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REVIEW

Expanding the malaria molecular diagnostic options: opportunities and challenges for loop-mediated isothermal amplification tests for malaria control and elimination

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ABSTRACT

Introduction: The loop-mediated isothermal amplification (LAMP) technique holds substantial promise as an alternative easy-to-use molecular test for malaria parasite detection. Several modifications to the initial malaria LAMP assay have been made in an effort to make the LAMP platform more field-friendly.

Areas covered: A PubMed literature search was performed using the following search terms: 'malaria,' 'loop mediated isothermal amplification', 'LAMP', 'molecular tests' and 'diagnostics'. The authors review the currently reported malaria LAMP assays and discuss what requirements would be needed to make malaria LAMP assays field-usable, especially in the context of malaria elimination.

Expert commentary: Expanding the malaria LAMP tests as options for use in malaria control programs will require addressing some important challenges such as the need for simplified sample preparation steps; ready to use kits that require no cold chain; the use of a non-subjective results readout and preferably cost-effectiveness. Two malaria LAMP kits are now CE-marked and commercially available: the Loopamp MALARIA kit and the *Illumigene* malaria LAMP. Malaria LAMP tests, like other molecular tests, will likely be utilized in very specific studies such as: to evaluate 'detect and treat' strategies; in controlled malaria infection trials or drug efficacy trials and as confirmatory test in reference laboratories.

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Malaria; diagnosis; elimination; LAMP; molecular tests; loop mediated isothermal amplification

1. Introduction

1.1. Global malaria situation

Malaria continues to be a major global public health threat. According to the World Health Organization (WHO) World Malaria Report, 2016, a total of 212 million new cases of malaria and an estimated 429,000 malaria deaths (range 235,000–639,000) were reported worldwide in 2015 [1]. These statistics represent a 21% decrease in the incidence of malaria and 29% decline in malaria mortality rates globally since 2010. Moreover, 5 countries eliminated malaria between the years 2007 and 2015, while 13 others reported zero indigenous cases during this period and another 10 countries are targeted for elimination by 2020 (http://www.who.int/malaria/areas/elimination/overview/en/). However, the 2017 world malaria report shows that after this unprecedented period of success in the global fight against malaria, progress has stalled [2]. While progress was made with existing malaria control interventions, new or improved diagnostic tools will help move the elimination process forward, as they will better enable the tracking of malaria infection in communities and help target interventions particularly in low-endemic settings and pre-elimination areas. In addition, whenever possible, appropriate tools to detect low-density malaria infection in non-endemic regions are needed to ensure prompt treatment and prevent reintroduction of malaria in countries with little to no malaria transmission. As reviewed by Landier et al., the early diagnosis and treatment of malaria infections is a mainstay of malaria control programs and the backbone of the elimination programs [3]. Typically, symptomatic persons will seek diagnosis and would receive treatment, therefore decreasing potential for continued transmission. However, individuals with asymptomatic infections are unlikely to seek diagnosis and thus not treated for their infection. These asymptomatic infections are often shown to be of low-density infections and are capable of contributing to ongoing transmission. Due to this potential problem, low-density, asymptomatic infections are of heightened importance in elimination programs. To address the problem of asymptomatic carriers require detect-and-treat approaches such as active case detection (ACD) [4] such as mass testing and treatment (MTaT) and focal testing and treatment strategies [5,6] and reactive case detection (RCD) studies [7] rather than relying solely on passive case detection. These approaches, however, require highly sensitive diagnostic tests.

