Chapter

Prompt and Accurate Diagnosis, A Veritable Tool in Malaria Elimination Efforts

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Abstract

The concept of malaria elimination is to get rid of local transmission of malaria parasites in a defined geographical area. Among the measures required for malaria elimination is prompt and accurate diagnosis. Malaria diagnostic tools currently in use: clinical diagnosis, Malaria Rapid Diagnostic Tests (mRDT) and molecular diagnosis, have limitations. Clinical diagnosis can be used as first step in making prompt malaria diagnosis, but cannot confirm cases. Malaria RDTs satisfies the need for prompt diagnosis but has low accuracy in confirming cases. Accuracy of microscopy depends on making good blood films, and accurate film interpretation. Molecular diagnosis required for species-specific diagnosis of malaria parasites, and determination of genes that confers drug resistance to *Plasmodium* species is not available for routine use. As part of elimination efforts, there is development of mRDT kits that utilize urine or saliva instead of blood specimen, microscopy digital image recognition and different technologies for molecular diagnosis. So far, none of these diagnostic tools has satisfied the need for prompt and accurate diagnosis. It is therefore recommended that more than one diagnostic tool is needed for malaria elimination to be achieved in a given area. This will ensure early detection and treatment of cases, as well as prevent the re-establishment of transmission.

Keywords: malaria, elimination, malaria diagnosis, clinical diagnosis, rapid diagnostic tests, microscopy, molecular diagnosis

1. Introduction

Malaria in humans is a parasitic disease caused by four species of *Plasmodium*: *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*; with increasing recognition of enzoonotic transmission of a simian species, *P. knowlesi*. In most cases, the malaria parasites are transmitted through the bite of infected female anopheline mosquitoes. The mosquitoes carry infective sporozoites of malaria parasite in their salivary glands, and they inoculate or transfer these sporozoites into the blood stream of humans during blood meals [1]. After inoculation, the individual(s) is /are said to be infected with malaria parasites. The disease, malaria begins once (i) the asexual parasite multiplies within the red blood cells and (ii) the host produces some immunological response against the parasites. On rare occasions, malaria parasite infection can be acquired by transfusion of blood from a malaria patient, also known

as transfusion malaria; transmission of infection to foetus in uterus through the placenta, also known as congenital malaria and by the use of contaminated syringes particularly among drug addicts [2].

Malaria results in a wide range of outcomes and pathologies with severity of the disease ranging from asymptomatic infection to rapidly progressive fatal illness. Generally, malaria is characterized by many signs and symptoms: fever, chills, headache, nausea, vomiting, sweating, chest or abdominal pain, diarrhea and cough. Severe infection may result in serious complications such as severe anemia, algid malaria, spleenomegaly, cerebral malaria, black water fever, hypoglycaemia, complications in pregnancy, respiratory distress, metabolic acidosis, pulmonary oedema, concomitant pneumonia, shock and coma [3]. Death due to malaria can occur as a result of delay in diagnosis and treatment of infected individuals. Thus malaria has remained a major threat to public health despite decades of control and elimination efforts.

2. Diagnosis of malaria

Prompt and accurate diagnosis is important in malaria elimination efforts. This will help in early detection of malaria cases before complications set in. Also, malaria cases are separated from other diseases that present with the same symptoms as malaria especially in cases of co-infections (with diseases such as typhoid fever, HIV, hepatitis, Covid-19 e. t. c.). Incorrect diagnosis of malaria will not only waste resources, but also delays treatment thereby resulting in poor outcome of patients. It may also contribute to increasing resistance of malaria parasite to available anti-malaria drugs, there by frustrating malaria elimination efforts. To achieve malaria elimination, different diagnostic tools are required. The malaria diagnostic tools could be broadly classified as provisional or clinical diagnosis and parasite-based diagnosis. These diagnostic tools have been used over the years; nevertheless, they have their limitations.

