Low sensitivity but high specificity of ParaHIT-f in diagnosing malaria among children attending outpatient department in Butimba District Hospital, Mwanza, Tanzania

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Abstract: Malaria is the leading cause of mortality among under-fives in the sub-Saharan Africa. The gold standard method for the diagnosis of malaria is by the use of microscopy which is time consuming and requires skilled technicians. Recently the cost effective antigen based rapid diagnostic tests (RDTs) such as ParaHIT-f have been introduced in various places for the diagnosis of malaria. The choice of these RDTs to use in a certain country and specific geographical locations is critical to malaria diagnosis, treatment and ultimately control of drug resistance. This study was conducted to evaluate the sensitivity and specificity of the cheapest and easily available RDT in the study area, using microscopy as gold standard. This cross-sectional study was conducted to determine the performance of ParaHIT-f in diagnosing malaria in 300 children attending outpatient department at Butimba district hospital, Tanzania. Two thick and one thin blood smear were stained using standard Giemsa stain. The prevalence of *Plasmodium falciparum* malaria was 5.7% and 15.7% by ParaHIT-f and microscopy, respectively; the difference was significant ($P<0.001$). The sensitivity and specificity of ParaHIT-f were 29.8% and 98.8% respectively. There was a strong association between parasitaemia of ≥1000parasites/µl and being positive for ParaHIT-f diagnostic test ($P <0.001$). There is a need to revisit the use of ParaHIT-f in Tanzania. Further studies on the sensitivity and specificity are needed. A good quality control system on the performance of ParaHIT-f and other RDTs is highly recommended.

Key words: Malaria, diagnosis ParaHIT-f, sensitivity, specificity, Tanzania

Malaria presents a diagnostic challenge in most tropical countries and its diagnosis still relies on clinical presentation and microscopic examination of blood smears. Microscopy is labour intensive, requires significant skills and time which causes delay in treatment and overdiagnosis (Reyburn *et al*., 2004). These limitations in diagnosing malaria can lead to fatal complications if not diagnosed and treated at appropriate time. Introduction of rapid diagnostic tests (RDTs) is a promising way forward in quick diagnosis of malaria and hence early treatment and reduction of complications (WHO, 2003). There are several studies that have evaluated RDTs in Tanzania (Mboera *et al.,* 2006; Reyburn *et al*., 2007; Kamugisha *et al*., 2008) and have come up with promising results. Worldwide results of different RDTs have differed a lot, with some reporting higher sensitivities and specificities while others reporting the opposite (Kamugisha *et al.,* 2008; Belizario *et al., 2005*). The availability and cost of RDTs are among factors that determine which test will be used in a particular setting. There is a need to evaluate the locally available RTD kits in the markets that are likely to be used in malaria diagnosis. This study was therefore carried out to compare ParaHIT-f with microscopy in diagnosing malaria among children at the outpatient clinic at Butimba District Hospital in Mwanza, Tanzania.

A cross-sectional study was conducted from June to July, 2008 to determine the performance of ParaHIT-f in diagnosing malaria in 300 children. All children aged between 1 and 120 months who attended outpatient department with history and clinical features suggestive of malaria were recruited serially until the sample size was reached. A finger-prick blood sample was collected aseptically after obtaining a written informed consent from caregivers. The sample was used to prepare blood smears and for testing with ParaHIT-f test.

Two thick and one thin blood smears were stained with 10% Giemsa (Sigma Aldrich, Nairobi) using standard procedures. The two thick smears were examined by two microscopists independently; one batch was examined at the field site (Butimba) and another batch at Bugando Medical Centre. The thin smears were used for identification of the malaria species. All discordant slides and 30 randomly selected slides were examined by the third expert for quality control. A slide was concluded to be negative when no parasites were observed after counting 200 white blood

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cells in different fields.

