Mini Review

Life Sciences, Medicine and Biomedicine
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A review on lab-on-chip as a potential diagnostic tool for early detection of *Plasmodium knowlesi*

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ABSTRACT

Massive elimination efforts have been done to control the malaria disease caused by the emergence of the fifth human malaria parasite known as *Plasmodium knowlesi*. Early detection of the parasite is important in treating malaria infection. Microscopic examination of Giemsa-stained thick and thin blood films is the gold standard for laboratory malaria diagnosis, while rapid diagnostic tests (RDTs), polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP) are significant diagnostic techniques to detect acute infection. However, these methods have several limitations in which it could delay the treatment. The potential of lab-on-chip (LOC) as a point-of-care diagnostic tool for malaria fulfils the requirement of limitations where it is able to produce early detection of malaria infection. This review discusses advantages and disadvantages of malaria diagnostic methods as well as new approaches that could be used for high speed, sensitive and reliable malaria detection to prevent the disease from causing severe complications and even fatal if left untreated.

Keywords: *Plasmodium knowlesi; diagnostic test; early detection; lab-on-chip*

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Received: 20 August 2020
Revised: 30 October 2020
Accepted: 5 November 2020
Published: 14 November 2020

doi
https://doi.org/10.28916/lsmb.4.9.2020.69

INTRODUCTION

Malaria is a worldwide public health challenge, in which tremendous efforts have been taken to subside the disease. Upon malaria eradication, the World Health Organization (WHO) has developed a Global Technical Strategy with a comprehensive target for malaria in 2030 by assessing the progress in the year 2020-2025 (WHO, 2019). Generally, malaria is caused by hemoprotozoa from the genus *Plasmodium*. The disease is spread to humans through the bite of infected female *Anopheles* mosquitoes from the Leucosphyrus group (Roncalés, Vidal, Torres, & Herreros, 2015). *Plasmodium* parasites are species-specific in which four of them (*P. falciparum, P. vivax, P. malariae* and *P. ovale*) are known to cause malaria in humans (Amir, Cheong, de Silva, Liew, & Lau, 2018).

A simian *Plasmodium* parasite known as *P. knowlesi* can also cause malaria in humans after large number of malaria cases caused by this species were discovered in 2004 in the Kapit Division of Sarawak, Malaysian Borneo. Wild long-tailed (*Macaca fascicularis*) and pig-tailed (*Macaca nemestrina*) macaques are the natural reservoirs for *P. knowlesi* (Amir et al., 2018; Divis et al., 2020).

EPIDEMIOLOGY OF MALARIA

In 2018, 228 million malaria cases throughout the world were reported by WHO, which mostly affected people in tropical and subtropical regions. India and 18 African countries were reported for nearly 85% of the global malaria cases. Based on the number of cases per 1,000 population of global malaria incidence rate, a reduction pattern from 71 cases to 57 cases were observed from the year 2010 to 2018. However, the rate of the declining cases remained at 57 cases in the year 2014 until 2018. Malaria death reported 405,000 cases in the year 2018 with approximately 67% of the cases affecting children below 5 years (WHO, 2019). *P. falciparum* was reported to be the most life-threatening among all human malaria parasites with almost 99% cases in sub-Saharan Africa followed by *P. vivax* causing 200 million cases outside Africa (Mohring et al., 2019). Meanwhile, only scarce cases were reported to be caused by other non-falciparum parasites (*P. ovale* and *P. malariae*) in western Africa, South America, Asia and western Pacific region (Lo et al., 2017; Okafor & Finnigan, 2019). However, *P. knowlesi* was reported to be widely spread across endemic areas in Southeast Asia including Indonesia, Singapore, Thailand, Cambodia, Philippines, Myanmar and Vietnam after its discovery cases at Malaysian Borneo (Barber, Rajahram, Grigg, William, & Anstey, 2017; Millar & Cox-Singh, 2015). Malaysia reported the highest clinical cases of *P. knowlesi* with a total of 7,745 cases in the year 2017 and 2018, which were mostly detected in Sabah and Sarawak. *P. knowlesi* cases were also detected in several states in Peninsular Malaysia such as Kelantan, Perak and Selangor (Divis et al., 2020; Mohamad & Abu-Bakar, 2019).

