



Medicines and Healthcare products
Regulatory Agency

NUMBER

Evaluation Report **MHRA 03137**

MONOLISA anti-HBc PLUS

**MHRA Evaluation Report
MHRA Report number 03137**



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MONOLISA anti-HBc PLUS

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Background

MONOLISA anti-HBc PLUS assay was evaluated to determine its ability to detect antibody to hepatitis B virus core antigen (anti-HBc). This investigation was undertaken by the Microbiological Diagnostics Assessment Service (MiDAS).

Evaluation panel

The evaluation panel consisted of 1,405 specimens. Of these, 500 specimens were from blood donors, 500 from injecting drug users (IDUs), 85 HBsAg positive/ IgM anti-HBc negative serum specimens, 50 antenatal serum specimens, 63 specimens from four commercial anti-HBc seroconversion panels, 15 specimens from one commercial anti-HBc performance panel, 59 specimens that were dilutions of ten positive anti-HBc specimens and replicates of a single 'total' anti-HBc quality control sample. A subset of the main panel was used to assess a second production lot of each assay.

Specificity findings

Specificity was determined using 492 anti-HBc negative blood donor specimens and 180 anti-HBc negative injecting drug user specimens. The MONOLISA anti-HBc PLUS assay had 6 repeatedly reactive specimens, giving a specificity of 99.1%.

Sensitivity findings

Randomly selected positives

Sensitivity was determined using 316 anti-HBc positive injecting drug user specimens. The MONOLISA anti-HBc PLUS assay detected 311 reactive injecting drug user specimens, to give the third highest sensitivity of 98.4%.

Seroconversion panels

Four anti-HBc seroconversion panels were tested and the seroconversion sensitivity of the anti-HBc assay was determined by employing two scoring systems. In the first system the number of positive specimens found for each of the seroconversion panels was added to obtain an aggregate score. The MONOLISA anti-HBc PLUS assay detected 28 anti-HBc positive specimens. To provide better discrimination in the seroconversion sensitivity a second approach was employed in which the highest score was awarded to the assay that first became positive with the highest level of activity, and the lowest score to the assay that became positive last and with the lowest reactivity. With this system the MONOLISA anti-HBc PLUS assay obtained a score of 14 and was the fourth most sensitive assay at detecting seroconversion.

Dilution series

Ten anti-HBc positive specimens were diluted from 1/50 to 1/500,000 in anti-HBc negative human serum and the analytical sensitivity of each of the anti-HBc assays was compared by employing two scoring systems as with seroconversion panels. By adding the number of positives found in each dilution series the MONOLISA anti-HBc PLUS assay detected 35 anti-HBc positive samples. The second scoring system awarded the highest score to the assay that remained positive to the highest titre and with the highest level of activity. The MONOLISA anti-HBc PLUS assay obtained the third highest score of 42.

Evaluation of assay cut-off

The delta values for the MONOLISA anti-HBc PLUS assay are evenly distributed (overall positive delta 2.89, overall negative delta -2.38), indicating that its cut-off has been set to optimise both sensitivity and specificity.

Comparison of two production lots

A subset of the main evaluation panel, consisting of 167 specimens, was used to compare two production lots of each anti-HBc assay. There was generally close agreement between results for the two lots. The only qualitative differences between lots were observed with the blood donor specimens. In the first production lot one reactive blood donor specimen was detected and none were found in the second. Upon repeat testing the one reactive sample in the first production was unreactive.

Conclusion

The sensitivity of the MONOLISA anti-HBc PLUS assay was 98.4% for the anti-HBc IDU specimens, ranking it the third most sensitive anti-HBc assay evaluated. The MONOLISA anti-HBc PLUS assay could have a role in HBV confirmatory testing algorithm, further to characterise HBsAg positive specimens and to assist in determining whether HBV infection is past or current. In many countries anti-HBc assays are used to screen blood donations. In this evaluation the MONOLISA anti-HBc PLUS assay had a specificity of more than 99% and would therefore be a good candidate for blood screening.

Antibody to Hepatitis B virus core antigen (anti-HBc) is a marker of past and current Hepatitis B virus (HBV) infection (1). It is the first HBV antibody marker to appear, and persists for many years following initial infection. During HBV infection, a complex and evolving array of antigens and antibodies may be detected in serum and other biological fluids. Uncomplicated acute HBV infection is characterised by the presence of HBV surface antigen (HBsAg) in serum and the development of IgM anti-HBc (2). During convalescence HBsAg is cleared and IgG anti-HBc, followed by anti-HBs (antibody to HBV surface antigen) develop. In chronic HBV infection HBsAg remains present and anti-HBc is present, but not anti-HBs. Occasionally anti-HBc may be the only marker detected. The finding of anti-HBc reactivity alone most often reflects a waning antibody response in a historic infection but, particularly when at high titre, it may be due to resolution of acute or chronic HBsAg carriage prior to the appearance of anti-HBs. As such, a donor whose specimen has such a profile may still be infectious (3,4).

In the clinical laboratory anti-HBc tests are employed as part of a HBV testing algorithm, used to identify past and current HBV infection, and further to characterise HBsAg positive specimens. Currently UK blood centres do not screen for anti-HBc and rely on highly sensitive HBsAg assays alone to identify donations that may transmit HBV. A number of other countries, including several European countries and the USA, screen blood donors for anti-HBc. Anti-HBc only positive blood donations, with no detectable HBsAg and anti-HBs, may be the source of post-transfusion HBV infection, which anti-HBc screening could prevent (5).

Radioimmunoassays (RIA) for anti-HBc have largely been replaced by enzyme immunoassays (EIA) which have been shown to have similar sensitivity and specificity (6). However, further studies have shown greater variation in the sensitivity and specificity between the anti-HBc EIAs (7, 8).

This report describes the evaluation of the MONOLISA anti-HBc PLUS. The evaluation focused on the sensitivity and specificity of the assay and results were compared with those obtained from the evaluation of other anti-HBc assays, the results of which have been published in previous MHRA evaluations (10).

Each assay was evaluated against the same serum specimen panel, which has been constructed to minimise potential bias. The panel included serum samples from blood donors to determine each assay's specificity. Serum specimens from injecting drug users and antenatal patients consisted of anti-HBc positive and negative sera to determine sensitivity and specificity. Seroconversion panels were used to test the kits' ability to detect anti-HBc at the time of seroconversion. Anti-HBc dilution series were included to test the analytical sensitivity of the kits. HBsAg positive/ IgM anti-HBc negative sera were added as a further challenge of the kits' ability to detect anti-HBc. Quality control sera were used throughout the evaluation to assess inter-run variation. A smaller panel of specimens was tested in a second lot of each kit to assess lot-to-lot reproducibility.

Description of the assay

A summary of the characteristics of the MONOLISA anti-HBc PLUS is provided in [table 1a](#). The table includes specific details of the kit presentation, assay stages and any additional equipment required. Quotations from the instructions supplied with the kit, including details about the performance of the assays and their limitations can be found in [table 1b](#). A digital photograph of the MONOLISA anti-HBc PLUS is shown in [figure 1](#).

The MONOLISA anti-HBc PLUS is an indirect enzyme immunoassay for the detection of total antibodies to Hepatitis B virus core in human serum or plasma. All kit components are stored at 2-8°C and must be brought to room temperature for 30 minutes before use. The substrate solution is diluted 1:11 in substrate buffer and is stable for 6 hours at 18-30°C in the dark. The wash solution is diluted 1:10 in distilled water and is stable for 2 weeks at 2-8°C.

The procedure for the MONOLISA anti-HBc PLUS assay involves adding sample diluent to every well and then adding sample or control to their allocated wells. The plate is left to incubate to allow any anti-HBc in the sample to bind to the rHBcAg coated wells. The plate is washed to remove any unbound material, then the conjugate (peroxidase-labelled goat antibody directed against human IgG and IgM) is added to every well and incubated to allow the conjugate to bind to any antibody present. The plate is again washed and substrate-TMB is added to every well, which will react with any bound conjugate present. The reaction is stopped by adding sulphuric acid and must be read at 450nm with 630nm reference filter within 30 minutes.

Figure 1: MONOLISA anti-HBc PLUS kit

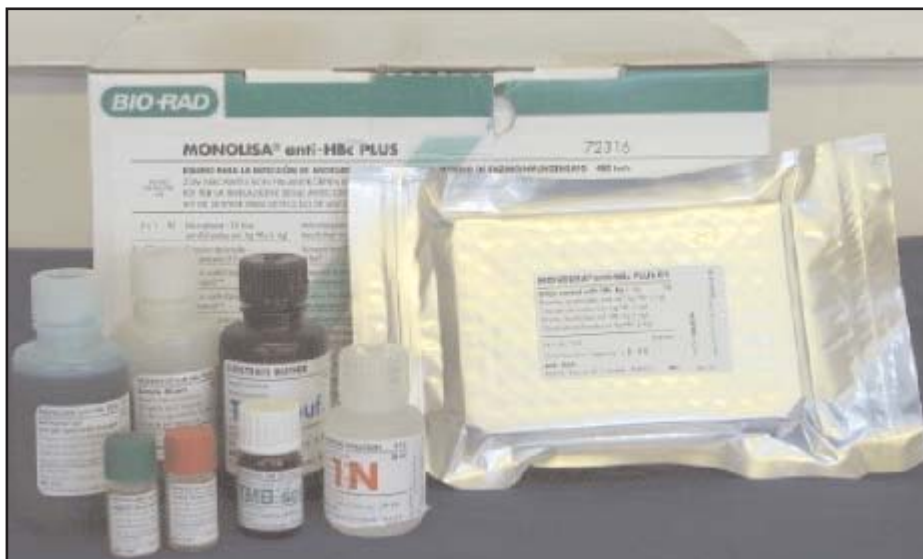


Table 1a: Assay information

General	
Assay name	MONOLISA anti-HBc PLUS
Manufacturer / UK agent	BIO-RAD
Product number	72315/ 72316
Number of tests per kit	96/ 480
UK launch date	
Presentation	
Assay type	Indirect microplate enzyme immunoassay
Sample volume	20µl
Solid phase	rHBcAg coated wells
Conjugate	Peroxidase-labelled goat antibody directed against human IgG & IgM
Substrate	Chromogen (TMB)
Controls per run	2 anti-HBc negative, 3 anti-HBc positive
Cut-off computation	mean positive control/ 5. (reactive >1.00)
Equivocal zone	values 10% below cut-off may be retested
Stages	
Preparation / sample loading	30 minutes/ 96 samples
Incubation status	Dry incubator
Sample incubation	30 minutes at 37°C
Number of washes	4
Conjugate incubation	60 minutes at 37°C
Number of washes	4
Substrate incubation	30 minutes at room temperature
Reading	450nm with 620nm reference filter
Total incubation times	120 minutes
Additional equipment required	
Dry incubator	
Plate washer	
Plate reader	
Micropipettes: 5-50µl	
Multichannel micropipettes: 50-200µl	
Disposable tips	
Disposable reagent troughs	
Distilled water	
Notes:	
TMB = 3, 3', 5, 5'-tetramethylbenzidine	
rHBcAg = Recombinant Hepatitis B virus core antigen	

Table 1b: Claims for the assay and its limitations (quoted from the kit insert)

Claims
<p>Sensitivity studies with MONOLISA anti-HBc PLUS test have been performed on positive samples from patients with chronic Hepatitis B or sensitivity panels with documented samples from patients recently infected by Hepatitis B virus. The MONOLISA anti-HBc PLUS test was shown to be at least as sensitive as the EIA used in reference. The sensitivity was evaluated using the Paul Ehrlich Institute IgG and IgM standards, and the limit of detection was estimated at 0.5 U PEI/ml and 8 U PEI/ml for the IgG and IgM respectively.</p> <p>The specificity of the test on non selected blood bank donors was 99.91% on 4274 tested samples.</p>
Limitations
<p>The colorimetric method for the sample and conjugate desposition verification does not allow to verify the accuracy of the dispensed volume of samples and conjugates. This method shows only the presence of sample and conjugate. The rate of wrong answers with this method is closely linked to the accuracy of the utilized system (cumulated coefficient of variation of dispensing and reading over 10% significantly decrease the quality of the verification).</p>

The evaluation was carried out according to a protocol agreed by the manufacturer (see [Appendix](#)). The manufacturer supplied two lots of the MONOLISA anti-HBc PLUS assay for evaluation (lot 2K1537, expiry 30/07/2003 for lot 1 testing and lot 3B0030, expiry 15/03/2004 for the second lot testing). The kits were tested against a panel of serum specimens designed to be minimally biased and comprised 500 specimens from injecting drug users and 500 specimens from blood donors to determine assay sensitivity and specificity. The panel also included commercially available anti-HBc seroconversion panels to challenge the ability of the anti-HBc assays to detect anti-HBc at the time of seroconversion. Anti-HBc dilution series were included to challenge the ability of the anti-HBc assay to detect increasing titres of anti-HBc. Quality control sera were added to each plate to monitor intraplate and interplate variation. Antenatal samples and HBsAg positive/ IgM anti-HBc negative samples were a final set of specimens added to challenge the ability of the assays to detect anti-HBc ([table 2a](#)). A smaller panel of specimens were tested on a second lot of the assay to assess lot-to-lot variation ([table 2b](#)).

