



Blood and Transplant



Evaluation of Abbott Architect HBsAg Assay Product code 6C36

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Introduction

The Microbiological Diagnostics Assessment Service (HPA-MiDAS) in conjunction with the National Transfusion Microbiology Reference Laboratory (NTMRL), North London Blood Centre carried out evaluations of six assays for the Abbott Architect i2000SR analyser. The analyser was installed at the NTMRL where all testing took place.

The object of the evaluations was to assess the ability of the Architect HBsAg, HIV Ag/Ab combo, Anti-HCV, Rubella IgG, anti-CMV and Syphilis TP assays to detect serological evidence of each respective marker in human serum and plasma specimens.

The results of the evaluation of the HBsAg assay are presented in this report. The kits were tested against a panel of serum/plasma samples found to be either reactive or unreactive by relevant screening assays used in Europe. In addition, several sequential blood collections from individuals undergoing seroconversion for the relevant marker (chosen to compare directly with a range of other assays), one commercial low-titre performance panel and four national quality control samples were incorporated in this evaluation.

Abbott Diagnostics provided all equipment, reagents and consumables required for this evaluation. They were responsible for the training of the operators in the use of the analyser and for the installation and ongoing maintenance and repair of any faulty equipment.

HPA-MiDAS was responsible for the testing of the evaluation panel which consisted of HBsAg positive and weakly positive samples, seroconversion panels and quality control samples. NTMRL was responsible for the specificity testing of a panel of ante-natal samples and sensitivity testing of a panel of samples used for the NBS Lot Release Testing program.

Description of the assay

The Abbott Architect HBsAg assay is a two-step sandwich chemiluminescent microparticle immunoassay (CMIA) for the detection of Hepatitis B surface antigen (HBsAg) in human serum or plasma. HBsAg present in the sample binds to two mouse monoclonal antibodies (IgM and IgG) coated onto paramagnetic particles. After a wash step, a goat IgG anti-HBsAg acridinium-labelled conjugate is added. Following a further wash step, pre-trigger solution (hydrogen peroxide) and trigger solution (sodium hydroxide) are added. The resulting chemiluminescent reaction is measured in relative light units (RLUs) which are directly proportional to the amount of HBsAg present in the sample.

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Table 1: Assay information

General	
Assay name	Architect HBsAg
Manufacturer/UK agent	Abbott Diagnostics
Product number	6C36
Number of tests per pack	100 / 400
Sample volume (including 'dead volume')	150µL

Presentation	
Assay type	Two-step chemiluminescent sandwich immunoassay
Solid phase	Paramagnetic microparticles coated with anti-HBs (mouse monoclonal, IgM and IgG)
Conjugate	Acridinium-labelled anti-HBs (goat IgG)
Substrate	Pre-trigger - hydrogen peroxide solution Trigger - sodium hydroxide solution
Negative control	1
Positive control	2
Reading wavelength	n/a - chemiluminescent
Cut-off computation	n/a - curve fit. 0.05IU/mL cut-off value
Equivocal zone	None

Stages	
Preparation/sample well loading	5 minutes
Specimen volume (excluding 'dead volume')	75µL
Incubation status	37°C
Sampling time - 1 sample	1 minute
Total time to completion (from initial loading of first sample)*	
- 1 sample	30 minutes
- 10 samples	33 minutes
- 100 samples	60 minutes

Additional equipment requirement	
Centrifuge	
Latex/nitrile gloves & personal protective equipment	

Note. *These data were observed timings by the evaluator.
Information provided by Abbott Diagnostics: Throughput approximately 100 tests in the first hour and 200 tests per hour after the first result is generated.

Evaluation panel and method

A total of 1380 samples were included in the evaluation panel, Table 2.

The main specificity study was carried out by the NTMRL, for which 1003 ante-natal patients' specimens previously screened and found negative for HBsAg were tested. Additionally, the NTMRL also tested a panel of 15 specimens which comprised the NBS Lot Release Testing panel. Fourteen of the 15 samples were positive for HBsAg and one was negative.

The evaluation panel used by the HPA-MiDAS totalled 362 specimens. Two hundred and one specimens were from HBsAg positive samples, nine were weakly reactive specimens from long-term carriers clearing antigenaemia and 20 HBsAg negative samples from ante-natal patients. Twelve seroconversion panels from commercial sources were also included. It was agreed with the NBS, Abbott and HPA-MiDAS that those panels for which Abbott already had data would not be included in the HPA-MiDAS panel. One panel was chosen to be also tested by HPA-MiDAS to allow for a limited comparison with the Abbott data for that panel. Abbott agreed in advance to share their data from the seroconversion panels that they had tested. In addition, four quality control samples, from the HPA and NIBSC, were included in the panel.

Table 2: Evaluation panel

Sample category	Number
NTMRL, NBS	
1. HBsAg negative - Ante-natal patients' sera	1003
2. Lot Release Testing Panel	15
HPA-MiDAS	
1. HBsAg Negative - Blood donors' sera	20
2. HBsAg positive samples	
a) HBsAg positive	201
b) weakly reactive (long term carriers clearing antigenaemia)	9
3. HBsAg seroconversion panels	
BBI: PHM 904	3
BBI: PHM 908	8
BBI: PHM 911	25
BBI: PHM 912	9
BBI: PHM 915	13
BBI: PHM 918	3
BBI: PHM 919	9
BBI: PHM 921	6
BBI: PHM 922	12
BBI: PHM 924	5
BCP: 6276	8
BCP: 6281	12
4. HBsAg performance panel	
BBI - PHA103 (low titre)	15
5. Quality control samples	
NIBSC Monitor Sample (0.05IU/ml)	3x 1
NIBSC Working Standard (0.2IU/ml)	3x 1
HPA HBsAg quality control serum QC1	3x 1
HPA HBsAg quality control serum QC2	3x 1
TOTAL (number of tests)	1380 (1388)
Notes:	
BBI = Boston Biomedica Inc (SeraCare Life Sciences); BCP = BioClinical Partners Inc (Zeptomatrix);	
HPA = Health Protection Agency	
NIBSC = National Institute for Biological Standards and Control.	

