



Evaluation of the Roche HBsAg II assay

Katrina Barlow, Keith Perry

Microbiological Diagnostics Assessment Service
Department for Evaluations, Standards and Training
Health Protection Agency - Centre for Infections
61 Colindale Avenue
London
NW9 5EQ

Report PER07003

October 2009

Background and description of the assay

HBsAg II is a sandwich principle immunoassay manufactured by Roche Diagnostics for the detection of hepatitis B surface antigen (HBsAg). Being a component of the external envelope of hepatitis B, HBsAg is the first immunological marker post-infection and is subject to selective pressure from the immune system or antiviral therapy; this leads to the generation of a range of escape mutants which could fail to be detected by some HBsAg assays. The HBsAg II assay has been developed to detect such mutants.

The assay characteristics are summarised in Table 1. The HBsAg II kit comprises of a single compact unit containing three reagent wells (M, R1 and R2); once any residue in the chamber lids is removed, the unit is loaded onto an automated analyser without the requirement for any further manipulations. The assay can be run on one of four Roche automated analysers which incorporate electrochemiluminescence (ECL) detection: a MODULAR ANALYTICS E170 automated immunoassay analyser, Elecsys 2010, cobas e411 or cobas e601. Briefly, the sample is mixed with antibody conjugates labelled with biotin or a ruthenium complex. The resulting Ab/Ag complexes are captured using streptavidin-coated magnetic microparticles, washed, and following application of a voltage in the measuring cell a chemiluminescent signal is produced and measured using a photomultiplier.

This study evaluated the use of HBsAg II on the MODULAR ANALYTICS E170 automated immunoassay analyser, using a panel of specimens consisting of HBsAg positives (including various disease stages, low positives and HBsAg mutants), HBsAg negatives, quality control specimens and seroconversion panels.



Modular Analytics E170-module

The Roche HBsAg II assay carries a CE mark and has therefore undergone testing described in the Common Technical Specification for Annex IIa related products and in accordance to the European Union *In Vitro* Diagnostic Medical Device Directive. This evaluation builds on the work already completed for CE marking by providing comparative performance information on a range of specimens with a particular focus on

seroconversion timing. The panel is moderately sized, recognising that a large number of specimens have already been tested as part of the CE Marking process.

This report specifically relates to the kit version and lot numbers supplied for this evaluation. We cannot guarantee that these will reflect the performance of other lot numbers or subsequent versions. Laboratories should always validate and monitor assay performance as part of an ongoing quality control program.

Table 1: Assay information

General	
Assay name	HBsAg II
Manufacturer / UK agent	Roche Diagnostics
Product number	04687787
Number of tests per pack	100
Specimen volume	150µL (30µL + 120µL 'dead' volume)

Presentation	
Assay type	HBsAg surface antigen detection
Solid phase	Streptavidin-coated microparticles (M)
Conjugate	Two biotinylated monoclonal anti-HBsAg antibodies (mouse) (R1); mono- and polyclonal HBsAg-specific antibodies labelled with ruthenium complex (R2)
Substrate	None – electrochemiluminescent detection following voltage application
Controls (supplied separately)	PreciControl HBsAg II (product no: 04687876)
Negative control	PC HBSAG1
Positive control	PC HBSAG2
Calibrators	Cal1 (HBsAg negative human serum) Cal2 (HBsAg at approx 0.5 IU/mL, in human serum)
Reading wavelength	620 nm
Cut-off computation	Performed by the MODULAR ANALYTICS E170 automated immunoassay analyser based on calibration values
Equivocal zone	Signal/cut-off values ≥ 0.90 to < 1.0

Stages	
Preparation/sample well loading (per five samples)	10 minutes (some laboratories may be able to load primary specimen vessels)
In MODULAR ANALYTICS E170 analyser: - Sample and conjugates mixed - Resulting complexes bind to streptavidin-coated microparticles - In measuring cell, microparticles are magnetically captured and washed - Voltage applied, resulting in chemiluminescence captured by a photomultiplier	
Approximate time to completion* - 1 sample - 10 samples - 100 samples	- 19 min - 25 min - 84 min

*Only one photocell was used in this evaluation due to its small scale – it is anticipated that using both cells would decrease these times

Additional equipment required	
Micropipettes	
Centrifuge	

Evaluation panel and methods

The evaluation panel comprised of 609 serum or plasma specimens (Table 2). Of these, 199 were HBsAg negative specimens from blood donors and 209 were HBsAg positive, including characterised specimens and those from long-term carriers clearing antigenaemia. Positive and negative specimens were interspersed where possible. A further 149 specimens from 20 seroconversion panels, 15 from a low titre performance panel, 33 from a panel of native mutant specimens generated by the Virus Reference Department of the HPA² and four quality control samples were tested. A subset of this panel was used to assess a second assay lot (Table 3).

The method outlined in the kit insert was strictly followed. Kit calibration specimens were tested at the start of the evaluation and following introduction of a new reagent lot. The calibration was repeated if a kit remained loaded on the machine for seven days. The two PreciControl specimens were tested as a minimum at the beginning of each day, at the start of each new kit and after calibration. Primary specimen tubes were not used for this evaluation and instead 150µL aliquots of plasma or serum were dispensed into barcode-labelled assay cups (Hitachi Standard) and loaded onto the MODULAR ANALYTICS E170 analyser.

The analyser calculated the cut-off based on the measurements of the calibrators Cal1 and Cal2. Specimens were interpreted using the criteria below as outlined in the kit insert.

Specimens with a cut-off index (signal/cut-off value) < 0.90 were classed as non-reactive, and considered negative for HBsAg. These specimens did not require further testing.

The kit insert states that specimens with a cut off index ≥ 0.90 and < 1.0 are considered borderline, and should be retested in duplicate; values of ≥ 0.90 in either of the retested specimens are considered to be repeat reactive.

Specimens with a cut-off index ≥ 1.0 were considered reactive and therefore positive for HBsAg. The kit insert recommended retesting all positives in duplicate but since the specimen status was known this was not performed in this evaluation.

