



Seroconversion sensitivity of ORTHO[®] Antibody to HCV Core Antigen ELISA Test System

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Report NBSR06002

May 2006

Description of the Assay

Ortho[®] Antibody to HCV Core Antigen ELISA Test System (Ortho HCV Core Ag EIA) is an enzyme-linked immunosorbent assay for the detection of hepatitis C core antigen in the absence of HCV antibodies in human serum or plasma. The assay utilises several monoclonal antibodies with specificity to different regions of the HCV core antigen. Monoclonals coated onto the microwell solid phase capture the antigen, and monoclonals conjugated to horseradish peroxidase detect the captured antigen. Further assay information is shown in Table 1.

Table 1: Assay information

General	
Assay name	Ortho [®] Antibody to HCV Core Antigen ELISA Test System
Manufacturer / UK agent	Ortho
Product number	933255 / 933260
Number of tests in one pack	192 / 480
Specimen volume	100µL
Sample addition monitoring?	Yes (green to blue)
Presentation	
Assay type	Direct antigen capture ELISA
Solid phase	12 x 8 microtitre plate wells
Coating	Murine monoclonal antibodies to hepatitis C core antigen
Conjugate 1	Anti-hepatitis C core antigen conjugated to horseradish peroxidase
Substrate	o-phenylenediamine.2HCl
Controls per plate	5 + 1 blank well
Negative control	3
Positive control	2
Reading wavelength	490 / 630nm
Cut-off computation	MEAN(Neg Control) + 0.04
Equivocal zone	n/a
Stages	
Preparation / sample well loading	30 minutes
Incubation status	Shaking (approx 700rpm) / static
Sample incubation	90 minutes, 37°C, Shaking
Conjugate incubation	30 minutes, 37°C, Static
Substrate incubation	30 minutes, room temperature
Number of washes	6
Reading	5 minutes
Total incubation times	150 minutes
Approximate time to completion	210 minutes
Number of optional procedures	none
Additional equipment required	
Incubator, type not specified (*Dry incubator and Jencons shaking incubator)	
Microplate spectrophotometer (* EL 808)	
Micropipettes: 40 - 200µL, 200 - 1000µL & 2 - 10mL	
Multichannel pipettes: 50 - 300µL	
Disposable tips	
Reagent troughs and bottles	
Measuring cylinder	
Distilled water	
Notes:	
* Equipment used in this evaluation.	

Evaluation Panel and Method

The assay was evaluated to determine its ability to detect HCV core antigen in seroconversion panels. The evaluation panel consisted of 220 specimens from 26 seroconversion panels and two quality control specimens that were each tested in triplicate. The evaluation was carried out using one kit lot (AGK156).

The method described in the kit insert was strictly followed. Briefly, 100µL of specimen diluent followed by 100µL of specimens or controls was added to each of the microplate wells. The wells were incubated at 37°C with shaking (700rpm) for 90 minutes then washed 6 times. 200µL of conjugate was added to each of the wells, which were then incubated for a further 30 minutes at 37°C. The microplate was washed 6 times then 200µL of substrate was added to each of the wells. The microplate was incubated in the dark for 30 minutes at room temperature (18 – 25°C). Finally 50µL of stop solution was added and the plate was read at 450/630nm.

Table 2: Evaluation panel (Lot AGK156)

Sample category	Number
HCV seroconversion panels	
BBI: PHV901	11
BBI: PHV904	7
BBI: PHV905	9
BBI: PHV906	7
BBI: PHV907	7
BBI: PHV908	13
BBI: PHV909	3
BBI: PHV910	5
BBI: PHV911	5
BBI: PHV913	4
BBI: PHV914	9
BBI: PHV915	4
BBI: PHV916	8
BBI: PHV917	10
BCP6211	15
BCP6212	9
BCP6213	12
BCP6214	13
BCP6215	4
BCP6216	7
BCP6222	8
BCP6229	8
BCP9041	8
BCP9044	6
BCP9045	8
BCP9047	10
Quality control samples	
HPA: HCV-QC1	3
NIBSC QC	3
TOTAL (number of specimens)	216
Notes:	
BBI = Boston Biomedica Inc; BCP = BioClinical Partners Inc (Zeptomatrix)	
HPA = Health Protection Agency - Centre for Infections	
NIBSC = National Institute for Biological Standards and Control.	

Results: Seroconversion Sensitivity

Number of positive results

When testing seroconversion panels, the number of positive results detected by a kit can be an indication of the sensitivity of that kit. It was possible to compare Ortho HCV Core Ag EIA with previous evaluation results for seven HCV antibody-only kits and one HCV combined antigen/antibody kit. Ortho HCV Core Ag EIA gave an aggregate score of 144 out of 202 making it the second most sensitive kit behind Monolisa HCV Ag/Ab (151/202). The most sensitive antibody only kit was Vitros Eci HCV scoring 99/202 (Table 3).

It should be noted that when scoring positive results it is difficult to make a direct comparison between the three different kit formats since they each give reactions at different points during seroconversion. The Ortho HCV Core Ag EIA was more likely to give reactions early in the panel, whilst the antibody-only kits gave reactions later in the panel. The Ag/Ab kit has the advantage of being able to detect early and late positives.

The Ortho HCV Core Ag EIA was originally developed as a donor screening tool to reduce the seroconversion “window period” and was to be used in conjunction with antibody testing. If the results of Ortho HCV Core Ag EIA are combined with those of Vitros *ECi* HCV, as they could be in a real diagnostic situation, then a much higher score of 184/202 is achieved. A full comparison of the 26 seroconversion panels tested by the different kit formats is shown in *Appendix 1*.