The method described in the kit insert was followed strictly. Abbott Architect i2000SR is a fully automated analyser; all processing steps are performed on the instrument. The Architect assay parameters are factory set and defined in the system software.

A daily maintenance program is followed each day, the steps for which are prompted on the display screen. Principally, Probe Conditioning Solution and sodium hypochlorite solution are loaded onto the analyser by the operator and the analyser completes the program automatically. This process takes approximately 20 minutes. In addition, a weekly maintenance program is also required in which the sample, reagent and wash probes are cleaned with cotton-wool swabs soaked in distilled water and the air filters are cleaned.

Prior to running the analyser, test reagents, pre-trigger solution, trigger solution, wash buffer and reaction vessels are loaded onto the analyser and automatically primed and loaded as appropriate. The latter two may also be added whilst the analyser is in 'Running' mode.

Prior to running a new batch of an assay, a calibration must first be performed. The calibrators are provided in dropper bottles and an appropriate volume is placed into sample cups and loaded onto the analyser. The calibration is valid for all subsequent tests using that particular lot number; it is not time limited. For the HBsAg assay, the calibrator pack consists of two calibrators with HBsAg concentrations of 0.5 and 250 IU/mL. A calibration curve is produced by the analyser against which the RLU reading from each sample is compared to give the number of international units per mL.

Three HBsAg kit controls are provided by Abbott. It is recommended that these are run at least once within every 24 hours that the test is in use. The HBsAg controls consist of a Negative Control (0.0IU/mL, range 0-0.04IU/mL), Positive Control 1 (0.25IU/mL, range 0.16-0.34IU/mL) and Positive Control 2 (175.0IU/mL, range 113.75-236.25IU/mL).

Samples may be loaded in their primary tubes, if suitable for the analyser, or aliquotted into Architect sample cups. Sample processing is initiated by the loading of the samples onto the analyser. The reactions occur in the following processing sequence: -

- A reaction mixture is formed combining sample and microparticles in the reaction vessel.
- After the first incubation is complete, the reaction mixture undergoes a wash step. A magnetic field is applied to retain the paramagnetic microparticles within the reaction vessel during the wash procedure.
- The anti-HBs/acridinium conjugate is then added and a further incubation takes place.
- Following a second wash step, pre-trigger (hydrogen peroxide) and trigger (sodium hydroxide) solutions are added to the reaction vessel
- The resultant chemiluminescent signal is measured and expressed as Relative Light Units.

The observed time from loading a sample to obtaining a result was 30 minutes for the HBsAg assay. Subsequent results are obtained approximately every 18 seconds, assuming continuous loading of samples. (18 seconds is a set cycle time and does not vary).

The cut-off sensitivity for the Abbott Architect HBsAg assay is set by the manufacturer at 0.05IU/mL. The results for this assay are presented as IU/mL in this report. Sample/cut-off values are not applicable for this assay, see calibration above.

Specificity

One thousand and three antenatal specimens were tested by the Architect HBsAg assay, lot number 50507LU00, at NTMRL. The specimens had been previously screened and found to be HBsAg negative by bioMérieux Hepanostika Ultra, product code 284133.

Nine hundred and ninety-eight samples were nonreactive in the Architect HBsAg assay, giving a specificity of 99.5% (95% confidence interval 98.85-99.83%), Table 3. Five specimens were initially reactive and were again reactive following retests in duplicate, initial and repeat reactive rate 0.5% (95% confidence interval 0.17-1.15%). The IU results for these specimens are shown in Table 4, along with the results of further testing where sufficient specimen volume was available.

Table 3: Specificity

Architect HBsAg result	Number	Number reactive	Reactive rate (95% confidence interval)	Mean IU/mL		Median IU/mL		Range IU/mL	
				Initial	Repeat	Initial	Repeat	Initial	Repeat
Negative	998	0	N/A	0.01	N/A	0.01	N/A	0.00-0.04	N/A
Reactive	5	5	N/A	0.15	0.39	0.09	0.16	0.05-0.31	0.06-1.11
Total	1003	5	0.5% (0.17-1.15%)	0.01	0.01	0.01	0.01	0.00-0.31	0.00-1.11

Table 4: Results of false positive samples

Sample number	Architect HBsAg			Biorad Ultra HBsAg S/CO	Murex HBsAg S/CO
	Initial IU/mL	Repeat IU/mL			
55	0.09	0.09	0.13	0.39/0.37	0.62/0.63
340	0.95	0.95	0.48	Insuff	Insuff
343	0.18	0.13	0.09	0.37/0.37	Insuff
760	1.11	0.68	0.21	0.38/0.37	Insuff
802	0.05	0.07	0.06	Insuff	0.57

Twenty HBsAg negative specimens were interspersed among the HBsAg positive specimens when tested by the HPA-MiDAS. All were nonreactive by the Architect HBsAg assay, Table 5.

Table 5: Specificity

Number tested	Number initially reactive	Initial reactive rate
20	0	0%

Sensitivity

HPA-MiDAS tested 201 HBsAg positive specimens by the Architect HBsAg assay (lot number 50507LU00). All 201 specimens were detected to give a sensitivity of 100% (95% confidence interval 98.2-100%). The results ranged from 1.64 - >250IU/mL with mean and median values of 211.84 and >250IU/mL, respectively. Table 6 shows the results of the Architect HBsAg assay along with sensitivity findings from 13 other HBsAg assays previously evaluated.