Table 2: Evaluation panel (Lot 1, 00151 238-03)

Specimen category	Number
1 HBsAg negative blood donors sera	199
2 HBsAg positive samples (N=209)	
a) Acute positive	31
b) Chronic positive	39
c) anti-e positive (SNBTS)	31
d) eAg positive (SNBTS)	8
e) subtype ad	39
f) subtype ay	17
g) Positive (uncharacterised)	35
h) weakly reactive (long term carriers clearing antigenaemia) (NHSBT)	9
3 HBsAg seroconversion panels (x20; N=149)	
PHM903 (subtype ad)	6
PHM904 (subtype ad)	3
PHM909 (subtype ad)	7
PHM910 (subtype ad)	6
PHM911 (subtype ad)	25
PHM914 (subtype ad)	6
PHM916 (subtype ay)	11
PHM917 (subtype IND)	3
PHM918 (subtype ad)	3
PHM919 (subtype ad)	9
PHM920 (subtype ad)	6
PHM921 (subtype ad)	6
PHM922 (subtype ad)	12
PHM923 (subtype ay)	4
PHM924 (subtype ad)	5
PHM925 (subtype IND)	5
BCP 6271	5
BCP 6274	7
BCP 6276	8
BCP 6281	12
4 HBsAg performance panels	
PHA103 (low titre)	15
5 Native HBsAg mutants panel	33
6 Quality control samples	
NIBSC Monitor Sample: 0.05IU/ml (subtype ad)	1 (x3)
NIBSC British Working Standard: 0.2IU/ml (subtype ad)	1 (x3)
HPA HBsAg QC1	1 (x3)
HPA HBsAg QC2	1 (x3)
Total number of specimens tested	609

Positive samples (unless indicated) and PHM/PHA panels from SeraCare (formerly Boston Biomedica Inc); SNBTS: obtained from the Scottish National Blood Service; NHSBT: NHS Blood and Transplant (North London Centre); BCP panels from Zeptomatrix (formerly BioClinical Partners Inc); HPA: Health Protection Agency; NIBSC: National Institute for Biological Standards and Control

Table 3: Evaluation panel (Lot 2, 00152 095-01)

Specimen category	Number
1 HBsAg negative blood donors sera	40
2 HBsAg positive samples	40
3 HBsAg seroconversion panels (x5; N=41)	
PHM914 (subtype ad)	6
PHM920 (subtype ad)	6
PHM922 (subtype ad)	12
PHM924 (subtype ad)	5
BCP 6281	12
4 Quality control samples	
NIBSC Monitor Sample: 0.05IU/ml (subtype ad)	1 (x3)
NIBSC British Working Standard: 0.2IU/ml (subtype ad)	1 (x3)
HPA HBsAg QC1	1 (x3)
HPA HBsAg QC2	1 (x3)
Total number of specimens tested	125

PHM/PHA panels from SeraCare (formerly Boston Biomedica Inc); BCP panels from Zeptomatrix (formerly BioClinical Partners Inc); HPA: Health Protection Agency; NIBSC: National Institute for Biological Standards and Control

Specificity findings

Of 199 HBsAg negative blood donor specimens, one was initially indeterminate and 198 negative (Table 4) to give an initial specificity for the HBsAg II assay of 99.50% (95% CI: 97.2-100%). Upon retesting in duplicate, the indeterminate specimen was negative to give a repeat specificity of 100% (95% CI: 98.2-100%).

Table 4: Specificity of Roche HBsAg II

	Result (N=199)			Specificity (95% CI)
	Negative	Borderline	Positive	
Initial	198*	1	0	99.5% (97.2-100)
Repeat	199	0	0	100% (98.2-100)

*This includes seven specimens which were reactive at a very low level, but were flagged by the MODULAR ANALYTICS E170 automated immunoassay analyser as the result of a potential carryover of the microparticles (requiring retesting). All of these were unreactive on retesting and hence were counted as negative in the calculation of initial specificity

Sensitivity findings

All 200 HBsAg positive specimens were reactive by the HBsAg assay to give an initial sensitivity of 100% (95% CI: 98.2-100%). The signal/OD values ranged from 44.89 to 14360 and values for each specimen category are presented below in Table 5 (the numbers are too small for meaningful analysis).

Table 5: Sensitivity of Roche HBsAg

Specimen category	No. of specimens (N=200)	Signal/CO		
		Mean	Median	Range
Acute positive	31	5086.28	3694.00	106.00 - 12588
Chronic positive	39	7249.99	8192.00	101.80 - 14360
Anti-e positive	31	6013.83	5929.00	109.20 - 12788
eAg positive	8	5185.75	5518.00	1264.00 - 7300
Subtype ad	39	6987.52	6118.00	915.10 - 12959
Subtype ay	17	4810.29	5339.00	1159.00 - 7327
Positive (uncharacterised)	35	4328.37	3574.00	44.89 - 14056

Distribution of initial reactivities

The distribution of the initial reactivities for the 199 HBsAg negative and 200 HBsAg positive specimens is shown in Figure 1. Assays with good discrimination have few or no samples wrongly classified and few reactivities close to the cut-off.

The HBsAg II assay had no false negatives and seven false positive results with a reaction just above the cut-off. These were flagged by the MODULAR ANALYTICS E170 analyser as specimens requiring retesting (due to a suspected carryover of microparticles) and were all unreactive on retesting in duplicate (Appendix 1); due to these being a potential problem with the MODULAR ANALYTICS E170 analyser, the retest values were used in the analysis. A further HBsAg negative specimen initially gave a borderline result (negative on retesting). The HBsAg positive specimens all gave high reactivities (>40).

