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CORRESPONDENCE

Verification and implementation of a commercial loop-mediated isothermal amplification (LAMP) assay for malaria testing in a public laboratory in New South Wales, Australia

To the Editor,

The current protocol for malaria screening in the majority of New South Wales Health Pathology (NSWHP) laboratories is to test the samples for malaria specific antigen using rapid diagnostic tests (RDT). These laboratories refer the RDT negative samples alongside the blood films for confirmatory testing at the malaria reference laboratory, Parasitology Unit, NSW Health Pathology - Institute of Clinical Pathology and Medical Research (ICPMR). Likewise, all blood samples and films with positive RDT are also referred to the malaria reference laboratory for confirmation and identification of the malaria species.

Although easy to perform, RDT tests suffer from lack of analytical sensitivity especially for non-falciparum malaria.[1] Furthermore, deletion of the histadine rich protein (HRP) gene by *Plasmodium falciparum* is an increasing problem when malaria diagnosis relies on detecting this antigen.[2]

While it is considered the reference gold standard for malaria detection, malaria diagnosis using blood-film microscopy has significant drawbacks.[3,4] This method of diagnosis is difficult to incorporate into laboratory automation systems. It is a labour intensive method and it is becoming more difficult to maintain the quality assurance, skills and numbers of trained microscopists as the number of malaria cases declines globally.[4] This is especially applicable in the context of non-endemic regions like NSW, Australia, which encounters very few positive cases of malaria (January 2018 to July 2022, 181 malaria notifications in NSW).[5] The declining skill in malaria microscopy increases the chance of missed cases of malaria especially when parasitaemia is low.[3]

Several molecular tests based on polymerase chain reaction (PCR) assays have been developed to amplify and detect malarial DNA. These tests have demonstrated higher sensitivity than microscopy,[6] however a time consuming DNA extraction may be required prior to PCR amplification. The turnaround time and skill requirements are significant hurdles for putting these assays to routine laboratory use. Also, a malaria PCR assay would need an elaborate laboratory set up with relatively expensive equipment.[7]

The loop-mediated isothermal amplification (LAMP) assay is a comparatively new molecular technique based on isothermal amplification of DNA. The sensitivity of LAMP assays has been demonstrated as comparable to PCR assays for various parasitic DNA detection including assays for malaria, filaria and strongyloidiasis.[8−10] In addition, LAMP assays can have a higher tolerance to inhibition when compared to PCR.[10]

A malaria LAMP commercial assay (Alethia Malaria LAMP assay; Meridian Bioscience, USA) has become available in Australia. This assay is supplied in a kit format with pre-dispensed reagents to detect malarial DNA at a genus level. It has the advantage of a simple extraction process prior to isothermal incubation with the whole reaction completed within an hour. In addition, this assay has stable reagents without the need for refrigeration. However, the test cannot distinguish between different *Plasmodium* species.

A verification study was conducted at the malaria reference laboratory, Parasitology Unit, NSWHP-ICPMR, Westmead Hospital, NSW, Australia. The objective of this study was to evaluate the performance of the Alethia malaria LAMP assay (referred as the LAMP assay hereinafter) in detecting malaria compared to blood-film microscopy and the BinaxNOW Malaria RDT (Abbott Laboratories, USA) in returned travellers. The other objective of the study was to consider the application of the LAMP assay in the malaria reference laboratory and the core microbiology section at ICPMR for malaria diagnostic testing. The study had ethical approval from the Western Sydney Local Health District - Human Research Ethics Committee (Approval No. AU RED LNR/18/WMEAD/139).

Blood samples (n=150) comprising 58 malaria microscopy positive and 92 malaria microscopy negative specimens were tested in this study (Table 1). The malaria microscopy positive samples comprised 28 *P. falciparum*, 18 *P. vivax*, seven *P. ovale*, two *P. malariae* and one *P. knowlesi* microscopy positive, with single species infection. An additional two positive malaria samples had mixed infections; one with *P. falciparum* and *P. vivax*, while the other had *P. falciparum* and *P. malariae.* Malaria microscopy was performed according to standard laboratory protocols,[11] and the RDT and the LAMP assay were performed according to the manufacturer's instructions.

All 58 samples where malaria parasite was detected by microscopy, tested positive by the LAMP assay. Malaria antigen was detected only on 47 of 58 microscopy positive samples using the RDT and 11 samples were classified as false negative for malaria antigen. Out of the 92 microscopy negative samples, seven samples tested positive for malarial DNA by the LAMP assay. Of note, all seven samples were from patients who had malaria microscopy positive results from the recent past, and this finding was indicative of residual malarial nucleic acids. Apart from these seven discrepant results, no false positive results were detected with the LAMP assay. Malaria antigen was detected in 12 of 92 malaria microscopy negative samples using the RDT. While eight of these 12 samples were follow-up samples from previous malaria microscopy positive cases, the other four samples suggested false positive RDT results. The respective positive predictive values for the RDT and the LAMP assay were 79.7% and 89.2%, while the negative predictive values for these assays were 87.9% and 100%.

The time required to process each sample from handling to transfer to the LAMP reader for incubation was less than 6−8 minutes. Results were obtained from the instrument reader 40 minutes after starting the run. This turnaround time was longer compared to the RDT (15−20 minutes), but significantly less than microscopy.

