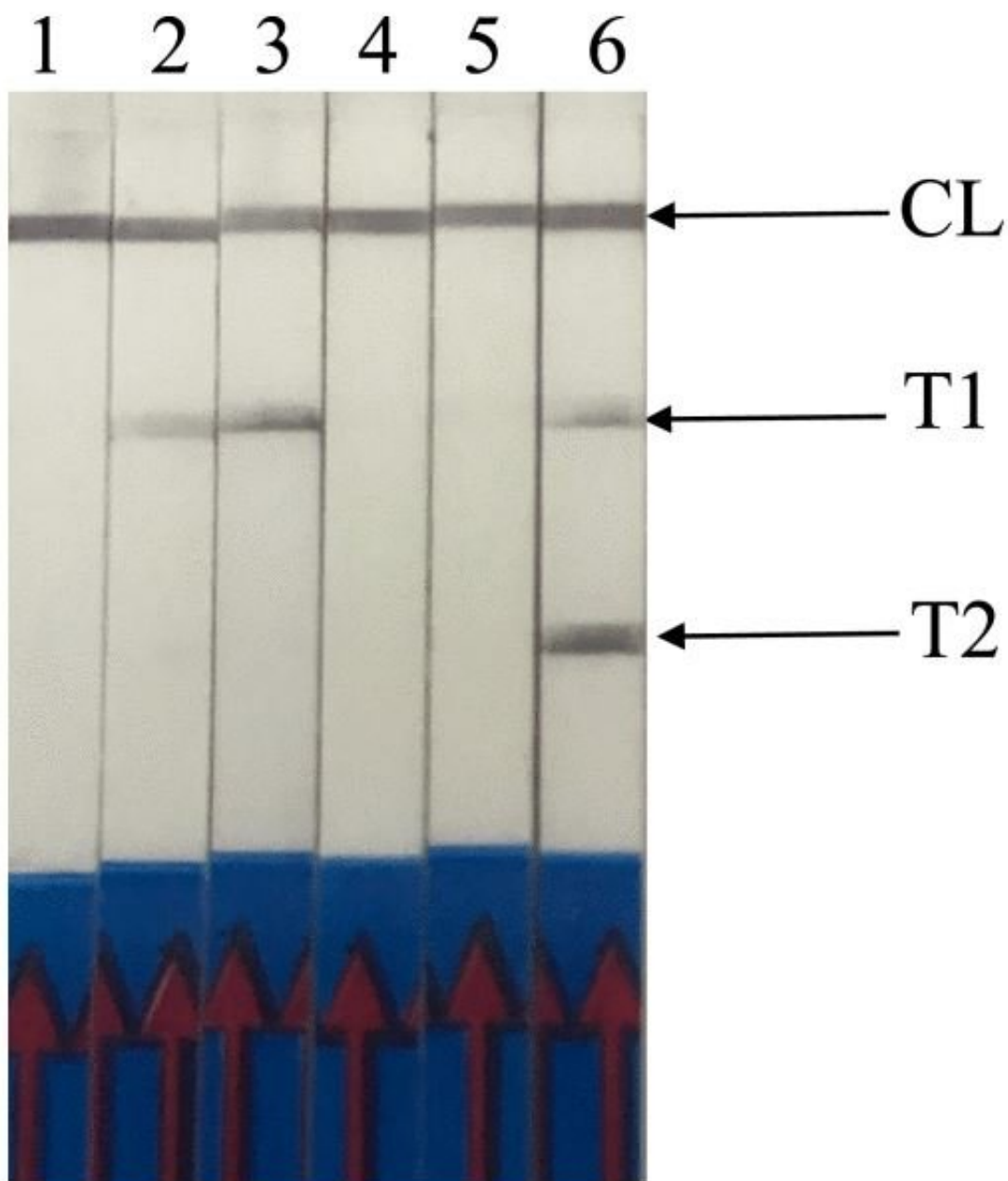


Developing rapid molecular method for the detection of *Plasmodium knowlesi*

March 13 2018



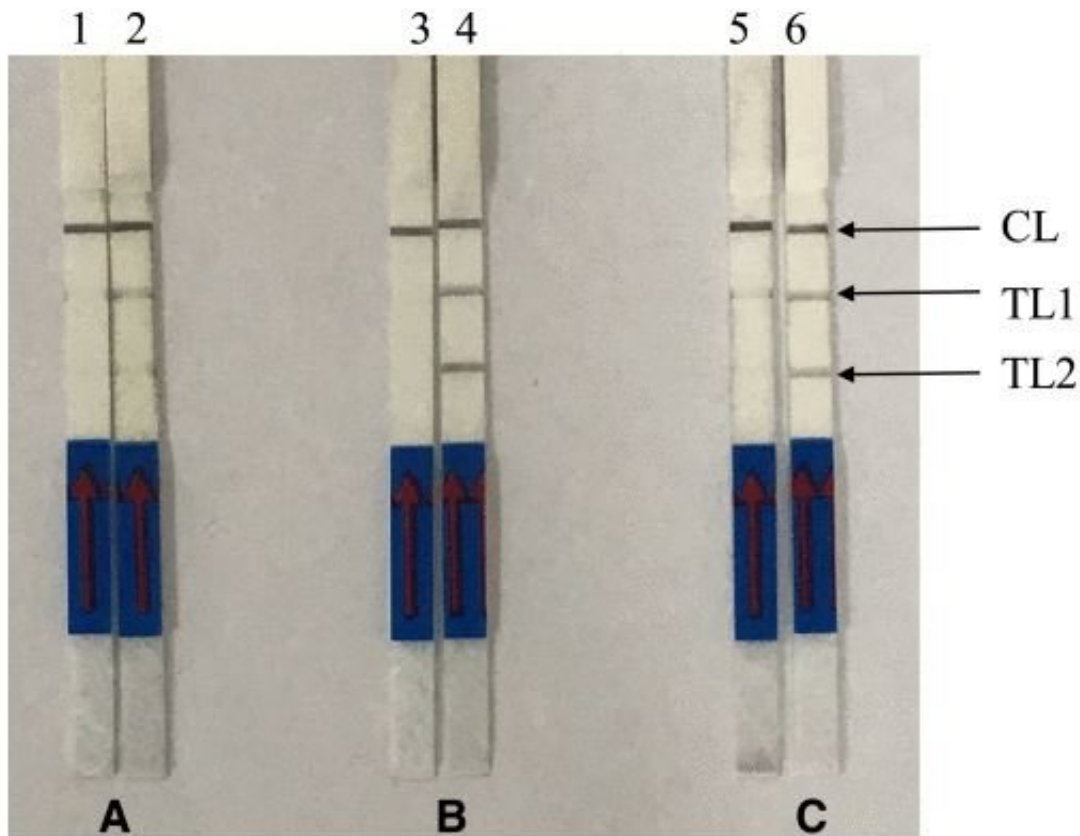
Detection of recombinase polymerase amplification (RPA) amplified product on lateral flow strip. TL1 indicates a genus-specific gene and TL2 shows Plasmodium knowlesi-specific gene detection. A sample was interpreted as negative if only the CL was visible. Representative image of specificity of RPA on genomic DNA extracted from patient samples. CL = control line; Strip 1 = negative control (distilled water); Strip 2 = Plasmodium vivax; Strip 3 = Plasmodium falciparum; Strip 4 = Toxoplasma gondii; Strip 5 = Sarcocystis sp. Strip 6 = P. knowlesi; TL1 = test line 1; TL2 = test line 2. Credit: *The American Journal of Tropical Medicine and Hygiene*

Malaria is a major public health threat in tropical and subtropical countries. In 2015, an estimated 212 million malaria cases occurred worldwide, resulting in 429000 deaths. Malaysia's aim to eliminate malaria by year 2020 is threatened by the emergence of the zoonotic species Plasmodium knowlesi. It is the predominant malaria species infecting humans in Malaysia and capable of producing severe disease. An early and accurate Point-of-Care (PoC) diagnosis can assist in patient management and reduce mortality.

PoC tests are simple and can be performed at the bedside. To date, PoC diagnosis of malaria is not available in Malaysia and most parts of the world. Researchers from University of Malaya aimed to develop a rapid (

The RPA assay was performed using specifically designed primers and probes. The P. knowlesi small subunit ribosomal RNA (18S rRNA) and Plasmodium genus-specific gene was employed as target gene and the primers and probes were designed. Endpoint detection of the amplification product was performed by lateral flow technology that required a specifically designed lateral flow (LF) probe. The probes consisted of an oligonucleotide backbone with a 5- FAM, a

tetrahydrofuran (THF) residue that replaces a nucleotide and a polymerase extension blocking group, C3-spacer at the 3' end.