Table 3: Aggregate seroconversion scores

Panel	Number of specimens in panel	Monolisa HCV AgAb ULTRA (cut-off = 0.5)	Monolisa HCV AgAb ULTRA (cut off = 1.0)	Ortho HCV Ag EIA	Vitros Eci anti-HCV	AxSYM® HCV version 3.0	Ortho HCV 3.0 with Enh Save (Short inc.)	Access® HCV Ab PLUS	PRISM™ HCV	IMx	Monolisa anti-HCV Plus
PHV901	11	9 (97)	9 (97)	2 (65)***	9 (97)	9 (97)	9 (97)	9 (97)	9 (97)	9 (97)	9 (97)
PHV904	7	4 (9)	4 (9)	4 (0)***	4 (9)	4 (9)	4 (9)	3 (14)	4 (9)	3 (14)	4 (9)
PHV907	7	7 (0)	7 (0)	6 (0)***	4 (13)	3 (18)	3 (18)	4 (13)	2 (21)*	1 (164)	2 (21)
PHV908	13	10 (11)	9 (13)	4 (11)***	9 (13)	10 (11)	8 (19)	8 (19)	8 (19)*	3 (41)	7 (25)
PHV909	3	3 (0)	3 (0)	3 (0)	2 (28)	0 (>30)	2 (28)	2 (28)	2 (28)*	0 (>30)	2 (28)
PHV910	5	5 (0)	5 (0)	5 (0)	3 (8)	3 (8)	3 (8)	3 (8)	3 (8)*	2 (11)	2 (11)
PHV913	4	4 (0)	4 (0)	4 (0)	3 (2)	0 (>9)	2 (7)	2 (7)	0 (>9)*	0 (>9)	2 (7)
PHV914	9	9 (0)	9 (0)	9 (0)	5 (16)	5 (16)	5 (16)	5 (16)	3 (24)*	3 (24)	4 (19)
PHV915	4	3 (5)	2 (12)	0 (>14)	2 (12)	3 (5)	1 (14)	0 (>14)	2 (12)*	2 (12)	1 (14)
6212	9	7 (14)	6 (23)	1 (23)***	8 (12)	8 (12)	8 (12)	6 (23)	8 (12)*	8 (12)	4 (32)
6213	12	5 (28)***	3 (37)	11 (2)	3 (37)	3 (37)	2 (43)	2 (43)	2 (43)*	2 (43)	2 (43)
6214	13	10 (0)***	6 (25)	12 (0)***	5 (30)	6 (25)	5 (30)	5 (30)	5 (30)*	3 (49)	4 (32)
6215	4	4 (0)	4 (0)	4 (0)	1 (20)	1 (20)	1 (20)	1 (20)	1 (20)*	1 (20)	1 (20)
6216	7	1 (23)	1 (23)	0 (>23)	1 (23)	1 (23)	1 (23)	1 (23)	1 (23)*	0 (>23)	0 (>23)
6222	8	6 (17)	6 (17)	6 (17)	1 (40)	1 (40)	1 (40)	1 (40)	1 (40)	0 (>43)	1 (40)
9041	8	6 (27)	6 (27)	7 (24)	4 (62)	4 (62)	4 (62)	4 (62)	4 (62)*	4 (62)	4 (62)
9044	6	6 (0)	6 (0)	6 (0)	2 (25)	3 (21)	2 (25)	2 (25)	2 (25)*	2 (25)	2 (25)
9045	8	8 (0)	7 (0)***	8 (0)	2 (37)	2 (37)	2 (37)	2 (37)	2 (37)*	2 (37)	1 (41)
9047	10	10 (0)	10 (0)	10 (0)	4 (28)	4 (28)	4 (28)	4 (28)	4 (28)*	4 (28)	3 (30)
Sub-Total**	148	117	107	102	72	70	66	64	63	47	55
PHV905	9	6 (11)	6 (11)	6 (4)***	5 (14)	5 (14)	5 (14)	3 (21)	NT	2 (25)	4 (18)
PHV906	7	7 (0)	7 (0)	7 (0)	7 (0)	7 (0)	7 (0)	7 (0)	NT	5 (7)	7 (0)
PHV911	5	5 (0)	5 (0)	5 (0)	3 (14)	3 (14)	3 (14)	3 (14)	NT	2 (21)	3 (14)
PHV916	8	8 (0)	7 (2)	8 (0)	3 (19)	4 (16)	2 (23)	2 (23)	NT	NT	2 (23)
PHV917	10	9 (13)	9 (13)	3 (13)	6 (85)	6 (85)	NT	6 (85)	NT	NT	NT
6211	15	11 (147)	10 (150)	13 (140)	3 (182)	3 (182)	3 (182)	2 (186)	NT	0 (>189)	2 (186)
Total	202	163	151	144	99	98	N/A	84	N/A	N/A	N/A
Product Number		72558	72558	933255	131 8450	3B44-20	9307401	34330	6A5248	3A99-20	72312
Lot Number		5B1513	5B1513	AGK156	0100	65113LU00	GECV028	194822	10143 HP00	12220 HP00	6C501.U

Notes:
 NT = not tested. NS = not scored, all panels had not been tested by the assay.
 * PRISM results were extracted from BBI / BCP data sheets
 **The total for each assay was calculated by summing the correct positive reactions for each of the panels. A higher score suggests higher sensitivity.
 The number in parenthesis is the number of days from the initial bleed to the first positive sample
 *** Panels marked began positive but had one or more negative results later on in the panel

Comparison with Monolisa HCV Ag/Ab

Monolisa HCV Ag/Ab is the most sensitive EIA currently available. When Ortho HCV Core Ag EIA was compared to the Monolisa kit we found that 12 of the 26 seroconversion panels tested gave the same results on both kits. For eight of the 26 panels tested, Ortho HCV Core Ag EIA detected HCV infection earlier than Monolisa HCV Ag/Ab. Of these, five panels remained positive after the initial positive and three became negative at some point later in the panel. The Ortho kit did not detect HCV Ag at all in two of the panels tested. The four remaining panels gave either a negative-positive-negative or a positive-negative results pattern by Ortho HCV Core Ag EIA and negative-positive or all positive results by Monolisa HCV Ag/Ab (Table 4).

It is this last group of panels that makes comparison between the two kits difficult because the Ortho kit may detect infection earlier than the Monolisa kit, but the negative results later in the panel mean that the test is only suitable for detection of early infection. A selection of these results is shown in Figures 1 - 7.

Table 4: Comparison of seroconversion panel results

All specimens positive by Ortho and Monolisa	Ortho and Monolisa both have 1st positive at specimen -03	Ortho detects positive earlier than Monolisa, and remains positive	All specimens negative on Ortho, some positives on Monolisa	Neg-Pos-Neg pattern on Ortho; Neg-Pos on Monolisa	Pos-Neg pattern on Ortho; Neg-pos on Monolisa	Pos-Neg pattern on Ortho; All specimens Pos on Monolisa
PHV906 PHV909 PHV910 PHV911 PHV913 PHV914 BCP6215 BCP6229 BCP9044 BCP9045 BCP9046	BCP6222	PHV916 BCP6211 BCP6213 BCP6214 BCP9041	PHV915 BCP6216	PHV901* PHV905 [§] PHV908 [§] PHV917 [†] BCP6212 [†]	PHV904	PHV907
				*Monolisa detects positive earlier		
				[§] Ortho detects positive earlier		
				[†] Both kits detect positive at same time		

