



Evaluation of Hepanostika HBsAg ULTRA

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Background and Description of the Assay

Hepanostika HBsAg ULTRA is an enzyme immunoassay based on the “sandwich” principle. The assay uses monoclonal antibodies selected to recognise the various subtypes of HBsAg. Upon completion of the assay, the development of colour indicates the presence of HBsAg, while no or low colour development suggests the absence of HBsAg. An additional feature of this assay is that it has Sample and Reagent Addition Monitoring, meaning that addition of samples and reagents can be measured colorimetrically. Further assay information is shown in Table 1.

The assay was evaluated to determine its ability to detect HBsAg. Limited sensitivity and specificity investigations were done at the Evaluations Unit, HPA - Colindale. The main specificity investigations were undertaken at the North London Blood Centre (Colindale). The kit has obtained a CE Mark.

Table 1: Assay Information

General	
Assay name	Hepanostika HBsAg Ultra
Manufacturer / UK agent	Biomerieux
Product number	284133
Number of tests in one pack	576
Specimen volume	100µL
Presentation	
Assay type	two-Step Sandwich ELISA
Solid phase	12 x 8 microtitre plate wells
Coating	Monoclonal anti-HBs antibodies (murine and human)
Conjugate	HRP-Labelled anti-HBs (ovine)
Substrate	TMB
Controls per calibration	4
negative control	3
positive control	1
Reading wavelength	450 / 630
Cut-off computation	(Mean of negative controls) + 0.04
Equivocal zone	n/a
Stages	
Preparation / sample well loading	30 minutes
Prewash of reaction plate	n/a
Incubation status	Static
Sample incubation	60 minutes 37°C
Conjugate incubation	60 minutes 37°C
Number of washes	6
Substrate incubation (time/temp)	30 minutes 18-25°C
Reading	5 minutes
Total incubation times	150 minutes
Approximate time to completion	185 minutes
Number of optional procedures	none
Additional equipment required	
Incubator, type not specified (*Dry incubator)	
Microplate spectrophotometer (* EL 808)	
Micropipettes: 40 - 200µL, 200 - 1000µL & 2 - 10mL	
Multichannel pipettes: 50 - 300µL	
Disposable tips	
Reagent troughs and bottles	
Measuring cylinder	
Distilled water	
Notes:	
* Equipment used in this evaluation.	

Evaluation Panel and Method

The evaluation panel consisted of 598 specimens (Table 2). Of these, 200 specimens were from HBsAg negative blood donors, 200 from HBsAg positive subjects and ten weakly reactive specimens from long-term carriers clearing their antigenaemia. A further 152 specimens were from 20 commercial seroconversion panels and 15 specimens were from a low titre performance panel. Four dilutions of four seroconversion panel members and replicates of five quality control samples were also tested.

Evaluation testing was carried out using the Ortho Summit™ System at the North London Blood Centre (Colindale). Specimens (100µL) and specimen diluent (25µL) were added manually. The ELISA plate was then transferred to the Summit™ for continuation with the test procedure. Briefly, wells were incubated at 37°C for 60 minutes. After 60 minutes 50µL of conjugate was added to each of the wells which were then incubated for a further 60 minutes at 37°C. The wells were washed six times and 100µL of TMB was added to all wells. The wells were then incubated at 15-30°C for 30 minutes. Finally, 100µL of 1M sulphuric acid was added to all wells. The reactions were read on an EL 808 plate reader at 450nm (with 630nm reference) using KC4 software. Samples with Optical Density (OD) ≥Cut-off were regarded as positive.

Table 2: Evaluation panel used (Lot B15HB)

Sample category	Number	
1. Blood donors' sera	200	
2. HBsAg positive samples		
a) HBsAg positive	200	
b) weakly reactive (long term carriers clearing antigenaemia)	10	
3. HBsAg seroconversion panels		
BBI: PHM 903	6	
BBI: PHM 904	3	
BBI: PHM 908	8	
BBI: PHM 909	7	
BBI: PHM 910	6	
BBI: PHM 911	25	
BBI: PHM 914	6	
BBI: PHM 916	11	
BBI: PHM 917	3	
BBI: PHM 918	3	
BBI: PHM 919	9	
BBI: PHM 920	6	
BBI: PHM 921	6	
BBI: PHM 922	12	
BBI: PHM 923	4	
BBI: PHM 924	5	
BCP: 6271	5	
BCP: 6274	7	
BCP: 6276	8	
BCP: 6281	12	
4. Seroconversion panel dilutions (diluted in negative human serum)		
BBI - PHM902-14 (1/20 to 1/1280)	4	
BBI - PHM907-09 (1/20 to 1/1280)	4	
BBI - PHM920-06 (1/20 to 1/1280)	4	
BBI - PHM922-09 (1/20 to 1/1280)	4	
5. HBsAg performance panels		
BBI - PHA103 (low titre)	15	
6. Quality control samples		
NIBSC 2nd British working standard	6x	1
NIBSC monitor sample (0.05IU/ml)	6x	1
NIBSC Working Standard (0.2IU/ml)	6x	1
HPA HBsAg quality control serum QC1	6x	1
HPA HBsAg quality control serum QC2	6x	1
TOTAL (number of specimens)	598	

Notes:

BBI = Boston Biomedica Inc; BCP = BioClinical Partners Inc;
 HPA = Health Protection Agency
 NIBSC = National Institute for Biological Standards and Control.