The MONOLISA anti-HBc PLUS tests were performed according to the manufacturer's instructions. The plates were read with a Tecan Columbus washer. The optical densities were read by a Bio-Tek EL808 ultra microplate reader linked to a computer with KC4 software. Before testing the specimen panel a washer efficiency test was conducted to check for carryover. This involved testing multiple replicates of a strong anti-HBc positive specimen interspersed with replicates of an anti-HBc negative specimen.

The assigned anti-HBc status of each specimen in the panel was decided during the first anti-HBc evaluation of the panel using four commercial anti-HBc assays. Any specimens that had given discordant results were retested in duplicate and if the results were repeatedly discordant the specimens were tested with HBsAg and anti-HBs supplementary assays to help determine the anti-HBc status of each specimen. Any specimen in this evaluation that differed from the previous consensus was retested in duplicate and tested by supplementary assays. To allow readers of this report to compare the performance of the MONOLISA anti-HBc PLUS assay to other commercial anti-HBc assays, a comparison with findings from the previous report has been included ([10](#)). The anti-HBc assays previously evaluated were AxSYM CORE (product number B7A410), Bioelisa anti-HBc (3000-1102), Murex anti-HBc total (8G21-01/ 8G21-02) and Vitros anti-HBc (849 6812).

A draft of this report was sent out to BIORAD in November 2003, allowing them the opportunity to comment prior to the publication of the final report. The company's response is reproduced in the [Appendix](#).

Table 2a: Main specimen panel for the evaluation of anti-HBc assays

Sample category	Number	
1. Blood donors'/healthy adults' sera		500
2. Negative human serum (NHS)	2x	1
3. Injecting drug users (IDUs)		500
4. HBsAg 'resolving carrier' specimens (NLBC and BPL)	2x	3
5. HBsAg positive, IgM anti-HBc negative		85
6. Seroconversion panels: total anti-HBc		
BBI - PHM935A		20
BCP - 6278		11
BCP - 6281		12
Profile - RP009		20
7. Performance panels: total anti-HBc		
BBI - PHE102 (IgM anti-HBc low titre)		15
8. Dilution of anti-HBc positive (9 specimens x 6 dilutions - 1 specimen x 5 dilutions)	2x	59
9. Quality control samples		
HPA - total anti-HBc QC serum: sample 1	6x	1
Three replicates of suitable controls on each plate (estimate)		60
10. Ante-natal specimens		50
TOTAL (number of tests)		1405

Notes:

BBI = Boston Biomedica Inc, USA;
 BPL = BioProducts Laboratory, UK
 BCP = BioClinical Partners, USA
 Profile = Pyramid Profile Diagnostics, USA
 NLBC = North London Blood Centre, UK
 HPA = Health Protection Agency, UK

Table 2b: Specimen panel for the assessment of a second batch of the anti-HBc assays

Sample category	Number	
1. Blood donors'/healthy adults' sera		50
2. Negative human serum (NHS)	2x	1
3. Injecting drug users (IDUs)		50
4. HBsAg 'resolving carrier' specimens (NLBC and BPL)	2x	3
5. HBsAg positive, IgM anti-HBc negative		10
6. Dilution of anti-HBc positive (3 specimens x 6 dilutions)	2x	18
7. Quality control samples		
PHLS - total anti-HBc QC serum: sample 1	3x	1
Three replicates of suitable controls on each plate (estimate)		10
TOTAL (number of tests)		167
Notes:		
BPL = BioProducts Laboratory, UK		
NLBC = North London Blood Centre, UK		
HPA = Health Protection Agency, UK		

Key to the presentation of results

The presentation of results is intended to allow readers to draw their own conclusions from the data on the assay. The data are presented as tables and figures summarising:

- Blood donor specimen results ([tables 3-5](#), [figure 2](#))
- Anti-HBc reactivities for specimens from injecting drug users ([tables 6-8](#), [figure 3](#))
- Assay cut-off evaluation ([table 9](#))
- Antenatal specimen anti-HBc reactivities ([Tables 10-11](#))
- Detection of anti-HBc in HBsAg positive/ IgM anti-HBc negative specimens ([table 12](#))
- Detection of anti-HBc in seroconversion panels ([tables 13-14](#), [figures 4-7](#))
- Detection of anti-HBc in dilution series ([Table 15](#))
- Summary of specificity findings ([table 16](#))
- Summary of sensitivity findings ([table 17](#))
- Quality control sera ([table 18](#), [figure 8](#))
- Manufacturer's kit controls ([table 19](#))
- Comparison of two production lots ([table 20](#), [figure 9](#))

Blood donor specimen results

The MONOLISA anti-HBc PLUS assay was evaluated against 500 blood donors' specimens to determine the assay specificity (table 3). The assay was tested on the same set of blood donor specimens as the Bioelisa and Vitros anti-HBc assays and the four specimens previously found to be positive for another HBV marker were removed from the specificity calculation. Any other specimens found to be falsely reactive were repeated in duplicate and tested by supplementary HBV assays (table 4). The MONOLISA testing revealed four more blood donor samples found to be positive for another HBV marker as they were anti-HBs positive and were also removed from the specificity calculation (table 5). Specimens that were confirmed as anti-HBc reactive were also anti-HBs reactive, indicating a recovered infection.

The MONOLISA anti-HBc PLUS assay had 7 initially false positive specimens, of which four were repeatedly reactive. This gave the assay a specificity of 99.2% with the blood donor specimens. This specificity is lower than the Bioelisa anti-HBc and Vitros anti-HBc assays, which both achieved 99.6%. Figure 2 shows the MONOLISA anti-HBc PLUS assay had 4 falsely reactive specimens, two specimens were just below the cut-off and the majority of specimens were far from the cut-off.

The density of anti-HBc positive specimens used in the MiDAS evaluation, where the anti-HBc negative blood donor specimens were interspersed among anti-HBc positive specimens, is unlikely to be encountered elsewhere, even in reference centres. The potential for carryover or cross contamination at centres other than MiDAS is therefore less.

Table 3: Specificity findings for the blood donor specimens

Assay	Total number tested*	Number initially negative	Number repeatedly negative	Specificity (%)**	95% Confidence interval (%)**	Range S/CO**	Mean S/CO**	Median S/CO**
AxSYM	496	496	496	100.0	99.3-100.0	0.44-0.81	0.54	0.54
Bioelisa	492	483	490	99.6	98.6-99.9	0.30-1.23	0.47	0.45
Monolisa	492	485	488	99.2	96.6-99.2	0.06-2.27	0.29	0.22
Murex	498	498	498	100.0	99.3-100.0	0.31-0.90	0.47	0.46
Vitros	492	489	490	99.6	98.6-99.9	0.24-1.31	0.33	0.31

Notes:
 * Confirmed positive sera were removed from total when calculating specificity
 ** Specificity and statistics are based on the repeat reactive rate

Table 4: Summary of results for blood donor specimens that were falsely positive by Monolisa anti-HBc

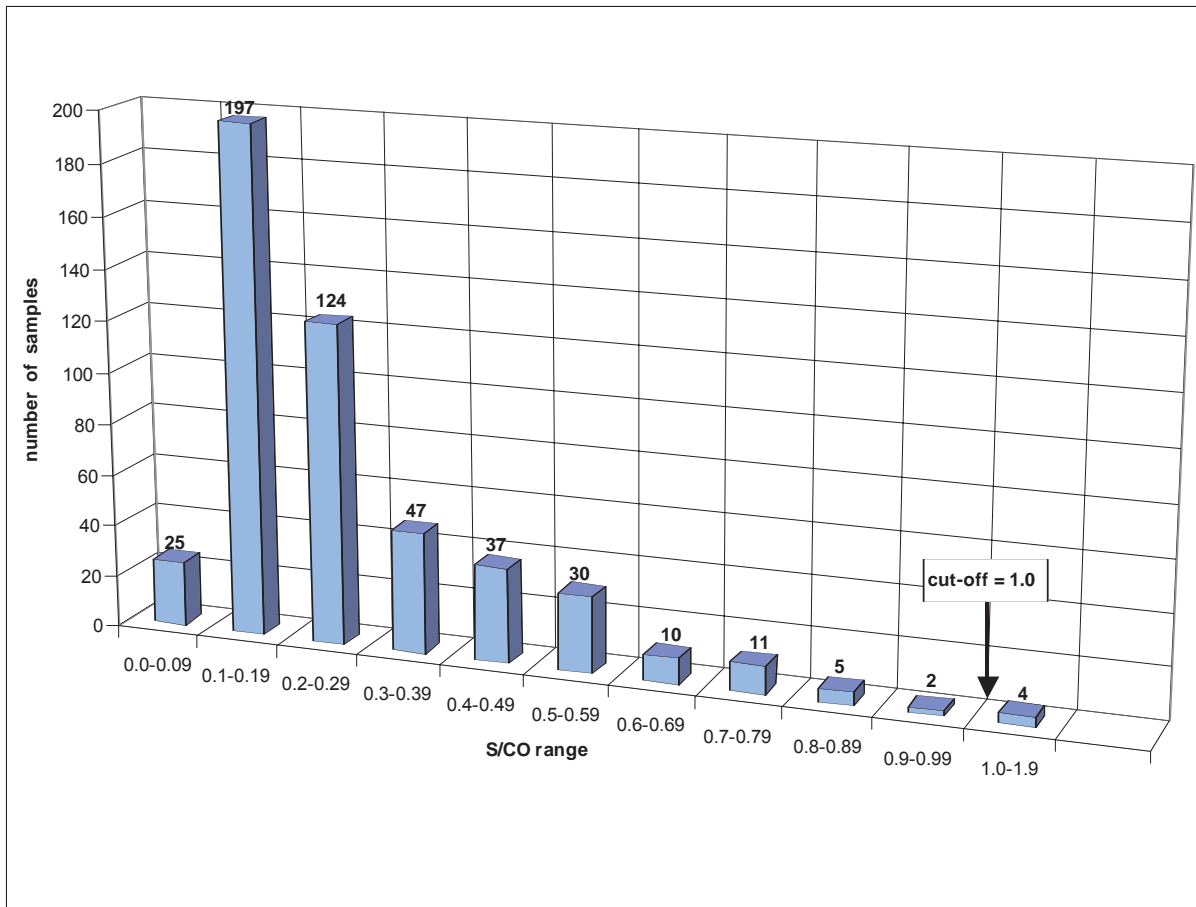
Sample ID	Monolisa testing		Anti-HBc assays			Supplementary assays	
	initial OD/CO	retest OD/CO	Bioelisa CO/OD	Vitros CO/OD	COMPRIA CO/OD	anti-HBs mIU/mL	HBsAg OD/CO
02N0029	1.48	0.65	0.46	0.33	0.27	0.0	0.289
02N0060	1.10	1.25	0.50	0.32	0.34	0.0	0.423
02N0075	1.30	1.22	0.56	0.32	0.29	0.0	0.456
02N0080	1.15	0.23	0.79	0.58	0.34	0.0	0.441
02N0093	1.24	0.49	0.54	0.32	0.28	0.0	0.578
02N0125	2.11	1.94	0.51	0.26	0.29	NT	0.411
02N0283	1.04	1.23	0.34	0.29	0.26	0.0	0.259

Note:
 Any Monolisa sample whose anti-HBc status changed after retesting is highlighted in grey
 NT = Not tested as not enough sample remaining

Table 5: Blood donor specimens assigned positive status after supplementary testing

Sample ID	Monolisa anti-HBc		Anti-HBc assays			Supplementary assays	
	initial OD/CO	retest OD/CO	Bioelisa CO/OD	Vitros CO/OD	COMPRIA CO/OD	anti-HBs mIU/mL	HBsAg OD/CO
0204572	2.28	1.94	0.40	0.29	0.32	403.8	0.441
0204589	1.43	1.63	0.40	0.29	0.30	126.2	0.913
0204622	1.31	1.89	0.46	0.32	0.26	4.1	0.548
02N0193	2.04	2.27	0.38	0.28	0.33	1000.0	0.776

Figure 2: Distribution of S/CO values for blood donor specimens tested by MONOLISA anti-HBc PLUS



Anti-HBc reactivities for specimens from injecting drug users

Specimens from 500 injecting drug users (IDU) consisted of 316 anti-HBc positive and 180 anti-HBc negative specimens and four specimens that were removed from the evaluation. Of the four specimens that were removed, no clear anti-HBc status could be established on two specimens, and no result could be obtained with a previous anti-HBc assay on two specimens.

