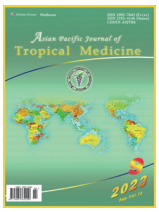

Letter to Editor

Asian Pacific Journal of Tropical Medicine

apjtm.org

doi: 10.4103/1995-7645.345946

Impact Factor: 3.1

A simple and purification-free nucleic acid extraction method for rapid diagnosis of malaria

Lee Phone Youth Zen[1], Meng Yee Lai[1], Mohd Hafizi Abdul Hamid[2], Jenarun Jelip[2], Rose Nani Mudin[2], Noor Hafizan Bt Mat Salleh[3], Netty Darwina Dawam[4], Nirzayanna Eyan Abdul Ajak[4], Mohd Ashrin Afiq Bin Zainudin[5], Yee Ling Lau[1]✉

[1]*Department of Parasitology, Faculty of Medicine, Universiti Malaya, 50603 Kuala Lumpur, Malaysia*

[2]*Vector Borne Disease Sector, Ministry of Health, Putrajaya, Malaysia*

[3]*Lipis District Health Office, Pahang, Malaysia*

[4]*State Health Department, Negeri Sembilan, Malaysia*

[5]*Microbiology Sector, Hospital Kuala Kubu Bharu, Selangor, Malaysia*

In a point of care setting for the diagnosis of malaria, DNA extraction using conventional methods are time-consuming and complicated. Therefore, in this study we aim to utilize a simple nucleic acid extraction method to directly extract DNA from blood. This would in turn reduce the time, cost and equipment needed to perform DNA extraction. This method is then coupled with LAMP assay for rapid diagnosis of malaria.

We obtained 77 malaria samples, of which 36 were *Plasmodium* (*P.*) *knowlesi*, 10 *P. vivax*, 10 *P. falciparum*, 1 *P. malariae* and 20 healthy blood samples from district hospitals from Selangor, Kelantan, Negeri Sembilan, Pahang, and Perak, from 2019 to 2021. All malaria samples tested by LAMP or nested PCR were collected prior to antimalarial treatment. All malaria samples collected were confirmed by microscope at the hospital and cross-checked by Medical Laboratory Technician at the District Health Office. The samples were confirmed by microscopic examination and nested PCR as described by Snounou *et al*[1] and Imwong *et al*[2].

The LAMP assay and primers were adapted from Lau *et al*[3]. The extraction method and buffers were adapted from Zou *et al*[4] with minor modifications. Blood samples of 60 μL, and 240 μL of lysis buffer [800 mM guanidine hydrochloride, 50 mM Tris (pH 8), 0.5% Triton™ X-100, 1% Tween-20, 40 μg/mL Proteinase K] was used. The tube consisting of blood and lysis buffer mixture was constantly inverted for until homogenous. A 6 mm diameter Whatman grade 1 qualitative filter paper was inserted into the tube was mixed constantly for 1 minute. The filter paper was then removed from the blood lysis mixture and washed in 1 mL of washing buffer [10 mM Tris (pH 8.0), 0.1% Tween-20]. The filter paper was then mixed constantly in the washing buffer for 1 minute. Following that, the filter paper was removed and dipped 5 times into the PCR tube consisting of the LAMP reaction before removing the filter paper. The LAMP assay was incubated in a Loopamp Real time turbidimeter LA 500 (Eiken Chemical Co., Ltd., Japan) at 65 ℃ for 60 minutes and inactivated at 80 ℃ for 2 minutes.

Limit of detection of the method was performed by using *P. knowlesi* strain A1H1 obtained from the Department of Parasitology, Faculty of Medicine, Universiti Malaya. *P. knowlesi* strain A1H1 culture blood was ten-fold serially diluted to parasitemia of 1%, 0.1%, 0.01%, 0.001% and 0.000 1% respectively with healthy blood, and was tested with the above method in triplicates.

The clinical sensitivity and specificity of LAMP assay was determined using microscopy as the reference standard methods. Sensitivity was calculated as (number of true positives)/(number of true positives+number of false negatives), and specificity was calculated as (number of true negatives)/(number of true

✉To whom correspondence may be addressed. E-mail: lauyeeling@um.edu.my

How to cite this article: Zen LPY, Lai MY, Hamid MHA, Jelip J, Mudin RN, Salleh NHBM, et al. A simple and purification-free nucleic acid extraction method for rapid diagnosis of malaria. Asian Pac J Trop Med 2023; 16(9): 428-430.

