

Evaluation of MONOLISA HCV Ag-Ab ULTRA

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Background and Description of the Assay

MONOLISA HCV Ag-Ab ULTRA is a two-step enzyme immunoassay for the detection of Hepatitis C virus (HCV) infection in human serum and plasma. The assay is the first HCV ELISA designed to detect both HCV antibody and antigen. It is based on the combination of an indirect test for antibody detection and a sandwich test for core antigen detection. Upon completion of the assay, the development of colour indicates the presence of HCV, while no colour development suggests the absence of HCV. The assay includes Sample and Reagent Addition Monitoring; thus the addition of samples and reagents can be measured colorimetrically. The kit has a CE Mark and further assay information is shown in Table 1. The assay was evaluated to determine its ability to detect HCV.

Table 1: Assay Information

General	
Assay name	MONOLISA HCV Ag-AB ULTRA
Manufacturer / UK agent	BIO-RAD
Product number	72556 / 72558
Number of tests in one pack	96 / 480
Specimen volume	50µL

Presentation	
Assay type	two-Step Sandwich / indirect ELISA
Solid phase	12 x 8 microtitre plate wells
Coating	Monoclonal anti-HCV Capsid Antibodies Two recombinant proteins from NS3 region: genotype 1 and 3a One recombinant antigen from non-structural region NS4 A mutated peptide from the HCV capsid
Conjugate 1	Biotinylated mouse anti-HCV capsid antibodies
Conjugate 2	Peroxidase labelled Mouse anti-Human IgG antibodies Peroxidase labelled Streptavidin
Substrate	TMB
Controls per plate	5
Negative control	1
Antibody positive control [Pos1]	3
Antigen positive control [Pos2]	1
Reading wavelength	450 / 630
Cut-off computation	Mean [Pos1] / 4
Equivocal zone	n/a

Stages	
Preparation / sample well loading	30 minutes
Prewash of reaction plate	n/a
Incubation status	Static
Sample + Conjugate 1 incubation	90 minutes 37°C
Conjugate 2 incubation	30 minutes 37°C
Number of washes	5
Substrate incubation (time/temp)	30 minutes 18-25°C
Stop solution incubation	4 minutes
Reading	5 minutes
Total incubation times	154 minutes
Approximate time to completion	190 minutes
Number of optional procedures	none

Additional equipment required	
Incubator, type not specified (*Dry 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 evaluation panel consisted of 599 specimens (Table 2). Of these, 200 specimens were from HCV negative blood donors', 200 from HCV positive subjects, 197 from twenty-five commercial seroconversion panels and two quality control samples. Eighty-eight specimens were tested against a second kit batch (Table 3).

The method described in the kit insert was strictly followed. Briefly, 100µL of conjugate 1 followed by 50µL of specimens or controls was added to each of the microplate wells. The wells were incubated at 37°C for 90 minutes then washed five times. 100 µL of conjugate 2 was added to all wells, which were then incubated for a further 30 minutes at 37°C. The microplate was washed five times then 80µL 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 the stop solution was added and the plates were read at 450/630nm.

Results were interpreted using two thresholds; a standard threshold of 1.0 where results with an OD/CO greater than 1.0 were classed as positive and a lower threshold where results with an OD/CO greater than 0.5 were classed as positive.

Table 2: Evaluation panel (Lot 5B1513)

Sample category	Number
1. Anti-HCV negative	200
2. Anti-HCV positive	200
3. 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	10
BCP6212	9
BCP6213	12
BCP6214	13
BCP6215	4
BCP6216	7
BCP6222	8
BCP9041	8
BCP9044	6
BCP9045	8
BCP9047	10
4. Quality control samples	
HPA: HCV-QC1	6x 1
NIBSC QC	6x 1
TOTAL (number of specimens)	599

Notes:

BBi = Boston Biomedica Inc; BCP = BioClinical Partners Inc (Zeptometrix)

HPA = Health Protection Agency

NIBSC = National Institute for Biological Standards and Control.

Table 3: Batch 2 evaluation panel (Lot: 5B1013)

Sample category	Number
1. Anti-HCV negative	19
2. Anti-HCV positive	19
3. HCV seroconversion panels	
BBI: PHV914	9
BCP6214	10
BCP9045	9
BCP9047	10
TOTAL (number of specimens)	76
Notes:	
BBI = Boston Biomedica Inc; BCP = BioClinical Partners Inc (Zeptometrix)	

Specificity Findings

Of the 200 HCV negative blood donor specimens there were no reactive specimens. A reactive rate of 0% (95% confidence interval, 0 – 1.8%) was therefore observed (Table 4). No retesting was carried out.

At the request of BioRad we also investigated how a positive / negative threshold of 0.5, instead of 1.0 would affect the specificity. In this case there was no difference in specificity between the two thresholds as the maximum OD/CO was 0.385. The distribution of the initial reactivities is shown in Figure 1.

Appendix 1 shows a table of false positive rates for 13 previously evaluated HCV screening kits.

Table 4: Specificity of Monolisa HCV Ag-Ab ULTRA

HCV negative blood donors	Number tested	Number reactive	Number reactive	Range OD/CO	Mean OD/CO	Median OD/CO	Specificity
Stored < 6 mths	200	Initial	0	0.013 - 0.385	0.067	0.054	100%
		Repeat	N/A	N/A	N/A	N/A	N/A

Sensitivity Findings (routine positives)

All of the 200 randomly selected HCV positive specimens were detected by the assay. A sensitivity of 100% (95% confidence interval, 98.2 – 100%) was therefore observed (Table 5, Figure 1).

Appendix 2 shows a table of sensitivities for 13 previously evaluated HCV screening kits.

Table 5: Sensitivity of Monolisa HCV Ag-Ab ULTRA

	Number tested	Number initially negative	Number repeatedly negative	Range OD/CO	Mean OD/CO	Median OD/CO
Anti -HCV Positive Specimens	200	0	0	2.00 - 7.73	6.61	6.88