1.2. Current malaria diagnostics

A summary of some malaria diagnostic tests is shown in Figure 1. The diagnostic toolbox for malaria has not changed dramatically over the decades: microscopy is still commonly utilized in many malaria-endemic countries for both clinical management and surveillance. Microscopy, when correctly used, is a valuable diagnostic tool for malaria diagnosis as it is the best method to

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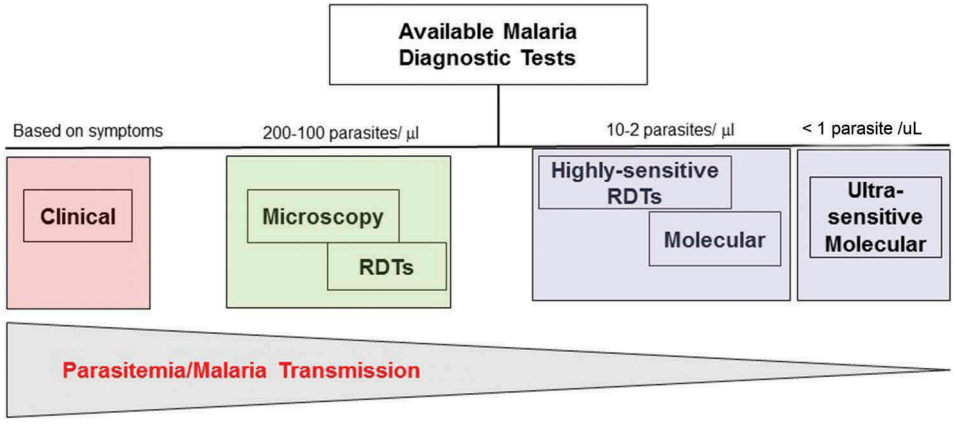

Figure 1. Diagnostic tools for malaria with changing transmission intensity.

The availability of rapid diagnostic tests (RDTs) for malaria diagnosis led to the paradigm shift from clinical diagnosis to a parasitological diagnosis of malaria prior to treatment. However, as transmission intensity declines or as more cases of low-density infections occur, more sensitive diagnostic tools will be required

identify different morphological stages of asexual blood-stage parasites and gametocytes. Furthermore, microscopy can be used to quantify parasite density. The operational sensitivity of microscopy in clinical settings is estimated to be between 50 and 100 parasites per microliter although expert microscopists can detect as low as 30 parasites per microliter [8]. Major drawbacks for microscopy, however, are that it requires very well-trained and experienced laboratory staff, a strong quality assurance and quality control system, and continuous supervision and retraining of staff, all of which are difficult to implement and assure in many malaria-endemic countries.

The availability of rapid diagnostic tests (RDTs) for malaria diagnosis led to the paradigm shift from reliance on clinical diagnosis to confirmation of a parasitological diagnosis of malaria prior to treatment as the standard of care for malaria case management being recommended by WHO since 2010. The use of RDTs averts overtreatment and has become a practical test, especially for use in Africa where the malaria burden is greatest. The operational sensitivity of RDTs has been estimated to be in the same range as that of microscopy, approximately 100 parasites per microliter, and although this level of sensitivity is appropriate for case management, it is not sufficient to detect low-density infections. In low-endemic settings, particularly in pre-elimination areas, low-density parasitemia at levels of around one to five parasites per microliter are common, and these are well below the limit detectable by microscopy and current RDTs. This is with the exception of the ultra-sensitive Alere malaria RDT that was recently made available for commercial use. This histidine-rich protein 2 (HRP2)-based RDT was reported to be 10 times more sensitive than the current HRP2-based RDTs, making it possible to use it for the detection of low-density infections [9] . However, another limitation of RDTs is the fact that many of them are only capable of detecting *Plasmodium falciparum* and non-falciparum (using the pan-antigens) infections, and the specific detection of the other species is limited. Recently, a bead-based immunoassay, capable of detecting HRP2 at sub-picogram levels and with high specificity, was described [10]. This 96-well assay was shown to be cost-effective as it allows for the fast processing and screening of large numbers of samples and has potential for use in the detection of low-density infections likely in surveillance studies.

