Reference list for Malaria-LAMP

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.

CONCLUSION:
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.

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 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.

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-S1-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/µl 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.

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 ≥ 2 parasites/μL, 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 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.

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.

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 LoopampTM 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.

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 µl. 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 µl 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.

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.

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.

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.

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.

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.

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.

CONCLUSION:

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.

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.

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/μL, 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.

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.

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.

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.