

MULTI-ANTIGEN-CHEMILUMINESCENT-ELISA FOR THE DIAGNOSIS OF
TRYPANOSOMA CRUZI INFECTION IN CHIHUAHUA, MEXICO

JOSE ANDREI OROZCO-ARROYO JR

Master's Program in Biological Sciences

APPROVED:

Rosa A. Maldonado, D.Sc., Chair

Igor C. Almeida, D.Sc.

German Rosas-Acosta, Ph.D.

Delfina Dominguez, Ph.D.

Charles Ambler, Ph.D.
Dean of the Graduate School

Copyright ©

by

Jose Andrei Orozco-Arroyo Jr.

2018

MULTI-ANTIGEN-CHEMILUMINESCENT-ELISA FOR THE DIAGNOSIS OF
TRYPANOSOMA CRUZI INFECTION IN CHIHUAHUA, MEXICO

by

JOSE ANDREI OROZCO-ARROYO JR, B.S.Micr.

THESIS

Presented to the Faculty of the Graduate School of

The University of Texas at El Paso

in Partial Fulfillment

of the Requirements

for the Degree of

MASTER OF SCIENCE

College of Science

THE UNIVERSITY OF TEXAS AT EL PASO

May 2018

ProQuest Number: 10813798

All rights reserved

INFORMATION TO ALL USERS

The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if material had to be removed, a note will indicate the deletion.



ProQuest 10813798

Published by ProQuest LLC (2018). Copyright of the Dissertation is held by the Author.

All rights reserved.

This work is protected against unauthorized copying under Title 17, United States Code
Microform Edition © ProQuest LLC.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

Table of Contents

Table of Contents	iv
List of Tables	vi
List of Figures	vii
Introduction	1
Chagas Disease	1
Epidemiology	1
Life cycle	3
Pathogenesis.....	5
Status in Mexico	6
Chapter 1: Multi-antigen Chemiluminescent-ELISA for diagnosis of <i>T. cruzi</i> infection.....	10
Hypothesis.....	12
Specific Aim	12
Materials and Methods.....	13
Epimastigote culture	13
Antigen preparation	13
Sample collection.....	14
Sera	14
Chemiluminescent Enzyme-Linked Immunosorbent Assay (CL-ELISA)	17
Results.....	18
Chemiluminescent Enzyme-Linked Immunosorbent Assay (CL-ELISA)	18
Statistical analysis.....	22
Discussion	24
Chapter 2: Immunoblot of seropositive samples to assess performance of EpM CL-ELISA as a diagnostic tool.	26
Hypothesis.....	31
Specific Aim	31
Materials and Methods.....	32
Parasite culture and antigen preparation	32
Electrophoresis and western blot	32
Results.....	33

TCT Y-strain immunoblot	33
Data analysis	36
Discussion	36
References	38
Vita	42

PREVIEW

List of Tables

Table 1. Flow chart of the procedures that were followed for the collection, handling and analysis of the donors' samples.	16
Table 2. Reproducibility of the EpM CL-ELISA	23

PREVIEW

List of Figures

- Figure 1. The life cycle of *Trypanosoma cruzi*. 1. Triatomine bug takes a blood meal releasing metacyclic trypomastigotes in the feces. 2. Metacyclic trypomastigotes enter the bite wound or mucosal membranes infecting nucleated cells. 3. Inside nucleated cells, the parasite transform into amastigotes and multiply by binary fission. 4. Amastigotes transform into trypomastigotes and burst out of the cell to infect new cells. 5. A triatomine bug takes a blood meal from infected mammalian host ingesting trypomastigotes. 6. Trypomastigotes transform into epimastigotes in the midgut. 7. Epimastigotes replicate by binary fission. 8. Epimastigotes travel to the hindgut where they transform into metacyclic trypomastigotes to be released in feces. (from Center for Disease Control and Prevention, 2015) [18]..... 4
- Figure 2. Titration curves for the EpM CL-ELISA. Sera were serially diluted (1:2) from 1:250 to 1:2000 in 1% BSA-PBS. (A) EpM CL-ELISA multiantigen at 4 ng/well. (B) EpM CL-ELISA multiantigen at 10 ng/well. (C) EpM CL-ELISA multiantigen at 40 ng/well. (D) EpM CL-ELISA multiantigen at 108 ng/well. (E) EpM CL-ELISA multiantigen at 120 ng/well. Positive control: pool of 10 Chagasic patients from Barcelona, -●-; negative control: pool of 10 healthy donors from Barcelona, -■-; serum sample of a known healthy donor -▲-. The highest differential reactivity between positive and negative sera was determined at (D) multiantigen concentration of 108 ng/well and sera dilution of 1:500. Titrations were performed in triplicates and the results represent the means of the replicates. 19
- Figure 3. Reactivity of EpM CL-ELISA using the multiantigen of *T. cruzi* epimastigotes (108ng/well). Donor samples (n=319) were diluted 1:500 in 1% BSA-PBS and tested in duplicates. Individual sera comprising the pools of Chagasic patients (n=10) and healthy donors (n=10) respectively, were diluted 1:500 in 1% BSA-PBS and assayed in triplicates to determine the cutoff value. Dotted line, a cutoff value corresponding to titer = 1.0. A sample is considered positive when its titer is equal to or higher than 1.0 and negative when its titer is less than 0.9. Samples with titers ranging from 0.9-0.99 are considered as inconclusive. The reported results represent the means of the replicates. 21
- Figure 4. Western blot analysis of EpM CL-ELISA seropositive samples. TCT lysate was resolved in 6% polyacrylamide gel and then transferred to a nitrocellulose membrane for Western blot analysis of EpM CL-ELISA seropositive patients. Positive control: pool of Chagasic patients (1:2000), negative control: naïve mice sera (1:1000) and patient's sera (1:1000) used as primary antibodies. Secondary antibody anti-human IgG (1:10,000) and the horse-radish peroxidase conjugated streptavidin (1:10,000). 35