3. The use of clinical diagnosis or provisional diagnosis to achieve malaria elimination

Clinical diagnosis is achieved based on signs and symptoms presented by a patient. This is because the release of malaria antigens, pigments and toxins give rise to a cascade of pathological events. The earliest symptoms: headache, fever, body pains and chills could be used in making prompt diagnosis for malaria parasite infection. [4] reported that 27.0% of mothers and caregivers practice home management of malaria because of the need for prompt diagnosis and treatment. The most commonly recognized and used symptom for clinical diagnosis is fever [5]. It has been used in making provisional diagnosis for malaria parasite infection in Nigeria and other malaria endemic regions. It gives prompt indication of malaria parasite infection in such area. A study in Nigeria reported 83.2% cases of malaria parasite infection among patients that presented with fever [6]. Another study in Southwestern Nigeria reported 66.8% cases of malaria parasite infection among febrile patients [7]. Even though these findings confirm that not all fever conditions can be attributed to malaria, but then a significant number of individuals would have been treated of malaria based on fever outcome. However, this may only be achievable in malaria endemic area because clinical diagnosis alone is not reliable and cannot be used to confirm malaria parasite infection. Its uncertainty and varied results makes it an inaccurate method for achieving malaria elimination. In malaria

endemic areas, under reporting of malaria cases (with inclusion of non-cases (false positive cases) and exclusion of actual cases (false negative cases)) using clinical diagnosis alone is most likely. A typical example can be presented by using the data in the work of [6]. In that study, 350 patients were examined for malaria parasite infection and 200 (57.14%) cases were confirmed using microscopy. However, clinical diagnosis showed 149 (42.57%) cases; 124 (35.43%) actual cases, 25 (7.14%) false positive cases and 76 (21.71%) missed cases or false negative cases. In the work of [8], clinical diagnosis recorded 74.30% prevalence of malaria as against 95.33% recorded with microscopy. This makes it clear that in malaria endemic areas, one can only use clinical diagnosis as a first step in making prompt or early malaria diagnosis and not to confirm malaria cases. In non-endemic areas, over reporting of cases may occur because diseases other than malaria may be the cause of common clinical signs observed in that area. Clinical diagnosis is therefore recommended for use alongside a parasite based diagnostic method.

3.1 Limitations of using clinical diagnosis in malaria elimination

There are issues in the use of clinical diagnosis alone to achieve malaria elimination.

a. Using clinical diagnosis alone, asymptomatic cases are unreported especially in *P. vivax* and *P. ovale* malaria where high parasitaemia is a prerequisite for mild and severe disease [9]. It is also applicable with *P. malariae* infection that is rarely fatal and the blood stage of the parasite persists for up to 40 years. In a study, [6] reported 37.8% malaria prevalence among non-febrile patients. This is to show that there might be missed cases of malaria parasite infection in a given population if one relies on clinical diagnosis alone.

b. There are inconsistencies in the choice of symptom for clinical diagnosis. While some people may focus on fever alone, some other may focus on chills, stomach upset, vomiting, body ache and headache [5]. This brings about uncertainty with this diagnostic tool.

c. The symptoms of malaria are non-specific. This makes precise calculation of malaria burden difficult especially in co-morbidity states. This may result in either under treatment of malaria cases or over treatment of malaria cases; none of which is good for malaria elimination. For example, [10] reported a case of *Plasmodium vivax* malaria and COVID-19 co-infection in Qatar. He presented the difficulty in distinguishing COVID-19 from malaria because COVID-19 presents with a variety of clinical manifestations including, but not limited to, fever, cough, diarrhea, vomiting, headache, myalgia and fatigue. This case was identified during the time of COVID-19 pandemic and it was possible that some other cases like this existed but more attention was given to COVID – 19 at the expense of other diseases including malaria. This is a typical scenario that could lead to missed cases of malaria, and malaria positive individuals still remaining in the environment serve as a source of infection and reinfection of other susceptible.

In the same way, malaria may be over reported and/or given more attention at the expense of other diseases in malaria endemic areas. In the work of [11], 155 participants tested for malaria and typhoid fever infection shows there were 64 (41.7%) cases of malaria and 60 (38.0%) cases of typhoid fever, with 40 (25.0%) cases that were mixed infection. If we look critically in that finding,

24 cases were malaria only whereas 20 cases were typhoid fever only. Then the total number of persons that would have been reported to have malaria based on clinical diagnosis alone is 84 (24 malaria only cases +20 typhoid fever only cases +40 cases of mixed infection). The implication will be excessive and indiscriminate use of antimalarial. When such happens, some strains of malaria parasites with genes that confer resistance to commonly used antimalarial will emerge and bring about treatment failure. This will definitely frustrate malaria elimination efforts because researchers will need to go back to the laboratory to develop another antimalarial that can take care of the emergent strain of malaria parasite. Of course, the researchers will not be able to develop a new product without identifying and characterizing the malaria parasite strain, using a parasite based diagnostic method.

