

# Publication List

## Malaria-LAMP





# Publication list for Malaria-LAMP

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## Application in elimination settings

### Prevalence of asymptomatic *Plasmodium* infections with sub-microscopic parasite densities in the northwestern border of Thailand: a potential threat to malaria elimination

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– *Malar J* (2018) 17:329 –

#### Abstract

##### Background

Asymptomatic infections with sub-microscopic *Plasmodium* serve as a silent reservoir of disease, critical to sustaining a low level of remanent malaria in the population. These infections must be effectively identified and targeted for elimination. The sensitivity of light microscopy, the traditional method used for diagnosing *Plasmodium* infections, is frequently insufficient for detecting asymptomatic infections due to the low density of parasitaemia. The objective of this study was to explore the current prevalence of asymptomatic sub-microscopic *Plasmodium* carriage to evaluate the parasite reservoir amongst residents from 7 hamlets in Tak Province in northwestern Thailand using a highly sensitive molecular method.

##### Methods

Malaria infection was screened in a real-world setting from 3650 finger-prick blood specimens collected in a mass cross-sectional survey using light microscopy and loop-mediated isothermal amplification (LAMP). LAMP results were later confirmed in a laboratory setting in Bangkok using nested PCR, restriction enzyme digestion and DNA sequencing. The association of malaria infection with demographic factors was explored.

##### Results

Parasite prevalence was 0.27% (10/3650) as determined by microscopy. Sub-microscopic infection prevalence was 2.33% (85/3650) by LAMP. Of these, 30.6% (26/85) were infected with *Plasmodium falciparum*, 52.9% (45/85) with *Plasmodium vivax*, 2.4% (2/85) with *Plasmodium malariae*, 4.7% (4/85) with mixed *P. falciparum* and *P. vivax*, and 9.4% (8/85) had parasite densities too low for species identification. Asymptomatic carriage ( $T < 37.5^{\circ}\text{C}$ ) accounted for 95% (76/80) of all sub-microscopic cases with the highest prevalence occurring in the subjects 31–45 years of age ( $p < 0.035$ ). Participants working on plantations or as merchants had an increased infection risk. Evaluation by microscopy identified 10.53% (10/95) of all *Plasmodium* infected participants.

##### Conclusion

Participants carrying asymptomatic *Plasmodium* infections with sub-microscopic parasite densities are considerable in this area. These findings provide the true disease burden and risk factors in this region. This information helps to direct policy makers towards better schemes and delivery of targeted interventions. Moreover, this is the first study to use LAMP in mass screening for sub-clinical and sub-microscopic infections in a field setting in Thailand. LAMP proves to be a sensitive and field-deployable assay suitable for national malaria control screening campaigns

## Subpatent malaria in a low transmission African setting: a cross-sectional study using rapid diagnostic testing (RDT) and loop-mediated isothermal amplification (LAMP) from Zambezi region, Namibia

Patrick McCreesh, Davis Mumbengegwi, Kathryn Roberts, Munyaradzi Tambo, Jennifer Smith, Brooke Whittemore, Gerard Kelly, Caitlin Moe, Max Murphy, Mukosha Chisenga, Bryan Greenhouse, Henry Ntuku, Immo Kleinschmidt, Hugh Sturrock, Petrina Uusiku, Roland Gosling, Adam Bennett, Michelle S. Hsiang

– *Malar J* (2018) 17:480 –

### Abstract

#### Background

Subpatent malaria infections, or low-density infections below the detection threshold of microscopy or standard rapid diagnostic testing (RDT), can perpetuate persistent transmission and, therefore, may be a barrier for countries like Namibia that are pursuing malaria elimination. This potential burden in Namibia has not been well characterized.

#### Methods

Using a two-stage cluster sampling, cross-sectional design, subjects of all age were enrolled during the end of the 2015 malaria transmission season in Zambezi region, located in northeast Namibia. Malaria RDTs were performed with subsequent gold standard testing by loop-mediated isothermal amplification (LAMP) using dried blood spots. Infection prevalence was measured and the diagnostic accuracy of RDT calculated. Relationships between recent fever, demographics, epidemiological factors, and infection were assessed.

#### Results

Prevalence of *Plasmodium falciparum* malaria infection was low: 0.8% (16/1919) by RDT and 2.2% (43/1919) by LAMP. All but one LAMP-positive infection was RDT-negative. Using LAMP as gold standard, the sensitivity and specificity of RDT were 2.3% and 99.2%, respectively. Compared to LAMP-negative infections, a higher portion LAMP-positive infections were associated with fever (45.2% vs. 30.4%,  $p = 0.04$ ), though 55% of infections were not associated with fever. Agricultural occupations and cattle herding were significantly associated with LAMP-detectable infection (Adjusted ORs 5.02, 95% CI 1.77–14.23, and 11.82, 95% CI 1.06–131.81, respectively), while gender, travel, bed net use, and indoor residual spray coverage were not.

#### Conclusions

This study presents results from the first large-scale malaria cross-sectional survey from Namibia using molecular testing to characterize subpatent infections. Findings suggest that fever history and standard RDTs are not useful to address this burden. Achievement of malaria elimination may require active case detection using more sensitive point-of-care diagnostics or presumptive treatment and targeted to high-risk groups.

## Loop-mediated isothermal amplification (LAMP) and Polymerase Chain Reaction (PCR) as quality assurance tools for Rapid Diagnostic Test (RDT) malaria diagnosis in Northern Namibia

Munyaradzi Tambo, Mary Mwinga, Davis R. Mumbengegwi

– *PLoS ONE* 13(12): e0206848 –

### Abstract

Malaria cases sometimes go undetected using RDTs due to their inaccurate use, poor storage conditions and failure to detect low parasitaemia (<50parasites/ $\mu$ L). This could result in continuous transmission of malaria and sustenance of parasite reservoirs. Molecular diagnostic tools are more sensitive and specific than RDTs in the detection of plasmodium parasites. However, the Polymerase Chain Reaction (PCR) is not routinely used because equipment and reagents are expensive and requires highly skilled personnel. Loop-mediated isothermal amplification (LAMP) is a relatively new molecular diagnostic tool for malaria with all the advantages of PCR (sensitive and specific) without the mentioned disadvantages. However, it has not been evaluated extensively as a point of care diagnostic in the field. One hundred and fifteen used RDTs were collected from health facilities in Northern Namibia in a blind study and PCR and LAMP were used to determine the presence of Plasmodium DNA. The sensitivities and PPV were 40.91% and 90% respectively for RDTs, 72.73% and 100% respectively for PCR with LAMP as the golden standard. In low malaria transmission settings, LAMP can be also be considered for use as a surveillance tool to detect all sources of malaria and determine proportion of low parasitaemia infections in order to eliminate them.



## Evaluation of loop-mediated isothermal amplification as a surveillance tool for malaria in reactive case detection moving towards elimination

Munyaradzi Tambo, Joyce R. Auala, Hugh J. Sturrock, Immo Kleinschmidt, Ronnie Bock, Jennifer L. Smith, Roland Gosling and Davis R. Mumbengegw

– *Malar J* (2018) 17:255 –

### Abstract

#### Background

As malaria transmission decreases, the proportion of infections that are asymptomatic at any given time increases. This poses a challenge for diagnosis as routinely used rapid diagnostic tests (RDTs) miss asymptomatic malaria cases with low parasite densities due to poor sensitivity. Yet, asymptomatic infections can contribute to onward transmission of malaria and therefore act as infectious reservoirs and perpetuate malaria transmission. This study compared the performance of RDTs to loop-mediated isothermal amplification (LAMP) in the diagnosis of malaria during reactive active case detection surveillance.

#### Methods

All reported malaria cases in the Engela Health District of Namibia were traced back to their place of residence and persons living within the four closest neighbouring houses to the index case (neighbourhood) were tested for malaria infection with RDTs and dried blood spots (DBS) were collected. LAMP and nested PCR (nPCR) were carried out on all RDTs and DBS. The same procedure was followed in randomly selected control neighbourhoods.

#### Results

Some 3151 individuals were tested by RDT, LAMP and nPCR. Sensitivity of RDTs and LAMP were 9.30 and 95.50%, respectively, and specificities were 99.27 and 99.92%, respectively, compared to nPCR. LAMP carried out on collected RDTs showed a sensitivity and specificity of 95.35 and 99.85% compared to nPCR carried out on DBS. There were 2 RDT samples that were negative by LAMP but the corresponding DBS samples were positive by PCR.

#### Conclusions

The study showed that LAMP had the equivalent performance as nPCR for the identification of *Plasmodium falciparum* infection. Given its relative simplicity to implement over more complex and time-consuming methods, such as PCR, LAMP is particularly useful in elimination settings where high sensitivity and ease of operation are important.

## Diagnostic accuracy of loop-mediated isothermal amplification (LAMP) for screening malaria in peripheral and placental blood samples from pregnant women in Colombia

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– *Vásquez et al. Malar J (2018) 17:262* –

### Abstract

#### Background

Pregnant women frequently show low-density *Plasmodium* infections that require more sensitive methods for accurate diagnosis and early treatment of malaria. This is particularly relevant in low-malaria transmission areas, where intermittent preventive treatment is not recommended. Molecular methods, such as polymerase chain reaction (PCR) are highly sensitive, but require sophisticated equipment and advanced training. Instead, loop mediated isothermal amplification (LAMP) provides an opportunity for molecular detection of malaria infections in remote endemic areas, outside a reference laboratory. The aim of the study is to evaluate the performance of LAMP for the screening of malaria in pregnant women in Colombia.

