

سامية محمد مصطفى



شبكة المعلومات الجامعية

بسم الله الرحمن الرحيم



سامية محمد مصطفى



شبكة المعلومات الجامعية



شبكة المعلومات الجامعية التوثيق الالكتروني والميكرو فيلم



سامية محمد مصطفى



شبكة المعلومات الجامعية

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

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



يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار



سامية محمد مصطفى



شبكة المعلومات الجامعية



بعض الوثائق الأصلية تالفة



سامية محمد مصطفى



شبكة المعلومات الجامعية



بالرسالة صفحات لم ترد بالأصل



Assessment of Two Quantitative Techniques for Hepatitis C Viraemia

A Thesis Submitted by

Ashraf Magdy Nassif

For fulfillment of

The Master Degree of Science in Biochemistry

Under the Supervision of

Zeinab

Dr. Zeinab Zaki El-Dardiri

Professor of Biochemistry

Biochemistry Department

Faculty of Science

Ain Shams University

Dr. Nadia Y. S. Morcos

Ass. Professor of Biochemistry

Biochemistry Department

Faculty of Science

Ain Shams University

Nadia

Dr. Hatem EL Sebai

Lecturer of Biochemistry

Biochemistry Department

Faculty of Medicine

Monufiya University

Dr. Hatem EL Sebai

Department of Biochemistry

Faculty of Science

Ain Shams University

2001

B

11010

Assessment of Two Quantitative Techniques for Hepatitis C Viraemia

A Thesis Submitted by

Ashraf Magdy Nassif

For fulfillment of

The Master Degree of Science in Biochemistry

Under the Supervision of

Dr. Zeinab Zaki El-Dardiri

Professor of Biochemistry

Biochemistry Department

Faculty of Science

Ain Shams University

Dr. Nadia Y. S. Morcos

Ass. Professor of Biochemistry

Biochemistry Department

Faculty of Science

Ain Shams University

Dr. Hatem EL Sebai

Lecturer of Biochemistry

Biochemistry Department

Faculty of Medicine

Monufiya University

Department of Biochemistry

Faculty of Science

Ain Shams University

2001

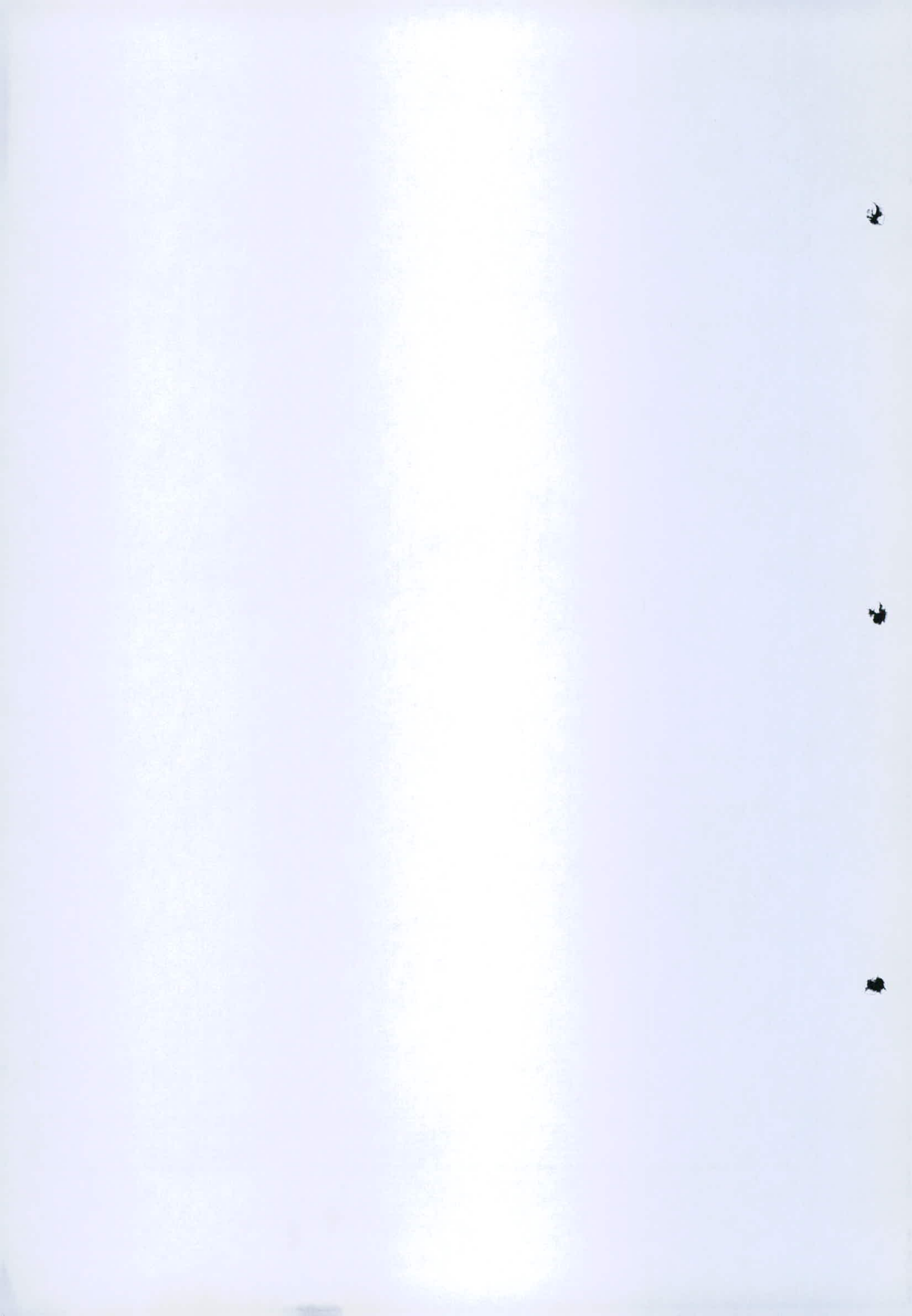
[illegible]

