OPEN ACCESS
Saudi Journal of Biomedical Research
Abbreviated Key Title: Saudi J Biomed Res
ISSN 2518-3214 (Print) |ISSN 2518-3222 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: http://scholarsmepub.com/sjbr/

Original Research Article

Evaluation of the Immunochromatographic Strip Method and Microscopic Method in the Identification of Malaria Parasites

Chukwurah Ejike Felix[1], Obeagu Emmanuel Ifeanyi[2*], Ututu Sunday Unahci[3]
[1]Department of Haematology and Immunology, Faculty of Clinical Medicine, Ebonyi State University, Abakaliki, Nigeria
[2]Department of Medical Laboratory Science, Faculty of Health Sciences, Imo State University, Owerri, Nigeria
[3]Department of Medical Laboratory Science, Faculty of Health Sciences, Ebonyi State University, Abakaliki, Nigeria

***Corresponding author:** Obeagu Emmanuel Ifeanyi
| **Received:** 15.03.2019 | **Accepted:** 24.03.2019 | **Published:** 31.03.2019
DOI:10.21276/sjbr.2019.4.3.6

Abstract

This project work evaluated the strip (rapid diagnostic test) method and microscopic method in the identification of malaria parasites. To evaluate the suitability of both methods and also to find the advantage and disadvantage of both methods. Two ml (2ml) blood sample was collected with a total number of 100 samples from children between the age of 5-14years presented with fever attending Federal Teaching Hospital Abakaliki, haematology/blood group serology unit into EDTA container in the basis of first come first serve were used. Out of the 100 patients under present study, 71% turned out to be positive as overall. Out of these, 59% were positive for strip method whereas 71% were positive for microscopic method (thick film). Both methods showed that the most pathogenic and predominant species in Abakaliki is plasmodium falciparum. Thick film was made in a clean grease free slide immediately after collection and allowed to air dry and stained with Giemsa stain diluted with buffered water (pH 7.2) for 20 minutes and the stained slide was viewed microscopically by focusing with XI0 and examine with XI00 objective (oil in immersion). Strip method (rapid diagnostic test) used is manufactured from standard diagnostics, INC. (SD). The test device was removed from the foil pouch, placed on a flat, dry surface. With the aid of a 5ul capillary pipette provided, draw whole blood to a black line and then transfer the drawn whole blood into the round sample well. Four drops of assay diluents was added vertically into the square assay diluent well. Result was read after 15 minutes, one line (control line) indicate negative result while two lines test and control line indicate positive result. Both methods are good in the identification of malaria parasites, but microscopy is the "gold standard". Microscopic method requires high skill manpower, a lot of training/expertise and are not encouraged in rural area with low/no power supply. Strip method is rapid, does not requires expertise/training and it does not consume time, but it is just a screening test, if used should be confirmed with the microscopic method.
Keywords: Immunochromatographic Strip Method, Microscopic Method, Malaria Parasites.

INTRODUCTION

Malaria is one of (he highest killer diseases affecting most tropical countries especially African. It affects over 500 million people worldwide and over one million children die annually from malaria [1]. Of all the human malaria parasites, *plusmodium falciparum (P. falcipanun)* is the most pathogenic and is frequently falal if untreated in time [2]. In India, according to Nandwani *et al.*,[2] 0.89 million cases of P. *falciparium* cases and a total of 1.82 million cases of malaria with 902 death were reported in the year 2002. Traditional practice for outpatients has been to treat presumptively for malaria based on a history of fever but, a significant proportion those treated may not have parasites cover 50% in many settings and hence waste a considerable amount of drugs. This old clinical based practice is still relevant today especially in infants where time spent on getting confirmatory laboratory diagnosis could lead to increased fatality.

World Health Organization (WHO) currently makes the tentative recommendation (hat parasite based diagnosis should be used in all cases of suspected malaria with the possible exception of children in high-prevalence areas and certain oilier situation [3], for this recommendation to be adhered to obviously, rapid and accurate laboratory finding or demonstration of malaria parasite should be established.

Malaria was original diagnosed by traditional microscopy presently other methods of diagnosis like RDT, polymerase chain reaction buffy coal, immune-chromatographic test etc. started evolving.

The traditional method of microscope identification of parasites however is not only daunting in poor power setting, but also time consuming and requiring a lot of expertise/training. Thus microscopy in Africa is generally, limited to large clinics/tertiary centres. This conventional staining of peripheral blood smears/microscopy However still remains the "gold standard" in laboratory diagnosis of malaria [2]. Rapid diagnosis tests (RDTs) for malaria could be considered for most patients in endemic regions, especially in poor power setting where there is shortage of qualified manpower in Africa [1]. However, there is very little evidence, especially from malaria endemic areas to guide decision makers on (he sensitivity and specificity of these Rapid Diagnostic Tests (RDTs).