#### Methods

This is a nested prospective study that uses data and samples from a larger cross-sectional project conducted from May 2016 to January 2017 in three Colombian endemic areas (El Bagre, Quibdó, and Tumaco). A total of 531 peripheral and placental samples from pregnant women self-presenting at local hospitals for antenatal care visits, at delivery or seeking medical care for suspected malaria were collected. Samples were analysed for *Plasmodium* parasites by light microscopy (LM), rapid diagnostic test (RDT) and LAMP. Diagnostic accuracy endpoints (sensitivity, specificity, predictive values, and kappa scores) of LM, RDT and LAMP were compared with nested PCR (nPCR) as the reference standard.

#### Results

In peripheral samples, LAMP showed an improved sensitivity (100.0%) when compared with LM 79.5% and RDT 76.9% ( $p < 0.01$ ), particularly in afebrile women, for which LAMP sensitivity was two-times higher than LM and RDT. Overall agreement among LAMP and nPCR was high (kappa value = 1.0). Specificity was similar in all tests (100%). In placental blood, LAMP evidenced a four-fold improvement in sensitivity (88.9%) when compared with LM and RDT(22.2%), being the only method, together with nPCR, able to detect placental infections in peripheral blood.

#### Conclusions

LAMP is a simple, rapid and accurate molecular tool for detecting gestational and placental malaria, being able to overcome the limited sensitivity of LM and RDT. These findings could guide maternal health programs in low-transmission settings to integrate LAMP in their surveillance systems for the active detection of low-density infections and asymptomatic malaria cases.

## Clinical consequences of submicroscopic malaria parasitaemia in Uganda

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– *Malar J* (2018) 17:67 –

### Abstract

#### Background

Submicroscopic malaria parasitaemia is common in both high- and low-endemicity settings, but its clinical consequences are unclear.

#### Methods

A cohort of 364 children (0.5–10 years of age) and 106 adults was followed from 2011 to 2016 in Tororo District, Uganda using passive surveillance for malaria episodes and active surveillance for parasitaemia. Participants presented every 90 days for routine visits ( $n = 9075$ ); a subset was followed every 30 days. Participants who presented with fever and a positive blood smear were treated for malaria. At all routine visits microscopy was performed and samples from subjects with a negative blood smear underwent loop-mediated isothermal amplification for detection of plasmodial DNA.

#### Results

Submicroscopic parasitaemia was common; the proportion of visits with submicroscopic parasitemia was 25.8% in children and 39.2% in adults. For children 0.5–10 years of age, but not adults, having microscopic and submicroscopic parasitaemia at routine visits was significantly associated with both fever (adjusted risk ratios [95% CI], 2.64 [2.16–3.22], 1.67 [1.37–2.03]) and non-febrile illness (aRR [CI], 1.52 [1.30–1.78], 1.26 [1.09–1.47]), compared to not having parasitaemia. After stratifying by age, significant associations were seen between submicroscopic parasitaemia and fever in children aged 2–< 5 and 5–10 years (aRR [CI], 1.42 [1.03–1.98], 2.01 [1.49–2.71]), and submicroscopic parasitaemia and non-febrile illness in children aged 5–10 years (aRR [CI], 1.44 [1.17–1.78]). These associations were maintained after excluding individuals with a malaria episode within the preceding 14 or following 7 days, and after adjusting for household wealth.

#### Conclusions

Submicroscopic malaria infections were associated with fever and non-febrile illness in Ugandan children. These findings support malaria control strategies that target low-density infections.

## Laboratory challenges of *Plasmodium* species identification in Aceh Province, Indonesia, a malaria elimination setting with newly discovered *P. knowlesi*

Farah N. Coutrier, Yusrifar K. Tirta, Chris Cotter, Iska Zarlinda, Iveth J. González, Alanna Schwartz, Cut Maneh, Jutta Marfurt, Maxwell Murphy, Herdiana Herdiana, Nicholas M. Anstey, Bryan Greenhouse, Michelle S. Hsiang, Rintis Noviyanti

– *PLoS Negl Trop Dis* 12(11): e0006924 –

### Abstract

The discovery of the life-threatening zoonotic infection *Plasmodium knowlesi* has added to the challenges of prompt and accurate malaria diagnosis and surveillance. In this study from Aceh Province, Indonesia, a malaria elimination setting where *P. knowlesi* endemicity was not previously known, we report the laboratory investigation and difficulties encountered when using molecular detection methods for quality assurance of microscopically identified clinical cases. From 2014 to 2015, 20 (49%) *P. falciparum*, 16 (39%) *P. vivax*, 3 (7%) *P. malariae*, and 2 (5%) indeterminate species were identified by microscopy from four sentinel health facilities. At a provincial-level reference laboratory, loop-mediated isothermal amplification (LAMP), a field-friendly molecular method, was performed and confirmed *Plasmodium* in all samples though further species-identification was limited by the unavailability of non-falciparum species-specific testing with the platform used. At a national reference laboratory, several molecular methods including nested PCR (nPCR) targeting the 18S small subunit (18S) ribosomal RNA, nPCR targeting the cytochrome-b (*cytb*) gene, a *P. knowlesi*-specific nPCR, and finally sequencing, were necessary to ultimately classify the samples as: 19 (46%) *P. knowlesi*, 8 (20%) *P. falciparum*, 14 (34%) *P. vivax*. Microscopy was unable to identify or mis-classified up to 56% of confirmed cases, including all cases of *P. knowlesi*. With the nPCR methods targeting the four human-only species, *P. knowlesi* was missed (18S rRNA method) or showed cross-reactivity for *P. vivax* (*cytb* method). To facilitate diagnosis and management of potentially fatal *P. knowlesi* infection and surveillance for elimination of human-only malaria in Indonesia and other affected settings, new detection methods are needed for testing at the point-of-care and in local reference laboratories.

## Loop mediated isothermal amplification (LAMP) accurately detects malaria DNA from filter paper blood samples of low density parasitaemias

Aydin-Schmidt B, Xu W, González IJ, Polley SD, Bell D, Shakely D, Msellem MI, Björkman A, Mårtensson A.

– *PLoS ONE* 9(8): e103905. doi:10.1371/journal.pone.0103905 –

### Abstract

#### Background

Loop mediated isothermal amplification (LAMP) provides an opportunity for improved, field-friendly detection of malaria infections in endemic areas. However data on the diagnostic accuracy of LAMP for active case detection, particularly low-density parasitaemias, are lacking. We therefore evaluated the performance of a new LAMP kit compared with PCR using DNA from filter paper blood spots.

#### Methods and findings

Samples from 865 fever patients and 465 asymptomatic individuals collected in Zanzibar were analysed for Pan (all species) and Pf (*P. falciparum*) DNA with the Loopamp MALARIA Pan/Pf kit. Samples were amplified at 65°C for 40 minutes in a real-time turbidimeter and results were compared with nested PCR. Samples with discordant results between LAMP and nested PCR were analysed with real-time PCR. The real-time PCR corrected nested PCR result was defined as gold standard. Among the 117 (13.5%) PCR detected *P. falciparum* infections from fever patients (mean parasite density 7491/μL, range 6-782,400) 115, 115 and 111 were positive by Pan-LAMP, Pf-LAMP and nested PCR, respectively. The sensitivities were 98.3% (95%CI 94-99.8) for both Pan and Pf-LAMP. Among the 54 (11.6%) PCR positive samples from asymptomatic individuals (mean parasite density 10/μL, range 0-4972) Pf-LAMP had a sensitivity of 92.7% (95%CI 80.1-98.5) for detection of the 41 *P. falciparum* infections. Pan-LAMP had sensitivities of 97% (95%CI 84.2-99.9) and 76.9% (95%CI 46.2-95) for detection of *P. falciparum* and *P. malariae*, respectively. The specificities for both Pan and Pf-LAMP were 100% (95%CI 99.1-100) in both study groups.

#### Conclusions

Both components of the Loopamp MALARIA Pan/Pf detection kit revealed high diagnostic accuracy for parasite detection among fever patients and importantly also among asymptomatic individuals of low parasite densities from minute blood volumes preserved on filter paper. These data support LAMPs potential role for improved detection of low-density malaria infections in pre-elimination settings.

## LAMP kit for diagnosis of non-falciparum malaria in *Plasmodium ovale* infected patients

Juan Cuadros, Alexandra Martin Ramírez, Iveth J. González, Xavier C. Ding, Ramon Perez Tanoira, Gerardo Rojo-Marcos, Peña Gomez-Herruz and Jose Miguel Rubio

– *Malar J* (2017) 16:20 –

### Abstract

#### Background

Microscopy and rapid diagnosis tests have a limited sensitivity in diagnosis of malaria by *Plasmodium ovale*. The LAMP kit (LoopAMP®) can be used in the field without special equipment and could have an important role in malaria control programmes in endemic areas and for malaria diagnosis in returned travellers. The performance of the Pan primer of the kit in detecting malaria by *P. ovale* was compared with the results of standard nPCR in samples of patients returning from *P. ovale* endemic areas.

#### Methods

*Plasmodium ovale* positive samples (29, tested by PCR and/or microscopy) and malaria negative specimens (398, tested by microscopy and PCR) were collected in different hospitals of Europe from June 2014 to March 2016 and frozen at –20 °C. Boil and spin method was used to extract DNA from all samples and amplification was performed with LoopAMP® MALARIA kit (Eiken Chemical, Japan) in an automated turbidimeter (Eiken 500). The results of LAMP read by turbidimetry and with the naked eye were compared.