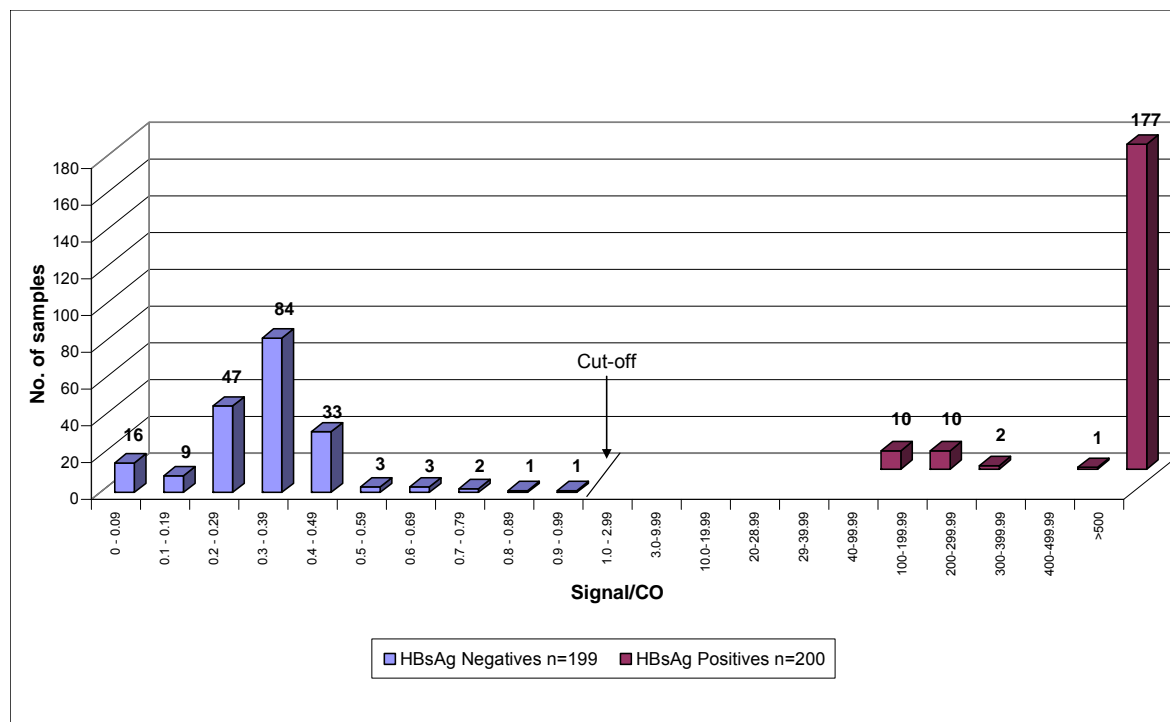


Figure 1: Distribution of initial reactivities

The scale for Signal/CO is not continuous

Weakly positive specimen panel: long-term carriers

A panel of nine weakly positive specimens from long-term carriers clearing their antigenaemia were tested by HPA-MiDAS. All nine (100%) of the panel tested were detected by the HBsAg assay, with a mean signal/CO value of 32.55, median of 10.69 (range: 1.53-231.80) (Table 6; Appendix 2).

Table 6: Comparative HBsAg kit results for 9 weakly positive samples

Specimen category	No. of specimens	Signal/CO		
		Mean	Median	Range
Weakly reactive (long term carriers clearing antigenaemia)	9	32.55	10.69	1.53-231.80

Seroconversion sensitivity

Twenty seroconversion panels were tested including 16 from SeraCare (formerly Boston Biomedica Inc) and four from Zeptometrix (formerly BioClinical Partners, Inc). Comparative data from 14 other HBsAg assays was available for 16 of these panels. The HBsAg II assay gave a score of 57 out of 112 making it the 6th most sensitive (Table 7). Detailed results of the individual panels are given in Appendix 3.

Table 7: Seroconversion assay comparison (18 panels)

Assay	Product no.	Cumulative score* (PHM903-924) N = 112	Rank
Abbott PRISM™ HBsAg	3A4748	72	1
AxSYM® HBsAg (V2)	7A40-22	65	2
Enzygnost® HBsAg 5.0	OQPW11/21	62	3
Monolisa HBsAg ULTRA	72346	60	4=
Murex HBsAg (version 3)	GE34/36	60	4=
Roche HBsAg II	4687787	57	6
Architect HBsAg	6C15-20	55	7=
Hepanostika HBsAg ULTRA	248133	55	7=
Vitros ECi HBsAg	843 5307	53	9
ETI-MAK-4	N0019	52	10
Bioelisa HBsAg colour	3000-1130	45	11
Auszyme monoclonal	1980-24	43	12
VIDAS HBsAg	30 300	40	13
DIA.PRO HBsAg One-Step	SAG1.CE	39	14
Access® HBs Ag	34220	36	15

*The score was calculated by summing the number of positive samples for each of the seroconversion panels; a higher score suggests a higher sensitivity. The position in this table is based on 16 seroconversion panels.

Comparative timing of detection

Timing of detection was analysed by assigning the most sensitive assay for each seroconversion panel a value of 'time zero', and any less sensitive assay a positive value based on the number of days after the most sensitive assay detected infection.

An overall mean and median delay was then calculated for the seroconversion panels tested (Table 8, Figure 2).

The mean delay can be 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 median delay is not affected in the same way. The median detection time for HBsAg II assay was 7 days, which ranks the assay joint fifth with five other assays out of 15.

Using mean values, the HBsAg II assay was the sixth most sensitive assay for the panels tested, and detected HBsAg infection approximately 0.73 days earlier than the next best kit (Architect HBsAg), and 6.13 days after the Abbott Prism HBsAg assay which was ranked first (Table 8, Figure 2).

