LB-5116 - Rapid extraction and detection of Leishmania donovani DNA from skin lesions of suspected cases in Sri Lanka

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S. Hansen: None.

Abstract
Leishmaniasis is a disease caused by a vector born protozoan affecting millions of people worldwide. In Sri Lanka, the cutaneous form of leishmaniasis is predominant, however, a few endogenous cases of mucosal and visceral forms are also reported. Geimsa-stained smear examination under light microscopy for the presence of amastigotes is the most widely used diagnostic method in hospitals of Sri Lanka. However, the reported clinical sensitivity of slit skin smears is low (35%). Furthermore, facilities for in vitro cultures and PCR are only available in a few parasitology laboratories attached to the Universities. All these diagnostic tools demand the services of highly trained doctors and/or technicians, as well as well-equipped laboratory facilities and high running cost. Therefore, there is a need for low cost, highly sensitive and specific screening test for diagnosis of leishmaniasis.

In this pilot study, the mobile suitcase laboratory applying novel extraction (SpeedXtract) and nucleic acid detection (recombinase polymerase amplification assay, RPA) methods were evaluated for the diagnosis of cutaneous leishmaniasis in Sri Lanka.

The developed assay was applied on three different sample formats (punch biopsy, slit skin smears and fine needle aspirates) in order to determine which sample would give the best results with RPA at point of need. The results showed that the 2 mm punch biopsy sample produced the best exponential amplification curve and early fluorescence signal in RPA assay. These experiments were carried out at a local hospital using six highly suspected cutaneous leishmaniasis samples. For comparison, the samples were sent to the central laboratory for testing using the gold standard (conventional PCR assay), which revealed the same results as the RPA operated in the field via the mobile suitcase laboratory. The whole procedure under the field condition took place in 35 minutes, while almost 8 hours were needed to finalize the extraction and detection in the laboratory.

The suitcase laboratory is a promising set up for rapid point of need detection of Leishmania donovani. All reagents were cold-chain independent and the mobile laboratory was operated by a solar power battery, which make it ideal for poor resource settings.