Of the 316 anti-HBc positive IDU specimens, the MONOLISA anti-HBc PLUS assay initially detected 308 anti-HBc positive specimens. Following retests 311 specimens were detected (table 6). This gave the assay an overall sensitivity of 98.4% with the IDU positive specimens, ranking it the third most sensitive anti-HBc assay with this category of specimen. The sensitivity figures quote the repeat reactive rate as retesting was conducted to confirm the HBV status, however specimens whose anti-HBc status changed from negative to positive would not normally be detected in a HBV testing algorithm.

Of the 180 anti-HBc negative IDU specimens, the MONOLISA anti-HBc PLUS assay initially detected 176 negative IDU specimens. Following retests 178 specimens were detected (table 7). This gave the assay a 98.9% specificity with the IDU negative specimens. Table 8 shows the S/CO ratios for the IDU specimens that were retested on the MONOLISA anti-HBc PLUS assay.

The distribution of the reactivities for IDU specimens tested against the MONOLISA anti-HBc PLUS assay can be seen in figure 3. When specimens were retested because of discordant initial results, the retest result was used for the analysis. Assays with good discrimination have few, or no, specimens wrongly classified and reactions in close proximity to the cut-off. The distribution graph shows the MONOLISA anti-HBc PLUS assay had 5 false negatives spread out below the cut-off and 2 false positives just above the cut-off.

Table 6: Sensitivity findings for 316 anti-HBc positive IDU specimens

Assay	Total number tested	Number initially positive	Number repeatedly positive	Sensitivity (%)*	95% confidence interval (%)*	Range S/CO*	Mean S/CO*	Median S/CO*
AxSYM	316	307	312	98.7	96.8-99.7	0.91-26.32	9.40	9.35
Bioelisa	316	293	295	93.4	90.0-95.8	0.41-546.10	114.88	30.55
Monolisa	316	308	311	98.4	96.3-99.5	0.46-9.84	6.04	6.27
Murex	316	315	314	99.4	97.7-99.9	0.83-21.35	9.33	9.74
Vitros	316	298	301	95.3	92.3-97.3	0.45-50.00	18.62	16.67
COMPRIA	316	306	306	96.8	94.3-98.5	0.48-43.93	10.42	9.26

Notes:
* figures based on the repeat reactive rate

Table 7: Specificity findings for 180 anti-HBc negative IDU specimens

Assay	Total number tested	Number initially negative	Number repeatedly negative	Specificity (%)*	95% confidence interval (%)*	Range S/CO*	Mean S/CO*	Median S/CO*
AxSYM	180	177	179	99.4	96.9-100.0	0.39-1.29	0.55	0.53
Bioelisa	180	177	179	99.4	96.9-100.0	0.32-1.10	0.46	0.44
Monolisa	180	176	178	98.9	96.0-99.9	0.04-2.10	0.26	0.18
Murex	180	180	180	100	98.0-100.0	0.32-0.92	0.49	0.47
Vitros	180	179	179	99.4	96.9-100.0	0.26-2.28	0.35	0.32
COMPRIA	180	180	180	100	98.0-100.0	0.23-0.63	0.31	0.29

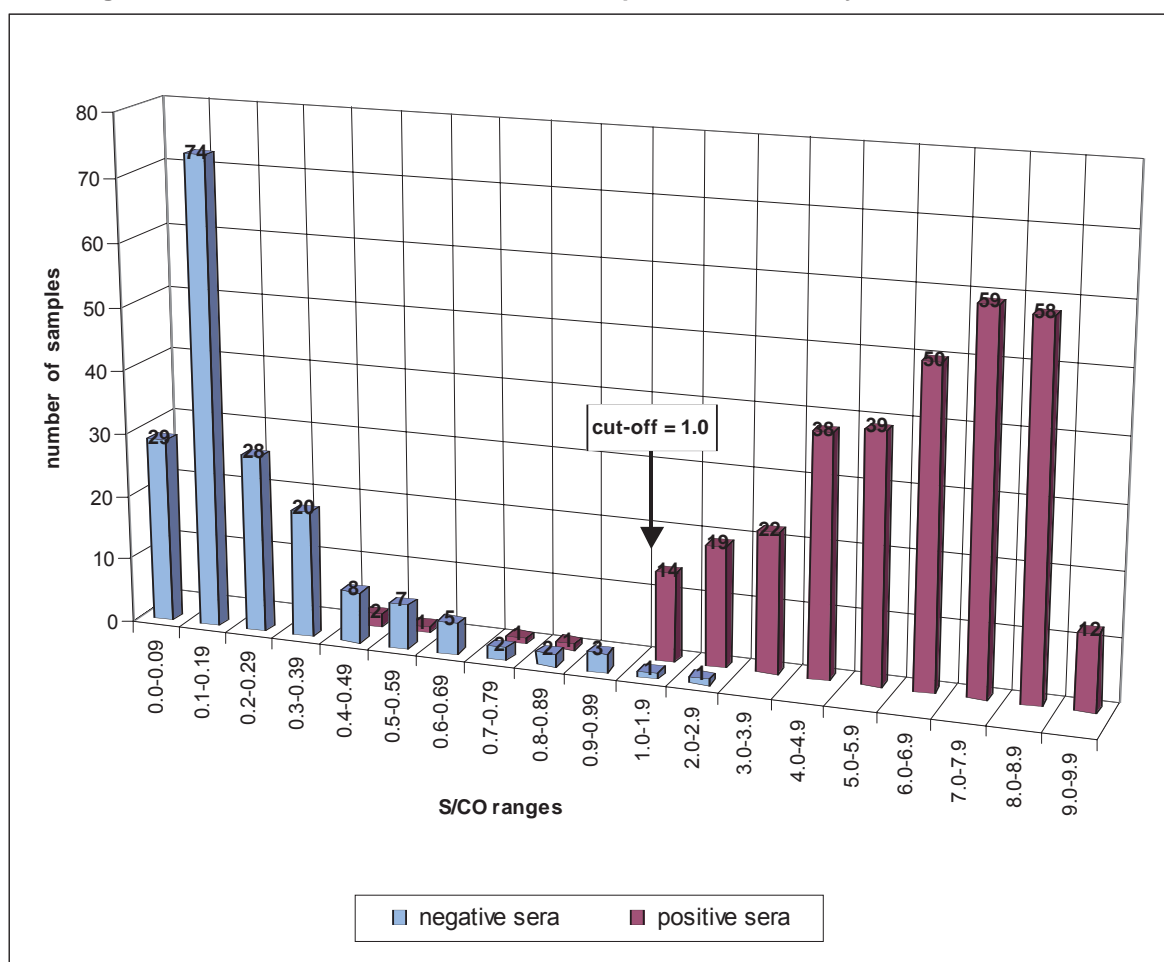
Notes:
* figures based on the repeat reactive rate

Table 8: S/CO ratios for IDU specimens that were retested by MONOLISA anti-HBc PLUS

Sample ID	Monolisa testing		anti-HBc testing				Supplementary tests*		Assigned status
	initial OD/CO	retest OD/CO	AxSYM CO/OD	Bioelisa CO/OD	Murex CO/OD	Vitros CO/OD	HBsAg OD/CO	anti-HBs OD/CO	
0200501	0.68	1.57	3.91	1.23	8.10	1.64	NT	NT	POS
0200512	0.53	0.61	2.00	0.65	2.45	0.81	2.44	1.92	POS
0200519	0.73	0.95	1.28	0.47	1.60	0.68	0.41	2.06	POS
0200529	0.51	2.53	3.22	1.24	3.81	0.97	0.35	24.62	POS
0200537	0.47	2.26	2.02	0.67	4.57	0.65	0.39	3.08	POS
0200545	0.80	0.59	0.91	1.39	2.73	1.68	0.37	1.84	POS
0200682	0.79	0.77	0.92	3.10	1.51	1.67	0.39	1.25	POS
0200527	0.64	0.46	1.21	0.67	1.10	0.72	0.63	0.45	POS
0200386	1.10	1.05	0.85	0.76	0.77	0.49	NT	NT	NEG
0200579	1.01	0.98	0.58	0.59	0.58	0.41	NT	NT	NEG
0200618	1.11	0.87	0.69	0.54	0.89	0.44	NT	NT	NEG
0200684	2.08	2.10	0.45	0.39	0.47	0.29	NT	NT	NEG

Note:
Any sample whose anti-HBc status changed after retesting by ORTHO HBc kit is highlighted in grey
* Supplementary test results taken from the MHRA report (reference 10) which used these results to assist in deciding the anti-HBc status. A sample was assigned positive status if all anti-HBc assays agreed, or were positive for another HBV marker, or the two most sensitive anti-HBc kits (AxSYM & Murex) agreed on the anti-HBc result.
NT = Not tested, as anti-HBc kits evaluated in a previous MHRA report (reference 10) all gave same result

Figure 3: Distribution of reactivities for IDU specimens tested by MONOLISA anti-HBc PLUS



Assay cut-off evaluation

Delta values are a statistical measure, for a particular assay, of the distribution of the population of reactivities given by specimens containing the marker sought (delta positive) and those lacking it (delta negative), benchmarked against the assay's cut-off. They provide an insight into whether the assay's threshold has been set at a level that provides a suitable balance of sensitivity and specificity. The delta values for the positive and negative anti-HBc specimens can be seen in [table 9](#). The delta values for the MONOLISA anti-HBc PLUS assay show a good balance between sensitivity and specificity. The delta values are quite evenly distributed (mean positive delta 2.89, mean negative delta -2.38), suggesting that this cut-off has been set to optimise both sensitivity and specificity.

Table 9: Delta values for 369 anti-HBc positive specimens and approximately 680 anti-HBc negative specimens

Assay	Anti-HBc positive specimens delta values			Anti-HBc negative specimens delta values		
	Injecting drug users (n=316)	HBsAg positive/IgM anti-HBc negative (n=53)	Overall positive delta value (n=369)	Injecting drug users (n=180)	Blood donor specimens (n~500)*	Overall negative delta value (n~680)*
AxSYM	2.50	2.25	2.46	-4.43	-7.38	-6.08
Bioelisa	1.60	2.64	1.65	-4.47	-4.13	-4.22
Monolisa	3.22	2.03	2.89	-2.21	-2.37	-2.38
Murex	3.70	3.27	3.63	-3.64	-4.84	-4.43
Vitros	1.77	2.3	1.81	-5.24	-5.63	-5.52
COMPRIA	2.17	N/A	2.17	-6.75	NT	-6.75

Notes:
 N/A = Not available
 NT = Not tested
 * The number of blood donor specimens and the total number of anti-HBc negative specimens for each assay are: AxSYM CORE 497 & 677, Bioelisa anti-HBc 492 & 676, MONOLISA anti-HBc PLUS 492 & 672 Murex anti-HBc (total) 498 & 678 and Vitros anti-HBc 492 & 676.

Antenatal specimen anti-HBc reactivities

Fifty antenatal specimens, obtained from an area of South London with a relatively high HBV prevalence, were tested against the MONOLISA anti-HBc PLUS assay. This set of antenatal specimens, which contained 8 anti-HBc positive specimens and 42 anti-HBc negative specimens, had also been tested by the Bioelisa and Vitros anti-HBc assays.

The MONOLISA anti-HBc PLUS assay gave a sensitivity of 100% for the positive antenatal specimens (table 10). One repeatedly false positive specimen was found with the negative specimens, to give a specificity of 97.6% (table 11). However, the sample size for the antenatal specimens is too low to make strong statements about sensitivity and specificity.

Table 10: Sensitivity findings for antenatal specimens

Assay	Number of positive samples tested	Total positive	Sensitivity (%)	95% confidence interval (%)	Range S/CO	Mean S/CO	Median S/CO
Antenatal specimens - set 1							
AxSYM	14	14	100.0	76.8-100.0	4.90-25.00	11.89	11.56
Murex	14	14	100.0	76.8-100.0	8.15-14.20	9.99	9.08
COMPRIA	14	14	100.0	76.8-100.0	2.87-18.06	7.75	7.58
Antenatal specimens - set 2							
Bioelisa	8	8	100.0	63.1-100.0	3.53-483.40	89.37	14.02
Monolisa	8	8	100.0	63.1-100.0	1.63-8.63	5.27	5.54
Vitros	8	8	100.0	63.1-100.0	2.13-25.00	13.96	13.33
COMPRIA	8	8	100.0	63.1-100.0	1.94-16.12	7.32	6.63

Table 11: Specificity findings for antenatal specimens

Assay	Number of negative samples tested	Total negative	Specificity (%)	95% confidence interval (%)	Range S/CO	Mean S/CO	Median S/CO
Antenatal specimens - set 1							
AxSYM	36	35	97.2	85.5-99.9	0.49-1.23	0.59	0.56
Murex	36	36	100.0	90.3-100.0	0.35-0.65	0.44	0.42
COMPRIA	36	36	100.0	90.3-100.0	0.27-0.52	0.34	0.31
Antenatal specimens - set 2							
Bioelisa	42	42	100.0	91.6-99.0	0.38-0.96	0.49	0.46
Monolisa	42	41	97.6	87.4-99.9	0.09-2.41	0.32	0.21
Vitros	42	42	100.0	91.6-100.0	0.27-0.78	0.35	0.31
COMPRIA	42	42	100.0	91.6-100.0	0.32-0.49	0.38	0.38

Detection of total anti-HBc in HBsAg positive/ IgM anti-HBc negative specimens

Eighty-five HBsAg positive/ IgM anti-HBc negative specimens were tested by the MONOLISA anti-HBc PLUS assay. Of these, 53 were anti-HBc positive by at least one anti-HBc assay. The MONOLISA anti-HBc PLUS assay detected 49 of the 53 anti-HBc total positive specimens, giving a sensitivity of 92.5% (table 12).

