

Evaluation of a Monoclonal-based EIA for the Detection of *Giardia lamblia* and the Identification of the Antigen

James Hunter Boone

Thesis submitted to the Faculty of Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science

in

Biology

Joseph O. Falkinham III, chair
Tracy D. Wilkins, co-chair
Noel R. Krieg

May 5, 1998
Blacksburg, Virginia

Keywords: *Giardia lamblia*, Enzyme Immunoassay, Cyst Wall Protein One

Copyright 1998, James H. Boone

Evaluation of a Monoclonal-based EIA for the Detection of *Giardia lamblia* and the Identification of the Antigen

James Hunter Boone

(STATEMENT OF THE OBJECTIVES)

I. A number of commercial enzyme immunoassay (EIA) tests are available for the diagnosis of giardiasis. In a time of rising health-care costs, there is a need for diagnostic tests that are rapid, specific, sensitive and inexpensive. In the first phase of this study, I developed a monoclonal-based EIA, the *GIARDIA TEST*, with these qualities in mind. This assay's performance characteristics were determined by a comparison study using conventional ova and parasite examination, immunofluorescence antibody test (IFA) and other commercial EIA tests. Studies were done in-house at TechLab, Inc. and at various U.S. medical facilities. Results were statistically analyzed to determine sensitivity (ability of the assay to detect a positive result), specificity (amount of crossreactivity), predictive positive value (the confidence in a positive result), predictive negative value (the confidence in a negative result) and overall correlation with the reference assay.

II. There remain many questions to be answered about the various antigens produced by *Giardia lamblia* and how they can be utilized as diagnostic markers. In the second phase of this study, I identified and partially characterized the antigen (Ct7 Ag) that reacts with the Ct7 monoclonal antibody (MAb). This MAb is an IgM class mouse immunoglobulin that is utilized by the *GIARDIA TEST* and by an immunofluorescence antibody test (IFA) which detects *Giardia* cysts in water and feces. The results of this study will provide physicians and researchers with detailed information about the Ct7 Ag and why it is a useful marker for giardiasis.

ACKNOWLEDGMENTS

I would like to thank my wife, Karen, whose love and support made the completion of this work possible. I am also thankful for my grandfather, Thomas D. Taylor, who built the computer that I used to write this thesis. In addition, I am grateful for the encouragement and advice received from my colleagues at TechLab, Inc., most importantly that given by Dr. David Lyerly, vice-president. I greatly appreciate the guidance and direction given by my advisory committee: Drs. Tracy D. Wilkins, Joseph O. Falkinham III and Noel R. Krieg. Finally, I would like to dedicate this work to my son, Jordan Thomas Boone, who like this degree, is a blessing from God.

TABLE OF CONTENTS

Statement of the Problem.....	ii
Acknowledgments.....	iii
Table of Contents.....	iv
List of Figures.....	v
List of Tables.....	vi

CHAPTER 1

Evaluation of a Monoclonal-based Enzyme Immunoassay (EIA) for the Detection of *Giardia lamblia* in Human Stool

Abstract.....	2
Introduction.....	3
Materials and Methods.....	9
Results.....	12
Discussion.....	23
References.....	25

CHAPTER 2

Identification of *Giardia lamblia* Cyst Wall Protein 1 Detected by the Ct7 Monoclonal Antibody Used in the *GIARDIA* TEST

Abstract.....	29
Introduction.....	30
Materials and Methods.....	32
Results.....	41
Discussion.....	55
References.....	58

VITA.....	61
-----------	----

LIST OF FIGURES

CHAPTER 1.

Figure 1. <i>Giardia</i> Life Cycle.....	4
Figure 2. <i>Giardia</i> Trophozoites.....	5
Figure 3. <i>Giardia</i> Cysts.....	6
Figure 4. Dose Response Curves of the <i>GIARDIA</i> TEST.....	20

CHAPTER 2.

Figure 1. SDS-PAGE Analysis of Ct7 Ag.....	47
Figure 2. Western Blot Analysis of Cyst Lysate, Culture Filtrate and Ct7 Ag.....	48
Figure 3. Western Blot Analysis of Ct7 Ag Using the 7D2 MAb.....	49
Figure 4. N-terminal Sequences of CWP1 and CWP2.....	50
Figure 5a. Western Blot Analysis of Culture Filtrate During Encystment.....	51
Figure 5b. Photo of a Encysting <i>Giardia</i> Trophozoite Following IFA Analysis.....	52
Figure 5c. Photo of a <i>Giardia</i> Cyst Following IFA Analysis.....	53
Figure 6. Western Blot Analysis of <i>Giardia</i> -positive Stools.....	54

LIST OF TABLES

CHAPTER 1.

Table 1. Commercially Available Tests for the Detection of <i>Giardia</i>	7
Table 2. Clinical Studies Comparing the New <i>GIARDIA</i> TEST and the Alexon Test....	15
Table 3. Clinical Study Comparing the <i>GIARDIA</i> TEST to O&P.....	16
Table 4. Clinical Study Comparing the Alexon Test to O&P.....	17
Table 5. In-house Study Comparing the <i>GIARDIA</i> TEST and the Remel Test.....	18
Table 6. Organisms that Do Not React in the <i>GIARDIA</i> TEST.....	19
Table 7. Inter-Assay Testing of the <i>GIARDIA</i> TEST.....	21
Table 8. Intra-Assay Testing of the <i>GIARDIA</i> TEST.....	22

CHAPTER 2.

Table 1. Assessment of the Starting Material for a Ct7 Ag Purification.....	44
Table 2. Immunopurification of the Ct7 Ag.....	45
Table 3. Recoveries of Various Steps of the Ct7 Ag Purification Scheme.....	46