#### Results

The kit showed a sensitivity of 100% and a specificity of 97.24% with positive and negative predictive values of 72.5 and 100%, respectively. Naked eyed readings were in accordance with turbidimetry readings (sensitivity, 92.5%, specificity, 98.96% and positive and negative predictive values, respectively, 90.24 and 99.22%). The limit of detection of LAMP assay for *P. ovale* was between 0.8 and 2 parasites/μl.

#### Conclusions

The Pan primer of the Malaria kit LoopAMP® can detect *P. ovale* at very low-levels and showed a predictive negative value of 100%. This tool can be useful in malaria control and elimination programmes and in returned travellers from *P. ovale* endemic areas. Naked eye readings are equivalent to automated turbidimeter readings in specimens obtained with EDTA.

## Malaria risk factor assessment using active and passive surveillance data from Aceh Besar, Indonesia, a low endemic, malaria elimination setting with *Plasmodium knowlesi*, *Plasmodium vivax*, and *Plasmodium falciparum*

Herdiana Herdiana, Chris Cotter, Farah N. Coutrier, Iska Zarlinda, Brittany W. Zelman, Yusrifar Kharisma Tirta, Bryan Greenhouse, Roly D. Gosling, Peter Baker, Maxine Whittaker, Michelle S. Hsiang  
– *Malar J.* 2016; 15(1): 468. –

### Abstract

#### Background

As malaria transmission declines, it becomes more geographically focused and more likely due to asymptomatic and non-falciparum infections. To inform malaria elimination planning in the context of this changing epidemiology, local assessments on the risk factors for malaria infection are necessary, yet challenging due to the low number of malaria cases.

#### Methods

A population-based, cross-sectional study was performed using passive and active surveillance data collected in Aceh Besar District, Indonesia from 2014 to 2015. Malaria infection was defined as symptomatic polymerase chain reaction (PCR)-confirmed infection in index cases reported from health facilities, and asymptomatic or symptomatic PCR-confirmed infection identified in reactive case detection (RACD). Potential risk factors for any infection, species-specific infection, or secondary-case detection in RACD were assessed through questionnaires and evaluated for associations.

#### Results

Nineteen *Plasmodium knowlesi*, 12 *Plasmodium vivax* and six *Plasmodium falciparum* cases were identified passively, and 1495 community members screened in RACD, of which six secondary cases were detected (one *P. knowlesi*, three *P. vivax*, and two *P. falciparum*, with four being asymptomatic). Compared to non-infected subjects screened in RACD, cases identified through passive or active surveillance were more likely to be male (AOR 12.5, 95 % CI 3.0–52.1), adult (AOR 14.0, 95 % CI 2.2–89.6 for age 16–45 years compared to <15 years), have visited the forest in the previous month for any reason (AOR 5.6, 95 % CI 1.3–24.2), and have a workplace near or in the forest and requiring overnight stays (AOR 7.9, 95 % CI 1.6–39.7 compared to workplace not near or in the forest). Comparing subjects with infections of different species, differences were observed in sub-district of residence and other demographic and behavioural factors. Among subjects screened in RACD, cases compared to non-cases were more likely to be febrile and reside within 100 m of the index case.

#### Conclusion

In this setting, risk of malaria infection in index and RACD identified cases was associated with forest exposure, particularly overnights in the forest for work. In low-transmission settings, utilization of data available through routine passive and active surveillance can support efforts to target individuals at high risk.

## Malaria diagnosis by loop-mediated isothermal amplification (LAMP) in Thailand

Ronja OCKER, Yongyut PROMPUNJAI, Salakchit CHUTIPONGVIVATE, Panagiotis KARANIS

– *Rev Inst Med Trop Sao Paulo*. 2016; 58: 27. –

### Abstract

The loop-mediated isothermal amplification method (LAMP) is a recently developed molecular technique that amplifies nucleic acid under isothermal conditions. For malaria diagnosis, 150 blood samples from consecutive febrile malaria patients, and healthy subjects were screened in Thailand. Each sample was diagnosed by LAMP, microscopy and nested polymerase chain reaction (nPCR), using nPCR as the gold standard. Malaria LAMP was performed using *Plasmodium* genus and *Plasmodium falciparum* specific assays in parallel. For the genus *Plasmodium*, microscopy showed a sensitivity and specificity of 100%, while LAMP presented 99% of sensitivity and 93% of specificity. For *P. falciparum*, microscopy had a sensitivity of 95%, and LAMP of 90%, regarding the specificity; and microscopy presented 93% and LAMP 97% of specificity. The results of the genus-specific LAMP technique were highly consistent with those of nPCR and the sensitivity of *P. falciparum* detection was only marginally lower.





## Diagnostic accuracy of a LAMP kit for diagnosis of imported malaria in Switzerland

Hanspeter Marti, Chris Stalder, Iveth J. Gonzalez

– *Travel Medicine and Infectious Disease* (2015) 13, 167e171 –

### Abstract

#### Background

The diagnosis of acute malaria in non-endemic countries is still carried out largely by microscopic examination of thick and thin smears or rapid diagnostic tests. Low-density infections might be missed, however, but the more sensitive PCR is more expensive, complex and requires considerable more time.

#### Method

We examined the suitability of a new loop-mediated isothermal DNA-amplification kit (LAMP) for malaria diagnosis in febrile returning travelers in comparison to qPCR and microscopic examination in a prospective study in a non-endemic setting at the Swiss TPH.

#### Results

Among 205 complete datasets, 43 samples were positive for malaria by microscopy, with *Plasmodium falciparum* (35 cases) being the most frequent species. All these samples were positive by both LAMP and qPCR, too. An additional 4 samples negative by microscopy were positive by both LAMP and qPCR. Three of these samples were follow-up samples taken after start of treatment in patients originally identified as positive by microscopy.

#### Conclusions

The LAMP performed exactly as did the qPCR and is a very valuable diagnostic alternative with a potential of being used also in endemic settings.

## Loop-mediated isothermal amplification (LAMP) for point-of-care detection of asymptomatic low-density malaria parasite carriers in Zanzibar

Jackie Cook, Berit Aydin-Schmidt, Iveth J González, David Bell, Elin Edlund, Majda H Nassor, Mwinyi Msellem, Abdullah Ali, Ali K Abass, Andreas Mårtensson and Anders Björkman

– *Malaria Journal* 2015: DOI 10.1186/s12936-015-0573-y –

### Abstract

#### Background

Asymptomatic, low parasite density malaria infections are difficult to detect with currently available point-of-care diagnostics. This study piloted a loop-mediated isothermal amplification (LAMP) kit for field-friendly, high-throughput detection of asymptomatic malaria infections during mass screening and treatment (MSAT) in Zanzibar, a malaria pre-elimination setting.

#### Methods

Screening took place in three known hotspot areas prior to the short rains in November. Finger-prick blood was taken for screening by rapid diagnostic test (RDT) and LAMP and collected on filter paper for subsequent polymerase chain reaction (PCR) analyses. LAMP results were compared to RDT and to PCR using McNemar's test.

#### Results

Approximately 1,000 people were screened. RDT detected ten infections (1.0% (95% CI 0.3-1.6)) whilst both LAMP and PCR detected 18 (1.8% (95% CI 0.9-2.6)) infections. However, PCR identified three infections that LAMP did not detect and *vice versa*. LAMP testing was easy to scale-up in field conditions requiring minimal training and equipment, with results ready one to three hours after screening.

#### Conclusions

Despite lower than expected prevalence, LAMP detected a higher number of infections than the currently used diagnostic, RDT. LAMP is a field-friendly, sensitive diagnostic test that could be useful for MSAT malaria campaigns which require quick results to enable prompt treatment.

## Field deployment of loop-mediated isothermal amplification for centralized mass-screening of asymptomatic malaria in Zanzibar: a pre-elimination setting

Ulrika Morris, Mwinyi Khamis, Berit Aydin-Schmidt, Ali K Abass, Mwinyi I Msellem, Majda H Nassor, Iveth J González, Andreas Mårtensson, Abdullah S Ali, Anders Björkman and Jackie Cook

– *Malaria Journal* 2015: DOI 10.1186/s12936-015-0731-2 –

### Abstract

#### Background

Molecular tools for detection of low-density asymptomatic *Plasmodium* infections are needed in malaria elimination efforts. This study reports results from the hitherto largest implementation of loop-mediated isothermal amplification (LAMP) for centralized mass screening of asymptomatic malaria in Zanzibar.

#### Methods

Healthy individuals present and willing to participate in randomly selected households in 60 villages throughout Zanzibar were screened for malaria by rapid diagnostic tests (RDT). In 50 % of the study households, participants were asked to provide 60 µL of finger-prick blood for additional LAMP screening. LAMP was conducted in two centralized laboratories in Zanzibar, by trained technicians with limited or no previous experience of molecular methods. The LAMP assay was performed with Loopamp™ MALARIA Pan/Pf Detection Kit (Eiken Chemical Company, Japan). Samples positive for *Plasmodium* genus (Pan)-LAMP were re-tested using *Plasmodium falciparum*-specific LAMP kits.

#### Results

Paired RDT and LAMP samples were available from 3983 individuals. The prevalence of asymptomatic malaria was 0.5 % (CI 95 % 0.1-0.8) and 1.6 % (CI 95 % 1.1-2.2) by RDT and Pan-LAMP, respectively. LAMP detected 3.4 (CI 95 % 2.2-5.2) times more *Plasmodium* positive samples than RDT. DNA contamination was experienced, but solved by repetitive decontamination of all equipment and reagents.

