سامية محمد مصطفى

شبكة المعلومات الجامعية

بسم الله الرحمن الرحيم

سامية محمد مصطفى

شبكة المعلومات الجامعية

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التوثيق الالكتروني والميكروفيلم

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سامية محمد مصطفى

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

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بالرسالة صفحات لم ترد بالأصل

EVALUATION OF DNA PROBING & PCR TECHNIQUE IN IDENTIFICATION OF SOME POSSIBLY ISOLATED VIRUSES

THESIS

Submitted in partial fulfillment
For The (M.D.)Degree In
Clinical &Chemical Pathology

BY

AMIRA ABD EL-KADER KELANY EL-HENDY
M.B.,B.Ch
M.Sc. Clinical &Chemicl Pathology

Supervisors

PROF. DR. IBRAHIM KHALIL ALY
Prof. of Clinical &Chemical Pathology,
Faculty of MedicineAin Shams University

PROF. DR. SAMIA HASSAN KANDEIL
Prof. and head of the Department of
Clinical Pathology Faculty of Medicine
Menoufiya University

PROF .DR. SAMIRA ABD EL-BAKY SHOEB
Prof. of Microbiology and Immunology Faculty of Medicine
Cairo University

ASSIS. PROF. DR. KHALIL EL-HALAFAWY
Ass. Prof. of Genetics Faculty of Agriculture
Menoufiya University

Faculty of Medicine
EL-MENOUFIYA UNIVERSITY

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TO MY MOTHER AND FATHER
TO MY LOVELY KIDS

HADIL
& AHMED

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CONTENTS

GLOSSARY

Base pair (bp) : A pair of complementary bases in DNA (A with T G,with C).

cDNA : Single - Stranded DNA complementary to m RNA.

DNA : (deoxyribonucleic acid).

Gene : A part of the DNA molecule which directs the synthesis of a specific polypeptide chain. It is composed of many codons : When the gene is considered as a unit of function in this way, the term cistron is often used.

Genomic DNA : DNA sequences in the chromosome.

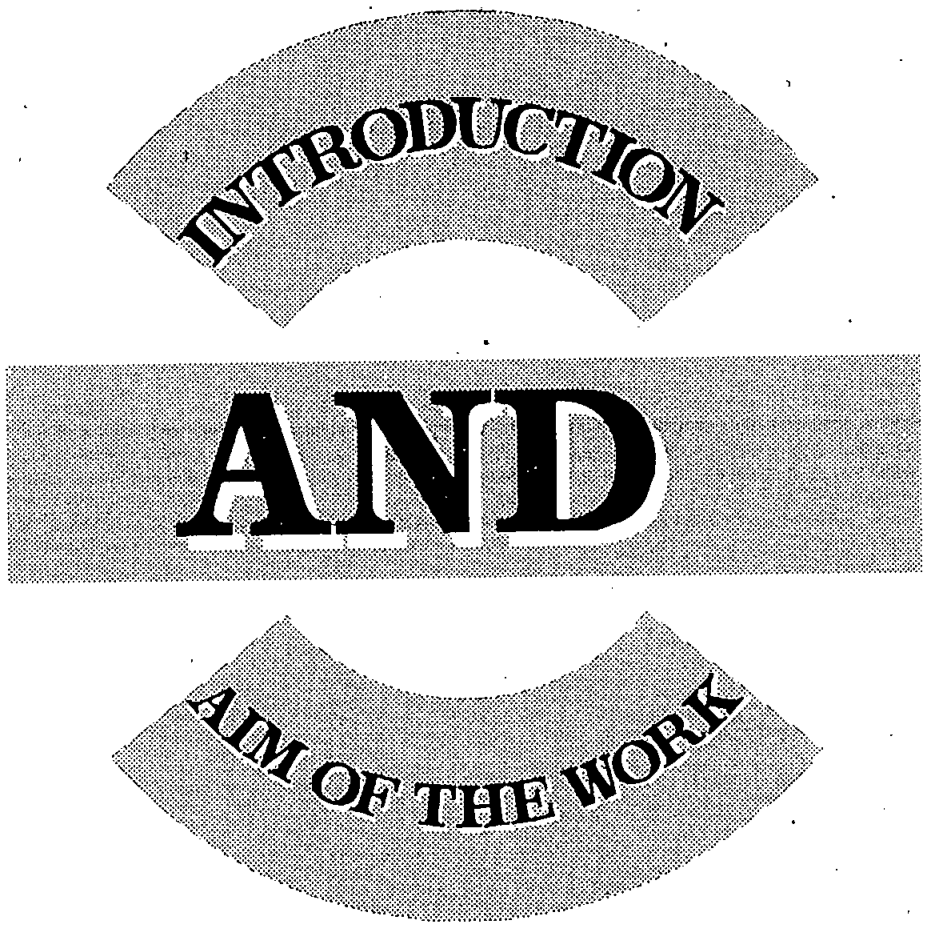
mRNA :Messenger - RNA transfers genetic information from the nucleus to the ribosomes in the cytoplasm and acts as a template for the synthesis of polypeptides.