4. The use of parasite-based diagnosis to achieve malaria elimination

Parasite based diagnosis is required to provide accurate result needed to achieve malaria elimination. The malaria case management guidelines released by WHO in 2009 recommended prompt parasitological confirmation of diagnosis either by microscopy or malaria rapid diagnostic test (RDT) in all patients with suspected malaria before treatment is administered [12]. The above statement supported the role of clinical or provisional diagnosis in making prompt and accurate diagnosis of malaria. Early indication of cases through clinical diagnosis (promptness) facilitates the use of parasite based diagnostic method (accuracy). On the other hand, malaria parasite infection has been reported among non-febrile individuals [6] and in line with making prompt diagnosis, frequent check-up or routine laboratory test using parasite based diagnostic method is required. This is to ensure that asymptomatic individuals are captured early enough before complications due to malaria set in. Parasite based diagnosis can also be achieved using molecular techniques.

5. The use of malaria rapid diagnostic test (mRDT) to achieve malaria elimination

The World Health Organization recommends the use of mRDTs as a good alternative method for malaria diagnosis. As such, it has become a primary tool for parasitological diagnosis or confirmation of malaria. Malaria Rapid Diagnostic Tests (mRDT) are immuno-chromatographic tests that target certain proteins produced by *Plasmodium* species. There are different brands of mRDT kits. They include: SD Bio Line®, Tell®, First Response®, Sky Tech®, optiMAL®, Care Stat®, Paracheck®, Marrow Care®, Binaxnow® Malaria, Fyodor® and so on. They come in different test formats: strips, cassette etc., for ease of use and safety. These mRDT kits detect specific antigens (proteins) produced by malaria parasites in the blood of infected individuals. To achieve malaria elimination, the commercial test kits are manufactured with different antigens targeted to suit the local malaria epidemiology of a given area [13]. Rapid Diagnostic Test (RDT) kits most widely used for malaria are based on the detection of the following target antigens of the parasite: Histidine-Rich Protein II (HRP2), *Plasmodium* Lactate Dehydrogenase (*P*LDH) and P-Aldolase [14]. Some of these antigens could be excreted in the urine or are found in the saliva of sick or infected individuals. Thus one may require any of blood, urine or saliva as specimen for conducting the test, depending on the mRDT brand in use. Nevertheless, blood has been the most widely used specimen because that is where the highest concentration of the malaria parasite antigens are found.

Most commonly used RDT devices have two bands (a control line and a test line) and are designed to detect *P. falciparum*. The test line targets either histidine-rich protein-2 (HRP-2) or *P. falciparum* specific parasite lactate dehydrogenase (*Pf*-pLDH), depending on the RDT brand. However, RDTs that distinguish *P. falciparum* from the three non - *P. falciparum* species are available. Such RDT device may have either three bands (a control line and two test lines) or four bands (a control line and three test lines). In the case of RDT test device with three bands, one test line targets *P. falciparum* specific antigen; the second test line targets antigens common to the four species, such as pan-*Plasmodium*-specific parasite lactate dehydrogenase (pan-pLDH) or aldolase. In the case of RDT test device with four bands, the third test line targets *Plasmodium vivax*-specific parasite lactate dehydrogenase (*Pv*-pLDH).

In general, HRP2-based mRDTs are more sensitive and stable than mRDTs based on other *Plasmodium* antigens, and so are the mRDTs of choice in most endemic countries where *P. falciparum* malaria predominates [15]. *Plasmodium* lactate dehydrogenase (*P*LDH) which is another antigen detected by mRDT has been less widely used but have higher specificity, mostly due to a much shorter time to become negative [16]. Emphasis on the malaria antigen (HRP2) being stable when excreted in urine, may have encouraged [17] to experiment using urine specimen to conduct malaria rapid diagnostic test with mRDT kit manufactured to use blood specimen. In that study, they found that mRDT using urine specimen could serve as a practical method for detection of malaria parasites even though the sensitivity is dependent on the level of parasitaemia. It was based on this, that Fyodor®, a non-invasive dipstick test that uses monoclonal antibody to target *P. falciparum* protein (Highly repetitive cognate poly histidine-rich protein2 (HRP2) excreted in urine was developed. Fyodor® has been reported to demonstrate nearly equivalent performance compared to available blood-based RDT for the diagnosis of malaria [6, 18, 19]. Fyodor® was found to be useful in malaria diagnosis with 76.9% sensitivity and 82.5% specificity, even when the parasitaemia level is as low as 260 parasites/μl of blood [6]. In addition to Fyodor®, [20] produced a prototype saliva-based RDT for *P. falciparum* gametocyte detection in carrier individuals. This is still in line with the efforts to advance malaria elimination strategies.

