Malaria Screening Using Front-Line Loop-Mediated Isothermal Amplification

Fourteen-Month Experience in a Nonendemic Regional Hub-and-Spoke Laboratory Setting

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ABSTRACT

Objectives: *We implemented front-line loop-mediated isothermal amplification (LAMP)–based malaria screening in our nonendemic multicenter health region to reduce reliance on microscopy without sacrificing diagnostic efficiency. We aimed to evaluate changes in test volumes, positivity rates, turnaround times, and approximate labor time savings resulting from implementation of LAMP-based malaria testing to assess the efficacy of the novel testing algorithm in our regional hub-and-spoke testing model.*

Methods: *We reviewed data generated from institutional malaria testing between 2016 and 2019, having implemented LAMP in October 2018 as a front-line screening test for all malaria investigations from our hub facility and investigations from satellite facilities with negative rapid diagnostic tests (RDTs) and microscopy.*

Results: *Blood film microscopy and RDT workloads decreased substantially in the year following LAMP implementation (by 90% and 46%, respectively,) despite similar numbers of patients tested and positivity rates for malaria compared with historical data. LAMP turnaround times (TATs) were comparable to historical TATs for RDTs, and TATs for RDTs and thick films did not increase with the change in workflow.*

Conclusions: *LAMP was successfully implemented in our multicenter health region malaria diagnostic algorithm, significantly reducing reliance on microscopic evaluations and RDT and providing substantial labor time savings without compromising TATs.*

Key Points

- Loop-mediated isothermal amplification (LAMP) is a technique to amplify target DNA without thermocycling. LAMP assays for malaria are available but are not in widespread use in North America.
- The assay's robust performance and high sensitivity, comparable to traditional polymerase chain reaction, suggest that LAMP-based malaria screening can replace blood film screening by microscopy.
- Algorithms incorporating front-line LAMP-based malaria screening can lead to meaningful reductions in the clinical laboratory workload without sacrificing turnaround times or diagnostic sensitivity.

Malaria, while not endemic in North America, remains an important cause of fever in travelers and has potential to cause severe morbidity and mortality without prompt treatment.[1] In 2018, there were an estimated 228 million cases of malaria worldwide, the majority of which occurred in the World Health Organization (WHO) African region.[1] Malaria is comparatively rarely diagnosed in Canada, with approximately 488 cases of malaria reported yearly.[2] In the province of British Columbia, the number of cases of malaria reported to the British Columbia Center for Disease Control in the most recent 5 years for which data are available averaged 35.4 cases yearly (ranging from 26 in 2014 to 54 in 2013).[3] Despite the low incidence, malaria testing is commonly ordered for fever in returning travelers.

Malaria diagnosis generally relies on microscopic examination of thick and thin preparations of Giemsa-stained blood films for parasites, an approach with a reported limit of detection (LOD) in the range of 50 to

100 parasites/μL of blood for typical microscopists (or as low as 5 parasites/μL of blood for highly skilled microscopists).[4-6] Accurate diagnosis by microscopic methods depends on the quality of blood films, degree of parasitemia, available equipment, and expertise of staff. Maintaining staff competency may be challenging in laboratories that encounter malaria infrequently, an issue that is compounded by low parasitemia in nonendemic settings.[5] Furthermore, repeated blood films are recommended if initial evaluations are negative, resulting in prolonged or repeated patient visits.[2] Rapid diagnostic tests (RDTs) capable of detecting *Plasmodium* antigens by immunochromatography can be implemented as a potential alternative or adjunct to microscopy and do not require specialized laboratory skills. Many RDTs are available for purchase and differ in *Plasmodium* species detected but are generally limited by a LOD similar to or less sensitive than that of microscopy for *P falciparum* (particularly at low parasitemia) and lower sensitivity to the presence of other *Plasmodium* species.[2,5] Detection of asymptomatic carriers of malaria parasites with extremely low parasitemia, an important group to identify in the setting of travel or immigration medical assessment, is difficult using microscopy or RDT testing and may be facilitated by molecular detection methods.[7]

