

# **Development of Immunodiagnostic Reagents for Hepatitis C**

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## **Abstract**

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Serologic tests are essential for the management of hepatitis C virus (HCV) infection in order to diagnose infection, select treatment and assess virologic response. As part of a study to construct an epitope map for type 4a HCV to aid in the development of improved immunodiagnostic reagents, we have selected four peptides of about 200 amino acids for expression and evaluation of immunodiagnostic potential. These peptides are derived from the non-structural (NS) regions, (NS2, NS3, NS4 and NS5) of the HCV polyprotein. The selected peptides were expressed as GST-fusions, and their immunoreactivity towards sera from patients infected with HCV-4a was assessed using Western and Dot blots as well as ELISA. The reactivity of the expressed peptides was compared with a commercially available ELISA kit. The four peptide antigens were well recognized by HCV-4a infected sera, but not by normal sera. Furthermore, three of the four peptides were more sensitive in detecting antibodies in patient sera than the commercial ELISA kit. The results described demonstrate the presence of epitopes on the selected peptides which are potentially useful for the development of improved immunodiagnostic reagents for HCV type 4.

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*This thesis has not been submitted  
for publication at this or any other  
university*

***Miral Mahmoud Refeat***

# 1. Introduction

## 1.1. Hepatitis C virus (HCV)

Viral hepatitis is an inflammation of the liver caused by at least six different, mostly unrelated hepatitis viruses (HAV, HBV, HCV, HDV, HEV and HGV). The hepatitis C virus (HCV) was identified in **1989** by **Choo *et al.*** as the major cause of most cases of non-A, non-B hepatitis (NANABH) (**Patel *et al.*, 2006**). Viral hepatitis causes acute and chronic liver infections (**Blumberg, 1997 and O'shea, 2004**). HCV belongs to the Flaviviridae family and is the only member of the Hepacivirus genus (**Choo *et al.*, 1989**).

## 1.2. Genotypes of Hepatitis C

One of the feature of HCV replication is the rapid generation of virus variants. In fact, based on the genomic variability in a small region of NS5B, HCV has been classified into at least six genotypes each with several subtypes (**Simmonds *et al.*, 1993**). Even within a patient HCV does not exist as a single entity but rather as a swarm of microvariants of a predominant 'master sequence', a phenomenon that has been referred to as quasispecies (**Holland *et al.*, 1992**).

The production of such a large number of variants is primarily due to the high error rate of the viral RdRp (**Ogata *et al.*, 1991; Okamoto *et al.*, 1992**). The high variation observed with HCV replication may also account for the fact that a significant fraction of virus genomes appear to be defective (**Martell *et al.*, 1992**).

Traditionally, viruses have been classified according to antigenic characteristics, but with recent advances in molecular biology, genotypic classification through the analysis of genomic variation is now possible. Variations in the HCV genome fall into a series of specific patterns that have been classified into genotypes (**Mahaney et al., 1994**). Among the different HCV genotypes, the sequence of the 5' non-coding (NC) region is relatively conserved and is most often applied for diagnosis of HCV infection by PCR. In contrast sequences of NS3/NS5 and the core regions are more variable and are therefore often used to define and distinguish them from each other (**Qui et al., 1994**). It has been reported that HCV is classified into 11 main genotypes, each of which is further divided into many subtypes. The prevalence of the different HCV genotypes is shown in Table 2 (**Michael,1999**).

**Table1: Areas of high prevalence of different HCV genotypes.**

<b>Genotype</b>	<b>Subtype</b>	<b>Area of high prevalence</b>
1	a,b,c	Central Africa, Europe, North America
2	a,b,c,k	Western Africa
3	a,b,k	Southeast Asia
4	a	Central Africa, Middle East
5	a	Central Africa
6	a,b,d,g,h,k	Southeast Asia

Genotype 4 is the principal genotype among prevalent in the Middle East and North Africa particularly Egypt where it is associated with a high population prevalence. It is also prevalent in central Africa, and is not frequently detected in populations outside these areas.

Isolate ED43 for HCV type 4a has an E2 protein smaller than in other genotypes. The amino terminus of this E2 forms the hypervariable region. Also, (**Chamberlain et al., 1997**) has been reported that the NS5A protein in isolate ED43 of genotype 4a is the smallest of all the HCV isolates with apparent deletions at several places in the protein, including a four amino acid deletion within the predicted interferon-sensitivity determining region (amino acid residues 2214-2250).