#### Conclusions

LAMP is a simple and sensitive molecular tool, and has potential in active surveillance and mass-screening programmes for detection of low-density asymptomatic malaria in pre-elimination settings. However, in order to deploy LAMP more effectively in field settings, protocols may need to be adapted for processing larger numbers of samples. A higher throughput, affordable closed system would be ideal to avoid contamination.

## Clinical evaluation of a loop-mediated amplification kit for diagnosis of imported malaria

Spencer D. Polley, Iveth J. González, Deqa Mohamed, Rosemarie Daly, Kathy Bowers, Julie Watson, Emma Mewse, Margaret Armstrong, Christen Gray, Mark D. Perkins, David Bell, Hidetoshi Kanda, Norihiro Tomita, Yutaka Kubota, Yasuyoshi Mori, Peter L. Chiodini, Colin J. Sutherland

– *J Infect Dis.* 2013 Aug 15;208(4):637-44 –

### Abstract

#### Background

Diagnosis of malaria relies on parasite detection by microscopy or antigen detection; both fail to detect low-density infections. New tests providing rapid, sensitive diagnosis with minimal need for training would enhance both malaria diagnosis and malaria control activities. We determined the diagnostic accuracy of a new loop-mediated amplification (LAMP) kit in febrile returned travelers.

#### Methods

The kit was evaluated in sequential blood samples from returned travelers sent for pathogen testing to a specialist parasitology laboratory. Microscopy was performed, and then malaria LAMP was performed using *Plasmodium* genus and *Plasmodium falciparum*-specific tests in parallel. Nested polymerase chain reaction (PCR) was performed on all samples as the reference standard. Primary outcome measures for diagnostic accuracy were sensitivity and specificity of LAMP results, compared with those of nested PCR.

#### Results

A total of 705 samples were tested in the primary analysis. Sensitivity and specificity were 98.4% and 98.1%, respectively, for the LAMP *P. falciparum* primers and 97.0% and 99.2%, respectively, for the *Plasmodium* genus primers. Post hoc repeat PCR analysis of all 15 tests with discrepant results resolved 4 results in favor of LAMP, suggesting that the primary analysis had underestimated diagnostic accuracy.

#### Conclusions

Malaria LAMP had a diagnostic accuracy similar to that of nested PCR, with a greatly reduced time to result, and was superior to expert microscopy.

## Molecular diagnosis for screening and elimination of malaria: performance of the first commercially-available malaria LAMP test

Iveth J González, Spencer Polley, Heidi Hopkins, Colin Sutherland, Yasuyoshi Mori, Mark Perkins, David Bell  
– *Malaria Journal* 2012: doi:10.1186/1475-2875-11-51-O30 –

### Background

The ability to screen for asymptomatic malaria infection at a field level is increasingly recognized as a key strategy in malaria elimination campaigns. However, molecular methods necessary to detect very low parasite density infections, such as PCR, are restricted to reference-level laboratories and require considerable training to perform. To be effective, such techniques must be close enough to the positive cases to enable rapid treatment. Loop-mediated isothermal DNA amplification (LAMP) is highly sensitive and specific, faster than PCR, requires minimal processing and instrumentation, and allows result detection with the naked eye.

### Materials and methods

FIND has been working with the Hospital for Tropical Diseases in London and Eiken Chemical Company (Japan) in the development of a simplified LAMP assay for the diagnosis of malaria. An optimized test targeting different sequences in the mitochondrial DNA was developed for the detection of parasitaemias below 1 parasite/ $\mu$ l of blood in less than 40 minutes. Prototypes of this test have been compared to PCR with samples from febrile patients in two clinical trials, one in London (travelers) and other in an endemic setting in Uganda.

### Results

Both clinical trials have demonstrated that LAMP is equivalent to nested PCR in sensitivity and specificity with faster time-to-results. In London with 705 samples, sensitivity and specificity of the LAMP *P. falciparum* primers were 98.4% and 98.1% respectively, and for the LAMP Pan primers, 97.0% and 99.2% respectively. In Uganda, 272 samples were tested with the LAMP *P. falciparum* primers and sensitivity and specificity were 93.3% and 85% respectively. This performance of the LAMP assay for malaria was achieved using two simple DNA extraction methods that take only 15 minutes per sample. The study in Uganda also demonstrated that technicians without molecular training could perform the test after a short training period in a simple laboratory space with basic equipment.

### Conclusions

This LAMP test has potential applications both as reference standard for other diagnostics, for primary diagnosis of returned travelers in non-endemic countries, and as a tool for population screening in malaria elimination campaigns. A high-throughput assay suited to large-scale screening studies is on development.

## Application in endemic settings

### Loop-Mediated Isothermal Amplification Assay for Identification of Five Human *Plasmodium* Species in Malaysia

Lau YL, Lai MY, Fong MY, Jelip J, Mahmud R

– *Am. J. Trop. Med. Hyg.*, 94(2), 2016, pp. 336 -339 –

#### Abstract

The lack of rapid, affordable, and accurate diagnostic tests represents the primary hurdle affecting malaria surveillance in resource- and expertise-limited areas. Loop-mediated isothermal amplification (LAMP) is a sensitive, rapid, and cheap diagnostic method. Five species-specific LAMP assays were developed based on 18S rRNA gene. Sensitivity and specificity of LAMP results were calculated as compared with microscopic examination and nested polymerase chain reaction. LAMP reactions were highly sensitive with the detection limit of one copy for *Plasmodium vivax*, *Plasmodium falciparum*, and *Plasmodium malariae* and 10 copies for *Plasmodium knowlesi* and *Plasmodium ovale*. LAMP positively detected all human malaria species in all positive samples (N = 134; sensitivity = 100%) within 35 minutes. All negative samples were not amplified by LAMP (N = 67; specificity = 100%). LAMP successfully detected two samples with very low parasitemia. LAMP may offer a rapid, simple, and reliable test for the diagnosis of malaria in areas where malaria is prevalent.

## Highly sensitive detection of malaria parasitemia in a malaria-endemic setting: performance of a new loop-mediated isothermal amplification kit in a remote clinic in Uganda

Hopkins H, González IJ, Polley SD, Angutoko P, Ategeka J, Asiimwe C, Agaba B, Kyabayinze DJ, Sutherland CJ, Perkins MD, Bell D.

– *J Infect Dis.* 2014 May 1;209(9):1494. –

### Abstract

#### Background

Current malaria diagnostic tests, including microscopy and antigen-detecting rapid tests, cannot reliably detect low-density infections. Molecular methods such as polymerase chain reaction (PCR) are highly sensitive but remain too complex for field deployment. A new commercial molecular assay based on loop-mediated isothermal amplification (LAMP) was assessed for field use.

#### Methods

Malaria LAMP (Eiken Chemical, Japan) was evaluated for samples from 272 outpatients at a rural Ugandan clinic and compared with expert microscopy, nested PCR, and quantitative PCR (qPCR). Two technicians performed the assay after 3 days of training, using 2 alternative blood sample-preparation methods and visual interpretation of results by fluorescence assay.

#### Results

Compared with 3-well nested PCR, the sensitivity of both LAMP and single-well nested PCR was 90%; the microscopy sensitivity was 51%. For samples with a *Plasmodium falciparum* qPCR titer of  $\geq 2$  parasites/ $\mu\text{L}$ , LAMP sensitivity was 97.8% (95% confidence interval, 93.7%-99.5%). Most false-negative LAMP results involved samples with parasitemia levels detectable by 3-well nested PCR but very low or undetectable by qPCR.

#### Conclusions

Malaria LAMP in a remote Ugandan clinic achieved sensitivity similar to that of single-well nested PCR in a United Kingdom reference laboratory. LAMP dramatically lowers the detection threshold achievable in malaria-endemic settings, providing a new tool for diagnosis, surveillance, and screening in elimination strategies.

## Loop-mediated isothermal amplification assay for rapid diagnosis of malaria infections in an area of endemicity in Thailand

Jetsumon Sattabongkot, Takafumi Tsuboi, Eun-Taek Han, Sirasate Bantuchai, Sureemas Buates

– *J Clin Microbiol.* 2014 Jul;52(7):2746. –

### Abstract

The loop-mediated isothermal amplification (LAMP) method, developed by our group for diagnosis of four human malaria parasites, was evaluated on a large scale at a remote clinic in Thailand where malaria is endemic. A total of 899 febrile patients were analyzed in this study. LAMP was first evaluated in 219 patients, and the result was compared to those of two histidine-rich protein (HRP)-2 rapid diagnostic tests (RDTs) and microscopy as a gold standard. LAMP DNA extraction was conducted by a simple boiling method, and the test results were assessed visually. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were 95.7%, 100%, 100%, and 98%, respectively, for LAMP and 98.6%, 98%, 95.8%, and 99.3%, respectively, for RDTs. Since RDT-positive results were based on one out of two RDTs, the sensitivity of RDTs was slightly higher than that of LAMP. However, LAMP tended to be more specific than RDTs. LAMP next was evaluated in 680 patients, and the result was compared to that of microscopy as a gold standard. Sensitivity, specificity, PPV, NPV, and diagnostic accuracy of LAMP were 88.9%, 96.9%, 92.2%, 95.5%, and 94.6%, respectively. Nested PCR was used to confirm the discrepant results. Malaria LAMP in a remote clinic in Thailand achieved an acceptable result, indicating that LAMP malaria diagnosis is feasible in a field setting with limited technical resources. Additionally, the rapid boiling method for extracting DNA from dried blood spots proved to be simple, fast, and suitable for use in the field.