Molecular malaria detection techniques based on traditional polymerase chain reaction (PCR) have the advantage of high sensitivity, with reported LOD of 0.5 to 5 parasites/μL of blood; however, their use may be limited by the requirement for molecular technical capacity.[4,5] Loop-mediated isothermal amplification (LAMP) is a nucleic acid amplification technique using polymerases and primers designed for rapid amplification of target DNA without the requirement for labor-intensive initial DNA extraction or thermocycling during the reaction; this substantially simplifies molecular detection of malaria and extends its accessibility beyond laboratories with dedicated molecular facilities.[8] The Alethia Malaria LAMP assay (known previously as Illumigene Malaria; Meridian Bioscience) has shown superior sensitivity and specificity for detection of malaria compared with microscopy in validation studies, with relative ease of implementation in clinical laboratories, robust detection of *Plasmodium* parasites at the genus level (without species discrimination), and reported LOD similar to that of traditional PCR.[9-11] The high sensitivity and negative predictive value suggests that a single negative result is sufficient to exclude a diagnosis of malaria.[12,13]

Our clinical laboratory operates in a large tertiary care hospital and serves as the regional malaria testing center for 10 satellite community hospitals and health centers. Historically, our method of malaria investigation has relied on microscopy and RDT testing performed throughout the region, with centralized speciation and reference laboratory confirmation. To reduce reliance on microscopy within our region without loss of diagnostic sensitivity, we designed a new malaria testing workflow incorporating front-line LAMP screening at our hub facility. Previous studies have validated the Alethia LAMP malaria detection assay for use in travelers returning to nonendemic areas and demonstrated excellent diagnostic performance.[12-16] Malaria testing algorithms incorporating front-line LAMP as a screening test in nonendemic regions have been devised by several groups; however, published data regarding a multicenter approach are lacking.[9,12,15] In this article, we describe our 14-month institutional experience with front-line LAMP testing in a multicenter setting—to our knowledge, the first report of its kind—and estimate impacts on workload relative to the preimplementation standard workflow.

Materials and Methods

Testing Sites

Our 1,000-bed tertiary care center acts as the hub facility for specialty hematology laboratory testing for 10 acute care satellite facilities in a hub-and-spoke regional model, serving a population of 1.25 million. Characteristics of the 10 satellite facilities are listed in **Table 1**. Each satellite facility has a variable on-site test menu, but all send samples to the hub site for additional testing and confirmation. Sample transport systems include air, ferry, and ground modalities.

Internal Validation of LAMP Testing

On-site validation of the Alethia Malaria LAMP assay with Illumipro-10 instruments (Meridian Biosciences) was performed before implementation. All hematology technologists at the hub site were trained in LAMP. LAMP was evaluated for accuracy against traditional Wright-Giemsa–stained thin film and Giemsa-stained thick film microscopy using 79 patient samples, 13 of which had been confirmed positive by PCR. These positive specimens included cases of *P falciparum*, *P vivax*, and *P ovale*, with known parasitemia ranging from less than 0.1% to 5.2%. The lower LOD of the LAMP assay was determined using serial dilutions of 7 specimens positive for malaria, prepared using ABO-compatible whole blood, to determine the lowest parasitemia resulting in a positive LAMP result. LOD represented as parasites/μL of blood was calculated using percentage of parasitemia by WHO approximations

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Table 1
Characteristics of Satellite Facilities Affiliated With the Hub Facility for Hematology Laboratory Testing

	Sat 1	Sat 2	Sat 3	Sat 4	Sat 5	Sat 6	Sat 7	Sat 8	Sat 9	Sat 10
Acute care capacity	92	264	228	21	46	20	4	33	14	15
24/7 laboratory	Yes	Yes	Yes	Yes[b]	Yes[b]	No	No	Yes[b]	Yes[b]	Yes[b]
RDT on-site	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No
Distance[a] from hub, km	10	15	10	65	70[c]	125	155	175[d]	430[d]	485[d]

RDT, rapid diagnostic test; Sat, satellite.
[a]Nearest 5 km.
[b]On-call technologist available overnight.
[c]Includes ferry.
[d]Includes air.

between these measures in severe *P falciparum* malaria.[17] The assay performed as expected in internal validations of specimen transport and stability with room temperature or frozen aliquot storage (including with positive specimens frozen for up to 6 months).

