# THE IMPACT OF HCV DIVERSITY ON DIAGNOSIS TOOLS FOR HCV INFECTION
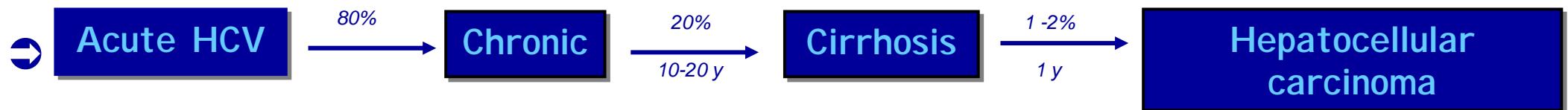
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HCV COLLABORATIVE TEAM OF RIIPIA

HCV-INFECTION IS A MAJOR HEALTH PROBLEM WORLDWIDE

⮞ HCV is a hepatotropic RNA virus and it is a major causative agent of human liver disease

⮞ | Acute HCV | →80%→ | Chronic | →20%, 10-20 y→ | Cirrhosis | →1-2%, 1 y→ | Hepatocellular carcinoma |
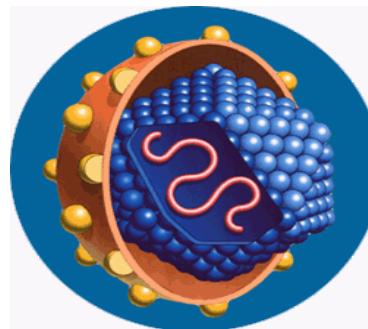
⮞ HCV infects over 170 million people worldwide and causes 476.000 deaths per year

⮞ Blood borne virus transmitted mainly parenterally

⮞ Current therapies for HCV infection (combination of pegylated IFN-α and ribavirin) show only limited efficacy and the development of a vaccine remains a major challenge
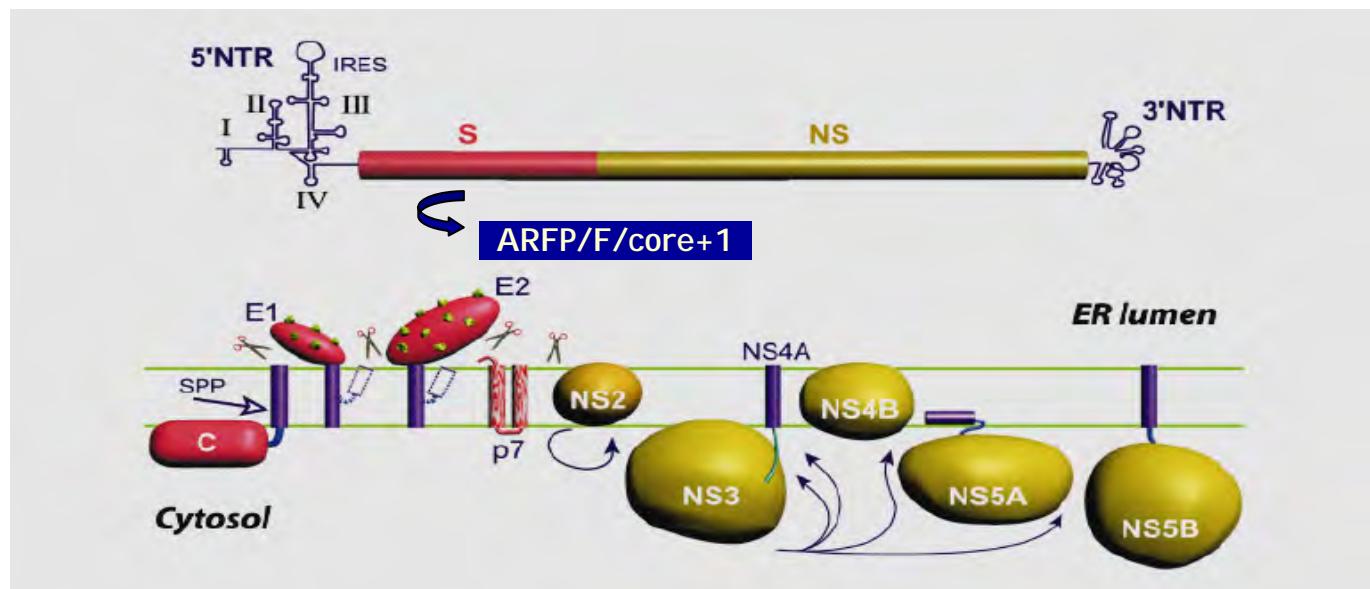
HCV: THE VIRUS