## *Plasmodium vivax*

### *Plasmodium vivax* molecular diagnostics in community surveys: pitfalls and solutions

Maria Gruenberg, Clara Antunes Moniz, Natalie Ellen Hofmann, Rahel Wampfer, Cristian Koepfli, Ivo Mueller, Wuelton Marcelo Monteiro, Marcus Lacerda, Gisely Cardoso de Melo, Andrea Kuehn, Andre M. Siqueira, Ingrid Felger

– *Malar J* (2018) 17:55 –

#### **Abstract**

A distinctive feature of *Plasmodium vivax* infections is the overall low parasite density in peripheral blood. Thus, identifying asymptomatic infected individuals in endemic communities requires diagnostic tests with high sensitivity. The detection limits of molecular diagnostic tests are primarily defined by the volume of blood analysed and by the copy number of the amplified molecular marker serving as the template for amplification. By using mitochondrial DNA as the multi-copy template, the detection limit can be improved more than tenfold, compared to standard 18S rRNA targets, thereby allowing detection of lower parasite densities. In a very low transmission area in Brazil, application of a mitochondrial DNA-based assay increased prevalence from 4.9 to 6.5%. The usefulness of molecular tests in malaria epidemiological studies is widely recognized, especially when precise prevalence rates are desired. Of concern, however, is the challenge of demonstrating test accuracy and quality control for samples with very low parasite densities. In this case, chance effects in template distribution around the detection limit constrain reproducibility. Rigorous assessment of false positive and false negative test results is, therefore, required to prevent over- or under-estimation of parasite prevalence in epidemiological studies or when monitoring interventions.

## On the epidemiology of *Plasmodium vivax* malaria: past and present with special reference to the former USSR

Anatoly V. Kondrashin, Lola F. Morozova, Ekaterina V. Stepanova, Natalia A. Turbabina, Maria S. Maksimova, Evgeny N. Morozov

– *Malar J* (2018) 17:346 –

### Abstract

Presently, many malaria-endemic countries in the world are transitioning towards malaria elimination. Out of the 105 countries with ongoing malaria transmission, 10 countries are classified as being in the pre-elimination phase of malaria control, and 9 countries are in the malaria elimination stage, whereas 7 countries are classified as being in the prevention of introduction phase. Between 2000 and 2015, 17 countries eliminated malaria (i.e., attained zero indigenous cases for 3 years or more). Seven countries were certified by the WHO as having successfully eliminated malaria. The purpose of this review was to analyse the epidemiological characteristics of vivax malaria during the various stages of malaria eradication (elimination) programmes in different countries in the past and present. Experiences of the republics of the former USSR with malaria are interesting, particularly since the data overwhelmingly were published in Russian and might not be known to western readers. Among the most important characteristics of *Plasmodium vivax* epidemiology at present are changes in the ratio of the short-incubation *P. vivax* to long-incubation *P. vivax*, the incidence of severe *P. vivax* cases, the increased numbers of asymptomatic *P. vivax* cases, the reduced response to anti-malarials and a few others. Various factors contributing towards the peculiarities of *P. vivax* epidemiology are discussed.

## Diagnosis and Treatment of *Plasmodium vivax* Malaria

J. Kevin Baird, Neena Valecha, Stephan Duparc, Nicholas J. White, and Ric N. Price

– doi:10.4269/ajtmh.16-0171. –

### Abstract

The diagnosis and treatment of *Plasmodium vivax* malaria differs from that of *Plasmodium falciparum* malaria in fundamentally important ways. This article reviews the guiding principles, practices, and evidence underpinning the diagnosis and treatment of *P. vivax* malaria.

## Comparison to other methods

### Loop-mediated isothermal amplification (LAMP) and Polymerase Chain Reaction (PCR) as quality assurance tools for Rapid Diagnostic Test (RDT) malaria diagnosis in Northern Namibia

Munyaradzi Tambo, Mary Mwinga, Davis R. Mumbengegwi

– *PLoS ONE* 13(12): e0206848 –

#### Abstract

Malaria cases sometimes go undetected using RDTs due to their inaccurate use, poor storage conditions and failure to detect low parasitaemia (<50parasites/ $\mu$ L). This could result in continuous transmission of malaria and sustenance of parasite reservoirs. Molecular diagnostic tools are more sensitive and specific than RDTs in the detection of plasmodium parasites. However, the Polymerase Chain Reaction (PCR) is not routinely used because equipment and reagents are expensive and requires highly skilled personnel. Loop-mediated isothermal amplification (LAMP) is a relatively new molecular diagnostic tool for malaria with all the advantages of PCR (sensitive and specific) without the mentioned disadvantages. However, it has not been evaluated extensively as a point of care diagnostic in the field. One hundred and fifteen used RDTs were collected from health facilities in Northern Namibia in a blind study and PCR and LAMP were used to determine the presence of Plasmodium DNA. The sensitivities and PPV were 40.91% and 90% respectively for RDTs, 72.73% and 100% respectively for PCR with LAMP as the golden standard. In low malaria transmission settings, LAMP can be also be considered for use as a surveillance tool to detect all sources of malaria and determine proportion of low parasitaemia infections in order to eliminate them.

## Costs and cost-effectiveness of malaria reactive case detection using loop-mediated isothermal amplification compared to microscopy in the low transmission setting of Aceh Province, Indonesia

Brittany W. Zelman, Ranju Baral, Iska Zarlinda, Farah N. Coutrier, Kelly C. Sanders, Chris Cotter, Herdiana Herdiana, Bryan Greenhouse, Rima Shretta, Roly D. Gosling and Michelle S. Hsiang

– Zelman et al. *Malar J* (2018) 17:220 –

### Abstract

#### Background

Reactive case detection (RACD) is an active case finding strategy where households and neighbours of a passively identified case (index case) are screened to identify and treat additional malaria infections with the goal of gathering surveillance information and potentially reducing further transmission. Although it is widely considered a key strategy in low burden settings, little is known about the costs and the cost-effectiveness of different diagnostic methods used for RACD. The aims of this study were to measure the cost of conducting RACD and compare the cost-effectiveness of microscopy to the more sensitive diagnostic method loop-mediated isothermal amplification (LAMP).

#### Methods

The study was conducted in RACD surveillance sites in five sub-districts in Aceh Besar, Indonesia. The cost inputs and yield of implementing RACD with microscopy and/or LAMP were collected prospectively over a 20 months study period between May 2014 and December 2015. Costs and cost-effectiveness (USD) of the different strategies were examined. The main cost measures were cost per RACD event, per person screened, per population at risk (PAR); defined as total population in each sub-district, and per infection found. The main cost-effectiveness measure was incremental cost-effectiveness ratio (ICER), expressed as cost per malaria infection detected by LAMP versus microscopy. The effects of varying test positivity rate or diagnostic yield on cost per infection identified and ICER were also assessed.

#### Results

Among 1495 household members and neighbours screened in 36 RACD events, two infections were detected by microscopy and confirmed by LAMP, and four infections were missed by microscopy but detected by LAMP. The average total cost of conducting RACD using microscopy and LAMP was \$1178 per event with LAMP specific consumables and personnel being the main cost drivers. The average cost of screening one individual during RACD was \$11, with an additional cost of diagnostics at \$0.62 and \$16 per person for microscopy and LAMP, respectively. As a public health intervention, RACD using both diagnostics cost an average of \$0.42 per PAR per year. Comparing RACD using microscopy only versus RACD using LAMP only, the cost per infection found was \$8930 and \$6915, respectively. To add LAMP as an additional intervention accompanying RACD would cost \$9 per individual screened annually in this setting.

The ICER was estimated to be \$5907 per additional malaria infection detected by LAMP versus microscopy. Cost per infection identified and ICER declined with increasing test positivity rate and increasing diagnostic yield.

### Conclusions

This study provides the first estimates on the cost and cost-effectiveness of RACD from a low transmission setting. Costs per individual screened were high, though costs per PAR were low. Compared to microscopy, the use of LAMP in RACD was more costly but more cost-effective for the detection of infections, with diminishing returns observed when findings were extrapolated to scenarios with higher prevalence of infection using more sensitive diagnostics. As malaria programmes consider active case detection and the integration of more sensitive diagnostics, these findings may inform strategic and budgetary planning.



## Subpatent malaria in a low transmission African setting: a cross-sectional study using rapid diagnostic testing (RDT) and loop-mediated isothermal amplification (LAMP) from Zambezi region, Namibia

Patrick McCreesh, Davis Mumbengegwi, Kathryn Roberts, Munyaradzi Tambo, Jennifer Smith, Brooke Whittemore, Gerard Kelly, Caitlin Moe, Max Murphy, Mukosha Chisenga, Bryan Greenhouse, Henry Ntuku, Immo Kleinschmidt, Hugh Sturrock, Petrina Uusiku, Roland Gosling, Adam Bennett, Michelle S. Hsiang

– *Malar J* (2018) 17:480 –

### Abstract

#### Background

Subpatent malaria infections, or low-density infections below the detection threshold of microscopy or standard rapid diagnostic testing (RDT), can perpetuate persistent transmission and, therefore, may be a barrier for countries like Namibia that are pursuing malaria elimination. This potential burden in Namibia has not been well characterized.

