Point of Care Testing for Malaria Using LAMP, Loop Mediated Isothermal Amplification

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(See the major article by Patel et al on pages 1180–7.)

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For over a century the laboratory diagnosis of malaria has relied on visualization of the parasite in blood smears. But microscopy is time and labor consuming and often inaccurate due to the considerable training and experience required. About 2 decades ago, immunochromatographic rapid diagnostic tests (RDTs) were introduced, providing a simpler and more rapid point of care diagnostic. However, RDTs, like blood smears, are limited in the detection of low-density infections. Nucleic acid amplification based assays offer markedly improved sensitivity, but standard polymerase chain reaction (PCR) diagnostics require hours of processing time, sophisticated technical skill, and expensive equipment to perform. Loop mediated isothermal amplification (LAMP) may offer the best of both worlds for malaria diagnostics, with the sensitivity of PCR but speed closer to that of RDTs.

Highly sensitive diagnostics may not be appropriate in all situations. Patients with symptomatic malaria generally have high-density infections that are detectable by blood smears or RDTs, and in resource-limited settings, use of a more sophisticated assay may not be practical. However, when the goal is to interrupt transmission or eliminate malaria, there is a need to detect all infections, including asymptomatic infections, which are usually of low density [1]. In these low-endemic settings, subpatent infections, or those below the detection level of microscopy or RDT, are estimated to result in 20%–50% of all transmission episodes [2]. A malaria program that is serious about elimination cannot ignore these infections [3].

LAMP was developed in 2000 as a simple method to amplify DNA with high sensitivity, specificity, efficiency, and speed under isothermal conditions [4]. LAMP uses a DNA polymerase with strand displacement properties, usually from Bacillus stearothermophilus, obviating the need for a thermocycler. The assay has high specificity because amplification only occurs when 6 separate regions of target DNA are recognized. Specifically, amplification requires 2 inner and 2 outer primers, plus 2 additional loop-primers, which anneal at the loop structure in LAMP amplicons. This assay design enhances the sensitivity of the reaction and accelerates the reaction time to less than an hour, compared to typical PCR runs of several hours. Magnesium pyrophosphate precipitates after the reaction and accelerates the reaction significantly, enabling visual detection. Alternatively, amplification can be detected as the loss of quenching of calcein, with emission of a fluorescent signal. The polymerases used for LAMP, which are less sensitive to inhibitors present in biological samples than some PCR polymerases, allow the use of simple and rapid DNA extraction methods such as “boil and spin” [5, 6].

Initially, LAMP was applied to pathogens causing food-borne disease, and kits to detect Salmonella, Legionella, Listeria, verotoxin-producing Escherichia coli, and Campylobacter have been commercialized. Promising assays have been developed for a variety of viruses including those causing severe acute respiratory syndrome (SARS), influenza, measles, human papilloma virus disease, and mumps. Methods have also been developed for diseases of resource-limited settings, including tuberculosis, human immunodeficiency virus (HIV) infection, and African trypanosomiasis. For malaria, the first reported primer sets for LAMP targeted the 18S ribosomal RNA gene [7]. Subsequent targeting of mitochondrial DNA provided...
improved sensitivity [8]. The development of prepackaged kits for DNA extraction and amplification [9–11], as well as automated platforms combining amplification and detection [12, 13], have improved the field-applicability of assays.

Patel et al, in this issue of the Journal of Infectious Diseases, report on the field evaluation of RealAMP, a new portable device that integrates LAMP targeting the 18S ribosomal RNA gene with real-time detection of fluorescence for simple and rapid detection of malaria parasites [11]. The study took place in low transmission areas of India and Thailand, where both Plasmodium falciparum and Plasmodium vivax are endemic. In India, 141 individuals presenting at governmental health facilities with fever were screened with microscopy and RealAMP. The results were 100% concordant, and so RealAMP did not provide an advantage for screening these symptomatic individuals with fairly high-density infections (median 1560 parasites/μL). Using nested PCR targeting the 18S Plasmodium rRNA gene as gold standard, the sensitivity and specificity of both techniques were 94.8% and 100%, respectively.