Table 6: Sensitivity

Assay	Product code	Number positive / number tested	Sensitivity % *	95% confidence interval %	Range S/CO	Mean S/CO	Median S/CO
Abbott Architect HBsAg [‡]	6C15-20	201/201	100.0	98.2 - 100	1.64 – >250 IU/mL	211.84 IU/mL	>250 IU/mL
AxSYM [®] HBsAg (V2)	7A40-22	491 / 491	100.0	99.3 – 100	1.20 – 291.55	170.77	196.81
Enzygnost [®] HBsAg 5.0	OQPW11/21	427 / 427	100.0	99.1 – 100	2.68 – 39.30	35.63	36.00
DIA.PRO HBsAg One-Step	SAG1.CE	422 / 422	100.0	99.1 - 100	2.00 – 61.28	49.15	55.77
Bioelisa HBsAg colour	3000-1130	150/150	100.0	97.6 - 100	3.07 – 44.02	35.63	37.51
Murex HBsAg Version 3	GE 34/36	298 / 298	100.0	98.8 – 100	5.63 – 24.59	22.98	23.08
ETI-MAK-4	N0019	286 / 286	100.0	98.7 – 100	2.57 – 120.00	69.98	75.00
Hepanostika HBsAg ULTRA	284133	200/200	100.0	98.2 - 100	4.71 – 155.77	136.07	144.28
MONOLISA HBs Ag ULTRA	72346	200/200	100.0	98.2 - 100	17.81 – 52.77	44.05	46.66
Auszyme monoclonal	1980-24	150/150	100.0	97.6 – 100	2.04 – 43.48	37.64	39.22
PRISM [™] HBsAg	3A47-48	145/145	100.0	97.6 – 100	4.48 – 813.58	426.35	470.69
VIDAS HBsAg	30 300	150/150	100.0	97.6 – 100	1.08 – 131.00	70.59	120.73
Vitros ECI HBsAg	843 5307	270/272	99.3	97.4 – 99.9	0.24 – 8740	3619.39	3695
Access [®] HBS Ag	34220	201/203	99.0	96.5 – 99.9	0.32§ – 1201.41	583.22	579.94

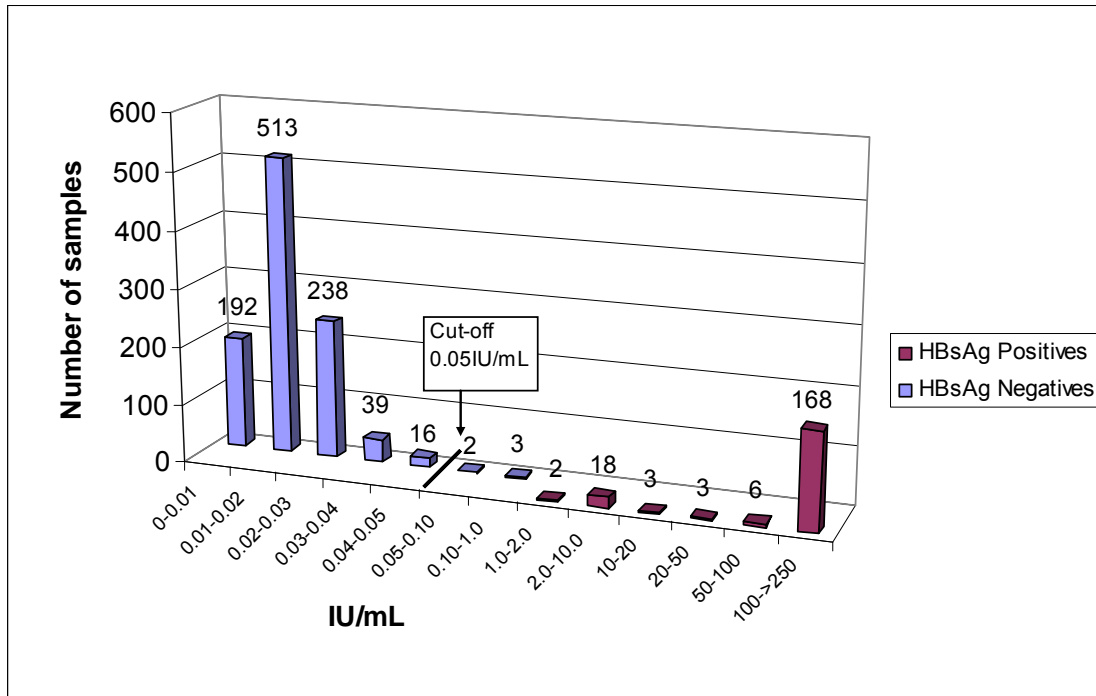
Notes:
[‡] The cut-off value for the Architect HBsAg assay is 0.05IU/mL
^{*} The sensitivity is based on initial reactive rates of HBsAg positive samples.
[§] The minimum value is for a specimen diluted 1 in 5 in normal human plasma (on repeat testing in duplicate S/CO ratios of 1.42 and 1.05 were obtained).

Distribution of initial reactivities

The distribution of reactivities for the 1003 HBsAg negative and 201 HBsAg positive samples is shown in Figure 1. Assays with good discrimination have few or no samples wrongly classified and few reactions close to the cut-off.

For the HBsAg negative specimens, the Architect HBsAg assay gave five initial reactive results which were again reactive on retesting. No false negative results were observed and the majority of the positives gave high reactivities

Figure 1: Distribution of reactivities



Note: The scale used for the IU/mL values is not continuous

Results of testing of NBS Lot Release Panel

Fifteen samples that comprise the NBS HBsAg lot release panel were tested at NTMRL. One sample was negative for HBsAg and 14 were HBsAg positive, with reactivities ranging from weak to strong positive. All 14 HBsAg positive samples were detected by the Architect HBsAg assay and the negative sample was nonreactive, Table 7.

Table 7: Results of NBS Lot Release Testing Panel

Panel/Ctrl Member	Expected Ratio	Expected Result	Architect HBsAg	
			IU/mL	Result
1	>2	Positive	0.21	Reactive
2	>2	Positive	0.26	Reactive
3	>1.5	Positive	0.25	Reactive
4	>1	Positive	0.26	Reactive
5	>2.5	Positive	0.36	Reactive
6	<1	Negative	0.00	Nonreactive
7	>1.5	Positive	0.56	Reactive
8	>1.5	Positive	0.20	Reactive
9	>3	Positive	0.58	Reactive
10	>2	Positive	2.83	Reactive
11	>1	Positive	0.13	Reactive
12	>1	Positive	0.13	Reactive
13	>1.5	Positive	0.35	Reactive
14	>2	Positive	0.70	Reactive
15	>3	Positive	>250	Reactive

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 Architect HBsAg assay, Table 8.