The diagnosis of malaria using ParaHIT-f (Span Diagnostics Ltd, Surat, India, Lot 9624) was done according to manufacturer's instruction. The ParaHIT-f kits were stored below 40⁰C as recommended by manufacturer. The microscopists were blinded of RDT results, and positive smear results were used for initiating treatment according to Tanzania Malaria Treatment Guidelines. In the analysis only positive slides by at least 2 readers were included. Data were analysed using SPSS version 11.5 (SPSS inc. Chicago). Sensitivity was calculated as true positive (TP)/ [TP+false negative (FN)], specificity as True negative (TN/ [(TN+false positive (FP)], Negative predictive value (NPV) as TN/(TN+FN) and Positive predictive value as TP/(TP+FP) (Neeru *et al*, 2005).

There was a good agreement with only 5 slides showing discordant results between the first two readers. A total of 47 slides were positive by two readers and were included in the analysis. The prevalence of asexual *Plasmodium falciparum* parasitaemia was 5.7% (14) and 15.7% (47) by ParaHIT-f and microscopy, respectively. The asexual parasite density ranged from 40 to 300,000parasites/µl. The sensitivity, specificity, positive predictive value (PPV), negative predictive values (NPV) of ParaHIT-f using microscopy as gold standard were 29.8% ,98.8% , 82.4% and 88.3%, respectively (Table 1). There was a strong association between being positive by ParaHIT-f diagnostic test and parasitaemia (cut off point ≥1000parasites/µl) (Fisher's Exact test, *P*<0.001), with ParaHIT-f picking only those with high parasite count.

Microscopy detected malaria in patients with parasitaemia ranging from 40 to

vomiting by 24.3%(73). Among children with fever only 46 (16.1%) had positive blood slide compared to only 1 (7.1%) of those children with no fever (Fisher's Exact test, *P*=0.325).

ParaHIT-f is a rapid, easily performed RDT with clear lines both in the control and in the test. Such a test and other RDTs are needed in the laboratories lacking trained microscopists in malaria endemic areas. RDTs are likely to facilitate early diagnosis and prompt treatment in endemic areas.

The results of this study show that ParaHIT-f which is already available for use in Tanzania has low sensitivity of 29.8%. Similar results have been reported recently from Philippines (Belizario *et al.,* 2005). The results however differ from what was reported in India where ParaHIT-f was reported to have higher sensitivity in areas with different epidemiological settings (Neeru *et al.,* 2005). Reasons for the low sensitivity in this study were not investigated. However, it has been documented that when quality of RDTs is good deletion mutation or diversity in Pf HRP2 is likely to be the reason. Deletion mutation of Pf-HRP2 has been reported by Baker *et al.* (2005). Other RDTs targeting HRP2 have performed well in other parts of Tanzania (Mboera *et al.,* 2006; Kamugisha *et al.,* 2008). The difference in sensitivities of RDTs targeting Pf-HRP2 within the same country has been reported recently and is due to diversity in Pf-HRP2 gene (Natacha *et al.,* 2008). Some repeats are associated with false negative results especially at low parasitaemia (Natacha *et al.,* 2008). The difference could also be explained by storage conditions, (Mboera *et al.,* 2006; McMorrow *et al.,* 2008). However, in this study storage, procedures and transport were accordingly to manufactures instructions.

Table 1: Sensitivity, Specificity, positive and negative predictive value (PPV, NPV) of paraHIT-f according to parasite density

Parasitaemia	Sensitivity, (95%) CI	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Overall	29.8 (24.6-35.0)	98.8 (97.6-100)	82.4 (78.1-86.7)	88.3 (84.7-92.0)
<1000/µl	8.3 (5.2-11.4)	99 (98.0-100)	17.6 (13.3-22.0)	100
1000+ / µl	100	98.8 (97.6-100)	64.7 (59.3-70.1)	88.3 (84.7-92.0)

300,000parasites/µl while ParaHIT-f detected only one patient with 160parasites/µl and others had parasitaemia above 600parasites/µl. Fever was the main presenting symptom reported by 95.3% (286) followed by cough 39%(117) and

Specificity of ParaHIT-f in this study was high 98.8% and the results were similar with other reports (Belizario *et al.,* 2005; Neeru *et al.,* 2005). In this study, ParaHIT-f also detected parasitaemia higher than 35,000parasites/µl

contrary to what was reported by other studies (Neeru *et al.,* 2005). The sensitivity of paraHIT-f was high at higher parasitaemia and there was a strong association between higher parasitaemia and being positive for ParaHIT-f (*P*<0.001). The same observation was reported by other studies; the main suggestions for their observations were the diversity in Pf-HRP2 gene, with certain repeats not able to be detected at parasitaemia less than 250parasites/μl (McMorrow *et al* 2008; Natacha *et al.,* 2008). In the present study, only 16.1% among children with fever had positive slides; this indicates the importance of reliable diagnostic tools to differentiate malaria and other causes of fever (Reyburn *et al*., 2004).