RDTs as diagnostic tools for malaria are simple to perform and provide quick result. It is a simple and fast way for health workers to test for malaria parasites in a patient's blood. It is easy to learn and people living in rural areas can access and use this form of test because it does not require electricity or any other special equipment. It is also simple to interpret the test result. Some (that require urine or saliva as specimens) are not invasive and so can be used closer to homes. Thus, it was developed for use in areas where the only realistic alternative is clinical diagnosis especially to tell if a feverish condition is caused by malaria parasite. This satisfies the need for prompt diagnosis in malaria elimination efforts. However, a major concern in the use of malaria RDTs is its low accuracy in reporting of malaria cases. In both malaria endemic and non-endemic areas, under reporting of malaria cases (with inclusion of non-cases (false positive cases) and exclusion of actual cases (false negative cases)) is likely. Using the same data in the work of [6], blood based mRDT reported 77 (22.0%) cases; 71 (20.29%) actual cases, 6 (1.71%) false positive cases and 129 (36.86%) missed cases or false negative cases. On the other hand, urine base mRDT reported 39 (11.14%) cases; 36 (10.29%) actual cases, 3 (0.86%) false positive cases and 164 (46.86%) missed cases or false negative cases. It could be deduced from the above that mRDT is more specific in reporting malaria cases than clinical diagnosis, even though its sensitivity seems low.

Using mRDT requires training of health workers on the importance of continuous quality control monitoring for the mRDT kits while they use them [21, 22].

The training should be carried out in connection with the brand of mRDT being used. The quality control can be achieved by checking its expiration date, storage conditions and using microscopy and/or PCR to assess the performance. In molecular epidemiology studies to assess the performance of mRDTs, RDT used strips or cassettes can be used as a source of DNA for molecular detection of malaria parasites [23].

5.1 Limitations of mRDTs in malaria elimination effort

Many factors may affect the performance of malaria RDTs in achieving malaria elimination. These include:

a. Number of different species of malaria parasites infecting a host: Some RDTs can detect only one species (*P. falciparum*) while others detect multiple species (*P. vivax*, *P. malariae* and *P. ovale*) of malaria parasites. Even with mRDTs that detect multiple species of malaria parasites, the species are poorly classified as *P. falciparum* and non *P. falciparum.*

b. This test does not also detect actual parasite, rather it detects parasite antigens; this makes it difficult to accurately identify the species of malaria parasites present or prevalent in a given locality. The RDTs indirectly tells whether a patient has malaria parasites are present or absent. Also, RDTs cannot be used to quantify malaria parasites that are present in the blood.

c. Level of parasitaemia: High level of parasitaemia is normally required for recording high level of sensitivity with mRDTs. This has been shown with the significant weak positive correlation between malaria parasite density of microscopy result and mRDT results as reported by [6]. It then makes the test limit of detection for RDTs low; as a result, false negative results occur. In the work of [7], 32.8% false negative results were recorded. The implication is that if such RDT is used alone for malaria diagnosis, approximately 33 in every 100 malaria cases will be missed and malaria elimination will be hard to achieve.

d. Variability in parasite antigenic structure: There is variability within the parasite antigen being detected by the mRDTs, which also leads to false negative results. While false-negative mRDT results have been attributed primarily to the tests' limit of detection, [24, 25] have confirmed that genetic variation of *P. falciparum* can also affect mRDT performance. The variability may be due to the (i) presence or absence of the target epitope, (ii) variation in the number of epitopes present in a particular parasite isolate [26]. Genetic diversity among malaria parasites may be particularly important for *Pf*HRP2-based RDTs, since the antigen consists of a number of alanine- and histidine - rich amino acid repeats [27] that vary in size among malaria parasite strains [28]. Comparison of the *Pf*HRP2 sequences from several parasite strains has shown differences in the number of tri- and hexa- peptide repeat units and rare amino acid variants [27, 29, 30]. An additional report showed that the amino acid sequence of *Pf*HRP2 in a Chinese isolate was different from that in South American (7G8) and Gambian (FCR3) isolates [31].