Rapid Diagnostic Tests (RDTs) are commercially available in kit forms with all necessary reagents and the ease of performance of the procedures; docs not require extensive training or equipments to perform or to interpret the results.

Results arc read in 12-13min [4]

Rapid Diagnostic Tests (RDTs) mainly come in Iwo forms. One is antigen based and normally requires the use of haemolyzed red blood cells while the other is antibody based and normally requires the use of extracted serum. Generally speaking, antibodies are better expressed in serum otherwise plasma could also stand in place of serum for antibody based method. The principles of lest stem from detection of malaria parasites protein histidine where antibody method is used, it means detection, of the presence of antibodies against histidine in the human serum and where whole blood is used, it implies detection of malaria parasites histidine on the red blood cells [4].

Aim

- To evaluate the strip and microscopic method for the detection of malaria parasites.

OBJECTIVES

- To screen for malaria parasite using rapid diagnostic tests (RTDs)
- To screen for malaria parasite using microscopic method (Thick Him)
- To compare there results.

MATERIALS AND METHODS

Study Site/Population

This project was carried out at Federal Teaching Hospital, haematology unit Abakaliki.

Other Materials/Reagents

The SD Bioline malaria Ag Pflcst contain following items to perform the assay.

- 25 test devices individually foil pouched with a dcsiccant.
- Assay diluents (IX5ml/vial)
- 5 disposable sample applicator (5ul).
- 25 safety lancets
- 5 Alcohol swab.
- 1 Instruction for use

Active ingredients of main component

- 1 test strip include: Gold conjugate; mouse monoclonal antibodies specific to P.F HRP-II-gold colloid (0.1 ± 0,02ug), Test line P.F: mouse monoclonal antibodies specific to P.F HRP-1I (0.5 ± 0.1ug) control line: Goal anfi mouse IgG (1.0±0.2ug).
- Assay diluents: Bovine serum albumin (q.S), tritonX 100 (q.S).

METHODS

Two ml (2ml) blood samples were collected into EDTA and plain tubes from a total of 100 patients who presented with fever for 1-3 days and were clinically diagnosed of malaria fever.

Thick and thin films were made in triplicates from EDTA sample within l0mins of collections while sera were harvested from the plain tubes as soon as clots were fully formed.

Thick film were stained by Giemsa's stain and diluted with 20 volume of buffered water (pH 7.2) for 20 minutes while the thin film were stained by Lieshinan's and diluted Giemsa's methods.

RDT based on antigen was carried out on aliquots of haemolysed whole blood in duplicates. Sera were tested in duplicates to detect malaria parasites antibody based on RDTs-antibody detection method.

Sampling and Sample Size

Two ml (2ml) of whole blood is collected from the vein puncture into EDTA container from children under the age of 5 to 14 years with history of fever intending federal teaching hospital, haematology/blood group serology unit, Abakaliki.

Ethical Approval

Before the commencement of this study Ethical approval was obtained from the ethical committee of the various hospitals and informed consent was obtained from the patients before the collection of the samples.

Microscopy

Techniques for Making Thick Blood Film

Thick film was made by placing a small drop of blood on the centre of a clean grease live-slide and spread out with the edge of another slide which served as spreader to cover an area about 4 times its original area at an angle of 45^0.

The film was allowed to air dry thoroughly for at least 30 minutes at 37^0C.

Take to staining rack and immerse the slide for 20 minutes in a staining jar containing Giemsa stain freshly diluted with 20 volumes of buffered water to give a final dilution of Giemsa stain

Wash in buffered water of $_p$H 7.2 for 3 minutes, then stand the slides upright to air dry. Do not blot.

View microscopically by focusing with xlO and examine .with xlOO objective (oil immersion).

Rapid Diagnostic Tests (RDTS)

Standard diagnostic, INC (SD)

Test Principle

The SD biolinc malaria antigen *p. faciparium* (AgPF) lost cassette contains a membrane strips which is procoated with mouse monoclonal antibodies specific to HRP=H of *p. faciparium* colloid gold conjugate react with the malaria *plasmodium faciparium* antigen in the sample the more along the membrane chromatographically to the lest region *faciparium* and form a visible line as the antibody-antigen antibody gold particle complex with high degree of sensitivity and specificity. Both the test line and control line in the result window are not visible before applying any sample. The control line is used for procedural control and should always appear it the test procedure is performed correctly.

Procedure

- Allow kit components and specimen to room temperature prior to testing.
- Remove the test device from the foil pouch, place it on a Hal dry surface.
- Clean the fingertip and prick the finger with lancet.
- With a 5ul capillary pipette, draw whole blood specimen lo black liwe and then transfer drawn whole blood into the round sample well.
- Add four (4) drops of assay diluents vertically into the square assay diluents well.
- Wait a minimum of 15 minutes (up to 30minules) and read result.

