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Evaluation of BID® plasmodium lactate dehydrogenase (pLDH) rapid test for detections of malaria parasite in Calabar, Nigeria.

A REPORT PRESENTED BY:

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Rapid test kits supplied by BUNDI International Diagnostics its BID rapid malaria pLDH cassette.

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BACKGROUND

Malaria is one of the nightmares facing the sub-Saharan Africa as it bears 90% burden of the global estimate of 300 to 500 million new infections and 1.5 to 3 million deaths annually. Children under 5 years, pregnant women and non-immune travelers are at risk (1, 2). Morbidity and mortality due to malaria can be significantly reduced with early and rapid diagnosis guiding prompt and effective treatment (3). This is more relevant now with widespread resistance to commonly used drugs and recent policy changes across the world to the use of the costlier artemisinin combination drugs (ACT) as first line treatment. Home treatment of malaria is also common in endemic malaria areas with increasing self diagnosis of malaria (4) and subsequent consuming of antimalarials from patent medicine vendors (PMV) or local drug peddlers before visiting a health care center if accessible to them.

The gold standard for diagnosis of malaria remains traditional microscopy though other more laborious, costly and highly technical procedures e.g. polymerase chain reaction (PCR), parasite identification by culture etc are also employed. Slide microscopy needs trained experts, constant supply of electricity, access to good quality reagents or stains (e.g. giemsa) and being meticulous to identify parasites especially when counts are low. It takes almost one hour to practically have the results even in field studies. These limits are used in rural areas and places where laboratory experts and amenities are lacking. There has been a recent concern on the reliability of blood film results due to above issues and bias of interpretation (5) though it remains a very reliable means of parasite identification.

Blood based malaria diagnosis with antigen specific rapid (immunochromatographic) diagnostic tests (RDT) gives hope of achieving definitive diagnosis of malaria in areas where microscopy is impracticable (6) and in self diagnosis.

MALARIA SITUATION IN NIGERIA

Nigeria is a West African country, one of the largest countries in the region with an estimated population of 130 million. It is classified as a third world country with 66% of its citizens living below the poverty line. Life expectancy is 52 years and child mortality (< 5yrs) is 183 per 1,000 (UNJCEF). The climate is tropical, humid in the southern costal zone and more arid in the north.

Nigeria is a hyper endemic zone of malaria with high and stable prevalent rates throughout the year in the more humid areas. Malaria is the most common cause of out-patient visits to health facilities and is one of the leading causes of mortality, accounting for up to 40% of deaths in children less than 5 years in age in Nigeria (6). *Plasmodium falciparium* infection accounts for more than 90% of all cases.

Malaria treatment policy is now in favor of artemisinin combination drags (ACT) and efforts geared towards making treatment widely available and affordable. We therefore evaluated the sensitivity and specificity of RDT for diagnosis of malaria compared to slide microscopy in Calabar, South East Nigeria.

OBJECTIVES

Primary Objective

- To determine the diagnostic sensitivity and specificity of RTD (BID®) against traditional microscopy for malaria.

Secondary Objective

- To determine the least level of parasitaemia per microlite of blood of RTD (BID®) sensitivity.
- To determine the percentage detection of mixed parasitaemia.

Study Population

The trial population consists of male and female patients of all age groups with signs and symptoms of acute uncomplicated malaria, presenting at the outpatient unit of the health facility and who gives informed consent for study inclusion (parents/guardian gives consent incase of children),

BASE LINE CHARACTERISTICS OF STUDY AREA

Ikot Ansa in Calabar Municipality of Cross River state in Eastern Nigeria lies within the tropical rain forest belt (Lat.4°N and 7°N). The council has a projected population size of about 222,100 with an under age 5 population of almost 44,420. Subsistence farmers of different ethnic groups mainly populate Ikot Ansa. Malaria is holoendemic in the study area with high and perennial transmission especially in the rainy season from April to November. Health care seeking behavior is poor with the people tending towards self/home treatment of common ailments like malaria and visits to healthcare facilities when this fails or treatment is subsidized.

Study Site

Primary health centre (PHC), Ikot Ansa, Calabar municipal council of Cross River State.

Ethical Considerations

Ethical approval for this study was applied for and obtained from the University of Calabar Teaching Hospital Ethical Committee. We also obtained informed (verbal) consent of the parent/caregiver prior to inclusion into the trial. Ws advised, treated, recommended treatment or referred all patients as appropriate.

Test procedure

We used a WHO protocol (6) modified and adapted into an ITDR&P standard protocol and with inputs from a protocol provided by the product manufacturers (Bundi International Diagnostics).

Screening for Eligibility
The clinician and nurse consecutively screened all potentially eligible participants who presented for treatment at the Health Centre for inclusion. Eligibility was based on the following criteria:

Entry criteria
Inclusion:
1. Presentation with signs of uncomplicated malaria,
2. Absence of severs malnutrition or signs of severe malaria
3. Presence of axillary temperature of ≥ 37.5℃ and/or history of fever in the preceding 24 hours.
4. Informed consent of patients (parent/guardian, in case of children)

Exclusion:
1. Presence of general danger signs
    - Not able to drink or breastfeed
    - Vomiting everything
    - Recent history of convulsion
    - Lethargic or unconscious state
    - Unable to sit or stand up
2. Signs of severe and complicated *falcliparum* malaria as defined by WHO
3. Febrile conditions caused by diseases other than malaria. ..

