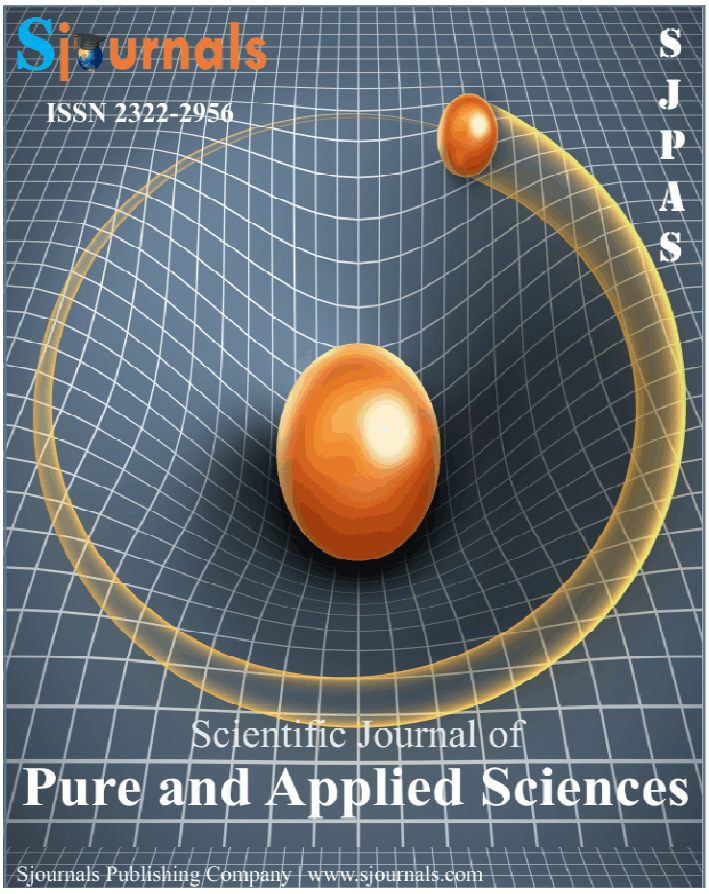

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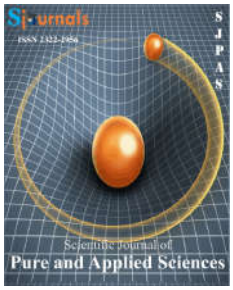

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Review article

Parasite-based confirmation of malaria with rapid diagnostic tests: Challenges and Advantages

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ABSTRACT

The key to effective case management of malaria is prompt and accurate diagnosis of malaria which is necessary to prevent morbidity and mortality. Accurate and practical malaria diagnostic such as immunochromatographic rapid diagnostic test can avert unnecessary treatments and save lives. Malaria presents a diagnostic challenge in most tropical countries. The urgency and importance of obtaining results quickly from the examination of blood samples from patient with suspected malaria is possible with the introduction of rapid malaria diagnostic test. Rapid diagnostic tests are useful in guiding therapeutic intervention, can be accessible to rural and urban populations, easy to perform and to interpret, affordable, requires no sophicated equipment and prompt treatment of malaria. The use of rapid diagnostic test in the case management of malaria has a lot of challenges which includes: persistent of histidine rich protein 2 in circulation after therapy and parasite death, effect of genetic diversity, lack of quality control on microscopy examination, poor transportation and storage condition, poor regulatory mechanism, lot-to-lot variation, parasite density and lack of extensive training prior to the use of rapid diagnostic test and supervision.

1. Introduction

Malaria is the leading parasitic disease causing about 515 million clinical cases of morbidity and mortality worldwide and 1 million deaths annually (Masaninga et al., 2013). Children in sub-Saharan Africa experience the highest burden of illness, accounting for 75% of all fatal cases (WHO, 2013). About a half of the world's population is at risk of malaria. 212 million cases and an estimated 429,000 malaria deaths were recorded in 2015 (WHO, 2016). Of these cases and deaths, the sub-Saharan region has the highest burden globally of 90% of cases and 92% of total deaths due to malaria (WHO, 2016). About 97% of the total population (approximately 173 million) are at risk of malaria infection (Dawaki et al., 2016). Also 60% of outpatient visits to the hospitals is attributed to malaria and approximately 11% maternal mortality and 30% child mortality especially among children less than five years (Dawaki et al., 2016). Five species of the *Plasmodium* species are known to cause malaria in humans i.e. *P falciparum, P vivax, P ovale, P malaria* and *P knowlesi* (occasionally by infection from plasmodium species that normally infect animals, yet there are no reports of such 'zoonotic infection' of malaria) (Herchline, 2018).

The WHO recommends prompt malaria diagnosis either by microscopy or malaria rapid diagnostic test in all patients suspected of having malaria before treatment is administered (WHO, 2009). Rapid diagnostic test is of immense importance in remote areas where there is limited access to sufficient microscopy-based diagnosis which is the mainstay in malaria diagnosis. In highly endemic areas, diagnosis based on clinical algorithm has been most employed but it has been it has been found to be unreliable and contributed to antimalarial drug wastage as a result of over-diagnosis (Oguonu et al., 2014). Accurate and prompt malaria diagnosis is a cornerstone of global control efforts. Malaria misdiagnosis in endemic areas is common, resulting in harm to vulnerable populations (Moges et al., 2012). Diagnosis of malaria must be carried out, as even a few hours delay in treatment can mean the difference between life and death. An estimate of 400 million unnecessary treatments and save 100,000 lives annually due to practical, timely and accurate diagnostic tests for malaria diagnosis have the potential to avert (Ratsimbasoa et al., 2012).

The way of presumptive treatment was easily and rapidly adopted by health workers, and this led to a situation in which the principle of proper diagnosis prior to treatment became an exception rather than the rule. The strategy of presumptive treatment of all fevers with anti-malarials leads clinicians to believe that all fevers are due to malaria, resulting in a massive over-diagnosis (Massika et al., 2006; Masanja et al., 2011), and more importantly to ignoring non-malaria causes of fever that have similar, or even higher case fatality rates than malaria (Ishengoma et al., 2011). The Lateral flow immunochromatographic devices, or rapid diagnostic tests (RDTs), offer the visibility of sensitive and specific field parasitological diagnosis of malaria through the detection of parasite antigen, such as histidine-rich protein 2. Quality assured MRDTs are reliable, simple, rapid, easy-to-use and affordable malaria RDTs allows a realistic switch from presumptive treatment to parasite-base confirmed diagnosis (WHO, 2010; Moges et al., 2012). This is especially important considering the trend of malaria decline in Africa, which leads to a strong reduction in the proportion of fevers due to malaria (D'Acremont et al., 2013). The use of malaria RDTs in the health facility has brought about the reduction in the use of antimalaria drugs. WHO policy recommends parasite-based confirmation before antimalaria drugs can be administered (WHO, 2010).