Table 12: Anti-HBc reactivity of 85 HBsAg positive/ anti-HBc IgM negative specimens

Assay	Number samples tested	Number anti-HBc positive	Number positives detected	Sensitivity (%)	95% confidence interval (%)	Range S/CO	Mean S/CO	Median S/CO
AxSYM	83	53	50	94.3	84.3-98.8	0.52-19.23	10.12	10.64
Bioelisa	83	53	51	96.2	87.0-99.5	0.74-527.70	406.89	527.70
Monolisa	83	53	49	92.5	81.8-97.7	0.17-9.79	7.44	8.56
Murex	83	53	52	98.1	89.9-100.0	0.80-15.92	9.04	9.29
Vitros	83	53	52	98.1	89.9-100.0	0.05-100.00	36.94	50.00
COMPRIA*	83	53	48	90.6	79.3-96.9			

Notes:
* Statistics are not given for RIA as results are only expressed as % inhibition

Detection of anti-HBc in seroconversion panels

Four seroconversion panels were included in the sensitivity evaluation of the MONOLISA anti-HBc PLUS assay to assess its ability to detect anti-HBc at the time of seroconversion. One of the seroconversion panels was from Boston Biomedica Inc, two were from BioClinical Partners Inc, and one was from Profile Diagnostics Inc. The number of positive specimens found for each of the seroconversion panels is added to obtain an aggregate score (table 13a). The most sensitive assays detect the most number of positive specimens. The MONOLISA anti-HBc PLUS assay detected 28 anti-HBc positive specimens.

Since there are only a few anti-HBc seroconversion panels a second scoring system was used to show more discrimination in the assay sensitivity. With each panel every assay was given a score. The highest score (6 points since 6 kits have been evaluated) awarded to assay that first became positive and with the highest level of activity. The lowest score (1) to the assay that became positive last with the lowest level of activity. For comparative purposes an aggregate score was obtained for each kit by adding the scores for each of the seroconversion panels (table 13b). The most sensitive assay is deemed to be the one that overall detected anti-HBc in the panels earlier than the other assays, as indicated by obtaining the highest score. The MONOLISA anti-HBc PLUS assay had a score of 14 and was the fourth most sensitive assay with the seroconversion panels. Figures 4-7 illustrate the detection of anti-HBc in the four seroconversion panels. Details of the detection of anti-HBc and the S/CO ratios can be found in the Appendix.

PHE102 is an anti-HBc IgM low titre performance panel containing 15 undiluted serum or plasma specimens from Boston Biomedica Inc. The panel has 14 specimens with reactivities near the sensitivity limits of four anti-HBc IgM tests currently approved by the FDA and one sample with negative reactivity. Table 14 shows the S/CO ratios for the MONOLISA anti-HBc PLUS assay. The MONOLISA anti-HBc PLUS assay detected 13 of the anti-HBc IgM positive specimens, with one false negative specimen.

Table 13a: Comparative detection of anti-HBc in four seroconversion panels (based on the number of reactive samples)

Assay	Seroconversion panels - number of positive samples and days since first bleed in brackets				Aggregate score
	BBI- PHM935A	BCP-6278	BCP-6281	PROFILE-RP009	
AxSYM	8 (66)	2 (37)	4 (41)	16 (29)	30
Bioelisa	8 (66)	3 (33)	4 (41)	15 (31)	30
Monolisa	8 (66)	2 (37)	2 (50)	16 (29)	28
Murex	6 (85)	1 (41)	3 (43)	16 (29)	26
Vitros	8 (66)	2 (37)	4 (41)	16 (29)	30
RIA	8 (66)	1 (41)	3 (43)	14 (36)	26

Table 13b: Comparative detection of anti-HBc in four seroconversion panels (based on the first reactive sample with the highest level of activity)

Assay	Seroconversion panels - highest points given to first positive sample with the highest level of activity				Aggregate score
	BBI- PHM935A	BCP-6278	BCP-6281	PROFILE-RP009	
AxSYM	3	4	4	4	15
Bioelisa	6	6	5	2	19
Monolisa	4	3	1	6	14
Murex	1	2	3	5	11
Vitros	5	5	6	3	19
RIA	2	1	2	1	6

