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TITLE: A Novel Field-Deployable Point-of-Care Diagnostic Test for Cutaneous Leishmaniasis

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14. ABSTRACT

Background. Leishmaniasis is caused by the protozoan *Leishmania* and is generally transmitted by the bite of sand flies of the genus *Lutzomyia* or *Phlebotomus*, The disease has significant global impact, producing 10-20 million cases of leishmaniasis worldwide. Cutaneous leishmaniasis (CL) is characterized by chronic skin ulcers that can impact the individual's functional status, lead to expensive and untimely treatment, and result in disfiguring scarring. Leishmaniasis causes a spectrum of diseases that include localized cutaneous leishmaniasis (LCL), and destructive nasal and oropharyngeal lesions of mucosal leishmaniasis (ML). LCL in the New World is most commonly caused by species of the *Viannia* subgenus (*L. braziliensis, L. panamensis, L. guyanensis, L. peruviana*) and to a lesser extent by species of the *Leishmania* subgenus (*L. mexicana*, *L. amazonensis*). Historically, the leishmaniases have had significant impact on military operations. Thousands of cases of visceral and cutaneous leishmaniasis occurred in soldiers in World Wars I and II. Military training and combat operations resulted in cases of CL in soldiers (USA, UK) deployed to Central America. More recently (2003-2004), CL was reported in almost 1,200 members of the U.S. Armed Forces deployed to Iraq and Afghanistan, and the infection is an ongoing concern in the OEF/OIF veteran population. Unpublished information indicates that the number of military personnel with cutaneous leishmaniasis could exceed 3,000.

Rationale. A major challenge in the diagnosis of leishmaniasis is that the disease occurs in remote and resource-limited areas of the world with poor or inexistent primary health infrastructure. This also could be true during military field operations and training exercises where sophisticated laboratory equipment and medical personnel are scarce or not available. For CL or ML, scrapings of dermal tissues or punch biopsies of the lesions are necessary and the diagnostic sensitivity by histopathology, microscopy of smears or culture could be unacceptably low (40-70%). The highly sensitive PCR method cannot be implemented in resource-poor settings due to the high costs, personnel training and need of sophisticated equipment. Therefore, novel methods to detect leishmaniasis at the POC are urgently needed. To date, there is no field-standardized molecular method based on DNA amplification coupled with Lateral Flow reading to detect leishmaniasis. Isothermal amplification by RPA (Recombinase Polymerase Amplification) is a novel strategy to diagnose infectious diseases that can be used at the POC because it is highly sensitive, fast, inexpensive and able to work at most ambient temperatures. Furthermore, RPA does not require refrigeration of reagents and can be adapted easily to lateral flow detection.

Hypothesis: *RPA coupled with a Lateral Flow test strip (RPA-LF) to detect Leishmania DNA will have high sensitivity and specificity to diagnose cutaneous leishmaniasis at the point of care in a field setting.*

Study Design. We propose to utilize for the first time an RPA-based assay coupled with lateral flow (LF) reading to diagnose cutaneous leishmaniasis. We will test novel approaches that could enhance the success of the RPA method in the field, including 1) isolation of DNA from clinical samples using a mini (portable) extractor at the POC or FTA Whatman filter paper specially designed to improve DNA preservation and purification at POC. **Aim 1: To optimize the analytical sensitivity and specificity of the genus- and complex-specific RPA-LF tests using *Leishmania* isolates and clinical samples from collaborating study sites.** We successfully developed *Leishmania spp.* primer sets for RPA that specifically amplified *Leishmania* kinetoplast DNA and were able to detect the equivalent of <10 parasites in spiked clinical specimens. We will compare the analytical sensitivity and specificity of RPA-LF with qPCR using a broad panel of clinical *Leishmania* isolates from the field sites (NAMRU-6 in Peru and NAMRU-3 detachment in Ghana). Clinical validation: A minimum of 20 retrospective convenience samples of clinical specimens known to be parasite positive or negative by PCR sent to UTMB from the field sites will be evaluated by RPA-LF. **Aim 2: To prospectively determine the diagnostic sensitivity and specificity of the RPA-LF test for diagnosis of cutaneous leishmaniasis.** Sub-aim 2.1. New World CL (NAMRU-6): A prospective field trial of the diagnostic test will be conducted in Puerto Maldonado, Madre de Dios, Peru. Based on estimated RPA-LF sensitivity of 95% and specificity of 99% we will enroll 184 positive, parasite confirmed individuals and 42 parasite negative controls to have adequate statistical power. The sensitivity and specificity will be determined using microscopy of dermal samples, and qPCR as the gold standard. Sub-aim 2.2. Old World CL (NAMRU-3): A similar prospective field trial will be conducted through the NAMRU-3 Ghana detachment, at the Noguchi Memorial Institute for Medical Research. Considerable effort will be taken to ensure consistency at the two sites. Patients will be enrolled principally from the villages in the Ho, HoHoe, and Kpando districts of the Volta Region where CL outbreaks due to *L. major* were previously recorded.