#### Methods

Using a two-stage cluster sampling, cross-sectional design, subjects of all age were enrolled during the end of the 2015 malaria transmission season in Zambezi region, located in northeast Namibia. Malaria RDTs were performed with subsequent gold standard testing by loop-mediated isothermal amplification (LAMP) using dried blood spots. Infection prevalence was measured and the diagnostic accuracy of RDT calculated. Relationships between recent fever, demographics, epidemiological factors, and infection were assessed.

#### Results

Prevalence of *Plasmodium falciparum* malaria infection was low: 0.8% (16/1919) by RDT and 2.2% (43/1919) by LAMP. All but one LAMP-positive infection was RDT-negative. Using LAMP as gold standard, the sensitivity and specificity of RDT were 2.3% and 99.2%, respectively. Compared to LAMP-negative infections, a higher portion LAMP-positive infections were associated with fever (45.2% vs. 30.4%,  $p = 0.04$ ), though 55% of infections were not associated with fever. Agricultural occupations and cattle herding were significantly associated with LAMP-detectable infection (Adjusted ORs 5.02, 95% CI 1.77–14.23, and 11.82, 95% CI 1.06–131.81, respectively), while gender, travel, bed net use, and indoor residual spray coverage were not.

#### Conclusions

This study presents results from the first large-scale malaria cross-sectional survey from Namibia using molecular testing to characterize subpatent infections. Findings suggest that fever history and standard RDTs are not useful to address this burden. Achievement of malaria elimination may require active case detection using more sensitive point-of-care diagnostics or presumptive treatment and targeted to high-risk groups.

## Characterizing microscopic and submicroscopic malaria parasitaemia at three sites with varied transmission intensity in Uganda

John Rek, Shereen Katrak, Hannah Obasi, Patience Nayebare, Agaba Katureebe, Elijah Kakande, Emmanuel Arinaitwe, Joaniter I. Nankabirwa, Prasanna Jagannathan, Chris Drakeley, Sarah G. Staedke, David L. Smith, Teun Bousema, Moses Kamya, Philip J. Rosenthal, Grant Dorsey, Bryan Greenhouse

– *Malaria Journal* 2016: DOI 10.1186/s12936-016-1519-8 –

### Abstract

#### Background

Parasite prevalence is a key metric used to quantify the burden of malaria and assess the impact of control strategies. Most published estimates of parasite prevalence are based on microscopy and likely underestimate true prevalence.

#### Methods

Thick smear microscopy was performed in cohorts of children (aged 6 month to 10 years) and adults every 90 days over 2 years, at three sites of varying transmission intensity in Uganda. Microscopy-negative samples were tested for sub-microscopic parasitaemia using loop-mediated isothermal amplification (LAMP). Generalized estimating equation models were used to evaluate associations between age and parasitaemia, factors associated with sub-microscopic infection and associations between parasitaemia and haemoglobin.

#### Results

A total of 9260 samples were collected from 1245 participants. Parasite prevalence among children across the three sites was 7.4, 9.4 and 28.8 % by microscopy and 21.3, 31.8 and 69.0 % by microscopy plus LAMP. Parasite prevalence among adults across the three sites was 3.1, 3.0 and 5.2 % by microscopy and 18.8, 24.2 and 53.5 % by microscopy plus LAMP. Among those with parasitaemia, adults and persons recently treated with anti-malarial therapy had the highest prevalence of sub-microscopic infection. Children with sub-microscopic or microscopic parasitaemia had lower mean haemoglobin levels compared to children with no detectable parasites.

#### Conclusions

Across a range of transmission intensities in Uganda, microscopy vastly underestimated parasite prevalence, especially among adults.

## Field Evaluation of Malaria Microscopy, Rapid Malaria Tests and Loop-Mediated Isothermal Amplification in a Rural Hospital in South Western Ethiopia

Juan Cuadros, Ramón Pérez-Tanoira, Laura Prieto-Pérez, Ines Martin-Martin, Pedro Berzosa, Vicenta González, Gebre Tisiano, Seble Balcha, José Manuel Ramos, Miguel Górgolas  
– *PLoS ONE* 10(11): e0142842. doi:10.1371/journal.pone.0142842 –

### Abstract

#### Background

In up to one third of the hospitals in some rural areas of Africa, laboratory services in malaria diagnosis are limited to microscopy by thin film, as no capability to perform thick film exists (gold standard in terms of sensitivity for malaria diagnosis). A new rapid molecular malaria diagnostic test called Loop-mediated isothermal DNA amplification (LAMP) has been recently validated in clinical trials showing exceptional sensitivity and specificity features. It could be a reliable diagnostic tool to be implemented without special equipment or training.

#### Objective

The objective of this proof of concept study was to confirm the feasibility of using LAMP technique for diagnosis of malaria in a rural Ethiopian hospital with limited resources.

#### Methodology/Principal Findings

This study was carried out in Gambo General Hospital, West Arsi Province (Ethiopia), from November 1st to December 31st 2013. A total of 162 patients with a non-focal febrile syndrome were investigated. The diagnostic capability (sensitivity, specificity, positive predictive and negative predictive values) of rapid malaria tests and microscopy by thin film was evaluated in comparison with LAMP. Eleven (6.79%) out of the 162 patients with fever and suspected malaria, tested positive for LAMP, 3 (1.85%) for rapid malaria tests and none of the eleven cases was detected by thin film microscopy.

#### Conclusions/Significance

LAMP can be performed in basic rural laboratories without the need for specialized infrastructure and it may set a reliable tool for malaria control to detect a low level parasitemia.



## Comparative evaluation of published real-time PCR assays for the detection of malaria following MIQE guidelines

Saba Alemayehu, Karla C Feghali, Jessica Cowden, Jack Komisar, Christian F Ockenhouse and Edwin Kamau  
– *Malar J.* 2013 Aug 8;12:277 –

### Abstract

#### Background

The use of malaria-specific quantitative real-time PCR (qPCR) is increasing due to its high sensitivity, speciation and quantification of malaria parasites. However, due to the lack of consensus or standardized methods in performing qPCR, it is difficult to evaluate and/or compare the quality of work reported by different authors for a cross-study and/or cross-platform assay analysis.

#### Methods

The performances of seven published qPCR assays that detect *Plasmodium* spp or *Plasmodium falciparum* were compared using standard DNA and samples from a clinical trial. Amplification and qPCR measurements were performed using the Applied Biosystems 7500 Fast Real-Time PCR System. All the analyses were automatically established using the default settings. For the TaqMan probe format, the assays were performed in the background of QuantiFast Probe Master Mix whereas in SYBR Green format, the assays were performed in the background of QuantiFast SYBR Green Master Mix and QuantiTect SYBR Green Master Mix background.

#### Results

Assays with high PCR efficiencies outperformed those with low efficiencies in all categories including sensitivity, precision and consistency regardless of the assay format and background. With the exception of one assay, all assays evaluated showed lower sensitivity compared to what have been published. When samples from a malaria challenge study were analysed, the qPCR assay with the overall best performance detected parasites in subjects earliest and with most consistency.

#### Conclusions

The data demonstrate the need for increased consensus and guidelines that will encourage better experimental practices, allowing more consistent and unambiguous interpretation of qPCR results.

## Simple, rapid, inexpensive platform for the diagnosis of malaria by loop mediated isothermal amplification (LAMP)

Rambabu Surabattula, Manju Pradeep Vejandla, Prudhvi Chand Mallepaddi, Konrad Faulstich, Rathnagiri Polavarapu

– *Exp Parasitol.* 2013 Jul;134(3):333-40 –

### Abstract

We attempted to improve the loop-mediated isothermal amplification (LAMP) method for malaria diagnosis by using a simple DNA extraction procedure, and a portable device performing both the amplification and detection of LAMP in one platform. Additionally, the device served as a heating block for the DNA preparation. We refer this method as LAMP-Tube scanner, and evaluated using 209 microscopically positive malaria samples and compared them to RDTs and LAMP-Thermocycler. Two most common human infecting *Plasmodium* species were detected. The LAMP-Tube scanner method is found to be simple and allowed real-time detection of DNA amplification. The time to amplification varied but was closely less than 60 min. Sensitivity and specificity of LAMP-Tube scanner in detecting *Plasmodium falciparum* were 95% and 93.3%, compared to microscopy and 98.3% and 100% respectively, compared to standard LAMP-Thermocycler. In addition, it showed a detection limit of 10 and 40 copies of the parasitemia for *Plasmodium vivax* and *P. falciparum*. Accordingly, in comparison to the results obtained by microscopy, the LAMP-Tube scanner had a less divergence in sensitivity and specificity, and yielded results similar to those of LAMP-Thermocycler. This method has the great potential as a field usable molecular tool for the diagnosis of malaria and is an alternative to conventional PCR-based diagnostic methods for field use.

## Evaluation of Loop-Mediated Isothermal Amplification (LAMP) for Malaria Diagnosis in a Field Setting

Jeeraphat Sirichaisinthop, Sureemas Buates , Risa Watanabe, Eun-Taek Han, Wachira Suktawonjaroenpon, Somporn Krasaesub, Satoru Takeo, Takafumi Tsuboi, Jetsumon Sattabongkot

– *Am. J. Trop. Med. Hyg.*, 85(4), 2011, pp. 594–596 –

### Abstract

We used the loop-mediated isothermal amplification (LAMP) method developed by our group for malaria diagnosis with genus-specific and species-specific primers for the four human malaria parasites at a field clinic in comparison with standard microscopy. Among 110 blood samples collected from the malaria clinic in Thailand, LAMP detected 59 of 60 samples positive by microscopy (sensitivity = 98.3%) and none of the 50 microscopy-negative samples (specificity = 100%). Negative predictive value (NPV) and positive predictive value (PPV) of LAMP were 98% and 100%, respectively. These results indicate that LAMP is an effective tool for malaria diagnosis at a field clinic in a field setting.