Figure 4: Detection of PHM935A seroconversion panel by anti-HBc assays

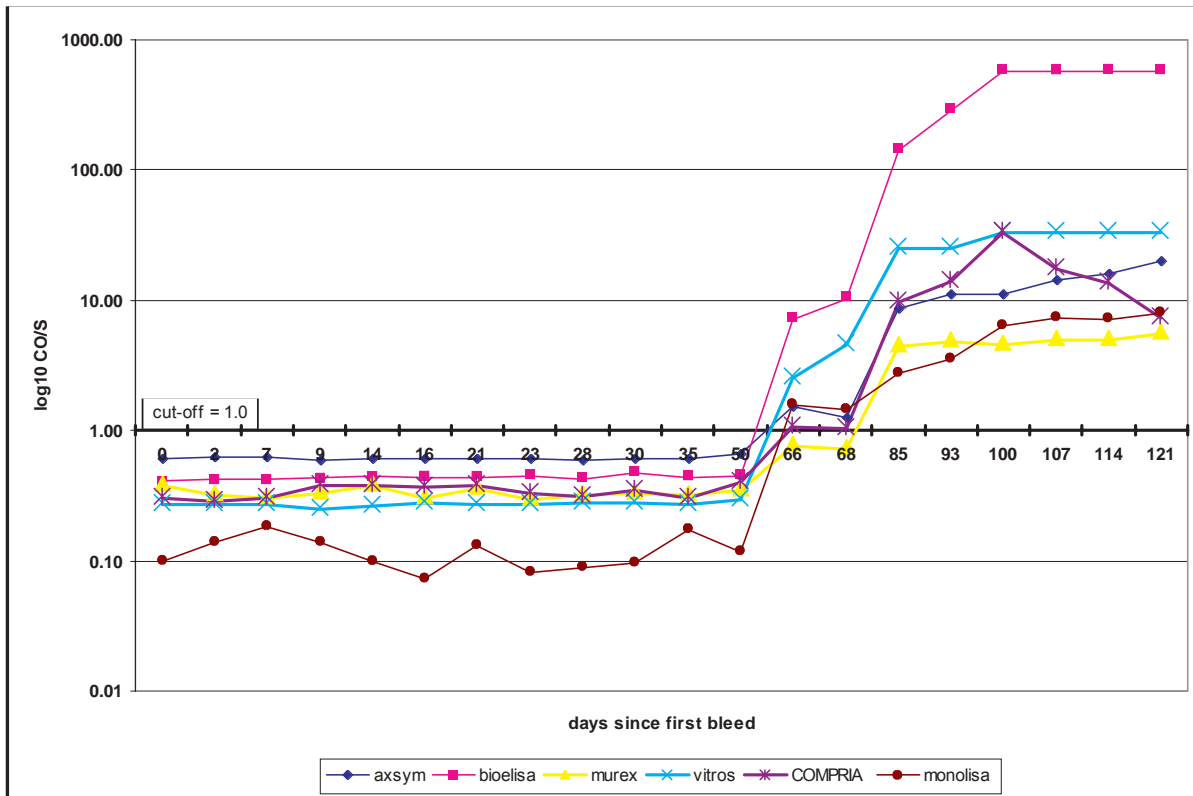


Figure 5: Detection of BCP-6278 seroconversion panel by anti-HBc assays

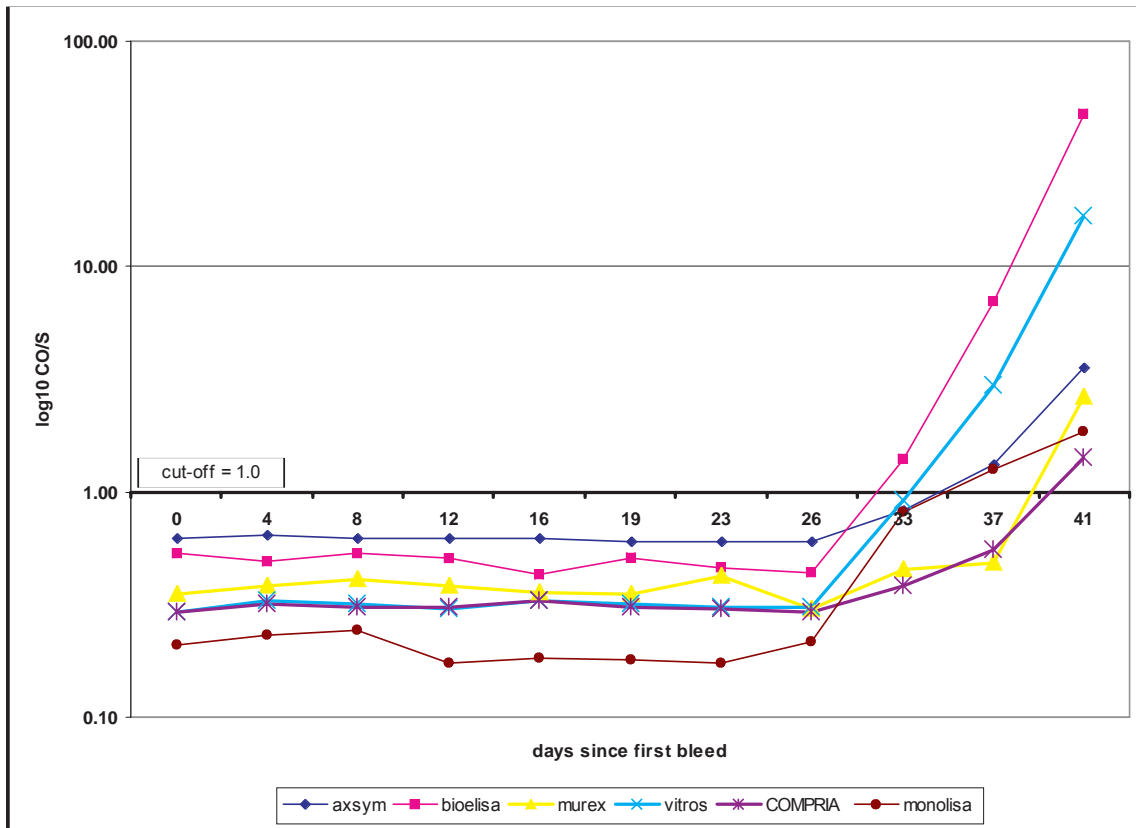


Figure 6: Detection of BCP-6281 seroconversion panel by anti-HBc assays

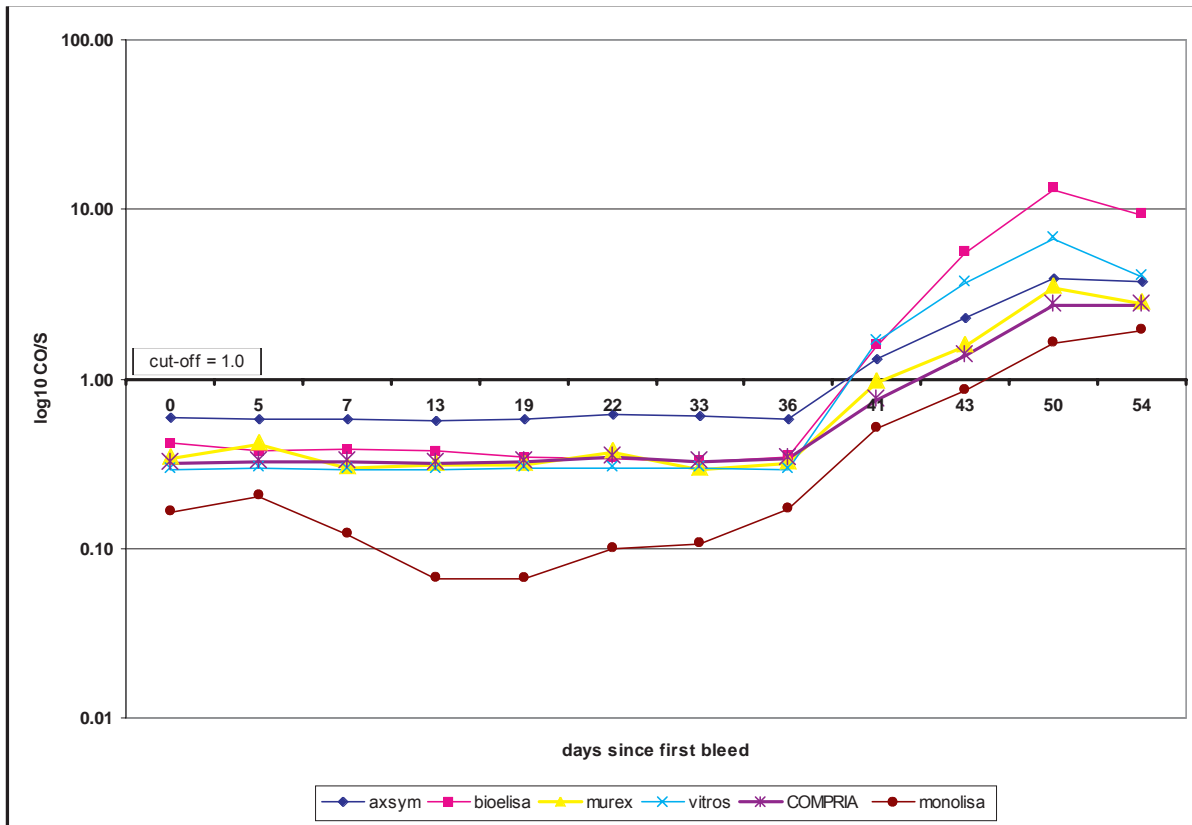


Figure 7: Detection of Profile-RP009 seroconversion panel by anti-HBc assays

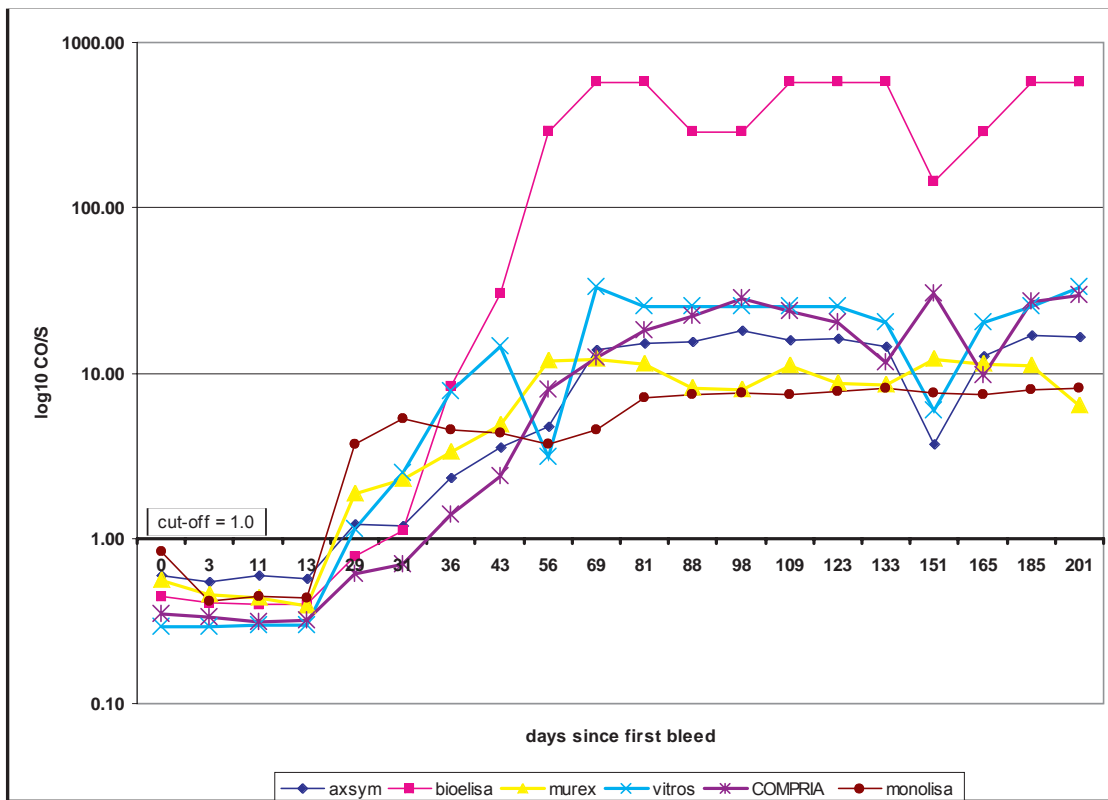


Table 14: S/CO ratios for the PHE102 performance panel tested by anti-HBc assays

Panel ID	Anti-HBc evaluation testing						BBI information sheet	
	AxSYM CORE	Bioelisa anti-HBc	Murex anti-HBc	Vitros anti-HBc	COMPRIA	Monolisa anti-HBc	Anti-HBc IgM Abbott EIA*	Anti-HBc IgM Abbott RIA*
	CO/S	CO/S	CO/S	CO/S	CO/S	S/CO	S/CO	S/CO
PHE102-01	1.50	6.45	1.08	3.70	1.61	0.83	1.5	1.5
PHE102-02	10.00	80.57	7.47	25.00	17.78	3.78	3.6	3.7
PHE102-03	14.71	483.40	6.00	33.33	22.81	7.46	1.7	1.7
PHE102-04	8.70	20.14	5.23	5.88	5.07	5.97	1.3	1.2
PHE102-05	16.95	483.40	7.95	50.00	35.91	6.86	1.3	1.1
PHE102-06	3.43	37.18	2.79	14.29	2.14	2.13	2.0	2.5
PHE102-07	0.57	0.45	0.38	0.27	0.27	0.36	0.1	0.1
PHE102-08	11.91	483.40	5.38	25.00	15.30	7.63	1.2	1.4
PHE102-09	3.25	24.17	3.18	10.00	3.98	2.67	2.3	2.3
PHE102-10	16.95	483.40	6.31	50.00	13.69	8.30	1.4	1.1
PHE102-11	2.27	26.86	2.90	7.14	2.10	1.58	0.6	0.7
PHE102-12	9.35	80.57	5.46	20.00	11.51	6.85	4.8	4.2
PHE102-13	11.63	483.40	7.04	33.33	13.53	7.93	1.6	1.6
PHE102-14	2.51	9.48	1.86	4.35	1.99	1.43	1.0	1.0
PHE102-15	7.30	80.57	6.20	8.33	15.30	6.57	2.9	4.1

Notes:
Results are expressed as specimen absorbance to cut-off ratios. Ratios equal to or greater than 1.0 are considered reactive.
* Data for both assays are taken from Procedure B in the BBI information sheet

Detection of anti-HBc in dilution series

Ten anti-HBc dilution series were included as part of the sensitivity rating to determine the analytical sensitivity of the assays to detect anti-HBc to the highest titres. The dilution series were prepared by using ten anti-HBc total positive specimens that were diluted in ten-fold steps from 1/50 to 1/500,000 in negative human serum. The number of positive specimens found by each assay in each dilution series was added to give an aggregate score (table 15a). The most sensitive assay detects the highest number of positive specimens. The MONOLISA anti-HBc PLUS assay detected 35 anti-HBc positive specimens in the ten dilution series. The AxSYM CORE and Murex anti-HBc (total) assays achieved higher scores of 37 and 36 respectively and three other assays obtaining a lower score of 34, placing the MONOLISA anti-HBc PLUS assay as the third most sensitive kit with the dilution series.

Previously evaluated assays have given close scores, therefore a second scoring system was used to show more discrimination. For each dilution series every assay was given a score. The highest score (6 points since 6 kits have been evaluated) given to the assay which remained positive to the highest titre and with the highest level of activity. The lowest score (1) to the assay which remained positive to the lowest titre with the lowest level of activity. An aggregate score was determined for each kit by adding the scores obtained in each dilution series (table 15b). The MONOLISA anti-HBc PLUS assay obtained a score of 42 and is the third most sensitive anti-HBc assay with the dilution series. Further details on the detection of anti-HBc and the S/CO ratios can be found in the [Appendix](#).

Table 15a: Comparative detection of anti-HBc in 10 dilution series (based on the number of reactive specimens)

Assay	Dilution series - one point given for every positive sample										Aggregate score
	9522246	9522256	9522283	9522286	9523427	9523434	9523437	9523442	9523443	9523458	
AxSYM	2	4	4	4	4	4	4	3	4	4	37
Bioelisa	2	4	3	4	4	4	3	3	4	3	34
Monolisa	1	4	4	4	5	3	4	3	4	3	35
Murex	2	4	4	4	4	4	4	3	4	3	36
Vitros	2	4	3	4	4	4	3	3	4	3	34
Compria	1	4	4	4	5	3	4	3	3	3	34

Table 15b: Comparative detection of anti-HBc in 10 dilution series (based on the highest titre)

Assay	Dilution series - Highest points given to the assay which remained positive to the highest titre and lowest points to the assay which remained positive to the lowest titre.										Aggregate score
	9522246	9522256	9522283	9522286	9523427	9523434	9523437	9523442	9523443	9523458	
AxSYM	5	5	6	5	4	3	3	5	6	6	48
Bioelisa	6	1	1	1	1	5	1	3	3	3	25
Monolisa	1	4	5	6	6	2	6	4	4	4	42
Murex	3	6	5	4	3	4	5	6	5	5	46
Vitros	4	2	2	2	2	6	2	2	3	2	27
Compria	2	3	3	3	5	1	4	1	1	1	24

Summary of specificity findings

An overall specificity rating was obtained by adding the number of anti-HBc negative blood donor and IDU specimens that were correctly identified by the anti-HBc kits. In [table 16](#) the kits are ranked according to the overall specificity, with the most specific at the top. The MONOLISA anti-HBc PLUS assay has an overall specificity of 99.1% and is ranked as the least specific anti-HBc assay.

Table 16: Summary of specificity findings for the anti-HBc assays

Assay	Number blood donors	Number IDU	number negative samples	Number initially negative	Number repeatedly negative	Specificity* (%)	Rank
Murex	498	180	678	678	678	100	1
AxSYM	496	180	676	673	675	99.9	2
Bioelisa	492	180	672	660	669	99.6	3
Vitros	492	180	672	668	669	99.6	3
Monolisa	492	180	672	661	666	99.1	5

Note:
* based on number repeatedly negative

Summary of sensitivity findings

An overall sensitivity rating was obtained by adding the number of anti-HBc positives for the IDU, HBsAg positive/ anti-HBc IgM negative specimens, seroconversion panels, performance panel and the dilution series. In [table 17](#) the kits have been ranked according to the overall sensitivity, with the most sensitive at the top. The MONOLISA anti-HBc PLUS assay had an overall sensitivity score of 436 and is ranked as the third most sensitive anti-HBc assay.

Table 17: Summary of the sensitivity findings for anti-HBc assays

Assay	IDU	HBsAg positive/ IgM anti-HBc negative	Serconversion panels	PHE102 Performance panel	Dilution series	Overall sensitivity score	Rank
AxSYM	312	50	30	14	37	443	1
Murex	314	52	26	14	36	442	2
Monolisa	311	49	28	13	35	436	3
Vitros	301	52	30	14	34	431	4
COMPRIA	306	48	26	14	34	428	5
Bioelisa	295	51	30	14	34	424	6

Quality control sera

Replicates of a HPA total anti-HBc quality control sera were tested by the MONOLISA anti-HBc PLUS assay to determine intra-plate variation. Replicates of this QC serum were also tested against a second lot (table 18). A QC specimen/ statistical assay control should have a reactivity within the linear dynamic range of the assay. When the evaluation began only one anti-HBc QC serum was available for testing, which had not been optimised to detect all anti-HBc assays. The QC sera fell within this range for the MONOLISA anti-HBc PLUS assay.