Table 8: Delay in detection of seroconversion

Assay*	Product number	Delay in detecting seroconversion in each panel compared with the most sensitive assay (days)		
		Mean	Median	Range
Abbott PRISM™ HBsAg	3A4748	3.67	0	0-36
AxSYM® HBsAg (V2)	7A40-22	7.27	5	0-36
Enzygnost® HBsAg 5.0	OQPW11/21	8.60	5	0-36
Murex HBsAg (version 3)	GE34/36	9.47	6	0-43
Monolisa HBsAg ULTRA	72346	8.87	7	0-36
Roche HBsAg II	4687787	9.80	7	0-43
Architect HBsAg	6C15-20	10.53	7	0-43
Hepanostika HBsAg ULTRA	248133	10.80	7	0-43
Vitros ECi HBsAg	843 5307	11.13	7	0-43
ETI-MAK-4	N0019	11.73	7	0-43
Bioelisa HBsAg colour	3000-1130	13.47	12	0-43
Auszyme monoclonal	1980-24	14.40	12	0-43
VIDAS HBsAg	30 300	15.60	12	0-43
DIA.PRO HBsAg One-Step	SAG1.CE	16.13	12	0-43
Access® HBs Ag	34220	17.00	14	0-43

Notes: Time 0 = earliest detection of HBsAg infection by any screening assay. The upper limit of the range is, to some extent, influenced by the intervals between bleeds for any individual panel. The mean and median values provide a better general guide to each assay's ability to detect seroconversion. When an assay failed to detect seroconversion in a panel it was given an arbitrary extra 3 days delay for that panel.

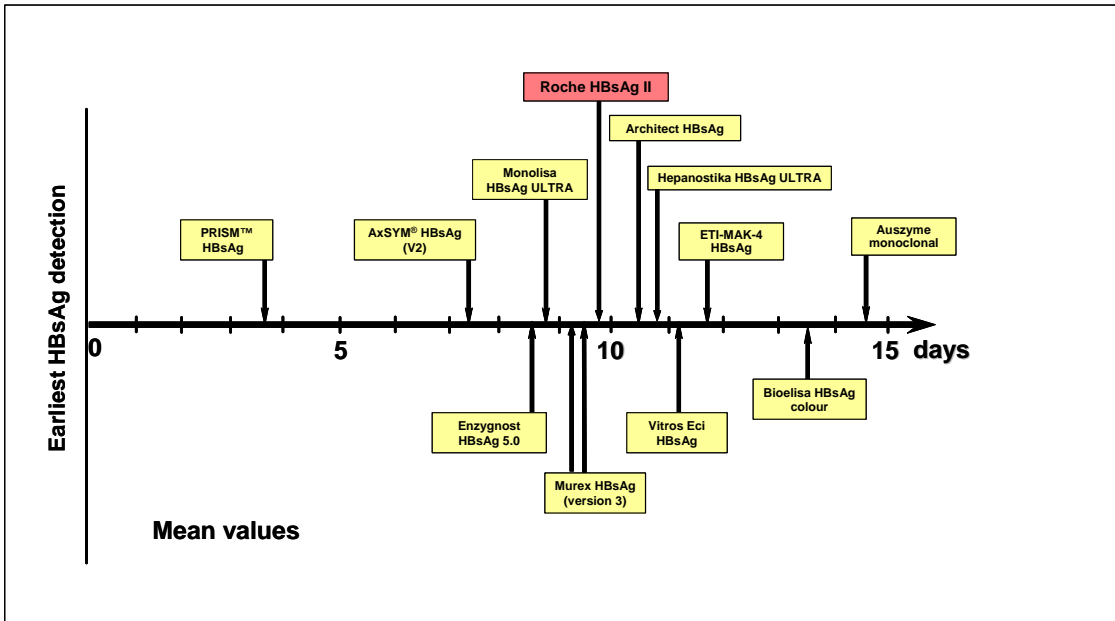


Figure 2a: Timing of detection for HBsAg detection assays (mean values)

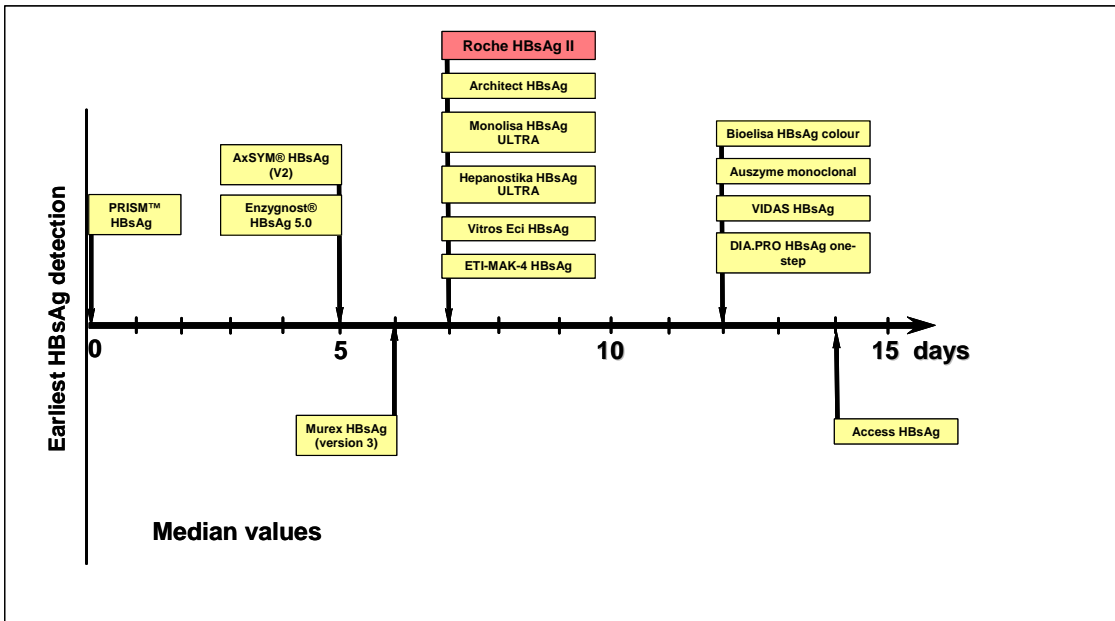


Figure 2b: Timing of detection for HBsAg detection assays (median values)

Proficiency panel

A low titre proficiency panel was assessed using the HBsAg assay (Table 9). All 14 positives from the panel of 15 specimens were reactive in the HBsAg assay with signal/CO values of between 1.06 and 11.55; PHA103-9 is a negative specimen.

