



شبكة المعلومات الجامعية

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ





شبكة المعلومات الجامعية



# شبكة المعلومات الجامعية

## التوثيق الالكتروني والميكرو فيلم

# جامعة عين شمس

التوثيق الالكتروني والميكرو فيلم

## قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها  
علي هذه الأفلام قد اعدت دون أية تغيرات



## يجب أن

تحفظ هذه الأفلام بعيداً عن الغبار

في درجة حرارة من 15 – 20 مئوية ورطوبة نسبية من 20-40 %

To be kept away from dust in dry cool place of  
15 – 25c and relative humidity 20-40 %



شبكة المعلومات الجامعية



# بعض الوثائق الأصلية تالفة



شبكة المعلومات الجامعية



بالرسالة صفحات  
لم ترد بالأصل

**Biochemical Studies For Evaluation and Molecular Characterization  
of a New Polymerase Chain Reaction-Enzyme-Linked  
Immunosorbent Assay (PCR-ELISA) for Detection of *Wuchereria  
bancrofti* DNA**

Submitted to Cairo University, Faculty of Science

In partial fulfillment of the Requirements

For the master degree

Of Science

(Biological Chemistry)

By

**Marwa Adly Abdalla**

Bachelor of Chemistry

Department of Chemistry

Faculty of Science

Cairo University

2001

B

1-02V

## Approval Sheet For Submission

Title of M.Sc. Thesis

Biochemical Studies For Evaluation and Molecular Characterization of a  
New Polymerase Chain Reaction-Enzyme-Linked Immunosorbent Assay  
(PCR-ELISA) for Detection of *Wuchereria bancrofti* DNA

Name of Candidate

**Marwa Adly Abdalla**

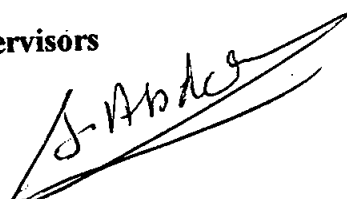
Submitted to the

Faculty of Science, Cairo University

This Thesis has been approved for submission by the supervisors

**1. Prof. Dr. Sadek El-Said Abdou**

Prof of Chemistry, Faculty of Science, Cairo University



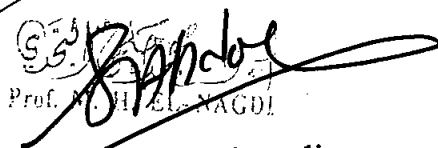
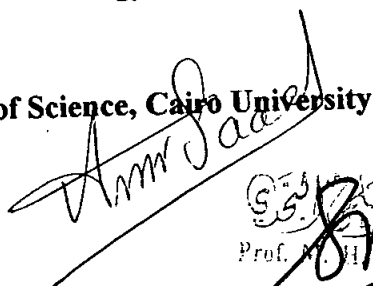
**2. Prof. Dr. Reda Rashad Ramzy**

Prof of immunology, and head of immunology Department, Nutrition Institute



**3. Dr. Amr Saad Mohamed**

Lecturer of Biochemistry, Faculty of Science, Cairo University



Prof. Dr. Mohammed Helmy Elnagdi

Prof Dr. Mohammed Helmy Elnagdi  
Chairman of Chemistry Department  
Faculty of Science, Cairo University

## **ABSTRACT**

**Name:** Marwa Adly Abdalla

**Title of M.Sc. Thesis:** Biochemical Studies For Evaluation and Molecular Characterization of a New Polymerase Chain Reaction-Enzyme-Linked Immunosorbent Assay (PCR-ELISA) for Detection of *Wuchereria bancrofti* DNA

**Degree:** Master of Science, Thesis, Faculty of Science, Cairo University (2001)

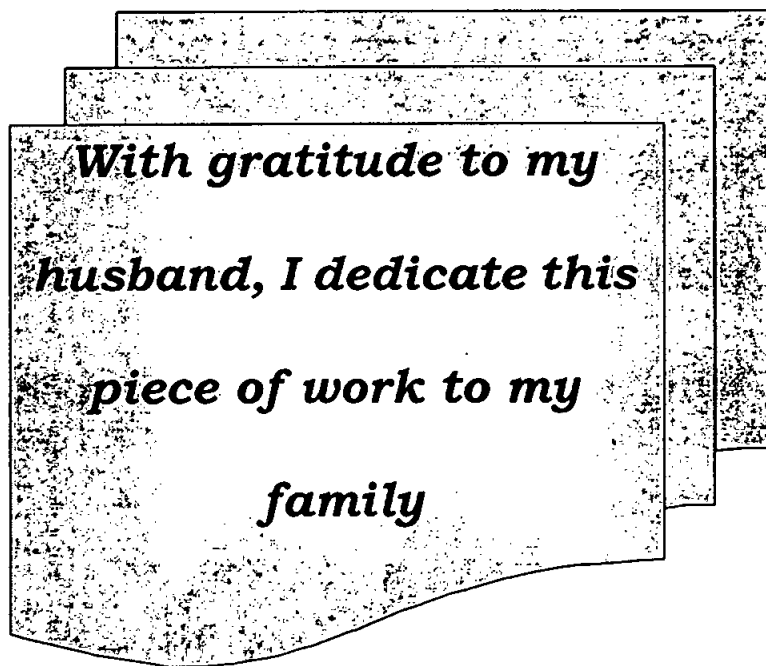
This work has been carried out to evaluate the performance, sensitivity and practical utility of the newly developed polymerase chain reaction-enzyme-linked immunosorbent assay (PCR-ELISA) in detecting *Wuchereria bancrofti* DNA in the mosquito vector in a low endemic village as a model for after treatment situation. The described assay that combines the conventional PCR with the use of an internal control and a very sensitive ELISA detection of PCR products can be successfully employed to screen large numbers of mosquitoes and calculate the infection rate of mosquitoes in low endemic areas. It was successfully employed to estimate the relative amount of *W. bancrofti* DNA present in wild caught mosquitoes providing semi-quantitative data. The method provides accurate description of the level of inhibition in mosquito pools and thus help preventing false negative results.