The 66 samples that were negative by thin and thick film microscopic evaluation were also negative by LAMP, and all 13 samples positive by microscopic evaluation also tested positive by LAMP. LAMP testing following serial dilutions of 3 different samples containing *P falciparum* yielded a lower LOD ranging from 0.5 to 5 parasites/μL of blood (parasitemia of 0.00001% to 0.0001%). For *P vivax*, 2.5 parasites/μL of blood (parasitemia of 0.00005%) were detectable by LAMP, and for *P ovale*, 3.4 parasites/μL of blood (parasitemia of 0.000067%) were detectable (where LODs for each non–*P falciparum* species were assessed by serial dilution of 1 positive sample).

Diagnostic Workflow for Malaria Investigations Before and After Implementation of LAMP

The diagnostic workflow for malaria investigation before implementation of LAMP is depicted in Figure 1. Before implementation of LAMP, test requests for malaria resulted in microscopic and RDT evaluation in all cases. Five thin and 4 thick films were prepared within 1 hour of blood collection. Two technologists reviewed the thin films (20-30 minutes) and issued a preliminary result to the ordering physician, and all satellite facilities then forwarded both thin and thick films to the hub for review by the pathologist (except satellite 2, where slides were reviewed by on-site pathologists). RDT for *P falciparum* (N.C.S. Malaria Rapid Test; Nova Century Scientific) was performed in all cases; for satellite facilities without RDT available in-house, RDT testing was performed at the hub facility. Malaria investigations originating from the hub facility followed an identical testing algorithm. Cases positive for malaria infection were sent to the provincial reference laboratory for confirmation by PCR. In the majority of cases where RDT and microscopy were negative, patients would be discharged with a requisition to have their second and third blood films performed at an outpatient laboratory.

On October 24, 2018, the testing algorithm was changed to incorporate LAMP as a front-line test for malaria investigation Figure 2. Satellite facilities are now instructed to forward a sample aliquot at room temperature to the hub site for LAMP testing, as well as thin and thick film slides (which are required for parasitemia calculation and speciation of positive cases). Given that there would be delays in issue of LAMP results because of transport time, 2 steps of the original workflow were maintained. First, all sites that can perform RDT maintain this test to quickly identify *P falciparum* infections and report results while awaiting LAMP. Second, wherever possible, technologists at satellite facilities perform a screen (5 minutes) of the thin film slide before sending it to the hub, with the intent of identifying obvious cases of non–*P falciparum* infection (where preliminary results are reported only when screening identifies parasites).

At the hub facility, LAMP serves as the initial malaria screening test. Positive LAMP results are followed by RDT (if not already performed), technologist review of thin films for determination of parasitemia, pathologist review of thick films for speciation, and referral to the reference laboratory for confirmation. Negative LAMP testing in all cases is considered confirmation of the absence of malaria infection, and no further investigations are undertaken.

Data Collection and Review

All instances of malaria testing requested at the hub facility and satellite facilities in the 14 months following LAMP implementation (November 2018 to December 2019, inclusive) and the 34-month period preceding LAMP implementation (January 2016 to October 2018, inclusive) were identified by query of the laboratory information system and reviewed. We tabulated the

