An assessment of the usefulness of a rapid immuno - chromatographic test, DetermineTMmalaria pf in evaluation of intervention measures in forest villages of central India.

Neeru Singh [ojcmrc@vsnl.com]
Manmohan Shukla [nsmrc@hotmail.com]

Abstract

Background: Plasmodium falciparum, is a major health problem in forested tribal belt of central India. Rapid and accurate methods are needed for the diagnosis of P. falciparum malaria. We performed a blinded evaluation of the recently introduced Determine™malaria pf test (Abbott, Laboratories, Japan) compared with microscopy and splenomegaly in children in epidemic prone areas of district Mandla to assess the impact of intervention measures.

Results: Using microscopy as the gold standard, the sensitivity and specificity for Determine rapid diagnostic test (RDT) were 91 and 80% respectively. The positive predictive values (PPV) and negative predictive values (NPV) of the test were
respectively 79 and 91%. On the contrary, the sensitivity and specificity of spleen were 57 and 75% respectively with PPV of 73% and an NPV of 59%.

**Conclusions:** Determine™ malaria rapid diagnostic test is easier and quicker to perform and has other advantages over microscopy in not requiring prior training of personnel or quality control. This study, although limited by small number of children highlights the usefulness of a rapid antigen test in assessing prevailing malaria situation in remote malarious areas and thus address one of the significant drawbacks to current antimalarial programme.
Background

Rapid and accurate methods are needed for the diagnosis of *Plasmodium* falciparum malaria [1,2] as malaria is estimated to kill between 1.5 and 2.7 million people each year, an average of one person (often a child aged < 5 years) every 12s [3]. *P. falciparum* infections in children may become life threatening making rapid diagnosis of extreme importance [4]. No clinical diagnostic criteria are both sensitive and specific for malaria, an undifferentiated febrile illness [5] which may result in the erroneous treatment of millions of non-malaria cases with antimalarial drugs in the absence of diagnostic malaria microscopy. Excessive antimalarial drug use is costly, propels the emergence of drug resistant parasites, speeds the obsolescence of affordable drugs and retards malaria control [6]. Prompt diagnosis and effective treatment of malaria improves prognosis [2]. In the absence of diagnostic microscopy in resource poor, remote villages of tribal forested belt of central India, the detection of *P. falciparum* histidine rich protein 2 (Pf HRP-2) in a blood specimen is one method by which expeditious diagnosis of *falciparum* malaria can be made on the spot [7,8]. Recently introduced rapid malaria test, Determine™malaria pf (Abbott Laboratories, Japan) have been evaluated for its effectiveness in detecting *P. falciparum* malaria in Philippines [9] and in central India [10].

This field study appraised the performance of the rapid diagnostic test (RDT) in assessing the impact of antimalarial intervention measures in villages of two adjacent Primary Health Centre’s of district Mandla, central India. For this study we performed
a blinded evaluation of RDTs compared with microscopy and splenomegaly in children as the endemicity of malaria is usually characterized by spleen and parasites rates in children [11].

**Results**

Spleen examination of 199 and 150 children respectively in Narayanganj and Bizadandi PHC’s are shown in Table 1. The spleen rate among children who lived in the villages of Narayanganj PHC (78%) was significantly higher (P<0.0001) than that among children of Bizadandi (24%). Fever was found in 18.6 and 3.3 % children in Narayanganj and Bizadandi PHC’s respectively without spleen enlargement. The sensitivity and specificity of spleen were 57 and 74 % respectively (Table.2). The PPV 73%, NPV 59% and accuracy were 65%. On the contrary, fever without spleen had relatively high sensitivity of 84% with specificity of 67% (Table.3). The PPV, NPV and accuracy were 88.6, 57 and 79.6% respectively.