Interpretation

- **Negative Result:** The presence of the colour hand ("e" control line) within the result window indicates negative result.
- **Positive result:** The presence of Iwo colour hand ("T" control line) within the result window no matter which hand appear first.

RESULT

Out of 100 patients under present study, 59 patients tested positive to strip method (rapid diagnostic test) and 41 patients test negative to rapid diagnostic lest (KTDS). In microscopic method, 71 samples out of 100 patients samples used tested positive to microscopic method ((hick film) and 29 out of the 100 tested negative to microscopic method. All patients that tested positive to strip method also tested positive to microscopic methods. These is represented in Table-1 below showing the number of positive, negative, positive predictive value (PPV), specificity and sensitivity.

Table-1: Test Result

Categories	Positive	Negative	Positive predictive value (PPV)	Sensitivity	Specificity
Strip method	59	41			
microscopic method	71	29	52%	31%	21%

Table-2: Describing the formular used for the calculation of sensitivity, specicity, positive

predictive value (PPV)	Disease-(number)	Non disease (number)	Total
Positive (number)	A (True positive)	B (False positive)	T (Test positive)
Negative (number)	C (False negative) T disease	D (True negative) T Non disease	T (Test negative Total)

DISCUSSION

There are four (4) principal methods for diagnosing malaria. These are symptomatic, microscopy, antigen test and molecular methods. Symptomatic diagnostic is the most common and people in poorer countries often use symptoms alone to diagnose malaria. In other areas, too, symptomatic diagnostic is often the initial one, followed by one of the other methods. However, it should be noted that many other diseases present symptoms very similar to malaria, and diagnosis by symptoms alone can be misleading and even harmful. Treating for malaria where other treatment is called for leaves the actual disease uncured and the patient in critical condition.

It is therefore imperative to follow up symptomatic diagnosis with one of the other more accurate methods. Onset of long periodic fevers, chills and bodily pain often taken together to be symptoms of malaria. However, this diagnostic is often wrong; so at limes is parasitemia, which means the concentration of parasites in they blood; both can be caused by other sorts of infections. It has been that retinopathy, the study of changes occurring in the retina of the eye, can give good indication of malaria, because the color and other aspects of retinas were changed as a result of particular diseases. A percentage parasitemia need

therefore be adopted to correlate with clinical presentation.

Microscopic examination of blood, ever since the singular discovery of I.averan, the French scientist who first identified the plasmodium parasites, is the most reliable method of diagnosing malaria. Therefore, a specimen of blood is observed under the microscopic for the presence of the malaria parasite.

Although, other bodily fluids like saliva or urine can also be used as less invasive methods, blood is preferred for higher concentration of the parasites.

World health organization (WHO) currently makes the tentative recommendation that parasites-based diagnosis should be used in all cases of suspected malaria with the possible exception of children in high-prevalence areas and certain oilier situations [3]. For this recommendation to be adhered lo obviously, rapid and accurate laboratory finding or demonstration of malaria parasite should be established. The introduction of high-cost antimalaria drugs such as ACT is encouraging in malaria-endemic countries in sub-Sahara Africa to reassess diagnostic practices Schillciiits *et al.,* 2008. This drive to have rapid and accurate method of malaria diagnosis led to carryout a comparative malaria detection between polymcrase chain reaction (PCR) and microscopy on 100 Indian patients [2]. They found the PCR method to be 96.8% sensitive's but took about 10-11 hours to complete whereas microscopy look an average of 40-45 minutes. PCT required electric power which costs a fortune and cannot easily be considered for use in Africa. Also it is very lime consuming and docs not meet our speed desire.

Immunochromatographic method to detect the presence of malaria parasite appears lo be most rapid and requires minimum or no training at all. Immunochromatographic method relies on the migration of liquid across the surface of a nitrocellulose membrane [4]. The test is based on the capture of parasite antigen from the peripheral blood using monoclonal antibodies prepared against malaria antigen target and conjugated lo either a liposomc containing selenium dye or gold particle in a mobile phase or reversed, where in place of monoclonal antibody lo capture the antigen, antigen is corporate into the cellulose to capture (he antibody in the serum or plasma. Our present study confirms that the immunochromatographic method represented as rapid diagnostic tests (RDTs) is the most rapid with the antibody method being the most rapid.