Nucleic acid-based tests (molecular tests) for malaria parasite detection have been shown to have high sensitivity and specificity. Different types of malaria molecular tests have been developed which include conventional gel-based PCR, many flavors of real-time qPCR, and even an ultra-sensitive high-throughput (HTP) test [11]. These different tests and an extensive discussion on the role of molecular tests in the changing landscape of malaria transmission have been discussed in several reviews [12–15] to which the reader is directed. In the 2014 WHO Evidence Review Group on Malaria Diagnosis in Low Transmission meeting, it was deemed that a molecular test (or any other non-molecular test) must be able to detect two parasites per microliter or fewer to be a 'significant improvement' over expert microscopy [16]. Molecular tests are well suited for detecting low-density infections as many of them have limits of detection equal to or less than two parasites per microliter. The high sensitivity of many molecular tests makes them the tests of choice for ACD and RCD approaches because RDTs or microscopy is incapable of detecting low-density infections. However, traditional PCR-based molecular tests require sophisticated laboratory infrastructure and therefore cannot be easily adopted for point-of-care settings or for field use in endemic countries. The advent of loop-mediated isothermal amplification (LAMP)-based tests presents the opportunity to develop molecular tests for clinical diagnosis in point-of-care settings as well as for use in a field environment as discussed further in this review.

2. Expanding the malaria molecular diagnostic options: the evolution of the malaria LAMP assays and platforms

Unlike PCR-based molecular tests, which require alternating temperature conditions, the LAMP assays amplify nucleic acids at a constant temperature (isothermal), typically around 62–65°C. As reviewed by Han et al. [17] and Oriero et al. [18], the malaria LAMP technique holds substantial promise as an

alternative molecular test for malaria parasite detection because it can be carried out using limited laboratory infrastructure and many of the described assays have similar sensitivities to PCR. It is likely that for clinical management of microscopically detectable levels of infection associated with acute malaria illness, both microscopy and conventional RDT [16] will continue to be the tests of choice. Therefore, in endemic countries, the role for molecular tests in malaria control and elimination will likely be to enable the detection of low-density infections for program use as opposed to the clinical management of malaria [16,19]. Therefore, LAMP-based tests and other nucleic acid amplification assays may be relevant for clinical diagnosis in non-endemic countries, focusing investigations in elimination settings, and detecting infection in high-risk groups such as pregnant women.

2.1. Desirable characteristics of a malaria LAMP assay

It is important that any malaria LAMP assay be an improvement to the currently available diagnostic tests. Therefore, meeting the two parasites per microliter limit of detection recommended by WHO [10] is paramount. Several of the more than 26 malaria LAMP assays developed to date meet this limit of detection [1,15–18]. For many of the assays, the limits of detection are not clearly defined, although a majority of the limits of detection reported are between one and five parasites per microliter (reviewed in [13]). High sensitivity alone does not make a test the optimum choice for use in all malaria endemic regions. Several technological modifications are required to make a test easy-to-use, rapid, and practical anywhere where it is needed while achieving its intended purpose. A good example of such a platform is the RDT that requires no technological infrastructure and is simple to use and rapid. Since the first malaria LAMP assay was described over 10 years ago, Abdul-Ghani et al. posed the question: 'Loop-mediated isothermal amplification (LAMP) for malarial parasites of humans: Would it come to clinical reality as a point-of-care test?' [20]. Several modifications to the initial LAMP assay [21] have been made by various investigators in an effort to optimize LAMP as a field-friendly molecular assay (Figure 2). In addition, two field-stable malaria LAMP kits are now CE-marked (certified to conform to health, safety, and environmental protection standards for products sold within the European Economic Area) and commercially available: the Loopamp MALARIA kit (Eiken Chemical Co) and the ***Illumi****gene* malaria LAMP (Meridian Bioscience). These efforts have moved the LAMP platform closer to the end user and are making the LAMP assays viable alternatives to the more sophisticated PCR assays. Some characteristics of the current malaria LAMP assays that make them field-friendly are reviewed below.