Nucleotide : Nucleic acid is made up of many nucleotides, each of which consists of a nitrogenous base, a pentose sugar and a phosphate group.

Oligonucleotide : A chain of Literally few nucleotides.

Probe : A labeled, single - stranded DNA fragment which hybridizes with and there by detects and Locates, complementary sequences among DNA fragments.

X - Linkage : Genes carried on the X chromosome.


INTRODUCTION
AND
AIM OF THE WORK

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Hepatitis B virus (HBV) infection can cause either acute or chronic hepatitis. In this respect, HBV is unusual :infection is associated with the production of large amounts of viral antigen that is readily detected in the serum. However, not all of the HBsAg in serum represents intact virions. Indeed, the majority of this antigen consists of HBsAg particles produced in great excess of complete HBV particles. Direct detection of HBV is difficult and is hampered by the lack of a simple tissue culture system for growing the virus. Neverthless, evidence for the presence of intact HBV can be demonstrated in some HBsAg - positive sera by molecular hybridization using recombinant radiolabeled HBV DNA probes. ***(Kaneko et al., 1990).***

Modern hybridization assays for HBV DNA are quite sensitive, detecting as little as 0.1 pg or 3×10^4 virus particles per serum sample ***(Berninger et al., 1982).*** Obviously, sera from patients with low titers of HBV in serum could have negative test results for HBV DNA. Indeed, not all HBsAg positive sera contain HBV DNA that is detectable by dot hybridization. For instance, during the course of acute hepatitis B, HBsAg appears in the Serum shortly before HBV DNA is detectable, and antigen persists for weeks to months after viral DNA is cleared. During chronic active hepatitis B, large amounts of HBV DNA are usually found early in the disease but viral DNA may no longer be detectable late in the course of illness. Hepatitis B virus DNA is usually not detected in patients who are "healthy carriers, i.e., patients who have HBsAg but not hepatitis Be antigen (HBeAg) in serum and no evidence of ongoing liver disease. The finding of HBsAg in the absence of detectable HBV DNA raises the questions whether there are no virions present or whether their presence is

merely below the levels measurable by current methodology. ***(Saiki et al., 1988).***

Recently a new method for detecting small amounts of DNA has been developed ; it relies on amplification of DNA using a heat stable polymerase. This method, called the polymerase chain reaction (PCR), has been used to detect extremely small quantities (i.e., attograms) of HBV DNA in serum when the sample is prepared by phenol/chloroform extraction and when the DNA is analyzed by electrophoresis and southern- blot hybridization ***(Kaneko et al., 1989 b).***

AIM OF THE WORK

The present work aims to evaluate PCR technique in diagnosis of hepatitis B virus infection and to compare the resuts with liver function tests as determined by serum albumin ,enzymatic levels as well as other hepatitis B markers includingHBVs antigens and HBV e antigens