Microscopy is the most widely tool used to diagnose malaria at the peripheral levels. In capable hands it is very sensitive for parasitemia $\leq$ 50/ul (0.001%) [2]. And it can give important information lo the clinician like species, parasites stages and parasites density. However, Good quality of microscopy is difficult lo implement and maintain. It is labour intensive and requires highly skilled personnel and regular qualify control. The use of malaria rapid diagnostic lest (RDTs) is recommended by World Health Organisation (WHO) when reliable microscopy is not available. In non-endemic sellings, where microscopic expertise is lacking due to low incidence, malaria RDTS arc of value for diagnosis of malaria and they provide information about the involvement of /'. *falciparum.* In a recent external quality control session, *72.2%* of 1X3 Belgian laboratories offering malaria diagnosis declared lo use RDTs as a tool for diagnosis, and their use is recommended it performed in conjugation with microscopy. Also showed that *P. falciparum, P. vivax and P. malariae* showed 94.65, 92.9% and 94.7% degree of sensitivity using RDTs in malaria parasites concentration of >1000/ul, it should naturally, be expected that their sensitivity will drop to almost zero at concentration of 0.001% (<50 ul) were also microscopy should be negative.

Malaria antigens currently targeted by RDTs are histidine rich protein-2 (IIRP-2, Parasite lactate dehydrogenase (PLDH) and plasmodium aldolase (PL-aldo) [4]. Demonstrated that plasmodium species secret these proteins thus the sensitivity and specificity of RDTs arc measured based on them. P. *falciparum* has been shown lo secret lots of HRP-2 more than HRP-I and IIRP-3 whereas PLDH and PL-aldo are found in other species of plasmodium.

Membranes of erythrocytes infected with human malaria parasite *P. falciparum* develop protrusions called knobs. These structures are essential for the survival of the parasite in the host, and their induction requires the synthesis of knob protein by the parasite. These knobs are rich in histidine. Histidine existing in man as an essential ammo acid, has a positively charged imidazole functional group. The imidazole makes it a common participant in enzyme catalyzed reactions. The unportonated imidalole is nueleophilie and can serve as a general base, while the prolonated form can sever as a general acid. The residue can also serve as a role in slabili/iiig (he folded structures of proteins. The histidine found on malaria parasites is an isomer and KDTs arc sensitive only lo that of malaria (HRP-2). This type is however (bund only in *P. falciparum* while the PLDH is found in the other species. But, most RDTs will not detect the presence of malaria parasites in mixed species infection. Our RDTs method was more sensitive to HRP-2 since *P. falciparum* is not the most fatal but also the most commonly found in this part of the world, then it is Justifiable. The microscopy, the gold standard also falls in line with this view. It must however be noted that since all patients under current study had antimalaria and were all relived of their ill-health, the possibility of other species of plasmodium may exist.

My work also shows clearly that (he antigen based method has a belter correlation with both (he gold standard therefore, microscopy and the clinical settings. The antibody based method as anticipated showed good level of sensitivity but, very unspecific.

Nigeria is a malaria endemic area, so antibodies against HRP-2 may be a common finding. In our present work, it was 100% and if this is extrapolated to our larger society, it means that virtually everyone that is febrile will test positive to the antibody method.

From this project work conclusion could be drawn that the rapid diagnostic lest (RUTs) for diagnosis of malaria is as reliable as microscopy but, only the antigen based method is suitable in Nigeria and perhaps other parts of the tropics where malaria is endemic. I also recommended that only the antigen based method kits be imported and, or used in Nigeria and perhaps other parts of the tropics with malaria endemicity.

CONCLUSION

From this project work conclusion could be drawn based *on* the present study that rapid diagnostic tests (RDTs) based on malaria antigen (whole blood) method is as specific as the traditional microscopy and even appears more sensitive than microscopy. The RDTs based on antibody (scrum) method is unspecific thus it should not be encouraged. It is most likely dial Africa being an endemic region, formation of certain levels of malaria antibody may not be uncommon. The present study also supports the opinion that a good number of febrile cases is not due to malaria.

We support WHO'S report on cost effectiveness of rapid diagnostic tests but, recommend that only the antigen based method should possibly, be adopted in Africa and malaria endemic regions of the world.

REFERENCES

1. Lu, A., Oo, E., Cca, A., Pc, U., Mi, S., Pjc, N., ... & Ao, A. (2009). Effects of the methanolic seeds extract of Carica Papaya on plasmodium Berghei infected mice. *Asian Pacific Journal of Tropical Medicine*, *2*(3), 1-6.
2. Nandwani, S., Mathur, M., & Rawat, S. (2005). Evaluation of the polymerase chain reaction analysis for diagnosis of falciparum malaria in Delhi, India. *Indian journal of medical microbiology*, *23*(3), 176-178.
3. World Health Organization. (2006). WHO/MAL/2000. 1091. New perspectives in
4. malaria diagnosis. World Health organization, Geneva, Switzerland.
5. Moody, A., Hunl-cooke, A., Jabbett, H., & Chiodini, P. (2000). Pcrlbrmance of ihe optimal (R) malaria antigen capture dipstick for malaria diagnosis and treatment monitoring at the hospital for tropical diseases, London. Mr. 1. Llaemalol, 109:891-894.