

Development of easy diagnostic tool CZC-dry-LAMP for tropical diseases

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Molecular diagnostic test such as polymerase chain reaction (PCR) is critical tool for detecting pathogens with high sensitivity and specificity. However, these techniques are seldom used in diseases endemic areas lacking adequate infrastructure. The loop-mediated isothermal amplification (LAMP) is one of the promising tools for rapid and sensitive disease diagnosis, which can amplify the pathogen genome at a constant temperature. However, it still required complicated sample preparation steps and a well-equipped laboratory which limits its use in resource-poor setting.

Human African Trypanosomiasis (HAT) is a neglected tropical disease caused by *Trypanosoma brucei rhodesiense* or *T. b. gambiense* and is transmitted through the bite of an infected tsetse fly. Early diagnosis and treatment before disease progression is crucial for the survival of HAT patients. Our study aimed at developing point-of-care sensitive HAT diagnostic tests, using improved LAMP method. The first essential improvement was that LAMP reagents were dried and stabilized in a single tube by incorporating trehalose as a cryoprotectant to prolong shelf life at ambient temperature. This dried LAMP has greater convenience for transportation. The second technical improvement was achieved by simplifying the sample preparation step so that DNA could be amplified directly from detergent-lysed blood. The developed LAMP assay (CZC-LAMP) system is now validated in Zambia and Malawi, to understand the epidemiology of trypanosoma parasite between human, domestic/wild animals, and tsetse fly vectors¹.

This technique has been also applied for other important tropical infectious diseases; Malaria²) and Chikungunya virus. Combining with portable sequencer MinION technology (Oxford Nanopore), On-site genotyping from the Chikungunya-LAMP products was also achieved. Our in-house RT-LAMP method and MinION sequencing system were validated with serum samples from recent outbreak chikungunya patients in Brazil. The obtained sequence data was informative to recognize recent outbreak genotype.

Our established on-site genome detection and sequencing system will be applicable for diagnosis of various tropical diseases. These CZC-LAMPs will provide new means for rapid, sensitive and reliable point-of-care diagnosis and will contribute to the disease control.

References

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2. K. Hayashida, K. Kajino, L. Hachaambwa, B. Namangala and C. Sugimoto. (2015) Direct Blood Dry LAMP: A Rapid, Stable, and Easy Diagnostic Tool for Human African Trypanosomiasis. *PLoS Neglected Tropical Diseases* 9(3): e0003578.