2.1.1. The use of a simplified sample preparation step

A key hurdle to making molecular tests field-friendly has been the sample preparation step: extraction of nucleic acids (DNA or RNA) from the blood samples (filter paper or whole blood). This relates to the need to achieve a balance between greater sensitivity of assays performed on larger volumes of whole blood, especially for low-density parasitemia not detectable by microscopy or conventional RDTs, and ease of sample collection, storage, and transport afforded by filter paper samples. Many of the conventional DNA extraction methods are time consuming (1 to 2 h) and require additional equipment such as water baths or heat blocks and centrifugation steps. The Eiken Loopamp Pan/*P. falciparum* method utilized by Aydin-Schmidt et al. [22] and Perera et al. [23] represents an effort to simplify this step by use of a vacuum-driven 96-well plate setup. Some assays have utilized the simple boil and spin method of DNA extraction [24–27]. While these options are simple, they still require a heat-block, a vacuum pump, and centrifugations steps, which in turn require a constant power supply. Sample preparation steps that require little to no manipulation to obtain amplifiable DNA/RNA for use in the LAMP test will facilitate its applicability in resource-limited settings. Efforts to simplify sample preparation have included use of a simple gravitation system that employs lysis buffers and columns in the ***Illumi****gene* malaria LAMP assays [28,29]. In this approach, whole blood is mixed with a lysis buffer and either used directly in the LAMP assay (simple filtration assay)

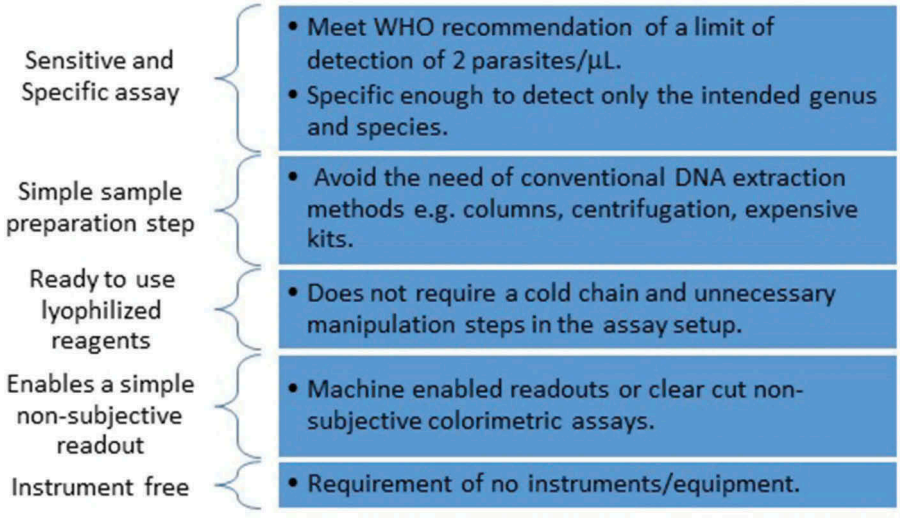

Figure 2. Desirable characteristics of a Malaria LAMP assay.

or passed through a column that purifies the DNA via gravity (***Illumi****gene* malaria PLUS). Recently, the use of unprocessed whole blood for the detection of *Plasmodium* spp. using the lyophilized CZC-LAMP kit was found to have high sensitivities in a field evaluation in Zambia [30]. However, the limit of detection of the CZC-LAMP was not reported. Nevertheless, this assay was shown to have superior sensitivities to the Hexagon Malaria Combi™ RDT test (Human Gesellschaft fur Biochemica und Diagnostica, Wiesbaden, Germany) used as a reference test.