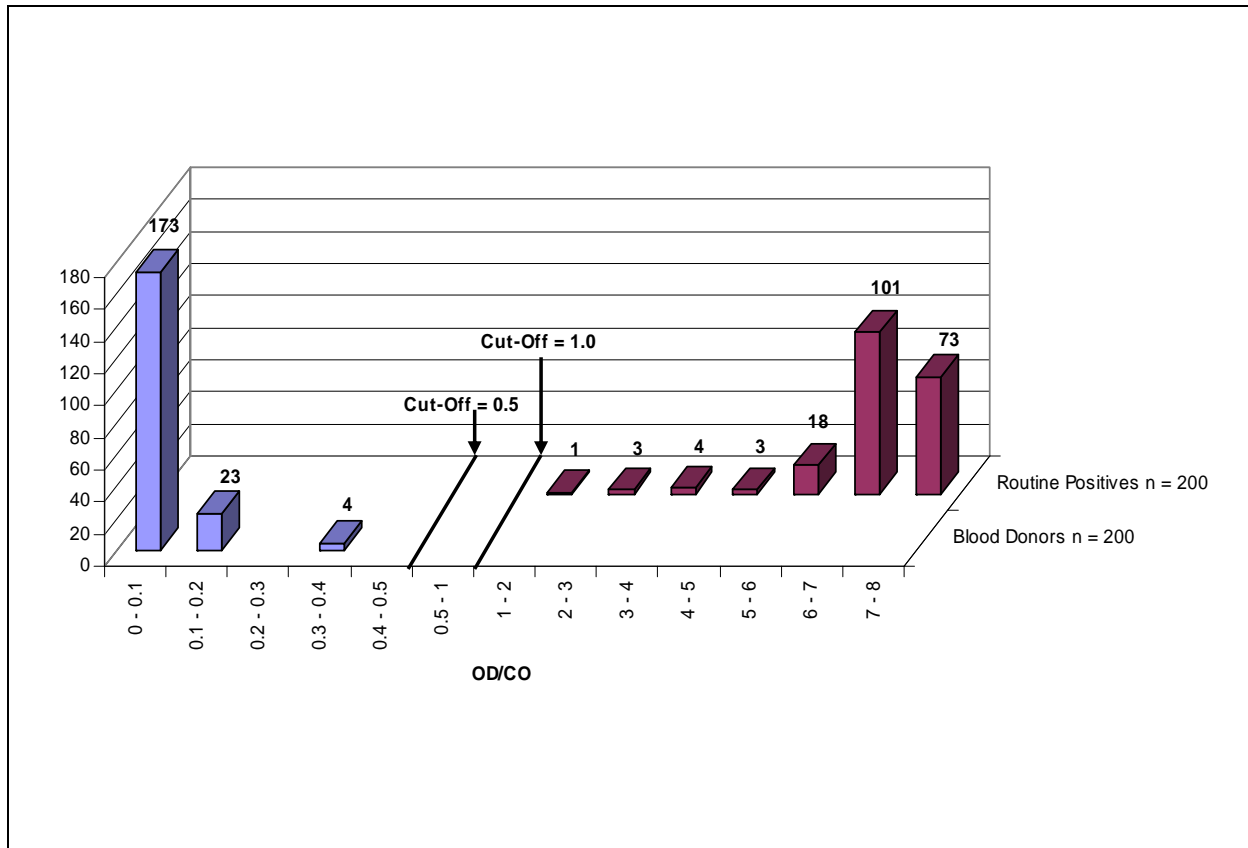


Figure 1: Distribution of initial reactivities

Seroconversion Sensitivity: Aggregate scores

The ability of the Monolisa HCV Ag-Ab ULTRA assay to detect early antigen or antibody in 19 seroconversion panels was compared with previous evaluation results for 8 other antibody-only HCV kits. When employing a positive/negative threshold of 0.5, the aggregate score for Monolisa HCV Ag-Ab ULTRA assay was 121 out of 164. When a threshold of 1.0 was employed the kit gave a score of 108 out of 164 and was the most sensitive kit. The next most sensitive kit was Vitros *ECi* anti-HCV with a score of 72 (Table 6).

It was also possible to compare results of 25 panels with three other HCV kits. In this case Monolisa HCV Ag-Ab ULTRA was still ranked as the most sensitive test (Table 7).

The OD/CO results for the 25 seroconversion panels as well as one additional panel (BCP6229) are shown in *Appendix 3*. Five of the 26 panels (PHV914, PHV917, BCP6214, BCP9045 and BCP9047) initially gave unexpected results with the Monolisa Ag/Ab kit. For each of these panels the result for the first bleed was positive, but one or two later bleeds gave negative results. This was observed when employing both the 1.0 and 0.5 thresholds. All of the panels were retested against a fresh aliquot that had been stored at -60°C . A fresh aliquot of BCP6213 was also tested as a control as this panel did not show any unexpected results when tested initially. The results of the repeat testing are described below and are shown in Table 8.

Upon retesting there was some variation in results compared with the initial test; in some instances there were no negative results after the first positive result but in other cases this still occurred. PHV914 initially had two negative results (bleeds -02 and -05) after the first positive, however when this panel was retested all members were positive. The same was observed for PHV917 where bleeds -03 and -04 were negative in the first aliquot but were positive when the second aliquot was tested.

BCP6213 did not give any unexpected results for the first aliquot, and the same results were observed for the second aliquot when the threshold of 1.0 was applied. With the 0.5 threshold there was one negative result (bleed -08) following the first positive. The OD/CO for this panel member was much lower than for the previous and following members suggesting that this is an anomalous result.

BCP6214 was negative until bleed -07 for both aliquots when the threshold of 1.0 was applied. When the sensitised threshold of 0.5 was applied, there were two negative specimens (bleeds -05 and -06) in the aliquot that had been stored at -40°C. The second aliquot tested gave three negative results (bleeds -05, -06 and -07) at the lower threshold. It should be noted that all OD/CO values were slightly lower for the second aliquot tested.

The first aliquot of BCP9045 was negative until bleed -06 when the 1.0 threshold was used. All panel members apart from bleed -06 were positive when using the 0.5 threshold. The second aliquot tested had only one negative specimen (bleed -06) and this became positive when a threshold of 0.5 was applied.

BCP9047 had one negative result (bleed -06) when the first aliquot was tested with a threshold of 1.0, there were no negatives when the second aliquot was tested.

All of the panels that were repeated initially showed an increasing trend in the OD/CO value, this value would then suddenly decrease, sometimes to below the cut-off, and would then continue increasing again (Figure 2). This trend was also seen for the panels that were not repeated, but here the drop in OD/CO did not affect the positive / negative status of the specimen. The decrease could indicate an earlier window period where antigen is no longer detectable and antibodies have not yet reached a high enough concentration to be detected by the kit giving a negative result midway through the panel. Comparison with an antigen-only kit could confirm this hypothesis. Repeating the tests has also shown that there was possibly some degradation of the panels that were stored at -40°C; this is particularly true for PHV917 and BCP9045.

Table 6: Combined Seroconversion Scores (19 panels)