The performance of RDT vs TBS are shown in Table 4. A good agreement was found indicating high sensitivity of 91% which is significantly higher than that of spleen (P<0.0001). However, specificity was 79.6%, which is not significant when compared with specificity of spleen (Table. 5). The PPV 79 % (P< 0.05), NPV 91 % (P< 0.0001) and accuracy 85% (P< 0.0001) were all significantly higher when compared with their respective values of spleen. In all 11 cases showed positive reaction by RDT for *P. falciparum* while blood smears were negative for malaria parasite. A prolonged re-examination of these slides who were positive by the RDT, but
apparently had negative slides, did not reveal the presence of *P. falciparum* asexual parasites. Further of the eight subjects with *P. falciparum* infection not detected by RDT, not all had low parasitaemia: five had parasite densities between 60-318/µl, but the three others had densities between 600-1000/µl. Out of 14 gametocytes of *P. falciparum*, only 8 (57%) were positive by RDT.

In addition, 2 subjects with *P. vivax* as detected by microscopy (2795.81±3.58/µl) were found positive for *P. falciparum* by RDT.

**Discussion**

Malaria diagnosis, is often made on the basis of presumptive symptoms although this is alarmingly inaccurate [15,16,1]. One of the earliest methods used for estimation of the amount of malaria in a given locality is that of determining the proportion of person with a palpable enlargement of the spleen. This method introduced by Dempster in India in 1848 is still used although it is admittedly a crude measure [17]. In this study large number of cases with enlarged spleen in Narayanganj PHC were asymptomatic, of which fairly large number were having malaria parasites. Thus, it appears that in this population the splenomegaly is a reliable predictor of malaria as recorded elsewhere [18]. This is an index, which can be used to monitor the impact of intervention measures. However, it requires the services of a medical specialist who is not certain whether the spleen is entirely due to malaria. On the contrary, RTD indicate the presence of *P. falciparum* easily and accurately and since it requires no laboratory or technical equipment, a diagnostic facility can be set up in the most
anterior areas and in the most rudimentary way which makes it ideal for monitoring large scale control programmes in resource poor areas where malaria is a serious problem. This diagnostic test also gives the general practitioner a means of immediate diagnosis, which would overcome the usual delay associated with dependence on medical laboratories. Thus RTDs could be a better prognostic indicator in \textit{P. falciparum} malaria than spleen and microscopy.

The fact that a small proportion of children (6\%) without \textit{P. falciparum} parasites in TBS were demonstrated to have detectable HRP-2 antigen does not reduce the value of the RTDs [19]. Positive results on the RDT, which can not be verified microscopically have been suggested to be due to circulating \textit{P. falciparum} HRP-2 antigen following treatment [20] or from sequestered parasites [3]. The objective was not, and the setting did not allow a systematic follow up of cases which might be interesting for monitoring the decline in antigaemia.

\textbf{In 4\% cases the RDTs gave false negative results compared to the TBS. Such cases may not have sufficient antigen in the blood for detection by RDT.} We have estimated in our earlier study that the threshold parasitaemia for detection by \textit{Determine test} to be about 500 parasites µl [10]. However, in this study RDT failed to detect 3 \textit{P. falciparum} subjects with ≤ 1000 µl of parasites. Unexplained false negative results on the RDTS have been reported in many studies [21,22,23] and rarely even with very high parasitaemia [24,25].
To determine whether the test could be widely applied to assess the performance of antimalarial measures for patients living in various levels of endemicity revealed important and not previously noted insights for policy makers responsible for disease management. In central India, the largest state in the country also contributing highest number of malaria cases (23%) in the country [26], the usual practice under NAMP is to provide radical treatment (Chloroquine and Primaquine) to all fever cases during monsoon and postmonsoon season in tribal villages due to inaccessibility. However, a significant change has taken place in the clinical and parasitological features of malaria infections as a results of the emergence of drug resistant malaria [27]. When Chloroquine (CQ) resistant *P. falciparum* infections are treated with CQ as CQ is still the first line of treatment in many tropical countries including India, these infections may be partially controlled with the persistance of low grade parasitaemia in the peripheral blood. Such patients experience no symptoms or barely symptomatic infections [28,29]. We contend that the asymptomatic infections which were recorded in this study were mainly infections of this type. From a transmission perspective it is these asymptomatic carriers who escape detection, form the most potent reservoir of infection. If undetected, such children are not only likely to lead to a growing reservoir of CQ resistant infection in the community but are also likely to suffer continued morbidity due to persistent infection. Even assuming that all these asymptomatic cases will become symptomatic weeks to months later and that the proportion of
parasitaemic cases detected by the clinical symptoms was high enough to terminate transmission, a sustained effort would be necessary to continue picking up new illnesses until the asymptomatic infectious reservoir had been eliminated by natural clearance. These asymptomatic carriers in remote villages are putting a strain on malaria control activities. Since the number of febrile patients presenting at most peripheral health facilities in central India far too high to test each patient for the presence of Plasmodium species, hence NAMP’s policy is to provide fever radical treatment. Thus collection and examination of blood smears from asymptomatic patients is not possible. This opens new possibilities for rapid screening of communities at risk particularly pregnant women and children [30,31] in whom correct diagnosis and treatment is especially important [32]. Whether a screening test is suitable for a specific task would depends on the predictive values in the given setting, which in turn depends on the prevalence of the condition to be detected [33]. Based on the sensitivity and specificity as determined by field staff, we could show that the test had PPV of 79% and an NPV of more than 90 %.