Table 8: Comparative HBsAg kit results for 9 weakly positive samples

Assay	Product number	Specimen number									Positive (%)
		1	2	3	4	5	6	7	8	9	
Abbott Architect HBsAg (IU/mL*)	6C15-20	0.075	0.521	9.956	0.214	0.465	0.707	1.149	0.071	0.368	9/9 (100)
Murex HBsAg Version 3 (S/CO)	GE34/36	2.32	8.39	21.74	4.46	9.05	12.25	17.75	1.29	5.63	9/9 (100)
PRISM™ HBsAg (S/CO)	3A4748	4.41	12.26	171.09	5.27	12.40	28.46	53.08	1.93	16.62	9/9 (100)
Monolisa HBsAg Ultra (S/CO)	72346	1.89	6.16	33.28	1.74	4.71	6.37	1.69	1.38	3.01	9/9 (100)
Enzygnost® HBsAg 5.0 (S/CO)	OQPW11/21	1.26	8.92	36.14	3.37	8.34	3.27	1.16	1.97	3.08	9/9 (100)
Hepanostika HBsAg ULTRA (B15BA) (S/CO)	284133	1.675	9.162	40.786	2.615	7.658	8.906	3.060	1.675	4.427	9/9 (100)
Hepanostika HBsAg ULTRA (B15HB) (S/CO)	284133	1.266	7.656	32.922	2.219	6.813	7.828	2.875	1.531	4.516	9/9 (100)

Notes:
 * The cut-off value for the Architect HBsAg assay is 0.05IU/mL
 Positive reactions are shown in bold type.
 Specimens 5 and 6 are serial bleeds from the same patient taken on 2/12/1991 and 29/1/1993, respectively.
 Specimens 4 and 8 are serial bleeds from the same patient taken on 22/2/1993 and 27/1/2000, respectively.

Seroconversion sensitivity

Twelve commercial seroconversion panels were tested by the HPA-MiDAS, 10 panels from Boston Biomedica Inc (BBI) and two from Zeptometrix (previously known as BioClinical Partners Inc, BCP).

For eight of the 10 BBI panels, comparative data were available for 13 other HBsAg assays. Of the total of 71 samples, 34 were detected by the Architect HBsAg assay, which was joint sixth most sensitive in the comparison, Table 9. Comparative data was available for six other assays for the two BCP panels. The Architect HBsAg assay detected eight of the total of 20 samples, Table 10. Data for all the panels tested by HPA-MiDAS may be seen in *Appendix Tables 14 and 16*.

Abbott Diagnostics provided their data for a further eight BBI panels which, when combined with data from the eight panels tested by HPA-MiDAS, comprised 142 samples. The Architect HBsAg assay detected 59 samples and was sixth most sensitive when compared with the other 13 assays, Table 11. The IU/mL values for each sample were not provided to HPA-MiDAS by Abbott Diagnostics therefore they are not presented in this report.

In addition, a commercial low titre performance panel comprising 14 positive and one negative specimen, was also tested. All 15 of the panel members were detected. The HBsAg positive samples gave values between 0.07-0.44IU/mL and the negative sample gave a value of 0.07IU/mL in the Architect HBsAg assay, *Appendix Table 15*.

Table 9: Comparative sensitivity for 8 BBI seroconversion panels (HPA-MiDAS data)

Assay	Product number	Number of positive samples and the number of days from initial bleed to first reactive sample (shown in parenthesis)								Score*
		PHM904 (n=3)	PHM908 (n=8)	PHM911 (n=25)	PHM918 (n=3)	PMH919 (n=9)	PHM921 (n=6)	PHM922 (n=12)	PHM924 (n=5)	
AxSYM [®] HBsAg (V2)	7A40-22	2 (7)	5 (13)	9 (65)	3 (0)	6 (12)	6 (0)	7 (16)	4 (23)	42
Abbott PRISM [™] HBsAg	3A4748	3 (0)	4 (15)	6 (77)	2 (7)	8 (5)	6 (0)	9 (9)	4 (23)	42
Monolisa HBsAg ULTRA	72346	2 (7)	4 (15)	6 (77)	2 (7)	6 (12)	6 (0)	8 (14)	4 (23)	38
Enzygnost [®] HBsAg 5.0	OQPW11/21	2 (7)	3 (20)	6 (77)	2 (7)	5 (14)	6 (0)	9 (9)¶	3 (29)	36
Murex HBsAg (version 3)	GE34/36	2 (7)	3 (20)	6 (77)	2 (7)	5 (14)	6 (0)	7 (16)	4 (23)	35
Abbott Architect HBsAg	6C15-20	2 (7)	3 (20)	6 (77)	2 (7)	5 (14)	6 (0)	7 (16)	3 (29)	34
Hepanostika HBsAg ULTRA	248133	1 (18)	3 (20)	6 (77)	2 (7)	5 (14)	6 (0)	7 (16)	4 (23)	34
Vitros [®] ECI HBsAg	843 5307	2 (7)	3 (20)	6 (77)	2 (7)	4 (19)	6 (0)	7 (16)	3 (29)	33
ETI-MAK-4	N0019	1 (18)	3 (20)	5 (79)	2 (7)	4 (19)	6 (0)	7 (16)	3 (29)	31
Bioelisa HBsAg colour	3000-1130	2 (7)	2 (33)	4 (84)	1 (12)	4 (19)	6 (0)	6 (21)	2 (35)	27
Auszyme monoclonal	1980-24	1 (18)	2 (33)	4 (84)	0 (>12)	4 (19)	6 (0)	6 (21)	3 (29)	26
DIA.PRO HBsAg One-Step	SAG1.CE	1 (18)	2 (33)	3 (86)	1 (12)	4 (19)	6 (0)	6 (21)	2 (35)	25
Access [®] HBs Ag	34220	1 (18)	2 (33)	3 (86)	1 (12)	3 (21)	6 (0)	6 (21)	2 (35)	24
VIDAS HBsAg	30 300	1 (18)	2 (33)	3 (86)	0 (>12)	4 (19)	6 (0)	6 (21)	2 (35)	24

Notes: * The score was calculated by summing the number of positive specimens detected for each panel. A higher score suggests higher sensitivity.

