after obtaining consent from heads of the communities and from the individuals or their guardians. The patients recruited complained of fever, aches, pains, loss of appetite, bitter taste and other malaria-associated sympotmatology. Some patients with non-specific complaints were also part of the subjects included in the project. Following an explanation and consent by the patient, a simple questionnaire was completed as regards age, sex, current medication, any recent blood transfusion and malaria associated signs and symptoms.

Microscopic Examination
The technique used for microscopic identification was as reported in our previous work [14,15,21]. The presence of malaria parasites using this microscopic technique was considered as the "Gold Standard" reference level for positively. Parasite enumeration and identification of the species were carried out by two, and in some cases, three microscopists for every specimen collected. This was for purposes of accuracy and confirmation of results.

The Packed Cell Volume (PCV) of each patient was determined as in previous work, using capillary tubes and haematocrit centrifuge. The results were read with a haematocrit reader.
Optimal 1_was supplied as the Diamed Optimal Test Kit and used as instructed by the Manufacturers. The principle has been enunciated (see introduction).

Briefly, in the procedure, a dipstick was introduced to lysed blood and this was followed by a buffer (supplied). After 12 minutes the strip was examined. A negative result showed on a reaction line, those of *P. ovale* (supplied) and *P. malariae* showed two positive lines while the presence of *P.falciparum* indicated 3 lines. Only viable parasites were said to produce lactate dehydrogenase.

The SD BIOLINE Malaria P.falciparum test device:
The Principle of this test has been explained in the introductory part of this paper. The test device was removed from the foil and placed on a flat, dry surface. Whole blood (20ul) was slowly added to the sample well and this was followed by 3 drops of the assay diluent. As the test began to work a purple colour moved across the result window in the centre of the test device. The result was interpreted within 5-20 minutes, but not after 20 minutes. The colour band appearing in the left section of the result window was the control and it indicated that the test was working properly. The presence of only one band within the result window indicated a negative result whilst the presence of two bands

within the result window, (regardless of which band appeared first) indicated a positive result.

Studies on the Disappearance of parasitaemia, Lactate dehydrogenase (LDH) and BIOLINE antibodies.

A Cohort Study was also undertaken to examine the test performance over a period of time in 10 patients known to be parasitaemic. Following positive diagnosis, patients were treated with therapeutic doses of Fansidar® or Chloroquine. Venous blood was subsequently collected on day 4 and day 7 by finger prick in order to follow the decline in parasitaemia and antigenaemia. This test was only carried out in ten (10) positive patients since it could not be done in all the patients as a result of limitation in the number of test strips.

Analysis of Results: Data were entered in the EPI-INFO 6.04 and analysed. Values were given in the text and tables as means ± SD where applicable. Values for different groups (age, etc) were compared using students t-test or chi squared test and P. values less than 0.05 were taken as significant. analysis of variance was performed for multiple group comparisons. The key variables were as follows:

$$\text{Sensitivity (\%)} = \frac{\text{No of True Positives (TP) X 100}}{\text{No of TP + No. of false Neg(FN)}}$$

$$\text{Specificity (\%)} = \frac{\text{No of True Negatives (TN) X 100}}{\text{No of TN + No of false Positives (FP)}}$$

$$\text{Positive Predictive Value (PPV)\%} = \frac{\text{No of TP X 100}}{\text{No TP + FP}}$$

$$\text{Negative Predictive value (NPV)} = \frac{\text{FP X 100}}{\text{FN + TN}}$$

$$\text{False Negative Rates (FNR)\%} = \frac{\text{FN X 100}}{\text{FN + TN}}$$

RESULTS

COMPARISON OF MALARIA PREVALENCE RATES USING THICK/THIN BLOOD FILM MICROSCOPY, OPTIMAL 1 AND SD-BIOLINE TECHNIQUES.