Specificity Findings

Of the 200 HBsAg negative blood donor specimens, four were initially reactive (Table 3) giving an initial reactive rate of 2%. After retesting none of the specimens were reactive and therefore the repeat reactive rate was 0% (95% confidence interval, 0 – 1.8%).

Appendix 1 shows a table of false positive rates for 30 previously evaluated HBsAg screening kits.

Table 3: Specificity

HBsAg negative blood donors	Number tested	Number reactive	Number reactive	Range OD/CO	Mean OD/CO	Median OD/CO	Specificity
Stored < 6 mths	200	Initial	4	0.328 - 4.094	0.458	0.411	98%
		Repeat	0	0.328 - 0.754	0.416	0.411	100%

More extensive specificity testing was carried out at the North London Blood Centre (Colindale). 864 serum and plasma specimens were tested on the first batch of the kit and 1040 were tested on the second batch (Table 4). Of the 1904 tested over the two batches, two were repeatedly reactive to give a repeat reactive rate of 0.1% (95% confidence interval, 0 – 0.4%) (Table 5).

The distribution of reactivities for each batch is shown in Figures 1a and 1b.

Table 4: Specimens tested in extended specificity evaluation

Lot Number	B15GA	B15HB
Serum	352	776
Plasma	512	264
Total	864	1040

Table 5: Initial and Repeat Reactive Rates for extended specificity evaluation

		Number IR (% IR)	Number RR (% RR)
B15GA	Serum	0 (0)	0 (0)
	Plasma	2 (0.4)	1 (0.2)
	Total	2 (0.23)	1 (0.12)
B15HB	Serum	1 (0.13)	1 (0.13)
	Plasma	0 (0)	0 (0)
	Total	1 (0.1)	1 (0.1)
Total (both batches)		3 (0.16)	2 (0.1)