Heat stability is an important factor to be considered when selecting a RDT, as it will often be used in situations where there is little or no refrigeration. It is also important to be aware that RDTs do not give as much information as microscopy as they are not quantitative and do not indicate what stages of the parasite are present in the blood. However, the implementation of malaria RDT at scale poses many challenges (Moges et al., 2012). Rigorous procedures to train and supervise clinicians, to strengthen procurement systems and to ensure quality assurance need to be established, scarcity of health facilities and personnel (Irene et al., 2012).

1.1. Malaria case management

Malaria is one of the most important parasite disease in the world. Approximately 1.5 million deaths occur each year, in sub-Saharan Africa (WHO, 2011). Children under the age of five years and pregnant women are at risk of the disease as a result of immature and lowered immunity respectively (Ratsimbasoa et al., 2012). The WHO guidelines for the treatment of malaria recommends confirmation of a diagnosis of malaria in all suspected cases before administration of treatment. This new recommendations emphasizes the importance of high-quality microscopy or, where not available, quality-assured MRDTs. It recognizes the latter as a valid alternative to microscopy for the diagnosis of falciparum malaria infection in malaria endemic areas (WHO, 2009).

Effective case management (ECM) remains a cornerstone for reduction of malaria morbidity and mortality in sub-Saharan Africa (WHO, 2011; WHO, 2013). However, early recognition of symptoms and signs enables ECM to function effectively. These are interpreted as a malaria episode and the clinical skills of a peripheral health care worker as there are often no resources for laboratory diagnosis in most malarious endemic areas (Greenwood, 2001). Perceived fever headache are signs most health workers use to diagnose clinical malaria. However, studies in areas of intense transmission have found reported fever or a history of fever to be an unreliable indicator of clinical malaria (Luxerburger et al., 1998).

The availability of a simple and accurate test could greatly assist in the diagnosis of malaria infection especially in remote areas and endemic areas where health facility coverage is low and the population is at high risk of contracting malaria. An ideal diagnostic test for malaria must fulfill various requirements before it is of utility in the field. It must be simple, able to be performed rapidly, easy to perform and interpret, do not require electricity, do not require highly trained personnel and discriminate between *P. falciparum* and other *Plasmodium species*. The test should be inexpensive so that it could be readily used in endemic areas where the disease is prevalent. Malaria rapid diagnostic tests are based on the detection of antigen derived from parasites in lysed patient blood, using a variety of immuno-chromatographic methods (Murray et al., 2009; Batwala et al., 2011). The WHO guidelines for the treatment of malaria, which were first published in 2006, provides global, evidence-based recommendations on the case management of malaria, targeted mainly at policy-makers at country level, providing a framework for the development of specific and more detailed national treatment protocols that take into account local antimalarial drug resistance patterns and health service capacity in the country (WHO, 2006; WHO, 2010).

Volunteer community health workers (CHWs) represent a potentially valuable human resource for expanding this technology to where it is most needed, remote rural communities in sub-Saharan Africa with limited health facilities and personnel. In order to administer the RDTs, the CHWs need to be given practical training, accurate information, and standardized guidelines to ensure the safety of their patients. By training more CHWs in malarial diagnosis and treatment, CHWs could expand the number of patients reached, encourage and provide early treatment, and gain additional skills for managing this epidemic (Batwala et al., 2011). Zambia was the first sub-Saharan country to deploy malaria RDT at the national scale in 2004 followed by Senegal in 2006 Since then, several countries have adopted laboratory confirmed diagnosis, even in highly endemic areas. However, the implementation of malaria RDT at scale poses many challenges. Rigorous procedures to train and supervise clinicians, to strengthen procurement systems and to ensure quality assurance need to be established. Strong monitoring and evaluation plans need to be put in place. The impact of large-scale implementation of malaria RDT needs to be carefully assessed in different settings and health systems to ensure that it actually reduces over-diagnosis, wastage of anti-malarial drugs and prevents patient suffering.

In the past centuries, the gold standard for the diagnoses of malaria has been microscopy to visualise parasites directly in a blood film. This entails the presences of a laboratory set-up with a good and standard microscope, reagents, slides and a trained/experienced microscopist. However, in many malaria-endemic countries, most of the smaller health facilities do not have laboratories, so malaria is commonly diagnosed on clinical grounds alone. This has resulted to many people been administered unnecessary drugs, since almost all fever cases are referred to as malaria cases. Majority of endemic countries have switched their national drug policy for first-line drug to artemisinin-based combination therapy (ACT). This strategy is more expensive than before, so accurate diagnosis of malaria is very paramount in drug administration (Murray et al., 2009). Unnecessary use of ACTs has crated greater interest in the use of RDTs, which test for the presence of malarial parasites, mostly *Plasmodium falciparum,* in blood samples. Malaria high prevalence countries (health departments), have increasingly adopted the usage of RDTs. Community health workers are used as a means of distributing the RDTs, which require no lab work, could break this barrier. However, in most countries in sub-Saharan Africa, CHWs are barred from handling blood because of concerns about the accidental transmission of Human Immunodeficiency Virus. Little research has been conducted to determine the ability of CHWs to properly, safely and effectively administer RDTs (WHO, 2013)

Despite the method or techniques used for malaria diagnosis, it is very important to have quality control and assurance systems in place to ensure that diagnosis is of a high standard. However, these are the benefits to have a clear parasitological diagnosis: improved clinical management; prevention of unnecessary treatment and therefore exposure to antimalarials (therefore reducing selection pressure for resistance) and improved disease reporting (WHO, 2013).

1.2. Rapid Diagnostic Test Technique

The major target and aim of controlling malaria is prompt and accurate diagnosis. Malaria RDTs are designed predominantly for use in malaria endemic area beyond the reach of good quality microscopy. Malaria RDTs are based on the detection of antigens derived from malaria parasites in a patient blood, using a variety of immuno-chromatographic methods (Moody, 2002; Murray et al., 2009). Rapid and accurate malaria diagnosis enables effective malaria control by eliminating malaria - associated morbidity and mortality in resource poor counties. In Africa, fevers are treated presumptively as malaria in the absence of laboratory confirmed diagnosis (Albino et al., 2014) which results in the extensive over use of anti- malaria drugs (Rolfes et al., 2012).