In view of the high level of resistance to CQ worldwide and increasing also to SP (second line), rational use of drugs is essential to postpone development of resistance to second line antimalarials. Since SP is not as cheap as CQ hence it can not be used only on presumptive symptoms. As RDT has the potential of enhancing speed and accuracy of the diagnosis of *P. falciparum* malaria, it could
greatly aid in the diagnosis of malaria. However, a diagnostic test which is to be used in peripheral areas of developing country has to be simple and fast to perform by unskilled staff. While comparing with other HRP-2, determine has some advanced features. The ParaSight F test requires six steps and 50µl of blood, followed by ICT which required 5 steps and 10µl of blood while determine assay require 2 steps and 2µl of blood. Though final reading time is designed as 30 min, which may be a disadvantage particularly during malaria outbreak, generally a clear red line in patient bar is seen within 10 minutes in mild to moderately parasitaemic cases. In our study the reliability of field worker in reading test results was excellent after only brief on-site training. Finally, the application of the RDT in the programme will be determined by cost effective analysis. The population and countries at highest risk from malaria are mainly poorest and the most disadvantaged [1]. The price of the test varies with quantity and source of purchase [32]. For example the costs of the ICT pf tests ranges from US$ 1.80 / test in developing countries [34] to US$ 27 for two test bought in developed world. The ParaSight F test sells for US$ 1.20 / test in Uganda [34], US$ 2.25 in south Africa [35] and US$10-13 in Europe. The OptiMAL strip currently sells for US$ 3.00 / test [3]. These prices appear prohibitive at first sight in a country which only spends a few dollars/person/year for health care. However the picture may look different when the total costs to disease management are considered for the community and the programme. The cost effectiveness advantage of RDT in the long term is the reduction of mistreatment
in terms of drugs, costs, toxicity and development of resistance [36].

Furthermore, cost effectiveness analysis must also take into account a range of factors. These include the costs of missed diagnosis including return visits to clinics and time off from work. An important advantage of the availability of the RDT that it does not depend on just one person but can be performed by all staff members of the health clinic.

Conclusions

In a situation like ours where laboratory facilities are poor or non-existent, RDTs is the best option to support malaria diagnosis for case management in malaria control programme. However, persistent positive reactions upto 2 weeks following treatment [3] would not justify the introduction of RDT as a screening test by village health workers in the region. It appears that sensitivity of the RDTs remains high on treated patients which is not a failure of the test performance, but indicates a period during which the test should not be used for assessment of the [37]. The observed diagnostic trends therefore mean that it will be important to take patients history into consideration during routine usage of the RDTs. Furthermore, in the study area, people are also subject to other 

Plasmodium  infections i.e. P. vivax, which are less common and initially indistinguishable. These non-falciparum plasmodial infections have important therapeutic and prognostic implications. Thus a second generation of RDT’s are
required which are intended to detect and distinguish \textit{P. vivax} from \textit{P. falciparum}.