Table 10: Comparative sensitivity for two BCP seroconversion panels (HPA-MiDAS data)

Assay	Product Number	Number of positive samples and the number of days from initial bleed to first reactive sample (shown in parenthesis)	
		6276 (n=8)	6281 (n=12)
Hepanostika HBsAg ULTRA	284133	2 (27)	7 (13)
Abbott Architect HBsAg	6C15	2 (27)	6 (19)
AxSYM [®] HBsAg V2	7A40-22	1 (29)	7 (13)
Enzygnost [®] HBsAg 5.0	OQPW11/21	2 (27)	6 (19)
Monolisa HBsAg ULTRA	72346	2 (27)	6 (19)
ETI-MAK 4	N0019	2 (27)	5 (22)
DIA.PRO HBsAg One-Step	SAG1.CE	2 (27)	4 (33)

Table 11: Comparative sensitivity for 16 seroconversion panels (data generated by HPA-MiDAS and Abbott Diagnostics)

Assay	Product number	No. of positive samples and the number of days from initial bleed to first positive sample (shown in parentheses)															Score* n = 120	
		PHM903 (n=6)	PHM904 (n=3)	PHM908 (n=8)	PHM909 (n=7)	PHM910 (n=6)	PHM911 (n=25)	PHM914 (n=6)	PHM916 (n=11)	PHM917 (n=3)	PHM918 (n=3)	PMH919 (n=9)	PHM920 (n=6)	PHM921 (n=6)	PHM922 (n=12)	PHM923 (n=4)		PHM924 (n=5)
Abbott PRISM™ HBsAg	3A4748	4 (6)	3 (0)	4 (15)	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)	76
AxSYM® HBsAg (V2)	7A40-22	4 (6)	2 (7)	5 (13)	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)	70
Enzygnost® HBsAg 5.0	OQPW11/21	4 (6)	2 (7)	3 (20)	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)	65
Monolisa HBsAg ULTRA	72346	4 (6)	2 (7)	4 (15)	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)	64
Murex HBsAg (version 3)	GE34/36	4 (6)	2 (7)	3 (20)	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)	63
Architect HBsAg	6C15-20	2 (14)	2 (7)	3 (20)	4 (9)	4 (35)	6 (77)	4 (151)	2 (34)	3 (0)	2 (7)	5 (14)	4 (26)	6 (0)	7 (16)	2 (15)	3 (29)	59
Hepanostika HBsAg ULTRA	248133	3 (10)	1 (18)	3 (20)	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)	58
ETI-MAK-4	N0019	3 (10)	1 (18)	3 (20)	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)	58
Vitros ECi HBsAg	843 5307	3 (10)	2 (7)	3 (20)	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)	56
Bioelisa HBsAg colour	3000-1130	2 (14)	2 (7)	2 (33)	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)	47
Auszyme monoclonal	1980-24	2 (14)	1 (18)	2 (33)	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)	45
VIDAS HBsAg	30 300	2 (14)	1 (18)	2 (33)	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)	42
DIA.PRO HBsAg One-Step	SAG1.CE	2 (14)	1 (18)	2 (33)	3 (14)	3 (42)	3 (86)	2 (158)	1 (69)	1 (43)	1 (12)	4 (19)	3 (35)	6 (0)	6 (21)	1 (22)	2 (35)	41
Access® HBs Ag	34220	2 (14)	1 (18)	2 (33)	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)	38

Notes:
 Results shown in green: data provided by Abbott Diagnostics. Results shown in tan: data generated by HPA-MiDAS
 * The score was calculated by summing the number of positive specimens detected for each panel. A higher score suggests higher sensitivity.
 † Panel PHM922 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.

Seroconversion Sensitivity: Comparative timing of detection

Timing of detection was determined using a method that assigns for each seroconversion panel the most sensitive test “time zero” and any test less sensitive, a positive value (based on the number of days after the most sensitive kit has detected infection). An overall mean and median delay is then calculated for all seroconversion panels tested.

The median delay is not affected in the same way as the mean delay which can be strongly influenced by outlying results from seroconversion panels for which the interval between the last negative and the first positive specimen is long. This can give rise to an artefact due to the timing of blood collection.

From the data generated for the 16 panels tested by HPA-MiDAS and Abbott Diagnostics, on the basis of the median value the Architect HBsAg was ranked the joint sixth most sensitive assay, with three other assays, with a median value of seven days for the panels tested. This resulted in the assay detecting HBsAg approximately seven days later than the kit ranked first, Abbott PRISM HBsAg, and 4.5 days after the second ranked assay, AxSYM HBsAg (V2), Table 12 and Figure 2a.

On the basis of the mean value the Architect HBsAg was ranked the third most sensitive assay for the panels tested. This resulted in the assay detecting HBsAg approximately 4.5 days later than the kit ranked first, Abbott PRISM HBsAg, and one day after the second ranked assay, AxSYM HBsAg (V2), Table 12 and Figure 2b.