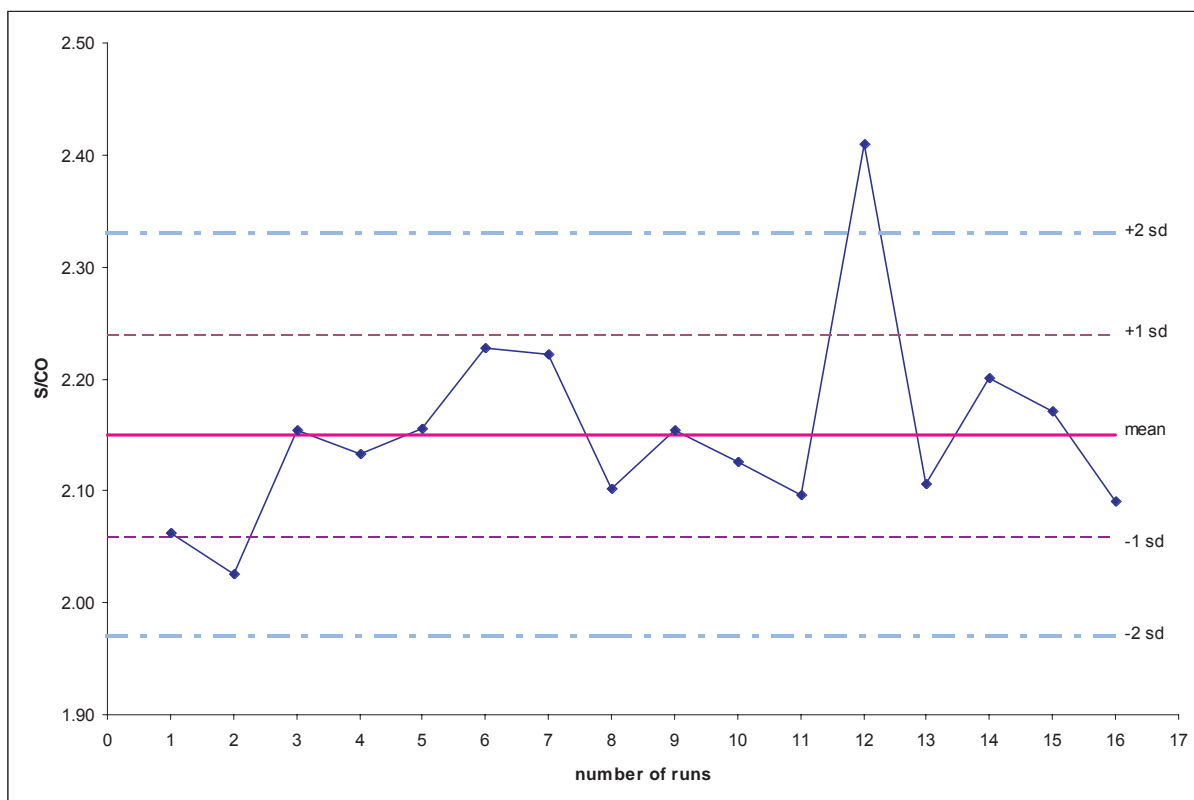
The inter-run reproducibility of the MONOLISA anti-HBc PLUS assay was determined using the anti-HBc QC serum. The QC serum was tested on three different locations on each plate and the mean value was calculated. The range of values detected is shown in figure 8. All values except one were within 2 standard deviations of the mean.

Table 18: S/CO ratios for the HPA Quality control sera tested by MONOLISA anti-HBc PLUS assay

Assay	LOT 1* - ANTI-HBc QC SERA						mean
	S/CO 1	S/CO 2	S/CO 3	S/CO 4	S/CO 5	S/CO 6	
Monolisa	1.96	2.30	2.26	2.13	2.15	2.12	2.15
Assay	LOT 2* - ANTI-HBc QC SERA						mean
	S/CO 1	S/CO 2	S/CO 3	S/CO 4	S/CO 5	S/CO 6	
Monolisa	1.50	2.06	2.14				1.90

Notes:
* lot numbers for each assay can be found in table 20

Figure 8 : Inter-run variation of the HPA anti-HBc quality control serum with the MONOLISA anti-HBc PLUS assay



Manufacturers kit controls

The results for the positive and negative controls provided by the manufacturer of the MONOLISA anti-HBc PLUS assay are shown in [table 19](#). All results are within the specified limits described in the kit inserts.

Table 19: Results for manufacturers kit controls

assay	Positive control				
	Number of assay runs used to calculate ranges	range S/CO	mean S/CO	median S/CO	% CV
Monolisa	17	5.00-5.00	5.00	5.00	0.00
assay	Negative control				
	Number of assay runs used to calculate ranges	range S/CO	mean S/CO	median S/CO	% CV
Monolisa	17	0.06-0.26	0.10	0.09	44.54

Comparison of two production lots

The full specimen panel (table 2a) was used to evaluate one production lot of the MONOLISA anti-HBc PLUS assay and a subset of the specimen panel (table 2b) was used to evaluate a second production lot. Table 20 shows a comparison of the number of specimens detected by each production lot. The S/CO values for both production lots can be found in the Appendix.

Fifty unreactive blood donor specimens were tested on both production lots of the assay. 49 were unreactive by lot 1 and all 50 were unreactive with lot 2, showing some inter-lot variation with the assays specificity. However, the one false reactive specimen in lot 1 was found to be unreactive on repeat testing of the assay.

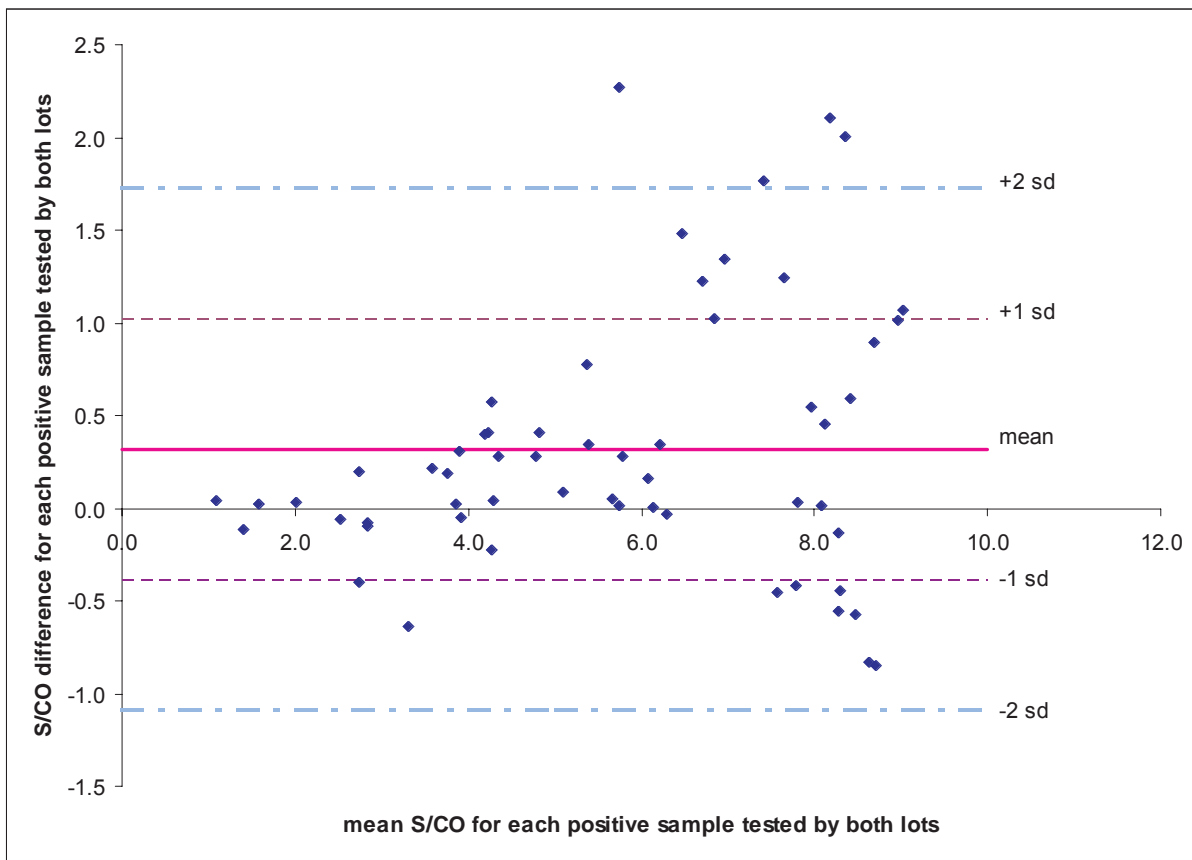
Fifty IDU specimens were tested on both production lots of the assay and 38 of these were reactive by both production lots. Three dilution series panels were tested by both production lots and the same number of positive specimens were detected by both production lots. Overall 58 anti-HBc positive specimens were detected by both production lots. The MONOLISA anti-HBc PLUS assay shows no inter-lot variation in the sensitivity of the assay.

All anti-HBc positive samples from both production lots were compared in a Bland Altman graph for each assay (figure 9). At lower S/CO ratios there is less difference than can be seen at higher S/CO ratios.

Table 20: Comparison of 2 production lots of the MONOLISA anti-HBc PLUS assay

Category	Number of samples	Monolisa anti-HBc	
		lot: 2K1537	lot: 3B0030
Blood donor sera	50	49	50
Negative human plasma	2*	2	2
Total negative samples:		51	52
Injecting drug users (IDU)	50	38	38
Dilution panel - 9522286	6	4	4
Dilution panel - 9523434	6	3	3
Dilution panel - 9523442	6	3	3
HBsAg resolving carrier - '0008381	2*	2	2
HBsAg resolving carrier - '0008382	2*	2	2
HBsAg resolving carrier - '0141532	2*	2	2
HBsAg +/-anti-HBc IgM negative	10	4	4
Total positive samples:		58	58
Notes:			
* samples were tested in duplicate			

Figure 9: Bland Altman comparison of mean and difference between the reactivities of 58 positive anti-HBc specimens tested by 2 production lots of MONOLISA anti-HBc PLUS assay



The MONOLISA anti-HBc PLUS kit instructions for using the reagents and running an assay were clear and easy to follow. The kit and reagent packaging were clearly identified and it was easy to prepare reagents that required reconstitution (wash buffer and substrate solution). 200µl of sample diluent was added before the addition of 20µl of sample. No colour change was observed on addition of sample. The washing stages were performed using a Tecan Columbus washer and the washer efficiency pre-evaluation test showed no carryover when applying the wash cycle specified in the kit instructions. Additional equipment used was a Jencons incubator and BioTek Ultramicroplate reader. For the evaluation, tests were processed manually and each 96 well plate took approximately 3 hours to complete.

Readers are encouraged to study carefully the results presented and to draw their own conclusions from them. We offer the following comments:

Specificity

The MONOLISA anti-HBc PLUS assay gave 6 repeatedly false positive specimens with the blood donor negative specimens and the IDU negative specimens. Overall the MONOLISA anti-HBc PLUS assay achieved a specificity of 99.1% and was the fifth most specific anti-HBc assay evaluated.

Sensitivity

The overall sensitivity rankings were obtained by adding the number of confirmed anti-HBc positive specimens that were detected by the anti-HBc assays. The anti-HBc positive specimens were from IDUs, commercial seroconversion and performance panels, dilutions series, and 'HBsAg positive/ IgM anti-HBc negative' specimens. Overall the MONOLISA anti-HBc PLUS assay is the third most sensitive anti-HBc assay evaluated, detecting 436 of the positive specimens.

Evaluation of assay cut-off determination

The delta values for the MONOLISA anti-HBc PLUS assay are evenly distributed (overall positive delta 2.89, overall negative delta -2.38), indicating that its cut-off has been set to optimise both sensitivity and specificity.

Quality control sera

A single HPA anti-HBc (total) quality control sera was used throughout the evaluation. Unlike evaluations for other markers (e.g. HIV, HBsAg) for which more quality control specimens have been available this was the only anti-HBc control available when the evaluation began. The MONOLISA anti-HBc PLUS assay was able to consistently detect the quality control sample. The inter-run reproducibility of the assays using the quality control sera showed that all values except one were within 2 standard deviations of the S/CO mean.

Presentation

The MONOLISA anti-HBc PLUS assay was well presented and easy to perform. The assay took 3 hours to complete one 96 well plate.

Application

The sensitivity of the MONOLISA anti-HBc PLUS assay was 98.4% for the anti-HBc IDU specimens and overall was the third most sensitive anti-HBc assay evaluated. The MONOLISA anti-HBc PLUS assay could have a role in HBV confirmatory testing algorithms, further to characterise HBsAg positive specimens and to assist in determining whether HBV infection is past or current. In many countries anti-HBc assays are used to screen blood donations. In this evaluation the MONOLISA anti-HBc PLUS assay had a specificity of more than 99% and would therefore be a good candidate for blood screening.

Acknowledgements

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North London Blood Centre, Colindale NW9

HPA London, Department of Virology, Kings College Hospital (Dulwich site), London SE22.

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10. **White R, Delieu E, Perry KP, Parry JP** (2003): Four anti-HBc assays. *Medicines and Healthcare products Regulatory Agency*: MHRA 03136.

Evaluation protocol

Protocol for evaluation of anti-HBc kits

Procurement of product for evaluation, duration of evaluation and training of evaluator

The evaluator will require for evaluation a package that contains sufficient anti-HBc kits to test a panel of specimens, together with ancillary reagents and consumables that are recommended by the manufacturer. Two batches of the kit are required to investigate lot-to-lot reproducibility. The kits will be used in conjunction with equipment, such as spectrophotometer and plate washers, or automated analyser, that is either provided by the manufacturer or is available at MiDAS and has been agreed to meet the requirements of the manufacturer's representative.

Before evaluation starts, the manufacturer will be invited to train the evaluator in the use of the kits and equipment and to satisfy themselves that the evaluator is properly trained.

Conduct of the evaluation

The product will be used in exactly the manner laid down in the manufacturer's instructions. Any modifications to the instructions provided with the kit that are described during the training period, or any subsequent changes must be confirmed in writing. All microtitre plates will be read on a plate reader eg Bio-Tek EL808 linked to a computer with KC4 software.

All data will be stored on the anti-HBc kit database for the duration of the evaluation. The data may subsequently be down-loaded to tape or other long-term back-up system. In addition the evaluators will keep clear records of the practical work. All original printouts from the plate reader or automated systems will be retained, together with any reader printouts from confirmatory assays.