HCV is a small enveloped RNA virus that is classified within the flaviviridae family
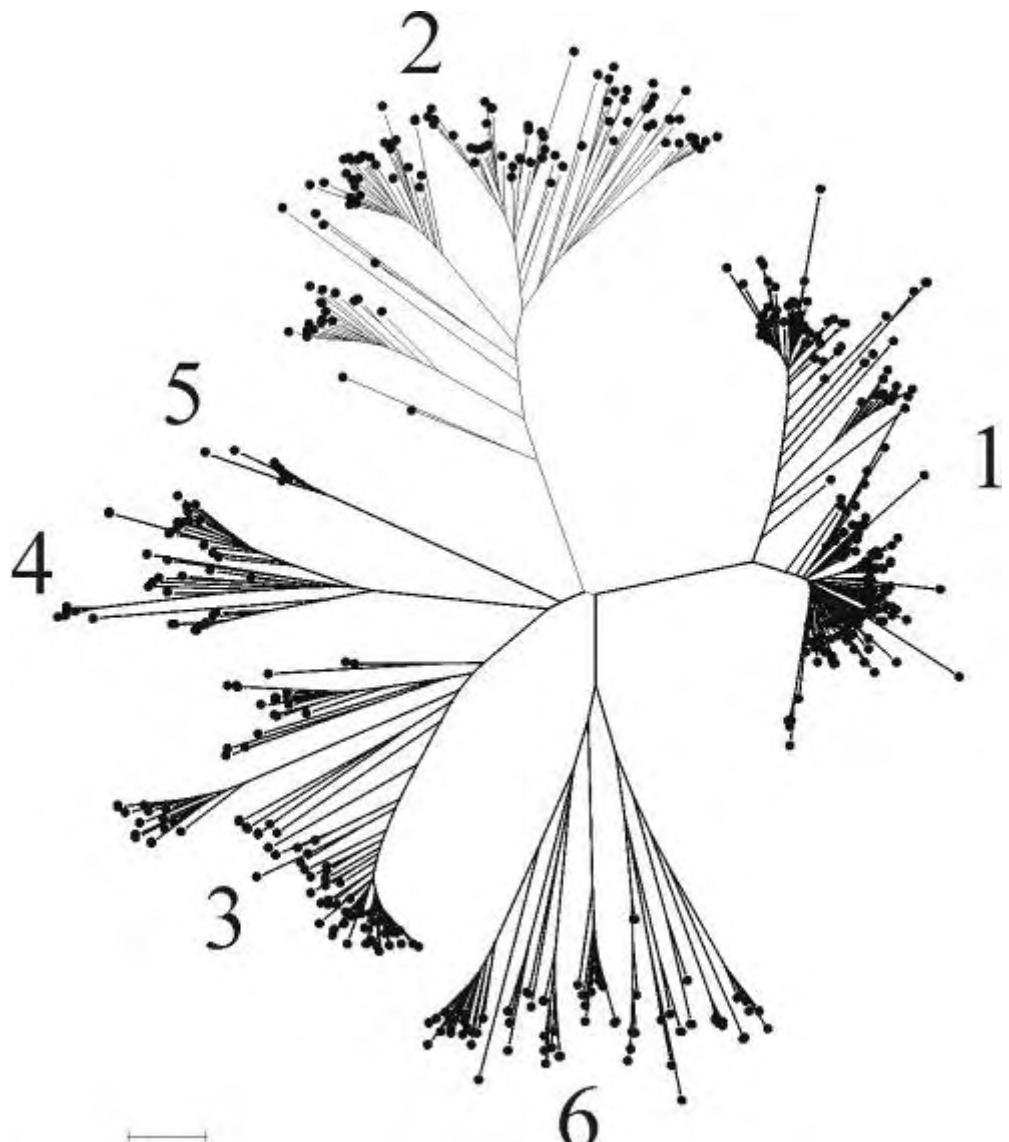
Exceptional degree of genetic heterogeneity

High propensity to establish chronic infection

HCV GENETIC VARIABILITY
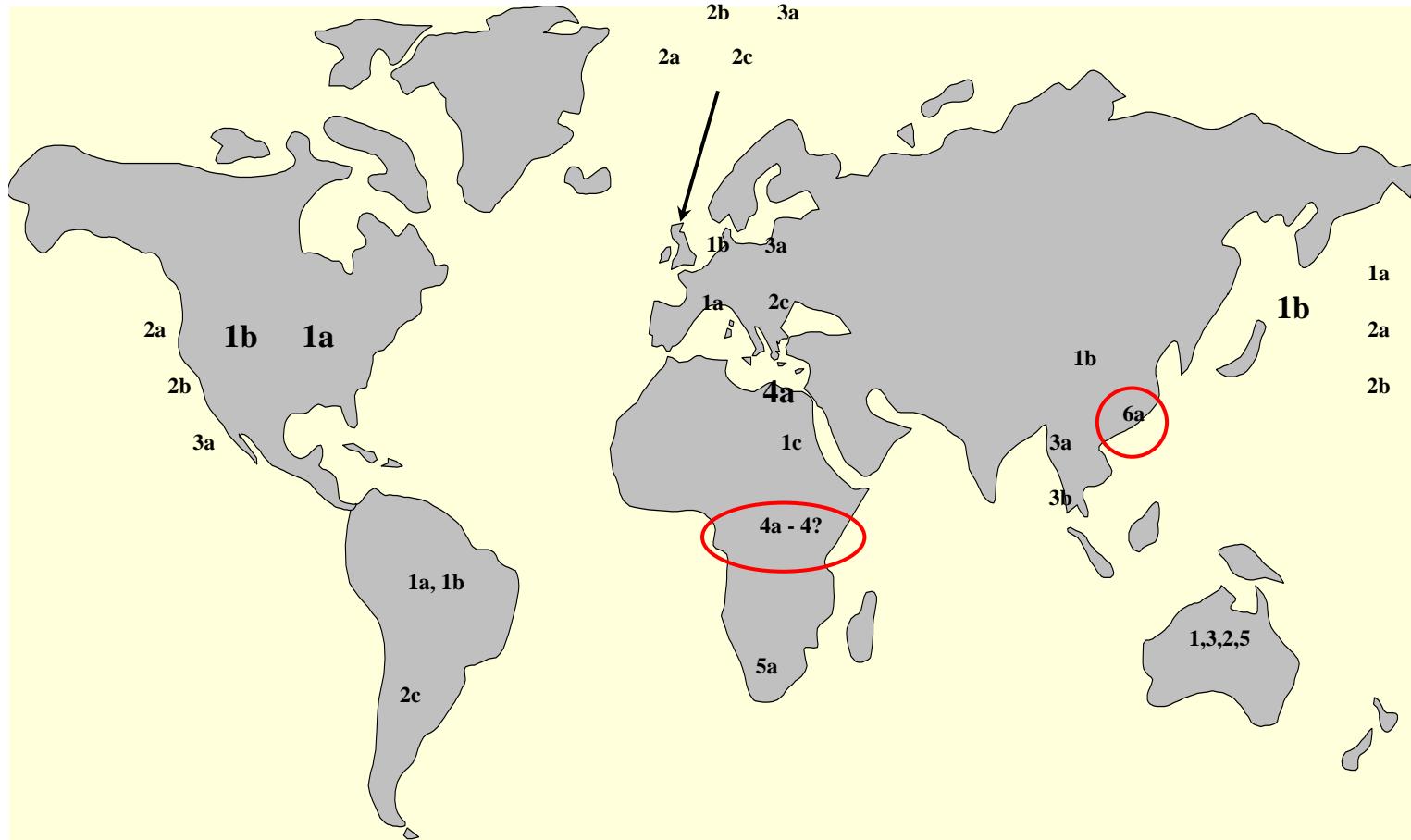
Evolutionary tree of known HCV genotypes/subtypes

- HCV can be classified into six genetically distinct genotypes/clades (Seq.divergence ~30%)
- HCV genotypes can be further subdivided into at least (90 subtypes Seq. divergence ~20%)
- Different HCV genotypes may exhibit differing phenotypic properties

  ▸ Severity of liver disease

  ▸ Response to antiviral therapy

  ▸ Antigenic heterogeneity/Impact in diagnostic assays and vaccine development

- Functional properties of viral proteins.