e. Persistence of the malaria antigens in the blood after treatment, thereby giving false positive results with mRDTs. False positive results occur because the mRDT devices can still detect the antigens of malaria parasites after the patient has been treated due to the persistence of HRP2 antigen in the blood for several days after infection. The device tests positive to infection when the patient is

actually free from the parasite infection. For instance, [7] reported 4.4% cases of false positive result with HRP2-based mRDTs. This limits their specificity and usefulness in accurate reporting of malaria cases.

f. Problem of storage. Even though RDTs are recommended for use in places electricity is poor or not available, it can be damaged by heat and humidity when not stored properly (in a cool dry place at a temperature of 2 °C to 8 °C). [32] reported that exposure to high temperature can damage the nitrocellulose membrane of the RDT test device or denature the antibodies in the test membrane, thereby causing poor performance of RDTs in the tropics. The effect is mostly high level of false negative results which implies high number of unreported malaria cases.

6. The use of malaria microscopy to achieve malaria elimination

Malaria is commonly diagnosed using microscopy of stained blood films. Although the use of rapid diagnostics test for malaria diagnosis is on the increase, microscopy has remains the gold standard and as well serves as a reference standard in the evaluation of new tools for malaria diagnosis [33]. Venous blood or capillary blood can be used for malaria microscopy. This method involves the staining of thick and thin blood films on a clean grease free glass slide, to visualize the malaria parasite. Thick blood film is used for malaria parasite detection while the thin blood film is used for malaria parasite species identification. Commonly used stains for the preparation of blood films are Giemsa stain, Leishmann stain, Fields stains A and B. The standard procedure for preparation and examination of blood films for malaria parasite detection and species identification are contained in the documents by [33, 34]. A good binocular (compound or digital) microscope is required for accurate reading of the blood films.

In malaria endemic areas, routine microscopy is usually done using binocular light microscope. Microscopy is cheaper than the use of RDTs, considering the cost of consumables alone. Using microscopy, malaria parasite stages are seen and can also be counted. Microscopy has the advantage of providing a quantitative assessment of peripheral blood parasitaemia and parasite stages as well as information on the other blood components [35]. Thus it gives accurate diagnosis of malaria when properly done. Due to its conventional use it helps to reduce cases of wrong administration of anti-malaria drugs to patients exhibiting non-malaria fever as a symptom. In Tanzania, this diagnostic method has help to reduce the wrong administration of malaria drugs to patients with non-malaria fevers [36].

The use of microscopy in malaria diagnosis will require continuous training, practice and experience. There is also need for assessment and supervision of microscopists (slide readers) for quality control of their test results. This is because detection, identification and quantification of malaria parasites using microscopy will depend on making good blood films, having a good microscope and accurate film interpretation by the laboratory scientist/technician. For improved microscopy needed to achieve malaria elimination, there is development of microscopy digital image recognition using artificial intelligence [37–43]. The concept is that a standard blood film is prepared using Giemsa stain or field stain. After that, a digital microscope or imaging scanner is used to capture the image viewed under the microscope. The captured image is stored in a computer and is used as an input for the image-recognition algorithms that will extracts useful visual features to locate, identify and count the malaria parasites. This is with the view to achieve better microscopy result because of inherent human factors in blood film preparation

and interpretation. When developed and certified, it will open new possibilities for automated recognition of malaria parasites in standard blood films and thus eliminate uncertainty about the quality of microscopic diagnoses worldwide.

6.1 Limitations of microscopy in malaria elimination

The challenges in the use of microscopy for malaria elimination are stated below:

a. Malaria microscopy requires specialized personnel for accurate detection and identification of malaria parasites. This is particularly important especially when the level of parasitaemia is low or in cases of mixed infection with different *Plasmodium* species. It's low sensitivity at low parasitaemia is a problem and can implicate the use of RDTs for false positive result.

b. Compared to mRDTs, this method is labour intensive and time consuming. Delay in its use may occur during blood film preparation or examination. In some cases, one may even have to prepare a new slide where poor staining or loss due to breaking of a slide occurs.

c. Variability in blood film preparation techniques and reading skills can account for inaccurate results. It may be due to slide differences because variation of parasite density within slides occurs even when prepared from a homogenous sample [44]. Accuracy of results obtained through microscopy also depends on individual technician performance in blood film preparation and examination, thereby making its standardization difficult.

d. Non accessibility of this method by rural areas due to lack of facilities is also a major issue. In malaria endemic countries where greater efforts are required for elimination, poor and high cost of electricity reduces the frequency of microscopy use for diagnosis. There could be a delay in providing results for patient especially when the blood films have to be made repeatedly.

e. Poor quality stains sold in the market can affect the quality of the thick and thin films, there by affecting the accuracy of the result.

f. The technology for automated microscopy using image-recognition algorithms has not been fully developed and certified. More so, the data base of the digital image to be analyzed is not yet robust to contain any distortion or unusual presentation of the malaria parasites in the blood films.