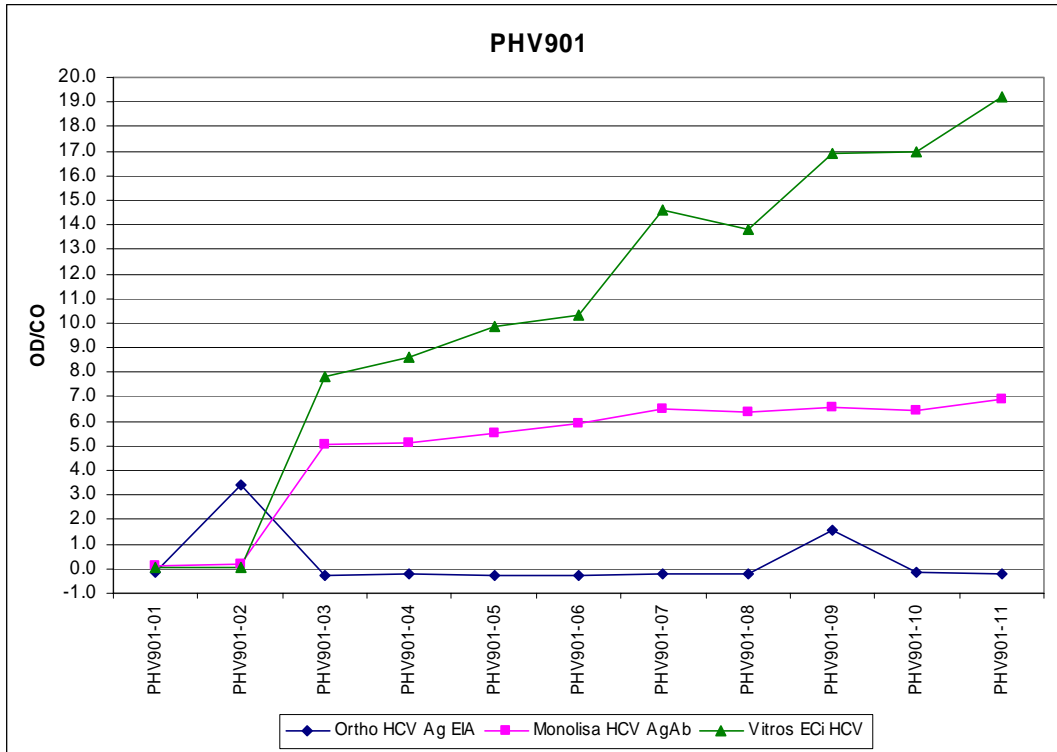


Figure 1: OD/CO values for PHV901. Monalisa and Vitros were positive from member -03, Ortho HCV Core Ag was positive for members -02 and -09.

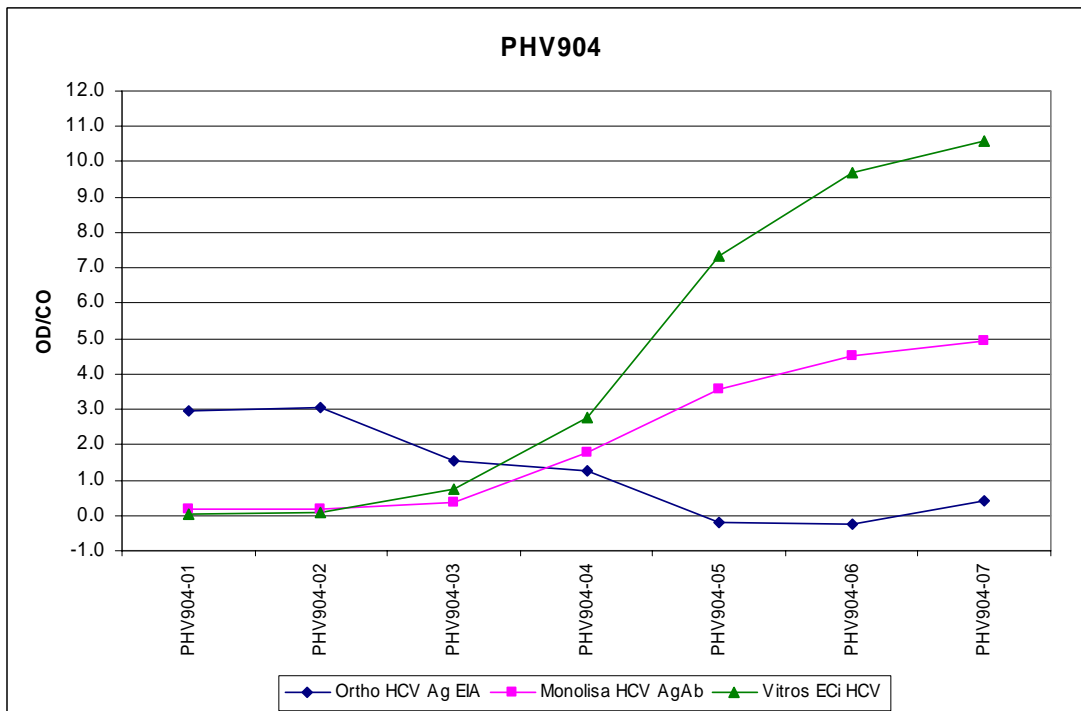


Figure 2: OD/CO values for PHV904. Ortho HCV Core Ag was initially positive, then became negative. Monalisa and Vitros were positive from member -04 onwards.