WORLDWIDE DISTRIBUTION OF HCV GENOTYPES

GOALS

To assess the impact of the HCV genotypes (particularly HCV-4 and HCV-6) on the detection of anti-HCV antibodies in human sera

EXPERIMENTAL OBJECTIVES

➲ To produce home-made HCV antigens for core, NS3 and NS4B from HCV isolates of selected HCV genotypes

● Peptides   ● Recombinant proteins

➲To generate a sera panel

➲To develop home-made ELISA assays and assess the antibody reactivity in HCV patient sera using type-homologous and type-heterologous antigens

CAMBODIAN PANEL: 90 plasma from Cambodian blood donors

58-Positive [RNA HCV (+) and Ab HCV (+)]

28 HCV-6

9 HCV-1a

20 HCV-1b

1 HCV-2

3 False Negative [RNA HCV (+) but Ab HCV (-)]
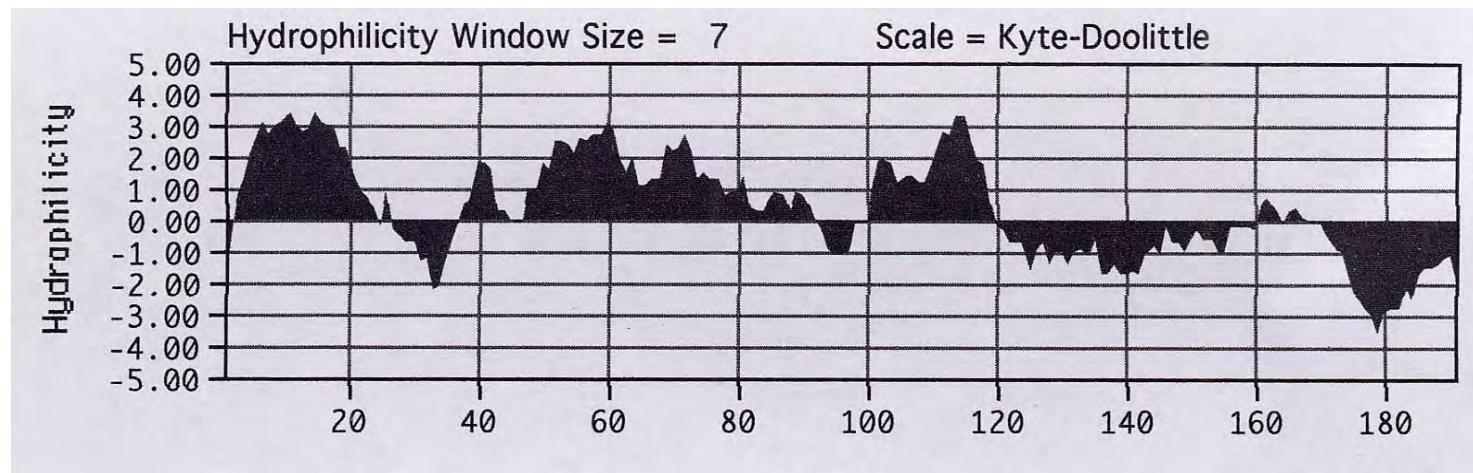
29 HCV-Negatives [RNA HCV (-) and Ab HCV (-)]

2 Controls (-)

1 HBsAg (+) HCV (-)

CORE PEPTIDE ANTIGENS
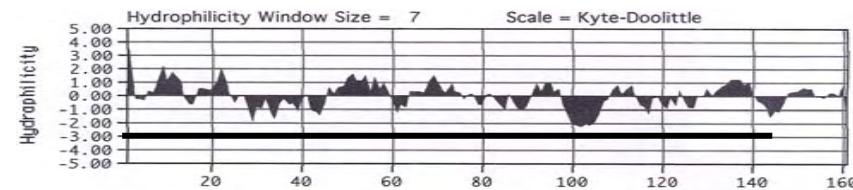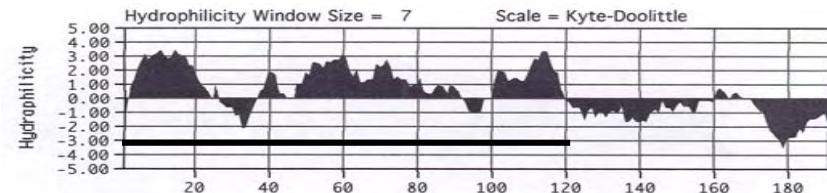
Peptides
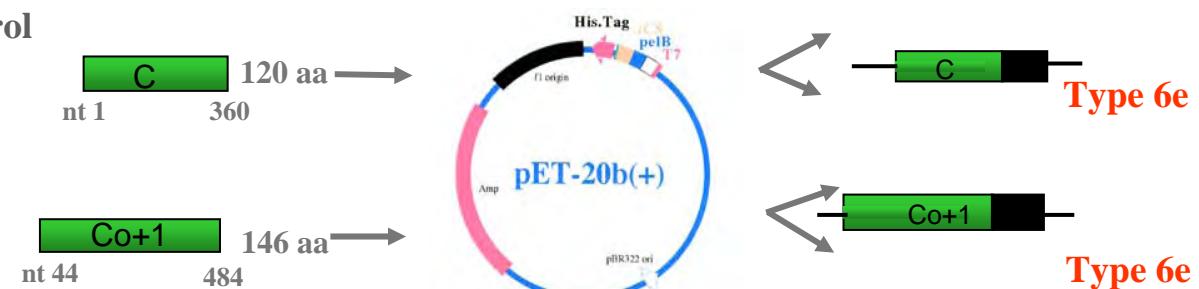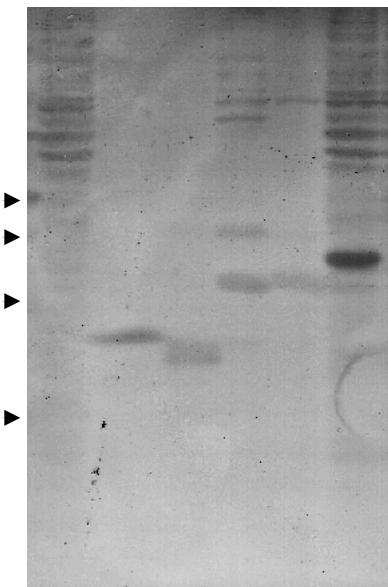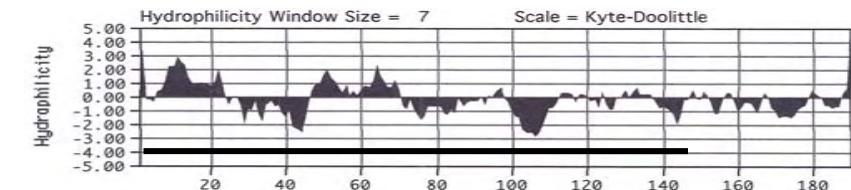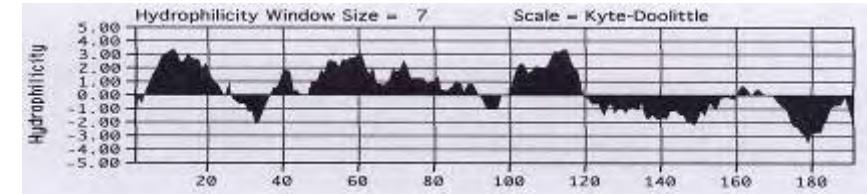
HCV-21	core: 1b	3-75*aa*
HCV-26	core: 2*a*	11-45*aa*
HCV-34	core: 1b	11-45*aa*

