Evaluation of an IS2404 LAMP protocol, a simple and rapid test for diagnosis of Buruli ulcer in low-resource settings

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Introduction

Buruli ulcer caused by *Mycobacterium ulcerans* is a devastating necrotic skin disease. PCR, recommended for confirmation of Buruli ulcer by WHO, requires an adequately equipped laboratory, often delaying diagnosis and treatment of patients in remote or humanitarian settings. We aimed to assess loop-mediated isothermal amplification (LAMP), which is a molecular assay for isothermal amplification of DNA suggested for timely diagnosis of Buruli ulcer in low-resource settings.

Methods

This study combines quantitative and qualitative methods. First, we evaluated a simple rapid syringe DNA extraction method (SM) in comparison with a conventional extraction method (CM), followed by a LAMP assay targeting IS2404 for the detection of *M ulcerans*, either using a pocket warmer (pw) or a heat block (hb) for incubation of the reaction. 83 clinical specimens (swabs and fine-needle aspirates from different centres in Ghana) were tested. We assessed sensitivity, specificity, and positive and negative predictive value (PPV and NPV). Second, we explored the diagnostic workflow for Buruli ulcer at a community-based health centre in rural Ghana, a potential target setting. We used observations and interviews with researchers and healthcare workers (HCWs) and community-based surveillance volunteers. We discuss evaluation results in relation to the target setting and requirements of a target product profile for Buruli ulcer diagnosis.

Ethics

This study was approved by the Institutional Review Board of the Noguchi Memorial Institute for Medical Research, University of Ghana, and by the Oxford Tropical Research Ethics Committee at University of Oxford.

Results

DNA extraction using SM followed by IS2404 PCR (IS2404 PCR^{SM}) identified *M ulcerans* DNA in 73 of 83 clinical specimens. The sensitivity, specificity, PPV, and NPV of IS2404 PCR^{SM} were 90.12%, 100%, 100%, and 65.21%, respectively, compared with the reference standard IS2404 PCR with the CM protocol. Evaluation of the LAMP assay on 64 SM DNA extracts showed a sensitivity, specificity, PPV, and NPV of 83.6%, 100%, 100%, and 50%, respectively, using either pw (pwLAMP^{SM}) or hb (hbLAMP^{SM}) for incubation, compared with the same reference standard. The limit of detection of both pwLAMP^{SM} and hbLAMP^{SM} was 30 target copies. Interviews confirmed that, despite great engagement from HCWs and volunteers, patients met challenges regarding transport and costs for initial diagnosis and follow-up and often sought alternative treatments first. Diagnostic confirmation via PCR in a reference laboratory led to a delay in the initiation of treatment. A diagnosis at the point of care, following clinical screening, was considered advantageous to prevent delays and loss to follow-up, therefore ensuring timely patient treatment.

Conclusion

Our findings support the potential use of pwLAMP for rapid diagnosis of Buruli Ulcer in patients with a suspected infection at the community or primary health-care level, with limited equipment and without reliable electricity supply such as found in humanitarian settings.

Conflicts of interest

All authors declare no competing interests.