The repeatability of the RPA-LF test will be determined in the NAMRU's field sites while the reproducibility will be determined in the central diagnostic lab at UTMB where a subset of samples (10%) of positive and negative individuals will be delivered by the investigators of NAMRU-3 and NAMRU-6.

Training: The project will provide training to field and laboratory personnel, as well as military personnel temporarily stationed in the field of endemic areas to ensure effective deployment of the POC test.

15. SUBJECT TERMS

Cutaneous leishmaniasis-diagnosis-point of care-DNA amplification-field applicable

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1. INTRODUCTION:

Leishmaniasis is caused by the protozoan *Leishmania* and is generally transmitted by the bite of sand flies of the genus *Lutzomyia* or *Phlebotomus,* The disease has significant global impact, producing 10-20 million cases of leishmaniasis worldwide. Cutaneous leishmaniasis (CL) is characterized by chronic skin ulcers that can impact the individual's functional status, lead to expensive and untimely treatment, and result in disfiguring scarring. Military training and combat operations resulted in cases of CL in soldiers (USA, UK) deployed to Central America. More recently (2003-2004), CL was reported in almost 1,200 members of the U.S. Armed Forces deployed to Iraq and Afghanistan, and the infection is an ongoing concern in the OEF/OIF veteran population. To date, there is no field-standardized molecular method based on sensitive DNA amplification coupled with Lateral Flow reading to detect leishmaniasis. Isothermal amplification by RPA (Recombinase Polymerase Amplification) is a novel strategy to diagnose infectious diseases that can be used at the POC because it is highly sensitive, fast, inexpensive and able to work at most ambient temperatures.

2. KEYWORDS:

Cutaneous leishmaniasis-diagnosis-point of care-DNA amplification-field applicable

3. OVERALL PROJECT SUMMARY:

- The analytical sensitivity and specificity of RPA-LF for species of the Leishmania Viannia subgenus was established.
- We determined that a different set of primers and probe that we designed for RPA-LF was capable of detecting L. major and L. enriettii, the two species implicated in cutaneous leishmaniasis in Ghana.
- The results obtained during this study period were communicated to the scientific community through presentations made at the Military Health System Research Symposium (MHSRS).Fort Lauderdale during August 17-20, 2015.

4. KEY RESEARCH ACCOMPLISHMENTS:

1. Successfully determined Genus and species of Leishmania samples from Peru (by PCR) that were sent to the Travi lab for analyses.

2. We completed the kickoff Coordination Meeting of participating institutions

3. We submitted protocols for local IRB approval and HRPO approval

4. We achieved local institution IRB approval at NAMRU-6 and the Noguchi institute in Ghana.

5. CONCLUSION:

PLANS

- Make sure that protocol approval for NAMRU-3 IRB and NMIMR Research Committee is in place in preparation to the second phase of the study. The Ghana Health Service Ethics Committee has issued a conditional approval of the protocol; a response to the committees concerns has been submitted and NAMRU-3 co-investigators are awaiting the response.
- Evaluate different DNA extraction methods that provide good nucleic acid quality for RPA amplification.
- Select the most practical DNA extraction method amenable to field application
- This objective is crucial to initiate field studies in both study sites in Peru and Ghana

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:

(1) Lay Press: Nothing to report
(2) Peer-Reviewed Scientific Journals: In preparation
(3) Invited Articles: Nothing to report
(4) Abstracts: One abstract as described below

Military Health System Research Symposium (MHSRS).Fort Lauderdale during August 17-20, 2015

Development of a Point-Of-Care Molecular Diagnostic Test for Cutaneous Leishmaniasis.

Bruno L. Travi, DVM, PhD1*, Omar A. Saldarriaga DVM, PhD1, Alejandro Castellanos, PhD1, Gerald C. Baldeviano, PhD2, Maxy B. De los Santos, PhD2, Peter C. Melby, MD1, Andrés G. Lescano, PhD2
1University of Texas Medical Branch, Galveston, TX; 2US Naval Medical Research Unit No. 6 (NAMRU-6), Lima, Peru.

7. INVENTIONS, PATENTS AND LICENSES: Nothing to report

8. REPORTABLE OUTCOMES:

The development of the diagnostic tool is in progress, and 90% of its laboratory evaluation has been completed.

