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Comparative assessment of malarial diagnosis using rapid diagnostic tests and peripheral smear

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Abstract
The study was planned in Department of Pathology in Darbhanga Medical College and Hospital, Darbhanga, Bihar from Jan 2017 to July 2017. Patients found positive for the malaria were undergone the Rapid Diagnosed Tests (RDT) and Peripheral smears tests.
The detection of malarial antigens by variety of Rapid diagnostic test available is of great value especially in severe and complicated malaria wherein blood smears may be negative. Hence, this study was planned to compare the gold standard i.e. peripheral blood smear examination and the newer rapid diagnostic test (malaria plasmodium falciparum/ plasmodium vivax antigen card) to know the diagnostic accuracy of rapid diagnostic kits.
The data generated from the present study concludes that peripheral smears are considered to be gold standard for diagnosis of malaria. RDTs can be more sensitive and specific than peripheral smears. Peripheral smears is time consuming as one test requires 60minutes, in comparison to that RDT is simple more objective, requires no equipment, but only drawback is it is quite expensive. RDT does not have any subject bias whereas in microscopy the results are affected by the skill and workload of the microscopists.

Keywords: rapid diagnostic tests, RST, peripheral smear, malaria

Introduction
Early and accurate diagnosis of malaria is essential for both rapid and effective disease management and surveillance. High-quality malaria diagnosis is important in all settings as misdiagnosis can result in significant morbidity and mortality. WHO recommends prompt malaria diagnosis either by microscopy or malaria rapid diagnostic test (RDT) in all patients with suspected malaria before treatment is administered. Diagnostic testing improves the overall management of patients with febrile illnesses, and may also help to reduce the emergence and spread of drug resistance by reserving anti-malaria's for those who actually have the disease [1].
The most economic, preferred, and reliable diagnosis of malaria is microscopic examination of blood films because each of the four major parasite species has distinguishing characteristics. Two sorts of blood film are traditionally used. Thin films are similar to usual blood films and allow species identification because the parasite's appearance is best preserved in this preparation. Thick films allow the microscopist to screen a larger volume of blood and are about eleven times more sensitive than the thin film, so picking up low levels of infection is easier on the thick film, but the appearance of the parasite is much more distorted and therefore distinguishing between the different species can be much more difficult. With the pros and cons of both thick and thin smears taken into consideration, it is imperative to utilize both smears while attempting to make a definitive diagnosis [2].
From the thick film, an experienced microscopist can detect parasite levels (or parasitemia) as few as 5 parasites/μL blood [6]. Diagnosis of species can be difficult because the early trophozoites ("ring form") of all four species look similar and it is never possible to diagnose species on the basis of a single ring form; species identification is always based on several trophozoites. Plasmodium malariae and P. knowlesi (which is the most common cause of malaria in South-east Asia) look very similar under the microscope. However, P. know lesiparasitemia increases very fast and causes more severe disease than P. malariae, so it is important to identify and treat infections quickly. Therefore, modern methods such as PCR or monoclonal antibody panels that can distinguish between the two should be used in this part of the world [3].
For areas where microscopy is not available, or where laboratory staff are not experienced at malaria diagnosis, there are commercial antigen detection tests that require only a drop of blood [8]. Immunochromatographic tests (also called: Malaria Rapid Diagnostic Tests, Antigen-Capture Assay or "Dipsticks") have been developed, distributed and field-tested. These tests use finger-stick or venous blood, the completed test takes a total of 15–20 minutes, and the results are read visually as the presence or absence of colored stripes on the dipstick, so they are suitable for use in the field. The threshold of detection by these rapid diagnostic tests is in the range of 100 parasites/μl of blood (commercial kits can range from about 0.002% to 0.1% parasitemia) compared to 5 by thick film microscopy. One disadvantage is that dipstick tests are

qualitative but not quantitative – they can determine if parasites are present in the blood, but not how many.

The first rapid diagnostic tests were using Plasmodium glutamate dehydrogenase as antigen [4]. PGluDH was soon replaced by Plasmodium lactate dehydrogenase (pLDH). Depending on which monoclonal antibodies are used, this type of assay can distinguish between different species of human malaria parasites, because of antigenic differences between their pLDH isoenzymes. Antibody tests can also be directed against other malarial antigens such as the P. falciparum specific HPR2.

Modern rapid diagnostic tests for malaria often include a combination of two antigens such as a P. falciparum. specific antigen e.g. histidine-rich protein II (HRP II) and either a P. vivax specific antigen e.g. P. vivax LDH or an antigen sensitive to all plasmodium species which affect humans e.g. pLDH. It should be noted that such tests do not have a sensitivity of 100% and where possible, microscopic examination of blood films should also be performed. Malaria rapid diagnostic tests (RDTs) assist in the diagnosis of malaria by providing evidence of the presence of malaria parasites in human blood. RDTs are an alternative to diagnosis based on clinical grounds or microscopy, particularly where good quality microscopy services cannot be readily provided.

