

## NOVEL CANDIDATE PEPTIDE VACCINE AGAINST HCV INFECTION

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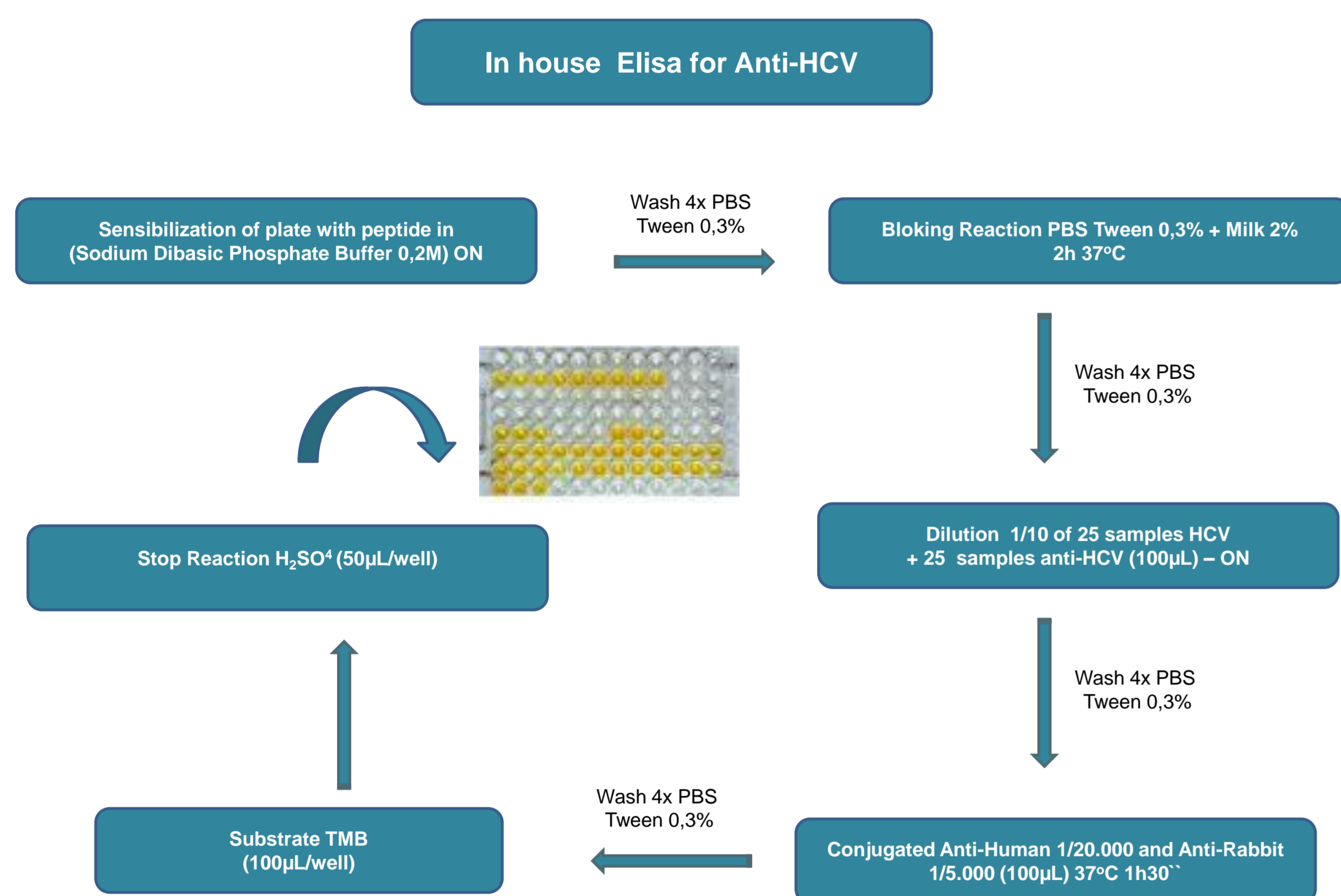
### INTRODUCTION

Hepatitis C Virus (HCV) is a worldwide public health problem that affects more than 70% of the estimated 170 million people with chronic hepatitis. It induces chronic hepatic lesions that lead to severe fibrosis and cirrhosis, hepatic failure, or hepatocellular carcinoma. The envelope glycoproteins E1 and E2 are the targets of neutralizing antibodies responses but are also the two of the most variable HCV proteins. Neutralizing monoclonal antibodies that target HCV epitopes encompassing amino acids (aa) 412 to 423 of the E2 protein. This study was designed to assess the immunogenic properties of genetically conserved (amino acid sequence; 412-419) peptides derived from the C-terminal region of the hypervariable region-1 (HVR-1) and to detect the presence of antibodies reacting with this small 412-423 sequence in natural infection.

### MATERIAL AND METHODS

One candidate peptide was selected based on sequence conservation among E2 sequences recorded on the HCV data base. Twenty-five serum samples from chronic HCV patients testing anti-HCV positive and 25 samples from healthy individuals who tested negative for anti-HCV antibodies were used to establish the reactivity of these sera with the small 412-419 synthetic peptide. Enzyme linked immunosorbent assay (ELISA) was developed in house calculating the cutoff value with mean absorbance value obtained from HCV negative sera plus three times the standard deviation. Immunized rabbit sera with E2-conserved synthetic peptide in the position 412-419 aminoacids conjugated with KLH generated high titers of anti-peptide antibody was used as positive control.