\textbf{Material and methods}

\textit{Study Area and Study Population}

This Study was undertaken in two Primary Health Centre’s (PHC) of district Mandla. The entire region is undulating and hilly. The villages are generally located on the slopes of the hillocks or on hill tops adjoining perennial streams. The inhabitants are (gond ethnic tribe) illiterate, poorly clothed, health ignorant and work in forest or road construction work etc. An outbreak of \textit{P. falciparum} was recorded in villages of Bizadandi PHC in 1995 (unpublished observation), while in villages of Narayanganj PHC in 1996 [12]. Appropriate intervention measures were undertaken in both the areas by N A M P which are described previously [13]. Briefly two rounds of focal spraying with DDT (1g/m²) along with prompt surveillance and treatment to bring the incidence of malaria under control.

For monitoring the impact of intervention measures in these two PHC’s, children aged 2-10 yrs with and without fever were examined for spleen enlargement [14] in three villages of each PHC during autumn season (December, 1999), by one medical specialist by establishing a mobile field clinic after obtaining informed consent from their parents. Treatment was given when appropriate as per Indian N.A.M.P. The study is approved by ethic committee of Malaria Research Centre (I.C.M.R), Delhi.
**Sampling and Data analysis**

From these children thick blood smears (TBS) were prepared from finger prick and read by a technician unaware of the spleen results. Parasites were counted against 200 white blood cells and converted into counts/µl assuming the average count is 8000µl. Simultaneously, RDTs were performed by a field lab attendant after only a brief on the spot training according to the manufacturer’s instructions taking about 30 minutes to provide one result without reference to the results of the TBS and spleen. The TBS were re-examined to discard a false negative results, combined infections or misdiagnosis of species by an experienced technician who was blinded to the previous results of TBS. The figures for specificity, sensitivity and predictive values were calculated as described previously [10] using microscopy as gold standard. Briefly, sensitivity was calculated as TP / (TP + FN), specificity as TN /(TN + FP), the positive predictive values (PPV) as TP / (TP + FP), and the negative predictive values (NPV) as TN / (FN + TN). The test accuracy, the proportion of all tests that gave correct result, was defined as (TP + TN) / numbers of all tests. The mixed infection of *P. vivax* and *P. falciparum* are treated as falciparum cases for the purpose of analysis. Likewise, the instances of gametocytes only are classified as false positives since gametocytes neither cause illness nor require treatment with blood stage schizonticidal drugs. The Z-test was used for comparison between proportions.

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Table 1

Results of examination of spleen of children from villages of two PHC, Mandla district.

| PHC       | Children examined | No. with enlarged spleen | Fever only | Mean class for enlarged spleen | Spleen rate (%) |
|-----------|-------------------|--------------------------|------------|-------------------------------|-----------------|
| Narayanganj | 199               | 155                      | 37         | 1.86                          | 78              |
| Bizadandi  | 150               | 36                       | 5          | 1.16                          | 24              |
Table 2

Comparison of the results of spleen examination in children and microscopic examination of thick blood smears (sensitivity and specificity of the spleen vs thick blood smears).

| Spleen                        | P. falciparum | Negative | Total |
|-------------------------------|---------------|----------|-------|
| Enlarged Spleen               | 109           | 82       | 191   |
| Spleen without Enlargement    | 41            | 117      | 158   |
Table 3

Comparison of the results of fever cases in children
with microscopic examination of thick blood smears

(sensitivity and specificity of the fever vs thick blood smear).

| Microscopy result                  | P. falciparum | Negative | Total |
|-----------------------------------|---------------|----------|-------|
| P. falciparum, asexual± sexual    | 31            | 6        | 37    |
| Negative                          | 4             | 8        | 12    |
| Total                             | 35            | 14       | 49    |
Table 4

Comparison of the results of Determine malaria Pf and microscopic examination for malaria for 191 children with enlarged spleen.