The use of lateral flow immunochromatographic assays for malaria diagnosis in the early 1990s has improved the management of tropical febrile disease (Premji et al., 1994). A local village community health worker could for the first time, promptly, rapidly and accurately distinguish between parasitaemic and non-parasitaemic febrile illness in areas where microscopy was impractical without relying predominantly on guess work. Although antigen-detecting rapid diagnostic tests have other applications, which includes the travel health market (Whitty et al., 2000), prevalence surveys and in complex emergencies (Hashizume et al., 2006; WHO, 2005), potentially the most powerful application of this technology is providing a rapid and accurate diagnostic service for malaria where a high-quality microscopy service cannot be sustained. For more than a decade or so, these assays were used rarely in malaria endemic areas, as a means to diagnose clinical malaria. This has led to the current upsurge in the use of these RDT (WHO, 2006).

Malaria RDTs in the open market are displayed in three main formats test strips a heavy paper housing (Waverly et al., 2007) and can be classified (dipsticks), plastic - encased cassettes and card tests (is based on the analyze they detect). The two major types of malaria RDTs are the antigen detection and the antibody detection RDTs (Nana et al., 2008). Three antigens utilized in RDTs are Histidine rich protein 2 (HRP2), *Plasmodium* lactate dehydrogenase (pLDH) and aldolase. The antibody utilize MRDTs is Merozoite Surface Protein (MSP).

2. Prospects of malaria RDTs in the case management of malaria

Malaria rapid diagnostic tests render significant potential to improve the detection of malaria parasites, and are playing an increasing role in malaria case management, control and elimination. Rapid Diagnostic Tests for malaria have become increasingly recognized as an essential components of efforts to improve diagnostic accuracy in areas where a large part of the population at risk of malaria. The tests are easy to perform and provide rapid results (in 15 to 20 min) without the need of electricity, expensive equipment or extensive training. RDTs have, therefore, great potential for rapid and accurate malaria diagnosis in most malaria-endemic areas. When malaria RDTs are used correctly, it can provide a useful guide to the presence of clinically significant malaria infection caused by the species of parasites they are designed to detect. RDTs can help in case management particularly when good quality microscopy-based diagnosis is unavailable.

2.1. The prospects of malaria rapid diagnostic tests are

- Prompt and timely diagnosis
- Easy to perform and interpret
- Accessible to rural and urban population
- Requires no laboratory sophistication
- Cost-effectiveness
- Guide to therapeutic interventions
- Availability of Product testing platform for quality control testing
- Availiability of Positive Control Wells (PCWs)

2.2. Prompt and timely diagnosis

Early, accurate and prompt diagnosis and treatment of malaria with efficient drugs is required for effective malaria control. A delayed diagnosis of malaria and the resultant death of a child for whom treatment was withheld owing to a false negative RDT result will undermine the confidence of both local health workers and the community in RDT - based diagnosis, negating the potential benefits of reduce unnecessary drug use and improved management of non-malaria illness. Malaria rapid diagnostic tests play a key role in rapid diagnosis and prompt

treatment of malaria in high transmission areas where resistance to chloroquine also necessitates the use of more expensive alternative therapy (Moody, 2002; Murray et al., 2008).

Malaria rapid diagnostic test is a device that detects malaria antigen and antibody in a small amount of blood usually 5-15µl, by immunochromatographic assay with monoclonal antibodies and antigens directed against the largest parasite antigen and antibodies, and impregnated on a test strip. The result usually a coloured test line, is obtained within 5-20 minutes. The major key to effective management of malaria is prompt and accurate diagnosis of *Plasmodium species*. Late and inaccurate diagnoses are major contribution to malaria mortality and mortality from non-malaria illness, particularly bacteria diseases, that malaria symptoms can mimic (Berkley et al., 2005; Carallo, 2005; Peter et al., 2004).

Immediate, accurate and timely detection of malaria parasite using RDT would reduce the indiscriminate use of drugs and this would also reduce the development of drug resistance a potential disaster given the dwindling armoury of effective anti malarial drug available (Duong et al., 2004; Towie et al., 2006). Malaria RDT could facilitate the early detection of malaria parasites and this would lead to the administration of an appropriate therapy in patients with complicated malaria thereby reducing mortality as recorded earlier (Singh et al., 2005; Nicastri et al., 2009).

2.3. Easy to perform and interpret

An ideal diagnostic test for malaria parasite detection must fulfill certain requirements before it is of utility in the field. It must be simple, able to be performed rapidly, accurate, easy to interpret and perform, should not require sophisticated technology and discriminate between *Plasmodium falciparum* and the other *Plasmodium species* (Piper et al., 1999). The malaria RDTs have been developed in different test formats like the dipstick, strip, card, pad, well, or cassette. Cassette format malaria RDTs have provided a more satisfactory device for safety and manipulation. Cassette are easier to use and reliable than dipstick MRDTs even though is usually 10-20% higher priced than dipstick malaria RDTs. Rapid diagnostic tests are relatively easy to use with minimal training required.

Malaria RDTs are easy to perform especially among trained community health workers. A study carried out by Harvey et al. (2008) and Kyabayinze et al. (2012), revealed that trained Community Health Workers (CHWs) found it easier to carry out the MRDT than the untrained ones. Fogg et al. (2008), assessed the ease of use among three new parasite lactate dehydrogenase (pan - pLDH) tests for diagnosis of uncomplicated malaria and evaluated that there were no significant differences between the tests. Different malaria RDTs revealed little advantages or disadvantages: for examples one had individual buffer sachets, considered to be an advantage, whereas another type had a delay of 60s between the blood application and buffer application, considered to be a disadvantage. Different MRDT comes with difference in structure of blood collection device, either incomplete loops, a full loop or a micropipette, led to differences in expected safety and ease of filling and emptying the device and ease of performing the MRDT technique (Fogg et al., 2008).

Ease to perform MRDTs can be achieved based on three criteria: Obtaining blood using dropper device in accordance with manufactures instructions, transferring blood without loss and releasing blood onto the MRDT cassette, judged by a clear blood stain on the cassette pad and absence of an intact blood film in the case of the loop, dropper, glass capillary and straw (Luchavez et al., 2007).

Malaria rapid diagnostic tests results are easy to interpret unlike microscopy result that requires specialized microscopist. Interpretation of MRDT test using the manufacturers instruction leads to accurate test result and trained CHW are known to give a better interpretation. The most common mistake was to read a faint positive or invalid result as negative. A research carried out by Tavrow et al. (2000), observed that 85% of all results read by end-users corresponded to the actual result indicated on the strip and that 15% were incorrectly interpreted. These misrepresentations were significantly reduced when the inserts were redesigned with pictorial aids (Singh et al., 2005).