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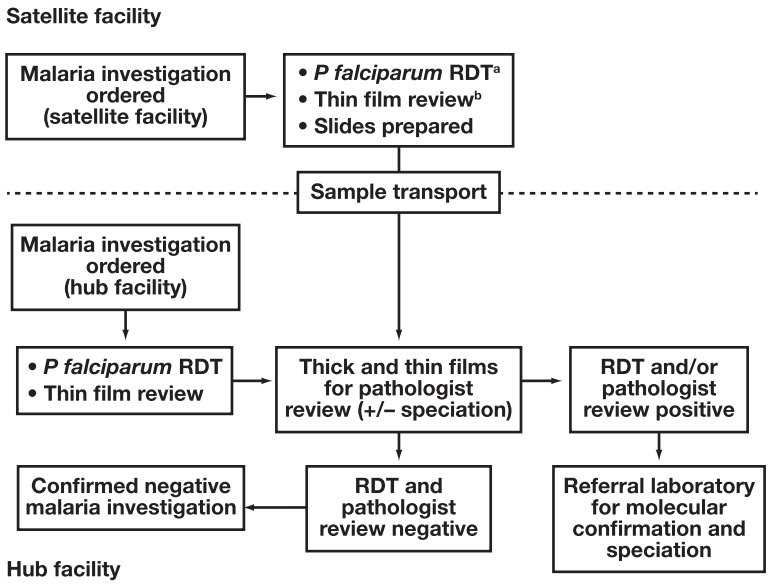

Figure 1 Workflow for malaria investigations ordered before implementation of loop-mediated isothermal amplification at the hub facility. [a]Rapid diagnostic tests (RDT) were performed at the hub facility for cases from satellite facilities without RDT capability on-site. [b]Thin film review refers to thorough (20- to 30-minute) technologist evaluation for parasites.

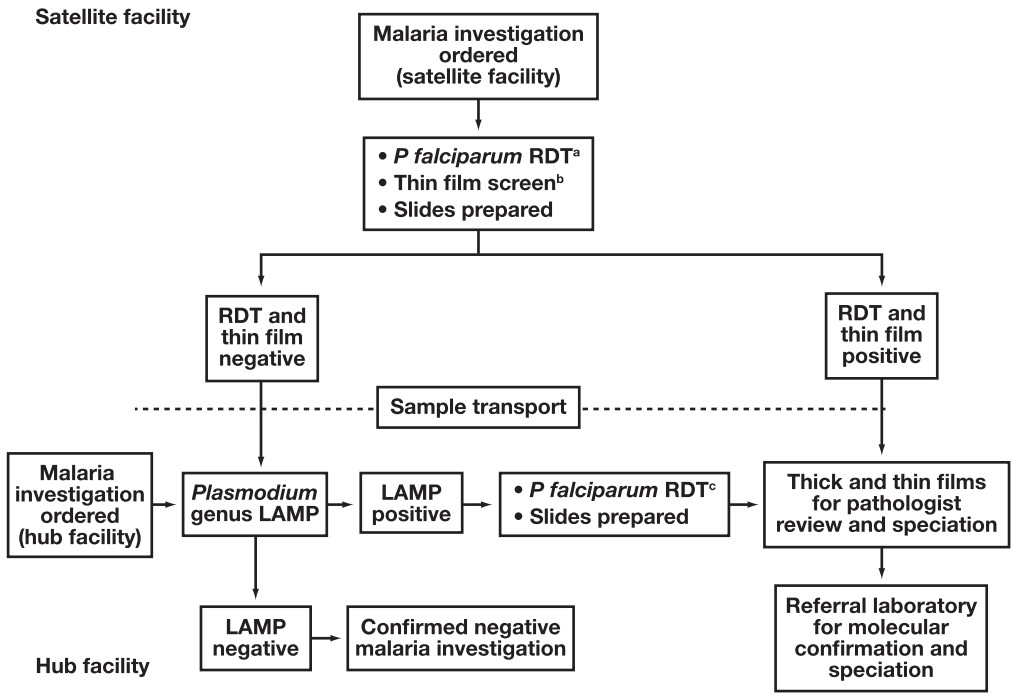

Figure 2 Workflow for malaria investigations after implementation of loop-mediated isothermal amplification (LAMP) at the hub facility. [a]For satellite facilities without rapid diagnostic tests (RDT) on-site, LAMP is performed at the hub facility instead (unless malaria is identified on thin film screening). [b]Thin film screen refers to rapid (<5-minute) technologist evaluation for parasites. [c]RDT is performed for all positive LAMP studies except when previously resulted as negative.