Table 12: Delay in detection of seroconversion

Assay	Product code	Delay in detecting seroconversion in each panel compared with the most sensitive assay		
		Range	Median	Mean
Abbott PRISM™ HBsAg	3A4748	0-36	0	3.31
AxSYM® HBsAg (V2)	7A40-22	0-36	2.5	6.81
Monolisa HBsAg ULTRA	72346	0-36	6	8.44
Enzygnost® HBsAg 5.0	OQPW11/21	0-36	6	8.50
Murex HBsAg (version 3)	GE34/36	0-43	6.5	9.31
Architect HBsAg	6C15-20	0-35	7	7.88
Hepanostika HBsAg ULTRA	248133	0-43	7	10.56
Vitros ECI HBsAg	843 5307	0-43	7	10.88
ETI-MAK-4	N0019	0-43	7	11.44

Figure 2a: Diagrammatic representation of median delay in detection of seroconversion

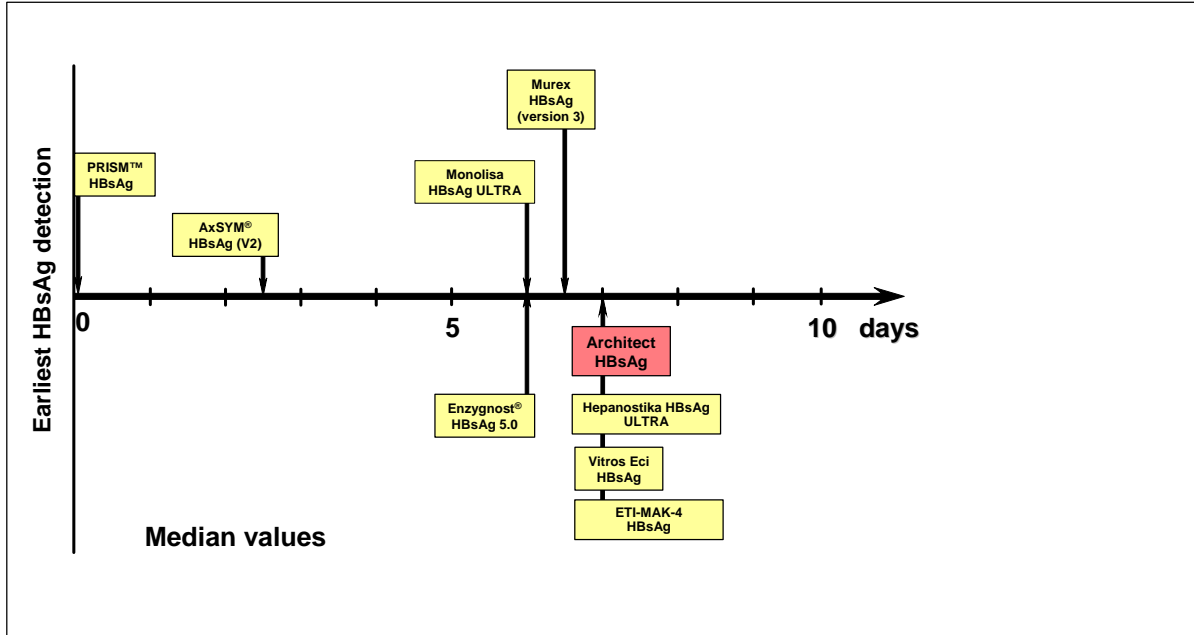
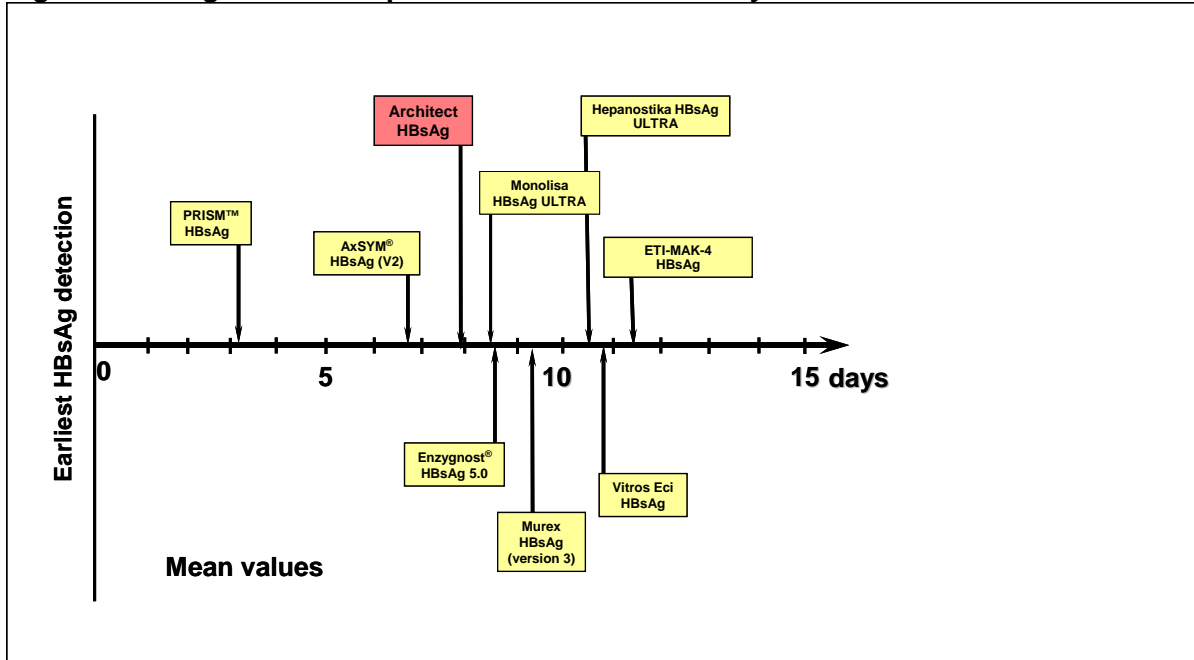


Figure 2b: Diagrammatic representation of mean delay in detection of seroconversion



Lot comparison

A subset of the main evaluation panel was tested in a second lot of the assay (Lot number 52234LU00). Forty positive specimens, 10 negative specimens and three seroconversion panels were compared, Tables 13a, 13b, 13c, and 16. The IU/mL results obtained from both lots were similar for all samples compared.

In addition, four quality control (QC) samples were tested in both lots of the assay, Table 13d. A QC specimen/statistical assay control should be chosen to have a reactivity within the linear dynamic range of the assay, typically these would give a value of 2 to 5 times the cut-off value. In this instance, although all four controls would be suitable for use in the Architect HBsAg assay, the HPA QC2 would be the most appropriate.