2.4. Accessible to rural and urban populations

Malaria rapid diagnostic tests are easily accessible to rural and urban population so far they are transported through a cool chain so that their performance wouldn't degrade due to exposure to high temperature. Malaria rapid diagnostic test have an important role to play in detection of malaria parasites in remote areas where good quality microscopy diagnosis cannot be provided. In transporting MRDTs to rural and urban population, temperature control of central storage facilities should be a basic and important requirement if storage is prolonged (WHO, 2010). In remote health facilities, simple and non-expensive evaporative cooling boxes may offer

a solution to the problem of high temperature during transportation and long-term storage, and thatch roofs are likely to be cooler than iron roof (Jorgensen et al., 2007)

Simple measures, during transport from manufacturer and within countries can help avoid exposure of MRDTs to high temperatures. These include notifying the shipper/air carrier of storage requirements, notification of arrival; avoid leaving MRDTs inside a vehicle in the sun or on an airport tarmac, and transporting the MRDTs in vehicles with air-conditioning whenever possible.

2.5. Cost

The introduction of more expensive antimalarial drugs makes an increase allocation of resources to both the development and application of accurate diagnostics a more attractive proposition, because of a reduction in drug wastage through earlier recognition of non-malaria febrile illness (Reyburn et al., 2004). Malaria rapid diagnostic tests comes in different formats namely; cards, dipstick, and cassette. Cassette formats MRDTs are usually 10-20% higher priced than dipstick MRDTs, although dipsticks sometimes require the procurer to provide wells, resulting in a similar total cost. When used by health workers, cassette MRDTs are probably more reliable than dipstick MRDTs, and so may provide savings through improved diagnosis (Agnamey et al., 2005; Yoel et al., 2008).

The cost effectiveness of MRDTs vary with malaria prevalence, MRDTs cost, cost of anti-malarial treatment, and the cost of treatment of other febrile illness when malaria has been ruled out. MRDTs become cost-effective as the price of antimalarials go up (Wongsrichanalai et al., 2007). Malaria rapid diagnostic tests can only be cost-effective if providers correctly perform all of the procedures, interpret the result accurately, and persuade clients to take appropriate actions based on the results (Tavrow et al., 2000). Initial data indicate that the cost effectiveness of rapid diagnostic tests is reasonable in an era of more expensive drugs such artemisinin combination treatment, and their use could result insignificant savings especially in areas of low transmission. (Coleman et al., 2004; Shillcut et al., 2007).

2.6. Requires no laboratory sophistication and electricity independent

Carrying-out MRDT test requires no elaborate or sophisticated equipment or technology, and can therefore be carried-out in any place unlike the microscopic method that requires sophisticated equipment and electricity (Swarthout et al., 2007). Thus, MRDT can be performed both in the rural and remote areas where malaria microscopy method in not available. Despite their apparent simplicity, previous studies indicate that MRDT will frequently be used in remote areas with little supervision or support and no sophisticated equipment is needed (Murray et al., 2008; Sina et al., 2013; Okangba et al., 2016). All it takes to carry-out MRDT is a comprehensive job aid to help the health worker performance.

2.7. Guiding therapeutic intervention

The urgency and importance of obtaining results quickly from the examination of blood samples from a patient with suspected acute malaria is now made possible with the introduction of RDT (WHO, 2000). Early detection of malaria in a febrile patient would help guide the use of antimalarials. The presumptive treatment of fever episodes as malaria results in significant over use of anti malaria and delays diagnosis of other illness (Chandramohan et al., 2002; Ndyomugyenyi et al., 2007). Therefore, an important potential gain from introducing a new diagnostic test is in reducing the proportion of febrile patient who receive unnecessary antimalarials treatment. This surely reduces the cost of giving unnecessary anti malarials, and may help avert morbidity associated with untreated non-malaria illness. A good or ideal RDTs should therefore have high specificity to avoid false-positive results that would prompt unnecessary antimalaria treatment. At the same time, it is critical that an RDT must have high sensitivity to ensure that true cases of malaria are detected and treated appropriately. Clinical diagnosis can be very insensitive, its limited specificity can lead to inappropriate treatment (Williams et al., 2009). As such clinician can no longer afford to provide treatment on the basis of clinical diagnosis alone. Therefore, MRDT plays an important role in diagnosis malaria and guiding therapeutic intervention.

In sub-Saharan Africa, management of febrile patients is typically characterized by over-prescription of antimalarial drugs (Ndiaye et al., 2009), as clinicians often do not have access to, or do not request, laboratory testing before prescribing antimalarials (no parasite confirmation before treatment) (Moon et al., 2009). Such practice were accepted, and even encouraged, when older, more affordable antimalarials, such as Chloroquine and Sulphadoxine - pyrimethamine were effective (Okangba et al., 2016). However, now that parasite resistance necessitates the introduction of new regimens such as artemisinin combination therapies (ACTs) (Agnamey et al.,

2005; Djimde et al., 2008), the strategy of presumptive treatment has become more problematic, as the new drugs are significantly more expensive and their safety profiles are not fully characterized. Use of RDTs to guide anti malarials therapy is increasingly advocated as a potentially safe and cost effective strategy for malaria case management (Sirima et al., 2009).

3. Challenges in the use of malaria rapid diagnostic tests in the case management of malaria

The differences in the performance of MRDTs may be associated to device related factors, operator factors, deterioration of the device, misinterpretation of the results and persistence related factors (Sani et al., 2013). Possible parasite factors include the level of parasitemia, variability in the target epitopes of the parasite antigen, or quantity of parasite antigen produced by the parasite or present in the peripheral blood (Baker et al., 2005). Studies have also shown that polymorphisms in the PfHRP2 protein may explain some of the variability in MRDT performance (Bell et al., 2006; Murray et al., 2008). The performance of the MRDTs is reported to be influenced by a multiple of factors like the type of the parasite and the level of parasitaemia; the type of test; the target antigen and the capture antibody; the expression of the target antigens on the parasite and the presence of several isomers; the presence of gametocytemia; persistent antigenemia or sequestration of the parasites; cross reaction, with other malaria species and with auto-antibodies and batch quality variation in test strip (Baker et al., 2005; Bell et al., 2006).