Panel	Number of specimens in panel	Monolisa HCV AgAb ULTRA (cut-off = 0.5)	Monolisa HCV AgAb ULTRA (cut-off = 1.0)	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	Abbott anti-HCV 3rd gen EIA
PHV901	11	9 (97)	9 (97)	9 (97)	9 (97)	9 (97)	9 (97)	9 (97)	9 (97)	9 (97)	9 (97)
PHV904	7	4 (9)	4 (9)	4 (9)	4 (9)	4 (9)	3 (14)	4 (9)	3 (14)	4 (9)	3 (14)
PHV907	7	7 (0)	7 (0)	4 (13)	3 (18)	3 (18)	4 (13)	2 (21)*	1 (164)	2 (21)	2 (21)
PHV908	13	10 (11)	9 (13)	9 (13)	10 (11)	8 (19)	8 (19)	8 (19)*	3 (41)	7 (25)	5 (32)
PHV909	3	3 (0)	3 (0)	2 (28)	0 (>30)	2 (28)	2 (28)	2 (28)*	0 (>30)	2 (28)	2 (28)
PHV910	5	5 (0)	5 (0)	3 (8)	3 (8)	3 (8)	3 (8)	3 (8)*	2 (11)	2 (11)	3 (8)
PHV913	4	4 (0)	4 (0)	3 (2)	0 (>9)	2 (7)	2 (7)	0 (>9)*	0 (>9)	2 (7)	2 (7)
PHV914	9	9 (0)	9 (0)	5 (16)	5 (16)	5 (16)	5 (16)	3 (24)*	3 (24)	4 (19)	4 (19)
PHV915	4	3 (5)	2 (12)	2 (12)	3 (5)	1 (14)	0 (>14)	2 (12)*	2 (12)	1 (14)	0 (>14)
6212	9	7 (14)	6 (23)	8 (12)	8 (12)	8 (12)	6 (23)	8 (12)*	8 (12)	4 (32)	2 (53)
6213	12	6 (28)***	3 (37)	3 (37)	3 (37)	2 (43)	2 (43)	2 (43)*	2 (43)	2 (43)	2 (43)
6214	13	13 (0)***	6 (25)	5 (30)	6 (25)	5 (30)	5 (30)	5 (30)*	3 (49)	4 (32)	3 (49)
6215	4	4 (0)	4 (0)	1 (20)	1 (20)	1 (20)	1 (20)	1 (20)*	1 (20)	1 (20)	1 (20)
6216	7	1 (23)	1 (23)	1 (23)	1 (23)	1 (23)	1 (23)	1 (23)*	0 (>23)	0 (>23)	1 (23)
6222	8	6 (17)	6 (17)	1 (40)	1 (40)	1 (40)	1 (40)	1 (40)	0 (>43)	1 (40)	0 (>40)
9041	8	6 (27)	6 (27)	4 (62)	4 (62)	4 (62)	4 (62)	4 (62)*	4 (62)	4 (62)	4 (62)
9044	6	6 (0)	6 (0)	2 (25)	3 (21)	2 (25)	2 (25)	2 (25)*	2 (25)	2 (25)	1 (29)
9045	8	8 (0)	8 (0)***	2 (37)	2 (37)	2 (37)	2 (37)	2 (37)*	2 (37)	1 (41)	0 (>41)
9047	10	10 (0)	10 (0)	4 (28)	4 (28)	4 (28)	4 (28)	4 (28)*	4 (28)	3 (30)	3 (30)
Total**	164	121	108	72	70	66	64	63	47	55	47
PHV905	9	6 (11)	6 (11)	5 (14)	5 (14)	5 (14)	3 (21)	NT	2 (25)	4 (18)	2 (25)
PHV906	7	7 (0)	7 (0)	7 (0)	7 (0)	7 (0)	7 (0)	NT	5 (7)	7 (0)	4 (10)
PHV911	5	5 (0)	5 (0)	3 (14)	3 (14)	3 (14)	3 (14)	NT	2 (21)	3 (14)	3 (14)
PHV916	8	7 (2)	7 (2)	3 (19)	4 (16)	2 (23)	2 (23)	NT	NT	2 (23)	NT
PHV917	10	9 (13)	9 (13)	6 (85)	6 (85)	NT	6 (85)	NT	NT	NT	NT
6211	10	11 (147)	10 (150)	3 (182)	3 (182)	3 (182)	2 (186)	NT	0 (>189)	2 (186)	2 (186)
Product Number		72558	72558	131 8450	3B44-20	9307401	34330	6A5248	3A99-20	72312	7A16-23
Lot Number		5B1513	5B1513	0100	65113LU00	GECV028	194822	10143 HP00	12220 HP00	6C501.U	12027 HP00

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

Table 7: Combined seroconversion scores (25 panels)

Panel	Number of specimens in panel	Monolisa HCV AgAb ULTRA (cut-off = 0.5)	Monolisa HCV AgAb ULTRA (cut-off = 1.0)	Vitros ECi anti-HCV	AxSYM® HCV version 3.0	Access® HCV Ab PLUS
PHV901	11	9 (97)	9 (97)	9 (97)	9 (97)	9 (97)
PHV904	7	4 (9)	4 (9)	4 (9)	4 (9)	3 (14)
PHV905	9	6 (11)	6 (11)	5 (14)	5 (14)	2 (21)
PHV906	7	7 (0)	7 (0)	7 (0)	7 (0)	7 (0)
PHV907	7	7 (0)	7 (0)	4(13)	3 (18)	4 (13)
PHV908	13	10 (11)	9 (13)	9 (13)	10 (11)	7 (19)
PHV909	3	3 (0)	3 (0)	2 (28)	0 (≥33)	2 (28)
PHV910	5	5 (0)	5 (0)	3 (8)	3 (8)	3 (8)
PHV911	5	5 (0)	5 (0)	3 (14)	3 (14)	3 (14)
PHV913	4	4 (0)	4 (0)	3 (2)	0 (≥12)	2 (7)
PHV914	9	9 (0)	9 (0)	5 (16)	5 (16)	5 (16)
PHV915	4	3 (5)	2 (12)	2 (12)	3 (5)	0 (>14)
PHV916	8	7 (2)	7 (2)	3 (19)	4 (16)	2 (23)
PHV917	10	9 (13)	9 (13)	6 (85)	6 (85)	6 (85)
6211	10	11 (147)	10 (150)	3 (182)	3 (182)	2 (186)
6212	9	7 (14)	6 (23)	8 (12)	8 (12)	6 (23)
6213	12	6 (28)**	3 (37)	3 (37)	3 (37)	2 (43)
6214	13	13 (0)**	6 (25)	5 (30)	6 (25)	5 (30)
6215	4	4 (0)	4 (0)	1 (20)	1 (20)	1 (20)
6216	7	1 (23)	1 (23)	1 (23)	1 (23)	1 (23)
6222	8	6 (17)	6 (17)	1(40)	1(40)	1 (40)
9041	8	6 (27)	6 (27)	4 (62)	4 (62)	4 (62)
9044	6	6 (0)	6 (0)	2 (25)	3 (21)	2 (25)
9045	8	8 (0)	8 (0)**	2 (37)	2 (37)	2 (37)
9047	10	10 (0)	10 (0)	4 (28)	4 (28)	4 (28)
Product Number		72558	72558	131 8450	3B44-20	34330
Lot Number		5B1513	5B1513	0100	65113LU00	194822
Score*	197	166	152	99	98	85

Notes:

*The score 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

Table 8: Data for seroconversion panels that gave unexpected results

Sample ID	Repeat results (-60°C aliquot)					Initial results (-40°C aliquot)		
	OD (2)	CO (2)	OD/CO (2)	Status (CO=1)	Status (CO=0.5)	OD (1)	CO (1)	OD/CO (1)
PHV914-01	0.527	0.331	1.595	Pos	Pos	0.415	0.339	1.224
PHV914-02	0.675	0.331	2.042	Pos	Pos	0.249	0.339	0.735
PHV914-03	0.639	0.331	1.933	Pos	Pos	0.592	0.339	1.746
PHV914-04	0.550	0.331	1.664	Pos	Pos	0.624	0.339	1.841
PHV914-05	0.602	0.331	1.822	Pos	Pos	0.225	0.339	0.664
PHV914-06	0.947	0.331	2.865	Pos	Pos	0.410	0.339	1.209
PHV914-07	1.366	0.331	4.133	Pos	Pos	0.851	0.339	2.510
PHV914-08	1.833	0.331	5.546	Pos	Pos	1.995	0.339	5.885
PHV914-09	1.961	0.331	5.933	Pos	Pos	2.069	0.339	6.103
PHV917-01	0.020	0.331	0.061	Neg	Neg	0.023	0.340	0.068
PHV917-02	0.962	0.331	2.911	Pos	Pos	0.461	0.340	1.358
PHV917-03	0.370	0.331	1.120	Pos	Pos	0.154	0.340	0.454
PHV917-04	0.561	0.331	1.697	Pos	Pos	0.261	0.340	0.769
PHV917-05	1.950	0.331	5.900	Pos	Pos	2.273	0.340	6.694
PHV917-06	1.855	0.331	5.613	Pos	Pos	2.185	0.340	6.434
PHV917-07	1.959	0.331	5.927	Pos	Pos	2.172	0.340	6.396
PHV917-08	1.970	0.331	5.961	Pos	Pos	2.421	0.340	7.129
PHV917-09	2.169	0.331	6.563	Pos	Pos	2.578	0.340	7.592
PHV917-10	2.220	0.331	6.717	Pos	Pos	2.559	0.340	7.536
6213-01	0.024	0.348	0.069	Neg	Neg	0.057	0.321	0.178
6213-02	0.035	0.348	0.101	Neg	Neg	0.033	0.321	0.103
6213-03	0.047	0.348	0.135	Neg	Neg	0.035	0.321	0.109
6213-04	0.025	0.348	0.072	Neg	Neg	0.024	0.321	0.075
6213-05	0.108	0.348	0.311	Neg	Neg	0.060	0.321	0.187
6213-06	0.085	0.348	0.244	Neg	Neg	0.081	0.321	0.252
6213-07	0.332	0.348	0.955	Neg	Pos	0.154	0.321	0.480
6213-08	0.138	0.348	0.397	Neg	Neg	0.240	0.321	0.748
6213-09	0.338	0.348	0.972	Neg	Pos	0.223	0.321	0.695
6213-10	0.793	0.348	2.280	Pos	Pos	0.673	0.321	2.097
6213-11	2.089	0.348	6.007	Pos	Pos	2.047	0.321	6.379
6213-12	2.062	0.348	5.930	Pos	Pos	2.120	0.321	6.606
6214-01	0.232	0.348	0.667	Neg	Pos	0.249	0.321	0.776
6214-02	0.223	0.348	0.641	Neg	Pos	0.210	0.321	0.654
6214-03	0.308	0.348	0.886	Neg	Pos	0.221	0.321	0.689
6214-04	0.174	0.348	0.500	Neg	Pos	0.175	0.321	0.545
6214-05	0.170	0.348	0.489	Neg	Neg	0.148	0.321	0.461
6214-06	0.126	0.348	0.362	Neg	Neg	0.125	0.321	0.390
6214-07	0.162	0.348	0.466	Neg	Neg	0.207	0.321	0.645
6214-08	0.355	0.348	1.021	Pos	Pos	0.386	0.321	1.203
6214-09	0.754	0.348	2.168	Pos	Pos	0.820	0.321	2.555
6214-10	1.467	0.348	4.219	Pos	Pos	1.493	0.321	4.652
6214-11	1.921	0.348	5.524	Pos	Pos	2.059	0.321	6.416
6214-12	1.939	0.348	5.576	Pos	Pos	2.050	0.321	6.388
6214-13	1.972	0.348	5.671	Pos	Pos	2.125	0.321	6.622
9045-01	0.493	0.348	1.418	Pos	Pos	0.294	0.321	0.916
9045-02	0.384	0.348	1.104	Pos	Pos	0.289	0.321	0.901
9045-03	0.485	0.348	1.395	Pos	Pos	0.219	0.321	0.682
9045-04	0.584	0.348	1.679	Pos	Pos	0.215	0.321	0.670
9045-05	0.500	0.348	1.438	Pos	Pos	0.192	0.321	0.598
9045-06	0.243	0.348	0.699	Neg	Pos	0.133	0.321	0.414
9045-07	0.957	0.348	2.752	Pos	Pos	0.834	0.321	2.599
9045-08	1.220	0.348	3.508	Pos	Pos	1.178	0.321	3.671
9047-01	1.113	0.348	3.201	Pos	Pos	0.757	0.321	2.359
9047-02	1.534	0.348	4.411	Pos	Pos	0.862	0.321	2.686
9047-03	0.861	0.348	2.476	Pos	Pos	0.489	0.321	1.524
9047-04	1.057	0.348	3.040	Pos	Pos	0.565	0.321	1.761
9047-05	0.719	0.348	2.068	Pos	Pos	0.351	0.321	1.094
9047-06	0.657	0.348	1.889	Pos	Pos	0.258	0.321	0.804
9047-07	1.032	0.348	2.968	Pos	Pos	0.801	0.321	2.496
9047-08	1.723	0.348	4.955	Pos	Pos	1.528	0.321	4.761
9047-09	2.135	0.348	6.140	Pos	Pos	2.023	0.321	6.304
9047-10	2.262	0.348	6.505	Pos	Pos	2.043	0.321	6.366

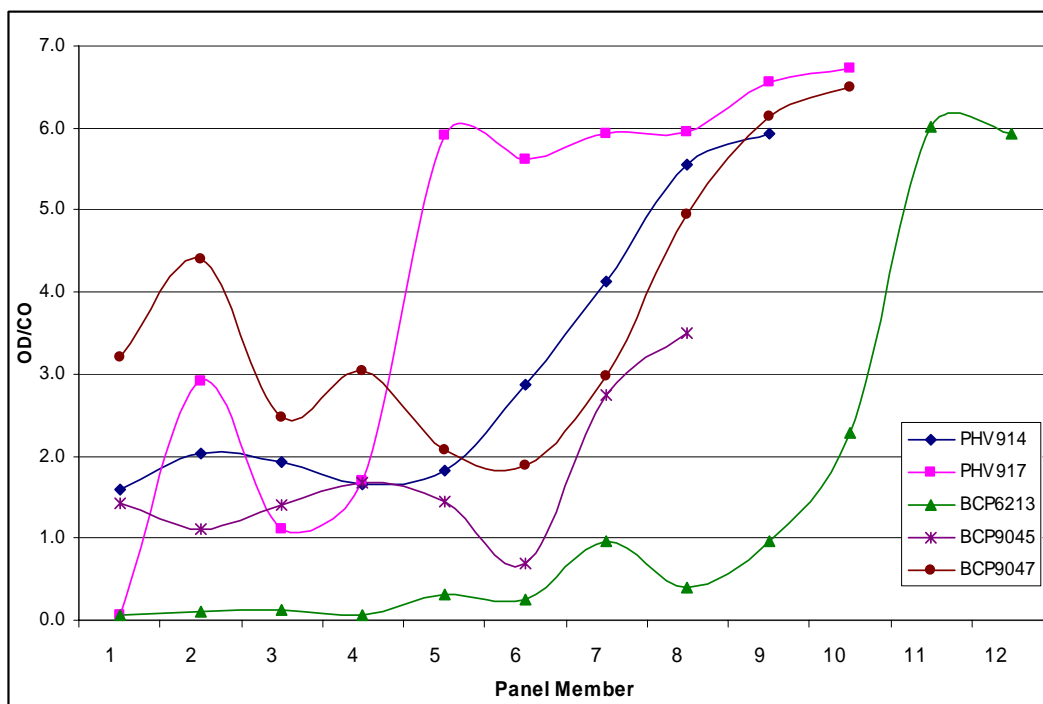


Figure 2: OD/CO values for repeated seroconversion panels

Seroconversion Sensitivity: 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 that Monolisa HCV Ag-Ab ULTRA detected HCV infection on average 12.1 days earlier than any other anti-HCV kit when the standard threshold of 1.0 was used. When a threshold of 0.5 was used Monolisa HCV Ag-Ab ULTRA detected HCV infection on average 14.8 days earlier than any other anti-HCV kit (Table 9, Figure 3). The median detection time for Monolisa HCV Ag/Ab was 0 days which was 20 days earlier than any other anti-HCV kit. The median delay is not affected in the same way as the mean delay which can be strongly influenced by outlying results from seroconversion panels for which the interval between the last negative and the first positive specimen is long. This can give rise to an artefact due to the timing of blood collection.