Data showed that of a total of 240 subjects examined by microscopy and the DiaMed Optimal (Optimal1), 86(35.8%) were Parasitaemic by microscopy while 67 (27.9%) were positive with Optimal 1 rapid assessment technique (Table I). Three (3) patients had P. Malariae which were identified by both techniques. With the removal of the P. Malariae, pevalence by microscopy was 34.6% while that of optimal 1 was 26.7%. mixed infections (3 in number) were also identified by both techniques.

The group investigating the performance of SD-Bioline undertook the test in 45 patients most of who were clinically malaria-positive. Prevalence rate of 55.6% using the BIOLINE and 68.9% using microscopy were obtained but these could not in fact be taken as true prevalence rates of parasitaemia in the area. Random investigation was considered a better technique for determination of prevalence.

Spill-Over effect of Antigenaemia:

Re-investigation of parasitaemia by light microscopy after anti-malarial therapy showed that the 10 randomly-selected previously-infected patients were negative on day 4 and on day 7 when they were re-examined Conversely on that 4th day, 2 (20.0%) and 3(30.0%) of the patients were still Optimal 1 and SD-BIOLINE positive respectively. Further test for antigenaemia using these rapid assessment techniques were not undertaken on day 7 and 14 as a result of the limited number of the test strips available. However, negative microscopy on day 4 for all the previously positive patients indicated that there was no antimalarial resistance in the patients. The positive

result obtained with the test strips after 4 days of chemotherapy in a number of patients mentioned above could only indicate the persistence of antibodies long after parasitaemia must have been cleared from the blood.

Table I: Sensitivity and Specificity of the DiaMed Optimal for the detection of *P falciparum* in 240 patients in Ibafo-Magboro Communities of Obafemi-Owode LGA, Ogun State.

		THICK BLOOD FILM MICROSCOPY		
DIAMED OPTIMAL 1		Positive	Negative	Total
	Positive	55	12	67
	Negative	31	142	173
	Total	86	154	240

Sensitivity: $\frac{55}{86} \times 100 = 63.95$

Specificity: $\frac{142}{154} \times 100 = 92.20$

Positive Predictive Value: $\frac{55}{67} \times 100 = 82.1$

Negative Predictive Value: $\frac{142}{173} = 82.1$

Table II: Sensitivity and Specificity of SD-BIOLINE for the detection of *P.falciparum* in-patients in Ibafo Communities of Obafemi-Owode LGA, Ogun State.

		THICK BLOOD FILM MICROSCOPY		
SD - BIOLINE		Positive	Negative	Total
	Positive	17	8	25
	Negative	14	6	20
	Total	31	14	45

Sensitivity: $\frac{17}{31} \times \frac{100}{1} = 54.84\%$

Specificity: $\frac{6}{14} \times \frac{100}{1} = 42.9\%$

Positive Predictive Value: $\frac{17}{31} \times \frac{100}{1} = 68.0\%$

Negative Predictive Value: $\frac{6}{20} \times \frac{100}{1} = 68.0\%$

SENSITIVITIES AND SPECIFICITIES OF THE DIAMED OPTIMAL AND SD-BIOLINE USING MICROSCOPY AS THE GOLD STANDARD

Result using the DiaMed Optimal gave a sensitivity, specificity, positive and negative predictive Values of 63.95%, 92.20%, 82.1% and 82.1% respectively. The SD-BIOLINE gave 54.84%, 42.9%, 68.0% and 68.0% respectively. In retrospect, the sensitivities shown by 3 other techniques (ICT, PSF and QBC) investigated by us were 94.60%, 91.17%, and 94.17% respectively (Table I). The SD-BIOLINE gave the worst comparative results and could not be recommended for use in Nigeria