Table 9: Performance of the HBsAg II assay using low titre proficiency panel PHA103

Panel member	Panel result (ng/ml)	HBsAg II result (signal/CO)
PHA103-9	0	0.15
PHA103-11	0.3	1.06
PHA103-4	0.3	2.32
PHA103-7	0.5	2.40
PHA103-13	0.6	2.73
PHA103-1	0.6	3.01
PHA103-8	0.8	3.39
PHA103-5	0.8	4.30
PHA103-15	0.8	5.04
PHA103-12	0.8	5.69
PHA103-2	0.9	6.76
PHA103-14	1.2	6.80
PHA103-6	1.2	8.40
PHA103-3	1.5	8.88
PHA103-10	1.7	11.55

Panel obtained from SeraCare; data shown in order of panel HBsAg result (ng/ml)

HBsAg mutant specimens

A panel of diverse native HBsAg mutant specimens generated by the Virus Reference Department of the HPA^{2,3} was assessed using the HBsAg II assay. The samples were tested at a concentration of 2.5ng/mL, determined by a reverse passive haemagglutination assay (Murex Hepatest) and subsequent dilution. Two of the specimens (Mutant 05 and Mutant 09) gave an error during the HBsAg II testing and insufficient volume was available for retesting; this error is likely to reflect either particulate matter in the specimen or an insufficient volume.

A positive and negative control were correctly identified, and 30 of the remaining 31 specimens were detected by HBsAg II (signal/CO ranging from 1.47 to 51.53; Appendix 4). One specimen (Mutant 20) containing five mutations (C100S, M103I, P142L, D144E, G145R) was negative (signal/CO of 0.80). Comparative data on the performance of these specimens was available for six other diagnostic assays^{2,3} and is shown in Appendix 4. Two kits detected all 31 mutants, three detected 30 and one detected 17.

Quality control reagents

Four quality control reagents were tested in triplicate (Table 10) - an ideal control is one with a dynamic range 2 to 3 times higher than the cut off. Two samples met this criterion: HPA HBsAg QC2 (hereafter QC2) and NIBSC HBsAg Monitor 0.05 IU. QC2 was selected as the run control and was tested at least once per day along with the test samples. The average signal/CO for each of the 15 runs of QC2 was 3.25 (median 3.36).

Table 10: Quality Control Reagent results

QC sample ID	Lot number	Signal/CO			Mean
		1	2	3	
HPA HBsAg QC1	07/B509-02	25.55	26.43	25.65	25.88
HPA HBsAg QC QC2*	07/B503-02	3.37	3.41	3.42	3.40
NIBSC Monitor 0.05 IU	01/286-001-WIL	3.17	3.27	3.19	3.21
NIBSC BWS 0.2 IU	01/476-013-WIL	6.27	6.27	6.31	6.28

*HPA HBsAg QC2 was selected to be the run control for this evaluation

Lot comparison

A subset of the specimens used for the main part of the evaluation was tested using a second kit lot (Lot number 152 095-01). This comprised of 40 HBsAg positives, 40 HBsAg negatives, 5 seroconversion panels (41 specimens) and 4 Quality Control samples.

All of the HBsAg-positive and negative specimens were correctly assigned by both assay lots. Three seroconversion panel specimens were positive in Lot 2 but not Lot 1 (Appendix 3a); two of these were borderline results and one high negative (S/CO values of 0.996, 0.948 and 0.815). These were not repeated.

Table 11: Comparison of two assay Lots

Specimen Category	No. of Specimens	No. of Reactive Specimens	
		Lot Number	
		1: 151 238-03	2: 152 095-01
HBsAg positive	40	40	40
HBsAg negative (blood donor sera)	40	0	0
HBsAg seroconversion panels (5; N=41)			
BBI – PHM914	6	4 (plus 1 borderline)	5
BBI – PHM920	6	4	4
BBI – PHM922	12	7	8
BBI – PHM924	5	3 (plus 1 borderline)	4
BCP 6281	12	6	6
Quality control samples (n=4)			
NIBSC Monitor Sample 0.05U/ml	1 (x3)	3	3
NIBSC BWS 0.2IU/ml	1 (x3)	3	3
HPA HBsAg QC1	1 (x3)	3	3
HPA HBsAg QC2	1 (x3)	3	3
TOTAL	121		

* one specimen was borderline (signal/CO: 0.98)

Technical appraisal

The HBsAg II assay instructions were clear and easy to follow, and the kit was very simple to load onto the MODULAR ANALYTICS E170 analyser. On a few occasions, the MODULAR ANALYTICS E170 analyser had some problems reading the barcodes, in which case samples were manually assigned to positions within racks. The reagents used for detection by the MODULAR ANALYTICS E170 analyser (ProCell and CleanCell) are only stable for one week once loaded, but this will not be an issue for laboratories with a high-throughput of samples.

Conclusion

The HBsAg II assay allows the detection of HBsAg surface antigen, including mutants generated as a result of immune pressure or antiviral therapy.

When used in combination with the MODULAR ANALYTICS E170 analyser, the HBsAg II assay showed excellent sensitivity when tested against a moderate number of routine positive specimens (100%: 95% CI: 98.2-100%), and it also detected weakly reactive specimens from long-term carriers clearing antigenaemia. A panel of native mutants were tested and the HBsAg II panel detected 30 of 31 of these, only missing one sample with five mutations. In terms of specificity, one indeterminate result was obtained on initial testing of 199 HBsAg negative blood donor specimens; this was negative on retesting to give a repeat sensitivity of 100% (95% CI:98.2-100%).

By employing various measures of seroconversion sensitivity the Roche HBsAg II was listed as the 6th most sensitive assay (using addition of reactive samples for 16 seroconversion panels); 6th most sensitive (using the timing of detection: mean method) and equal fifth with five other HBsAg kits (timing of detection: median). By the timing of detection (mean) method it detected HBsAg a mean of 0.73 days earlier than the next best kit, and 6.13 days after the Abbott PRISM HBsAg assay.

The HBsAg II assay is easy to use and suitable for laboratories wishing to use the MODULAR ANALYTICS E170 analyser range of assays.