BIOLOGY OF *P. KNOWLESI*

P. knowlesi has been misdiagnosed as *P. malariae* due to the similarities in morphology (Herman et al., 2018). This was revealed after Singh *et al.* investigated the large *P. knowlesi* cases that were mistakenly misdiagnosed as *P. malariae* at Kapit, Sarawak in 2004 (Singh et al., 2004). *P. knowlesi* has also been reported to be confused with *P. falciparum* and *P. vivax* in which the error could be fatal if the treatment to patients is delayed (Herman et al., 2018; Nuin et al., 2020). *In vitro* culture of *P. knowlesi* has brought many challenges to researchers. This is due to the requirement of the parasite to grow in macaque blood (Amir et al., 2016; Grüring et al., 2014). This has restricted *in vitro* studies since access to macaques or macaque blood is a challenge. However, continuous researches on *P. knowlesi* are now possible after several publications showed successful adaptation of the parasite to grow and proliferate in an *in vitro* culture by using human blood (Grüring et al., 2014; Moon et al., 2013; Noulin et al., 2014).

Even though researches on *P. knowlesi* in *in vitro* culture are now expanding and the number of malaria cases show a significant decrease, malaria remains a public health threat, and the malaria control and elimination strategy need to be improved. *P. knowlesi* infection could lead to severe complications such as hepatic dysfunction, renal failure, acute respiratory distress syndrome and shock if there is a delay in treatment (Mohamad & Abu-Bakar, 2019; Zaw & Lin, 2014). Thus, rapid diagnosis with reliable sensitivity and specificity for *P. knowlesi* detection is important. Therefore, this review focuses on the importance of development in diagnostic methods for early detection of *P. knowlesi.*

METHODS FOR DETECTION AND IDENTIFICATION OF *P. KNOWLESI*

Microscopic examination of thick and thin blood films stained with Giemsa is the gold standard for malaria detection (Berzosa et al., 2018; Mathison & Pritt, 2017). It is a common laboratory test for malaria diagnosis for *P. knowlesi* endemic countries including Malaysia (Nuin et al., 2020). This method helps to identify different species of malaria parasites at a low cost. It also allows the quantification of parasite density performed by experts (Berzosa et al., 2018). However, this method has several limitations including low sensitivity (50-500 parasites/µl) depending on the expertise, difficulty to recognise the species due to the morphological similarities of *P. knowlesi* with other species at certain stages, high probability to misdiagnose due to mixed infection or low parasitaemia and lack of expertise (Berzosa et al., 2018; Herman et al., 2018).

An alternative method known as rapid diagnostic test (RDT) has been developed to overcome the limitations caused by microscopic examination. This method does not require skilled personnel and can be used as a screening tool to detect malaria infection. RDT is an immunochromatographic test used to detect the presence of parasite antigens in blood at a low level of parasitaemia (Talapko, Škrlec, Alebić, Jukić, & Včev, 2019). This method is based on the sandwich ELISA principle, which allows the detection of mono-infection or co-infection of different species (Mukry et al., 2017). Although the RDT is more specific as compared to microscopic examination but the sensitivity of RDT can vary depending on the commercial devices and also could be reduced due to high temperature and humidity during the diagnosis (Fançony, Sebastião, Pires, Gamboa, & Nery, 2013). Thus, the results obtained from RDT are recommended to be confirmed by microscopic examination (Talapko et al., 2019). RDT devices are based on the detection of histidine-rich protein II (HRP2) and parasite lactate dehydrogenase (pLDH) enzyme of *P. falciparum* and other *Plasmodium* species, respectively (Mukry et al., 2017). However, this test can lead to false-positive as HRP2 remains in the blood for a few days only after infection clearance, while false-negative could be due to the HRP2 gene deletions. Hence, these may lead to inaccurate diagnosis for non-*P. falciparum* infections (Berhane et al., 2017; Berzosa et al., 2018).