DISCUSSION

We have investigated the efficacy of two rapid techniques for the diagnosis of P falciparum in 240 patients from Ibafo and Magboro Communities, which are rural areas of Ogun State. in this context, we have shown that the SD-BIOLINE was not able to detect some

positive cases which Optimal 1 (DiaMed Optimal) and microscopy could detect. However one interesting aspect of SD-Bioline is that the manufacturers used two surface proteins, namely, merozoite surface protein (MSP-1) and circumsporozoite protein (CSP) which have previously been documented to induce potent antibodies. They have been characterized and have been put forward as strong candidates for malaria vaccine development (23,24,25). It would have been interesting if this rapid assessment dipstick (BIOLINE) had surpassed all others in the detection of Plasmodium falciparum since it would have been seen as playing a dual role in our quest for methods of controlling malaria. Nevertheless, we are still investigating why the sensitivity and specificity of SD-BIOLINE were as low as 54.84% and 42.9% respectively while those of Optimal 1 now marketed as DiaMed Optimal by some companies were 63.95% and 92.20% respectively. The expiry date of the former was unfortunately not boldly printed on the packet. Comparative results using our previous work in 12 sites (manuscript accepted in journal of Malaria in Africa) has not shown the present work with Optimal 1 to be the best rapid assessment technique for malaria diagnosis. The immuno-chromatographic Test (ICT), ParaSight® F (PSF) and Quantitative Buffy Coat (QBC) techniques gave mean sensitivities as 88.63%, 89.95% 87.6% respectively. The mean specificities were 94.60%, 91.17% and 91.7% respectively. These, especially ICT, seemed to be better than Optimal 1 although no statistical significance emerged.

One advantage of Optimal 1 is its ability to detect and distinguish between the various isoforms of the metabolic enzyme, Lactate dehydrogenase (pLDH) of the various species of malaria parasites. However the work using the DiaMed Optimal (Optimal 1) and SD-BIOLINE was done in just one section of Obafemi – Owode LGA. It is possible that when more kits become available and a larger number of patients are tested in various communities and sites as in PSF and ICT, the main benefits, especially of Optimal 1 will emerge.

ACKNOWLEDGEMENT

We are grateful to the village heads of Magboro and Ibafo group of communities who gave us access to these villages. We also acknowledge the help of our Chief Nursing Officer (Mrs. Sowemimo-Coker). This work was sponsored by the Nigerian Institute of Medical Research, Yaba.

REFERENCES:

1. Ross R. An improved method for microscopical diagnosis of Intermittent fevers. Lancet, 1903; i., 86-87.
2. World Health Organization A global strategy for malaria control. Report of the World Health Organization Expert Committee. WHO, Geneva, 1993.
3. World Health Organization. Implementation of the global malaria control strategy. WHO Tech. Rep. Ser., No. 839. WHO, Geneva, 1993.
4. World Health organization (AFRO): Roll Back Malaria in the African Region: the inception meetings. In: MALARIA, Liaison Bulletin of the malaria programme WHO/Afro:1999; 2(1), 2-3.
5. Tharavanji S.New development in malaria diagnostic techniques. Southeast Asian Journal of Tropical Medicine and Public Health. 1990; 21:3-16.