Figure 1a: Distribution of reactivities for Batch B15GA

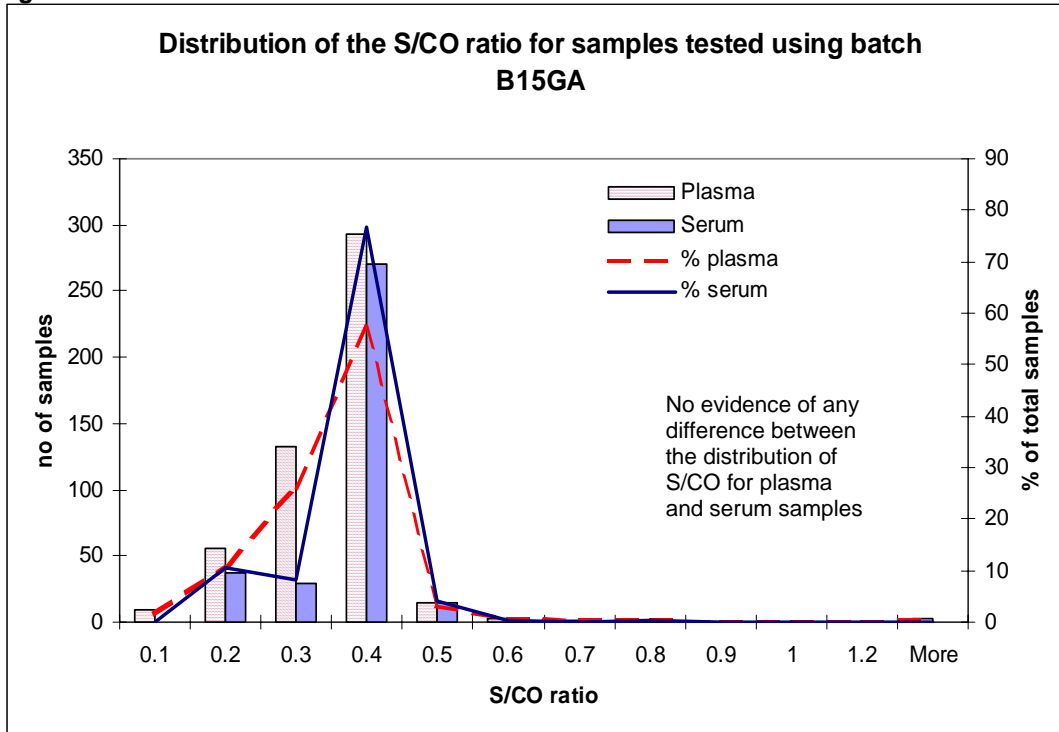


Figure 1b: Distribution of reactivities for Batch B15HB

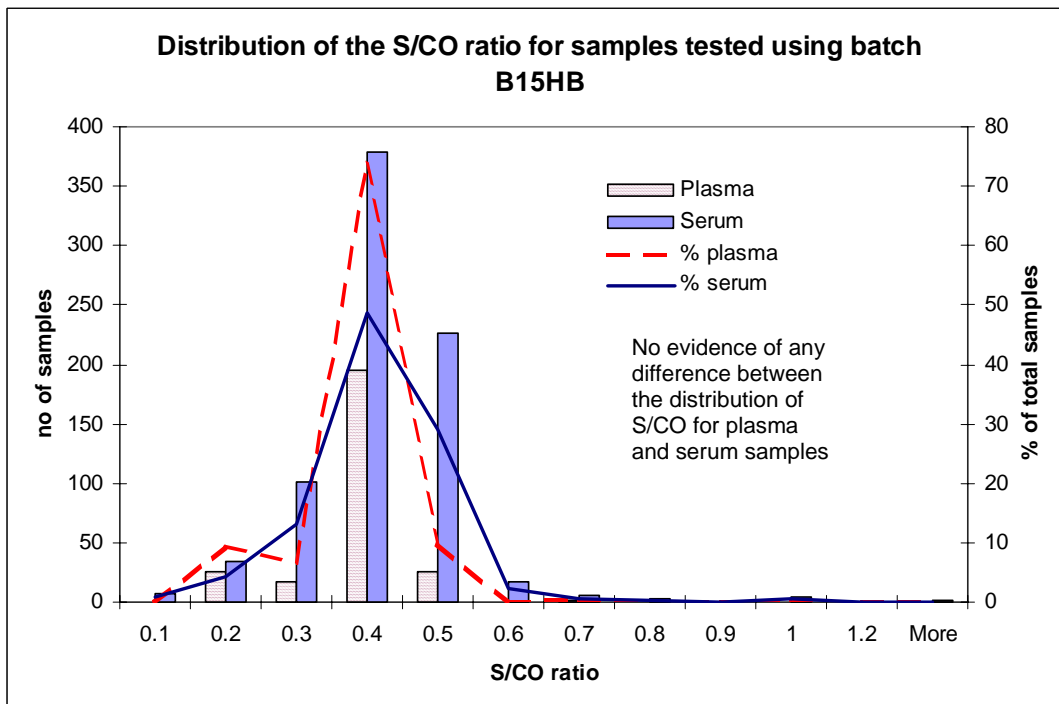


Table 7: Weakly Positive Specimens

Assay	Product number	Specimen number										Positive (%)
		1	2	3	4	5	6	7	8	9	10	
Murex HBsAg Version 3	GE34/36	1.09	2.32	8.39	21.74	4.46	9.05	12.25	17.75	1.29	NT	9/9 (100)
AxSYM [®] HBsAg (V2)	7A40-22	1.17	1.48	5.38	59.01	3.16	4.36	6.15	8.46	NT	NT	8/8 (100)
Monolisa HBsAg Ultra	72346	NT	1.89	6.16	33.28	1.74	4.71	6.37	1.69	1.38	NT	8/8 (100)
Enzygnost [®] HBsAg 5.0	OQPW11/21	NT	1.26	8.92	36.14	3.37	8.34	3.27	1.16	1.97	NT	8/8 (100)
PRISM [™] HBsAg	3A4748	1.29	4.41	12.26	171.09	5.27	12.40	NT	NT	1.93	NT	7/7 (100)
Hepanostika HBsAg ULTRA (B15BA)	284133	0.855	1.675	9.162	40.786	2.615	7.658	8.906	3.060	1.675	4.427	9/10 (91)
Hepanostika HBsAg ULTRA (B15HB)	284133	0.859	1.266	7.656	32.922	2.219	6.813	7.828	2.875	1.531	4.516	9/10 (91)
ETI-MAK-4	N0019	0.74	1.28	7.49	61.98	1.91	4.35	8.93	4.42	NT	NT	7/8 (87.5)
Auszyme monoclonal	1980-24	0.30	1.30	6.26	40.80	2.77	7.24	NT	NT	NT	NT	5/6 (83.3)
Monolisa Ag HBs 2nd gen.	72204	0.67	1.20	7.60	20.91	2.06	3.67	NT	NT	NT	NT	5/6 (83.3)
Murex HBsAg	GE14/15/16	0.66	1.22	3.67	25.66	3.07	4.27	NT	NT	NT	NT	5/6 (83.3)
Murex HBsAg (version 2)	GE14/15/16	0.67	1.07	2.69	19.61	2.04	5.62	NT	NT	NT	NT	5/6 (83.3)
Ortho HBsAg Test System 3	931801	0.77	1.26	6.94	51.25	2.14	3.16	NT	NT	NT	NT	5/6 (83.3)
Vitros EC ⁱ HBsAg	843 5307	0.47	2.39	14.60	115.00	2.25	3.27	NT	NT	NT	NT	5/6 (83.3)
ETI-MAK-3	P3142	0.33	0.21	5.92	28.55	1.15	3.73	NT	NT	NT	NT	4/6 (66.7)
Hepanostika HBsAg Uni-form II	6019/6023	0.60	0.81	1.77	5.46	1.21	1.58	NT	NT	NT	NT	4/6 (66.7)
DIA.PRO HBsAg One-Step	SAG1.CE	NT	1.02	NT	17.71	0.73	2.82	1.92	0.27	NT	NT	4/6 (66.7)
Bioelisa HBsAg colour	3000-1100	0.52	0.87	3.77	29.23	1.10	2.72	NT	NT	0.74	NT	4/6 (66.7)
Access HBsAg	34220	0.36	0.90	3.65	7.72	0.69	1.93	NT	NT	NT	NT	3/6 (50)
Amerlite HBsAg II	LAN.2212	0.19	0.92	5.44	103.10	0.40	2.37	NT	NT	NT	NT	3/6 (50)
Bioelisa HBsAg	3000-1080	0.02	0.62	3.09	19.20	0.36	1.77	NT	NT	NT	NT	3/6 (50)
COBAS Core HBsAg II	07/5350/5	0.50	0.86	5.15	42.40	0.86	7.75	NT	NT	NT	NT	3/6 (50)
Enzygnost HBsAg monoclonal II	OQC110/11	0.63	0.84	2.26	10.20	0.73	1.80	NT	NT	NT	NT	3/6 (50)
Enzymun-Test HBsAg	1288989	-0.37	-0.02	1.97	80.01	0.09	1.09	NT	NT	NT	NT	3/6 (50)
Heprofile HBsAg	M450	0.48	0.88	2.19	2.75	0.46	1.01	NT	NT	NT	NT	3/6 (50)
IMx HBsAg	2228-20	0.44	0.69	1.35	16.32	0.98	1.06	NT	NT	NT	NT	3/6 (50)
Monolisa Ag HBs plus (method 3)	72314	0.17	0.64	2.23	6.13	0.53	1.18	NT	NT	NT	NT	3/6 (50)
VIDAS HBsAg	30 300	0.23	0.77	2.39	10.92	0.54	1.69	NT	NT	NT	NT	3/6 (50)
MicroTrak II HBsAg	8HB29	0.19	0.15	0.64	3.52	0.19	0.35	NT	NT	NT	NT	2/6 (33.3)