Content of the evaluation / specimen panel

The object of this evaluation is to assess the ability of total anti-HBc kits to detect, with a high degree of sensitivity and specificity, anti-HBc in human serum and plasma.

To do this, the kits will be tested against an unbiased panel of plasma specimens obtained from injecting drug users and blood donors. In addition, this evaluation will incorporate commercially available anti-HBc performance panels and quality control samples and standards (Table 2).

Specimens previously tested because of false positive screening reactions will not be included in this study because of the potential bias that could be introduced against particular assays.

Storage of samples

Aliquots of each serum specimen will be distributed into plastic tubes with screw-cap lids with sealers. The aliquots will be stored at -20°C or below until required and at 4°C for the duration of the evaluation. Thawing will be carried out at room temperature.

Other aspects of the evaluation

The following features of the kits will be noted and may be remarked on in the report:

- | packaging and labelling of the materials
- | clarity of the operating instructions
- | ease of use and reliability of the products, including equipment supplied for the evaluation
- | health and safety considerations.

Table 2a: Panel for the evaluation of anti-HBc kits (lot 1).

Sample category	Number	
1. Blood donors/healthy adults' sera		500
2. Negative human serum (NHS)	2x	1
3. Injecting drug users (IDUs)		500
4. HBsAg 'resolving carrier' specimens (NLBC and BPL)	2x	3
5. HBsAg positive, IgM anti-HBc negative		85
6. Seroconversion panels: total anti-HBc		
BBI - PHM935A		20
BCP - 6278		11
BCP - 6281		12
Profile - RP009		20
7. Performance panels: total anti-HBc		
BBI - PHE102 (IgM anti-HBc low titre)		15
8. Dilution of anti-HBc positive (9 specimens x 6 dilutions - 1 specimen x 5 dilutions)	2x	59
9. Quality control samples		
HPA - total anti-HBc QC serum: sample 1	6x	1
Three replicates of suitable controls on each plate (estimate)		60
10. Ante-natal specimens		50
TOTAL (number of tests)		1405
Notes:		
BBI = Boston Biomedica Inc, USA; BPL = BioProducts Laboratory, UK NLBC = North London Blood Centre HPA = Health Protection Agency, UK;		

Table 2b: Panel for the evaluation of anti-HBc kits (lot 2).

1. Blood donors/healthy adults' sera		50
2. Negative human serum (NHS)	2x	1
3. Injecting drug users (IDUs)		50
4. HBsAg 'resolving carrier' specimens (NLBC and BPL)	2x	3
5. HBsAg positive, IgM anti-HBc negative		10
6. Dilution of anti-HBc positive (3 specimens x 6 dilutions)	2x	18
7. Quality control samples		
HPA - total anti-HBc QC serum: sample 1	3x	1
Three replicates of suitable controls on each plate (estimate)		10
TOTAL (number of tests)		167
Notes:		
BBI = Boston Biomedica Inc, USA; BPL = BioProducts Laboratory, UK NLBC = North London Blood Centre HPA = Health Protection Agency, UK		

Discordant results

A discordant result will arise when the kit under evaluation gives a result that disagrees with the observed consensus. If this occurs tests will be repeated in duplicate on the same aliquot of the serum. If the result is still discordant further investigations will be undertaken at the Sexually Transmitted and Blood Borne Virus Laboratory. This will include testing by other commercial or 'in-house' anti-HBc kits, other hepatitis B serological markers (eg anti-HBs, HBsAg, HBeAg, anti-HBe, IgM anti-HBc, as appropriate) and HBV DNA assays.

Analysis of results and evaluation report

Raw data will be transferred from the laboratory computer onto a database specifically prepared for these evaluations. The data entry will be checked by a second person.

A detailed report will be prepared for publication by the MHRA. The manufacturers' will be given the opportunity to comment on the results of the evaluation of their product before the MHRA evaluation report is published. Results obtained in other manufacturers' tests undergoing evaluation will not be disclosed at this time. Manufacturers' written comments, where relevant, will be appended to the report.

S/CO ratios for 4 seroconversion panels

Four seroconversion panels were included in the sensitivity testing to assess the ability of an assay to detect anti-HBc at the time of seroconversion. Details of the S/CO ratios for each panel are given in tables 21-24. Each table shows comparative information taken from the seroconversion panels data sheets on other relevant HBV markers tested for. Each seroconversion panel is a set of undiluted plasma samples from serial bleeds from a single plasma donor collected during a period of Hepatitis B seroconversion.

Table 21: BBI - PHM935A panel

Days since first bleed	anti-HBc evaluation testing						Data from panel information sheets			
	AxSYM CO/S	Bioelisa CO/S	Murex CO/S	Vitros CO/S	COMPRIA CO/S	Monolisa S/CO	HBV DNA Roche PCR	HBsAg Abbott IMx	IgM Abbott IMx	IgM Corzyme-M
0	0.61	0.43	0.38	0.27	0.30	0.10	BLD	0.4	0.0	0.1
2	0.63	0.44	0.32	0.27	0.28	0.14	BLD	0.4	0.0	0.1
7	0.63	0.45	0.30	0.27	0.30	0.18	BLD	0.4	0.0	0.1
9	0.59	0.46	0.33	0.25	0.37	0.14	6x10 ²	0.4	0.0	0.1
14	0.61	0.47	0.38	0.26	0.37	0.10	8x10 ²	0.4	0.0	0.1
16	0.60	0.46	0.30	0.28	0.37	0.07	5x10 ²	0.4	0.0	0.1
21	0.61	0.46	0.36	0.27	0.38	0.13	9x10 ³	0.5	0.0	0.1
23	0.60	0.48	0.29	0.27	0.33	0.08	8x10 ³	0.7	0.0	0.1
28	0.59	0.44	0.32	0.28	0.31	0.09	8x10 ⁴	2.3	0.0	0.1
30	0.61	0.49	0.33	0.28	0.35	0.10	1x10 ⁵	3.5	0.1	0.1
35	0.60	0.46	0.32	0.27	0.30	0.17	4x10 ⁵	11.7	0.1	0.1
50	0.66	0.47	0.35	0.29	0.40	0.12	2x10 ⁷	39.4	0.1	0.1
66	1.53	7.53	0.75	2.50	1.07	1.56	5x10 ⁶	27.7	0.7	3.7
68	1.27	10.63	0.71	4.55	1.02	1.45	>4x10 ⁷	29.1	1.0	6.4
85	8.70	148.80	4.41	25.00	9.78	2.77	>4x10 ⁷	33.9	2.7	6.2
93	10.99	297.60	4.75	25.00	13.79	3.57	1x10 ⁷	41.8	2.7	5.6
100	10.99	595.20	4.52	33.33	33.28	6.29	2x10 ⁶	43.2	2.6	5.5
107	14.29	595.20	4.88	33.33	17.27	7.32	9x10 ⁴	54.8	2.5	4.8
114	15.87	595.20	4.94	33.33	13.48	7.08	3x10 ⁴	57.2	2.5	>6.6
121	20.00	595.20	5.54	33.33	7.41	7.89	2x10 ⁴	49.8	2.5	>6.6

Notes:
BLD = Below limit of detection

Table 22: BCP - 6278 panel

Days since first bleed	anti-HBc evaluation testing						Data from panel information sheets			
	AxSYM CO/S	Bioelisa CO/S	Murex CO/S	Vitros CO/S	COMPRIA CO/S	Monolisa S/CO	Research use PCR	HBsAg Abbott IMx	HBsAg VIDAS *	aHBc IgM Abbott
0	0.62	0.56	0.35	0.29	0.29	0.21	100	0.48	0.02	0.08
4	0.64	0.52	0.38	0.33	0.32	0.23	100	0.52	0.03	0.08
8	0.62	0.56	0.41	0.32	0.31	0.24	500	0.45	0.05	0.08
12	0.62	0.53	0.38	0.30	0.31	0.17	1,000	1.13	0.26	0.07
16	0.62	0.45	0.36	0.33	0.33	0.18	40,000	4.06	1.40	0.06
19	0.60	0.54	0.35	0.32	0.31	0.18	120,000	16.23	6.32	0.07
23	0.60	0.48	0.42	0.31	0.3	0.17	1,200,000	37.47	12.55	0.09
26	0.60	0.46	0.30	0.31	0.29	0.21	6,900,000	56.43	13.62	0.08
33	0.83	1.46	0.45	0.92	0.38	0.82	50,000,000	82.25	12.76	0.34
37	1.32	7.26	0.48	2.94	0.55	1.25	>50,000,000	88.30	11.25	0.93
41	3.56	49.60	2.62	16.67	1.41	1.86	>50,000,000	72.76	14.06	3.62

Notes:
* >0.13 = reactive

Table 23: BCP - 6281 panel

Days since first bleed	anti-HBc evaluation testing						Data from panel information sheets			
	Axsym CO/S	Bioelisa CO/S	Murex CO/S	Vitros CO/S	COMPRIA CO/S	Monolisa S/CO	Research use PCR	HBsAg VIDAS	Roche Cobas HBsAgII	Abbott CORE-M
0	0.59	0.42	0.34	0.29	0.318	0.162	100	0.12	0.13	0.22
5	0.58	0.38	0.41	0.30	0.321	0.203	200	0.14	0.24	0.19
7	0.58	0.39	0.30	0.29	0.323	0.122	500	0.11	0.22	0.19
13	0.57	0.38	0.31	0.29	0.319	0.066	200	0.28	0.50	0.21
19	0.58	0.35	0.31	0.30	0.323	0.066	1,700	0.36	0.67	0.22
22	0.62	0.34	0.37	0.30	0.346	0.100	6,500	1.34	1.50	0.20
33	0.60	0.33	0.29	0.30	0.321	0.107	27,000	3.36	5.46	0.20
36	0.58	0.35	0.32	0.29	0.341	0.170	20,000	4.31	7.33	0.20
41	1.30	1.55	0.94	1.64	0.748	0.512	8,100	9.82	17.20	0.57
43	2.27	5.55	1.55	3.70	1.362	0.844	4,700	2.21	3.59	1.62
50	3.94	13.04	3.42	6.67	2.737	1.618	<100	0.07	0.37	2.47
54	3.75	9.31	2.76	4.00	2.701	1.931	<100	0.08	0.02	2.30

Table 24: Profile - RP009 panel

Days since first bleed	anti-HBc evaluation testing						Data from panel information sheets	
	Axsym CO/S	Bioelisa CO/S	Murex CO/S	Vitros CO/S	COMPRIA CO/S	Monolisa S/CO	HBsAg Abbott Auszyme	IgM Abbott Corzyme-M
0	0.60	0.47	0.56	0.29	0.349	0.826	0.79	0.13
3	0.54	0.43	0.46	0.29	0.331	0.420	9.58	0.15
11	0.59	0.42	0.44	0.30	0.315	0.446	36.17	0.14
13	0.57	0.42	0.39	0.30	0.319	0.435	68.00	0.14
29	1.22	0.82	1.87	1.14	0.602	3.730	91.04	0.20
31	1.18	1.16	2.28	2.50	0.691	5.329	91.67	0.19
36	2.34	8.63	3.32	7.69	1.403	4.518	91.67	0.63
43	3.58	31.33	4.87	14.29	2.361	4.286	72.67	2.92
56	4.74	297.60	11.84	3.09	7.885	3.697	54.94	13.38
69	13.70	595.20	12.17	33.33	12.379	4.563	91.67	13.08
81	15.15	595.20	11.23	25.00	17.955	7.124	91.21	13.47
88	15.39	297.60	8.11	25.00	22.116	7.460	61.11	14.18
98	17.86	297.60	7.96	25.00	28.116	7.570	91.46	14.64
109	15.87	595.20	10.95	25.00	23.552	7.452	87.42	12.15
123	16.13	595.20	8.59	25.00	20.150	7.710	86.96	7.62
133	14.49	595.20	8.42	20.00	11.442	8.027	29.63	5.60
151	3.68	148.80	12.17	5.88	29.975	7.651	3.54	4.40
165	12.50	297.60	11.23	20.00	9.776	7.452	0.71	3.68
185	16.95	595.20	10.95	25.00	27.067	7.928	0.46	2.48
201	16.67	595.20	6.35	33.33	29.730	8.090	0.21	2.83

S/CO ratios for 10 dilution series

Ten anti-HBc dilution series were included as part of the sensitivity rating to determine the ability of the assays to detect anti-HBc at lower concentrations. The dilution series were prepared using ten anti-HBc total positive specimens that were diluted in ten-fold steps from 1/50 to 1/500,000 in negative human serum. [Tables 25a-b](#) show the S/CO ratios for each dilution series.