9. OTHER ACHIEVEMENTS: Nothing to report

10. REFERENCES: Nothing to report

11. APPENDICES:

STATEMENT OF WORK – October 4, 2013
PROPOSED START DATE April 1, 2014

Site 1: University of Texas Medical Branch
301 University Blvd, Galveston
TX 77555

PI: Bruno L. Travi

Site 2: NAMRU-6

Av. Venezuela Cdra. 36 S/N,
Bellavista – Callao
Centro Medico Naval
Lima, Peru
ZIP: Callao 02

Partnering PI: Andres G. Lescano

Specific Aim 1(specified in proposal)	**Timeline**	**UTMB Galveston Texas**	**NAMRU-6 Peru**	**NAMRU-3 Ghana detachment**
<u>Aim 1</u>: To use simulated field conditions to optimize and produce the established RPA lateral flow diagnostic test for POC deployment.	Months			
<u>Sub-Aim 1.2</u>: To determine if a simple DNA extraction method will provide adequate sensitivity for optimal test function under field conditions. Comparison of DNA yield, sufficient for RPA-LF test using a DNA mini-extractor vs. Whatman FTA filter paper utilizing dermal tissues spiked with *Leishmania* grown in the lab	1-3	Drs.Travi & Castellanos		
<u>Sub-Aim 1.3</u>: To determine if subgenus- and/or species-specific primer-probe sets can achieve the same analytical sensitivity and specificity as the genus-specific primer-probe set using *Leishmania* isolates and clinical specimens from the field sites.	3-12	Drs.Travi, Castellanos	Drs. Lescano & Baldeviano	Drs. Adams & Puplampu
Kickoff Coordination Meeting of participating institutions	3	Drs. Melby, Travi, Castellanos	Drs. Lescano & Baldeviano	Drs. Adams & Puplampu
Protocol submission for local IRB approval and HRPO approval	3	Drs. Melby & Travi	Dr. Lescano	Dr. Adams
Milestone Achieved: Local IRB and HRPO approved protocols	6	Dr. Melby & Travi	Dr. Lescano	Dr. Adams

Milestone(s) Achieved: • Coordination meeting completed • Approvals of IRBs in place to initiate field studies in human populations • RPA-Lateral Flow test fully adapted for field application	12	Drs.Travi, Castellanos	Dr. Lescano & Baldeviano	Drs. Adams & Puplampu
<u>Aim 2</u>: To prospectively determine the diagnostic sensitivity and specificity of the RPA-lateral flow test for diagnosis of cutaneous leishmaniasis.				
<u>Sub-aim 2.1</u>. To recruit suspicious cutaneous leishmaniasis patients and evaluate the sensitivity/specificity of RAP-Lateral Flow vs. standard kDNA PCR at NAMRU-6; Lima and Puerto Maldonado, Madre de Dios, Peru Delivery of subset of positive and negative clinical samples (10%) from NAMRU-6 to UTMB for reproducibility testing	12-36	Drs. Castellanos,& Travi,	Dr. Lescano	Dr. Adams
Technical meeting at NAMRU-3, Ghana	14	Drs. Travi, Melby	Drs. Lescano, Baldeviano	Drs. Adams, Puplampu & Boakye
<u>Sub-aim 2.2.</u> To recruit suspicious cutaneous leishmaniasis patients and evaluate the sensitivity/specificity of RAP-Lateral Flow vs. standard PCR at NAMRU-3, Ghana detachment, Noguchi Memorial Institute for Medical Research, Ho Volta region Delivery of subset of positive and negative clinical samples (10%) from NAMRU-3 to UTMB for reproducibility testing	12-36	Drs. Castellanos, & Travi,		Dr. Adams & Puplampu
Technical meeting at NAMRU-6, Peru	14	Drs. Travi, Melby	Drs. Lescano, Baldeviano	Drs. Adams & Puplampu

Milestone(s) Achieved: Primary milestone: Validated RPA-Lateral Flow test for Point of Care utilization Secondary milestones: Updated epidemiological assessment of cutaneous leishmaniasis in the endemic areas of Peru and Ghana New point-of-care diagnostic test for cutaneous leishmaniasis ready for submission to obtain FDA clearance Final Report to DoD and scientific publications of results	36	Drs. Travi, Melby, Castellanos	Drs. Lescano & Baldeviano	Dr. Adams & Puplampu

Projected human Subject Enrollment

	Year 2				Year 3			
Target Enrollment (per quarter)	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
NAMRU-3	33	32	32	32	33	32	32	
NAMRU-6	33	32	32	32	33	32	32	
Target Enrollment (cumulative)	66	130	194	258	324	388	452	

Note: no human subjects will be recruited during the first year of the study

Note: The Government reserves the right to request a revised SOW format and/or additional information.