7. The use of molecular diagnosis to achieve malaria elimination

Molecular diagnosis of malaria is a laboratory techniques developed to detect and characterize the malaria parasites. It may not be used in routine diagnosis because of cost and time; but its use has increased the analytical sensitivity of assays for malaria parasites. In places where treatment failure occurs and drug resistance is suspected, molecular diagnosis is required. It is needed for species specific diagnosis of malaria parasites, determination of genes that confers drug resistance to the *Plasmodium* species and in selecting treatment options. For instance, there is growing concern on the antigen variability expressed by *P. falciparum* the most prevalent malaria parasite species in the Sub-Saharan Africa that accounted for 99.7% of estimated malaria cases in 2018 [45].

Molecular diagnostic tool has the ability to detect low level parasitaemia and allows accurate identification of malaria parasite species. It is therefore a confirmatory test used for the diagnosis of malaria when microscopy shows negative result. Molecular diagnosis can also detect mixed infection [46] and identify asymptomatic malaria carriers who may be targeted for treatment [47]. With molecular diagnosis, large number of specimens can be processed simultaneously using standard protocols and equipment [48]. In the general protocol, DNA of *Plasmodium* species is extracted using DNA extraction kit from a given manufacturer. The extracted DNA is subjected to selective amplification of the target gene. This can be achieved by using more than 65 primer sets with at least five molecular targets that can be used to test as many as five human *Plasmodium* species: *P. falciparum, P. ovale*, *P. malariae*, *P. vivax* and *P. knowlesi* [49]. After the DNA amplification, electrophoresis is run and the bands interpreted in order to know the result of the test [50]. This basically explains Polymerase Chain Reaction (conventional PCR, nested PCR, real-time PCR) and Restriction Fragment Length Polymorphism (RFLP) as diagnostic tools for malaria. Polymerase Chain Reaction (PCR) was used to confirm parasitaemia using *P. falciparum* Merozoite Surface Protein 2 (MSP2) as a marker while Restriction Fragment Length Polymorphism (RFLP) was used to identify *P. falciparum* Sulfadoxine – Pyrimethamine (SP) resistance molecular markers at codons 51, 59, 108, 164 of dihydrofolate reductase (dhfr), and codons 437, 540, 581 and 431 of dihydropteorate synthetase (dhps) genes [51]. Nested-Polymerase Chain Reaction (nPCR) was used to detect *P. falciparum* DNA in blood and saliva of febrile patients in Cameroon [52].

Other molecular diagnostic techniques includes: Loop – Mediated Isothermal Amplification (LAMP), Flow Cytometry (FC) assay techniques, Nucleic Acid Sequence Based Amplification (NASBA), Luminex Xmax Technology and so on. These techniques can greatly improve detection, species-specific identification and precise parasite count by using species-specific primers or probes [49]. Loop-Mediated Isothermal Amplification (LAMP) technique detected the conserved 18 s RNA gene of *P. falciparum*, *P. vivax*, *P. ovale*, *P. malaria* [46] and *P. knowlesi* [47]. Lamp has certain advantages compared to conventional PCR in the diagnosis of malaria because of its ability to perform the reaction and react without opening the tubes. It also has potential application for clinical diagnosis and surveillance of infectious diseases without the need for sophisticated equipment and skilled personnel in developing countries. Flow cytometric technique was developed to detect and quantify *P. falciparum* in the laboratories [53]. Flow cytometry was proposed as a malaria rapid diagnostic tool that counts the number of parasites and evaluate the malaria infected red blood cells [54]. FC is a very powerful tool in malaria research because it can use the nucleic acid content to identify various developmental stages of *P. falciparum* without being impaired by changes in the morphology of the parasite developmental stages [55]. Nucleic Acid Sequence Based Amplification (NASBA) is a homogenous, sensitive, isothermal and transcription based amplification system that uses three specific enzymes (Reverse transcriptase from avian myeloblastosis virus, T_7 RNA polymerase and Ribonuclease H) [56]; and do not require expensive thermal-cycling equipment [57]. Prevalence and density of *Plasmodium* gametocytes has been determine using quantitative NASBA (QT-NASBA) that has the ability to detect as low as 0.02–0.1 gametocytes per microliter of blood [58]; it is also used increasingly to detect both *P. falciparum* and *P. vivax* gametocytes [59]. Luminex Xmax Technology approach is very useful for the diagnosis of parasitic diseases and it is based on bead flow cytometry assay. It has been used to detect all the blood stage of the four human *Plasmodium* species [60]. Luminex Technology can improve the speed, the accuracy and reliability of other PCR methods because it eliminates the need for gel electrophoresis, and samples can be handled simultaneously and continuously through 96-well plate format from DNA extraction [61].