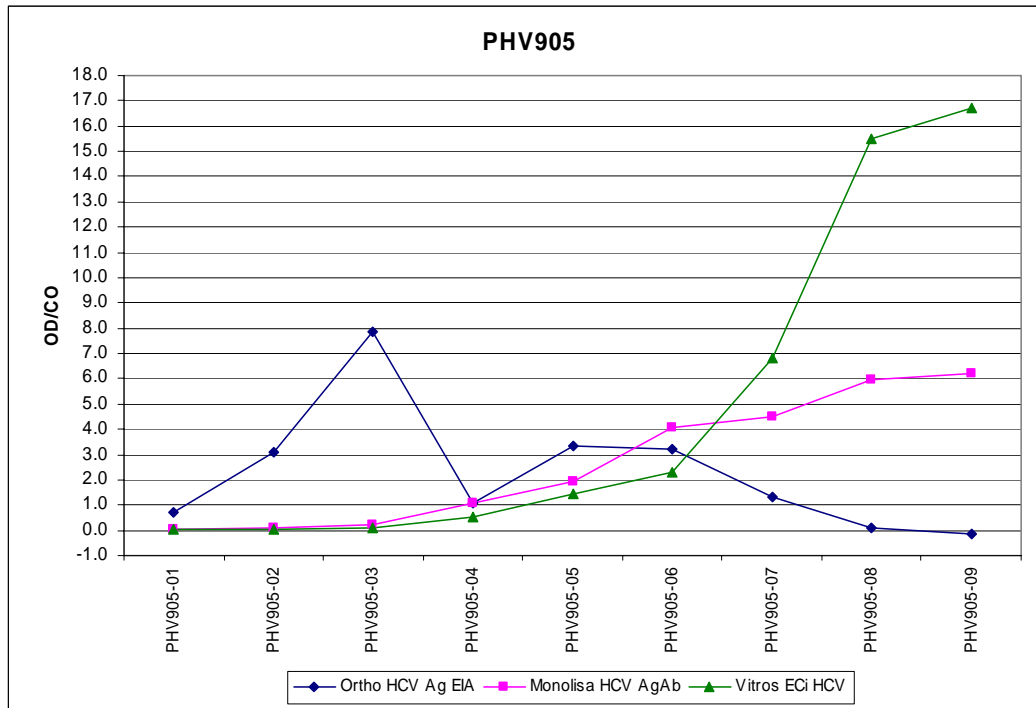


Figure 3: OD/CO values for PHV905. Ortho HCV Core Ag went from being negative to positive and back to negative. Monolisa and Vitros began negative and were both positive by member -05.

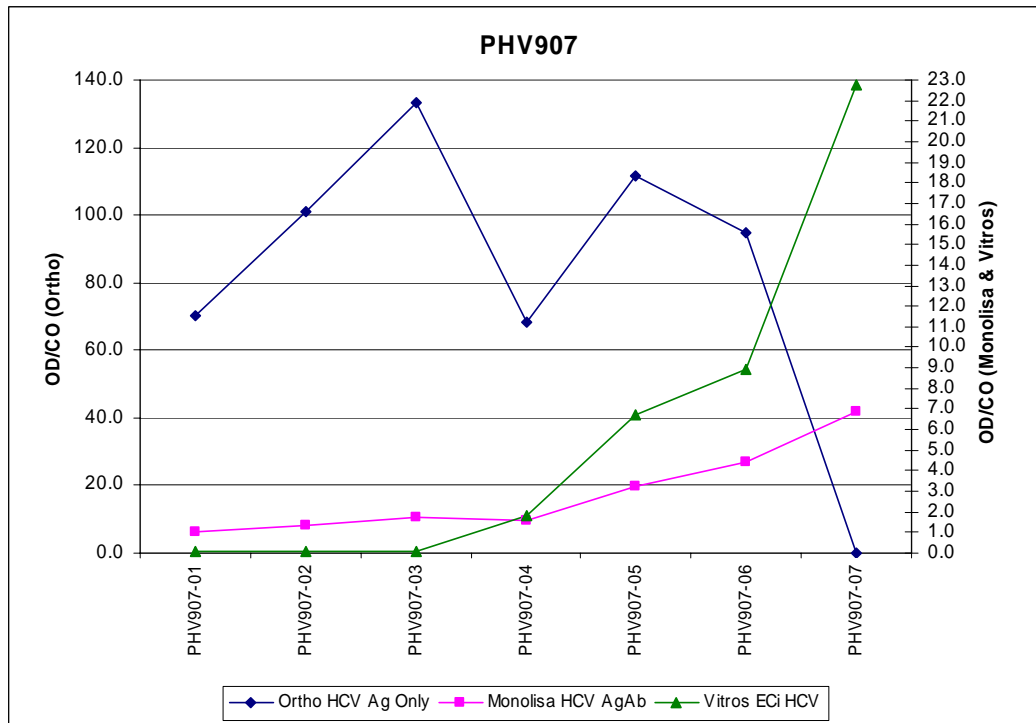


Figure 4: OD/CO values for PHV907. Ortho HCV Core Ag was strongly positive until the last member. Monolisa was positive for the whole panel and Vitros was positive from member -04.

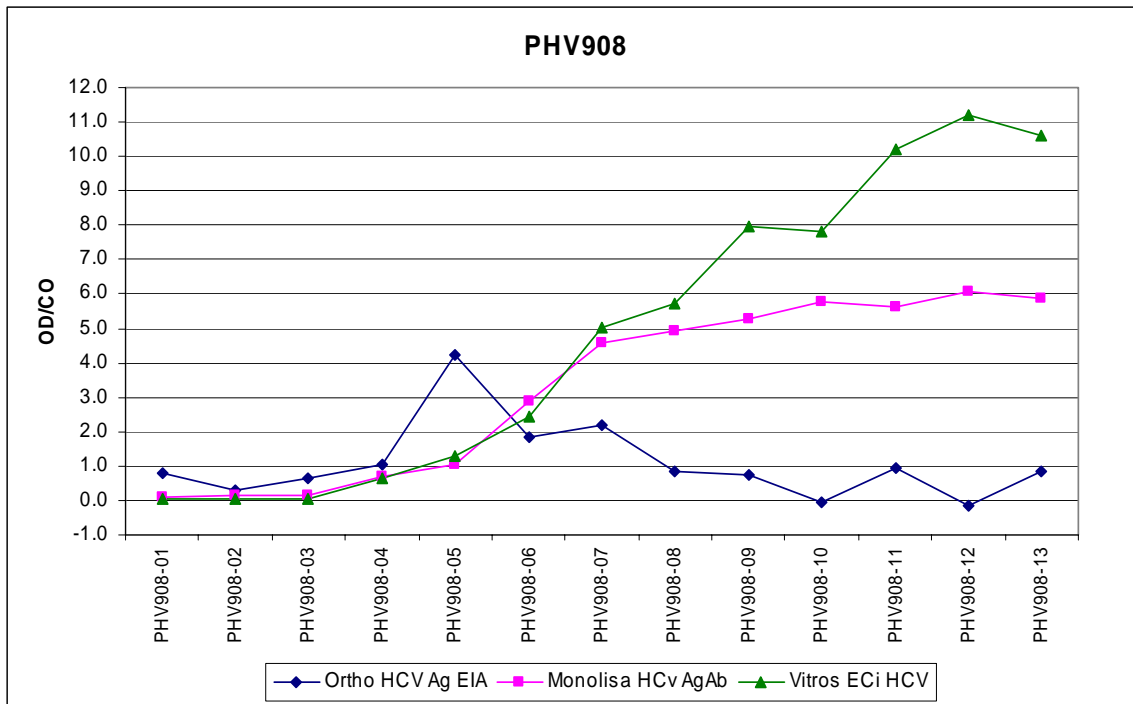


Figure 5: OD/CO values for PHV908. Ortho HCV Core Ag went from being negative to positive and back to negative. Monolisa and Vitros began negative and were both reactive from member -05.