Article history: Received 22 January 2022 Revision 26 April 2022
Accepted 12 May 2022 Available online 29 September 2023

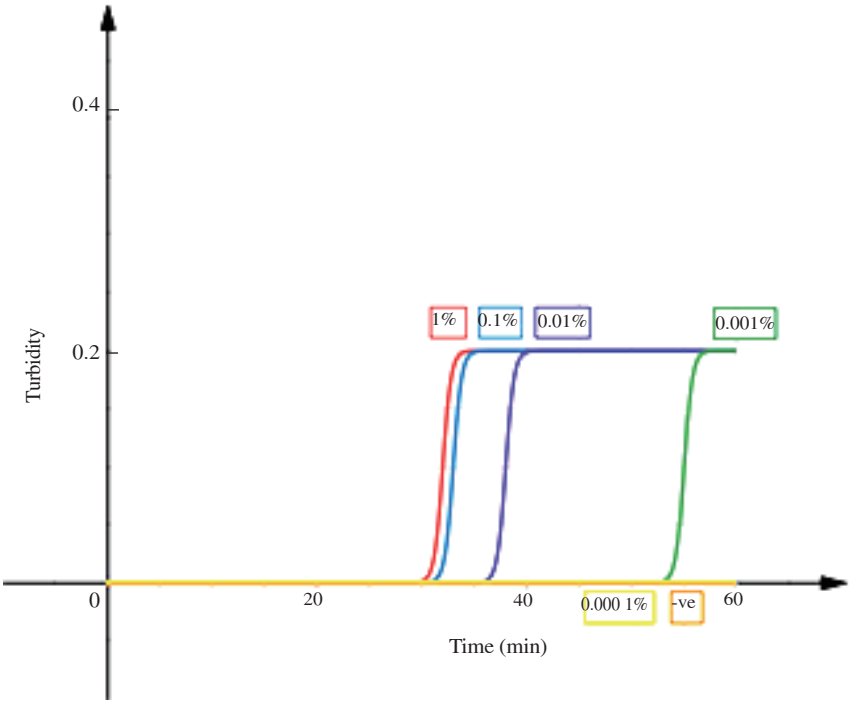

Figure 1. Limit of detection of the DNA extraction method coupled with loop mediated isothermal amplification. A graph was plotted based on the results obtained from the Loopamp Real time turbidimeter LA 500 (Eiken Chemical Co., Ltd., Japan). The X-axis represents time whereas, the Y-axis represents the turbidity. The labels are as follows, 1%: 1% parasitemia *Plasmodium knowlesi* A1H1 culture; 0.1%: 0.1% parasitemia *Plasmodium knowlesi* A1H1 culture; 0.01%: 0.01% parasitemia *Plasmodium knowlesi* A1H1 culture; 0.001%: 0.001% parasitemia *Plasmodium knowlesi* A1H1 culture; 0.000 1%: 0.000 1% parasitemia *Plasmodium knowlesi* A1H1 culture; -ve: double distilled H_2O (no target control).

negatives+number of false positives).

The DNA extraction method coupled with LAMP has a detection limit of 0.001% parasitemia (5 parasites/μL of blood) (Figure 1). A highly trained technician using microscopy can reliably detect as few as 50 parasites/μL of blood, while the published limit of detection of laboratory PCR methods is 0.5 to 5 parasites/μL[5]. When conducting the limit of detection, all samples with parasitemia higher than 0.001% was able to amplify successfully, whereas there was a 1/3 positive amplification at 0.000 1% parasitemia. Clinical sensitivity of LAMP was compared with the results from conventional nested PCR and microscopy. A total of 77 samples were diagnosed using both nested PCR and microscopy. Within the 77 samples diagnosed using both nested PCR and microscopy, there were *P. knowlesi* (*n*=36), *P. falciparum* (*n*=10), *P. vivax* (*n*=10), *P. malariae* (*n*=1) and 20 negative *Plasmodium* samples. Among 36 *P. knowlesi*, 3 were not detected using the alternative DNA extraction method coupled with LAMP. This method has successfully amplified all *P. falciparum* (*n*=10), *P. vivax* (*n*=10) and *P. malariae* (*n*=1) positive samples. The 20 healthy blood samples did not show any amplification. With this, the DNA extraction method coupled with LAMP showed 94.7% sensitivity and 100% specificity when compared with microscopy.

The alternative DNA extraction method coupled with LAMP has a clinical sensitivity of 94.7% and a 100% clinical specificity when compared with microscopy. Three samples failed to amplify by LAMP may be due to degradation of DNA. In order to confirm the degradation of DNA, these three samples were subjected to nested PCR and no amplification was observed. These samples were kept at -20 ℃ for more than a year. Short-term storage at room temperature, 4 ℃ and -20 ℃ will affect the yield of DNA greatly[6].