## General

### Combination of PURE-DNA extraction and LAMP-DNA amplification methods for accurate malaria diagnosis on dried blood spots

Jeanne Perpétue Vincent, Kanako Komaki-Yasuda, Moritoshi Iwagami, Satoru Kawai and Shigeyuki Kano  
– *Malar J* (2018) 17:373 –

#### Abstract

##### Background

Malaria is one of the most important parasitic infectious diseases for which almost half of the world's population is at risk. Although several diagnostic methods are now available to detect the infection, more sensitive and applicable tests are still required in the field. The loop-mediated isothermal amplification (LAMP) method is a DNA amplification tool in which the DNA amplification can be achieved by incubation at a stable temperature. A malaria detection kit based on this methodology has already been commercialized and is being used in some countries.

The kit includes two reaction tubes: one targeting the common *Plasmodium* genus (Pan tube) and the other specifically targeting *Plasmodium falciparum* (Pf tube). In parallel, a simple DNA extraction method, the procedure for ultra rapid extraction (PURE), which can produce a DNA solution suitable for the LAMP reaction without the use of a centrifuge, has also become available. In this study, the sensitivity of the combination of the PURE and LAMP methods (PURE–LAMP) was evaluated with archived dried clinical blood samples of imported malaria cases, including *P. falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium malariae*.

##### Results

Using a nested PCR as the reference, 117 samples including 46 *P. falciparum*, 7 *P. vivax*, 9 *P. ovale*, 4 *P. malariae*, and 51 negative cases were tested. The PURE–LAMP Pan correctly identified 64 of the 66 positives and the 51 negatives. Among the Pan-positive samples 45 *P. falciparum* were also detected with the PURE–LAMP Pf. The PURE–LAMP Pan and PURE–LAMP Pf had respective sensitivities of 96.96% (95% CI 89.47–99.63) and 97.82% (95% CI 88.47–99.94) and common specificity of 1.

##### Conclusion

The PURE–LAMP system is accurate when used with dried blood spots and extendable to the field.

## Strategy to improve malaria surveillance system preventing transfusion-transmitted malaria in blood banks using molecular diagnostic

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– *Malar J* (2018) 17:344 –

### Abstract

#### Background

Malaria can be transmitted by blood transfusion through donations collected from asymptomatic or parasitic donors. The parasites are released into the bloodstream during its life cycle and will therefore be present in donated blood by infected individuals. All cases of transfusion-transmitted malaria (TTM) notified since 2005 in Brazil were fatal. A good screening tool for *Plasmodium spp.* detection in blood units must have a high detection threshold, and the prevention of TTM relies entirely on the exclusion of potentially infected donors. However, in Brazilian blood banks, the screening test relies on blood thick smears examination.

#### Methods

The molecular diagnostic based on mitochondrial DNA (mtDNA) using real time PCR (mt-qPCR) was improved to detect *Plasmodium falciparum*, *Plasmodium vivax*, and standardized for use in *Plasmodium malariae*. The analytic sensitivity of this mt-qPCR methodology was performed using a sample of *P. vivax*.

#### Results

The mt-qPCR was highly efficient, and the analytic sensitivity for *P. vivax* was determined (0.000006 parasites/ $\mu$ L). This method was tested to detect *P. vivax* and *P. falciparum* in individuals from two malaria-endemic areas in Brazil, Amazon region (Pará and Rondônia states), the samples were collected in 10 reference units of two blood banks (Pará/nine cities and Rondônia/Porto Velho), and parasites mtDNA were detected in 10 of 2224 potential blood donors (0.45%). In all 10 positive samples, only *P. vivax* was detected.

#### Conclusion

Molecular diagnostic using mt-qPCR was effective in revealing infected potential donors with good perspectives to be applied as screening routine of asymptomatic carriers for preventing transfusion-transmitted malaria in blood banks.

## Laboratory challenges of *Plasmodium* species identification in Aceh Province, Indonesia, a malaria elimination setting with newly discovered *P. knowlesi*

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– *PLoS Negl Trop Dis* 12(11): e0006924 –

### Abstract

The discovery of the life-threatening zoonotic infection *Plasmodium knowlesi* has added to the challenges of prompt and accurate malaria diagnosis and surveillance. In this study from Aceh Province, Indonesia, a malaria elimination setting where *P. knowlesi* endemicity was not previously known, we report the laboratory investigation and difficulties encountered when using molecular detection methods for quality assurance of microscopically identified clinical cases. From 2014 to 2015, 20 (49%) *P. falciparum*, 16 (39%) *P. vivax*, 3 (7%) *P. malariae*, and 2 (5%) indeterminate species were identified by microscopy from four sentinel health facilities. At a provincial-level reference laboratory, loop-mediated isothermal amplification (LAMP), a field-friendly molecular method, was performed and confirmed *Plasmodium* in all samples though further species-identification was limited by the unavailability of non-falciparum species-specific testing with the platform used. At a national reference laboratory, several molecular methods including nested PCR (nPCR) targeting the 18S small subunit (18S) ribosomal RNA, nPCR targeting the cytochrome-b (cytb) gene, a *P. knowlesi*-specific nPCR, and finally sequencing, were necessary to ultimately classify the samples as: 19 (46%) *P. knowlesi*, 8 (20%) *P. falciparum*, 14 (34%) *P. vivax*. Microscopy was unable to identify or mis-classified up to 56% of confirmed cases, including all cases of *P. knowlesi*. With the nPCR methods targeting the four human-only species, *P. knowlesi* was missed (18S rRNA method) or showed cross-reactivity for *P. vivax* (cytb method). To facilitate diagnosis and management of potentially fatal *P. knowlesi* infection and surveillance for elimination of human-only malaria in Indonesia and other affected settings, new detection methods are needed for testing at the point-of-care and in local reference laboratories.

## High proportions of *pfhrp2* gene deletion and performance of HRP2-based rapid diagnostic test in *Plasmodium falciparum* field isolates of Odisha

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– *Malar J* (2018) 17:394 –

### Abstract

#### Background

With the documentation of cases of falciparum malaria negative by rapid diagnostic tests (RDT), though at low frequency from natural isolates in a small pocket of Odisha, it became absolutely necessary to investigate the status of HRP-2 based RDT throughout the state and in different seasons of the year.

#### Methods

Suspected individuals were screened for malaria infection by microscopy and RDT in 25/30 districts of Odisha, India. Discrepancies in results were confirmed by PCR. False negative RDT samples for *Plasmodium falciparum* mono-infection were evaluated for detection of HRP2 antigen in ELISA and genotyped for *pfhrp2*, *pfhrp3* and their flanking genes. Multiplicity of infection was ascertained based on *msp1* and *msp2* genotyping and parasitaemia level was determined by microscopy.

#### Results

Of the total 1058 patients suspected for malaria, 384 were microscopically confirmed for *P. falciparum* mono-infection and RDT failure was observed in 58 samples at varying proportion in different regions of the state. The failure in detection was due to undetectable level of HRP-2. Although most of these samples were screened during rainy season (45/345), significantly high proportion (9/17) of RDT negative samples were obtained during the summer compared to rainy season ( $P = 0.0002$ ;  $OR = 7.5$ ). PCR genotyping of *pfhrp2* and *pfhrp3* in RDT negative samples showed 38/58 (65.5) samples to be *pfhrp2* negative and 24/58 (41.4) to be *pfhrp3* negative including dual negative in 17/58 (29.3). Most of the RDT negative samples (39/58) were with single genotype infection and high proportions of *pfhrp2* deletion (7/9) was observed in summer. No difference in parasitaemia level was observed between RDT positive and RDT negative patients.

#### Conclusion

High prevalence of parasites with *pfhrp2* deletion including dual deletions (*pfhrp2* and *pfhrp3*) is a serious cause of concern, as these patients could not be given a correct diagnosis and treatment. Therefore, HRP2-based RDT for diagnosing *P. falciparum* infection in Odisha is non-reliable and must be performed in addition to or replaced by other appropriate diagnostic tools for clinical management of the disease.

## Detection of foci of residual malaria transmission through reactive case detection in Ethiopia

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– *Malar J* (2018) 17:390 –

### Abstract

#### Background

Sub-microscopic and asymptomatic infections could be bottlenecks to malaria elimination efforts in Ethiopia. This study determined the prevalence of malaria, and individual and household-level factors associated with *Plasmodium* infections obtained following detection of index cases in health facilities in Jimma Zone.

#### Methods

Index malaria cases were passively detected and tracked in health facilities from June to November 2016. Moreover, family members of the index houses and neighbours located within approximately 200 m from the index houses were also screened for malaria.

#### Results

A total of 39 index cases initiated the reactive case detection of 726 individuals in 116 households. Overall, the prevalence of malaria using microscopy and PCR was 4.0% and 8.96%, respectively. Seventeen (43.6%) of the index cases were from Doyo Yaya kebele, where parasite prevalence was higher. The majority of the malaria cases (90.74%) were asymptomatic. Fever (AOR = 12.68, 95% CI 3.34–48.18) and history of malaria in the preceding 1 year (AOR = 3.62, 95% CI 1.77–7.38) were significant individual-level factors associated with detection of *Plasmodium* infection. Moreover, living in index house (AOR = 2.22, 95% CI 1.16–4.27), house with eave (AOR = 2.28, 95% CI 1.14–4.55), area of residence (AOR = 6.81, 95% CI 2.49–18.63) and family size (AOR = 3.35, 95% CI 1.53–7.33) were main household-level predictors for residual malaria transmission.