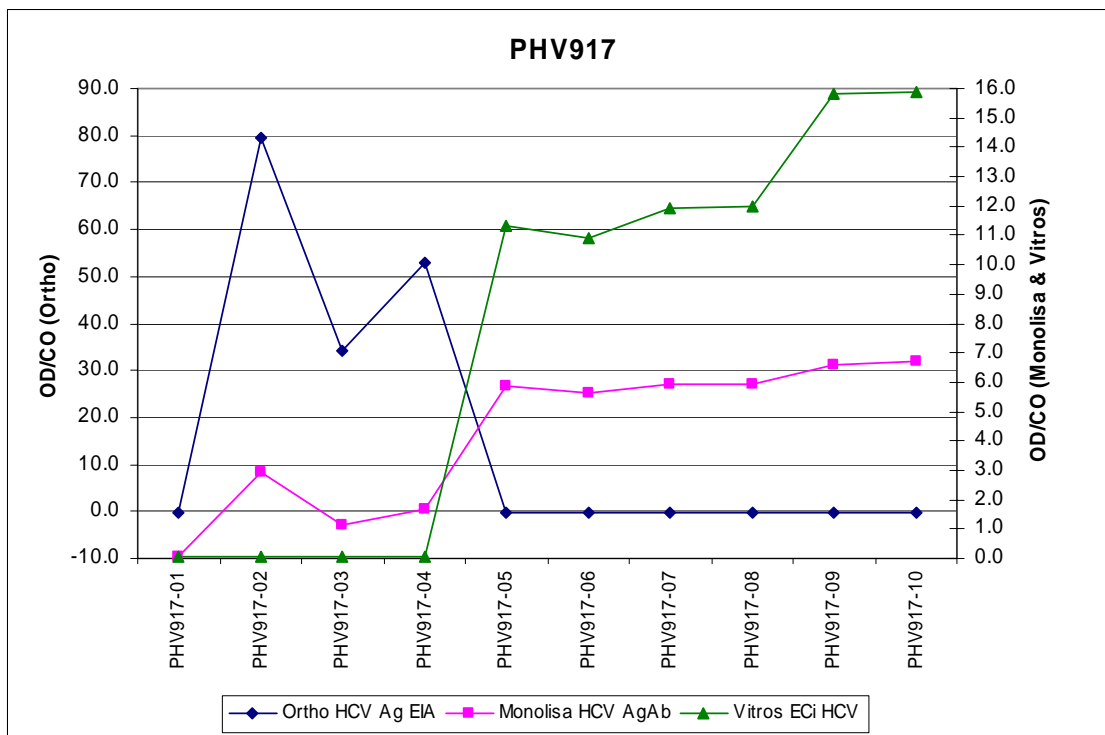


Figure 6: OD/CO values for PHV917 Ortho HCV Core Ag went from being negative to positive and back to negative. Monolisa and Vitros both began negative and became positive; Monolisa was reactive before Vitros.

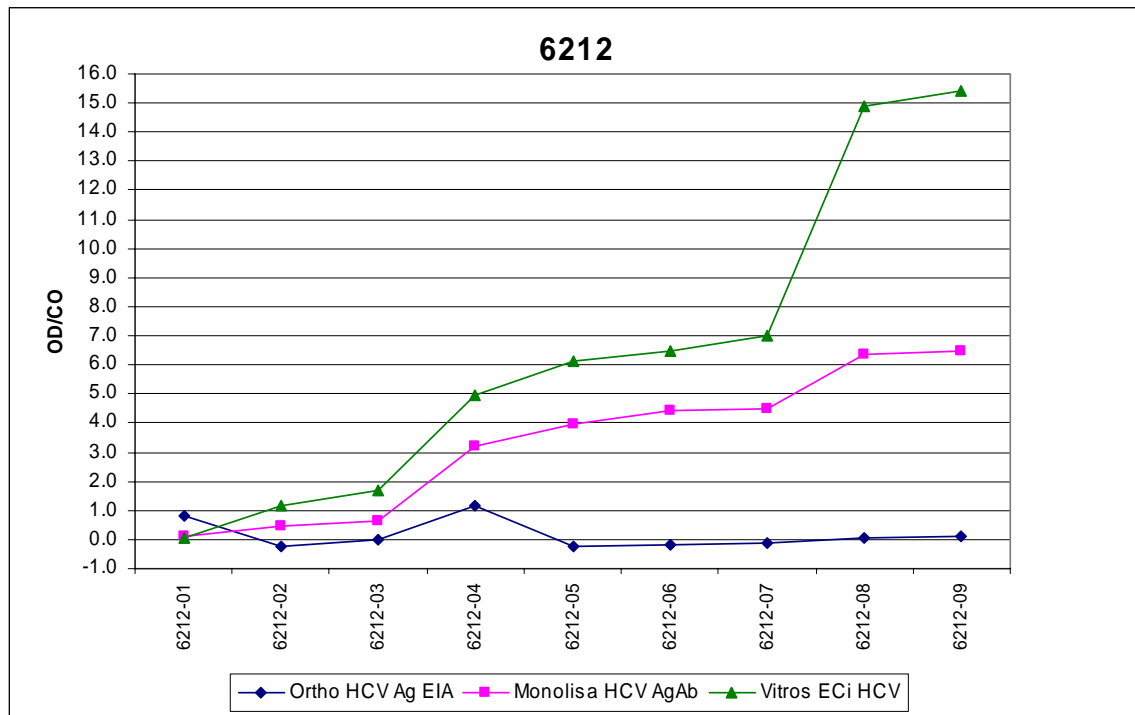


Figure 7: OD/CO values for 6212. Ortho HCV Core Ag was only reactive at member -04. Monolisa and Vitros both began negative and became positive.

Comparative timing of detection

Using a method that assigns the most sensitive test “Time zero” and any test less sensitive a positive value, we found Ortho HCV Core Ag EIA detected HCV infection on average 2.8 days later than PCR and was the most sensitive EIA (Table 5, Figure 8). It is important to note that this analysis only examines the time taken to detect the first positive specimen; it does not take into account any results after the first positive. This is significant when analysing antigen-only kits since these will usually detect positive specimens early in seroconversion but then become negative due to suppression of antigen by the antibody response.