STUDY DESIGN AND ENROLLMENT
Between 25th October and 19th November 2005, we enrolled individuals at the health center located at Ikot Ansa. The population was sensitized through the news media (radio and television), church announcements and by town criers through instructions from clan and family heads, asking them to bring anyone with fever for treatment. Individuals were eligible for inclusion into the study if they have clinically apparent uncomplicated malaria, live within the study area with axillary body temperature ≥37.5℃ or history of a fever the past 24 hours, without danger sign (see above) and individuals/parents or guardian give informed consent.

LABORATORY INVESTIGATION
The rapid diagnostic test was done with blood sample from a finger prick with a sterile lancet. The sample and buffer solution were dropped on the test cassette per protocol and read after 20 minutes as negative, *P. Falciparium*, mixed parasitaemia or *non-falciparium* infection.

Thick and thin blood smears were prepared on the same slide with blood sample collected from a finger prick with a sterile lancet, stained in 3% Giemsa solution for 30 minutes. The smears were read to 100 fields with quantification of *P. falciparum* asexual parasitaemia on the thick smear. Parasites were counted against 200 white blood cells (WBC). The parasite density (Per µl of blood) was calculated, assuming a normal level of 8,000 WBC/µl of blood. Slides were declared negative if 100 high power fields were examined and no parasites seen.

Packed cell Volume (PCV) was measured with sample collected on a capillary tube and centrifuged for 5 minutes at 10,000g with a micro centrifuge and read on a heamatocrit reader.

Temperature was measured with a digital thermometer, weight taken with a bathroom weighing scale and height measured with an apparatus calibrated in centimeters.

NOTE: Slide microscopy and RDT were done by different laboratory personnel who were also blind to the outcome of the other Investigation.

Data Management and Analysis
Data generated were recorded in a register and double-entered into EPI-Info version 6.4. Sensitivity, specificity, negative and positive predictive values were calculated using the standard formulae. Percentages were manually calculated.

RESULT:
248 patients with suspected uncomplicated malaria were recruited from 25th October to 19th November, 2005 following criteria stated in the protocol. Blood samples were tested with the BID rapid diagnostic test (RDT) kit and compared with the gold standard in malaria diagnosis - slide microscopy. RDT was assessed as positive or negative while slide microscopy was assessed as positive or negative with significant level based on blood parasitaemia of 100 parasites/µl of blood (6), determined by expert microscopists / parasitologists.

The test showed true positive in 68 patients, false positive in 7, true negative in 166 and false negative in 7. Sensitivity was 90.6% (68/75) and specificity 95.9% (166/173). The positive predictive value (PPV) was 90.5% (68/75) awl the negative predictive value (NPV) 95.9% (166/173).

Of all positive samples with RDT (75), 37 (49.3%) was *P. falciparium* alone, 32 (42.7%) was mixed infection and 6 (8.0%) was positive for non-*P. falciparium* malaria. The lowest level of parasitaemia detected by the RDT was 204 parasites/µl of blood.

Table 1: Comparison of RDT (BID®) test result with slide microscopy

	Microscopy			
RDT		Positive	Negative	Total
	Positive	68	7	75
	Negative	7	166	173
	Total	75	173	248

DISCUSSION:
Regional and global efforts at malaria control will be enhanced by the advent of new and efficient methods of diagnosis as a complement or substitution to the traditional slide microscopy. RDT easily fills this gap as it can be conducted quickly with the patient and caregiver waiting and decisions on treatment taken promptly. This is more so as current drugs for malaria treatment are costly and there is need to improve the immediate morbidity state, especially in severe malaria where time for diagnosis and treatment is of essence (7).

BID® has a sensitivity and specificity of 90.6% and 96.0% respectively, comparable to studies of RDTs across other endemic regions of the world (8, 9). This calls for the need to standardize its storage and use as a potential screening and diagnostic tool. Its availability to all symptomatic patients will reduce treatment delay and save some resources for empirical treatment of malaria in the wake of the current high cost of treatment.

The unit cost of BID® rapid malaria kit is about US $1.00, cheaper than microscopy, especially when time to diagnosis is factored in. More so, post trial interactions with the study team revealed that the rapid test was easy to perform and can be taught to individuals with much less laboratory expertise. This will encourage increased effectiveness of home and self diagnosis in a situation where home/self treatment is

rife as well as enhancing to community-based management of malaria if RDTs are made more accessible.

CONCLUSION AND RECOMMENDATION:

Implications for Practice: Accurate and rapid diagnosis of malaria is important for individual case management and in the reduction of the burden of the disease. BID® has demonstrated the ability to achieve this and therefore needs to be evaluated further with a view to popularize its use in malaria infested areas. The manufacturers of BID® should be encouraged to seek approval from the National Agency for Food and Drug Administration (NAFDAC) for its use in diagnosing malaria in Nigeria.

Implications for policy: Effective malaria management is under review globally. Regional policy makers, especially the national malaria control program of Nigeria, should see BID® as a timely tool to combat malaria and gear efforts towards exploring its use to save our country from further parasite resistance to drugs and to reduce patient turn around time in the health facilities.

Implications for research. There is need to further evaluate BID® in a multi-centre trial and especially to compare it with other RDT products for sensitivity, specificity, cost benefit and easiness of test performance. Conditions under which its sensitivity varies need to be explored, especially when it is expected to be used by individuals and in remote areas.

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