#### Conclusion

The number of index cases per kebele may enhance RACD efforts to detect additional malaria cases in low transmission settings. Asymptomatic and sub-microscopic infections were high in the study area, which need new or improved surveillance tools for malaria elimination efforts.



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

Naomi W. Lucchi, Daouda Ndiaye, Sumudu Britton & Venkatachalam Udhayakumar

– *Expert Review of Molecular Diagnostics* 2018, 18:2, 195-203 –

### 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.

## LAMP kit for diagnosis of non-falciparum malaria in *Plasmodium ovale* infected patients

Juan Cuadros, Alexandra Martin Ramírez, Iveth J. González, Xavier C. Ding, Ramon Perez Tanoira, Gerardo Rojo-Marcos, Peña Gomez-Herruz, Jose Miguel Rubio

– *Malar J* (2017) 16:20 –

### Abstract

#### Background

Microscopy and rapid diagnosis tests have a limited sensitivity in diagnosis of malaria by *Plasmodium ovale*. The LAMP kit (LoopAMP®) can be used in the field without special equipment and could have an important role in malaria control programmes in endemic areas and for malaria diagnosis in returned travellers. The performance of the Pan primer of the kit in detecting malaria by *P. ovale* was compared with the results of standard nPCR in samples of patients returning from *P. ovale* endemic areas.

#### Methods

*Plasmodium ovale* positive samples (29, tested by PCR and/or microscopy) and malaria negative specimens (398, tested by microscopy and PCR) were collected in different hospitals of Europe from June 2014 to March 2016 and frozen at –20 °C. Boil and spin method was used to extract DNA from all samples and amplification was performed with LoopAMP® MALARIA kit (Eiken Chemical, Japan) in an automated turbidimeter (Eiken 500). The results of LAMP read by turbidimetry and with the naked eye were compared.

#### Results

The kit showed a sensitivity of 100% and a specificity of 97.24% with positive and negative predictive values of 72.5 and 100%, respectively. Naked eyed readings were in accordance with turbidimetry readings (sensitivity, 92.5%, specificity, 98.96% and positive and negative predictive values, respectively, 90.24 and 99.22%). The limit of detection of LAMP assay for *P. ovale* was between 0.8 and 2 parasites/μl.

#### Conclusions

The Pan primer of the Malaria kit LoopAMP® can detect *P. ovale* at very low-levels and showed a predictive negative value of 100%. This tool can be useful in malaria control and elimination programmes and in returned travellers from *P. ovale* endemic areas. Naked eye readings are equivalent to automated turbidimeter readings in specimens obtained with EDTA.

## Novel molecular diagnostic tools for malaria elimination: a review of options from the point of view of high-throughput and applicability in resource limited settings

Sumudu Britton, Qin Cheng and James S. McCarthy

– *Malar J* (2016) 15:88 –

### Abstract

As malaria transmission continues to decrease, an increasing number of countries will enter pre-elimination and elimination. To interrupt transmission, changes in control strategies are likely to require more accurate identification of all carriers of *Plasmodium* parasites, both symptomatic and asymptomatic, using diagnostic tools that are highly sensitive, high throughput and with fast turnaround times preferably performed in local health service settings. Currently available immunochromatographic lateral flow rapid diagnostic tests and field microscopy are unlikely to consistently detect infections at parasite densities less than 100 parasites/ $\mu\text{L}$  making them insufficiently sensitive for detecting all carriers. Molecular diagnostic platforms, such as PCR and LAMP, are currently available in reference laboratories, but at a cost both financially and in turnaround time. This review describes the recent progress in developing molecular diagnostic tools in terms of their capacity for high throughput and potential for performance in non-reference laboratories for malaria elimination.

## Diagnosis and Treatment of *Plasmodium vivax* Malaria

J. Kevin Baird, Neena Valecha, Stephan Duparc, Nicholas J. White, and Ric N. Price

– doi:10.4269/ajtmh.16-0171. –

### Abstract

The diagnosis and treatment of *Plasmodium vivax* malaria differs from that of *Plasmodium falciparum* malaria in fundamentally important ways. This article reviews the guiding principles, practices, and evidence underpinning the diagnosis and treatment of *P. vivax* malaria.

## Molecular-based isothermal tests for field diagnosis of malaria and their potential contribution to malaria elimination

Oriero EC, Jacobs J, Van Geertruyden JP, Nwakanma D, D'Alessandro U

– *J Antimicrob Chemother.* 2015 Jan;70(1):2-13 –

### Abstract

In countries where malaria transmission has decreased substantially, thanks to the scale-up of control interventions, malaria elimination may be feasible. Nevertheless, this goal requires new strategies such as the active detection and treatment of infected individuals. As the detection threshold for the currently used diagnostic methods is 100 parasites/ $\mu\text{L}$ , most low-density, asymptomatic infections able to maintain transmission cannot be detected. Identifying them by molecular methods such as PCR is a possible option but the field deployment of these tests is problematic. Isothermal amplification of nucleic acids (at a constant temperature) offers the opportunity of addressing some of the challenges related to the field deployment of molecular diagnostic methods. One of the novel isothermal amplification methods for which a substantial amount of work has been done is the loop-mediated isothermal amplification (LAMP) assay. The present review describes LAMP and several other isothermal nucleic acid amplification methods, such as thermophilic helicase-dependent amplification, strand displacement amplification, recombinase polymerase amplification and nucleic acid sequence-based amplification, and explores their potential use as high-throughput, field-based molecular tests for malaria diagnosis.



## Loop-mediated isothermal amplification (LAMP) for malarial parasites of humans: would it come to clinical reality as a point-of-care test?

Abdul-Ghani R, Al-Mekhlafi AM, Karanis P.

– *Acta Trop.* 2012 Jun;122(3):233-40 –

### Abstract

Loop-mediated isothermal amplification (LAMP) is a novel molecular method that accelerates and facilitates DNA amplification and detection under isothermal conditions. It represents a revolution in molecular biology by reducing the high cost, turnaround time and technicality of polymerase chain reaction and other amplification methods. It has been applied for the diagnosis of a variety of viral, bacterial, parasitic and other diseases in the biomedical field. LAMP has been involved in studies concerning the diagnosis of malaria which is still a major cause of morbidity and mortality in different parts of the world. For the success attained with this technology to diagnose human malaria, is it time to think that LAMP-based point-of-care diagnostics come to application to support the diagnosis of clinical malaria cases? The present review deals with the use of LAMP in the diagnosis of malaria and related investigations to make a view on what has been investigated and highlights the future perspectives regarding the possible applications of LAMP in diagnosis of the disease.



## Mitochondrial DNA Targets Increase Sensitivity of Malaria Detection Using Loop-Mediated Isothermal Amplification

Spencer D. Polley, Yasuyoshi Mori, Julie Watson, Mark D. Perkins, Iveth J. González, Tsugunori Notomi, Peter L. Chiodini, Colin J. Sutherland

– *JOURNAL OF CLINICAL MICROBIOLOGY*, Aug. 2010, p. 2866–2871 –

### Abstract

Loop-mediated isothermal amplification (LAMP) of DNA offers the ability to detect very small quantities of pathogen DNA following minimal tissue sample processing and is thus an attractive methodology for point-of-care diagnostics. Previous attempts to diagnose malaria by the use of blood samples and LAMP have targeted the parasite small-subunit rRNA gene, with a resultant sensitivity for *Plasmodium falciparum* of around 100 parasites per  $\mu\text{l}$ . Here we describe the use of mitochondrial targets for LAMP-based detection of any *Plasmodium* genus parasite and of *P. falciparum* specifically. These new targets allow routine amplification from samples containing as few as five parasites per  $\mu\text{l}$  of blood. Amplification is complete within 30 to 40 min and is assessed by real-time turbidimetry, thereby offering rapid diagnosis with greater sensitivity than is achieved by the most skilled microscopist or antigen detection using lateral flow immunoassays.



## Loop-mediated isothermal PCR (LAMP) for the diagnosis of falciparum malaria

Daniel H. Paris, Mallika Imwong, Abul M. Faiz, Mahtabuddin Hasan, Emran Bin Yunus, Kamolrat Silamut, Sue J. Lee, Nicholas P. J. Day, and Arjen M. Dondorp

– *Am. J. Trop. Med. Hyg.*, 77(5), 2007, pp. 972–976 –

### Abstract

A recently described loop-mediated isothermal polymerase chain reaction (LAMP) for molecular detection of *Plasmodium falciparum* was compared with microscopy, PfHRP2-based rapid diagnostic test (RDT), and nested polymerase chain reaction (PCR) as the "gold standard" in 115 Bangladeshi in-patients with fever. DNA extraction for LAMP was conducted by conventional methods or simple heating of the sample; test results were either assessed visually or by gel electrophoresis. Conventional DNA extraction followed by gel electrophoresis had the highest agreement with the reference method (81.7%, kappa = 0.64), with a sensitivity (95% CI) of 76.1% (68.3-83.9%), comparable to RDT and microscopy, but a specificity of 89.6% (84.0-95.2%) compared with 100% for RDT and microscopy. DNA extraction by heat treatment deteriorated specificity to unacceptable levels. LAMP enables molecular diagnosis of falciparum malaria in settings with limited technical resources but will need further optimization. The results are in contrast with a higher accuracy reported in an earlier study comparing LAMP with a non-validated PCR method.