Table 5: Comparative timing of detection (19 panels)

Anti-HCV assay	Product number	Overall delay in detecting seroconversion compared with the most sensitive assay		
		Range (days)	Mean (days)	Median (days)
PCR	N/A	0 - 9	0.5	0
Ortho HCV Ag EIA**	933255	0 - 23	2.8	0
Monolisa HCV Ag-Ab (0.5 threshold)	72558	0 - 32	5.3	0
Monolisa HCV Ag-Ab (1.0 threshold)	72558	0 - 35	8.0	0
Vitros <i>ECi</i> anti-HCV	1318450	0 - 38	20.1	20
AxSYM [®] HCV version 3.0	3B44-20	0 - 38	20.2	20
Ortho [®] HCV 3.0 ELISA Enhanced SAve (short procedure)	9307401	0 - 41	21.3	20
PRISM [™] anti-HCV	6A52-48	0 - 41	22.0	23
Access [®] HCV Ab PLUS	34330	0 - 41	22.2	23
Monolisa [®] anti-HCV Plus EIA	72312	3 - 41	23.7	25
Abbott HCV EIA 3rd generation	7A16-23	0 - 53	26.7	28
IMx [®] HCV	3A99-20	3 - 164	32.7	26

Notes:

The upper limit of the range and the mean are, to some extent, influenced by the intervals between bleeds for any individual panel. The median value provides a better general guide to each assay's ability to detect seroconversion.

When any assay failed to detect seroconversion by the last sample available in a panel, an arbitrary extra three days delay was allocated to that kit's result for that panel.

** It should be noted that a number of panels tested on Ortho HCV EIA began positive but then became negative. This is not taken into account when calculating the time taken to detect the first positive.

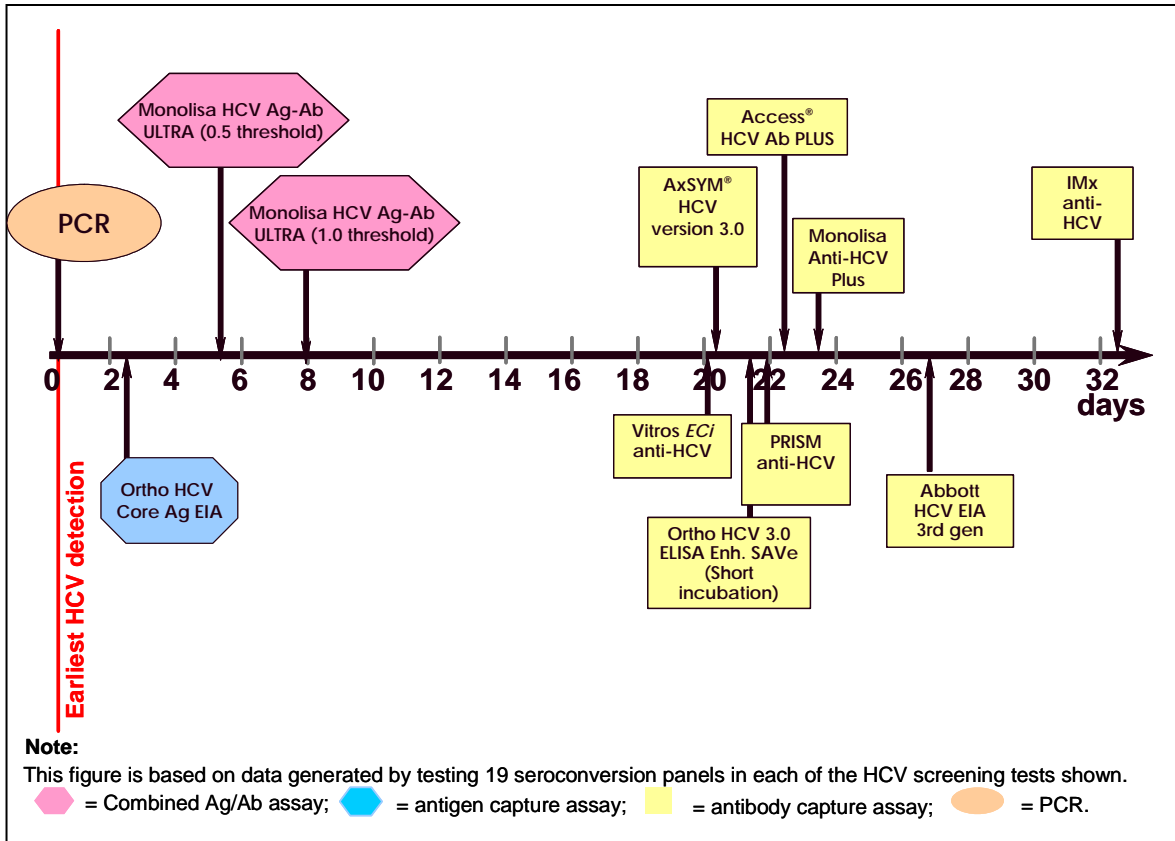


Figure 8: Mean delay of HCV detection. Ortho HCV Ag EIA detected HCV infection on average 2.5 days later than PCR. There is a possibility that if earlier samples were available for some seroconversion panels PCR may detect infection earlier (this will also apply to Ag-only and Ag-Ab EIAs).

Discussion

The seroconversion sensitivity of Ortho HCV Core Ag EIA was assessed by testing 26 commercially available seroconversion panels. When the timing analysis was employed we found that Ortho HCV Core Ag EIA was the most sensitive kit when compared to nine previously evaluated EIAs. It detected seroconversion on average 5.5 days earlier than the Monolisa HCV Ag-Ab kit and 17.6 days earlier than the most sensitive antibody-only EIA. Although by this analysis Ortho HCV Core Ag EIA was the most sensitive kit for detecting seroconversion, the Monolisa HCV Ag-Ab actually detected a greater number of positive reactions overall.

We did not assess the sensitivity of this test using our panel of routine positive specimens because the majority are only positive for anti-HCV antibodies. A few were tested but as expected all gave negative results. A number of specimens that were reported to be PCR positive were also tested and these too gave negative results (data not shown). It is possible that any antigen present may have degraded during long-term freezer storage.

The Ortho HCV Core Ag EIA was originally developed as a donor screening tool to reduce the seroconversion “window period” and was to be used in conjunction with antibody testing. The test was launched around the same time as nucleic acid testing which has since taken over as the method of choice. However, we have shown that this antigen-only EIA could still be suitable for laboratories that do not have the resources for nucleic acid testing providing it is used in an algorithm that includes an antibody detection method.