PROKARYOTIC EXPRESSION OF THE HCV Δ CORE AND Δ CORE+1 PROTEINS TYPE 1a OR 6e

Genotype 1a

Genotype 6e

Core

Core+1

171 aa core type 1a control

146 aa **core+1** type 6e
146 aa **core+1** type 1a
120 aa **core** type 1a
120 aa **core** type 6e

29
24
20
14,4

C 120 aa
nt 1   360

Co+1 146 aa
nt 44   484

His.Tag

pET-20b(+)
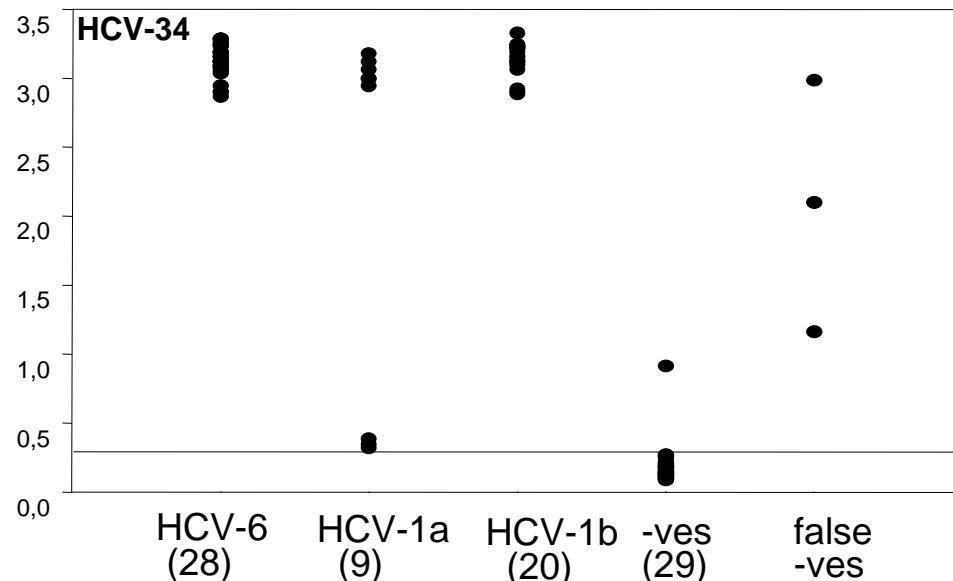
C   Type 6e