In conclusion, although ParaHIT-f is highly specific in malaria diagnosis, it is less sensitive in the study area. The use of ParaHIT-f in Tanzania should therefore be revisited and countrywide studies are recommended to determine the sensitivity, specificity and other parameters of ParaHIT-f to ensure quality of the test.

References

Baker, J., McCarthy, J. Gatton, M., Kyle, D.E., Belizario, V., Luchavez, J., Bell, D. & Chang, Q. (2005) Genetic diversity of *Plasimodium falciparum* histidine-rich protein 2 (*Pf*HRP2) and its effect on the performance of *Pf*HRP2 - based rapid diagnostic test. *Journal of Infection and Disease* 192,870-877.

Belizario, V.Y., Pasay, C.J., Bersabe, M.J., WU de Leon, Guerrero, D.M. & Bugaoisan, V.M., (2005) Field evaluation of malaria rapid diagnostic tests for the diagnosis of *P. falciparum* and non-*P. falciparum* infections. *Southeast Asian Journal of Tropical Medicine and Public Health* 36, 552-561.

Kamugisha, M.L., Msangeni, H., Beale, E., Malecela, E.K., Akida, J., Ishengoma, D.R.S. & Lemnge, M.M., (2008) Paracheck Pf compared with microscopy for diagnosis of *Plasmodium falciparum* malaria among children in Tanga City, north-eastern Tanzania. *Tanzania Journal of Health Research* 10, 14-19

Mboera, L.E.G., Fanello, C.I., Malima, R.C., Talbert, A., Fogliati, P., Bobbio, F. & Molteni, F. (2006) Comparison of the Paracheck-Pf test with microscopy, for the confirmation of *Plasmodium falciparum* malaria in Tanzania. *Annals of Tropical Medicine and Parasitology* 100, 115-22.

McMorrow, M.L., Masanja, M.I., Abdulla, S.M.K., Kahigwa, E. & Kachur, P. (2008) Challenges in routine implementation and quality control of rapid diagnostic tests for Malaria-Rufiji District, Tanzania. *American Journal of Tropical Medicine and Hygiene* 78, 385-390.

Natacha, M., Celine, B., Christian, B., Magali, T. & Didier, M. (2008) Country-wide assessment of the genetic polymorphism in *Plasmodium falciparum* and *Plasmodium vivax* antigens detected with rapid diagnostic tests for malaria. *Malaria Journal* 7:219

Neeru, S., Mishara, A.K., Shukla, M.M., Chand, S.K. & Praveen, K.B. (2005) Diagnostic and prognostic utility of an inexpensive rapid onsite malaria diagnostic test (ParaHIT-f) among ethnic tribal population in areas of high, low and no transmission in central India. *BMC Infectious Disease* 5, 50.

Reyburn, H., Mbakilwa, H., Mwangi, R., Mwerinde, O., Olomi, R., Drakeley, C. & Whitty, C. J. (2007) Rapid diagnostic tests compared with malaria microscopy for guiding outpatient treatment of febrile illness in Tanzania: randomized trial. *BMJ* 334, 403.

Reyburn, H., Mbatia, R., Drakeley, C., Carneiro, I., Mwakasungula, E., Mwerinde, O., Saganda, K., Shao, J., Kitua, A., Olomi, R., Greenwood, B.M. & Whitty, C.J. (2004) Overdiagnosis of malaria in patients with severe febrile illness in Tanzania: a prospective study. *BMJ* 329, 1212.

WHO (2003) Malaria rapid diagnosis, making it work. RS/2003/GE/05(PHL).