Specific, sensitive and rapid detection of human Plasmodium knowlesi infection by loop-mediated isothermal amplification (LAMP) in blood samples

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Abstract (provisional)

Background
The emergence of Plasmodium knowlesi in humans, which is in many cases misdiagnosed by microscopy as Plasmodium malariae due to the morphology similarity, has contributed to the needs of detection and differentiation of malaria parasites. Up to date, nested PCR targeted to Plasmodium sporozite genes is described as the most sensitive and specific method for Plasmodium detection. However, this methodology is costly and requires trained personnel for its implementation. Loop-mediated isothermal amplification (LAMP), a novel nucleic acid amplification method was developed for the clinical detection of P. knowlesi. The sensitivity and specificity of LAMP was evaluated in comparison to the results obtained via microscopic examination and nested PCR.

Methods
LAMP assay was developed based on P. knowlesi genetic material targeting the apical membrane antigen-1 (AMA-1) gene. The method uses six primers that recognize eight regions of the target DNA and it amplified DNA within an hour under isothermal conditions (65 degrees C) in a water-bath.

Results
LAMP is highly sensitive with the detection limit for AMA-1 as low as ten copies. LAMP detected malaria parasites in all confirm cases (n=13) of P. knowlesi infection (sensitivity, 100%) and none of the negative samples (specificity, 100%) within an hour. LAMP demonstrated higher sensitivity compared to nested PCR by successfully detecting a sample with very low parasitaemia (<0.01%).

Conclusions
With continuous efforts in the optimization of this assay, LAMP may provide a simple and reliable test for detecting P. knowlesi malaria parasites in areas where malaria is prevalent.

The complete article is available as a provisional PDF. The fully formatted PDF and HTML versions are in production.