Co+1   Type 6e

ELISA DATA WITH CORE PEPTIDES
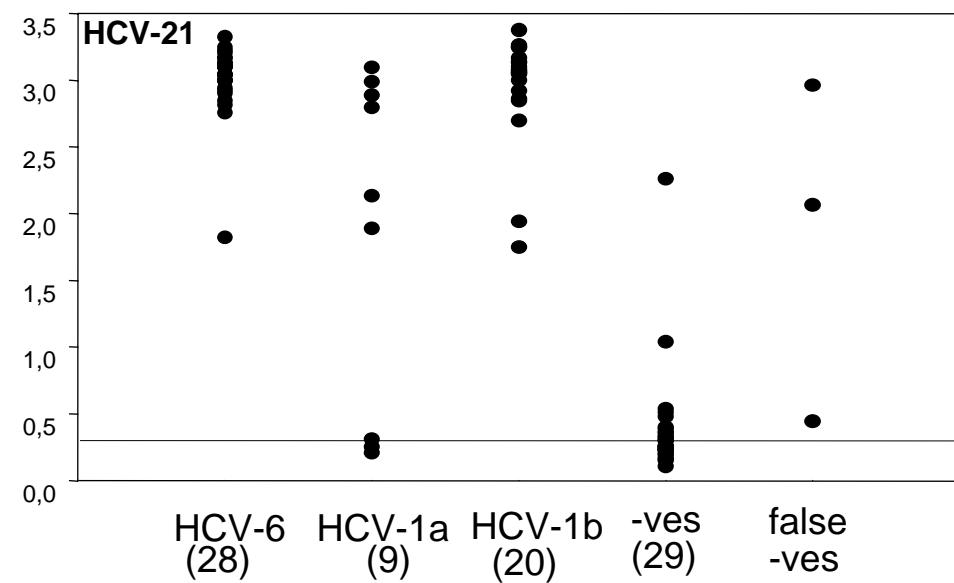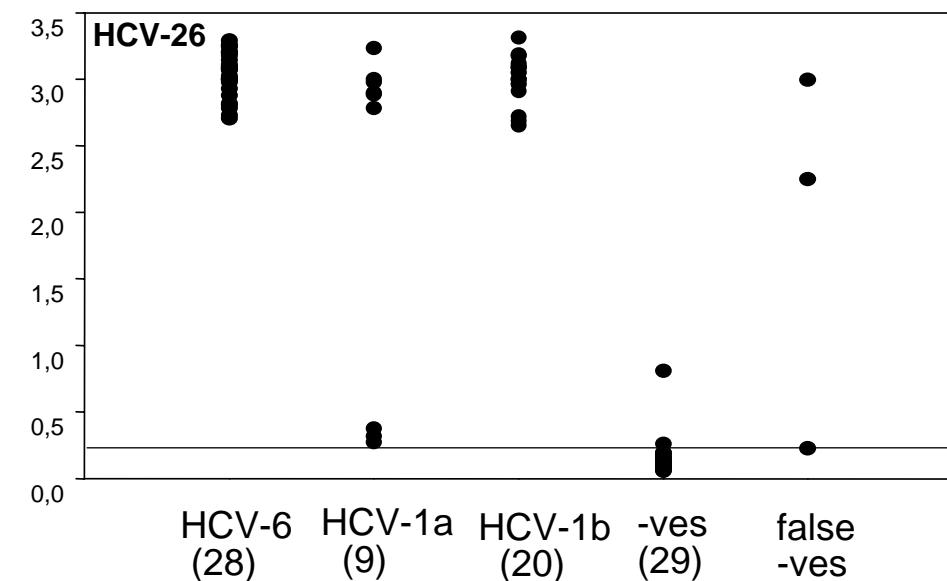
HCV-34 (11-45aa/1b)
57/57 +ves (100%)
28/29 -ves (97%)
3/3 false -ves

HCV-26 (11-45aa/2a)
57/57 +ves (100%)
28/29 -ves (97%)
3/3 false -ves

**HCV-21 (3-75aa/1b)**
55/57 +ves (96%)
16/29 -ves (55%)
3/3 false -ves
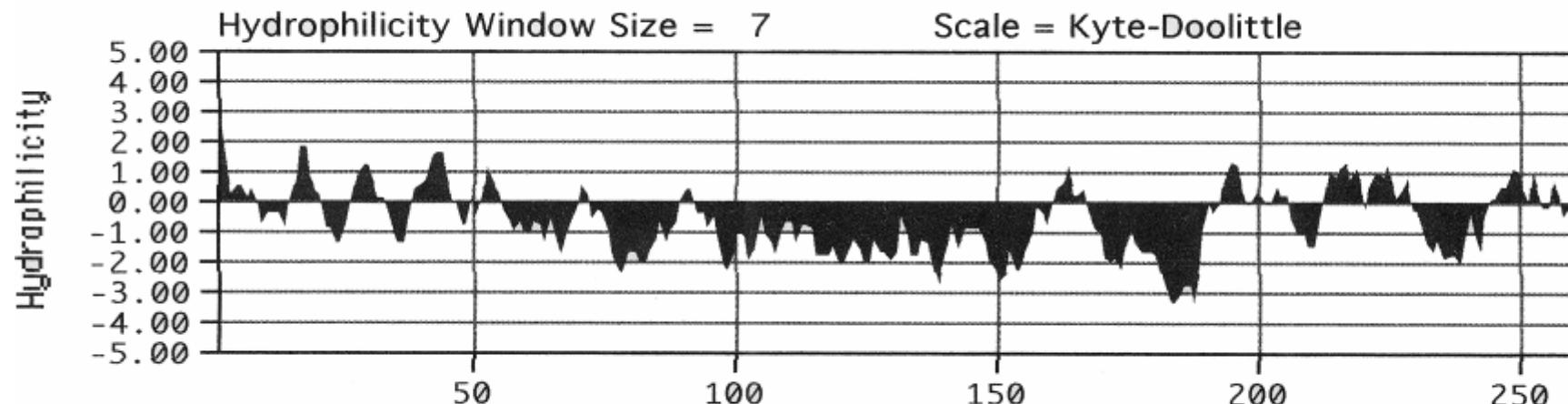
CONCLUSIONS