Table 13a: Comparison of 40 HBsAg positive samples

HBsAg Positive	Lot 1	Lot 2
	50507LU00	52234LU00
Number tested	40	40
Number reactive	40	40
Mean IU/mL	134.22	137.57
Median IU/mL	210.41	>250
Range IU/mL	1.64 - >250	1.73 - >250

Table 13b: Comparison of 10 HBsAg negative samples

HBsAg negative	Lot 1	Lot 2
	50507LU00	52234LU00
Number tested	10	10
Number reactive	0	0

Table 13c: Comparison of 3 seroconversion panels

Panel (number of samples)	Number reactive	
	Lot 1 50507LU00	Lot 2 52234LU00
PHM908 (8)	3	3
PHM922 (12)	7	7
PHM924 (5)	3	3
Total (25)	13	13

Table 13d: Results for 4 quality control samples

Quality control sample	Lot 1 50507LU00				Lot 2 52234LU00			
	IU/mL				IU/mL			
	Test 1	Test 2	Test 3	Mean	Test 1	Test 2	Test 3	Mean
NIBSC 0.05IU	0.09	0.08	0.09	0.08	0.07	0.08	0.08	0.08
NIBSC 0.2IU	0.28	0.29	0.33	0.30	0.30	0.31	0.33	0.31
HPA QC1	1.11	1.15	1.17	1.14	1.27	1.30	1.33	1.30
HPA QC2	0.11	0.12	0.11	0.11	0.12	0.12	0.13	0.12

Mutant panel

Permission was kindly given to reference in this report the results of the testing of a panel of specimens containing HBsAg mutants¹. Forty diverse native HBsAg mutants were tested in six HBsAg assays, including the Architect HBsAg assay. The concentration of the samples was determined by a reverse passive haemagglutination assay (Murex Hepatest) and subsequently diluted to a concentration of 2.5ng/mL prior to testing in the six HBsAg assays.

The Abbott Architect HBsAg assay detected 39 of the HBsAg mutants, however the sample containing both T123A and M133I mutations was not detected, *Appendix* Table 17. This mutant was also not detected by the Abbott Prism HBsAg but was detected by the four other assays. The Hepanostika HBsAg Ultra and Murex HBsAg GE34 detected all 40 mutants, the Biorad HBsAg Ultra detected 39 and the Bayer Centaur HBsAg detected 20.

Conclusion

The Architect HBsAg assay had an initial and repeat reactive rate of 0.5%, whereby five HBsAg negative specimens were repeatedly reactive with readings of 0.05 – 1.11IU/mL

All 201 HBsAg positive samples tested were positive in the assay to give a sensitivity of 100%. The majority of specimens were strongly positive with just two samples giving values of between 1 and 2IU/mL

Eight seroconversion panels were tested and, when combined with the results of eight further panels for which Abbott Diagnostics provided the data, the assay was ranked 6th most sensitive of a total of 31 assays previously evaluated. When assessed for the delay in seroconversion detection, the Architect HBsAg assay was joint sixth most sensitive when based on the median value and third most sensitive when based on the mean value.

A small lot comparison was undertaken in which both lots of the Architect HBsAg assay gave very similar IU/mL results for all samples tested.

Reference

1. Beale M. The Impact of Hepatitis B Surface Antigen Mutations on α -determinant Structure and Diagnostic Screening, September 2007. A Thesis submitted in partial fulfillment of the MSc Biomedical Science, School of Pharmacy & Biomedical Science, University of Portsmouth.

Appendix

Table 14: Results of seroconversion panels for Lot 1

Panel	Architect HBsAg IU/mL	Panel	Architect HBsAg IU/mL
PHM904-1	0.03	918-1	0.01
PHM904-2	0.05	918-2	0.05
PHM904-3	0.35	918-3	0.31
PHM911-1	0.01	919-1	0.01
PHM911-2	0.01	919-2	0.01
PHM911-3	0.01	919-3	0.02
PHM911-4	0.01	919-4	0.03
PHM911-5	0.01	919-5	0.05
PHM911-6	0.01	919-6	0.15
PHM911-7	0.02	919-7	0.29
PHM911-8	0.01	919-8	1.19
PHM911-9	0.02	919-9	1.42
PHM911-10	0.01	921-1	0.29
PHM911-11	0.01	921-2	0.79
PHM911-12	0.02	921-3	1.38
PHM911-13	0.02	921-4	5.54
PHM911-14	0.02	921-5	9.64
PHM911-15	0.01	921-6	46.07
PHM911-16	0.01	6281-1	0.01
PHM911-17	0.01	6281-2	0.02
PHM911-18	0.02	6281-3	0.01
PHM911-19	0.02	6281-4	0.03
PHM911-20	0.06	6281-5	0.05
PHM911-21	0.09	6281-6	0.16
PHM911-22	0.13	6281-7	0.49
PHM911-23	0.23	6281-8	0.70
PHM911-24	0.49	6281-9	1.29
PHM911-25	0.97	6281-10	0.35
PHM912-01	0.00	6281-11	0.01
PHM912-02	0.00	6281-12	0.01
PHM912-03	0.01	6276-1	0.01
PHM912-04	0.00	6276-2	0.01
PHM912-05	0.01	6276-3	0.01
PHM912-06	0.01	6276-4	0.01
PHM912-07	0.03	6276-5	0.01
PHM912-08	1.71	6276-6	0.01
PHM912-09	7.14	6276-7	0.06
PHM915-01	Insuff	6276-8	0.08
PHM915-02	0.01		
PHM915-03	0.02		
PHM915-04	0.04		
PHM915-05	0.03		
PHM915-06	0.03		
PHM915-07	0.05		
PHM915-08	0.06		
PHM915-09	0.07		
PHM915-10	0.09		
PHM915-11	0.10		
PHM915-12	0.27		
PHM915-13	0.20		