2.1.2. Availability of ready-to-use kits that require no cold chain

Ready-to-use kits for malaria LAMP tests in which the required reagents (buffers, Bst polymerase, and primers) are premixed are ideal as they reduce the number of steps to be carried out in the assay setup, thus saving time and avoiding potential contamination opportunities. The requirement of a cold chain to store and retain the integrity of the LAMP reagents greatly limits the use of many of the LAMP assays described to date, and kits are particularly appealing if they do not require a cold chain. Only a few of the malaria LAMP assays exist with ready-to-use lyophilized reagents which are stable when stored at room temperature: the two commercially available LAMP assays are Loopamp Malaria Pan/Pf kit from Eiken Chemical Company, Japan, and ***Illumi****gene* Malaria LAMP assays from Meridian Biosciences. DNA is added directly into the tubes and the LAMP assay performed as per protocol. Another format of 'ready-to-use' LAMP was recently described, initially for the detection of Human African Trypanosomiasis, in which the LAMP primers and a fluorescence indicating dye are dried in the bottom of PCR tubes and the Bst polymerase and dNTPs on the PCR tube lid. The stability of these reagents was tested for up to 7 months at 40°C; there was no significant loss of sensitivity [31]. A similar format was subsequently shown to be successful for detection of malaria parasites [30].

2.1.3. Generation of a simple, nonsubjective results readout

A feature of the LAMP chemistry is the fact that amplification of the nucleic acids is typically accompanied by the formation of white precipitate of magnesium pyrophosphates that can be visualized with the naked eye as turbidity in the positive tubes (negative tubes remain clear). Turbidity has been used as a readout in many LAMP assays, especially when the assay was still novel [21,24,32]. However, because of the potential subjectivity of using turbidity as a readout, several colorimetric- [33–35] and fluorescence-based [27,30,36–38] assays have been developed. Fluorescence-based assays require specialized equipment such as the handheld LED illuminators [30], the use of UV light-source to enable the readout of the fluorescence dyes, or the use of the ESE-tube scanner to read the fluorescence intensity in real time [27,36]. Equipment such as turbidimeters have been used to objectively quantify the turbidity after the LAMP run [25,26]. Recently, a malaria LAMP assay that uses a lateral-flow dipstick (LFD) to allow visualization of the LAMP results was described [39,40]. The use of the LFD requires manipulation of the LAMP products post-amplification and the use of specialized reagents to visualize the results, thereby increasing the complexity and potential risk for contamination. The use of specialized equipment for the visualization of the LAMP results (readout) reduces the versatility of the LAMP technique as a simple tool for use in endemic countries and tends to increase the total cost of the LAMP assays. Colorimetric approaches for LAMP product readout can help overcome this limitation; however, these need to provide a clear, nonsubjective result. LAMP colorimetric assays have utilized dyes, such as the malachite green which produces a blue-green color in positive samples and is colorless in negative samples [35]; hydroxynaphthol-blue which turns from violet to blue in positive samples [33,34,38,41], and SYBR Green I (naked eye) [42].

2.1.4. Instrument-free LAMP

The success of the lateral-flow immunochromatographic malaria RDTs lies in the fact that these are completely instrument- and electricity-free. They can, therefore, be performed in any setting where they are required. Many of the malaria LAMP assays described to date use some type of equipment to perform the amplification and, as described above, to visualize LAMP results. These steps, amplification and readout, are often performed using a single equipment, or the amplification is carried out in a simple heat-block and the results are visualized using a separate instrument. To date, no malaria LAMP assay has been described that is completely instrument free except the non-instrumented nucleic acid (NINA)-LAMP assay when combined with a visual readout [43–45]. The NINA-LAMP was evaluated in a field setting in 2015 in Ethiopia [44] and for the detection of imported malaria cases in Canada [45]. A complete visual inspection of turbidity or color (as recently described for the diagnosis of filarial infections [46]) with no additional instruments is an option to couple with the NINA-LAMP assay to make it a completely instrument-free assay. While this may come with the disadvantage of subjectivity (as described above), adequate test accuracy was achieved by Mohon et al. who reported only one discrepant readout out of the 140 tests performed in which two of three readers agreed [45], implying that visual inspection of turbidity might not be a problem.

