Diagnostics and biomarkers

**OC - (8173) - RAPID DETECTION OF MYCOBACTERIUM ULCERANS BY RECOMBINASE POLYMERASE AMPLIFICATION**

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**Background**

There are no primary measures to prevent people from contracting Buruli ulcer, mainly due to poor understanding of its epidemiology. The current control strategy emphasizes early diagnosis and prompt treatment, with the goal of avoiding the complications associated with advanced stages of the disease. There is no diagnostic test for the disease appropriate for use at the primary health care level where most cases are detected and treated. Diagnosis based on clinical signs is unreliable in inexperienced hands and complicated by infections that have similar presentations. This study was to develop and evaluate the use of recombinase polymerase amplification assay for the detection of Mycobacterium ulcerans (Mu) at the point of patient care.

**Methods**

Specific fragment of IS2404 of Mu was amplified in 15 minutes at a constant 42°C using the RPA assay and analyzed on a portable fluorometre. The method was tested for sensitivity and specificity with molecular standard of IS2404 DNA fragment, various Mu strains, other mycobacteria and environmentally associated bacteria. Additionally, the assay performance as a diagnostic tool was tested with archived DNA from symptomatic patients. All results were compared with that of a highly sensitive IS2404 PCR.

**Results**

The detection limit was 50 copies of IS2404 in 15 minutes using plasmid standard and 125 fg with genomic Mu DNA equivalent 25 genomic copies. The assay was highly specific in detecting all strains of Mu with no observed cross reactivity with other mycobacteria and common skin colonizing bacteria. The clinical sensitivity and specificity of the BU-RPA assay using clinical samples was 86% and 100% respectively.

**Conclusion**

We have developed a real-time isothermal RPA assay for the detection of M. ulcerans as a cheaper alternative to PCR. Combining this assay with a simple extraction protocol will maximize its use as point of care test for Buruli ulcer.