number of malaria tests performed, the frequency of positive results, malaria parasite species identified, test turnaround times (TATs; defined as time from sample collection to time of result entry), and estimated labor savings (including technical staff and pathologists) resulting from the change in the testing algorithm following implementation of LAMP. Median TATs were compared between calendar years using Kruskal-Wallis

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Table 2

Number of Tests Performed per Calendar Year at the Hub Facility and Satellite Facilities (Combined) and Identified Malaria Infections[a]

	2016	2017	2018	2019
Patients tested	284	309	275	293
Patients positive for malaria, No. (%)	10 (3.5)	10 (3.2)	13 (4.7)	9 (3.1)
LAMP tests performed	NA	NA	49	302
RDTs performed	371	383	300	191
No. of positive RDTs (patients)	3 (2)	15 (9)	9 (9)	6 (5)
Thin and thick films resulted for all patients	390	402	302	36
Thin and thick films resulted for patients with malaria	29	21	31	23

LAMP, loop-mediated isothermal amplification; NA, not applicable; RDT, rapid diagnostic test.
[a]LAMP testing was implemented in October 2018.

testing with post hoc Dunn pairwise comparison (with $\alpha = 0.0083$ after correction for multiple comparisons by the Bonferroni method).

Results

Testing Performed per Calendar Year During the Study Period

Malaria investigations performed within the region during the study period are depicted in Table 2. After implementation of LAMP as the front-line screening test in October 2018, there were a total of 351 LAMP tests performed (including 302 in the calendar year 2019) for 339 patients; 11 patients had at least 1 repeat LAMP test performed. Despite similar numbers of patients investigated for malaria (with similar rates of positive investigations) before and after implementation of LAMP, RDT testing performed decreased substantially following the introduction of the new workflow, from a mean of 351 RDTs per year between 2016 and 2018 to 191 in 2019 (a 46% decrease). This reduction reflects the discontinuation of RDTs performed at the hub site, where a negative LAMP result precludes further testing. The number of thin and thick films reported decreased markedly in the same period, from a mean of 365 per year between 2016 and 2018 to 36 in 2019 (a 90% decrease). This difference reflects the change in policy such that full microscopic assessment is performed only after a positive LAMP or RDT result.

Speciation of positive cases identified from the hub facility and satellite facilities is presented in Table 3. The most frequently detected species was *P falciparum* (60% of cases), followed in order by *P vivax* (21%), *P ovale* (12%), and *P malariae* (7%). No cases of *P knowlesi* were detected. LAMP was positive in 2 patients with malaria infections where parasites were either very difficult to detect or were not detectable at all by microscopy (1 case of *P malariae* and 1 case that could not be speciated). Both cases were confirmed positive by the reference laboratory.

TATs for Testing Per Calendar Year During the Study Period

Median TATs for RDT testing, thick and thin film review, and LAMP testing performed at the hub and satellite facilities were reviewed Table 4. Median TATs for RDTs decreased slightly from previous years after implementation of LAMP ($P < .0001$), whereas TATs for results to be issued for thick films remained similar ($P > .5$). TATs for results to be issued for thin films increased as a consequence of the new diagnostic workflow ($P = .001$ to $P = .004$), wherein results were issued for thin films only on positive cases identified by LAMP or RDT testing, incorporating sample transport time to the TAT in the majority of cases.

The median TATs for LAMP testing in 2019 from each originating site were determined to evaluate the effect of sample transport time on result reporting Table 5. In this period, the hub facility was able to provide LAMP results with median TATs only slightly longer than historical median TATs for RDTs. Median TATs for LAMP testing from satellite facilities varied widely (ranging from a minimum of 6.3 hours to a maximum of >20 hours), largely depending on geographic proximity and mode of transport.