The method described above looks at the average time of detection of infection for each test. The difference between each test can then be calculated by simple subtraction. Using a more sophisticated method the Statistics Unit at HPA – Centre for Infections analysed the data and calculated the average time for detection of HCV infection when compared to PCR. This was done by calculating the difference in the time to first positive between Monolisa HCV Ag-Ab ULTRA and PCR for each individual seroconversion panel. The average of the differences was then taken. This method was also used to compare Monolisa HCV Ag-Ab ULTRA with Vitros ECi anti-HCV (Table 10a).

Monolisa HCV Ag-Ab ULTRA was shown to detect HCV infection on average, 6 days later than PCR when the 1.0 threshold was used; and to 4 days when using the sensitised threshold of 0.5. The test was also shown to detect HCV infection at least 15 days earlier than Vitros ECi anti-HCV, increasing to 17 days when the sensitised threshold was used.

A second statistical analysis was also carried out, this analysis takes into account the fact that the first positive would actually occur in the interval between the last negative result and the first positive result and therefore gives a more accurate estimation of the detection of seroconversion.

The results showed that Monolisa HCV Ag-Ab detects infection on average, 6 days later than PCR when the 1.0 threshold was used; and to only 3 days when using the sensitised threshold of 0.5 (Table 10b).

Table 9: Comparative timing of detection

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 - 0	0.0	0
Monolisa HCV Ag-Ab (0.5 threshold)	72558	0 - 32	4.8	0
Monolisa HCV Ag-Ab (1.0 threshold)	72558	0 - 32	7.5	0
Vitros <i>ECi</i> anti-HCV	1318450	0 - 38	19.6	20
AxSYM [®] HCV version 3.0	3B44-20	0 - 38	19.7	20
Ortho [®] HCV 3.0 ELISA Enhanced SAVe (short procedure)	9307401	0 - 38	20.8	20
PRISM [™] anti-HCV	6A52-48	0 - 38	21.6	23
Access [®] HCV Ab PLUS	34330	0 - 38	21.7	23
Monolisa [®] anti-HCV Plus EIA	72312	3 - 41	23.3	25
Abbott HCV EIA 3rd generation	7A16-23	0 - 53	26.3	28
IMx [®] HCV	3A99-20	3 - 164	32.3	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.

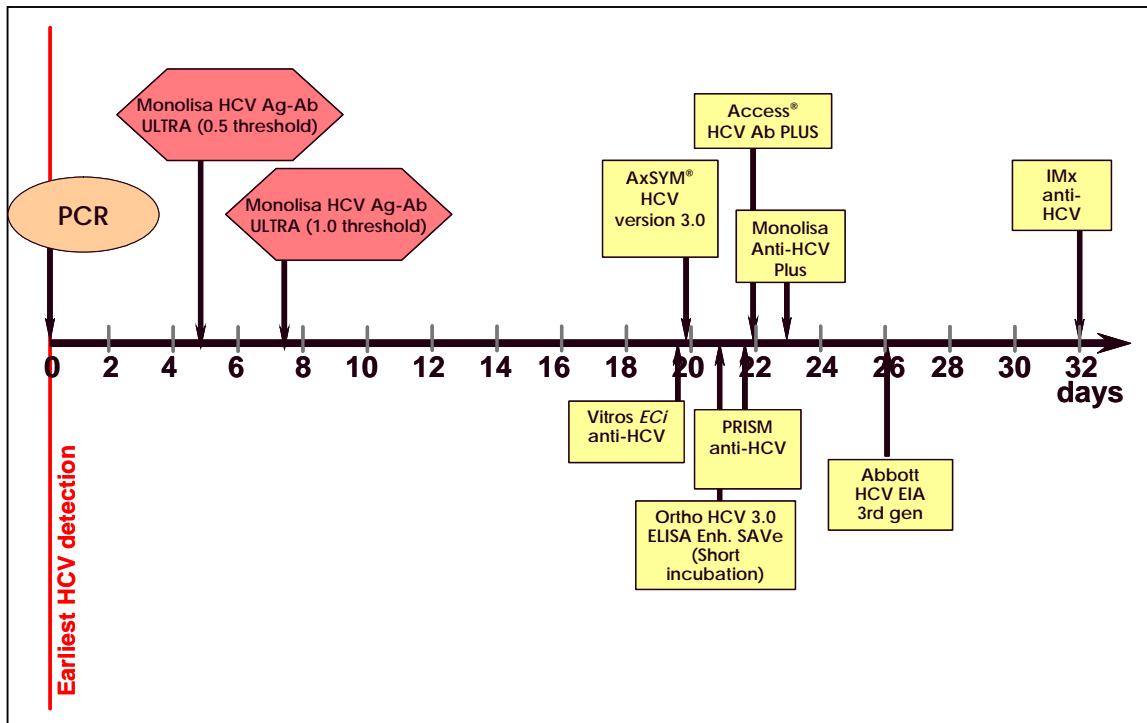


Figure 3: Mean Delay of HCV Detection. Monolisa HCV Ag-Ab ULTRA detected HCV infection on average 4.8 days later than PCR. There is a possibility that if earlier samples were available for some seroconversion panels PCR may detect infection earlier (this will also apply to Ag-Ab ELISAs).

Table 10a: Statistical analysis - Average difference in time to detection of first positive

	[Monolisa] – [Vitros] Average absolute difference [95%CI]; p-value	[Monolisa] – [PCR] Average absolute difference [95%CI]; p-value
Monolisa (threshold = 1.0)	-15 [-22, -7]; p<0.0005	6 [2, 10]; p=0.003
Monolisa (threshold = 0.5)	-17 [-24, -10]; p<0.0005	4 [1, 7]; p=0.01

Table 10b: Statistical analysis - Average difference in time to detection of first positive assuming that the 'actual' first positive is in the interval between the last negative result and the first positive result

	[Monolisa] – [Vitros] Average absolute difference [95%CI]; p-value	[Monolisa] – [PCR] Average absolute difference [95%CI]; p-value
Monolisa (threshold = 1.0)	-10 [-15, -6]; p<0.0005	6 [2, 9]; p=0.001
Monolisa (threshold = 0.5)	-13 [-17, -8]; p<0.0005	3 [1, 4]; p=0.002

Batch Comparison

Two batches of Monolisa HCV Ag-Ab ULTRA were tested to examine variation. The comparison showed that there was no difference in the number of positive specimens detected by the two batches when a threshold of 1.0 was used. When the threshold was set at 0.5 the second batch (5B1013) was found to be slightly more sensitive (Table 11).