➢ All three core peptides are immunoreactive with all HCV positive sera

➢ All three core peptides are immunoreactive with the 3 false-negative sera

➢ The shorter core peptides (aa 11-45) exhibit better specificity

NS4 PEPTIDE ANTIGENS

Hydrophilicity Window Size = 7   Scale = Kyte-Doolittle
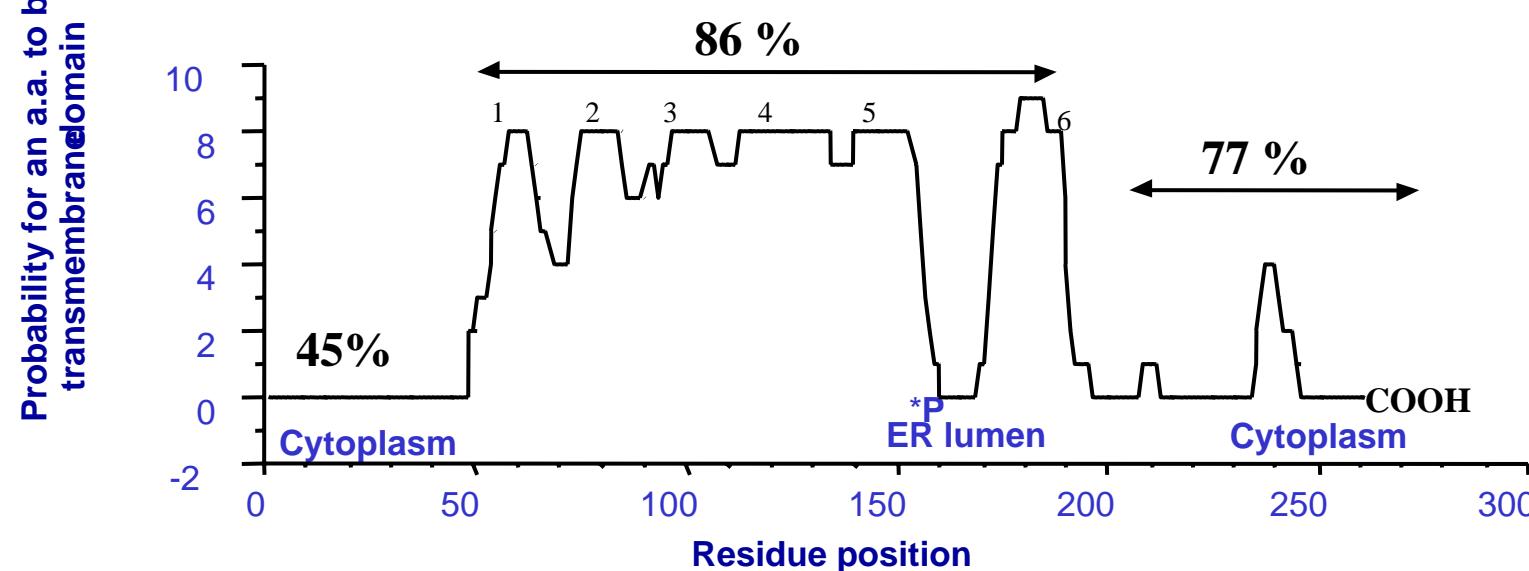