Notes:

NT = not tested

Positive reactions are shown in bold type. *results of two tests

5, 6 and 10 are serial bleeds from the same patient taken on 2/12/1991, 29/1/1993 and 24/03/2004 respectively.

4 and 8 are serial bleeds from the same patient taken on 22/2/1993 and 27/1/2000 respectively.

Detection of HBsAg in a dilution series

Dilutions of four specimens, one from each of four seroconversion panels (PHM902-14, PHM907-09, PHM920-06 and PHM922-09), were made in four fold steps from 1/20 to 1/1280 in human serum negative for HBsAg and anti-HBs. These dilutions were tested by the Hepanostika HBsAg Ultra assay (Table 8)

Unfortunately the dilution panels are now low in volume and the original BBI panels are no longer available at source. This means we are unable to compare the full dilution series results for Hepanostika HBsAg Ultra with other kits as they had previously been given a score for detection in six panels rather than the four that were available for this evaluation (see *discussion*).

Table 8: Detection of HBsAg in a dilution series

Sample ID	OD	CO	OD/CO
902-14 1:20	0.115	0.068	1.691
902-14 1:80	0.051	0.068	0.750
902-14 1:320	0.038	0.068	0.559
902-14 1:1280	0.030	0.068	0.441
907-09 1:20	1.281	0.068	18.838
907-09 1:80	0.527	0.068	7.750
907-09 1:320	0.060	0.068	0.882
907-09 1:1280	0.060	0.068	0.882
920-06 1:20	1.827	0.068	26.868
920-06 1:80	0.480	0.068	7.059
920-06 1:320	0.138	0.068	2.029
920-06 1:1280	0.067	0.068	0.985
922-09 1:20	0.200	0.068	2.941
922-09 1:80	0.068	0.068	1.000
922-09 1:320	0.051	0.068	0.750
922-09 1:1280	0.034	0.068	0.500

Seroconversion Sensitivity

The ability of the Hepanostika HBsAg Ultra assay to detect early antigen in 16 seroconversion panels was compared with that of 29 other HBsAg kits. When the aggregate scores were calculated, the Hepanostika HBsAg Ultra assay was ranked 6th with a score of 58 out of 120. Abbott PRISM HBsAg was the most sensitive kit with a score of 76/120. AxSYM[®] HBsAg V2 was the 2nd most sensitive kit (72/120), while Enzygnost HBsAg 5.0 was ranked third (66/120) (Table 9).

It was also possible to compare results of 20 panels with eight other HBsAg kits. In this case MONOLISA HBsAg ULTRA was ranked 4th out of ten. However Abbott PRISM was not included in this comparison as not all 20 panels had been tested by the PRISM assay (Table 10).

Hepanostika HBsAg Ultra detected HBsAg on average 9.8 days later than the earliest potential detection of HBsAg by any of the assays (Table 11, Figure 3). The median detection time was 7 days. The mean delay can be strongly influenced by outlying results from one or two seroconversion panels for which the interval between the last negative and the first positive specimen is long, giving rise to an artefact due to the timing of blood collection. However, this does not affect the median score.

A mixed titre performance panel consisting of 10 positive specimens with known subtype, 5 indeterminate specimens and one negative specimen was also tested. Hepanostika HBsAg Ultra correctly identified all of the positive and negative specimens and found all but one of the indeterminate specimens to be positive (Appendix 3).

Table 9: Combined seroconversion scores (16 panels)