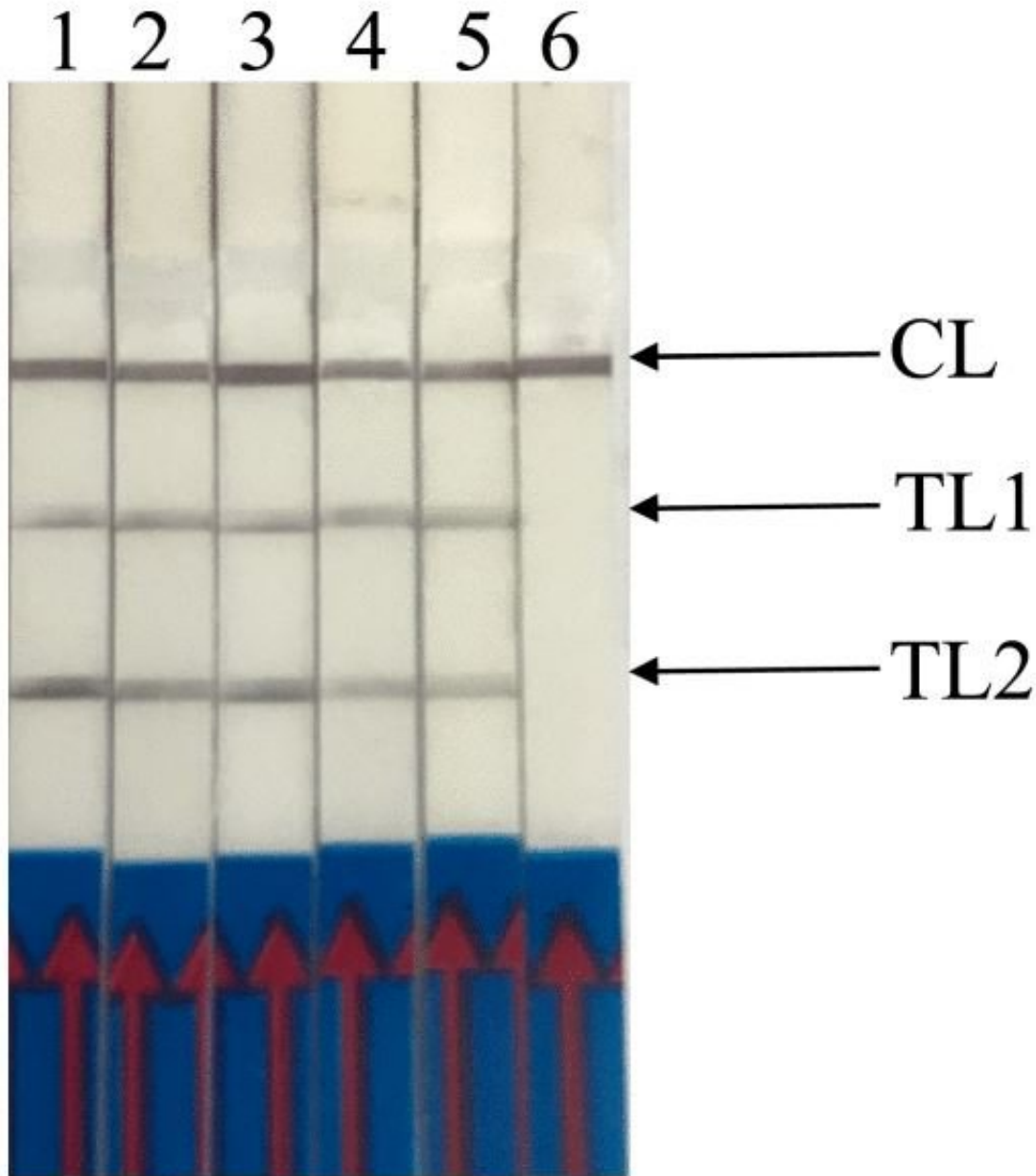
Effects of different concentration of Tris in running buffer A (50 mM Trizma-HCl and 150 mM NaCl), buffer B (50 mM Trizma-HCl, 150 mM NaCl, 0.05% Tween-20), and buffer C (25 mM Trizma-HCl, 150 mM NaCl, pH 7.2.) on recombinase polymerase amplification product. Strip 1, 3, and 5: Negative control (distilled water); 2, 4, and 6; *Plasmodium knowlesi* sample. Credit: *The American Journal of Tropical Medicine and Hygiene*

For detection of *Plasmodium* species genus, the LF probe was used with a biotin-labelled reverse amplification primer. Another oligonucleotide primer involved in the reaction is a conventional primer. To detect the P.

knowlesi genus-specific gene, the LF probe designed is similar to Plasmodium genus gene. However, this probe was used together with digoxigenin-labelled reverse amplification primer at the 5' end.