Table 11: Batch Comparison

Specimen Category	Number of specimens	Number of reactive specimens (threshold = 1.0)		Number of reactive specimens (threshold = 0.5)	
		Batch Number		Batch Number	
		5B1513	5B1013	5B1513	5B1013
HCV positive	19	19	19	19	19
PHV914	9	9	9	9	9
BCP6214	10	6	6	11	12
BCP9045	9	2	2	7	8
BCP9047	10	9	9	10	10
Total	57	45	45	56	58

Conclusion

The Monolisa HCV Ag-Ab Ultra kit allows simultaneous detection of HCV core antigen and anti-HCV. This significantly reduces the window period of HCV detection when compared to currently available ELISA kits. The kit showed excellent sensitivity and specificity when tested against a small number of routine positive and negative specimens.

When seroconversion sensitivity was assessed using a statistical method that takes into account the fact that the first positive would actually occur in the interval between the last negative result and the first positive result we found there was a 10 day improvement in detection of early infection when compared to antibody-only kits. This improvement increased to 13 days when the sensitised threshold of 0.5 was used. When compared to PCR, Monolisa HCV Ag-Ab was shown to detect infection between 3 and 6 days later, depending on the threshold used. This study does not address how use of the Monolisa Ag/Ab assay with single specimens compares with PCR detection on specimen pools of 48, as currently employed by the NBS.

The test was very easy to use and did not require a great deal of hands on time. This kit is suitable for any laboratory currently using an antibody-only EIA for HCV screening and for laboratories that are considering replacing PCR for a cheaper and simpler alternative.

Appendix 1: False positive rates for 13 previously evaluated HCV screening kits

Assay	Product code	Number tested	Number initially reactive (IR)	Initial reactive rates (95% confidence intervals)	Number repeatedly reactive (RR)	Repeat reactive rates (95% confidence intervals)
Abbott HCV EIA 3rd generation	7A16-23	199	0	0.00% (0 – 1.8%)	0	0.00% (0 – 1.8%)
Access [®] HCV	34310	181	2	1.10% (0.1 – 3.9%)	0	0.00% (0 – 2.0%)
Access [®] HCV Ab PLUS	34330	373	2	0.54% (0.1 – 1.9%)	0	0.00% (0 – 1.0%)
AxSYM [®] HCV version 3.0	3B44-20	377	3	0.80% (0.2 – 2.3%)	0	0.00% (0 – 1.0%)
Monolisa HCV Ag-Ab ULTRA	72558	200	0	0.00% (0 – 1.8%)	0	0.00% (0 – 1.8%)
Monolisa [®] anti-HCV Plus v2 Lot 9M512T	72317/18	1232*	0	0.00% (0 – 0.3%)	0	0.00% (0 – 0.3%)
Monolisa [®] anti-HCV Plus v2 Lot 9M513U	72317/18	1056*	0	0.00% (0 – 0.3%)	0	0.00% (0 – 0.3%)
Ortho [®] HCV 3.0 ELISA enh. SAVe (standard incubation - MiDAS data)	9307401	262	0	0.00% (0 – 1.4%)	0	0.00% (0 – 1.4%)
Ortho [®] HCV 3.0 ELISA enh. SAVe (short incubation)	9307401	1993*	2	0.10% (0 – 0.4%)	1	0.10% (0 – 0.3%)
PRISM [™] anti-HCV	6A52-48	9743*	20	0.20% (0.1 – 0.3%)	17	0.20% (0.1 – 0.3%)
Monolisa [®] anti-HCV Plus EIA (version 1)	72312	2090*	10	0.48% (0.20 – 0.90%)	6	0.29% (0.10 – 0.60%)
IMx [®] HCV	3A99-20	176	4	2.27% (0.6 – 5.7%)	1	0.57% (0 – 3.1%)
Vitros [®] ECI anti-HCV	1318450	310	2	0.65% (0.08 – 2.31%)	2	0.65% (0.08 – 2.31%)
Note:						
* = data from blood centre specificity evaluations						

Appendix 2: Sensitivity for 13 previously evaluated HCV screening assays

Assay	Product code	Number tested	Number positive (Sensitivity)	95% confidence interval %	Range S/CO	Mean S/CO	Median S/CO
Abbott HCV EIA 3rd generation	7A16-23	230	228 (99.1%)	96.9 – 99.9	0.19 – 6.49	4.68	4.62
Access [®] HCV	34310	177	175 (98.9%)	96.0 – 99.9	0.32 – 447.73	132.67	129.78
Access [®] HCV Ab PLUS	34330	499	499 (100%)	99.3 - 100	1.09 - 11.32	9.28	9.85
AxSYM [®] HCV version 3.0	3B44-20	500	500 (100%)	99.3 – 100	1.92 – 138.32	79.76	86.94
IMx [®] HCV	3A99-20	103	103 (100%)	96.5 – 100	1.84 – 54.22	31.15	30.26
Monolisa HCV Ag-Ab ULTRA	72558	200	200 (100%)	98.2 - 100	2.00 - 7.73	6.61	6.88
Monolisa [®] anti-HCV Plus version 1	72312	101	101 (100%)	96.4 – 100	1.21 – 10.65	8.50	8.73
Monolisa [®] anti-HCV Plus version 2 lot 9M512T	72317/18	40	40 (100%)	91.2 - 100	7.09 - 12.43	9.95	10.01
Monolisa [®] anti-HCV Plus version 2 lot 9M513U	72317/18	40	40 (100%)	91.2 - 100	8.00 - 13.10	11.06	11.35
Ortho [®] HCV 3.0 ELISA enhanced SAve (standard incubation)	9307401	215	215 (100%)	98.3 – 100	1.34 – 5.03	4.88	4.99
Ortho [®] HCV 3.0 ELISA enhanced SAve (short incubation)	9307401	215	215 (100%)	98.3 – 100	1.06 – 9.12	8.71	9.09
PRISM [™] anti-HCV	6A52-48	114	114 (100%)	96.8 – 100	1.22 – 6.47	4.59	4.75
Vitros <i>ECi</i> anti-HCV	1318450	433	433 (100%)	99.2 – 100	4.17 – 38.2	27.07	26.00