Assay	Product number	Aggregated score* PHM903 - 924 n = 120	Rank
Abbott PRISM™ HBsAg	3A4748	76	1
AxSYM® HBsAg (V2)	7A40-22	72	2
Enzygnost® HBsAg 5.0	OQPW11/21	66	3
Monolisa HBsAg ULTRA	72346	64	4
Murex HBsAg (version 3)	GE34/36	63	5
Hepanostika HBsAg ULTRA	284133	58	6
Vitros ECi HBsAg	843 5307	56	7
ETI-MAK-4	N0019	55	8
Bioelisa HBsAg colour plus	3000-1155	53	9=
Monolisa Ag HBs 2nd gen	72204	53	9=
Ortho HBsAg Test System 3	931801	49	11
Bioelisa HBsAg (colour)	3000-1100	46	12
COBAS Core HBsAg II	07/5350/5	47	13=
ETI-MAK-3	P3142	47	13=
Enzygnost HBsAg monoclonal II	OQC110/11	46	15=
Monolisa Ag HBs Plus	72314	46	15=
Auszyme monoclonal	1980-24	45	17=
Murex HBsAg	GE14/15/16	45	17=
Bioelisa HBsAg	3000-1080	44	19
Hepanostika HBsAg Uni-form II	6019/6023	42	20=
VIDAS HBsAg	30 300	42	20=
Heprofile HBsAg	M450	42	20=
Labsystems HBsAg EIA Plus	61 10 800/802	41	24=
DIA.PRO HBsAG One-Step	SAG1.CE	41	24=
Amerlite HBsAg II	LAN.2212	39	25
Murex HBsAg (version 2)[F107710]	GE14/15/16	38	26=
Access® HBs Ag	34220	38	26=
IMx HBsAg	2228-20	37	28
Enzymun-Test HBsAg	1288989	34	29
MicroTrak II HBsAg	8HB29	19	30
Notes:			
* The score was calculated by summing the number of positive specimens detected for each panel.			

Table 10: Combined seroconversion scores (20 panels)

Assay	Product Number	Score* PHM903-924** n = 120	Score* BCP6271 - 6276*** n = 20	Score* BCP6281 n = 12	Aggregated score n = 152
AxSYM® HBsAg	7A40-22	72	9	5	86
Enzygnost® HBsAg 5.0	OQPW11/21	66	12	6	84
Monolisa HBsAg ULTRA	72346	64	12	6	82
Hepanostika HBsAg ULTRA	284133	58	12	7	77
ETI-MAK 4	N0019	55	11	5	71
Bioelisa HBsAg colour plus	3000-1155	53	10	5	68
Monolisa Ag HBs Plus	72314	46	5	6	57
Hepanostika HBsAg Uni-form II	6019/6023	42	7	5	54
DIA.PRO HBsAg One-Step	SAG1.CE	41	7	4	52
Labsystems HBsAg EIA Plus	61 10 800/802	41	6	4	51

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

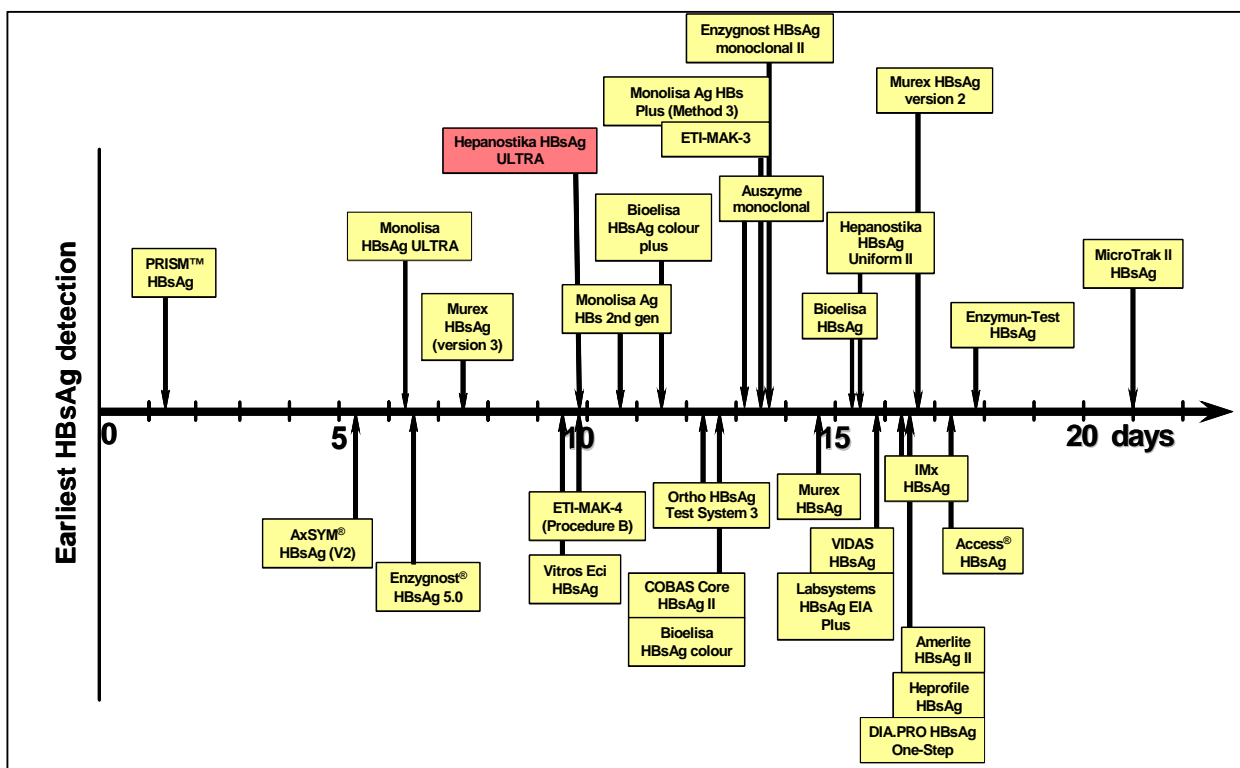
Table 11: Comparative timing of detection

