سامية محمد مصطفى



شبكة المعلومات الحامعية

بسم الله الرحمن الرحيم



-Caro-

سامية محمد مصطفي



شبكة العلومات الحامعية



شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم





سامية محمد مصطفى

شبكة المعلومات الجامعية

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسو

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات



يجب أن

تحفظ هذه الأقراص المدمجة يعيدا عن الغيار



سامية محمد مصطفي



شبكة المعلومات الجامعية



المسلمة عين شعور المسلمة عين شعور المسلمة عين شعور المسلمة عين شعور المسلمة ا

سامية محمد مصطفى

شبكة المعلومات الحامعية



بالرسالة صفحات لم ترد بالأصل



A Study on The Development of Immunological Method(s) For The Detection of Fascioliasis

A Thesis

SUBMITTED IN THE PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHARMACEUTICAL SCIENCES (MICROBIOLOGY & IMMUNOLOGY)

By

Yasser Mohammed El-Sayed Metwally Abdel-Rahman

ADVISORY COMMITTEE

Dr. Hussien Abdel-Baky ShoebProfessor of Microbiology & Immunology

Dr. Abdel-Hameed A. Abdel-HameedAsst. Professor of Microbiology & Immunology

Dr. Ali Kholify AhmadyAsst. Professor of Microbiology & Immunology

DEPARTEMENT OF MICROBIOLOGY & IMMUNOLOGY
FACULTY OF PARMACY
CAIRO UNIVERSITY
2001

B 10-V1 مبسم الله الرحمين الرحسي

TO MY FATHER & MOTHER WITH MY LOVE

Approval sheet

A study on the development of new immunological method(s) for the detection of fascioliasis

By

Yasser Mohammed El-Sayed Metwally Abdel-Rahman

Approved by:

(Committee in charge)

Date ../../2001

Acknowledgment

I would like to express my profound gratitude and deep appreciation to Dr. Hussein Shoeb, Professor of Microbiology and Immunology, Department of Microbiology and Immunology, Faculty of Pharmacy, Cairo University, for his advice in suggesting the point of reseach. I am also sincerely thankful to him for his overwhelming help in suggestion of many technical solutions in dealing with various scientific stop points throughout the thesis. I feel personal gratitude to the great efforts provided by Dr. Hussein in writing this thesis and accomplishing this work.

I am also very grateful to Dr. Abdel-Hameed A. Abdel-Hameed, Associate Professor of Microbiology and Immunology, Department of Microbiology and Immunology, Faculty of Pharmacy, Cairo University, for his sincere and kind help, encouragement, and for offering his deep experience to push me forward in an exceptionally friendly environment. I am also grateful to Dr. Abdel-Hameed for his supervision, criticism, great help in revising this thesis and support throughout the work.

I am also thankful for Dr. Ali Kholify Associate Professor of Microbiology and Immunology, Department of Microbiology and Immunology, Faculty of Pharmacy, Cairo University, for his support throughout this thesis.

I am grateful for Dr. Bahagat Associate Professor of Microbiology and Immunology, Department of Microbiology and Immunology, Faculty of Pharmacy, Cairo University, for supplying me with instruments that were needed in some experiments.

Special thanks to veterinarians at the EL-Bassateen abattoir, Cairo, for their help in collection of Fasciola worms.

I am obliged to the RESEARCH INSTITUTE OF VETERINARY VACCINES AND SERA (Abbaseia, Cairo), for helping with the lyophilization of the worm extracts.

I am sincerely grateful for the RESEACH INSTITUTE FOR TROPICAL MEDICINE for supplying me with most of the sera used in this thesis, especially Dr. Amaal noor El-hoda, Head of the Department of Parasitology at the research institute for tropical medicine.

I am greatly obliged to Ramy Karam, my colleagues at the Department of Microbiology and Immunology, Faculty of Pharmacy, Cairo University, for supplying me with the nitrocellulose membranes for western blotting as a gift.

A special word of thanks to Millipore Inc., USA and PALL Corporation, USA, for supplying me with the membranes and all other components of the lateral flow device as a gift.

I am also indebted and thankful to my colleagues, staff members, and to all workers in the Department of Microbiology and Immunology, Faculty of Pharmacy, Cairo University for their friendly cooperation.

