Title

Rest ASSURED: development of an isothermal diagnostic test for Cutaneous Leishmaniasis.

Abstract

In the NTD Roadmap to 2030, the WHO has outlined the lack of affordable diagnostics as a priority research area for Cutaneous Leishmaniasis (CL). Previous WHO guidelines such as the Target Product Profile and the ASSURED acronym list the desired characteristics: **A**ffordable, **S**ensitive, **S**pecific, **U**ser friendly, **R**apid/Robust, **E**quipment-Free, and **D**eliverable to end users. Furthermore, two particular case studies highlight additional gaps in the field. Firstly, the emergence of drug resistance to *L. tropica* in Pakistan points to the need to develop a differential diagnostic to distinguish *L. tropica* from the other endemic species such as *L. major*. Secondly, the failure of both the Loopamp and CL Detect rapid diagnostic tests in field trials in Ethiopia shows the need for a diagnostic test with greater sensitivity to *L. aethiopica*.

Isothermal amplification techniques such as LAMP offer a promising solution, being both highly sensitive and very cheap to implement. However, the rapid emergence of miniature diagnostic devices following the Covid-19 pandemic has not been translated to leishmaniasis, with most of the devices being tested primarily against bacterial and viral infections.

In this work we have developed a differential assay for *L. tropica* and *L. major*, as well as a highly sensitive assay for *L. aethiopica*. We have also begun work translating the assays into two of the newly developed diagnostic devices, the VH6 (VIDIIA Ltd) and qByte. The *L.major*-*L.tropica* differential assay has been tested on axenically cultured parasites, macrophages infected *in vitro* and *L. major*-infected mice. The *L. aethiopica* assay has been tested on the reference strain as well as five clinical isolates, all cultured axenically. We have also sequenced our primer targets in the reference strain and clinical isolates to assess whether genetic diversity in wild strains could affect assay sensitivity. Finally, we have tested some simplified DNA extraction methods to demonstrate the feasibility of the whole assay workflow, from sample collection to result.

Disclosure

This work was supported by a MRC Impact Acceleration Award (MR/X502947/1; PWD); a studentship to JJ from the EPSRC Molecular Sciences for Medicine Doctoral Training Partnership (EP/S022791/1; PWD); the UKRI - Global Challenges Research Fund, ‘A Global Network for Neglected Tropical Diseases’ (MR/ P027989/1; PWD); BSP Student Travel Award.