HBsAg assay	Product number	Delay in detecting seroconversion in each panel compared with the most sensitive assay		
		Range (days)	Mean (days)	Median (days)
PRISM HBsAg	3A4748	0-12	1.3	0.0
AxSYM HBsAg (V2)	7A40-22	0 - 35	5.3	2.5
Monolisa HBsAg ULTRA	72346	0 - 35	6.2	3.5
Enzygnost® HBsAg 5.0	OQPW11 / 21	0 - 35	6.3	5.0
Murex HBsAg Version 3	GE34/36	0 - 35	7.4	6.5
Vitros HBsAg EIA	843 5307	0 - 35	9.4	7.0
Hepanostika HBsAg ULTRA	284133	0 - 35	9.8	7.0
ETI-MAK-4	N0019	0 - 35	9.8	7.0
Monolisa Ag HBs 2nd gen	72204	0 - 35	10.8	7.0
Bioelisa HBsAg colour plus	3000-1155	0 - 35	11.4	7.5
Bioelisa HBsAg Colour	3000-1100	0 - 35	13.4	12.0
Monolisa Ag HBs Plus (short incubation)	72314	0 - 40	14.4	12.0
Enzygnost HBsAg monoclonal II	OQC110/11	0 - 40	14.6	12.0
Bioelisa HBsAg	3000-1080	0 - 40	15.2	12.0
Labsystems HBsAg EIA Plus	61 10 800/802	0 - 42	15.8	12.0
DIA.PRO HBsAg One-Step	SAG1.CE	0 - 45	16.4	12.0
Ortho HBsAg Test System 3	931801	0 - 35	13.3	13.0
COBAS Core HBsAg II	07/5350/5	0 - 35	13.4	13.0
Auszyme monoclonal	1980-24	0 - 35	14.1	13.0
ETI-MAK-3	P3142	0 - 40	14.4	13.0
Murex HBsAg	GE14/15/16	0 - 40	14.8	13.0
Hepanostika HBsAg Uni-form II	6019/6023	0 - 42	15.4	13.0
VIDAS HBsAg	30 300	0 - 40	15.8	13.0
Heprofile HBsAg	M450	0 - 42	16.4	13.0
IMx HBsAg	2228-20	0 - 42	16.2	13.5
Access HBsAg	34220	0 - 45	17.3	14.0
Amerlite HBsAg II	LAN.2212	0 - 42	16.4	14.5
Murex HBsAg (version 2)	GE14/15/16	0 -40	16.6	15.0
Enzymun-Test HBsAg	1288989	0 - 42	17.8	16.5
MicroTrak II HBsAg	8HB29	7 - 46	21.0	19.0

Notes:

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 any assay failed to dete

Figure 3: Comparative timing of detection



Quality Control Results

Our findings suggest that the NIBSC 0.05 IU/ml Monitor sample or HPA HBsAg QC2 are suitable Internal IQCs for the Hepanostika HBsAg Ultra kit (Table 12).

Table 12: Quality Control Results

QC sample ID	Hepanostika HBsAg ULTRA							
	Batch number: B15HA				Batch number: B15BA			
	OD/CO 1	OD/CO 2	OD/CO 3	Mean	OD/CO 1	OD/CO 2	OD/CO 3	Mean
HPA HBsAg QC1	22.995	21.319	21.527	21.947	23.624	23.863	23.453	23.647
HPA HBsAg QC2	4.436	1.787	2.220	2.814	2.274	2.086	2.086	2.148
NIBSC 2nd Working Standard	9.973	10.670	9.718	10.120	NT	NT	NT	
NIBSC 0.05 IU/ml Monitor Sample	1.883	16.654*	1.979	1.931	NT	NT	NT	
NIBSC 0.2 IU/ml Monitor Sample	3.942	4.245	4.532	4.240	NT	NT	NT	

* Sample probably contaminated by carry-over during washing step, not included in mean calculation.
NT = Not tested

Conclusion

Hepanostika HBsAg ULTRA showed good sensitivity when tested against a panel of 200 known HBsAg positive specimens. The assay also detected 9 of 10 weakly positive specimens from resolving carriers. The kit showed a 5 - 6 day improvement in detection of early antigen when compared to a previous Hepanostika HBsAg Uni-Form II kit version. However, Hepanostika HBsAg ULTRA was less sensitive than some other kits (eg PRISM HBsAg, Monolisa HBsAg ULTRA, Murex HBsAg v3) for early detection of antigen.

An initial reactive rate of 2% was resolved upon repeat testing to give a repeat reactive rate of 0% (95% confidence limits: 98.2 – 100%). Testing of 1904 blood donor specimens over two lot numbers at North London blood centre showed a repeat reactive rate of 0.1% (95% confidence limits: 99.6 – 100%).

A dilution series of seroconversion panel members has been included in the HBsAg evaluation panel for several years but is now becoming depleted. The value of creating a new set of dilutions for HBsAg kit evaluations will need to be discussed.