### **1.3. Prevalence**

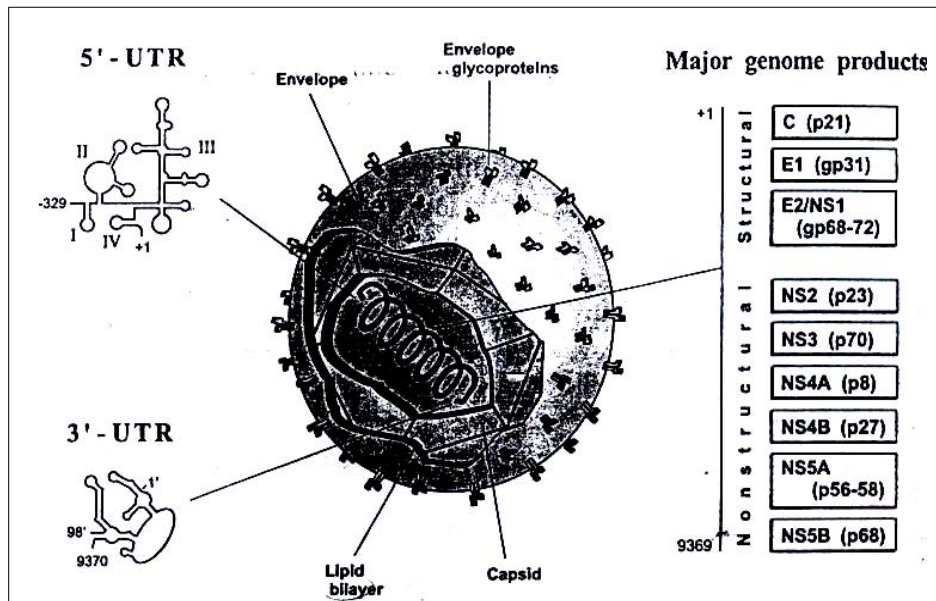
In 2000, the World Health Organization (WHO) declared hepatitis C a global health problem, with approximately 3% of the world's population (roughly 170-200 million people) infected with HCV (**Mezban and Wakil, 2006**).

Egypt has the highest prevalence of hepatitis C in the world. The national prevalence rate of HCV antibody positivity has been estimated to be between 10-13% (**Mohamed, 2004**). Since 30-40% of individuals clear the infection shortly after exposure (based on national studies and village studies in Egypt), the estimated adjusted national prevalence rate of chronic hepatitis C infection is 7.8% or 5.3 million people in 2004 (**Mohamed, 2004**).

### **1.4. HCV viral particle**

It is a spherical particle, 55-65 nm in diameter and possesses a lipoprotein envelope with small delicate spike glycoprotein projections (approx. 6 nm), which surrounds a 30-35 nm nucleocapsid (core particle) (**Takahashi et al., 1992 and Ishida et al., 2006**).

Molecular anatomy of HCV virion is described in **Figure 1**



**Figure 1: Molecular anatomy of the HCV virion.**

The predicted secondary structure models of the 5' and 3' UTR and the genomic products from structural and nonstructural domains of HCV genome are represented. Roman numbers in the 5' UTR indicate the structural domains, nucleotides 1' to 98' in the 3' UTR indicate the highly conserved terminal segment. The coding genes and the corresponding products are boxed (numbers within parenthesis indicate the size of each protein or glycoprotein). Nucleotide positions correspond to the prototype strain HCV-J of subtype 1b (Kato *et al.*, 1993 and Specter, 1999).

## 1.5.HCV genome and proteins

The HCV genome consists of a 9.6-kb positive-strand RNA that comprises a long open reading frame flanked by 5' and 3' noncoding regions (NCR) also named 5' untranslated region (UTR) or 5' non-translated region (NTR). The 5' NCR is highly conserved among different HCV isolates and contains an IRES. **(Tellinghuisen & Rice, 2002 and Brass *et al.*, 2006)**. Both 5' and 3' NCR have highly conserved RNA structures essential for polyprotein translation and genome replication **(Penin *et al.*, 2004)**.

Translation of the HCV genome leads to a polyprotein precursor that is co- and post-translationally processed by cellular and viral proteases to produce the mature structural and nonstructural proteins **(Rice, 1996; Bartenschlager, 1997 and Luo *et al.*, 2000)**. The N-terminal one-third of the polyprotein harbors the structural proteins core, E1 and E2 that form the viral particle. The structural region is followed by the p7 polypeptide which may be involved in ion channel formation (see below).

The nonstructural proteins 2-5B coordinate viral replication by the formation of a membrane-bound replication complex. Processing of the polyprotein at the core/E1, E1/E2, E2/p7, and p7/NS2 sites by the host cell signal peptidase produces all structural proteins and p7. Two viral proteases are responsible for the maturation of the nonstructural proteins. The NS2-3 autoprotease cleaves at the NS2/NS3 junction while all downstream sites are processed by the NS3-4A serine protease **(Tellinghuisen & Rice, 2002 and Brass *et al.*, 2006)**.