Peptides tested:

|		
HCV-6	NS4:1b	1921-1940 aa
HCV-45	NS4:1b	1689-1738 aa
HCV-452	NS4:2a	1688-1740 aa
HCV-645	NS4:1b	1689-1738 aa + 1921-1940aa
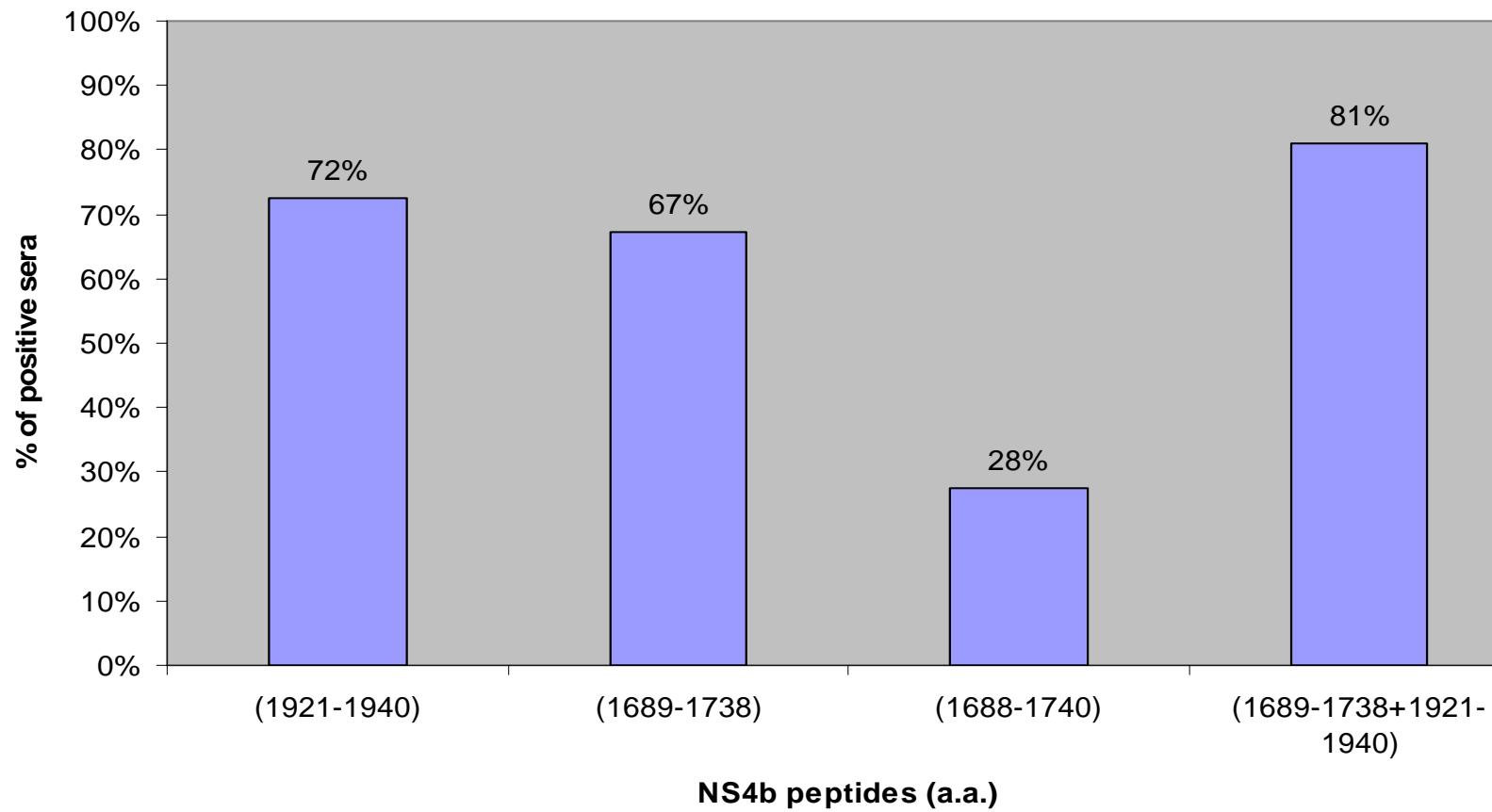
Hydrophobicity profile of NS4B
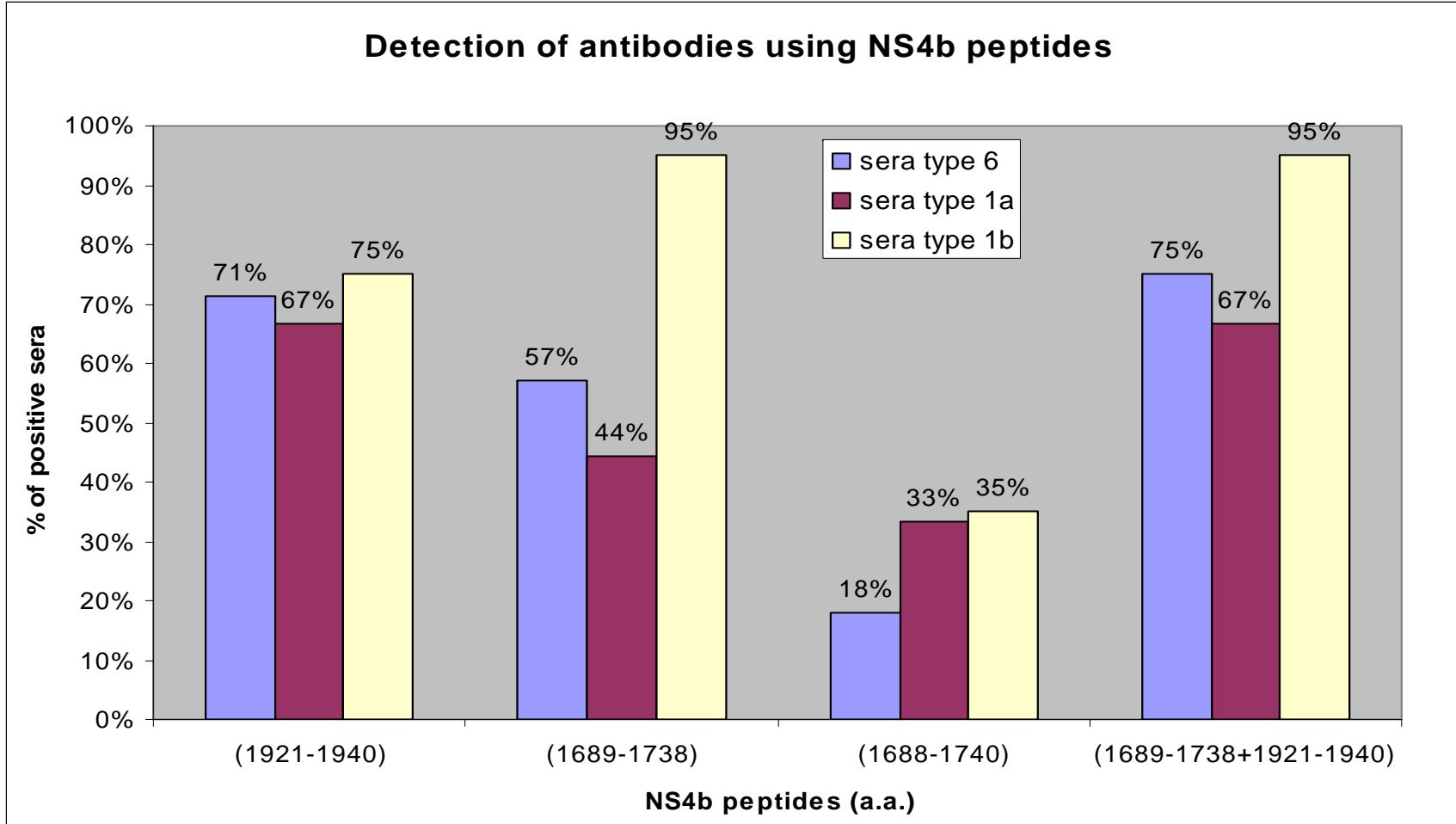
# Prediction of transmembrane domains