Discussion

Testing for malaria is ordered relatively frequently, with a positivity rate of less than 5% in our region. Before implementation of LAMP, the facilities in our region experienced significant workload burdens associated with performing microscopy on mostly negative samples, and maintaining staff competency in the morphologic identification of parasites was challenging. Implementation of LAMP as a front-line screening test resulted in a reduction

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Table 3

Malaria Species Identified During the Study Period by Calendar Year by Testing Performed on Specimens Originating From the Hub and Satellite Facilities

	2016	2017	2018	2019
Malaria cases identified (total)	10	10	13	9
Malaria cases originating from hub facility	Total: 6 *P ovale*: 5 *P vivax*: 1	Total: 5 *P falciparum*: 4 *P vivax*: 1	Total: 13[a] *P falciparum*: 9 *P vivax*: 4 *P malariae*: 1	Total: 4 *P falciparum*: 3 *P malariae*: 1
Malaria cases originating from satellite facilities	Total: 4 *P falciparum*: 2 *P vivax*: 2	Total: 5 *P falciparum*: 5	Total: 0	Total: 5 *P falciparum*: 2 *P vivax*: 1 *P malariae*: 1 Unspeciated: 1[b]

P, *Plasmodium*.
[a]Including 1 case of coinfection involving *P falciparum* and *P vivax*.
[b]Only detected by molecular methods and unable to be speciated.

Table 4

Median TATs for All Testing Performed by Satellite Facilities or the Hub Facility (Combined) per Calendar Year, Excluding Satellite 8 for Which Data Were Not Available[a]

	2016	2017	2018	2019
RDT TAT	1.4 (1.0-2.3)	1.5 (1.0-2.3)	1.4 (1.0-2.3)	1.1 (0.8-2.0)
Thin film TAT	2.3 (1.7-3.5)	2.4 (1.8-3.7)	2.4 (1.8-3.6)	3.2 (2.0-7.4)
Thick film TAT	21.5 (14.6-27.7)	24.0 (16.2-35.0)	22.1 (13.2-34.8)	23.7 (15.0-29.7)
LAMP TAT	NA	NA	4.2 (2.2-7.7)	5.0 (2.2-8.3)

LAMP, loop-mediated isothermal amplification; NA, not applicable; RDT, rapid diagnostic test; TAT, turnaround time.
[a]Data are shown as median (interquartile range).

Table 5

Median TATs for LAMP Testing Performed in the First Full Calendar Year Following Test Implementation[a]

	Sat 1	Sat 2	Sat 3	Sat 4	Sat 5	Sat 6	Sat 7	Sat 8	Sat 10	Hub
LAMP TAT, median (IQR), h	6.3 (5.1–7.4)	7.5 (5.6-13.1)	6.8 (5.3-10.5)	9.2 (7.4-12.5)	11.4 (9.3-16.2)	10.3 (7.2-24.5)	11.3 (9.0-19.7)	21.3 (16.7-31.2)	9.2 (7.4-12.5)	2.0 (1.7-2.8)
No. of tests	26	34	61	22	3	11	3	8	22	133

IQR, interquartile range; LAMP, loop-mediated isothermal amplification; Sat, satellite; TAT, turnaround time.
[a]No LAMP tests originated from satellite 9.

in microscopy workload at all our regional facilities, without a noticeable loss in test sensitivity. Although we observed the lowest rate of positivity for malaria infections (3.1%) in the year following implementation, we found that LAMP detected 2 malaria infections with low parasitemia that would have been missed by microscopy and RDT. Therefore, although we did not directly compare LAMP in parallel with microscopy, we have confidence that our new process did not miss cases of malaria. Previous investigators have reported that LAMP is more sensitive than microscopy. Cheaveau et al[9] prospectively tested returning travelers using LAMP and found 7 infections identified by LAMP that were missed by microscopy; they reported overall sensitivity and specificity of 100% for identification of malaria using LAMP. De Koninck et al[12] compared LAMP with microscopy and RDT on prospective and retrospective samples and identified 3 false-negative RDTs and 1 false-negative microscopic evaluation, all of which were detected by LAMP.[12] Importantly, skill in microscopy remains an integral component of malaria diagnosis when LAMP screening is positive because parasitemia cannot currently be quantified by LAMP, and the pan-specific *Plasmodium* assay in use in our laboratory is incapable of speciation.