[illegible]

[illegible]

[illegible]

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



ACKNOWLEDGEMENT

I wish to express my deepest respect and thanks to Prof. Dr. Zeinab Zaki El Dardiri, Professor of Biochemistry, Faculty of Science, Ain Shams University, for her help, kindness, continues guidance, supervision and her motherly attitude.

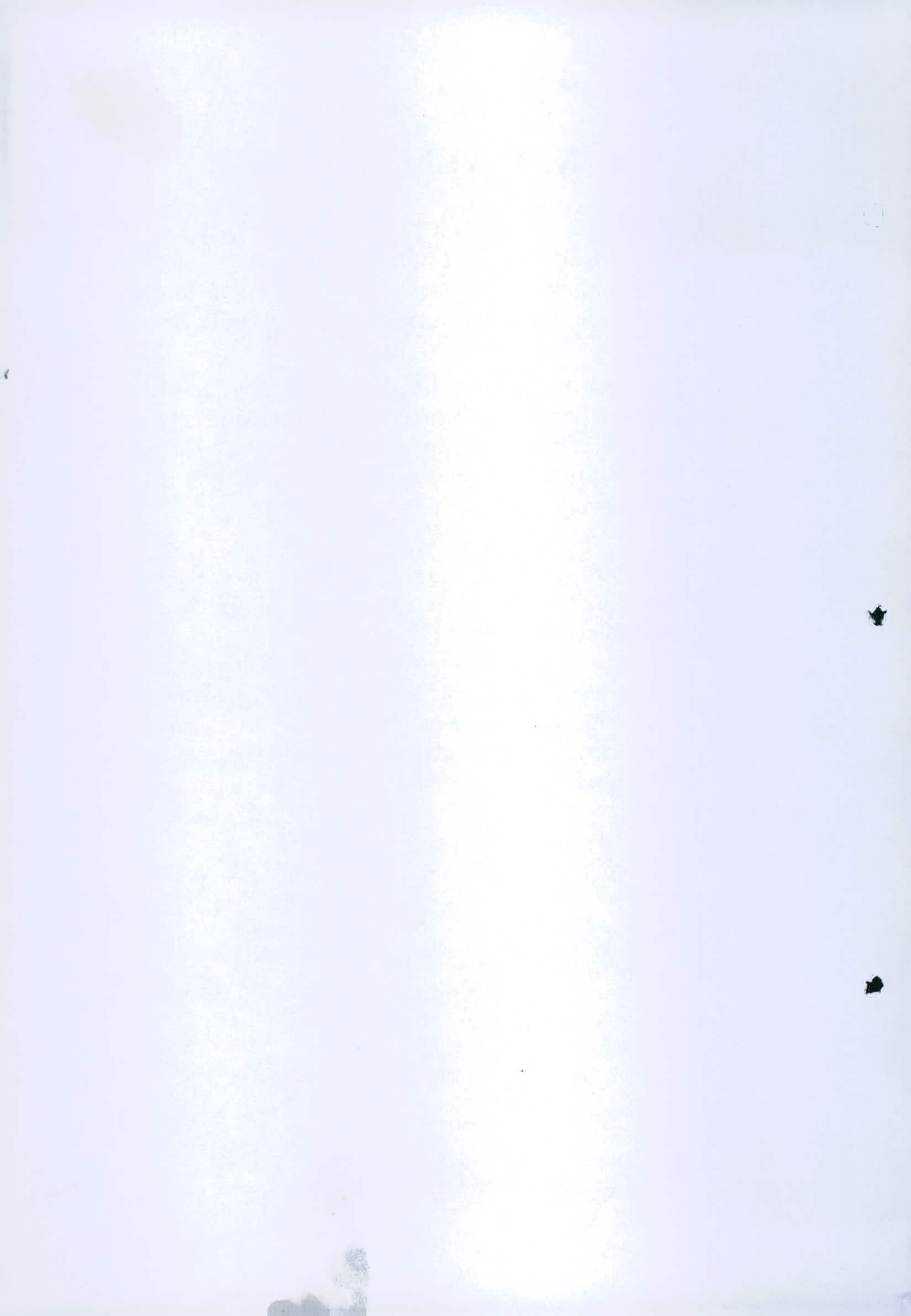
I would like to express my thanks to Dr. Nadia Y.S. Morcos, Ass. Professor Faculty of Science, Ain Shams University, I am grateful for her helpful, kind support and constant advice during this work. It is difficult for me to find the appropriate words that would do her favors.