Variations occur between products, such as targets and formats, though the principles of the tests are similar. Malaria RDTs detect specific antigens (proteins) produced by malaria parasites in the blood of infected individuals. Some RDTs can detect only one species (*Plasmodium falciparum*) while others detect multiple species (*P. vivax, P. malariae* and *P. ovale*). Blood for the test is commonly obtained from a finger-prick.

RDTs are lateral flow immuno-chromatographic antigen-detection tests, which rely on the capture of dye-labeled antibodies to produce a visible band on a strip of nitro-cellulose, often encased in plastic housing, referred to as cassettes. With malaria RDTs, the dye-labeled antibody first binds to a parasite antigen, and the resultant complex is captured on the strip by a band of bound antibody, forming a visible line (T - test line) in the results window. A control line (C- control line) gives information on the integrity of the antibody-dye conjugate, but does not confirm the ability to detect parasite antigen [5].

Table 1: Comparison of Peripheral Blood Smear Examination and RDTs for Malaria

	Peripheral Smear	**Rapid Diagnostic Tests**
Format	Slides with blood smear	Test strip
Equipment	Microscope	Kit only
Training	Trained microscopist	'Anyone with a little training'
Test duration	20-60 minutes or more	5-30 minutes
Test result	Direct visualization of the parasites	Color changes on antibody coated lines
Capability	Detects and differentiates all plasmodia at different stages	Detects malaria antigens (PfHRP2/ PMA/pLDH) from asexual and/or sexual forms of the parasite
Detection threshold	5-10 parasites/µL of blood	1 00-500/µL for P. *falciparum*, higher for non-falciparum
Species differentiation	Possible	Cannot differentiate among non-falciparum species; mixed infections of *P.falciparum* and non-falciparum appear as *P. falciparum*
Quantification	Possible	Not possible
Differentiation between sexual and asexual stages	Possible	Not possible
Disadvantages	Availability ofequipment and skilled microscopists, particularly at remote areas and odd hours	Unpredictable efficiency at low and very high parasitemia; cross reactions among plasmodial species and with auto-antibodies; persistence of antigens
Status	Gold standard	Not yet approved by the FDA

The detection of malarial antigens by variety of Rapid diagnostic test available is of great value especially in severe and complicated malaria wherein blood smears may be negative. Hence, this study was planned to compare the gold standard i.e. peripheral blood smear examination and the newer rapid diagnostic test (malaria plasmodium falciparum/ plasmodium vivax antigen card) to know the diagnostic accuracy of rapid diagnostic kits.

Methodology

The study was planned in Department of Pathology in Darbhanga Medical College and Hospital, Darbhanga, Biharfrom Jan 2017 to July 2017. Patients found positive for the malaria were undergone the Rapid Diagnosed Tests (RDT) and Peripheral smears tests.

For all the clinically suspected cases of WHO defined malaria fulfilling the eligibility criteria on basis of predefined inclusion and exclusion criteria (given vide infra) were subjected to rapid diagnostic test (RDT) and peripheral smear examination.

Following was the inclusion and exclusion criteria for the present study.

Inclusion criteria: Between ages of 15years to 40 years of age were enrolled in the study. • Patients whose parents or guardians were willing to give consent were included in the study.

Exclusion criteria: Patients presenting with signs and symptoms suggestive of diseases which could give false positive card test results eg: rheumatoid arthritis, immune compromised status were excluded from the study.

Rapid diagnostic Test: The samples were randomly selected and Rapid Diagnostic test was performed using Antigen based Pf (HRP-II) and PV (pLDH) specific kit. Procedure was

performed as per manufacturer's instructions. About 5 ŀl of blood was put in sample well with the help of disposable loop provided with the kit. 4 drops of assay diluent provided with the kit was added to second well. Results were interpreted after 15 -20 minutes. Results were interpreted as negative when only control band appeared with two negative test bands and as mixed infection when control band and two test bands appeared. It was interpreted as Plasmodium Vivax infection when PV band appeared along with control band. Plasmodium Falciparum was diagnosed when Pf band and control band appeared.

Peripheral smears Test: About 20 µl of blood was used in preparing thick smears of blood in a clean slide, which were dried and stained with 5% Giemsa stain for 30 min. Dried slides were viewed at x1000 with oil immersion by the certified Medical Laboratory Scientists. Parasites were counted against 200 white blood cells (WBCs) from the thick film. Parasite density was estimated assuming total WBC count of 10,000/mL. [20] The pictures of the slides were captured and results were recorded electronically. Slides were stored in a secure slide box and were reconfirmed by another scientist. The laboratory personnel were blinded of the first response results.