To enhance malaria elimination, molecular diagnosis should be used alongside microscopy in the quality control (QC) of mRDTs. With proper storage and handling of mRDTs, the QC may have two foci in determining sensitivity and specificity of mRDTs: (i) the ability of the test devices to detect malaria parasite if present in a blood specimen. Here, different drops of blood from the same specimen are used to perform the two tests respectively. (ii) the ability of the test devices to detect malaria parasite if present in the same drop of blood collected from a specimen. Microscopy alone can be used to achieve item (i) and not item (ii), since the same drop of blood used for microscopy cannot be used for mRDT. One can pick a drop of blood for Microscopy and it contains malaria parasite and another drop picked for mRDT from the same specimen may not contain malaria parasite antigen; and vice versa. Even, two slides prepared from the same blood specimen may not have the same microscopy readings. This is an issue relating to non-homogenous distribution of malaria parasites and the probability of picking malaria parasite in a drop of blood from a given blood specimen. Non-homogenous distribution of malaria parasites in the blood also affects PCR as much as microscopy and mRDTs. Nevertheless, PCR is a more robust tool for QC of mRDTs since it can achieve items (i) and (ii). To achieve item (ii), already used Rapid Diagnostic Test cassettes or strips are sources of DNA to detect malaria parasite by PCR [23]. Using the same cassette or strip, one can score the mRDT device based on what it picks from the specimen, rather than scoring it from another blood sample of the same specimen as is the case with microscopy. This approach is good for the overall quality control of mRDTs i. e. control for sensitivity, specificity, expiration, storage and handling.

7.1 Limitations in the use of molecular diagnosis for malaria elimination

a. Molecular diagnosis is still confined to few and special laboratories, thus it is not available for routine use.

b. PCR molecular assays are not feasible for field settings as it can be contaminated easily there by affecting the accuracy of the test result.

c. PCR also requires post amplification protocols like electrophoresis and it takes 3–4 hours in order to know the result [50]. Even the real-time PCR (with reduced risk of contamination) developed to replace the conventional nested and semi-nested PCR is difficulty and time consuming [62]. In other words, it does not give prompt diagnosis.

8. Conclusion

A significant challenge in the global malaria elimination effort is inadequacy of the tools needed for prompt and accurate diagnosis. In asymptomatic condition, clinical diagnosis lacks both promptness and accuracy. In symptomatic condition, clinical diagnosis provides prompt indication of malaria but still lacks accuracy. Following routine or frequent check-up in asymptomatic condition, Microscopy and RDTs which are the most widely used parasite based diagnostic tools techniques provides more prompt and accurate result than clinical diagnosis. In symptomatic condition, Microscopy and RDTs provides accuracy to complement the promptness of clinical diagnosis. Between the two, RDT provides more prompt diagnosis than microscopy whereas microscopy provides more accurate result than RDT. Molecular diagnosis is not prompt, but has higher level of accuracy needed to detect, identify and characterize malaria parasite.

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No one diagnostic tool has satisfied the need for prompt and accurate diagnosis. For malaria elimination to be achieved, serious attention should be given to the performances of the diagnostic tools. Where one tool fails, another one should be used to complement, rather than replace it. Clinical diagnosis is the basis for suspected malaria cases, even though routine check-up is highly encouraged. Confirmation of cases should be done using parasite based diagnostic tools. There is also need for scale up on the quality of parasite-based diagnostic methods in use. It is therefore recommended that more than one diagnostic tool is needed for malaria elimination to be achieved in a given area.

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