#### Schematic diagram of the protocol in house Elisa anti-HCV



### RESULTS AND CONCLUSIONS

Only two samples of patients reacted with the peptide 412-419 BSA in different dilutions. These samples were identified HCV/101 negative for anti-HCV test and the sample identified HCV/122 positive for anti-HCV by standard test. E2-peptides were able to recognize specific immunoglobulins in some chronic HCV patients. These results indicate that the selected epitope was able to induce humoral immune responses during the infection in a small proportion (8%) of the studied patients and that E2 conserved peptide used represent essential components of a candidate peptide vaccine against HCV infection. Not protection against HCV may be associated with genetic variations particularly within the hypervariable region-1 (HVR-1) and low titers of anti E2 antibodies or interference of non-neutralizing antibodies with the function of neutralizing antibodies. These findings suggest the detection of anti E2-peptide immunoglobulin in chronic HCV patients as potential therapeutic and/or prophylactic vaccines against HCV infection since most antiviral therapies fail and anti-HCV vaccine is not currently available.

### RESULTS

Table 1: Detection anti-HCV peptide 412-419 BSA of chronic positive HCV patients

Samples HCV	D 1/10 C 1/20.000	D 1/10 C 1/30.000	D 1/100 C 1/20.000	D 1/100 C 1/30.000	ANTI-HCV ST	DO-Cutoff
1/06	0,89	1,08	0,29	0,64	P	107,63
2/06	0,61	1,25	0,20	0,47	P	102,93
8/06	0,37	0,98	0,11	0,21	P	130,52
11/06	0,78	1,44	0,32	0,51	P	138,9
12/06	0,48	0,75	0,12	0,14	P	114
15/06	0,65	1,22	0,21	0,27	P	132,61
27/06	0,53	0,86	0,21	0,30	P	112,25
31/06	0,36	0,58	0,10	0,13	P	141,11
40/06	0,36	0,77	0,13	0,20	P	137,74
41/06	0,68	1,23	0,29	0,44	P	140,95
46/06	1,12	1,34	0,38	0,44	P	130,06
48/06	0,54	0,99	0,30	0,32	P	120,12
62/06	0,38	0,47	0,18	0,26	P	126,89
82/06	0,43	0,84	0,19	0,30	P	137,54
85/06	0,69	1,04	0,36	0,43	P	123,64
88/06	0,94	1,58	0,38	0,54	P	125,32
89/06	0,54	0,84	0,30	0,39	P	114,94
90/06	0,81	1,31	0,28	0,38	P	136,07
111/06	0,60	0,74	0,17	0,20	P	106,2
113/06	1,02	1,39	0,28	0,35	P	102,93
122/06	1,77	2,51	1,15	1,38	P	113,03
128/06	1,11	1,87	0,27	0,37	P	139,86
155/06	0,63	1,10	0,21	0,30	P	109,22
159/06	0,40	0,71	0,13	0,18	P	139,19
160/06	0,49	0,82	0,14	0,23	P	110,17
346/02	0,41	0,31	0,09	0,07	P	NR
680/03	0,37	0,31	0,09	0,07	P	NR
867/02	0,28	0,21	0,07	0,07	P	NR
1555/03	1,07	0,81	0,16	0,12	P	NR

NR: Not Realized  
D: Dilution of tested human serum  
C: Dilution of anti-human conjugated with peroxidase  
ST: Standard tests

Table 2: Detection anti-peptide 412-419 in not reactive HCV patients

Samples HCV	D 1/10 C 1/20.000	D 1/10 C 1/30.000	D 1/100 C 1/20.000	D 1/100 C 1/30.000	ANTI-HCV ST	DO-Cutoff
26/06	0,80	1,43	0,23	0,29	N	0,43
28/06	1,06	1,66	0,31	0,39	N	0,38
29/06	0,53	0,82	0,12	0,21	N	0,4
34/06	1,28	2,26	0,21	0,24	N	0,38
36/06	0,70	1,12	0,22	0,47	N	0,43
39/06	0,54	0,92	0,21	0,49	N	0,41
64/06	0,58	0,78	0,20	0,20	N	0,47
80/06	0,43	0,67	0,21	0,31	N	0,41
99/06	0,61	0,84	0,24	0,29	N	0,37
100/06	0,33	0,75	0,17	0,25	N	0,39
101/06	1,50	2,37	0,67	0,84	N	0,34
103/06	0,43	0,53	0,20	0,23	N	0,42
105/06	0,78	1,18	0,29	0,67	N	0,42
108/06	0,75	1,07	0,17	0,19	N	0,37
126/06	0,61	0,72	0,18	0,25	N	0,58
129/06	0,39	0,78	0,14	0,19	N	0,35
133/06	0,40	0,81	0,09	0,12	N	0,43
149/06	0,68	1,17	0,20	0,40	N	0,38
150/06	0,38	0,70	0,10	0,15	N	0,44
151/06	0,32	0,66	0,11	0,18	N	0,4
153/06	0,24	0,45	0,08	0,13	N	0,28
154/06	0,88	1,42	0,17	0,28	N	0,42
161/06	1,06	1,10	0,26	0,32	N	0,39
162/06	1,04	1,17	0,21	0,19	N	0,42
163/06	0,46	0,46	0,22	0,09	N	0,43
Serum 1	0,22	0,52	0,30	0,23	N	NR
Serum 2	0,63	0,29	0,09	0,08	N	NR
Serum 3	0,67	0,57	0,10	0,10	N	NR
Serum 4	0,79	0,59	0,13	0,11	N	NR

### FINANCIAL SUPPORT