Appendix 1: False positive rates for 30 previously evaluated HBsAg screening kits

HBsAg kit	Product code	Number tested	Number initially reactive	Number repeatedly reactive	Repeat reactive rate	95% confidence interval	MHRA Reference
Monolisa Ag HBs Plus (method 3)	71314	1285	2	0	0.00	0.00 – 0.30	2000/16
Labsystems HBsAg EIA Plus	61 10 800/802	547	1	0	0.00	0.00 – 0.67	01165
Vitros <i>ECi</i> HBsAg	843 5307	368	3	0	0.00	0.00 – 1.00	2000/33
Hepanostika HBsAg ULTRA	284133	200	4	0	0.00	0.00 - 1.80	NA
MONOLISA HBs Ag ULTRA	72346	200	7	0	0.00	0.00 - 1.80	NA
Amerlite HBsAg II	LAN.2212	97*	0	0	0.00	0.00 – 3.73	95/52
Bioelisa HBsAg	3000+1080	97*	1	0	0.00	0.00 – 3.73	95/52
Enzygnost HBsAg monoclonal II	OQC110/11	97*	2	0	0.00	0.00 – 3.73	95/52
Enzymun-Test	1288989	97*	2	0	0.00	0.00 – 3.73	95/52
ETI-MAK-3	P3142	97*	0	0	0.00	0.00 – 3.73	95/52
Heprofile HBsAg	M450	97*	0	0	0.00	0.00 – 3.73	95/52
IMx HBsAg	2228-20	97*	0	0	0.00	0.00 – 3.73	95/52
MicroTrak II HBsAg	8HB29	97*	0	0	0.00	0.00 – 3.73	95/52
Monolisa Ag HBs 2nd gen	72204	97*	2	0	0.00	0.00 – 3.73	95/52
Murex HBsAg	GE14/15/16	97*	0	0	0.00	0.00 – 3.73	95/52
Ortho HBsAg Test System 3	931801	97*	4	0	0.00	0.00 – 3.73	95/52
VIDAS HBsAg	30 300	97*	1	0	0.00	0.00 – 3.73	95/52
PRISM™ HBsAg	3A4748	9563	4	1	0.01	0.00 – 0.03	97/52
Murex HBsAg Version 3	GE34/36	3187	13	3	0.09	0.02 – 0.28	2000/55
Bioelisa colour	3000-1100	51133**	NA	34	0.10	0.00 - 0.10	99/68
Murex HBsAg (v2)	GE14/15/16	2000	6	2	0.10	0.01 – 0.36	98/39
ETI-MAK-4	N0019	499	6	1	0.20	0.00 – 1.10	01121
Enzygnost® HBsAg 5.0	OQPW11/21	500	3	1	0.20	0.00 – 1.10	03044
Bioelisa HBsAg colour plus	3000-1155	2262	27	6	0.30	0.10 - 0.60	02096
Access® HBs Ag	34220	500	5	2	0.40	0.00 – 1.40	98/75
AxSYM® HBsAg (V2)	7A40-22	500	5	2	0.40	0.00 – 1.40	01125
Auszyme monoclonal	1980-24	97*	2	1	1.03	0.03 – 5.60	95/52
Hepanostika HBsAg Uni-form II	6019/6023	97*	3	1	1.03	0.03 – 5.60	95/52
COBAS Core HBsAg II	07/5350/5	97*	3	2	2.06	0.25 – 7.24	95/52
DIA.PRO HBsAg One-Step	SAG1.CE	408	12	10	2.50	1.20 - 4.50	03031
Notes: * Assays evaluated using the same 97 specimens. ** Figures from NBA Monthly Donation Testing Report. NA = not available Highlighted cells = HBsAg kits on the list of kits suitable for use in the NBS Refer to previous MHRA reports for details of assays listed.							

Appendix 2: Sensitivity for 30 previously evaluated HBsAg screening kits

Assay	Product code	Number positive / number tested	Sensitivity % **	95% confidence interval %	Range S/CO	Mean S/CO	Median S/CO
AxSYM® HBsAg (V2)	7A40-22	491 / 491	100.0	99.3 – 100	1.20 – 291.55	170.77	196.81
Labsystems HBsAg EIA Plus	61 10 800/802	445 / 445	100.0	99.2 - 100	1.17 - 29.13	23.54	26.21
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 plus	3000-1155	408 / 408	100.0	99.1 - 100	1.13 - 53.36	43.92	45.19
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
Bioelisa HBsAg Colour	3000+1100	150/150	100.0	97.6 – 100	3.07 – 44.02	35.63	37.51
COBAS Core HBsAg II	07/5350/5	150/150	100.0	97.6 – 100	1.66 – 73.22	71.3	73.22
Enzygnost HBsAg monoclonal II	OQC110/11	150/150	100.0	97.6 – 100	1.10 – 54.80	49.62	50.76
ETI-MAK-3	P1342	150/150	100.0	97.6 – 100	2.35 – 58.82	56.12	57.69
Hepanostika HBsAg Uni-Form II	6019/6032	150/150	100.0	97.6 – 100	1.08 – 35.96	24.18	21.9
IMx HBsAg	22280-20	150/150	100.0	97.6 – 100	1.26 – 102.4	68.19	72.35
Monolisa Ag HBs 2nd gen	72204	150/150	100.0	97.6 – 100	2.14 – 83.33	72.23	71.43
Murex HBsAg	GE 14/15/16	150/150	100.0	97.6 – 100	1.56 – 27.27	23.24	25.86
Murex HBsAg (v2)	GE 14/15/16	153/153	100.0	97.6 – 100	1.45 – 25.00	21.3	23.44
Ortho HBsAg Test System 3	931801	150/150	100.0	97.6 – 100	1.60 – 96.77	85.34	88.24
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
Monolisa Ag HBs Plus (method 3)	72313 / 72314	458/459	99.8	98.8 – 100	0.87 – 61.22	55.11	60.00
Vitros ECi HBsAg	843 5307	270/272	99.3	97.4 – 99.9	0.24 – 8740	3619.4	3695
Amerlite HBsAg II	LAN.2212	149*/150	99.3	96.3 – 100	0.32 – 1711.00	1192.7	1254
Bioelisa HBsAg	3000+1080	149*/150	99.3	96.3 – 100	0.83 – 61.30	54.97	56.25
Enzymun-Test	1288989	149*/150	99.3	96.3 – 100	0.71 – 114.93	98.84	102.03
Heprofile HBsAg	M450	149*/150	99.3	96.3 – 100	0.70 – 58.82	27.28	26.55
MicroTrak II HBsAg	8HB29	149*/150	99.3	96.3 – 100	0.22 – 25.86	21.61	21.58
Access® HBS Ag	34220	201/203	99.0	96.5 – 99.9	0.32§ – 1201.41	583.22	579.94