Laboratory and field trials have a major role in determining the suitability for use of the rapidly expanding range of tests available. Field trial of RDTs show high variability in performance, incorrect storage and handling, poor preparation and interpretation and sometimes poor study methods, analysis and reporting. However, malaria RDT trials face inherent difficulties in design and execution. The apparent accuracy of any RDT in detecting malaria parasites will depend on various factors, including: the concentration of the target antigen in host blood; the mechanics of antigen and antibody flow along the nitrocellulose strip; the physical condition of the RDT, including the integrity of antibodies and conjugate; the availability of target epitopes to bind antibodies in the tests (that is, variation in antigen structure); the quality of test preparation and interpretation; and the accuracy of the reference standard (Baker et al., 2005). The trial design and the interpretation of trial results must take into account the likely conditions of intended use, the limitations of comparative standards and specific characteristics of malaria epidemiology (Baker et al., 2005).

3.1. Parasite related - challenges

- Persistence of Parasite antigen in circulation after Parasite death
- Prozone effect
- Inability of MRDTs to monitor the outcome of treatment
- HRP-2 variation and its impact on MRDTs results
- The effect of Genetic diversity on MRDTs sensitivity
- Parasitaemia versus antigenaemia relationship
- Effect of stored samples on test performance
- Sequestration of *Plasmodium falciparum*
- Lack of waste management
- Irregular product supply and procurement

3.2. Persistence of parasite antigen in circulation after parasite death

Persistence of parasite antigen leads to false positive even after the clinical symptoms of malarial has disappeared and the parasite have apparently been cleared from the host (Moody, 2002). The potential disadvantage of MRDTs, is the persistence of detectable circulating antigen for up to three weeks or more after malaria parasites have been eradicated (Guthmann et al., 2002), this can reduce the importance of HRP2-based assays in areas of high malaria transmission. Plasmodium lactate dehydrogenase based RDTs are relatively less sensitive but are more specific than HRP2 ones, as the antigen is rapidly cleared from the bloodstream within seven days (Lubell et al., 2008).

Studies from Asian countries have also reported HRP2 antigen persistence after malaria clinical cure and the levels reported have varied widely, ranging from 29-65% on day-7 (Huong et al., 2005; Swarthout et al., 2007). In a

study carried out by Singh *et al.* (2005), persistent positivity of RDT was detected in 24.4% of treated patients without asexual parasitaemia on day 10. In a study, carried by Kyabayinze et al. (2009) in Uganda, longer durations (42 days) of persistent antigenicity were reported and were shown to be associated with high parasite density. In general, parasite densities are significantly higher in Africa than in Asia and may explain why similar studies in Asia have not found such a high level of prolonged false positivity. These results show that the high parasitaemia is directly proportional to persistent antigenicity and support what Swarthout and others found in the DR Congo, another hyperendemic region in Africa (Swarthout et al., 2007).

Monitoring the success of anti-malarial drug therapy particularly in the presence of fever, it is important to accurately differentiate between a new episode of malaria and another cause. Due to the increase in drug resistant infections, it has been suggested that pLDH-based tests are more useful for this purpose, since they become negative soon after parasite clearance from the blood (Murray et al., 2008). However, the presence of gametocytes could give a false-positive result, especially if high gametocytaemia persists. ACT could modify this situation because of its rapid effect and anti-gametocyte action (Mueller et al., 2007).

Humar et al. (1997), detected circulating HRP 2 antigen in 68% of treated patient on day 7, and 27% on day 28. The action of antimalaria therapy on the parasite may influence persistence of the HRP2. Schiff et al. (1993), noted that 10% of patients treated with sulphadoxine pyrimethamine had detectable HRP2 antigen on day 14. Karbwang et al. (1996), also detected persistent HRP2 antigen during and after artemether therapy, acknowledging that the HRP2 signal was of no value during the first week of treatment but appeared to be a precise indicator of treatment failure under field conditions, when it was detected on day 14 post-treatment.

Potential cause of persistent antigenemia after malaria therapy include persistent viable asexual-stage parasitemia below the detection limit of microscopy, delayed clearance of circulating antigen (free or in antigen-antibody complex (Tjitra et al., 2005), rheumatoid factors (Singh et al., 2002), and detection of circulating sexual stages in convalescence (Farcas et al., 2003).

3.3. Prozone effect

The prozone effect (or high doses-hook phenomenon) consists of false-negative or false-low results in immunological tests, due to an excess of either antigens or antibodies. Although frequently cited as a cause of false-negative results in malaria rapid diagnostic tests, especially at high parasite densities of *Plasmodium falciparum*, it has been poorly documented. Despite being frequently cited in literature on RDTs, there is only a single original report in which the prozone effect in RDT is unequivocally demonstrated by appearance of the test line upon dilution of the sample (Risch et al., 1999).

It occurs particularly in one step immunoassays, such as agglutination tests, for which serial dilutions are advised to trace the effect (Forney et al., 2001). In the case of hyperparasitaemia in RDTs, high antigen concentrations will block all available binding sites of both the detection and the capture antibodies, thereby preventing the binding of the antigen-detection antibody-conjugate complex to the capture antibody, with failure of signal generation. Simple dilution of the sample will correct this effect.

3.4. Inability of malaria RDTs to monitor the outcome of treatment

Although HRP-2 based immunochromatographic tests permit rapid diagnosis of *P. falciparum* malaria, their clinical usefulness for monitoring of the therapeutic response is limited. HRP-2 antigen is known to persist at detectable levels for more than 28 days, after therapy and disappears of symptoms (Murray et al., 2008). It cannot be useful for monitoring the response to treatment. Aldolase and pLDH rapidly fall to undetectable levels after initiation of effective therapy, but all of these antigens are expressed in gametocytes, which may appear after the clinical infection is cleared (Mueller et al., 2007). Therefore, none of these assay is useful for monitoring the response to treatment. The close correlation between pLDH detection and viable *P. falciparum* parasitaemia makes this more useful for treatment monitoring than HRP2 detecting RDTs (Moody, 2002; Bell et al., 2006), but potential detection of pLDH from gametocyte after elimination of asexual stages and inability to detect very low parasite densities limits the applicability of RDTs for this purposes.

3.5. HRP 2 variation/effect of genetic diversity and its impact on MRDT results

One possible factor contributing to variable test performance is the diversity of parasite antigens. The ability to reliably diagnose malaria infections is fundamental to both the management of individual patients as well as public health efforts to control the disease.

