Design of a Hepatitis C Virus Antigen and Antibody Combination ELISA for Detection of HCV Infection

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ABSTRACT

HCV infection can be detected by testing for viral specific RNA, antigen or antibodies in patient samples. The HCV antibody assay is the most widely used test for blood screening at the present time. Although the efficacy of these assays have been demonstrated by significantly reducing the incidence of non-A, non-B hepatitis infection after blood transfusion, the relatively wide seroconversion window (~80 days) after HCV infection still remains as the major concern of blood banks and the plasma manufacturing industry. New HCV NAT assays will significantly reduce the seroconversion window. However, one cannot be sure that the possible ~15% HCV RNA non-reactive but HCV antibody positive samples found are not infectious. For this reason, there is a need to improve the HCV serological assay.

A prototype combination enzyme immunoassay for the detection of hepatitis C virus core antigen and antibodies to NS3/4a was developed. We incorporated double sandwich immunoassays for the detection of HCV core antigen and anti-NS3 antibodies on the same well. Anti HCV core monoclonals and NS3/4a recombinant antigen were coated onto microtiter plates. HCV core antigen was detected by another monoclonal antibody conjugated to HRP. In the same microwell, antibodies to HCV were detected by the c33c recombinant SOD fusion antigen pre-formed with anti-SOD monoclonal conjugated to HRP. No specimen treatment was required. In conclusion, this one well assay can detect HCV antigen and antibody and has shown early detection from 7–10 days ahead of current licensed HCV antibody tests.

In some of the panels, the HCV Ag/Ab combo assay can close the gap between core antigen reactive and antibody reactive in some early seroconversion panels.

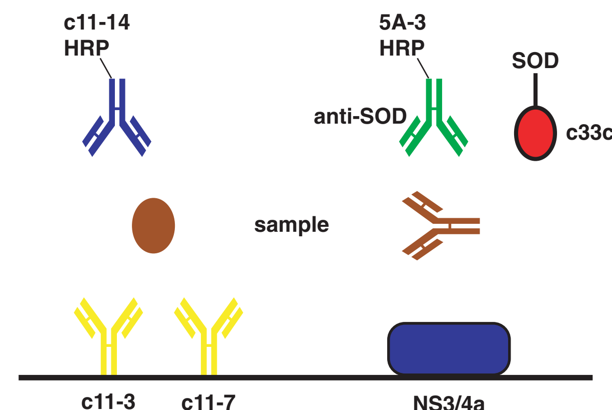
HCV Ag/Ab COMBO ASSAY DESIGN GOALS

- Develop new lysis buffer compatible for Combo assay
- Close gaps within seroconversion bleed dates
- Monoclonal antibody and NS3/4a antigen solid phase co-coat compatibility
- Signal detection compatibility
- Maintain assay run time of 150 minutes

HCV COMBO ASSAY FORMAT

- Add 100 µL enhanced lysis buffer + 100 µL sample
- Incubate 1 hr @ 40°C with shaking
- Wash
- Add 200 µL conjugate (c11-14-HRP + c33c pre-formed with mouse anti-SOD-HRP)
- Incubate 45 minutes @ 40°C with shaking
- Wash
- 200 µL OPD Substrate solution
- Stop and Read at 492 nm

HCV COMBINATION PROTOTYPE ASSAY



RESULTS

HCV SEROCONVERSION PHV 907 (s/co)

Bleed #	Date	Days	Abbott HCV Ab PRISM	Ortho 3G Ab Assay	Roche Amplicor copies/mL	Ortho 1G Ag Assay	Chiron Ag/Ab
1	4/6/96	0	0.1	0.0	**>5 x 10⁵**	**18.6**	**2.8**
2	4/10/96	4	0.1	0.0	**>5 x 10⁵**	**19.0**	**3.1**
3	4/13/96	7	0.1	0.0	**>5 x 10⁵**	**22.3**	**1.5**
4	4/19/96	13	0.3	0.1	**>5 x 10⁵**	**26.2**	**1.7**
5	4/24/96	18	**1.3**	0.4	**>5 x 10⁵**	**15.9**	**1.2**
6	4/27/96	21	**2.2**	**1.0**	**>5 x 10⁵**	**11.3**	**1.5**
7	9/17/96	164	**4.2**	**4.4**	**4 x 10⁴**	0.11	**2.5**

HCV SEROCONVERSION PHV 917 (s/co)

Bleed #	Date	Days	Abbott HCV Ab PRISM	Ortho 3G Ab Assay	Roche Amplicor copies/mL	Ortho 1G Ag Assay	Chiron Ag/Ab
1	5/29/96	0	0.1	0.0	BLD	0.11	0.5
2	6/11/96	13	0.1	0.0	**>5 x 10⁵**	**44.0**	**3.0**
3	6/18/96	20	0.1	0.0	**>5 x 10⁵**	**24.2**	**1.3**
4	6/20/96	22	0.3	0.0	**>5 x 10⁵**	**29.2**	**1.6**
5	8/22/96	85	**5.4**	**4.7**	BQR	0.06	**1.1**
6	10/7/96	131	**4.3**	**4.7**	BQR	0.09	**1.0**
7	10/11/96	135	**4.6**	**4.7**	**3 x 10³**	0.09	**1.2**
8	10/14/96	138	**5.5**	**4.7**	BLD	0.08	**1.2**
9	10/22/96	146	**5.9**	**4.7**	BLD	0.11	**2.1**
10	10/28/96	152	**5.2**	**4.7**	BQR	0.07	**1.8**

CONCLUSIONS

- We have developed a prototype HCV Combo immunoassay to detect core antigen and antibodies to NS-3 in the same reaction well.
- Because of NS3/4a's ability to detect low affinity seroconversion panels, we can close gaps between bleed dates where core antigen cannot be detected.