### **Key words**

Lymphatic filariasis, *Wuchereria bancrofti*, *Culex pipiens*, quantitative PCR, PCR-ELISA, internal control, false negatives.







## **Acknowledgement**

*I would like to express my deepest thanks and appreciation to my mentor Dr. Reda M. Rashad Ramzy, Professor of Immunology and Head of Immunology Unit, Nutrition Institute, for his invaluable scientific advice, non-ending support and precious time he kindly dedicated to this work.*

*I wish to extend my sincerest gratitude to Dr. Amr Saad Mohamed, Lecturer of Biochemistry, Faculty of Science, Cairo University for his encouragement and invaluable advice throughout thesis writing.*

*Thanks are also due to Dr. Sadek El- Said Abdou, Prof of Chemistry, Faculty of Science, Cairo University for his kind encouragement.*

*Warmest gratitude is due to Dr. Ibrahim Hassan Kamal, Assistant Professor of Biochemistry, Faculty of Science, Ain Shams University for his never-ending assistance and invaluable guidance.*

*Sincerest appreciation goes to Dr. Hanan Helmy, Researcher, Research and Training on Vectors of Diseases, Ain Shams University, for her non-ending moral support and invaluable advice.*

*My heartfelt thanks go to my colleagues at the Blue Lab, Research and Training Center on Vectors of Diseases, Ain Shams University, for their never-ending support.*

*I would like to sincerely thank my colleagues at the Research and Training Center on Vectors of Diseases, Ain Shams University, for their constant support and kind hospitality.*

*I would like to sincerely thank my colleagues of the filariasis field group of Research and Training Center on Vectors of Diseases, Ain Shams University, for the constant supply of biological materials.*

# Contents

	Page
<b>Abstract</b> .....	
<b>Acknowledgment</b> .....	
<b>List of figures</b> .....	
<b>List of tables</b> .....	
<b>I. Introduction</b> .....	1
<b>II. Review of literature</b> .....	7
© <b>A. The Parasite</b> .....	7
<b>B. Distribution</b> .....	8
<b>C. Clinical Manifestations</b> .....	9
1. Asymptomatic amicrofilaraemia.....	9
2. Asymptomatic microfilaraemia.....	10
3. Acute manifestations.....	10
4. Chronic manifestations.....	11
<b>D. Transmission of the Disease</b> .....	11
<b>E. Diagnosis</b> .....	13
1. Parasitological methods.....	13
2. Lymphangiography and Lymphosintigraphy.....	13
3. Immunological assays.....	14
a. Antigen detection assay.....	14
b. Filariasis card test.....	14

c. Antibody detection assay.....	15
4. Polymerase chain reaction (PCR).....	16
<b>F. Detection of <i>W. bancrofti</i> larvae in the mosquito vectors.....</b>	<b>17</b>
<b>1. Traditional dissection techniques.....</b>	<b>17</b>
<b>2. Nucleic acid probes.....</b>	<b>18</b>
<b>3. Polymerase chain reaction (PCR).....</b>	<b>19</b>
a. PCR Components.....	20
a.1. Single stranded DNA (Template).....	20
a.2. <i>Taq</i> DNA polymerase and reaction buffer.....	21
a.3. Deoxyribonucleoside triphosphates (dNTPs).....	22
a.4. Primers.....	23
b. PCR Technical Steps.....	24
1. Denaturation.....	24
2. Annealing.....	25
3. Extension .....	25
Cycles number.....	25
c. General aspects concerning PCR.....	26
c.1. Primer-dimer artifacts.....	26
c.2. False positive results.....	26
c.3. False negative results.....	27
d. Detection of PCR product.....	29
d.1. Direct visualization using gel electrophoresis.....	29
d.2. Detection using DNA probes.....	29

<b>4.Quantitative PCR</b> .....	30
a. Limiting dilution.....	30
b. External standards.....	31
c. Endogenous standards.....	32
d. Competitive PCR.....	33
a. The internal standard.....	34
b. Detection and analysis of PCR products.....	37
<b>III. Materials and methods</b> .....	39
<b>A. Biological Materials</b> .....	39
<b>1. Laboratory studies</b> .....	39
<b>2. Field studies</b> .....	39
a. Study area.....	39
b. Mosquito collection.....	40
<b>B. Laboratory Materials</b> .....	40
1. Mosquito DNA extraction buffers.....	40
2. Electrophoresis buffers.....	42
3. Wb-QC-PCR-ELISA buffers.....	43
<b>C. Samples assessment</b> .....	45
<b>1. Mosquito DNA extraction</b> .....	45
a. Preparation of mosquitoes.....	45
b. Alkaline lysis of crushed mosquitoes.....	46
c. DNA binding and washing of the pellet.....	46
d. Elution of DNA.....	46
<b>2. Polymerase chain reaction (PCR)</b> .....	47