Appendix 3a: Seroconversion Panels BBI: PHV901 – PHV907

Sample ID	OD/CO	Status (CO=1)	Status (CO=0.5)	RNA Detection	Test used at BBI
PHV901-01	0.120	Neg	Neg	Neg	In house PCR method (BBI)
PHV901-02	0.184	Neg	Neg	POS	
PHV901-03	5.047	Pos	Pos	POS	
PHV901-04	5.154	Pos	Pos	POS	
PHV901-05	5.489	Pos	Pos	POS	
PHV901-06	5.943	Pos	Pos	Neg	
PHV901-07	6.499	Pos	Pos	Neg	
PHV901-08	6.385	Pos	Pos	Neg	
PHV901-09	6.600	Pos	Pos	POS	
PHV901-10	6.456	Pos	Pos	POS	
PHV901-11	6.886	Pos	Pos	POS	
PHV904-01	0.172	Neg	Neg	6×10^5	AMPLICOR HCV monitor test
PHV904-02	0.200	Neg	Neg	1×10^5	
PHV904-03	0.375	Neg	Neg	2×10^5	
PHV904-04	1.796	Pos	Pos	2×10^5	
PHV904-05	3.570	Pos	Pos	2×10^4	
PHV904-06	4.494	Pos	Pos	3×10^4	
PHV904-07	4.933	Pos	Pos	1×10^5	
PHV905-01	0.055	Neg	Neg	4×10^5	AMPLICOR HCV monitor test
PHV905-02	0.129	Neg	Neg	5×10^5	
PHV905-03	0.230	Neg	Neg	4×10^5	
PHV905-04	1.065	Pos	Pos	8×10^4	
PHV905-05	1.906	Pos	Pos	3×10^5	
PHV905-06	4.068	Pos	Pos	3×10^5	
PHV905-07	4.516	Pos	Pos	2×10^5	
PHV905-08	5.983	Pos	Pos	7×10^4	
PHV905-09	6.186	Pos	Pos	2×10^4	
PHV906-01	4.562	Pos	Pos	2×10^6	AMPLICOR HCV monitor test
PHV906-02	4.853	Pos	Pos	1×10^6	
PHV906-03	5.692	Pos	Pos	1×10^6	
PHV906-04	6.760	Pos	Pos	4×10^6	
PHV906-05	6.545	Pos	Pos	1×10^6	
PHV906-06	7.094	Pos	Pos	2×10^6	
PHV906-07	6.818	Pos	Pos	1×10^6	
PHV907-01	1.065	Pos	Pos	3×10^6	AMPLICOR HCV monitor test
PHV907-02	1.360	Pos	Pos	2×10^6	
PHV907-03	1.776	Pos	Pos	1×10^6	
PHV907-04	1.578	Pos	Pos	1×10^6	
PHV907-05	3.224	Pos	Pos	1×10^6	
PHV907-06	4.407	Pos	Pos	1×10^6	
PHV907-07	6.906	Pos	Pos	N/K	

Appendix 3b: Seroconversion Panels BBI: PHV908 – PHV915

Sample ID	OD/CO	Status (CO=1)	Status (CO=0.5)	RNA Detection	Test used at BBI
PHV908-01	0.118	Neg	Neg	2×10^5	AMPLICOR HCV monitor test
PHV908-02	0.133	Neg	Neg	3×10^5	
PHV908-03	0.124	Neg	Neg	3×10^5	
PHV908-04	0.711	Neg	Pos	1×10^5	
PHV908-05	1.047	Pos	Pos	2×10^5	
PHV908-06	2.897	Pos	Pos	1×10^5	
PHV908-07	4.561	Pos	Pos	5×10^4	
PHV908-08	4.935	Pos	Pos	8×10^4	
PHV908-09	5.289	Pos	Pos	1×10^5	
PHV908-10	5.776	Pos	Pos	2×10^4	
PHV908-11	5.614	Pos	Pos	1×10^4	
PHV908-12	6.056	Pos	Pos	2×10^4	
PHV908-13	5.864	Pos	Pos	2×10^5	
PHV909-01	2.056	Pos	Pos	1×10^4	AMPLICOR HCV monitor test
PHV909-02	5.941	Pos	Pos	4×10^4	
PHV909-03	5.903	Pos	Pos	2×10^4	
PHV910-01	1.779	Pos	Pos	$>5 \times 10^5$	AMPLICOR HCV monitor test
PHV910-02	1.425	Pos	Pos	$>5 \times 10^5$	
PHV910-03	4.504	Pos	Pos	$>5 \times 10^5$	
PHV910-04	5.345	Pos	Pos	$>5 \times 10^5$	
PHV910-05	5.708	Pos	Pos	$>5 \times 10^5$	
PHV911-01	6.201	Pos	Pos	$>5 \times 10^5$	AMPLICOR HCV monitor test
PHV911-02	3.817	Pos	Pos	$>5 \times 10^5$	
PHV911-03	4.740	Pos	Pos	$>5 \times 10^5$	
PHV911-04	5.797	Pos	Pos	$>5 \times 10^5$	
PHV911-05	6.469	Pos	Pos	$>5 \times 10^5$	
PHV913-01	1.587	Pos	Pos	$>5 \times 10^5$	AMPLICOR HCV monitor test
PHV913-02	2.988	Pos	Pos	$>5 \times 10^5$	
PHV913-03	5.844	Pos	Pos	$>5 \times 10^5$	
PHV913-04	5.817	Pos	Pos	$>5 \times 10^5$	
PHV914-01	1.595*	Pos	Pos	$>5 \times 10^5$	AMPLICOR HCV monitor test
PHV914-02	2.042*	Pos	Pos	$>5 \times 10^5$	
PHV914-03	1.933*	Pos	Pos	$>5 \times 10^5$	
PHV914-04	1.664*	Pos	Pos	$>5 \times 10^5$	
PHV914-05	1.822*	Pos	Pos	$>5 \times 10^5$	
PHV914-06	2.865*	Pos	Pos	$>5 \times 10^5$	
PHV914-07	4.133*	Pos	Pos	$>5 \times 10^5$	
PHV914-08	5.546*	Pos	Pos	$>5 \times 10^5$	
PHV914-09	5.933*	Pos	Pos	$>5 \times 10^5$	
PHV915-01	0.124	Neg	Neg	1×10^4	AMPLICOR HCV monitor test
PHV915-02	0.587	Neg	Pos	1×10^3	
PHV915-03	1.622	Pos	Pos	2×10^4	
PHV915-04	2.906	Pos	Pos	2×10^4	

* = Results from the second aliquot of PHV914 that was tested. Original results are shown in Table 8.

Appendix 3c: Seroconversion Panels BBI: PHV916 – PHV917, BCP6211 – 6212

Sample ID	OD/CO	Status (CO=1)	Status (CO=0.5)	RNA Detection	Test used at BBI
PHV916-01	0.507	Neg	Pos	3×10^5	AMPLICOR HCV monitor test
PHV916-02	1.248	Pos	Pos	$>5 \times 10^5$	
PHV916-03	1.419	Pos	Pos	$>5 \times 10^5$	
PHV916-04	2.148	Pos	Pos	$>5 \times 10^5$	
PHV916-05	1.445	Pos	Pos	$>5 \times 10^5$	
PHV916-06	3.230	Pos	Pos	$>5 \times 10^5$	
PHV916-07	4.555	Pos	Pos	4×10^5	
PHV916-08	4.649	Pos	Pos	2×10^5	
PHV917-01	0.061*	Neg	Neg	BLD	AMPLICOR HCV monitor test
PHV917-02	2.911*	Pos	Pos	$>5 \times 10^5$	
PHV917-03	1.120*	Pos	Pos	$>5 \times 10^5$	
PHV917-04	1.697*	Pos	Pos	$>5 \times 10^5$	
PHV917-05	5.900*	Pos	Pos	BQR	
PHV917-06	5.613*	Pos	Pos	BQR	
PHV917-07	5.927*	Pos	Pos	3×10^3	
PHV917-08	5.961*	Pos	Pos	BLD	
PHV917-09	6.563*	Pos	Pos	BLD	
PHV917-10	6.717*	Pos	Pos	BQR	
6211-29	0.236	Neg	Neg	2×10^6	In house PCR method (BCP)
6211-30	0.525	Neg	Pos	2×10^6	
6211-31	1.696	Pos	Pos	$> 5 \times 10^6$	
6211-32	3.808	Pos	Pos	$> 5 \times 10^6$	
6211-33	4.466	Pos	Pos	$> 5 \times 10^6$	
6211-34	4.398	Pos	Pos	$> 5 \times 10^6$	
6211-35	6.808	Pos	Pos	$> 5 \times 10^6$	
6211-36	5.906	Pos	Pos	$> 5 \times 10^6$	
6211-37	4.858	Pos	Pos	$> 5 \times 10^6$	
6211-38	5.201	Pos	Pos	$> 5 \times 10^6$	
6211-39	6.127	Pos	Pos	$> 5 \times 10^6$	
6211-40	7.044	Pos	Pos	$> 5 \times 10^6$	
6212-01	0.083	Neg	Neg	8×10^5	In house PCR method (BCP)
6212-02	0.457	Neg	Neg	2×10^5	
6212-03	0.640	Neg	Pos	9×10^4	
6212-04	3.215	Pos	Pos	1×10^5	
6212-05	3.941	Pos	Pos	5×10^4	
6212-06	4.454	Pos	Pos	8×10^4	
6212-07	4.487	Pos	Pos	8×10^4	
6212-08	6.339	Pos	Pos	2×10^5	
6212-09	6.460	Pos	Pos	4×10^5	

* = Results from the second aliquot of PHV917 that was tested. Original results are shown in Table 8.