Acknowledgements

We would like to thank Mathew Beale, Prof Richard Tedder and Dr Samreen Ijaz for access to the panel of HBsAg mutants and comparative data of their performance, Mohammed Surti and Li Fu for their assistance with the specimen preparation, and the HPA's Quality Control Reagents Unit (QCRU) for the provision of quality control reagents. We would also like to thank members of the Virus Reference Department for testing and specialist advice.

References

1. **Barlow K, Perry K.** (2007): Evaluation of the Roche HIV combi assay. *PER07004*, p1-21. Available from www.hpa-midas.org.uk/reports
2. **Beale M.** (2007): The Impact of Hepatitis B Surface Antigen Mutations on α -determinant Structure and Diagnostic Screening. A Thesis submitted in partial fulfillment of the MSc Biomedical Science, School of Pharmacy & Biomedical Science, University of Portsmouth
3. **Burgess C, Perry K, Newham J, Kitchen A.** Evaluation of Abbott Architect HBsAg Assay Product code 6C36. *NBSR7002*, p1-18. Available from www.hpa-midas.org.uk/reports

Appendix

Appendix 1: Initially reactive results from HBsAg negative specimens

MiDAS specimen ID	Result	Initial signal/CO	Repeat signal/CO values
08 N0148	Borderline	0.946	0.266, 0.222
08 N0163	HBsAg positive	1.020	0.501, 0.366
08 N0155	HBsAg positive	1.200	0.253, 0.273
08 N0152	HBsAg positive	1.270	0.314, 0.306
08 N0164	HBsAg positive	1.270	0.292, 0.254
08 N0157	HBsAg positive	1.300	0.203, 0.218
08 N0153	HBsAg positive	1.680	0.226, 0.192
08 N0141	HBsAg positive	1.920	0.473, 0.229

Appendix 2: Comparative HBsAg kit results for 9 weakly positive samples (long-term carriers)

Assay	Product no.	Specimen number									Positive (%)
		1	2	3	4	5	6	7	8	9	
HBsAg II	04687787	1.87	10.69	4.27	11.09	13.15	2.48	231.80	16.04	1.53	9/9 (100)
Abbott Architect HBsAg (IU/mL*)	6C15-20	0.075	0.521	0.214	0.465	0.707	0.169	9.956	1.149	0.071	9/9 (100)
PRISM™ HBsAg (S/CO)	3A4748	4.41	12.26	5.27	12.40	28.46	4.11	171.09	53.08	1.93	9/9 (100)
Hepanostika HBsAg ULTRA (B15BA) (S/CO)	284133	1.675	9.162	2.615	7.658	8.906	0.444	40.786	3.060	1.675	8/9 (100)

* The cut-off value for the Architect HBsAg assay is 0.05 IU/mL; negative reactions are shown in grey. Specimens 3 and 4 are serial bleeds from the same patient taken on 2/12/1991 and 29/1/1993 respectively. Specimens 6, 7 and 8 are serial bleeds from the same patient taken on 10/04/1992, 22/2/1993 and 27/1/2000, respectively

Appendix 3a: HBsAg seroconversion panels - raw data

Panel	Signal/CO	
	Lot 1	Lot 2
BCP6271.01	0.293	-
BCP6271.02	0.481	-
BCP6271.03	1.480	-
BCP6271.04	8.800	-
BCP6271.05	78.630	-
BCP6274.01	0.816	-
BCP6274.02	1.760	-
BCP6274.03	4.200	-
BCP6274.04	7.130	-
BCP6274.05	28.160	-
BCP6274.06	65.510	-
BCP6274.07	216.600	-
BCP6276.01	0.260	-
BCP6276.02	0.097	-
BCP6276.03	0.078	-
BCP6276.04	0.152	-
BCP6276.05	0.204	-
BCP6276.06	0.194	-
BCP6276.07	0.890	-
BCP6276.08	1.640	-
BCP6281.01	0.137	0.355
BCP6281.02	0.091	0.375
BCP6281.03	0.119	0.388
BCP6281.04	0.645	0.721
BCP6281.05	1.150	1.080
BCP6281.06	4.720	3.040
BCP6281.07	18.850	11.830
BCP6281.08	25.710	15.930
BCP6281.09	40.600	30.910
BCP6281.10	10.330	8.450
BCP6281.11	0.008	0.292
BCP6281.12	0.050	0.248
PHM903.01	0.318	-
PHM903.02	0.382	-
PHM903.03	0.692	-
PHM903.04	1.590	-
PHM903.05	4.830	-
PHM903.06	11.200	-
PHM904.01	0.364	-
PHM904.02	0.988	-
PHM904.03	8.100	-
PHM909.01	0.304	-
PHM909.02	0.366	-
PHM909.03	0.600	-
PHM909.04	1.880	-
PHM909.05	6.550	-
PHM909.06	20.130	-
PHM909.07	47.610	-
PHM910.01	0.244	-
PHM910.02	0.329	-
PHM910.03	3.870	-
PHM910.04	26.440	-
PHM910.05	46.510	-
PHM910.06	69.620	-

Panel	Signal/CO	
	Lot 1	Lot 2
PHM911.01	0.238	-
PHM911.02	0.195	-
PHM911.03	0.159	-
PHM911.04	0.135	-
PHM911.05	0.131	-
PHM911.06	NT	-
PHM911.07	0.308	-
PHM911.08	0.123	-
PHM911.09	0.227	-
PHM911.10	0.225	-
PHM911.11	0.265	-
PHM911.12	NT	-
PHM911.13	0.340	-
PHM911.14	0.174	-
PHM911.15	0.165	-
PHM911.16	0.155	-
PHM911.17	0.213	-
PHM911.18	0.305	-
PHM911.19	0.439	-
PHM911.20	1.390	-
PHM911.21	2.360	-
PHM911.22	3.860	-
PHM911.23	6.330	-
PHM911.24	12.610	-
PHM911.25	28.290	-
PHM914.01	0.180	0.410
PHM914.02	0.996	1.180
PHM914.03	1.310	1.590
PHM914.04	2.090	2.160
PHM914.05	3.930	3.750
PHM914.06	6.510	7.380
PHM916.01	0.106	-
PHM916.02	0.178	-
PHM916.03	0.075	-
PHM916.04	0.154	-
PHM916.05	0.210	-
PHM916.06	0.194	-
PHM916.07	0.161	-
PHM916.08	0.229	-
PHM916.09	0.866	-
PHM916.10	3.090	-
PHM916.11	13.630	-
PHM917.01	0.246	-
PHM917.02	0.649	-
PHM917.03	6.100	-
PHM918.01	0.268	-
PHM918.02	1.780	-
PHM918.03	10.790	-
PHM919.01	0.337	-
PHM919.02	0.361	-
PHM919.03	0.288	-
PHM919.04	0.636	-
PHM919.05	0.930	-
PHM919.06	2.780	-
PHM919.07	5.120	-
PHM919.08	17.280	-
PHM919.09	24.180	-

