# Comparison of Recombinase Polymerase Amplification Assay based Mobile Suitcase Laboratory as a point of need test with existing parasitological diagnostic methods to diagnose cutaneous leishmaniasis in Sri Lanka

**By**

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**By**

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"The work described in this thesis was carried out by me under the supervision of Dr. Shalindra Ranasinghe, Professor Aresha Manamperi, Professor Renu Wickremasinghe and Senior Professor Nilanthi de Silva and a report on this has not been submitted in whole or in part to university or any other institution for another Degree / Diploma"

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# ABBREVATIONS

| | |
|---|---|
| BC: | Before Christ |
| CL: | Cutaneous Leishmaniasis |
| CSF: | Cerebospinal fluid |
| DAT: | Direct agglutination test |
| ELISA: | Enzyme Linked Immuno sorbant assay |
| FBS: | Fetal Bovine Serum |
| H and E: | Hemotoxyline-eosine |
| IC-RDT: | Immuno-Chromatographic Rapid Diagnostic tests |
| IFAT: | Indirect fluorescent antibody test |
| ITS1: | Internal Transcribed Spacer 1 |
| kDNA: | kinetoplast DNA |
| LD bodies: | Leishman - Donovan bodies |
| LST: | Leishmania skin test |
| MCL: | Muco-Cutaneous Leishmaniasis |
| NNN: | Nicolle, Novey and McNeil medium |
| PCR: | Polymerase Chain reaction |
| PKDL: | Post-Kala-azar Dermal Leishmaniasis |

| | |
|---|---|
| RDT: | Rapid Diagnostic Tests |
| RES: | Reticulo-Endothelial System |
| RPA: | Recombinase Polymerase Amplification |
| SDM: | Schneider's Drosophila Medium |
| SSB: | single-stranded DNA binding protein |
| SSS: | Slit Skin Smear |
| VL: | Visceral Leishmaniasis |
| WHO: | World Health Organization |

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# ABSTRACT

Introduction:

Leishmaniasis is a disease caused by a protozoan parasite belonging to the genus *Leishmania*. There are three main clinical forms of the disease: cutaneous, mucocutaneous and visceral. In Sri Lanka, the cutaneous form of the disease is predominant, which is usually diagnosed using Giemsa-stained Slit Skin Smear (SSS) examination and by histology. However, the sensitivity of SSS and histology are reportedly low. Moreover, facilities for the highly sensitive polymerase chain reaction (PCR) are available only in a few highly-equipped parasitology laboratories. Therefore, there is a need for low cost, sensitive and specific screening test for diagnosis of leishmaniasis at points of need.

Objective:

A mobile suitcase laboratory applying novel DNA extraction (SpeedXtract) and isothermal amplification and detection (recombinase polymerase amplification assay, RPA) method was evaluated for the diagnosis of CL in Sri Lanka in comparison with existing tests. The efficacy of ATL buffer and RNAlater as transport and storage medium for PCR to diagnosis of cutaneous leishmaniasis was also compared.

Methodology:

Two SSS and three 2 mm punch biopsy specimens were collected from 151 patients with clinically suspected CL skin lesions. RPA based mobile suitcase laboratory detection of parasite DNA was performed at point of collection. PCR (in samples stored in ATL buffer and RNA later) was carried out using ITS 1 primers. Giemsa stained SSS were examine under light microscope in the laboratory.

Results:

Sixty patients gave positive result from 151 tested patients with SpeedXtract/RPA assay at point of collection with a diagnostic sensitivity of 65.5%. The best diagnostic sensitivity of 92.4% (86) was with ATL buffer-PCR and only 54 became positive with RNAlater-PCR showing a diagnostic sensitivity of 63.4%. The Giemsa-stained slit skin smear delivered the worst clinical sensitivity (32.2%, 30). Fifty-seven samples were negative in all detection methods.

Conclusions:

The SpeedXtract/RPA method which was done on skin biopsies for the first time under field conditions took 35 min, while almost 8 h were needed to finalize the extraction and detection by PCR in the laboratory. The SpeedXtract/RPA method proven to effective for the diagnosis of CL in Sri Lanka. However, there is a need for a standardization of type of specimen and nucleic acid extraction methods for further improve the test in point of care. ATL buffer shown be better than RNAlater as a transport and storage medium for *Leishmania* DNA detection.