Appendix

Appendix 1a:

Sample ID	Bleed day	Ortho HCV Ag Only	Monolisa HCV AgAb	Vitros EciHCV	PCR
		OD/CO	OD/CO	S/CO	Status
PHV901-01	0	-0.17	0.12	0.05	Neg
PHV901-02	65	3.38	0.18	0.05	POS
PHV901-03	97	-0.30	5.05	7.85	POS
PHV901-04	99	-0.23	5.15	8.62	POS
PHV901-05	104	-0.30	5.49	9.84	POS
PHV901-06	106	-0.30	5.94	10.30	Neg
PHV901-07	131	-0.20	6.50	14.60	Neg
PHV901-08	139	-0.20	6.39	13.80	Neg
PHV901-09	159	1.60	6.60	16.90	POS
PHV901-10	166	-0.13	6.46	17.00	POS
PHV901-11	203	-0.20	6.89	19.20	POS
PHV904-01	0	2.97	0.17	0.05	Pos
PHV904-02	2	3.07	0.20	0.07	Pos
PHV904-03	7	1.53	0.37	0.75	Pos
PHV904-04	9	1.27	1.80	2.78	Pos
PHV904-05	14	-0.20	3.57	7.35	Pos
PHV904-06	21	-0.23	4.49	9.71	Pos
PHV904-07	23	0.42	4.93	10.60	Pos
PHV905-01	0	0.73	0.06	0.04	Pos
PHV905-02	4	3.10	0.13	0.04	Pos
PHV905-03	7	7.87	0.23	0.07	Pos
PHV905-04	11	1.10	1.07	0.50	Pos
PHV905-05	14	3.33	1.91	1.44	Pos
PHV905-06	18	3.23	4.07	2.31	Pos
PHV905-07	21	1.33	4.52	6.81	Pos
PHV905-08	25	0.10	5.98	15.50	Pos
PHV905-09	28	-0.17	6.19	16.70	Pos
PHV906-01	0	89.37	4.56	5.51	Pos
PHV906-02	2	65.60	4.85	6.31	Pos
PHV906-03	7	43.93	5.69	6.01	Pos
PHV906-04	10	60.77	6.76	9.02	Pos
PHV906-05	14	34.03	6.54	11.00	Pos
PHV906-06	17	47.60	7.09	12.50	Pos
PHV906-07	21	26.60	6.82	13.00	Pos
PHV907-01	0	70.47	1.06	0.05	Pos
PHV907-02	4	101.27	1.36	0.05	Pos
PHV907-03	7	133.37	1.78	0.05	Pos
PHV907-04	13	68.17	1.58	1.83	Pos
PHV907-05	18	111.77	3.22	6.68	Pos
PHV907-06	21	94.83	4.41	8.90	Pos
PHV907-07	164	0.20	6.91	22.80	not done
PHV908-01	0	0.80	0.12	0.06	Pos
PHV908-02	3	0.30	0.13	0.05	Pos
PHV908-03	5	0.63	0.12	0.06	Pos
PHV908-04	11	1.03	0.71	0.65	Pos
PHV908-05	13	4.23	1.05	1.30	Pos
PHV908-06	19	1.83	2.90	2.43	Pos
PHV908-07	25	2.17	4.56	5.04	Pos
PHV908-08	27	0.83	4.94	5.74	Pos
PHV908-09	32	0.77	5.29	7.97	Pos
PHV908-10	35	-0.03	5.78	7.83	Pos
PHV908-11	41	0.93	5.61	10.20	Pos
PHV908-12	45	-0.13	6.06	11.20	Pos
PHV908-13	48	0.87	5.86	10.60	Pos

Appendix 1b:

Sample ID	Bleed day	Ortho HCV Ag Only	Monolisa HCV AgAb	Vitros EciHCV	PCR
		OD/CO	OD/CO	S/CO	Status
PHV909-01	0	17.37	2.06	0.42	Pos
PHV909-02	28	16.30	5.94	11.30	Pos
PHV909-03	30	3.17	5.90	11.50	Pos
PHV910-01	0	2.90	1.78	0.16	Pos
PHV910-02	4	2.57	1.42	0.57	Pos
PHV910-03	8	6.70	4.50	7.04	Pos
PHV910-04	11	2.63	5.35	12.90	Pos
PHV910-05	15	13.80	5.71	15.00	Pos
PHV911-01	0	104.40	6.20	0.05	Pos
PHV911-02	3	95.77	3.82	0.06	Pos
PHV911-03	14	133.50	4.74	6.48	Pos
PHV911-04	21	36.43	5.80	16.40	Pos
PHV911-05	24	34.87	6.47	17.10	Pos
PHV913-01	0	32.67	1.59	0.09	Pos
PHV913-02	2	36.23	2.99	1.13	Pos
PHV913-03	7	59.70	5.84	7.76	Pos
PHV913-04	9	41.17	5.82	8.08	Pos
PHV914-01	0	101.20	1.59	0.05	Pos
PHV914-02	5	53.77	2.04	0.06	Pos
PHV914-03	9	63.93	1.93	0.07	Pos
PHV914-04	12	47.87	1.66	0.23	Pos
PHV914-05	16	31.23	1.82	5.88	Pos
PHV914-06	19	34.80	2.87	8.70	Pos
PHV914-07	24	24.73	4.13	14.20	Pos
PHV914-08	30	7.87	5.55	18.60	Pos
PHV914-09	33	10.20	5.93	19.50	Pos
PHV915-01	0	-0.10	0.12	0.11	Pos
PHV915-02	5	-0.33	0.59	0.67	Pos
PHV915-03	12	-0.13	1.62	2.32	Pos
PHV915-04	14	-0.16	2.91	4.71	Pos
PHV916-01	0	17.61	0.51	0.05	Pos
PHV916-02	2	40.08	1.25	0.05	Pos
PHV916-03	7	24.65	1.42	0.05	Pos
PHV916-04	9	51.91	2.15	0.04	Pos
PHV916-05	16	25.53	1.45	0.38	Pos
PHV916-06	19	33.95	3.23	1.44	Pos
PHV916-07	23	19.01	4.55	4.32	Pos
PHV916-08	28	3.00	4.65	5.39	Pos
PHV917-01	0	-0.11	0.06	0.05	Neg
PHV917-02	13	79.60	2.91	0.05	Pos
PHV917-03	20	34.21	1.12	0.05	Pos
PHV917-04	22	52.77	1.70	0.05	Pos
PHV917-05	85	-0.11	5.90	11.30	Neg
PHV917-06	131	-0.11	5.61	10.90	Neg
PHV917-07	135	-0.20	5.93	11.90	Pos
PHV917-08	138	-0.08	5.96	12.00	Neg
PHV917-09	146	-0.17	6.56	15.80	Neg
PHV917-10	152	-0.11	6.72	15.90	Neg

Appendix 1c:

Sample ID	Bleed day	Ortho HCV Ag Only	Monolisa HCV AgAb	Vitros EciHCV	PCR
		OD/CO	OD/CO	S/CO	Status
6211-26		-0.14	NT	NT	Neg
6211-27	121	0.00	NT	NT	Neg
6211-28	140	9.53	NT	NT	Pos
6211-29	143	3.22	0.24	NT	Pos
6211-30	147	10.01	0.53	NT	Pos
6211-31	150	35.52	1.70	0.08	Pos
6211-32	154	95.97	3.81	0.07	Pos
6211-33	157	84.06	4.47	0.08	Pos
6211-34	161	118.85	4.40	0.06	Pos
6211-35	164	125.66	6.81	0.06	Pos
6211-36	168	111.25	5.91	0.07	Pos
6211-37	171	116.69	4.86	0.06	Pos
6211-38	182	100.51	5.20	2.00	Pos
6211-39	186	88.40	6.13	6.18	Pos
6211-40	189	98.24	7.04	8.96	Pos
6212-01	0	0.78	0.08	0.04	Pos
6212-02	12	-0.23	0.46	1.19	Pos
6212-03	14	-0.03	0.64	1.68	Pos
6212-04	23	1.14	3.22	4.98	Pos
6212-05	26	-0.26	3.94	6.11	Pos
6212-06	32	-0.20	4.45	6.49	Pos
6212-07	37	-0.13	4.49	7.01	Pos
6212-08	53	0.03	6.34	14.90	Pos
6212-09	55	0.13	6.46	15.40	Pos
6213-01	0	-0.23	0.07	0.08	Neg
6213-02	2	3.65	0.10	0.08	Neg
6213-03	8	7.01	0.14	0.09	Neg
6213-04	11	3.62	0.07	0.09	Pos
6213-05	15	3.82	0.31	0.09	Pos
6213-06	18	6.00	0.24	0.07	Pos
6213-07	28	14.05	0.95	0.09	Pos
6213-08	30	2.02	0.40	0.08	Pos
6213-09	35	16.04	0.97	0.15	Pos
6213-10	37	20.67	2.28	2.54	Pos
6213-11	43	14.67	6.01	15.10	Pos
6213-12	46	12.16	5.93	16.00	Pos
6214-01	0	15.59	0.67	0.06	Pos
6214-02	2	15.88	0.64	0.09	Pos
6214-03	8	16.17	0.89	0.06	Pos
6214-04	10	11.25	0.50	0.07	Pos
6214-05	16	12.69	0.49	0.06	Pos
6214-06	18	8.90	0.36	0.06	Pos
6214-07	23	10.24	0.47	0.06	Pos
6214-08	25	24.85	1.02	0.11	Pos
6214-09	30	8.48	2.17	1.92	Pos
6214-10	32	19.86	4.22	4.62	Pos
6214-11	49	2.64	5.52	12.10	Pos
6214-12	53	0.20	5.58	12.10	Pos
6214-13	56	5.15	5.67	12.30	Pos
6215-01	0	79.30	3.91	0.05	Pos
6215-02	3	74.15	3.13	0.04	Pos
6215-03	10	60.49	1.77	0.10	Pos
6215-04	20	18.52	3.93	11.50	Pos

Appendix 1d:

Sample ID	Bleed day	Ortho HCV Ag Only	Monolisa HCV AgAb	Vitros EciHCV	PCR
		OD/CO	OD/CO	S/CO	Status
6216-01	0	-0.23	0.12	0.11	Neg
6216-02	3	-0.20	0.12	0.11	Neg
6216-03	8	-0.26	0.17	0.07	Neg
6216-04	10	0.16	0.11	0.11	Neg
6216-05	15	-0.20	0.12	0.17	Neg
6216-06	17	-0.23	0.14	0.10	Neg
6216-07	23	-0.23	2.56	9.93	Pos
6222-01	0	-0.07	0.07	0.05	Neg
6222-02	2	-0.16	0.08	0.06	Neg
6222-03	17	17.15	1.25	0.07	Pos
6222-04	19	25.83	2.16	0.05	Pos
6222-05	24	73.89	4.10	0.05	Pos
6222-06	26	59.67	3.54	0.05	Pos
6222-07	36	76.21	4.87	0.39	Pos
6222-08	40	42.91	5.83	4.62	Pos
6229-01	0	61.09	4.56	NT	Pos
6229-02	3	100.77	4.01	NT	Pos
6229-03	7	69.73	4.16	NT	Pos
6229-04	10	101.30	5.88	NT	Pos
6229-05	17	53.30	3.23	NT	Pos
6229-06	20	83.16	4.58	NT	Pos
6229-07	24	63.11	5.18	NT	Pos
6229-08	28	38.30	5.57	NT	Pos
9041-01	0	-0.20	0.05	0.05	Neg
9041-02	24	6.82	0.30	0.05	Pos
9041-03	27	45.26	1.67	0.05	Pos
9041-04	31	71.09	2.29	0.05	Pos
9041-05	62	24.91	6.73	16.70	Pos
9041-06	64	22.92	6.95	17.50	Pos
9041-07	69	8.87	6.99	18.30	Pos
9041-08	71	2.54	7.08	20.30	Pos
9044-01	0	80.67	2.24	0.06	Pos
9044-02	4	30.91	1.55	0.06	Pos
9044-03	17	44.61	1.95	0.06	Pos
9044-04	21	44.61	2.24	0.87	Pos
9044-05	25	24.13	4.42	5.30	Pos
9044-06	29	26.45	5.18	6.71	Pos
9045-01	0	39.03	1.42	0.06	Pos
9045-02	2	43.66	1.10	0.05	Pos
9045-03	7	35.28	1.39	0.05	Pos
9045-04	9	34.47	1.68	0.06	Pos
9045-05	26	29.32	1.44	0.06	Pos
9045-06	32	20.58	0.70	0.05	Pos
9045-07	37	24.55	2.75	2.30	Pos
9045-08	41	3.46	3.51	4.71	Pos
9047-01	0	89.30	3.20	0.05	Pos
9047-02	2	97.85	4.41	0.07	Pos
9047-03	10	63.59	2.48	0.06	Pos
9047-04	12	79.37	3.04	0.07	Pos
9047-05	19	46.77	2.07	0.05	Pos
9047-06	21	34.63	1.89	0.06	Pos
9047-07	28	15.51	2.97	2.67	Pos
9047-08	30	28.23	4.95	8.11	Pos
9047-09	35	27.56	6.14	13.80	Pos
9047-10	37	16.79	6.50	15.60	not done