Appendix 3a (continued): Seroconversion panels - raw data

Panel	S/CO	
	Lot 1	Lot 2
PHM920.01	0.220	0.285
PHM920.02	0.236	0.311
PHM920.03	3.330	3.310
PHM920.04	63.510	60.280
PHM920.05	134.600	132.100
PHM920.06	766.500	870.700
PHM921.01	6.420	-
PHM921.02	18.460	-
PHM921.03	33.960	-
PHM921.04	137.500	-
PHM921.05	393.100	-
PHM921.06	1156.000	-
PHM922.01	0.145	0.430
PHM922.02	0.417	0.404
PHM922.03	0.537	0.612
PHM922.04	0.529	0.771
PHM922.05	0.815	1.510
PHM922.06	1.410	3.110
PHM922.07	8.120	18.680
PHM922.08	30.520	44.180
PHM922.09	112.800	192.700
PHM922.10	513.100	456.500
PHM922.11	3307.000	1977.000
PHM922.12	2459.000	6707.000

Panel	S/CO	
	Lot 1	Lot 2
PHM923.01	0.252	-
PHM923.02	0.430	-
PHM923.03	2.070	-
PHM923.04	3.380	-
PHM924.01	0.218	0.435
PHM924.02	0.948	1.170
PHM924.03	2.250	2.250
PHM924.04	4.740	4.450
PHM924.05	15.440	14.930
PHM925.01	0.350	-
PHM925.02	0.733	-
PHM925.03	1.520	-
PHM925.04	5.280	-
PHM925.05	3.710	-

Negative reactions are indicated in grey; borderline in dark grey. Panel BCP6281 is an acute to recovered disease panel; members 11 and 12 had <100 copies by PCR. Two members of panel PHM911 failed during testing due to insufficient volume or the presence of particulate matter (numbers 6 and 12) – these were counted as negative in the analysis

Appendix 3b: Comparative results of seroconversion panels PHM903-924

Assay	Product number	No. of positive samples and the number of days from initial bleed to first positive sample (shown in parentheses)														Score [†] N = 112	
		903 (N=6)	904 (N=3)	909 (N=7)	910 (N=6)	911 (N=25)	914 (N=6)	916 (N=11)	917 (N=3)	918 (N=3)	919 (N=9)	920 (N=6)	921 (N=6)	922 (N=12)	923 (N=4)		924 (N=5)
Abbott PRISM™ HBsAg	3A4748	4 (6)	3 (0)	6 (4)	6 (0)	6 (77)	5 (146)	3 (31)	2 (36)	2 (7)	8 (5)	4 (26)	6 (0)	9 (9)	4 (0)	4 (23)	72
AxSYM® HBsAg (V2)	7A40-22	4 (6)	2 (7)	4 (9)	4 (35)	9 (65)	4 (151)	3 (31)	2 (36)	3 (0)	6 (12)	4 (26)	6 (0)	7 (16)	3 (7)	4 (23)	65
Enzygnost® HBsAg 5.0	OQPW11/21	4 (6)	2 (7)	4 (9)	4 (35)	6 (77)	5 (146)	3 (31)	2 (36)	2 (7)	6 (12)	4 (26)	6 (0)	8 (14)	2 (15)	4 (23)	62
Monolisa HBsAg ULTRA	72346	4 (6)	2 (7)	4 (9)	4 (35)	6 (77)	5 (146)	3 (31)	2 (36)	2 (7)	5 (14)	4 (26)	6 (0)	7 (16)	2 (15)	4 (23)	60
Murex HBsAg (version 3)	GE34/36	4 (6)	2 (7)	5 (7)	4 (35)	6 (77)	4 (151)	3 (31)	1 (43)	2 (7)	5 (14)	4 (26)	6 (0)	9 (9) [‡]	2 (15)	3 (29)	60
Roche HBsAg II^a	4687787	3 (10)	2 (7)	4 (9)	4 (35)	6 (77)	5 (146)	2 (34)	1 (43)	2 (7)	5 (14)	4 (26)	6 (0)	7 (16)	2 (15)	4 (23)	57
Architect HBsAg [‡]	6C15-20	3 (10)	2 (7)	4 (9)	4 (35)	6 (77)	4 (151)	2 (34)	1 (43)	2 (7)	5 (14)	4 (26)	6 (0)	7 (16)	2 (15)	3 (29)	55
Hepanostika HBsAg ULTRA	248133	3 (10)	1 (18)	4 (9)	4 (35)	6 (77)	3 (153)	3 (31)	1 (43)	2 (7)	5 (14)	4 (26)	6 (0)	7 (16)	2 (15)	4 (23)	55
Vitros ECi HBsAg	843 5307	3 (10)	2 (7)	3 (14)	4 (35)	6 (77)	3 (153)	3 (31)	1 (43)	2 (7)	4 (19)	4 (26)	6 (0)	7 (16)	2 (15)	3 (29)	53
ETI-MAK-4	N0019	3 (10)	1 (18)	4 (9)	4 (35)	5 (79)	4 (151)	2 (34)	1 (43)	2 (7)	4 (19)	4 (26)	6 (0)	7 (16)	2 (15)	3 (29)	52
Bioelisa HBsAg colour	3000-1130	2 (14)	2 (7)	3 (14)	4 (35)	4 (84)	2 (158)	2 (34)	1 (43)	1 (12)	4 (19)	4 (26)	6 (0)	6 (21)	2 (15)	2 (35)	45
Auszyme monoclonal	1980-24	2 (14)	1 (18)	3 (14)	4 (35)	4 (84)	2 (158)	2 (34)	1 (43)	0 (>12)	4 (19)	4 (26)	6 (0)	6 (21)	2 (15)	2 (35)	43
VIDAS HBsAg	30 300	2 (14)	1 (18)	3 (14)	4 (35)	3 (86)	2 (158)	2 (34)	1 (43)	0 (>12)	4 (19)	3 (35)	6 (0)	6 (21)	1 (22)	2 (35)	40
DIA.PRO HBsAg One-Step	SAG1.CE	2 (14)	1 (18)	3 (14)	3 (42)	3 (86)	2 (158)	1 (38)	1 (43)	1 (12)	4 (19)	3 (35)	6 (0)	6 (21)	1 (22)	2 (35)	39
Access® HBs Ag	34220	2 (14)	1 (18)	3 (14)	3 (42)	3 (86)	1 (160)	1 (38)	1 (43)	1 (12)	4 (19)	3 (35)	6 (0)	6 (21)	0 (>22)	1 (43)	36