Our testing algorithm shares similarities with published proposals in that LAMP is used as a front-line test, and no further testing is required for negative results.[9,11,12] Notable differences in our algorithm's design are incorporated to facilitate timely identification of malaria infections in our geographically dispersed multicenter health region, including earlier use of RDT and microscopic screening in satellite facilities to ensure rapid detection of *P falciparum*

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and non–*P falciparum* infections with high parasitemia before referral for centralized testing.

During validation studies, no false-negative or false-positive tests were observed, and we are not aware of any patient who tested negative by LAMP and subsequently tested positive at another laboratory, consistent with the previously reported near-perfect sensitivity and specificity for the assay kit.[9-11,13] The lower LOD identified by serial dilution of malaria-infected whole blood specimens was found to be as low as 0.5 parasites/μL of whole blood for *P falciparum*, and 2.5 or 3.4 parasites/μL of whole blood for *P vivax* and *P ovale*, respectively—findings comparable to LOD studies performed previously by other groups.[4,5] Relative to reported LODs of 50 to 100 parasites/μL of blood for average microscopists reviewing thin and thick films or to LODs generally greater than 200 parasites/μL for RDTs (depending on the assay in use), LAMP provides substantial improvement in sensitivity, rivaling that reported for molecular testing using traditional PCR.[4,5]

Implementation of LAMP as a front-line test at the hub facility has not increased TATs for the overall process of malaria diagnosis to a clinically relevant degree, as indicated by the similarity between LAMP TATs in the first calendar year after implementation and historical RDT TATs. For satellite facilities, timely identification of *P falciparum* infections remains primarily dependent on RDT. TATs for LAMP in malaria investigations originating from satellite facilities with negative RDT testing and thin film screens varied depending on the distance from the site to the hub facility and the mode of transport. Notably, median TATs for LAMP testing originating from satellite facilities were shorter than historical median TATs for pathologist thick film interpretation: negative investigations not requiring thick film review in the new algorithm have finalized results issued with shorter TATs, whereas positive investigations have finalized thick film reviews with TATs comparable to those in the pre-LAMP testing framework. The overall result is an algorithm with greater diagnostic efficiency and no undue delay in provision of results to clinicians despite test centralization.

We expected that implementing LAMP in routine malaria diagnosis would reduce laboratory staff workload. Assuming 300 malaria investigations are ordered in a typical year with a positive malaria identification rate of 3% (ie, 10 patients), 290 patients with initial negative investigations would ultimately result in a total of 870 thin and thick film reviews for definitive exclusion of malaria in the pre-LAMP workflow. With full microscopic evaluation requiring approximately 40 cumulative minutes of technologist time and 15 minutes of pathologist time, yearly microscopy workload times are estimated at 34,800 minutes (580 hours) of technologist time and 13,050 minutes (217 hours) of pathologist time. The technologist time commitment required for LAMP set up is approximately 15 minutes, for a total of 4,350 minutes of technologist time to test 290 patients. Consequently, the net savings would be an estimated 30,450 minutes (507 hours) of technologist time and 13,050 minutes (217 hours) of pathologist time. In addition to these savings, unnecessary patient interactions with the health care system for follow-up slide reviews are minimized.

A limitation of this study is that we did not encounter cases of *P knowlesi* (there are no reported cases to date in our region) and cannot comment on the detection rate of this species using our algorithm. Our hub-and-spoke testing model is reliant on several modes of transportation to transport specimens to the hub facility, and transport delays (due to weather or other unexpected disruptions) could delay the diagnosis of infections with non—*P falciparum* species; however, this has not been an issue we have experienced to date. We experienced no "invalid" LAMP results during the study period, an issue reported as an infrequent occurrence by other investigators.[9,11]

Conclusions

We successfully implemented a LAMP-based malaria investigation workflow in our regional multicenter laboratory model in a malaria nonendemic area. Thin and thick film microscopic review and RDT testing were substantially reduced, facilitating meaningful labor savings for technical staff and pathologists while maintaining diagnostic sensitivity and not sacrificing TAT in malaria diagnosis. The relatively low technical requirements and rapid TAT make LAMP-based malaria screening an excellent option for diagnostic laboratories, particularly in the North American nonendemic setting, where maintenance of proficiency in microscopy may be challenging.

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