Table 25a: Dilution series

Sample ID	Dilution	Axsym CO/S	Bioelisa CO/S	Murex CO/S	Vitros CO/S	COMPRIA CO/S	Monolisa S/CO
9522246a	neat	2.10	17.34	2.19	3.65	5.49	1.63
9522246b	1/50	1.22	2.27	1.31	1.34	0.69	0.74
9522246c	1/500	0.75	0.60	0.51	0.55	0.37	0.30
9522246d	1/5,000	0.60	0.44	0.40	0.35	0.38	0.17
9522246e	1/50,000	0.56	0.45	0.43	0.33	0.33	0.21
9522256a	neat	9.71	312.13	9.43	33.33	16.97	8.43
9522256b	1/50	10.48	33.60	6.64	12.50	8.16	7.15
9522256c	1/500	8.14	8.17	7.35	5.13	2.80	5.58
9522256d	1/5,000	3.32	1.39	6.09	1.60	1.60	3.07
9522256e	1/50,000	0.84	0.47	0.87	0.45	0.53	0.73
9522256f	1/500,000	0.60	0.41	0.39	0.33	0.34	0.23
9522283a	neat	17.88	468.20	7.95	41.67	9.76	9.06
9522283b	1/50	18.83	117.05	7.55	33.33	6.22	7.58
9522283c	1/500	11.12	5.29	7.88	8.33	4.21	5.04
9522283d	1/5,000	1.46	0.57	1.76	0.77	1.01	2.01
9522283e	1/50,000	0.62	0.46	0.43	0.34	0.43	0.36
9522283f	1/500,000	0.55	0.45	0.44	0.33	0.34	0.22
9522286a	neat	16.51	351.15	7.28	50.00	49.77	7.81
9522286b	1/50	14.69	84.54	6.54	25.00	9.27	7.34
9522286c	1/500	10.45	21.07	6.78	13.39	7.32	5.68
9522286d	1/5,000	5.35	2.27	5.63	2.57	1.90	3.84
9522286e	1/50,000	0.98	0.48	0.93	0.50	0.71	0.99
9522286f	1/500,000	0.63	0.41	0.45	0.34	0.34	0.23
9523427a	neat	9.85	468.20	8.26	50.00	24.89	7.52
9523427b	1/50	12.93	34.73	6.70	25.00	39.29	7.64
9523427c	1/500	9.09	6.68	5.91	14.29	9.45	6.11
9523427d	1/5,000	3.69	1.17	5.35	5.13	8.25	3.93
9523427e	1/50,000	0.91	0.51	0.86	0.75	1.18	1.31
9523427f	1/500,000	0.63	0.42	0.52	0.38	0.42	0.32

Table 25b: Dilution series

Sample ID	Dilution	Axsym CO/S	Bioelisa CO/S	Murex CO/S	Vitros CO/S	COMPRIA CO/S	Monolisa S/CO
9523434a	neat	1.54	91.97	5.23	29.17	10.71	6.88
9523434b	1/50	4.55	26.48	3.48	13.39	3.08	2.48
9523434c	1/500	2.31	8.75	2.27	6.46	1.43	1.59
9523434d	1/5,000	1.07	1.79	1.23	2.11	0.74	0.93
9523434e	1/50,000	0.65	0.48	0.65	0.53	0.41	0.34
9523434f	1/500,000	0.60	0.42	0.41	0.36	0.33	0.17
9523437a	neat	12.90	468.20	11.43	18.33	18.22	8.27
9523437b	1/50	12.06	26.78	11.44	29.17	10.24	6.98
9523437c	1/500	8.07	2.41	12.19	8.71	7.97	4.95
9523437d	1/5,000	1.47	0.50	4.19	0.88	3.69	2.67
9523437e	1/50,000	0.68	0.40	0.52	0.37	0.45	0.65
9523437f	1/500,000	0.61	0.38	0.48	0.34	0.35	0.30
9523442a	neat	11.63	284.20	11.44	25.00	35.97	6.14
9523442b	1/50	6.88	9.87	9.17	5.88	4.87	3.86
9523442c	1/500	3.12	1.92	4.09	1.74	1.27	2.03
9523442d	1/5,000	0.93	0.55	0.92	0.60	0.52	0.68
9523442e	1/50,000	0.59	0.49	0.49	0.38	0.39	0.30
9523442f	1/500,000	0.58	0.47	0.37	0.34	0.36	0.21
9523443a	neat	8.63	568.40	8.82	29.17	31.88	4.34
9523443b	1/50	8.80	35.53	8.32	13.39	8.61	4.33
9523443c	1/500	5.50	6.54	9.17	4.36	2.39	2.89
9523443d	1/5,000	1.68	1.10	1.93	1.19	0.74	1.34
9523443e	1/50,000	0.65	0.48	0.50	0.43	0.40	0.30
9523443f	1/500,000	0.58	0.44	0.36	0.34	0.36	0.22
9523458a	neat	10.37	568.40	9.77	20.00	25.05	7.25
9523458b	1/50	8.14	11.73	6.15	6.27	4.65	4.55
9523458c	1/500	4.07	2.06	5.34	1.69	1.42	2.89
9523458d	1/5,000	1.05	0.51	0.93	0.53	0.51	0.71
9523458e	1/50,000	0.63	0.45	0.41	0.39	0.34	0.28
9523458f	1/500,000	0.55	0.44	0.35	0.35	0.33	0.22

S/CO ratios for two production lots

A smaller specimen panel that includes 50 blood donor specimens, 50 injecting drug user specimens and 3 dilution series were tested on a second production lot. [Tables 26-31](#) show the S/CO ratios for specimens tested in both lots.

Table 26: Blood donor specimens

Sample ID	Lot 1	Lot 2
	OD/CO	OD/CO
02N0001A	0.19	0.16
02N0002A	0.12	0.09
02N0003A	0.14	0.11
02N0004A	0.19	0.13
02N0005A	0.19	0.19
02N0006A	0.65	0.34
02N0007A	0.41	0.29
02N0008A	0.14	0.08
02N0009A	0.30	0.21
02N0010A	0.45	0.46
02N0011A	0.13	0.06
02N0012A	0.22	0.12
02N0013A	0.16	0.15
02N0014A	0.34	0.14
02N0015A	0.38	0.45
02N0016A	0.22	0.12
02N0017A	0.13	0.11
02N0018A	0.18	0.10
02N0020A	0.35	0.32
02N0021A	0.76	0.17
02N0022A	0.12	0.05
02N0023A	0.25	0.10
02N0024A	0.19	0.13
02N0025A	0.74	0.15
02N0026A	0.38	0.11
02N0027A	0.12	0.07
02N0028A	0.27	0.15
02N0029A	1.48	0.29
02N0030A	0.15	0.15
02N0031A	0.08	0.06
02N0032A	0.22	0.15
02N0033A	0.60	0.26
02N0034A	0.14	0.11
02N0035A	0.21	0.12
02N0036A	0.24	0.12
02N0037A	0.12	0.10
02N0038A	0.57	0.37
02N0039A	0.07	0.03
02N0040A	0.17	0.20
02N0042A	0.09	0.17
02N0043A	0.40	0.27
02N0044A	0.12	0.15
02N0045A	0.27	0.32
02N0046A	0.09	0.09
02N0047A	0.13	0.17
02N0048A	0.13	0.15
02N0049A	0.21	0.17
02N0050A	0.10	0.10
02N0051A	0.19	0.20
02N0052A	0.19	0.16

Table 27: Injecting drug user specimens

Sample ID	Lot 1	Lot 2
	OD/CO	OD/CO
0200342e	1.34	1.45
0200343c	0.16	0.12
0200344c	6.28	6.31
0200345c	0.17	0.15
0200346d	3.89	3.95
0200347c	5.13	5.05
0200348c	0.11	0.04
0200349c	0.35	0.27
0200350c	8.21	8.34
0200351c	4.92	4.64
0200352c	7.63	6.28
0200353e	2.78	2.86
0200354c	8.28	6.51
0200355c	0.68	0.24
0200356c	0.47	0.46
0200357c	5.56	5.21
0200358c	5.75	5.74
0200359c	8.07	8.52
0200360c	7.35	6.32
0200361c	0.46	0.24
0200362e	2.78	2.87
0200363c	6.13	6.13
0200364c	4.42	4.01
0200365c	4.29	4.25
0200366e	2.53	2.92
0200367c	2.98	3.61
0200368c	7.58	7.99
0200369e	2.83	2.63
0200370c	5.91	5.63
0200371c	4.56	3.98
0200372c	4.04	3.74
0200373c	5.75	4.98
0200374c	0.79	0.75
0200375c	0.62	0.66
0200376c	0.32	0.23
0200377c	4.38	3.98
0200378c	7.20	5.72
0200379c	3.69	3.47
0200380c	4.48	4.21
0200381c	4.14	4.37
0200382c	8.07	8.07
0200383c	7.98	8.54
0200384c	0.43	0.37
0200385c	8.18	8.75
0200386c	1.10	1.06
0200387c	0.34	0.32
0200388c	8.34	7.89
0200389c	6.37	6.03
0200390c	5.02	4.61
0200391c	7.30	6.08

Table 28: Dilution series

	Sample ID	Lot 1		Lot 2	
		S/CO1	S/CO2	S/CO1	S/CO2
neat	9522286A	7.85	7.77	7.75	7.80
1/50	9522286B	7.43	7.24	7.29	8.30
1/500	9522286C	5.57	5.78	5.71	5.54
1/5,000	9522286D	3.82	3.86	3.63	3.67
1/50,000	9522286E	1.01	0.98	0.77	0.93
1/500,000	9522286F	0.24	0.23	0.20	0.24
neat	9523434A	7.02	6.73	4.58	4.62
1/50	9523434B	2.48	2.48	2.54	2.54
1/500	9523434C	1.52	1.65	1.58	1.55
1/5,000	9523434D	0.95	0.91	1.02	0.93
1/50,000	9523434E	0.34	0.35	0.24	0.21
1/500,000	9523434F	0.18	0.16	0.11	0.10
neat	9523442A	6.24	6.05	5.82	6.16
1/50	9523442B	3.85	3.86	3.81	3.85
1/500	9523442C	2.00	2.05	2.06	1.93
1/5,000	9523442D	0.67	0.68	0.56	0.55
1/50,000	9523442E	0.32	0.27	0.15	0.16
1/500,000	9523442F	0.23	0.20	0.14	0.14

Table 29: Negative Human Plasma

Sample ID	Lot 1		Lot 2	
	OD/CO 1	OD/CO 2	OD/CO 1	OD/CO 2
0217152	0.18	0.10	0.20	0.13

Table 30: HBsAg resolving carriers

Sample ID	Lot 1		Lot 2	
	OD/CO 1	OD/CO 2	OD/CO 1	OD/CO 2
008381	9.36	7.35	9.22	7.12
008382	8.27	7.02	8.23	7.68
0141532	8.28	9.13	8.20	9.03

Table 31: HBsAg positive/ anti-HBc IgM negative specimens

Sample ID	Lot 1	Lot 2
	OD/CO	OD/CO
7	9.45	8.44
8	8.69	8.10
9	0.23	0.13
14	9.13	8.23
16	0.55	0.55
23	0.23	0.21
28	0.75	0.27
30	0.28	0.10
33	9.55	8.48
41	0.29	0.07

Contact details for manufacturer and UK agent

MONOLISA anti-HBc PLUS

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Bio-Rad House
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Herts
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Manufacturer's comments

BIO-RAD
Research Direction
JF.Delagneau (Chief Scientist)
CD/03-183

Marnes, December 22, 2003

MIDAS - Evaluation and standards laboratory
Health Protection Agency
To Dr Keith R.Perry
61 Colindale Avenue
LONDON NW9 5HT

Re : Evaluation report MHRA/MIDAS Monolisa anti HBc Plus

Dear Dr Perry,

I would like to thank you and the associated evaluators for the implementation of your well-documented comparative study and for the supply of a clear and objective Monolisa anti-HBc Plus drafted evaluation report. In this evaluation, the Monolisa anti-HBc Plus assay was shown as one of the three most sensitive assays for the detection of anti-HBc antibodies. This was especially illustrated when the same seroconverter panels and the very same diluted samples were tested. Your sensitivity data strengthen our previous and independent studies and conclusion.

However, we believe that the Monolisa anti-HBc antibody plus assay true specificity (when normal blood donor samples are tested) is significantly higher than the one you reported when only 492 of such samples were tested at Colindale.

Independent specificity studies were conducted in French blood centers and a 99.9 % specificity was reported when 4274 samples were tested (and following roughly the same algorithm for the supplemental investigation of discrepant results).

We also noticed that you did not test the same collection of blood donor specimens with the 5 kits listed in table 3 (only Vitros, Bioelisa and Monolisa assays were evaluated with the same 492 member panel). We are not sure that the readers will consider this minus bias.

Nevertheless, we mostly agree with your conclusion and are confident with the use of our product in HBV confirmatory testing algorithm and in investigating whether HBV infection in patients is early or past.

We are also looking for large scale specificity assessments in different countries.

Again, we thank the MHRA/MIDAS representatives for having given us the opportunity to review and comment their drafted report prior to the publication of the final report.

With our best regards.

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