[†] The score was calculated by summing the number of positive specimens detected for each panel. A higher score suggests higher sensitivity. [‡] data for panels 903, 909, 910, 914, 916, 917, 920 and 923 was derived by HPA-MiDAS for this study. [¶]: Panel 922 members 04 and 06-12 were positive; panel members 04, 05 and 06 had OD/CO ratios of 1.04, 0.93 and 2.54, respectively.

Appendix 4: Results of testing the native HBsAg Mutant strain panel

Mutant Name	Spec. no.	Roche HBsAg II (Signal/CO)	Assay A	Abbott Architect*	Assay B	Assay C	Assay D	Assay E
4 aa insertion of TTST between 118+119, W196L and Y206F	1	13.11	14.180	12.9 (0.64)	18.607	12.340	11.934	9.690
T118A	2	10.07	16.600	9.9 (0.49)	18.064	11.585	10.957	8.890
P120S	4	9.62	14.080	10.1 (0.51)	9.771	9.404	8.120	15.680
P120S, W196L	6	5.43	8.300	5.7 (0.28)	10.929	11.277	8.938	1.460
P120S	7	20.51	24.710	20.8 (1.04)	17.271	18.830	16.035	33.730
T123A, M133I	10	30.30	0.390	0.7 (0.03)	32.700	29.702	23.132	31.950
G130N, I208T, S210K	11	3.48	4.600	3.4 (0.16)	6.900	4.436	4.500	4.240
N131P	12	6.57	10.490	6.5 (0.33)	8.521	7.702	7.957	6.730
T189I, M133I	13	13.53	23.620	17.3 (0.85)	23.043	17.064	12.593	36.620
F134I, T143M	15	8.01	10.590	7.4 (0.35)	10.914	8.936	9.508	0.830
F134I, T140L	16	7.47	8.350	6.0 (0.31)	13.393	8.989	8.818	10.610
F134L, D144V, L173F, P203R	17	2.57	4.770	3.2 (0.16)	5.736	3.138	4.058	5.480
C139Y	19	3.08	3.970	2.9 (0.15)	4.136	3.564	3.713	3.690
C100S, M103I, P142L, D144E, G145R	20	0.80	6.460	3.4 (0.17)	1.764	3.053	5.450	0.310
P142L, G145R	21	1.81	14.830	10.3 (0.5)	16.707	10.649	10.345	<0.1
P46I, V47E, N59K, C76Y, T143L, V190A	22	6.15	7.730	4.2 (0.21)	6.543	5.840	6.496	<0.1
T143L	23	11.14	14.620	7.3 (0.37)	10.986	9.809	10.651	<0.1
T143L	24	8.66	12.080	5.7 (0.28)	9.471	8.245	10.159	<0.1
R79H, T143L, N207R	25	15.70	16.100	11 (0.58)	14.686	13.649	13.705	0.200
D144A, A166G, I91T	26	2.01	16.030	12.2 (0.59)	21.857	10.968	14.407	<0.1
D144A	27	2.23	5.000	4 (0.21)	6.529	3.840	3.895	<0.1
D144E, I195M , P203Q, S204G, F220L	28	2.69	7.840	5.8 (0.28)	10.586	5.085	6.217	0.130
F145A, I195M , M197T, S210K, F220	30	6.52	11.180	9.8 (0.48)	9.914	7.170	6.260	6.900
G145R, I195T	31	24.83	23.590	17.9 (0.89)	22.643	24.585	17.492	28.350
G145R	32	2.17	7.770	7.5 (0.39)	6.657	7.234	6.376	0.330
M133I, F134H, D144V	34	1.47	2.650	1.0 (0.05)	0.429	1.106	4.143	<0.1
M133I, F134N, P142S, S143L, G145L	35	2.12	9.980	10.2 (0.49)	3.700	2.500	7.473	<0.1
T118A, E164D , I195M	36	15.86	19.750	15.6 (0.76)	20.307	14.181	11.287	31.110
W196L , P203R	37	18.11	22.590	17.5 (0.88)	18.614	19.043	14.837	28.560
I195M	38	9.63	14.900	9.6 (0.47)	11.507	11.298	9.039	10.910
D144, G145R	39	7.90	11.760	13.4 (0.68)	16.214	10.234	13.942	0.390
Positive control (genotype F, wild type)		51.53	63.810	68.900	34.679	36.138	25.589	117.560
Negative human plasma		0.174	0.370	0.000	0.414	0.532	0.504	0.540

Amino acids in blue indicate mutations associated with antiviral resistance. Non-reactive results are shown in grey. Results are as sample/CO except for Roche HBsAg II. *Results of the Architect HBsAg are presented as an approximate S/CO to allow for comparison and analysis; the IU/mL result is shown in parentheses