Therefore, molecular diagnostic techniques are required for accurate detection of *P. knowlesi* and other species. In Malaysia, multiplex real-time polymerase chain reaction (RT-PCR) has been used to confirm the presence of nucleic acid of the malaria parasites (Nuin et al., 2020). This method has been reported to have a high sensitivity for different malaria parasites: 0.125, 0.7, 1.5 and 40 parasites/µl for *P. knowlesi, P. falciparum, P. ovale* and *P. vivax,* respectively (Mathison & Pritt, 2017; Nuin et al., 2020). Another alternative technique for malaria detection at low cost is loop-mediated isothermal amplification (LAMP). This technique is portable due to less equipment needed, but it requires high temperature to start the amplification loop of mixed cell lysates with DNA polymerase and primers. It is also vulnerable to impurities and contamination (Hochstetter, 2020). Although the molecular diagnostic technique is the most sensitive method, it requires a longer time to produce results; therefore, it is not suitable for urgent cases. It is also more expensive and complex to perform in the laboratory, which is not suitable for malaria diagnosis in endemic areas (Berzosa et al., 2018; Mathison & Pritt, 2017).

FUTURE POTENTIAL DIAGNOSTIC TEST

Despite being a promising technique to diagnose malaria, the molecular diagnostic technique is not suitable to be implemented in endemic areas based on several limitations such as its dependence on equipment, requirement of well-trained technicians and challenges in maintenance of reagents (Berzosa et al., 2018). Therefore, rapid, sensitive and reliable diagnostic tools are urgently needed for *P. knowlesi* detection. One of the technologies known as lab-on-chip (LOC) has numerous advantages to fulfil the requirements of the potential diagnostic device. It is a device that combined several techniques producing high-speed detection, self-contained, low cost, portable and requires small sample volumes targeted to point-of-care (Hochstetter, 2020; Taylor et al., 2014). LOC device is able to detect and amplify DNA or RNA. The microfluidic principle used in the LOC device allows enrichment of the malaria parasites from the blood by segregating it through the sidewalls of microchannels (Hochstetter, 2020). This separation technique does not require external electrical and magnetic fields (Kong et al., 2015; Warkiani et al., 2015). Thus, the efficiency of malaria parasite enrichment from patient's blood facilitates the reliability and PCR specificity of malaria detection. Prominently, LOC device has a high sensitivity in which it can detect as low as 0.0005% of ring stage malaria parasites from peripheral blood (Kong et al., 2015).

Thus, it provides a significant benefit for malaria diagnosis especially in a low level of parasitaemia. However, the antigen level produced by the LOC is unable to determine whether it is a postinfection or not. This is because the HRP2 remains even after the infection clearance. Despite the limitation, the high-throughput LOC device is suitable for symptomatic infection and case management (Kolluri, Klapperich, & Cabodi, 2018). In conclusion, the LOC is a potential alternative method, which detects nucleic acids of malaria parasites in a short period and applicable in the field where malaria is endemic. Therefore, treatment can be administered within 24 hours after the onset of the first symptom to prevent malaria death.

DISCLOSURE

The authors declare no conflict of interest in this work.

ACKNOWLEDGEMENT

We would like to thank Universiti Sains Malaysia (USM) for providing the financial support under the Research University Grant (USM RUI Grant No. 1001/PPSK/812201).

REFERENCES

Amir, A., Cheong, F. W., de Silva, J. R., Liew, J. W. K., & Lau, Y. L. (2018). Plasmodium knowlesi malaria: current research perspectives. Infection and Drug Resistance, 11, 1145. https://doi.org/10.2147/IDR.S148664

Amir, A., Russell, B., Liew, J. W. K., Moon, R. W., Fong, M. Y., Vythilingam, I., Subramaniam, V., Snounou, G., & Lau, Y. L. (2016). Invasion characteristics of a Plasmodium knowlesi line newly isolated from a human. Scientific Reports, 6(1), 1-8. https://doi.org/10.1038/srep24623

Barber, B. E., Rajahram, G. S., Grigg, M. J., William, T., & Anstey, N. M. (2017). World Malaria Report: time to acknowledge Plasmodium knowlesi malaria. Malaria Journal, 16(1), 1-3. https://doi.org/10.1186/s12936-017-1787-y