Table of contents

		Page
	Introduction	1
	PART-I LITERATURE REVIEW	5
I	FASCIOLA (THE LIVER FLUKE)	5
1.	The Adult worms	5
2.	Epidemiology and mode of transmission	6
3.	Life cycle	8
4.	Pathogenesis	9
5.	Pathology	10
6.	Clinical presentation	11
	6.1. Incubation period	11
	6.2. Phases of infection	11
	6.2.1. Invasive or acute (prepatent) phase	12
	6.2.2. Latent phase	12
	6.2.3. Obstructive phase	12
	6.3. Ectopic fascioliasis	13
7.	Diagnosis	14
	7.1. Parasitological diagnosis	14
	7.2. Clinical diagnosis	14
	7.3. Laboratory findings	15
	7.4. Serological diagnosis of fascioliasis	16
•	7.4.1. Antigenic preparations	17
	7.4.2. Serodiagnostic methods for Fasciola	
	infections	18
	a) Detection of circulating antigens	18
	b) Detection of coproantigens in stool.	19
	c) Detection of circulating antibodies	21

		·	Page
		i) Whole worm antigen preparation	21
		ii) Excretory - Secretory antigen preparation	26
	7	.4.3. Conclusion	34
		a) Detection of antigens	34
		b) Detection of antibodies in serum	34
8.	Enz	symatic activity of Fasciola ES preparation	35
9.	Sim	ilarity between both species of Fasciola	38
Π	RA	PID TESTS	40
1.	PA	RTICLE BASED ASSAYS	41
	1.1.	Direct latex agglutination assays	41
1	1	.1.2. Light scatter immunoassay	42
	1	.1.3. Passive latex agglutination assay	42
	1	.1.4. Dry latex spot test	43
	1.	1.5. Magnetic latex assays	43
[1.2.	Sensitization of latex particles	44
L	1.	2.1. Adsorption	44
	1	.2.2. Covalent coupling of proteins on to surface of	
		microspheres	45
2.	ME	MBRANE BASED IMMUNOASSAY	48
	2.1.	Types of Membranes	48
	2.2.	Flow-Through Immunoassays and Lateral-Flow	
	j	mmunoassays	50
L	2.	2.1. Flow-Through Immunoassays	50
	2.	2.2. Lateral-Flow immunoassays	53
	2.3.	The Importance of Protein Binding	54
L	2.	3.1. Factors That Influence Protein Binding	56
		2.3.1.1. Capture Reagents	56

		Page	
	2.3.1.2. Ambient Humidity	56	
	2.3.1.3. Optimizing the Application Buffer	57	
2.4.	Optimizing Membrane Flow	59	
2.	4.1. Blocking Reagents	60	
2.	4.2. Blocking Methods	60	
2.5.	Conjugate pads	61	
2.6. Examples of different lateral flow devices actually			
formulated by developers 2.6.1 Unidirectional one-step lateral flow devices			
2.	6.1. Unidirectional one-step lateral flow devices	63	
	1. Enzyme Immunochromatography for		
	quantitative immunoassay.	63	
	2. A three-minute test strip for detection of urinary		
	trypsinogen-2 as a tool to detect Endoscopic		
	Retrograde Cholangio-Pancreatography induced		
	pancreatitis	65	
	3. Diagnosis of adenoviral conjunctivitis on		
	conjunctival swap by 10-minute		
	Immunochromatography	65	
.	4. Immunochromatographic strip for Indian		
	4. Immunochromatographic strip for Indian visceral leishmaniasis	66	
	Viscorar roisimilamasis	00	
	5. Simple Saliva-Based Test for Detecting		
	Antibodies to Human Immunodeficiency Virus	66	
	6. One step strip for the detection of sulfadimidine		
	residues	67	

		Page
	2.6.2. Bi-directional reversed lateral flow devices	68
	1. Immunochromatographic test for malaria diagnosis	68
	2. Detection of serum antibodies to Helicobacter pylori	69
	3. Immunochromatographic strip for diagnosis of Dengue virus	69
	PART-II MATERIALS AND METHODS	71
1.	CHEMICALS	71
	1.1. Chemicals Obtained From Sigma Inc., USA	71
	1.2. Chemicals Obtained From Aldrich Inc., USA	71
	1.3. Chemicals From Other Companies	71
	1.4. Biologicals, Special Chemicals And Polystyrene	
•	Beads	72
	1.5. Water	73
2.	PIPETTES	73
3.	INSTRAMENTS	74
	METHODS	75
1.	CRUDE ES PREPARATION	75
	1.1. Worm Collection	75
!	1.2. Lyophilization Of Worm Crude Antigens	76
2.	DETERMINATION OF PROTEIN CONTENT BY	
	BRADFORD COOMASSIE BRILLIANT BLUE	
	METHOD (Bradford, 1976)	76