Extracting genomic DNA by conventional methods such as isopropanol precipitation, formamide lysate method, nonorganic solvent extraction, and glass particle adsorption is a time-consuming process while, the phenol and chloroform method uses toxic reagents that are not fit for on field diagnostics. Majority of commercial DNA extraction kits require multiple liquid handling steps[7] which is not suitable for point of care. Another approach of the DNA extraction method is by using Fitzco/Flinder Technology Agreement (FTA) paper, a cellulose based DNA extraction method. FTA paper is an absorbent cellulose-based paper that has been treated with a proprietary mix of chemicals that allows good preservation and storage capabilities. However, FTA based DNA

extraction method is still considered costly when compared to alternatives that use untreated filter paper. Based on the Whatman price catalog[8], a single use FTA blood kit costs 10.48 USD, whereas one 70 mm diameter Whatman grade 1 qualitative filter paper for the proposed extraction method only costs 0.11 USD for multiple samples. A paper published by Zou *et al.*[4] found that cellulose based filter paper can be used to rapidly bind nucleic acids, retain them during a short washing step to remove contaminants, followed by elution directly into the amplification reaction. They then adapted the filter paper into a dipstick and was able to extract nucleic acids from a wide range of biological samples in less than 30 seconds without the need of any specialised equipment[4]. We adapted this method in this study for the diagnosis of malaria. A 6-mm diameter filter paper was used instead so that the bigger surface area would aid the binding of the DNA. The volume of the washing buffer was increased to 1 mL instead of 200 μL, as we found that a larger volume helped the diffusion of contaminants from the filter paper. The filter paper was then dipped into a LAMP assay allowing *Plasmodium* parasite DNA templates to be present in the assay.

In conclusion, the method presented here allows amplification of *Plasmodium* DNA in a time and cost-effective manner. Diagnosis of malaria is more accessible and affordable with this method, so it will be useful in resource limited areas.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Ethical approval

This study was approved by the Medical Ethics Committee of UMMC (MEC reference No. 817.18 and 908.11) and National Medical Research Registry (reference No. NMRR-12-1105-13079).

Acknowledgements

We sincerely thank the local health officers and the hospital staff from Hospital Kuala Lipis and Klinik Kesihatan Betau (Pahang); Hospital Sungai Siput, Hospital Permaisuri Bainun, Hospital Kuala Kangsar (Perak); Vector lab (Negeri Sembilan); Hospital Gua Musang, Hospital Sultan Ismail Petra and Hospital Jeli (Kelantan); and Hospital Kuala Kubu Bahru (Selangor) who had helped in sampling.

Funding

This study was supported by Long Term Research Grant Scheme (LRGS), LRGS/1/2018/UM/01/1/4 from the Ministry of Higher Education, Malaysia.

Authors' contributions

LPYZ and YLL conceptualized the study. LPYZ curated the data, formally analysed, investigated, validated and wrote the original draft. YLL acquired funding and administered the project. YLL and MYL supervised the project. MHAH, JJ, RNM, NHMS, NDD, NEAA and MAABZ contributed resources. All the authors were involved in the writing review and editing.

References

[1] Snounou G, Singh B. Nested PCR analysis of *Plasmodium* parasites. *Mol Biochem Parasitol* 1993; **61**(2): 315-320.

[2] Imwong M, Tanomsing N, Pukrittayakamee S, Day NPJ, White NJ, Snounou G. Spurious amplification of a *Plasmodium vivax* small-subunit RNA gene by use of primers currently used to detect *P. knowlesi*. *J Clin Microbiol* 2009; **47**(12): 4173-4175.

[3] Lau YL, Lai MY, Fong MY, Jelip J, Mahmud R. Loop-mediated isothermal amplification assay for identification of five human *Plasmodium* species in Malaysia. *Am J Trop Med Hyg* 2016; **94**(2): 336-339.

[4] Zou Y, Mason MG, Wang Y, Wee E, Turni C, Blackall PJ, et al. Nucleic acid purification from plants, animals and microbes in under 30 seconds. *PLoS Biol* 2017; **16**(5): e2003916.

[5] Mbanefo A, Kumar N. Evaluation of malaria diagnostic methods as a key for successful control and elimination programs. *Trop Med Infect Dis* 2020; **5**(2): 102.

[6] Bulla A, De Witt B, Ammerlaan W, Betsou F, Lescuyer P. Blood DNA yield but not integrity or methylation is impacted after long-term storage. *Biopreserv Biobank* 2016; **14**(1): 29-38.

[7] Tan SC, Yiap BC. DNA, RNA, and protein extraction: The past and the present. *J Biomed Biotechnol* 2009; **2009**: 574398.

[8]General Electric Healthcare. *Whatman price catalog. 2014.* [Online]. Avaliable from: https://www.iitk.ac.in/centralstores/data/price/15%20Whatman_Pricelist_2014_INR.pdf/. [Accessed on 22nd August 2021].

Publisher's note