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Table 1 Comparison of the RDT and the LAMP assays in reference to malaria microscopy

		BinaxNow malaria antigen (*n*=150)		Commercial malaria LAMP (*n*=150)	
		Positive	Negative	Positive	Negative
Malaria microscopy (*n*=150)	Positive (*n*=58)	47	11	58	0
	Negative (*n*=92)	12	80	7	85
Sensitivity % (95% CI)		81.0 (68.6−90.1)		100.0 (93.8−100.0)	
Specificity % (95% CI)		87.0 (78.3−93.1)		92.4 (85.0−96.9)	
Positive predictive value % (95% CI)		79.7 (69.5−87.1)		89.2 (80.3−94.4)	
Negative predictive value % (95% CI)		87.9 (80.9−92.6)		100.0	

CI, confidence interval; LAMP, loop-mediated isothermal amplification; RDT, rapid diagnostic test.

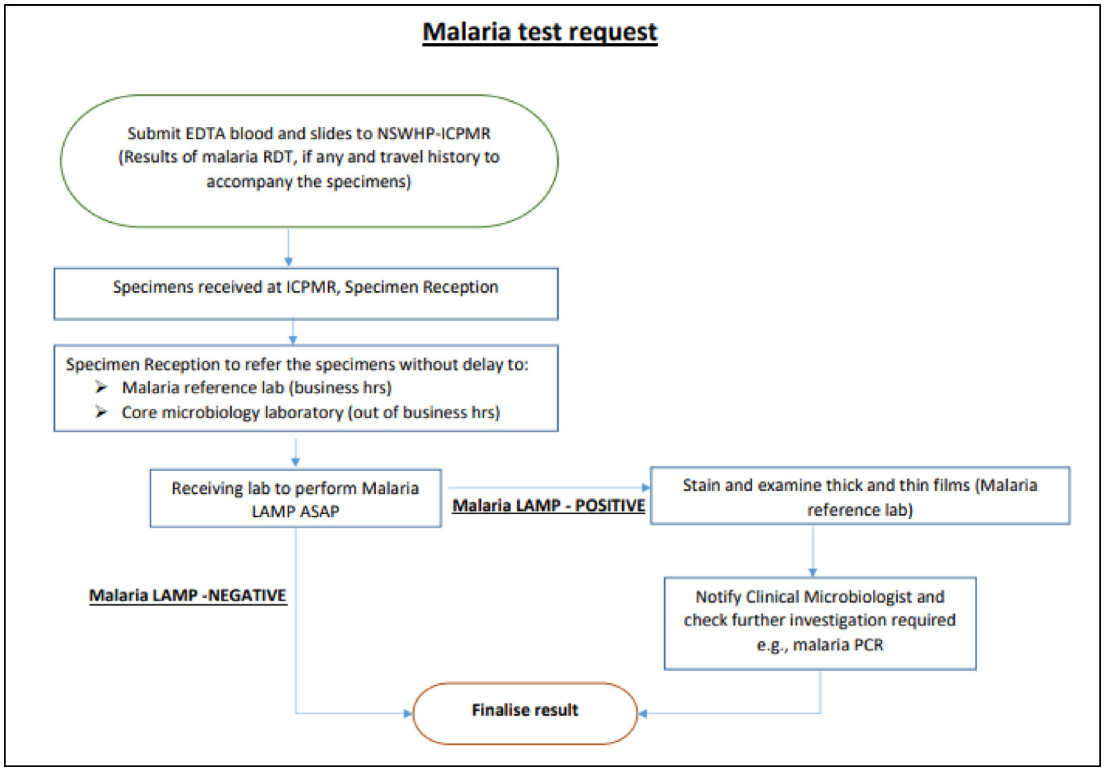

Fig. 1 Designed workflow incorporating the commercial malaria LAMP assay at NSWHP-ICPMR.

In our experience, this is an efficient and fast molecular assay to detect malaria. The LAMP assay was able to detect the five major human *Plasmodium species* with greater sensitivity and specificity than the RDT. This was in agreement with other similar studies published.[12] The LAMP assay will be useful when experienced microscopists are not available. In a malaria non-endemic region like Australia, this test suits the current laboratory workflow as a rapid molecular test with a relatively rapid screening time for malaria diagnosis and exclusion.

While an early diagnosis of malaria can be achieved using the LAMP assay, it cannot differentiate between malaria species. Thus, further testing (malaria microscopy or malaria PCR) would be required for species identification. Similarly, this assay cannot identify mixed malaria infections where more than one *Plasmodium* species may be present in the sample. This information is usually desired for the treatment and management of malaria. The prolonged circulation of *Plasmodium* spp. DNA renders the malaria LAMP assay unsuitable as a test for clinical cure against malaria.

The LAMP assay is being adopted by the Parasitology Unit, NSWHP-ICPMR, to replace the microscopy confirmation of negative RDT test samples and a corresponding testing algorithm has been developed (Fig. 1). With the implementation of the LAMP assay into the laboratory workflow, there will be increased speed and accuracy of malaria diagnosis in the laboratory.

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