Introduction

The genus *Trypanosoma* consists of flagellated protozoa parasites that cause infections in humans and animals. The most relevant infectious diseases to humans are African trypanosomiasis and Chagas disease caused by *Trypanosoma brucei*, *Trypanosoma cruzi*, respectively, which are considered as neglected tropical diseases (NTDs) [1]. Most affected by these parasitic diseases are low-income populations of countries located in tropical and subtropical areas of the world.

CHAGAS DISEASE

Chagas disease (ChD) or American Trypanosomiasis is caused by the protozoan parasite *Trypanosoma cruzi*. The parasite has a complex life cycle, which alternates between triatomine vectors and mammals, including humans. The disease can cause cardiac disorders, digestive or neurological alterations. *T. cruzi* is genetically diverse; it has been classified in six discrete typing units (DTU)s: TcI, TcII, TcIII, TcIV, TcV, and TcVI, which present different antigenicity, eco-epidemiological, clinical, and geographic associations [2]. It is considered a neglected tropical disease with endemic regions located in 21 Latin American countries, where it is mostly vector-borne when humans come across the feces or urine of the infected triatomine bug [3, 4].

Epidemiology

According to the World Health Organization (WHO), about 6 to 7 million people worldwide are estimated to be infected with ChD. Over the past half of the 20th century, there has been an increased migration of Latin Americans to non-endemic countries, greatly modifying the epidemiology of the disease. In the last decades, it has been detected in the U.S and Canada. Even in the Old World, *T. cruzi* has been detected in countries such as Japan, Australia and most of the European continent, where an approximate large number of immigrants infected with the protozoan live in Spain and Italy [3, 5]. Solely looking at the U.S., there are an estimated 300,000

infected individuals with *T. cruzi* in the states of New Mexico, Texas, Georgia, Louisiana and California [6, 7]. It is estimated that most individuals with ChD are immigrants that came from El Salvador, Guatemala, Honduras, and Mexico that acquired the infection in their countries of birth; the last accounting for around 174,388 infected individuals living in the U.S. [8-10]

Mexico is a country with immense climatic variety and vast biodiversity, providing the conditions for the development of etiological agents of all kinds, including *T. cruzi*. There are at least 30 species of triatomines reported in the country, all known to be potential vectors [11]. ChD remains as the most important parasitic disease in the country. As of 2010, it was estimated that 876,458 people were infected with *T. cruzi* and 70,117 people had chagasic cardiopathy [12]. The poverty in the country has forced people from endemic areas of the country to migrate to industrialized cities like Mexico City, in search of jobs. Carabarin-Lima et. al. report that the children under 5 years of age infected with ChD are distributed regularly in urban rather than rural areas, suggesting that ChD is becoming urbanized in Mexico [13].

In Mexico, there are 18 endemic areas located in the southeast comprising the states of Oaxaca, Jalisco, Yucatan, Chiapas, Veracruz, Puebla, Guerrero, Hidalgo and Morelos. A high prevalence is observed in the northeastern region of the country, which corresponds to the central area of a tropical region known as La Huasteca [13]. But the highest prevalence (1.94% - 1.68%) was observed in the US neighbor states of Nuevo Leon and Tamaulipas [13, 14].

In the southeast of the state of Chihuahua, near the limits of the states of Sonora and Sinaloa there is an area known as Barrancas de la Sierra Tarahumara, where there have been reports of triatomines infected with *T. cruzi*. In a study conducted by the Immunoparasitology Laboratory of the Medical School at the Autonomous University of Chihuahua, a sample of *Triatoma recurva* collected in the Chihuahuan municipality of Urique was found to be infected with *T. cruzi* [15].