Results & Discussion

Malaria is a parasitic infection of global importance and is a major public health problem in India. The surveillance activities against malaria are aimed at early diagnosis and prompt treatment of cases to reduce attributable morbidity and mortality. There are four principal methods for diagnosing malaria. These are symptomatic, microscopy, antigen test and molecular methods.

Table 1: Showing comparison of Peripheral smears and Rapid Diagnostic Tests diagnoses

Results	Peripheral smears	Rapid Diagnostic tests
Total Cases Tested	**330**	**330**
Positive cases	29	32
Plasmodium Vivax	26	29
Plasmodium Falciparum	02	02
Mixed infection	01	01
Negative	301	298

Microscopic examination remains the "gold standard" for laboratory confirmation of malaria. These tests should be performed immediately when ordered by a health-care provider. They should not be saved for the most qualified staff to perform or batched for convenience. In addition, these tests should not be sent out to reference laboratories with results available only days to weeks later. It is vital that health-care providers receive results from these tests within hours in order to appropriately treat their patients infected with malaria. A Rapid Diagnostic Test (RDT) is an alternate way of quickly establishing the diagnosis of malaria infection by detecting specific malaria antigens in a person's blood.

Accurate diagnosis and early treatment of malaria is essential to reduce mortality and morbidity due to malaria. The various modalities to diagnose malaria are conventional peripheral smear, Quantitative Buffy coat, antigen based Rapid diagnostic kits and Molecular studies (PCR). As per 2011 WHO report, the sensitivity of microscopic examination is less than 75%. It is a common practice in many parts of India to treat febrile patients with antimalarial drugs even after negative microscopic examination which has resulted in resistance to commonly used drug chloroquine. Now the concern is emergence of drug resistance to artemisinin therapy if empirical therapy is followed and this may not be cost effective also as artemesisnin is more expensive than chloroquine [6].

There are many rapid tests available on the global market, and although they have shown variable results in the past, the newer-generation assays have been refined, particularly foracute *P. falciparum* infections [7, 8]. Because *P. falciparum* malaria is the most common life-threatening infection in travellers, and because the health of infected persons may deteriorate rapidly [9-10], outpatient physicians must be able to reasonably exclude *P. falciparum* before allowing outpatient treatment in nonimmune patients. Therefore, a rapid test with a high negative predictive value would be of particular use in treating outpatients if a physician must decide in a timely manner if a nontoxic-appearing, nonimmune patient with exposure to *P. falciparum* should be hospitalized. Such a test with a high negative predictive value may fundamentally alter the current approach to treating the febrile returning traveller as an outpatient.

Evaluation of the stability level of RDTs by Bell and Peeling demonstrated that PLDH and aldolase had lower stability in comparison to PfHRP-2 Whereas the temperature also increased and immediately lowered their sensitivity in uncontrollable conditions [11]. False-negative cases were reported in 2003 (WHO), for instance, in very low level of parasite (< 100/µL), the kit is corrupted or damaged and the sensitivity is lost. Reported false-positive cases are due the presence of rheumatoid factor, incomplete treatment, and delay in clearance of blood circulating antigens that are either free or complex. Singh *et al*. by studying 344 patients with symptomatic P. falciparum and P. vivax revealed that sensitivity and specificity were 97.5% and 88% for P. falciparum and 72% and 99% for P. vivax, respectively [12].

The performance of the RDTs is reported to be influenced by a multitude of factors like the type of the parasite and the level of parasitemia; the type of test; the target antigen and the capture antibody; the expression of the target antigens on the parasites and the presence of several isomers; the presence of gametocytemia; persistent antigenemia or sequestration of the parasites; cross-reactions with other malaria species and with autoantibodies; batch quality variations in test strips; prozone phenomenon; and prior treatment. The interpretation of the color changes to identify the malaria infection is influenced by the level of training, the type of instructions, and in case of self-use, by the state of the patient. The inability to quantify and differentiate between the sexual and asexual parasitemia could pose problems in the areas of high transmission and in cases of incomplete treatment.

The sensitivity and specificity of the RDTs, and hence the diagnosis and treatment of malaria based on the RDTs, are influenced by the positive results due to causes other than malaria antigenemia, and the negative results due to causes other than low parasitemia. Therefore, the identification of the

color changes on the RDT strips may look simple but the interpretation of the result would require the knowledge of the malarial dynamics and of the possible errors with the RDTs. Otherwise, the RDTs may raise more questions than answers, and the insufficient accuracy of the RDTs could increase the number of incorrect malaria diagnoses [13].

Conclusion

The data generated from the present study concludes that peripheral smears are considered to be gold standard for diagnosis of malaria. RDTs can be more sensitive and specific than peripheral smears. Peripheral smears is time consuming as one test requires 60minutes, in comparison to that RDT is simple more objective, requires no equipment, but only drawback is it is quite expensive. RDT does not have any subject bias whereas in microscopy the results are affected by the skill and workload of the microscopists.

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