2.2. Additional considerations for a malaria LAMP assay

2.2.1. HTP assays

A platform that enables the processing of a large number of samples easily and with a fast turnaround time to results would be the ideal HTP test. Only a few HTP malaria LAMP assays have been described: two colorimetric HTP LAMP assays; the HtLAMP by Britton et al. capable of evaluating 96 samples simultaneously and is both genus specific (detects all *Plasmodium*) and species specific for *P. falciparum* [34], *P. vivax* [33], and *P. knowlesi* [47]; and the genus-specific malachite green LAMP described by Lucchi et al. [35], capable of testing 40 samples simultaneously. Recently, the previously described PURE extraction system [25,26] was modified to enable the processing of 96 whole-blood or dried blood spot samples followed by the performance of the Eiken Loopamp Pan/Pf LAMP (32 samples per run) [23]. This improvement in

conjunction with a LAMP assay that can test a large number of samples simultaneously will help move the LAMP tests for use in large-scale surveillance studies.

2.2.2. Cost of the malaria LAMP test

Only a few malaria LAMP assays described to date have provided a cost associated with the test; reported costs range from $0.11 to $5.05 per sample [34,35,45]. The least-expensive LAMP test appears to be the NINA-LAMP assay with a startup cost of $13 for the NINA device and a $0.11 cost per sample [45], a cost that is competitive with both RDTs and microscopy. However, due to the basic requirements needed to perform a LAMP assay (e.g. need for Bst polymerase, buffers, and heating device), a LAMP test will be most likely be more expensive than both microscopy and RDTs. Since cost can be a determining factor of whether or not to utilize a test, the advantages of using a more expensive LAMP test as compared to microscopy and/or RDTs will have to be considered.

3. Where does LAMP fit in the changing landscape of malaria transmission?

The role of malaria molecular tests will likely be to enable the detection of low-density infections for research, for the clinical diagnosis in non-endemic countries, to focus investigations in elimination settings, and for detecting infection in high-risk groups such as pregnant women, use as opposed to the clinical management of malaria in endemic countries.

3.1. Detection of imported malaria cases

The rise in globalization and immigration has led to an increase in the movement of people across the globe, which has resulted in an increase in the number of malaria cases reported in previously malaria-free or non-endemic countries [5–7,48–51]. Malaria in these countries is typically due to imported malaria cases in travelers, immigrants, or people returning from visiting friends and relatives in endemic countries. A majority of these infections (especially in the USA, Canada, and Europe) are caused by *P. falciparum* and originate from Africa [6]; however, non-falciparum imported malaria has also been reported [6,52]. The prompt detection and treatment of imported malaria infections in malaria-free countries or regions are an important part of malaria control and elimination agenda among other required approaches that need to be considered [53]. In most countries, the diagnosis of imported malaria cases is performed using microscopy or the country-approved RDTs. It has been proposed that given the lack of experience in malaria microscopy diagnosis by laboratory technicians living in non-endemic countries, the accurate diagnosis, especially of non-falciparum malaria, can be compromised [52]. Accurate diagnosis is further complicated by the fact that the majority of malaria RDTs available are based on the *P. falciparum*-specific HRP2 combined with a *Plasmodium* pan antigen (typically lactate dehydrogenase [LDH] or aldolase) which do not distinguish among the non-falciparum species. Because of these factors, the use of molecular tests to diagnose imported malaria cases may be an appropriate alternative.

The Eiken Loopamp Pan/Pf LAMP assay was shown to be a sensitive and specific alternative assay for the diagnosis of imported malaria cases in the UK [26]. Recently, the ***illumi****gene*-malaria LAMP tests were utilized in Canada with high sensitivities and specificities [29]. Several European laboratories that respond to suspected imported malaria cases have also investigated the use of the ***illumi****gene* malaria LAMP assays with promising results (unpublished data), and the NINA-LAMP assay was shown to be a highly sensitive and specific point-of-care tests for the diagnosis of imported malaria [45]. In all these studies, the malaria LAMP tests were shown to be both easy to perform and cost effective compared to the traditional PCR-based assay, but with similar sensitivities and specificities, making the LAMP platform a viable option for use in the diagnosis of imported malaria cases. These commercially available malaria LAMP tests can be used by anyone who can afford to buy the kits. This will likely be diagnostic laboratories in developed countries for the diagnosis of imported malaria cases or private diagnostic clinics.