6. Wardlaw SC and Levine RA., Quantitative buffy coat analysis: a new laboratory tool functioning as a screening complete blood cell count. Journal of the American Medical Association, 1983: 246:617-630.
7. Kawamoto F. Rapid diagnosis of malaria by fluorescence microscopy with light microscopy and interference filter. Lancet, 1991; 1:200-202.
8. Howard JR, Uni S, Aikawa M, Aley SB, Leech JH, Lew AM, Wellems TE, Renner J and Taylor DW. Secretion of a malarial histidine-rich protein – 2 (PFHRP 11) from Plasmodium falciparum-infected erythrocytes. Journal of Cell Biololgy, 1986; 103: 1267-1277.
9. Karbwang J, Tansanor O, Kanda T., Wattanagoon Y ParaSight® test for the detection of treatment failure in multidrug resistant P.falciparum malaria. 1996 Trans Roy. Soc. Trop. Med. And Hyg; 90:513-515.
10. Shiff CJ, Premji Z, Minjas JN. The rapid Manual ParaSight® F test: A new diagnostic tool for Plasmodium falciparum infection. Trans Roy. Soc. Trop. Med. And Hyg. 1993; 87:646-649.
11. Dietze R. The diagnosis of Plasmodium falciparum infection using a new antigen detection system. Am. J. Trop. Med. Hyg. 1995; 52:45-49.
12. Mbarakurnwa S. Trial of ParaSight® F Test for Malaria Diagnosis: The Primary Health Care, Zimbabwe. In: Malaria and infectious Diseases in Africa No. 4 (SmithKline Beecham Pharmaceuticals, France). 1996.
13. Beadle C, Long GW and Weiss WR. Diagnosis of malaria by detection of Plasmodium falciparum HRP-2 antigen with a Rapid Dipstick Antigen Capture assay. Lancet 1994; 343:564-568.
14. Agomo PU, Akindele SK and Salako LA. Limitation of ParaSight® F as a Rapid dipstick antigen capture assay for the detection of Plasmodium falciparum in Nigeria. Nigerian Journal of Parasitology, 1995; 16:55-64.
15. Agomo P. U., Akindele S. K., Asianya V. N. and Okonkwo C. A. The main Benefits of Histidine-rich protein-II Antigen capture Assay (ParaSight® F) in various Health Centres in Nigeria. Nigeria Quarterly Journal of Hospital Medicine, 1998; 8:9-13.
16. Bailey W. Latest Developments in the laboratory Diagnosis of Malaria. Africa Health, 1998; 20 (4): 16-20.
17. Cavallo JD, Hemandez E, Gerome F, Plotten N Debord T and Le Vangueresse, S. Serum HRP-2 antigens and imported Plasmodium falciparum malaria: Comparison of ParaSight® F and ICT malaria. Med. Trop. Mars 1997; 57 (4), 353-356.
18. Valecha, N, Sharma VP and Devi CU. A rapid immunochromatographic Test (ICT) for diagnosis of *Plasmodium falciparum*. Diag. Microbiol. Infect. Dis. 1998; 30 (4): 257-260.
19. Singh N., Valecha N, sharma VP. Malaria diagnosis by Field Worker using an immunochromatographic test. Trans. Roy. Soc. Trop. Med. Hyg. 19971 91 (4): 396-397.
20. Asianya VN, Agomo PU, Okonkwo CA, Mafe AG, Akindele SK, Agomo CO, Aina OO, Okoh HI, and Omoruyi SO. Evaluation of a new immunochromatographic Test

(ICT) for rapid diagnosis of *Plasmodium falciparum* malaria in Nigeria. The Nigerian Journal of Parasitology 1999; 20:19-26.

21. Agomo PU, Okonkwo CA, Asianya VN, Aina OO, Okoh HI, Nebe OJ. Comparative Evaluation of Immunochromatographic Test (ICT) and ParaSight® F for the rapid diagnosis of falciparum malaria in Nigeria. African Journal of Clinical and Experimental Microbiology, 2001; 2:43-51.

22. Palmer CJ, Lindo JF, Klaskala WI, Quesada JA, Kaminsky R, Baum MK, and Ager AL. Evaluation of the optimal test for rapid diagnosis of Plasmodium vivax and Plasmodium falciparum malaria. J. Clinical Microbiol. 1998; 36(1): 203-206.

23. Nardin, E., Zavala, F. Nussenzweig, RS. Pre-erythrocytic malaria vaccine: Mechanisms of protective immunity and human vaccine trials. Parasitologia 1999; 41:397-402.

24. Holder, A.A. Guevara Patino J.A., Uthaipibull, C. Syed, S.E.H. Ling, I.T. Scott-Finnigan T. Blackman, M.J. Merozoite surface protein 1, Immune evasion, and vaccines against asexual blood stage malaria. Parasitologia 1999; 41:409-414.

25. Target, G. Vaccine efficacy, and immunity affecting transmission. Parasitologia, 1999; 41:433-436.