Table 15: Results of low titre performance panel

Panel	ng/ml	Architect HBsAg IU/mL
103-1	0.6	0.17
103-2	0.9	0.27
103-3	1.5	0.40
103-4	0.3	0.07
103-5	0.8	0.18
103-6	1.2	0.40
103-7	0.5	0.11
103-8	0.8	0.15
103-9	0	0.07
103-10	1.7	0.44
103-11	0.3	0.09
103-12	0.8	0.15
103-13	0.6	0.14
103-14	1.2	0.36
103-15	0.8	0.30

Table 16: Results of lot comparison for three seroconversion panels

Lot 1: 50507LU00		Lot 2: 52234LU00	
Panel	Architect HBsAg IU/mL	Panel	Architect HBsAg IU/mL
908-1	0.02	908-1	0.00
908-2	0.00	908-2	0.01
908-3	0.01	908-3	0.01
908-4	0.02	908-4	0.01
908-5	0.03	908-5	0.02
908-6	0.07	908-6	0.07
908-7	0.64	908-7	0.70
908-8	1.63	908-8	1.88
922-1	0.01	922-1	0.01
922-2	0.01	922-2	0.01
922-3	0.02	922-3	0.02
922-4	0.02	922-4	0.02
922-5	0.03	922-5	0.03
922-6	0.05	922-6	0.06
922-7	0.68	922-7	0.70
922-8	1.43	922-8	1.53
922-9	7.50	922-9	7.93
922-10	19.36	922-10	19.85
922-11	89.51	922-11	91.41
922-12	>250	922-12	>250
924-1	0.00	924-1	0.01
924-2	0.04	924-2	0.03
924-3	0.09	924-3	0.09
924-4	0.20	924-4	0.22
924-5	0.75	924-5	0.69

Table 17. Results of testing of HBsAg Mutant strain panel

Mutant Name	Specimen number	Abbott Prism	Abbott Architect*	Biorad Ultra	Hepanostika Ultra	Murex GE34	Bayer Centaur	Mean S/CO**
4 aa insertion of TTST between 118+119, W196L and Y206F	1	14.180	12.900	18.607	12.340	11.934	9.690	13.275
T118A	2	16.600	9.900	18.064	11.585	10.957	8.890	12.666
P120S	4	14.080	10.100	9.771	9.404	8.120	15.680	11.193
P120T, P70L, S31N, W196S	5	23.160	22.700	15.700	24.287	19.244	2.740	17.971
P120S, W196L	6	8.300	5.700	10.929	11.277	8.938	1.460	7.767
P120S	7	24.710	20.800	17.271	18.830	16.035	33.730	21.896
T123A	9	7.070	5.900	6.621	5.968	5.430	9.020	6.668
T123A, M133I	10	0.390	0.700	32.700	29.702	23.132	31.950	29.371
G130N, I208T, S210K	11	4.600	3.400	6.900	4.436	4.500	4.240	4.679
N131P	12	10.490	6.500	8.521	7.702	7.957	6.730	7.983
M133I, T189I	13	23.620	17.300	23.043	17.064	12.593	36.620	21.707
F134I, T143M	15	10.590	7.400	10.914	8.936	9.508	0.830	9.469
F134I, T140L	16	8.350	6.000	13.393	8.989	8.818	10.610	9.360
F134L, D144V, L173F, P203R	17	4.770	3.200	5.736	3.138	4.058	5.480	4.397
C139Y	19	3.970	2.900	4.136	3.564	3.713	3.690	3.662
C100S, D144E, G145R, M103I, P142L	20	6.460	3.400	1.764	3.053	5.450	0.310	4.025
G145R, P142L	21	14.830	10.300	16.707	10.649	10.345	< 0.1	12.567
C76Y, N59K, P46I, T143L, V190A, V47E	22	7.730	4.200	6.543	5.840	6.496	< 0.1	6.162
T143L	23	14.620	7.300	10.986	9.809	10.651	< 0.1	10.673
T143L	24	12.080	5.700	9.471	8.245	10.159	< 0.1	9.131
N207R, R79H, T143L	25	16.100	11.000	14.686	13.649	13.705	0.200	13.828
A166G, D144A, I91T	26	16.030	12.200	21.857	10.968	14.407	< 0.1	15.092
D144A	27	5.000	4.000	6.529	3.840	3.895	< 0.1	4.653
D144E, F220L, I195M, P203Q, S204G	28	7.840	5.800	10.586	5.085	6.217	0.130	7.106
F220, F145A, M197T, S210K, I195M	30	11.180	9.800	9.914	7.170	6.260	6.900	8.537
G145R, I195T	31	23.590	17.900	22.643	24.585	17.492	28.350	22.427
G145R	32	7.770	7.500	6.657	7.234	6.376	0.330	7.107
W172stop	33	23.600	17.600	18.029	17.543	12.291	15.830	17.482
M133I, F134H, D144V	34	2.650	1.000	0.429	1.106	4.143	< 0.1	2.225
M133I, F134N, P142S, S143L, G145L	35	9.980	10.200	3.700	2.500	7.473	< 0.1	6.771
E164D, I195M, T118A	36	19.750	15.600	20.307	14.181	11.287	31.110	18.706
P203R, W196L	37	22.590	17.500	18.614	19.043	14.837	28.560	20.191
I195M	38	14.900	9.600	11.507	11.298	9.039	10.910	11.209
D144, G145R	39	11.760	13.400	16.214	10.234	13.942	0.390	13.110
M133I, F134N, P142S, S143L, G145L	40	8.610	9.900	3.693	2.372	7.775	0.210	6.470
Pos Ctrl		63.810	68.900	34.679	36.138	25.589	117.560	57.779
NHP		0.370	0.000	0.414	0.532	0.504	0.540	0.393
Note								
Non-reactive results are shown in bold								
* Results of the Architect HBsAg are presented as S/CO, <u>not as IU/ml</u>								
**Mean Result of all POSITIVE reactions (S/CO) for each sample								