3.2. Detect and treat studies

The call for malaria elimination, encouraged by the declining prevalence and transmission of malaria [1], has led to the development of detect-and-treat approaches such as ACD including MTaT and focal testing and treatment strategies [54,55] and RCD studies [56]. Many of these studies rely on the use of RDTs or microscopy for screening and identifying infected individuals who will be targeted to receive the intervention (treatment). However, these tools are incapable of detecting low-density infections known to be a key reservoir of infection in both low- and high-transmission settings [57–59] and reviewed by [60]. Therefore, their use in detect-and-treat approaches may compromise the potential gains of such programs in that persons with undetectable/asymptomatic infections will not receive the interventions and may continue to transmit malaria [59]. This was demonstrated in a study carried out in Zanzibar, where malaria elimination is targeted in the near to mid-term, in which treatment of the RDT-positive individuals was not shown to reduce subsequent malaria incidence compared with control areas, probably because RDT greatly underestimated the proportion of individuals with low-density parasitemia [61]. Operationally, a highly sensitive RDT test would be an ideal test for this purpose; and one such test has been developed. However, this test relies on detecting the HRP2 protein, which, in some countries, is reported to be missing in some parasite populations including some in Africa [62–64]. This test may therefore have some limitation for use in such settings. Again, simpler and highly sensitive molecular assays such as LAMP could provide viable alternative tests for research and program use [22,65–68]. Meeting the requirements outlined in Figure 2 will make the current LAMP assays operational for use in field settings. Some of the HTP LAMP assays described to date provide practical alternatives for detect-and-treat studies as they allow for the processing of larger numbers of samples and require no additional equipment to determine the results [22,23,33–35,47]; however, further

improvements such as lyophilized kit formats and simpler sample preparation steps will be required.

3.3. Special situations

Finally, the availability of a reliable, easy-to-perform malaria LAMP assay can provide a test of choice for case management in facilities that lack skilled microscopy or quality microscopes or in regions where the use of HRP2-based RDTs is inappropriate due to the reported high levels of HRP2 and HRP3 deletion and where the second-generation RDTs that utilize *P. falciparum* LDH antigen are not available.

4. Conclusion

New tools to support the fight against malaria towards elimination are needed [19]. These should include more accurate, sensitive, specific, and faster detection tools and finding new ways to access the hardest to reach and highest risk populations in overcrowded and resource-limited settings. This will require addressing the highlighted desired characteristics of a malaria LAMP assay for use in any malaria control program. In addition, further field evaluations will be required to establish the utility of any malaria LAMP assays as malaria control and elimination tools, which is an important step in moving any test/assay from a research tool to a public health tool.

5. Expert commentary

The early diagnosis and treatment of malaria infections is crucial for malaria control programs and is the backbone of the elimination programs. Therefore, new or improved diagnostic tools that enable the detection of low-density infections in both malaria-endemic countries and non-endemic regions will help target interventions whenever possible. Many malaria molecular tests have high sensitivity and specificity; however, their use is limited by the requirement of sophisticated equipment and technical expertise not available in many malaria-endemic countries. The advent of the LAMP-based tests presented an opportunity to develop molecular tests that only require simple everyday laboratory equipment such as a water-bath or heat-block for the amplification of nucleic acids. The last 10 years have witnessed great improvements in the malaria LAMP assays culminating in the commercialization of two malaria LAMP kits. However, expanding the malaria LAMP tests as options for use in malaria control programs will require addressing some important challenges/hurdles associated with the current formats of the malaria LAMP assays. Conceptualization of the optimal role for LAMP, either as a point-of-care diagnostic tool or as a HTP tool for the purposes of surveillance to support malaria elimination activities, is likely to determine the ultimate direction that LAMP assay development takes. This is likely to involve consideration of the acceptable balance between analytical sensitivity required to interrupt transmission in low prevalence settings, feasibility of sample collection and transport to facilitate achievement of optimal test sensitivity, and cost and turnaround time for assay such that timely and effective transmission interrupting interventions can be administered based on test results. Other features of the ideal LAMP assay will include lack of requirement for a cold chain, minimal requirement for electricity or additional equipment including expensive consumables, and technical simplicity of performing the assay reliably with minimal training. Many of the LAMP assays described to date are still in the research phase, and additional field evaluation will be required to establish their utility as malaria control and elimination tools, a critical part in moving any test/assay from a research tool to a public health tool. Therefore, further field evaluation of LAMP assays will be an important step in determining if the described test meets the important conditions required for a field-friendly yet sensitive tool for malaria control and elimination.