Berhane, A., Russom, M., Bahta, I., Hagos, F., Ghirmai, M., & Uqubay, S. (2017). Rapid diagnostic tests failing to detect Plasmodium falciparum infections in Eritrea: an investigation of reported false negative RDT results. Malaria Journal, 16(1), 1-6. https://doi.org/10.1186/s12936-017-1752-9

Berzosa, P., de Lucio, A., Romay-Barja, M., Herrador, Z., González, V., García, L., Fernandez-Martinez, A., Santana-Morales, M., Ncogo., P., & Valladares, B. (2018). Comparison of three diagnostic methods (microscopy, RDT, and PCR) for the detection of malaria parasites in representative samples from Equatorial Guinea. Malaria Journal, 17(1), 333. https://doi.org/10.1186/s12936-018-2481-4

Divis, P. C., Hu, T. H., Kadir, K. A., Mohammad, D. S., Hii, K. C., Daneshvar, C., Conway, D. J., & Singh, B. (2020). Efficient Surveillance of Plasmodium knowlesi Genetic Subpopulations, Malaysian Borneo, 2000-2018. Emerging Infectious Diseases, 26(7), 1392. https://doi.org/10.3201/eid2607.190924

Fançony, C., Sebastião, Y. V., Pires, J. E., Gamboa, D., & Nery, S. V. (2013). Performance of microscopy and RDTs in the context of a malaria prevalence survey in Angola: a comparison using PCR as the gold standard. Malaria Journal, 12(1), 284. https://doi.org/10.1186/1475-2875-12-284

Grüring, C., Moon, R. W., Lim, C., Holder, A. A., Blackman, M. J., & Duraisingh, M. T. (2014). Human red blood cell-adapted Plasmodium knowlesi parasites: a new model system for malaria research. Cellular Microbiology, 16(5), 612-620. https://doi.org/10.1111/cmi.12275

Herman, L. S., Fornace, K., Phelan, J., Grigg, M. J., Anstey, N. M., William, T., Moon, R. W., Blackman, M. J., Drakeley, C.J., & Tetteh, K. K. (2018). Identification and validation of a novel panel of Plasmodium knowlesi biomarkers of serological exposure. PLoS Neglected Tropical Diseases, 12(6), e0006457. https://doi.org/10.1371/journal.pntd.0006457

Hochstetter, A. (2020). Lab-on-a-Chip Technologies for the Single Cell Level: Separation, Analysis, and Diagnostics. Micromachines, 11(5), 468. https://doi.org/10.3390/mi11050468

Kolluri, N., Klapperich, C., & Cabodi, M. (2018). Towards lab-on-a-chip diagnostics for malaria elimination. Lab on a Chip, 18(1), 75-94. https://doi.org/10.1039/C7LC00758B

Kong, T. F., Ye, W., Peng, W. K., Hou, H. W., Preiser, P. R., Nguyen, N.-T., & Han, J. (2015). Enhancing malaria diagnosis through microfluidic cell enrichment and magnetic resonance relaxometry detection. Scientific Reports, 5, 11425. https://doi.org/10.1038/srep11425

Lo, E., Nguyen, K., Nguyen, J., Hemming-Schroeder, E., Xu, J., Etemesi, H., Githeko, A., & Yan, G. (2017). Plasmodium malariae prevalence and csp gene diversity, Kenya, 2014 and 2015. Emerging Infectious Diseases, 23(4), 601. https://doi.org/10.3201/eid2304.161245

Millar, S., & Cox-Singh, J. (2015). Human infections with Plasmodium knowlesi-zoonotic malaria. Clinical Microbiology and Infection, 21(7), 640-648. https://doi.org/10.1016/j.cmi.2015.03.017

Mohamad, F., & Abu-Bakar, N. (2019). Towards successful adaptation of Plasmodium knowlesi to long-term in-vitro culture in human erythrocytes. International Journal of Pharmaceutical Sciences and Research, 10(6), 2663-2669. http://dx.doi.org/10.13040/IJPSR.0975-8232.10(6).2663-69

Mohring, F., Hart, M. N., Rawlinson, T. A., Henrici, R., Charleston, J. A., Benavente, E. D., & Campino, S. (2019). Rapid and iterative genome editing in the malaria parasite Plasmodium knowlesi provides new tools for P. vivax research. Elife, 8, e45829. https://doi.org/10.7554/eLife.45829