Detection of antibodies using NS4b peptides

% of positive sera

- (1921-1940): 72%
- (1689-1738): 67%
- (1688-1740): 28%
- (1689-1738+1921-1940): 81%
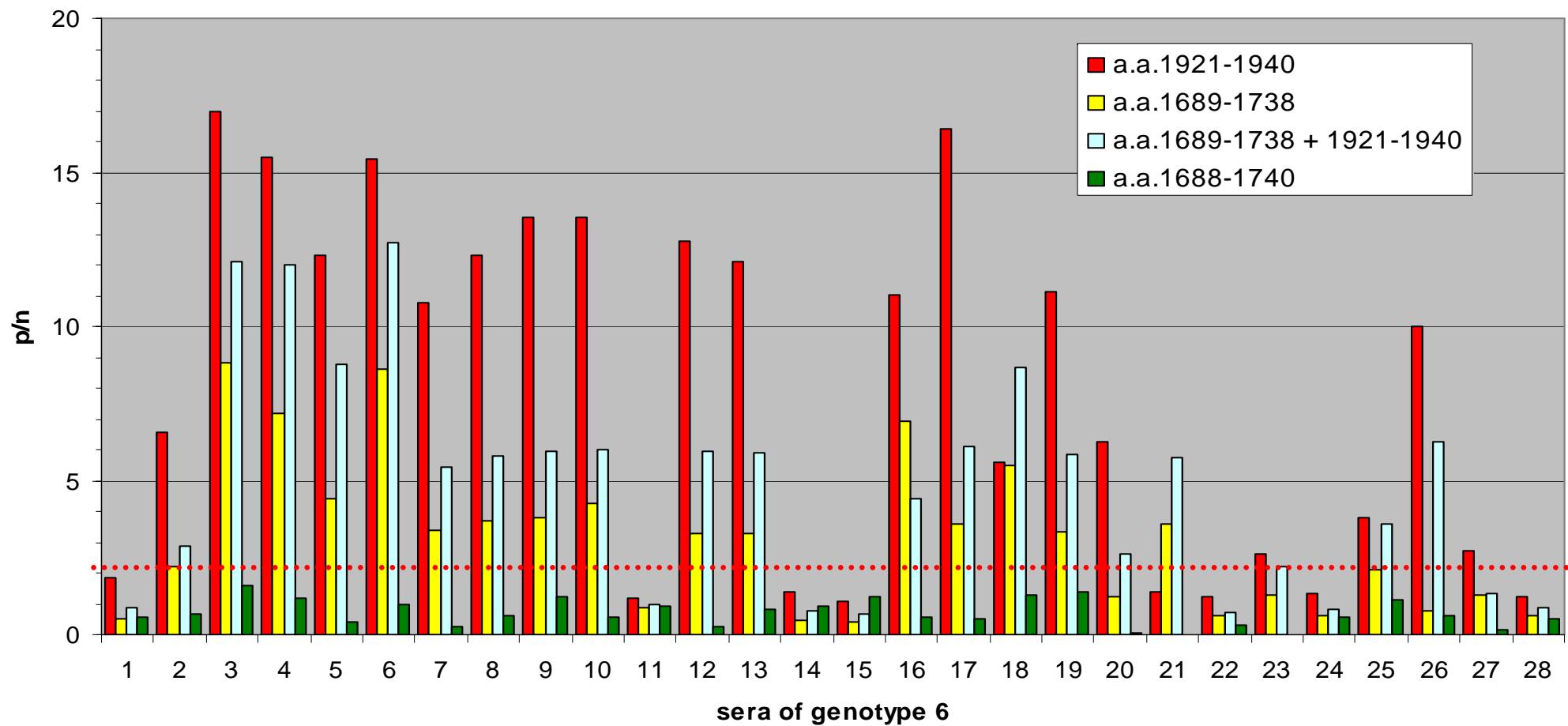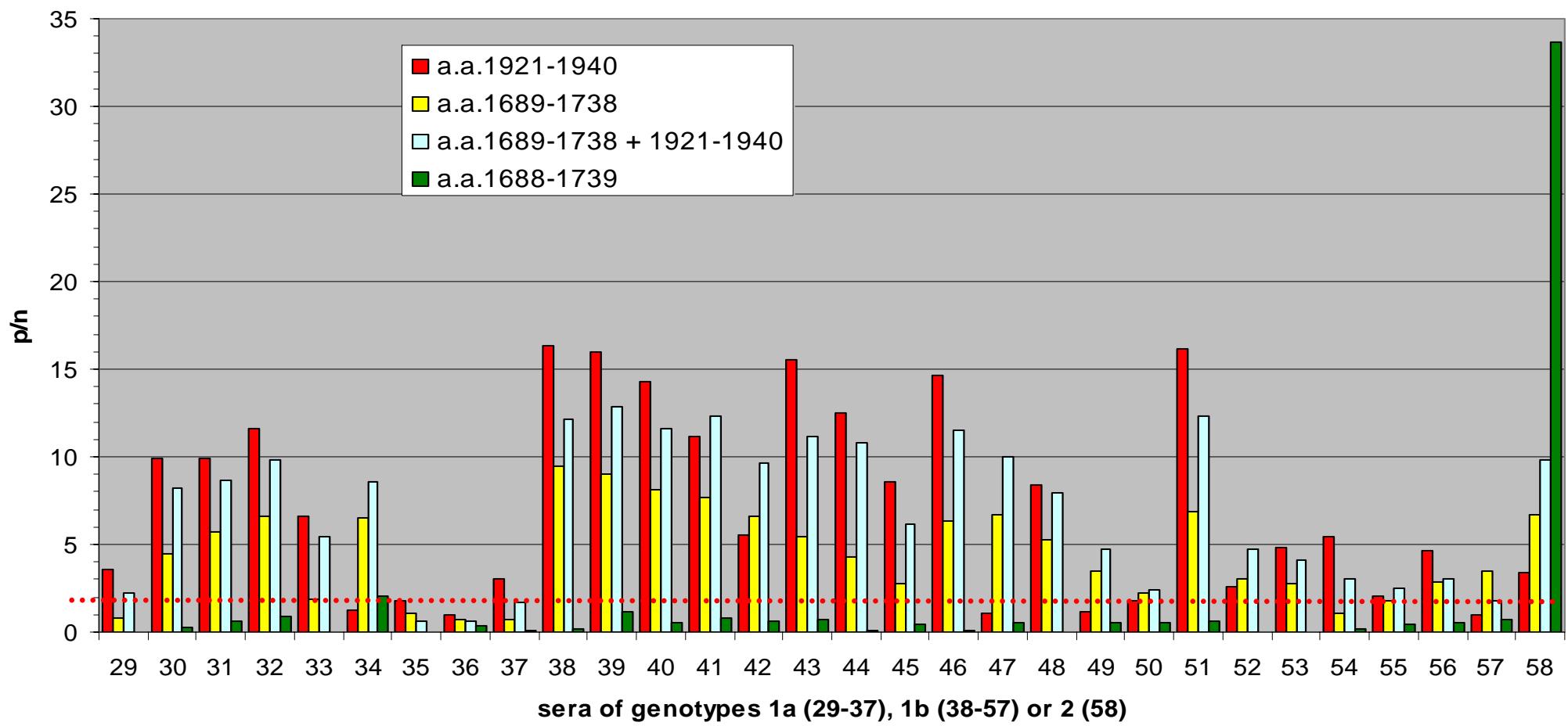
NS4b peptides (a.a.)

Detection of antibodies using NS4b peptides

Detection of antibodies using NS4b peptides

Legend:
- a.a.1921-1940
- a.a.1689-1738
- a.a.1689-1738 + 1921-1940
- a.a.1688-1740

p/n

sera of genotype 6

Detection of antibodies using NS4b peptides

Legend:
- a.a.1921-1940
- a.a.1689-1738
- a.a.1689-1738 + 1921-1940
- a.a.1688-1739

p/n

sera of genotypes 1a (29-37), 1b (38-57) or 2 (58)

CONCLUSIONS

- ➢ NS4 peptides recognize both HCV-1 and HCV-6 genotypes

- ➢ NS4 peptide HCV-645 (αα 1689-1738+1921+1940/1b) has the highest positivity rate (80%)

Implications of HCV diversity in the diagnosis and pathogenesis of virus infection in SE Asia, Eastern Europe and Central Africa (PTR126)

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