6. Five year view

Both microscopy and quality-assured RDTs will continue to be the main diagnostic tools for case management of suspected clinical malaria and for routine malaria surveillance in all epidemiological situations mainly because these tests have good performance in detecting clinical malaria, are relatively inexpensive compared to molecular tests, and are available in many malaria-endemic countries. Therefore, the next five years in malaria diagnosis are likely to see a push towards the development and possible deployment of highly sensitive RDTs such as the Alere™ RDT that was recently evaluated. In addition, given the recent studies confirming the presence of *P. falciparum* parasites with deleted HRP2 in Africa, efforts are also likely going to be put towards the development of non-HRP2-based RDTs. On this backdrop, malaria molecular tests will continue to be used for very specific studies such as: to evaluate 'detect-and-treat' strategies (MTaT, focal testing and treatment, and RCD studies); in controlled malaria infection trials; in drug efficacy trials; and as confirmatory test in reference laboratories.

Ten years after the development of the first malaria LAMP assay, PCR-based tests are still the molecular tests of choice for many laboratories and investigators. We speculate that most likely this is due to the fact that widespread awareness of the potential of malaria LAMP assays is lacking; in addition, many of the described malaria LAMP assays are still under investigation, and many do not meet the desired characteristics for a field-deployable LAMP assay. However, the two commercially available LAMP assays, the Loopamp MALARIA kit (Eiken Chemical Co) and the ***Illumi****gene* malaria LAMP (Meridian Bioscience), are well poised as viable alternatives to the sophisticated PCR-based molecular tests, and it is hoped that the next five years will see an uptake in the usage of these tests in both malaria-endemic countries and non-endemic developed countries.

Key issues

- Molecular tests with superior sensitivities than RDTs and microscopy will better enable the tracking of malaria infections in communities and help target interventions wherever they are needed.

- In general, malaria LAMP tests are simpler molecular tests/tools that can provide feasible alternatives to the commonly used PCR-based tests.
- Expanding the malaria LAMP tests as options for use in malaria control programs will require addressing some important hurdles associated with the current LAMP formats.
- Some desirable characteristics that would help make a malaria LAMP assay field-deployable include: the use of a simplified sample preparation step; availability of ready to use kits that require no cold chain; the use of a non-subjective results readout; non-instrumentation (if possible) and preferably cost-effective.
- High throughput LAMP platforms will be required if the LAMP assays are to be used in large-scale epidemiological studies.
- Two field-stable malaria LAMP tests are now CE-marked and commercially available: the Loopamp MALARIA kit (Eiken Chemical Co) and the ***Illumi****gene* malaria LAMP (Meridian Bioscience).
- The commercially available malaria LAMP tests were shown to be appropriate tools for the detection of imported malaria in non-endemic regions. However, the current cost of these tests may be too expensive for routine programmatic use.
- Malaria LAMP tests, like other molecular tests, will likely be utilized in very specific studies such as: to evaluate 'detect and treat' strategies such as mass testing and treatment (MTaT), focal testing and treatment and reactive case detection (RCD) studies; in research studies involving controlled malaria infection trials or drug efficacy trials and as confirmatory test in reference laboratories.

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Declaration of interest

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