Variable test performance has been observed when panels of blood samples have been tested using different tests targeting PfHRP2 as well as when the same test has been evaluated in different locations (Mason et al., 2002). Although there have been reports of these MRDTs failing to detect infections with high-level parasitemia (Bechem et al., 1999; Iqbal et al., 2001; Bells et al., 2006), most of the variation has occurred with a relatively low level of parasitemia (100 to 500 parasites/µl) and misinterpretation of the test results (Mason et al., 2002; Forney et al., 2003). Possible parasite factors include the level of parasitemia, variability in the target epitopes of the parasite antigen, or quantity of parasite antigen produced by the parasite or present in the peripheral blood. Variation is likely to influence the sensitivity of HRP-2 detecting MRDTs at parasite densities below 500p/µl. Some HRP-2 detecting RDTs are likely to cross react with other HRP-2 like antigens, and this cross-reactivity may modulate the effect of HRP 2 variation or MRDT sensitivity.

Extensive variation has been reported in HRP2 structure from *P. falciparum* isolates within and between countries from which these parasites are examined, and that this HRP 2 variation, or at least the range of variation, may vary geographically (WHO, 2006). The target epitopes of most existing anti - HRP2 monoclonal antibodies (Mabs) are yet to be defined. Establishing the target epitopes and mapping these epitopes on a range of *falciparum* malaria isolates from different regions will provide useful guidance concerning the potential for existing Mabs, or combination of Mabs, to be used in MRDTs. It is also necessary to determine the appropriateness of parasites samples and isolates, and use of recombination HRP2 for assessment of MRDT sensitivity.

The extent of variation in pLDH and parasite aldolase is less well defined. Plasmodium lactate dehydrogenase are known to vary less than HRP2, but that various isomers exist in some non-falciparum parasites that many potentially influence the sensitivity of pLDH - detecting MRDTs (Baker et al., 2005). In a study carried out by Baker et al. (2005), 75 *Plasmodium falciparum* culture lines and isolates from 19 different malaria endemic countries, showed an extensive diversity of PfHRP-2 nucleotide sequences. The translated Pf HRP 2 protein sequences consisted of various amino acid repeats which vary in composition, number and order. Detection of a sub-set of these parasite isolates with two different MRDTs revealed that the detection of relatively low parasitaemias (<.250p/µl of blood, parasite/µl) was highly dependent on the Pf HRP 2 sequences, while all isolates were well detected at parasitaemias higher than 100p/µl.

It was observed that HRP2 sequence variation affects MRDT performances, and this was further supported by a study demonstrating variable reactivity of HRP2-specific monoclonal antibodies (MABs) with geographically distinct *P. falciparum* isolates and different (MABS) recognizing different epitopes. *Plasmodium falciparum* HRP2 sequences, was determined in a total number of 373 parasite samples originating from Africa, Asia, South - East Asia, the Western Pacific, South America and the Caribbean. The resulting pf. HRP2 sequences was classified according to their predicted detection sensitivity by pf HRP2 - based MRDTs , Type A and B are predicted to be well detected at parasitaemias below 250p/µl, while the probability of detection of types C and borderline below this parasitaemia is predicted to below.

In all investigated geographical areas, the major and predominant HRP2 variant was of type B. Type C and borderline HRP 2 variants represented at least 30% of the parasite samples in Asian countries and the Western Pacific, while they were much less prevalent in African (15%) and were not detected in South and Central America (WHO, 2008).

3.6. Parasitaemia versus antigenaemia relationship

The relationship between antigen concentration and parasite density can vary with the degree of sequestration of parasite, the stage of parasite growth, and the persistence of antigen after reduction or elimination of the parasite population (Lon et al., 2007). The antigen concentration of quality control (QC) samples with a given parasite density may therefore vary within certain limits, and the parameters used for preparing QC sample must take this into account. Wild parasite rather than culture parasite are used for preparing the QC sample for the same reason, as the relationship between culture parasite density and antigen concentration of the culture medium is likely to vary significantly from the expected *in-vivo*.

Variation in the structure of some parasite antigens affects binding to antibodies (Baker et al. 2005). This variation should be taken into account when interpreting failure of tests against samples with low parasite density. This can also affect the performance of RDTs. The relationship between parasite density and antigen activity vary in samples of both cultured and wild parasites. Antigen production varies with the stage of the parasite (Desakorn et al., 2005), and this is crucial for the test sensitivity of synchronous parasites samples such as those derived from culture. The differing growth conditions of cultured and wild parasites can also influence antigen production.

Antigen can be detected from sequestrated parasites *in vivo* although few parasites might be present in peripheral blood samples (Singer et al., 2004).

Various factors can affect antigen content variation at a given parasite density. Some are of a technical nature e.g. eventual effects of blood sample components (donor blood used for dilution, anticoagulants) or inaccuracies during sample preparation (Microscopy, pipetting, mixing) Antigen content is also influenced by the parasite stage (particularly in the case of pLDH), the duration of the infection, the inherent antigen expression level of the parasite and antigen variant type (in the case of HRP-2), variation in the parasite load (parasite sequestration in the case of *P. falciparum*), and persistence of antigen after parasite elimination (particularly HRP2). Standard Operating Procedures (SOPs) of the sample preparation process in on ground to minimize the effect of these factors (WHO, 2013).

At a given parasite density, the antigen concentration will depend on:

1. The total parasite load (sequestered and circulating parasites: affects *Plasmodium falciparum only*)
2. The developmental stage of parasites
3. The accumulation of persistent antigens (HRP2) with duration of infections
4. Antigen expression by the parasite; and
5. Persistence of antigen after elimination of parasites

In parasite panels, the relationship may be further influenced by technique (including microscopy accuracy, dilution accuracy and mixing). Possible variation in donor blood, possible effects of preventives or anticoagulants measurement of the relationship may be further influenced by the Enzyme Linked Immunoassasy technique (ELISA) used for quantification of antigen, and the affinity of monoclonal antibodies (Mabs) in the ELISA kit to bind with the antigen of the particular parasite (isolate or strain). Assessment of antigen concentration using quantitative ELISA on a limited number of parasite samples indicates a wider variation in the antigen - parasite relationship with HRP2 than pLDH (Baker et al., 2005).

The relationship between parasite density and antigen activity will vary in samples of both cultured and wild parasites, and this is crucial for the rest sensitivity of synchronous parasite samples such as those derived from culture. The differing growth conditions of cultured and wild parasites can also influence antigen production. Antigen can be detected from sequestered parasites *in vivo* although few parasites might be present in peripheral blood samples (Bechem et al., 1999; Desakorn et al., 2005).