I was fortune to carry out this work under the guidance of Dr. Hatem Mahmoud Sami El-Sebai Lecturer of Biochemistry, Faculty of Medicine Monufiya University.

Last but not least, my thanks and best regards to the staff members of Biochemistry Department Faculty of Science, Ain Shams University, to my professors and colleagues, for their cooperation and giving me the chance to learn in the department.

Thanks

Ashraf Magdy Nassif



CONTENTS

Abstract	X
Aim of work	XI
I- INTRODUCTION	
Background	1
Hepatotropic Viruses	4
HCV	9
A. Historical view for Hepatitis C virus	9
B. Mode of Transmission	10
i) Percutaneous transmission	11
ii) Sporadic transmission	13
iii) Interfamilial transmission	13
iv) Sexual transmission	14
v) Perinatal transmission	14
vi) Patient to patient transmission	15
C. Genomic organization of HCV	16
• The different regions of the HCV genome	19
i) 5' UTR translation and replication	19
ii) The core region	20
iii) The envelope region E1,E2,NS1	21
iv) The NS2 and NS3 proteins	22
v) The NS4	23
vi) Internal ribosomal entry site (IRES)	24
vii) The 3' UTR	25
D. Sequence variation and genotyping	26
E. Epidemiology of Hepatitis C Virus	30
i) Hepatitis C Seroprevalence	30
ii) Hepatitis C in Egypt	31
1. Diagnostic Tests for Hepatitis C Virus	35
A. Screening assays for anti-HCV	35
B. Supplemental tests for anti-HCV	38
C. Qualitative tests for HCV RNA	38
D. Quantitative tests for HCV RNA	40
2. Viral Load Assays: Methodologies, Variables, And Interpretation	42
A. Viral load	43
B. Analyte compartment and technology	43
C. Variables that affect assay performance	44

1) Preanalytical variables	44
i) Sample processing	44
ii) Effects of vaccination and concurrent microbial infection	45
iii) Biologic and diurnal variation	45
2) Analytical Variables	46
• Different technologies	46
i) Branched DNA signal amplification	46
ii) RT-PCR	48
iii) QC-PCR	48
iv) Nucleic acid sequence based amplification	49
3) Key Assay performance issue	50
i) Linearity	50
ii) Accuracy	51
iii) Precision	51
iv) Tolerance limit	52
v) Sensitivity	52
vi) Specificity	53
vii) Subtype genetic variation	54
viii) Methodology strength and challenges	54
4) Post Analytical Variables	55
i) Affecting assay result output variability	55
ii) Interpretation	56
3. Treatment	57
II- SUBJECTS AND METHODS	62
1. Detection of Anti-HCV Ab by ELISA	63
2. Qualitative Polymerase Chain Reaction (PCR)	65
3. Quantitative Polymerase Chain Reaction (PCR)	66
4. Quantitative Branched DNA (bDNA)	79
5. Determination of Serum enzyme levels	86
A. Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST)	86
B. Alkaline Phosphatase Activity (ALP)	88
C. Gamma Glutamyl Transpeptidase (GGT)	89
• Statistical Analysis	89