| Microscopy Result                  | Results of Determine Test |
|-----------------------------------|---------------------------|
|                                   | $P. falciparum$ | Negative | Totals |
| $P. falciparum$, asexual ± sexual$^a$ | 80             | 8        | 88     |
| $P. vivax$, asexual ± sexual       | 2              | 5        | 7      |
| $P. falciparum$, ± sexual only     | 8              | 6        | 14     |
| Negative                          | 11             | 71       | 82     |
| Total                             | 101            | 90       | 191    |

$^a$asexual ± sexual, asexual stages, with or without sexual stage parasites.
Table 5

Performance characteristics of Determine Malaria Pf (RDT) relative to those of microscopy for patients with enlarged spleen and fever.

| Test       | Sensitivity % (95 % C.I.) | Specificity % (95 % C.I.) | PPV % (95 % C.I.) | NPV % (95 % C.I.) | Accuracy % (95 % C.I.) |
|------------|---------------------------|---------------------------|-------------------|-------------------|------------------------|
| RDT        | 90.91 (85.02-96.98)       | 79.61 (72.28-93.7)        | 79.21 (71.06-100) | 91.11 (85.08-100) | 84.82 (79.73-94.92)    |
| Spleen     | 57.07 (57.88-62.26)       | 74.05 (69.45-78.6)        | 72.67 (67.99-77.3) | 58.79 (53.6-63.95) | 64.76 (59.75-79.76)    |
| Fever      | 83.78 (73.46-94.1)        | 66.67 (53.47-79.9)        | 88.57 (79.66-97.5) | 57.14 (43.3-70.99)  | 79.59 (68.30-90.80)    |
An assessment of the usefulness of a rapid immuno-chromatographic test, DetermineMalaria pf in evaluation of intervention measures in forest villages of central India.

Neeru Singh [oicmrc@vsnl.com]
Manmohan Shukla [nsmrc@hotmail.com]

Mariles Craig

The author(s) report on a study evaluating the DetermineMalaria rapid immunographic test kit for detecting malaria HRP-2 antigen, against gold standard of thick film microscopy and spleen rates in children in rural India. The Determine Malaria test kit has not been evaluated much and this manuscript is therefore worth publishing, but would benefit from some editing.

The study is a relatively simple evaluation. The authors discuss at great length issues concerning presumptive treatment, transmission, drug resistance etc that have been discussed in detail by many other authors, and this paper could be shortened significantly, with only brief re-iteration of well-known arguments. The authors should perhaps concentrate more on those findings that are unique to this study, or that are particular to the Indian situation, or on arguments that have not been made before. Are spleen rates used routinely for clinical diagnosis in India? Perhaps some more comment on the significance on this?

The materials and methods section is in the wrong place. There are several grammatical errors which presumably the editor will address. The frequent use of acronyms makes reading difficult; terms like PPV, NPB, NAMP, PHC, TBS etc should be spelt out, perhaps even "rapid test" instead of RDT, and acronyms used only where they make reading easier. Check that the "Determine Malaria" test, where spelt out, does not become "determine malaria".

Pg 2, par 1, line 3: Reference 3 is not the best to cite on global malaria burden. A more abt reference would be for instance the WHO, 1994, "World malaria situation in 1991", Bull WHO 72:160-164 or something more recent.
Pg 3, par 2, line 6: "the sensitivity and specificity of spleen..." add "in detecting malaria infections"

line 7&8: It is not entirely clear what Table 3 is all about. What is shown in the rows? columns?

Pg 4, par 3, line 8&9: This statement contradicts the findings of this study, where the sensitivity, specificity and accuracy of spleen rates in detecting malaria parasitaemia was not very good at all.

Pg 5, par 1, line 1: anterior?

line 5: prognostic indicator? - "diagnostic method" perhaps?

par 2: what is known about the cross-reactivity with the rheumatoid factor in Determine Malaria test? This has been a problem in several chromatographic malaria test kits, including an early version of the DeterminMalaria test if I am not mistaken.

Pg 9, par 2, line 3: persistent positive reactions for 2 weeks following treatment does not mean that use of a rapid test cannot be justified. Its benefits may vastly outweigh this disadvantage. It just means that it would need to be used intelligently, especially straight after large-scale drug intervention.

Advice: Accept after revision, which I do not need to see