Another oligonucleotide primer involved is a forward amplification primer. RPA assay was conducted using the commercial TwistAmp kit. The reaction tube was then incubated in a heating block with constant temperature at 37 °C for approximately 15 min. For a positive sample, LF-RPA amplicon was observed as two test lines (TL1 and TL2) on the lateral flow strips (Figure 1). TL1 indicates a genus-specific gene and TL2 shows *P. knowlesi*-specific gene detection. A control line, which is immobilized with anti-rabbit antibodies serves as the RPA assay control.

For the evaluation of the signal enhancement, amplified RPA products were tested in different dilution buffers. The researchers found that buffer B (50 mM Trizma-HCl, 150 mM NaCl, 0.05% Tween-20) was the most suitable dilution buffer as the band of amplified RPA product was more visible with less background compared to other dilution buffers. DNeasy Blood and Tissue kit were used to extract and purify the DNA from *P. knowlesi* strain A1H1 culture for detection limit of the developed LF-RPA assay in this study. The detection limit of *P. knowlesi* RPA in this work was as low as 10 copies (Figure 3).



Determination of the detection limit of *Plasmodium knowlesi* recombinase polymerase amplification (RPA). A 10-fold serial dilution of the stock (10⁵ P. knowlesi parasites (p)/ μ L to 1 p/ μ L) was performed with sterile distilled water. The DNA template from each of the serial dilutions was tested in duplicate and repeated twice. Strip 1, 2, 3, 4, 5, and 6 indicate RPA assay using 10⁵ to 1 p/ μ L as template. Credit: The American Journal of Tropical Medicine and Hygiene

The specificity of LF-RPA was tested using DNA templates from other non-*P. knowlesi* parasites such as *Toxoplasma gondii*, *Sarcocystis* sp., *P. falciparum*, *P. vivax* and *P. ovale*. The results shown that LF-RPA was 100% specific for all *P. knowlesi* samples whereby both test lines and control line were present within 5 min of incubation at room temperature (Figure 1). Meanwhile, only TL1 and control line were present when tested with non-*P. knowlesi* strains (11 *P. falciparum*, 12 *P. vivax* and 2 *P. ovale*). In further experiments, DNA extracted from eight healthy donors were used as template in the LF-RPA reaction. The LF-RPA did not detect any of the negative DNA samples.

With incubation at 37 °C, the 18S rRNA gene of *P. knowlesi* was successfully amplified within 12 min. By adding a specifically designed probe to the reaction solution, the amplified RPA product can be visualized on a lateral flow strip. The RPA assay exhibited high sensitivity with limits of detection down to 10 parasites/ μ l of *P. knowlesi*. Nonetheless, it was demonstrated that all *P. knowlesi* (n = 41) and other *Plasmodium* sp. (n = 25) were positive while negative samples (n = 8) were negative. Therefore, a combination of RPA and lateral flow strip detection is a highly promising approach with the potential to be suitable for use in resource-limited settings.

With the PoC test, detection of malaria infection can be done easily and rapidly in the field or bedside (by finger-prick), thus can rapidly control the outbreaks. The use of the PoC device is applicable to remote area where no lab setup is required to conduct the testing. Only patient detected positive of malaria will be hospitalised for advanced treatment, thus reduces the number of patients under monitoring and unnecessary hospitalization. This testing methodologies has won a Gold medal at the 4th Korea Creative Invention Contest CiC 2017. The researchers plan to develop LF-RPA assay on detection of five human malaria *Plasmodium* sp (*P. knowlesi*, *P. ovale*, *P. falciparum*, *P. vivax* and *P. malariae*) simultaneously. There may be an opportunity to develop the device

further for personalised medicine, whereby self-testing is possible even at home without the need of supervision of medical officer.

More information: Meng-Yee Lai et al. Rapid Detection of Plasmodium knowlesi by Isothermal Recombinase Polymerase Amplification Assay, *The American Journal of Tropical Medicine and Hygiene* (2017). [DOI: 10.4269/ajtmh.17-0427](https://doi.org/10.4269/ajtmh.17-0427)

Meng-Yee Lai et al. Recombinase Polymerase Amplification Combined with a Lateral Flow Strip for the Detection of Plasmodium knowlesi, *The American Journal of Tropical Medicine and Hygiene* (2017). [DOI: 10.4269/ajtmh.17-0738](https://doi.org/10.4269/ajtmh.17-0738)

Provided by University of Malaya

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