3.7. Effect of stored samples on test performance

Antigen activity can be lost when blood is stored at room temperature and can still deteriorate slowly at low temperatures, although some antigen (for example HRP2) are relatively stable. Repeated freeze-thawing can accelerate antigen denaturation and change blood flow properties, but can also improve MRDT sensitivity in some cases, possibly owing to antigen release through parasite lysis. Cold blood samples can also flow differently than samples at room temperature. Prolonged storage of unfrozen blood can increase the deposition of some target antigens on the wall of containers and low absorbance containers are therefore preferable (WHO, 2004; WHO, 2008)

3.8. Sequestration of *Plasmodium falciparum*

RDT can yield an apparent “false-positive” diagnostic result in case of *P. falciparum* malaria where the parasites are sequestered out of a patient’s circulation, but circulating antigen remains in the blood, and when microscopy of peripheral blood is used as a reference standard. Although, this is unlikely to have a significant impact on a large MRDT trial, it can become significant in cases where there is sequestration of parasite in the placenta during pregnancy (Tagbor et al., 2008). In this scenario, MRDTs could potentially have significant greater sensitivity than reference peripheral blood microscopy; however, when analysed in field trials, the results varied between studies, perhaps owing to variation in the MRDT used and in the quality of the reference standard (Tagbor et al., 2008).

3.9. Regulatory mechanism - challenges

- Poor National Regulation of Manufacturer’s
- Poor transportation and storage conditions of MRDTs

- Absence of Positive Control Wells
- Suitability of MRDTs product/instruction

3.9.1. Poor National Regulation of Manufacturer's

National regulatory processes should provide safeguards for the safety and effectiveness of RDTs used nation-wide. Lack of governmental regulatory requirements for RDTs in developing countries is of great challenge to the use of RDTs in malaria diagnosis, unlike the tightening of governmental regulatory requirements for drugs in developing countries has done much to improve the standardization and quality of drug trials, in which efficacy and adverse effects are assessed and compared.

Unfortunately, regulatory standards are often lacking for diagnostic tests especially those targeting diseases that are uncommon in industrialized countries. As a result, diagnostic tests are often sold in the developing world without any formal evaluation of their performance and effectiveness.

WHO/TDR conducted a global survey of regulatory practices for diagnostic test in 2001. A questionnaire was sent to all 191 WHO member states to enquire whether clinical trials were required for regulatory approval. Of the 85 countries that responded, less than half (48%) reported that they regulated *in vitro* diagnostic for infectious disease (Cunningham et al., 2004). A greater number of countries in the developed world regulated *in vitro* diagnostic compared with the number of the developing world.

Even when clinical trials are mandated by regulatory authorities, there is lack of national and international guidelines for the evaluation of diagnostic tests for disease that are prevalent in developing countries standards for the evaluation of diagnostic tests are set by regulatory bodies such as US food Drugs Administration (FDA), National Agency for Food and Drugs Administration Control (NAFDAC).

Data from clinical/field trials designed to evaluate the performance characteristic of diagnostic tests are often found on product inserts or they remain in the company files. In many countries the lack of regulatory oversight on the design and conduct of diagnostic evaluation has led to inflated claims of test performance in product inserts.

This underscores the need for a set of international standards to regulate MRDT diagnostic for malaria diseases. There are plans for international standards for regulatory approval of malaria diagnostic tests of public health importance in the developing world are still in the distant future.

3.9.2. Poor transportation and storage conditions of MRDTs

The storage and use of MRDTs in remote areas presents a new challenge to many health systems. These devices, with limited shelf life and subject to degradation by the environment, are increasingly being deployed to remote and poorly resourced areas where users have limited supervision. In these settings, health personnel relies on the RDTs to make potentially life - saving treatment decisions.

Like other biological tests, MRDTs are degraded by heat and moisture and gradually deteriorate, even in ideal conditions. However, poorly controlled transport and storage conditions, and infrequent re-supply, necessitate a high level of reliability and stability. Most MRDTs manufacturers specify storage at less than 30 ^{0}C, but exposure to temperatures above this level is often unavoidable in tropical endemic areas Exposure of MRDTs to high temperature pose a great challenge in the use of MRDTs in endemic areas and has the potential to degrade MRDTs. Most manufacturers recommend, storage between 4 ^{0}C-30 ^{0}C, and shelf life is based on this assumption but refrigeration and air-conditioning is commonly unavailable in malaria endemic areas where MRDTs are intended for use (Jorgensen et al., 2006). A study carried out by Jorgensen et al. (2006), observed that most MRDTs transported from the manufacturers to the end-users where exposed to excessive heat during transportation. Exposure of MRDTs to more extreme conditions can be expected in other malaria endemic areas, for example, northern parts of Nigeria where seasonal temperatures can reach 45-50 ^{0}C, and exposure to such conditions may lead to reduced sensitivity or failure of MRDTs (Mason et al., 2002).

The susceptibility of immunochromatographic devices to degradation by heat and moisture mandates the need to avoid exposure to high temperature and damage to packaging during transport and storage. Freezing (e.g. during air transport) may also degrade some MRDTs. Such environmental exposure may have been responsible for low sensitivity reported in field trials and operational use (Murray et al., 2003). Malaria rapid diagnostic tests are to be treated with the care normally used for biological substances, used for diagnostic and therapeutic purposes. This demands the followings:

1. Careful coordination of transport to avoid unnecessary delays in customs, on airport tarmac or in non-air conditioned road transport
2. Avoidance of exposure to direct sunlight
3. Use of air-conditioned storage where possible
4. Temperature monitoring of storage facilities
5. Rejection of RDTs where packaging is significantly damaged and it is likely that moisture - proofing of envelopes or canisters is lost

Chemical-based temperature monitor presently used from vaccine transport, could be developed for use with MRDTs. It is recommended that manufacturers consider the inclusion of temperature-monitors in RDT boxes. WHO, should consider this further and assess likely costs of inclusion. The health service designated person responsible for overseeing Quality assurance (QA) of MRDT should coordinate the organization of "cool chain" transport and storage. Although longer storage, times are required for MRDTs to the end user, coordination of delivery to these areas with vaccination delivery should be possible.

3.9.3. Manufacturers of malaria RDTs

Manufacturers of MRDTs are faced with a number of challenges in producing high quality products. These include lack of established standards for sensitivity, specificity, stability and lack of access to good quality reference materials for use in assessing compliance with such standards. National regulatory authorities in endemic countries face similar problems in designing and implementing appropriate quality (WHO, 2007) Procuring agencies require high quality comparative performance data to determine appropriate products for the intended area of use.

