

## **Loop-mediated isothermal amplification (LAMP) assays for detection of malaria parasites**

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Loop-mediated isothermal amplification (LAMP) is a novel method that rapidly amplifies target DNA with high specificity under isothermal conditions. It has been applied as a diagnostic tool for several infectious diseases including viral, bacterial, and parasitic diseases, including human malaria.

Naturally acquired human infections with a macaque malaria parasite, *Plasmodium knowlesi*, have now been referred to as the most convincing zoonotic simian malaria. Recently, we developed a LAMP method for the molecular diagnosis of *P. knowlesi* infection (*PkLAMP*) and evaluated its sensitivity, specificity, and clinical applicability. We designed three sets of *PkLAMP* primers for the species-specific  $\beta$ -*tubulin* gene. The primer sets for *PkLAMP* specifically amplified the autologous DNA extracts of *P. knowlesi*, and the sensitivity of the test was much higher than single-PCR assay. These results indicate that our *PkLAMP* method can be used to efficiently distinguish between *P. knowlesi* and other malaria parasites. To evaluate the feasibility of using *in vivo* materials, comparisons of *PkLAMP* and the conventional nested PCR (nPCR) method and microscopic examination were made with blood samples from two experimentally infected monkeys. These studies showed that *PkLAMP* can be identified in the infectious course of *P. knowlesi* much earlier than with nPCR and microscopy.

In conclusion, LAMP assays can be considered as an efficient candidate for the molecular diagnosis of malaria infection in endemic areas. Moreover, a recent study of the LAMP method showed that it is able to detect both *Plasmodium* oocysts and sporozoites from an “all-in-one” template using whole mosquito bodies (Aonuma H., et al. 2008). These observations further emphasize the potential usefulness of the LAMP method as a diagnostic and new epidemiological surveillance tool for malaria.