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Detection of Hepatitis C Virus genotype-4 using nucleic acid aptamers

A thesis
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To whom I owed my deepest graditude

My family

My father & mother

My brothers & sisters

My wife

My lovely daughter

"Arwa"

&

My sincere friends

Acknowledgment

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Abstract

Egypt has the highest prevalence of Hepatitis C Virus (HCV) infection with 92.5% of patients infected with genotype-4. This study aimed to clone and express the Core gene from HCV genotype-4 and used the purified proteins to develop a highly sensitive, specific and cost-effective diagnostic assay for detection of HCV infection. Using synthetic HCV genotype-4 Core gene and pET15b as *E. coli* expression vector, the HCV Core protein (MW 17 kDa) was expressed in the form of inclusion bodies (IBs). The expressed protein was purified and refolded *in vitro* using rapid dilution method. The recombinant Core protein was purified successfully using weak cation exchange liquid chromatography. The immunogenicity of the purified protein was tested using 129 archived serum samples by ELISA and it was also used as a reference standard for detection of HCV Core antigen using the HCV Core aptamer colorimetric assay. Results showed that, the HCV Core aptamer assay had 100% sensitivity and specificity where as the in-house anti-HCV Core assay had ~98.55% sensitivity and ~98.33% specificity as judged by qRT-PCR. In conclusion, the sensitivity, specificity and correlation of

the developed HCV Core aptamer colorimetric assay to qRT-PCR are higher than those for the commercially available ELISA assays, and can be used as a screening and quantification assay for detecting HCV infection.

Keywords: HCV, Core protein, inclusion bodies, refolding, ELISA, aptamer.

List of abbreviations

Abbreviated name	Full name
aa	Amino acid
Abs	Antibodies
Ags	Antigens
AuNPs	Gold nanoparticles
ALT	Alanine aminotransferase
CDC	Centers for Disease Control and Prevention
cDNA	Complementary DNA
CHC	Chronic hepatitis C
CIA	Chemiluminescence immunoassays
DAAs	Direct-acting antivirals
DTT	Dithiothreitol
EDAC	1-Ethyl-3-(3-dimethyl-aminopropyl) carbodiimide
EDTA	Ethylenediamine tetraacetic acid
E1	HCV envelop1
E2	HCV envelop2
ELISA	Enzyme Linked Immunosorbent assay

ELONA	Enzyme linked oligonucleotides assay
ER	Endoplasmic reticlume
F	Forward
FRET	Fluorescence resonance energy transfer
G	Genotype
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus
HCV coreAg	HCV Core antigen
HIV	Human Immunodeficiency Virus
HRP	Horse raddish peroxidase
HTAs	Host-targeted agents
IBs	Inclusion bodies
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
IDUs	Intravenous drug users
IEC	Ion exchange chromatography
IFN	Interferon
IMAC	Immobilized metal ion affinity chromatography
IPTG	Isopropyl β-D-1-thiogalactopyranoside

lac	lactose
LOD	Limit of detection
MCS	Multiple cloning sites
MNPs	Magnetic nanoparticles
MWCO	Molecular weight cut-off
NANBH	The non-A, non- B Hepatitis
NATs	Nucleic acid amplification techniques
NCR	Noncoding region
NHS	N-hydroxysuccinimide
Ni-NTA agarose	Nickel-nitrilotriacetic acid coupled to a cross- linked agarose resin
NS1-NS5	Nonstructure protein (1-5)
nts	Nucleotides
OD	Optical density
OPD	O-phenylenediamine dihydrochloride
Ori	Origin of replication
ORF	Open reading frame
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
PDA	Polydiacetylene
PDGF	Platelet derived growth factor