3.9.4. Absence of positive control wells with MRDTs

Malaria rapid diagnostic tests are a potential breakthrough in the provision of accurate diagnosis in remote areas, but wide scale use is hampered by uncertainty over accuracy under field conditions. Positive control wells, which contain recombinant malaria parasite antigen, are a novel method for addressing this need for quality assurance. The potential of a commercially available positive control well, reconstituted with blood, was assessed for use in routine monitoring of MRDT sensitivity in a remote malaria-endemic region. When maintained at 4 ^{0}C, the wells produced a consistent level of parasite lactate dehydrogenase antigen activity, as detected by pLDH-detecting MRDTs, but activity reduced after cumulative exposure to temperatures likely to be encountered over a few months in a malaria-endemic area. This limitation was successfully overcome in the field through centralized, controlled storage.

Monitoring of MRDT sensitivity was successfully incorporated into routine supervisory visits to remote clinics. However, improved temperature stability of the wells would enhance their potential, even though they are still under construction. The threshold at which the wells' signal reduced MRDT sensitivity requires further investigation. The wells shows potential to overcome an important obstacle to the wide implementation of accurate parasite-based diagnosis and appropriate treatment. Further assessment of their place in malaria management is warranted (Lon et al., 2005).

Maintaining transport and storage of MRDTs less than 30 ^{0}C may be difficult in many malaria endemic countries and in field trials in the tropics and for many end-users, health workers who will be using the products operationally (Jorgensen et al., 2006). Therefore, apart from ensuring and monitoring proper storage, it is very important to constantly test the sensitivity of the MRDTs. Test wells containing recombinant parasite antigen are commercially produced and undergo stages of development, whereas comparison with microscopy in-representative sentinel sites may be a useful alternative (Lon et al., 2000).

3.9.5. Training and choice of technicians

End-users of MRDTs are likely to be remote health workers often volunteers with limited training. The technicians performing evaluations are commonly highly trained, experienced malaria control personnel. The performance of technicians in a trial environment can therefore differ significantly-positively or negatively from that of the intended end-user. Preparation and interpretation can also be affected by manual dexterity, visual acuity and available lighting. To perform the tests in a realistic test environment, trials should document the training and previous experience of technicians using either the intended end-users.

Various studies have documented significant variation between technicians both RDT preparation and interpretations (Funk et al., 1999; Kilian et al., 1999; Trachler et al., 1999; Fryauff et al., 2000; Jelinek et al., 2000; Tavrow et al., 2000; Whitty et al., 2000; Bell et al., 2001; Mayxay et al., 2004; Rennie et al., 2008). The accuracy of MRDTs can be affected by incorrect blood volume and reagent (buffer) volume. Blood volume and antigen load are expected to affect the sensitivity of MRDTs, while excess blood volume may cause background staining and obscure weak test (Luchavez et al., 2007). Therefore, using separate technicians for different products in comparative MRDT studies can bias the results. Multiple blinded readings and/or rotation of technicians will reduce the likelihood of bias. The duration of time between preparation and reading must be documented.

Various blood-transfer devices are provided with MRDTs. As the accuracy and consistency of performance of these device can vary, the mode of blood transfer can cause these variations.

3.9.6. Suitability of the MRDT product/instruction

Trials aimed at assessing the local suitability of products must address the suitability of the instructions and the technical requirements of the product to the proposed end-users and to patients. As test sensitivity is dependent on user technique this information is important in assessing MRDTs suitability (Bell et al., 2001; Mayxay et al., 2004). The quality of product instructions and training can be documented quantitatively and qualitatively.

4. Conclusion

Malaria rapid diagnostic tests are increasingly important aspect of malaria case management in remote rural settings where good and adequate microscopy is difficult to maintain. Malaria rapid diagnostic tests also provide an alternative to microscopy for inexperienced personnel. Malaria rapid diagnostic tests are relatively simple to perform, do not require electricity and provide results quickly for making treatment decisions. However, the accuracy and application of MRDTs results depends on several factors such as quality of the MRDTs, storage, transport, end-user performance and manufacturing defects. Malaria diagnostic tests needs to be highly accurate because false negative and false positive diagnoses have medical, social and economical consequences such as prolongation illness, increase in morbidity and mortality and loss in credibility of health services.

Malaria rapid diagnostic tests offers a realistic practical chance to move the diagnosis of malaria away from the laboratory and nearer to the patient. The assurance that life-threatening parasitaemia with *P. falciparum* will not be missed is welcome, particularly for inexperienced laboratory staff during night calls. The ability to detect the majority of the non-*falciparum* malaria cases also makes these tests ideally suited as major back-up procedures for malaria diagnosis.

Currently, WHO (2010), recommends parasite-based confirmation for all cases of suspected malaria before the administration of antimalarial. Malaria RDT is an effective tool in parasite-base diagnosis and hence, must be quality assured RDTs. Despite, this, Malaria RDTs devices have limitations, such as batch-to-batch quality variation, species and density determination, persistent positivity, accuracy, poor storage facility and transportation and operational issues. Other issues include prozone effect and rheumatoid factors. The genetic diversity of PfHRP2 and its relationship with the ability of the Mabs to bind presents also a serious challenge to the future testing and development of MRDTs that target HRP2 for the diagnosis of *P. falciparum* infection. And the issue of reported false negative results with MRDTs in the presence of high parasitaemia. The usefulness of MRDTs in higher transmission areas of Africa remains to be determined, and comparative cost effectiveness studies are needed.

Malaria rapid diagnostic test storage and distribution should include a quality assurance system including monitoring of sensitivity, a cool chain where possible, appropriate instructions and training, and supervision. The cost-effectiveness of rapid tests should be evaluated locally prior to widespread adoption. Test performance, under field conditions, should be evaluated prior to adoption, and if possible, each batch should be evaluated in a reference laboratory. Assays may be susceptible to heat and humidity. Inclusion of temperature monitors to control temperature is a basic requirement if storage is prolonged. Simple measures during transport from manufacturers and within countries can help avoid exposure of MRDT to high temperatures.

In conclusion, malaria RDTs are important because, while presumptive treatment of fever with malarial treatments has led to a decline of malaria in many areas in African countries, misdiagnosis of fever can prove extremely harmful. “With policies that recommend presumptive treatment of fever, health workers and caretakers are less likely to look for other causes of fever, leading to delay in appropriate treatment and higher case fatality rates among non-malaria fevers than in malaria fevers.” Moreover, presumptive diagnosis may lead to the

unnecessary, excessive use of expensive drugs and the development of drug resistance. As a result, RDTs are being used as an alternative to microscopy testing to improve diagnosis of febrile children in areas where malaria is prevalent.

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