Appendix 3d: Seroconversion Panels BCP6213 – 6222

Sample ID	OD/CO	Status (CO=1)	Status (CO=0.5)	RNA Detection	Test used at BBI
6213-01	0.069*	Neg	Neg	$< 1 \times 10^2$	In house PCR method (BCP)
6213-02	0.101*	Neg	Neg	$< 1 \times 10^2$	
6213-03	0.135*	Neg	Neg	$< 1 \times 10^2$	
6213-04	0.072*	Neg	Neg	1×10^5	
6213-05	0.311*	Neg	Neg	4×10^5	
6213-06	0.244*	Neg	Neg	3×10^6	
6213-07	0.955*	Neg	Pos	$> 5 \times 10^6$	
6213-08	0.397*	Neg	Neg	$> 5 \times 10^6$	
6213-09	0.972*	Neg	Pos	2×10^6	
6213-10	2.280*	Pos	Pos	2×10^6	
6213-11	6.007*	Pos	Pos	4×10^6	
6213-12	5.930*	Pos	Pos	5×10^6	
6214-01	0.667*	Neg	Pos	2×10^6	In house PCR method (BCP)
6214-02	0.641*	Neg	Pos	$> 5 \times 10^6$	
6214-03	0.886*	Neg	Pos	$> 5 \times 10^6$	
6214-04	0.500*	Neg	Pos	2×10^6	
6214-05	0.489*	Neg	Neg	5×10^6	
6214-06	0.362*	Neg	Neg	2×10^6	
6214-07	0.466*	Neg	Neg	$> 5 \times 10^6$	
6214-08	1.021*	Pos	Pos	$> 5 \times 10^6$	
6214-09	2.168*	Pos	Pos	$> 5 \times 10^6$	
6214-10	4.219*	Pos	Pos	3×10^6	
6214-11	5.524*	Pos	Pos	1×10^6	
6214-12	5.576*	Pos	Pos	1×10^6	
6214-13	5.671*	Pos	Pos	8×10^5	
6215-01	3.914	Pos	Pos	$> 5 \times 10^6$	In house PCR method (BCP)
6215-02	3.132	Pos	Pos	$> 5 \times 10^6$	
6215-03	1.767	Pos	Pos	$> 5 \times 10^6$	
6215-04	3.929	Pos	Pos	$> 5 \times 10^6$	
6216-01	0.125	Neg	Neg	$< 1 \times 10^2$	In house PCR method (BCP)
6216-02	0.115	Neg	Neg	$< 1 \times 10^2$	
6216-03	0.171	Neg	Neg	$< 1 \times 10^2$	
6216-04	0.109	Neg	Neg	$< 1 \times 10^2$	
6216-05	0.125	Neg	Neg	$< 1 \times 10^2$	
6216-06	0.137	Neg	Neg	$< 1 \times 10^2$	
6216-07	2.558	Pos	Pos	5×10^2	
6222-01	0.071	Neg	Neg	bld	In house PCR method (BCP)
6222-02	0.080	Neg	Neg	bld	
6222-03	1.251	Pos	Pos	3×10^5	
6222-04	2.162	Pos	Pos	6×10^5	
6222-05	4.100	Pos	Pos	7×10^5	
6222-06	3.543	Pos	Pos	8×10^5	
6222-07	4.867	Pos	Pos	2×10^6	
6222-08	5.826	Pos	Pos	5×10^5	

* = Results from the second aliquot of 6213 and 6214 that were tested. Original results are shown in Table 8.

Appendix 3d: Seroconversion Panels BCP6229 – 9047

Sample ID	OD/CO	Status (CO=1)	Status (CO=0.5)	RNA Detection	Test used at BBI
6229-01	4.556	Pos	Pos	8.3 x 10 ⁵	AMPLICOR HCV monitor test
6229-02	4.010	Pos	Pos	7.1 x 10 ⁵	
6229-03	4.157	Pos	Pos	6.5 x 10 ⁵	
6229-04	5.883	Pos	Pos	1.6 X 10 ⁶	
6229-05	3.231	Pos	Pos	5.2 x 10 ⁵	
6229-06	4.581	Pos	Pos	9.2 x 10 ⁵	
6229-07	5.176	Pos	Pos	1.1 X 10 ⁶	
6229-08	5.565	Pos	Pos	7.1 x 10 ⁵	
9041-01	0.047	Neg	Neg	<0.2	Chiron bDNA (MEq/ml)
9041-02	0.299	Neg	Neg	15.78	
9041-03	1.667	Pos	Pos	63.72	
9041-04	2.293	Pos	Pos	>120	
9041-05	6.734	Pos	Pos	72.27	
9041-06	6.952	Pos	Pos	66.27	
9041-07	6.989	Pos	Pos	21.8	
9041-08	7.077	Pos	Pos	11.24	
9044-01	2.237	Pos	Pos	105.9	Chiron bDNA (MEq/ml)
9044-02	1.549	Pos	Pos	52.71	
9044-03	1.954	Pos	Pos	77.18	
9044-04	2.237	Pos	Pos	71.52	
9044-05	4.422	Pos	Pos	72.82	
9044-06	5.182	Pos	Pos	48.83	
9045-01	1.418*	Pos	Pos	17.73	Chiron bDNA (MEq/ml)
9045-02	1.104*	Pos	Pos	16.42	
9045-03	1.395*	Pos	Pos	36.19	
9045-04	1.679*	Pos	Pos	38.07	
9045-05	1.438*	Pos	Pos	44.86	
9045-06	0.699*	Neg	Pos	17.78	
9045-07	2.752*	Pos	Pos	18.36	
9045-08	3.508*	Pos	Pos	2.32	
9047-01	3.201*	Pos	Pos	65.95	Chiron bDNA (MEq/ml)
9047-02	4.411*	Pos	Pos	81.76	
9047-03	2.476*	Pos	Pos	54.85	
9047-04	3.040*	Pos	Pos	64.03	
9047-05	2.068*	Pos	Pos	55.45	
9047-06	1.889*	Pos	Pos	42.97	
9047-07	2.968*	Pos	Pos	9.132	
9047-08	4.955*	Pos	Pos	26.58	
9047-09	6.140*	Pos	Pos	22.77	
9047-10	6.505*	Pos	Pos	nd	

* = Results from the second aliquot of 9045 and 9047 that were tested. Original results are shown in Table 8.

MEq/ml = Milli Equivalentents / ml