Moon, R. W., Hall, J., Rangkuti, F., Ho, Y. S., Almond, N., Mitchell, G. H., Pain, A., Holder, A. A., & Blackman, M. J. (2013). Adaptation of the genetically tractable malaria pathogen Plasmodium knowlesi to continuous culture in human erythrocytes. Proceedings of the National Academy of Sciences, 110(2), 531-536. https://doi.org/10.1073/pnas.1216457110

Mukry, S. N., Saud, M., Sufaida, G., Shaikh, K., Naz, A., & Shamsi, T. S. (2017). Laboratory diagnosis of malaria: comparison of manual and automated diagnostic tests. Canadian Journal of Infectious Diseases and Medical Microbiology, 2017. https://doi.org/10.1155/2017/9286392

Noulin, F., Manesia, J. K., Rosanas-Urgell, A., Erhart, A., Borlon, C., Van Den Abbeele, J., d'Alessandro, U., & Verfaillie, C. M. (2014). Hematopoietic stem/progenitor cell sources to generate reticulocytes for Plasmodium vivax culture. PLoS One, 9(11), e112496. https://doi.org/10.1371/journal.pone.0112496

Nuin, N. A., Tan, A. F., Lew, Y. L., Piera, K. A., William, T., Rajahram, G. S., Jelip, J., Dony, J. F., Mohammad, R., Cooper, D. J., & Barber, B. E. (2020). Comparative Evaluation of Two Commercial Real-time Pcr Kits (Quantifast™ and Abtes™) for the Detection of Plasmodium knowlesi and Other Plasmodium Species in Sabah, Malaysia. https://doi.org/10.21203/rs.3.rs-30644/v1

Okafor CN, Finnigan NA. Plasmodium Ovale Malaria. [Updated 2020 Aug 31]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK519021/

Roncalés, M., Vidal, J., Torres, P. A., & Herreros, E. (2015). In vitro culture of Plasmodium falciparum: Obtention of synchronous asexual erythrocytic stages. Open Journal of Epidemiology, 5(01), 71. https://doi.org/10.4236/ojepi.2015.51010

Singh, B., Sung, L. K., Matusop, A., Radhakrishnan, A., Shamsul, S. S., Cox-Singh, J., thomas, A., & Conway, D. J. (2004). A large focus of naturally acquired Plasmodium knowlesi infections in human beings. The Lancet, 363(9414), 1017-1024. https://doi.org/10.1016/S0140-6736(04)15836-4

Talapko, J., Škrlec, I., Alebić, T., Jukić, M., & Včev, A. (2019). Malaria: the past and the present. Microorganisms, 7(6), 179. https://doi.org/10.3390/microorganisms7060179

Taylor, B. J., Howell, A., Martin, K. A., Manage, D. P., Gordy, W., Campbell, S. D., Jin, D., Polley, S. D., & Samuel, R. A. (2014). A lab-on-chip for malaria diagnosis and surveillance. Malaria Journal, 13(1), 179. https://doi.org/10.1186/1475-2875-13-179

Warkiani, M. E., Tay, A. K. P., Khoo, B. L., Xiaofeng, X., Han, J., & Lim, C. T. (2015). Malaria detection using inertial microfluidics. Lab on a Chip, 15(4), 1101-1109. https://doi.org/10.1039/C4LC01058B

WHO. (2019). World Malaria Report. Geneva: World Health Organization.

Zaw, M. T., & Lin, Z. (2014). Methods for Detection and Identification of Plasmodium knowlesi: A Review Article. International Journal of Collaborative Research on Internal Medicine & Public Health, 6(1), 0-0.

Citation:

Maketar, S., & Abu Bakar, N. . (2020). A review on lab-on-chip as a potential diagnostic tool for early detection of *Plasmodium knowlesi*. *Life Sciences, Medicine and Biomedicine*, *4*(9). https://doi.org/10.28916/lsmb.4.9.2020.69

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Life Sciences, Medicine and Biomedicine
ISSN: 2600-7207