In Thailand, 127 asymptomatic individuals were screened as part of standard active surveillance activities. The reported values for diagnostic accuracy are difficult to interpret, as pooled PCR, which was used as a gold standard, used a slightly lower template volume than RealAMP and missed several infections that were positive by both RealAMP and microscopy. Others have also found that pooling can compromise PCR sensitivity [14]. More importantly, the RealAMP system clearly provided improved sensitivity, compared to microscopy, for low-density asymptomatic infections (median 4 parasites/μL). Using the pooled PCR as gold standard, the sensitivity of RealAMP was 1.7-fold higher than expert microscopy and 2.2-fold higher than local microscopy. An additional small pilot of the RealAMP system in a rural clinic in Thailand showed that it performed better than microscopy and was operationally feasible.

Thus, the RealAMP system was field friendly in a resource-limited setting. The system was sensitive enough to double the detection of asymptomatic infections compared to microscopy—a convincing argument for application in active surveillance, particularly in the context of malaria elimination programs.

LAMP seems poised for an important role in active surveillance programs in malaria eliminating countries. A survey among eliminating countries in the Asia-Pacific found that all implemented active surveillance activities [15]. In Thailand, the current active surveillance program, which mainly uses microscopy, is very resource and time intensive, and few positives are found. In areas with ongoing malaria transmission, villagers receive monthly scheduled screening, reactive case detection (screening in response to a local index case), and additional screening for outbreaks. High-risk, hard to reach populations are screened at international borders and in mobile malaria clinics. Reactive case detection is also implemented in areas where malaria was recently eliminated when there is concern for reintroduction [16]. The availability of a highly sensitive and convenient diagnostic test for these purposes would be useful for Thailand and other eliminating countries.

Despite the promise of LAMP for malaria diagnosis, some challenges stand in the way of routine implementation of this technology. First, a wide range of methods have been reported, and we do not yet have standardized methodology regarding sample collection, DNA extraction, amplification, or detection [17]. Second, for comparisons of assays, gold standards are not standardized. As such, even with use of the same nucleic acid based amplification assay, data may not be comparable. In Uganda, a field evaluation of the LoopAmp Malaria Pan/Pf detection kit, which targets mitochondrial DNA, reported a lower specificity (85%) than that seen in this study [11], but the gold standard used (positivity in at least 1 out of 3 PCR reactions) was likely more sensitive [9]. Third, false positive LAMP reactions can occur from contamination with small amounts of DNA [10, 18]; as for PCR great care must be taken to avoid contamination. Finally, if LAMP is to be widely applicable, platforms that are high throughput and capable of detecting all human malaria species are needed.

How can LAMP technology move from the realm of research to implementation? Learning from experience with RDTs, for which wide scale uptake was hampered by a bloated market of different RDTs and lack of global and regional quality assurance systems [19], LAMP development will require a coordinated effort to focus on a small number of accurate, practical, and cost-effective systems. To ensure that supply chains are reliable and that implementation is successful, global, regional, and national policies should be clear regarding appropriate use of point of care tests [20]. Because of cost, 1-hour processing time, infrastructure and expertise needs, and the risk of detracting from confidence in standard diagnostic modalities (microscopy and RDTs), LAMP should probably not be used as a point of care diagnostic for symptomatic individuals in most resource-limited settings. Rather, LAMP should generally be reserved for active surveillance of subpatent infections [21]. Operational research should take place in resource-limited laboratories to inform the refinement of LAMP by evaluating accuracy, usability, and effectiveness. Finally, consideration should be given for other emerging technologies, such as highly sensitive RDTs [22].

The article by Patel et al offers a promising approach, using LAMP for malaria surveillance in low endemic settings. Hopefully, it will stimulate a rapid and coordinated effort to make LAMP available as a new point of care diagnostic to support malaria elimination efforts.

Notes

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