Notes:

- * The same specimen gave a negative result in the five assays indicated and gave weak positive results in other assays tested.
- ** 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).

Highlighted cells = HBsAg kits suitable for use in the NBS

Appendix 3: Performance panel data

Product name	Product number	Panel ID	PHA103-01	PHA103-02	PHA103-03	PHA103-04	PHA103-05	PHA103-06	PHA103-07	PHA103-08	PHA103-09	PHA103-10	PHA103-11	PHA103-12	PHA103-13	PHA103-14	PHA103-15
		Subtype	Ind	ad	ad	Ind	ad	ad	ad	ay	Neg	ad	Ind	Ind	ay	ad	ad
Access® HBs Ag	34220	S/CO	0.62	1.75	1.11	0.49	0.61	1.83	0.68	0.71	0.23	1.20	0.53	0.61	0.79	3.00	1.22
AxSYM® HBsAg (V2)	7A40-22	S/CO	2.02	3.02	3.55	1.14	2.10	4.02	1.28	1.67	0.65	3.99	1.00	2.47	1.81	4.27	2.78
Bioelisa HBsAg colour	3000-1130	OD/CO	1.07	3.21	2.41	0.93	1.97	3.90	1.25	1.75	0.30	4.00	0.98	1.20	1.39	3.53	2.18
Bioelisa HBsAg colour plus (A-5900)	3000+1130	OD/CO	2.26	5.76	3.08	2.41	3.00	5.96	2.57	2.44	0.51	5.77	1.29	2.51	2.15	5.85	4.49
Bioelisa HBsAg colour plus (B-3200)	3000+1130	OD/CO	1.55	3.92	2.57	2.17	2.40	4.91	1.95	2.20	0.45	5.08	1.10	2.42	1.89	4.99	3.70
ETI- MAK 4	N0019	OD/CO	1.79	5.83	5.93	1.52	3.07	6.90	1.66	3.03	-0.10	7.72	1.03	4.03	3.10	8.00	5.45
Hepanostika HBsAg ULTRA	284133	OD/CO	2.044	5.206	2.074	3.176	2.691	5.706	1.838	2.265	0.500	5.971	1.588	0.838	2.515	6.368	4.250
Hepanostika HBsAg Uni-Form II	6019/6023	OD/CO	0.66	1.27	1.22	0.55	0.79	1.42	0.85	0.99	0.27	0.42	0.56	0.81	1.08	1.82	1.64
Labsystems HBsAg EIA Plus	6110 802	OD/CO	0.69	1.41	0.77	0.57	0.91	1.50	0.58	0.86	0.43	1.55	0.63	1.15	0.90	2.04	1.30
Monolisa Ag HBs Plus / Method 3 (37°C for 60)	72314	OD/CO	0.98	3.11	1.04	0.64	1.40	2.69	1.04	1.42	0.20	2.82	1.13	1.58	1.62	3.78	2.56
Monolisa Ag HBs Plus / Method 4 (37°C for 120)	72314	OD/CO	1.51	6.42	2.77	1.49	2.91	7.33	2.16	3.58	-0.05	4.91	1.19	3.49	3.42	8.33	4.79
Monolisa HBsAg ULTRA	72346	OD/CO	1.23	6.69	3.47	1.85	3.97	9.10	2.74	3.55	0.48	6.08	1.81	4.03	5.42	9.88	6.88
Murex HBsAg	GE14/15/16	OD/CO	0.88	1.20	1.38	0.57	0.83	1.55	0.68	0.77	0.45	1.50	0.59	0.89	0.76	1.48	1.08
Murex HBsAg (version 2) ¹	GE14/15/16	OD/CO	1.39	1.74	1.95	0.91	1.40	2.43	0.95	1.22	0.49	3.23	0.83	1.57	1.18	2.87	1.95
Murex HBsAg (version 2) ²	GE14/15/16	OD/CO	0.96	1.42	1.59	0.80	0.91	1.48	0.85	1.00	0.61	1.40	0.84	1.16	0.89	1.78	1.42
Murex HBsAg (version 3)	GE34/36	OD/CO	3.62	6.79	5.64	1.76	3.66	8.81	2.50	3.16	0.54	10.25	1.80	3.90	2.94	8.89	4.95
PRISM™ HBsAg	3A4748	S/CO	3.16	7.96	6.38	2.98	4.03	9.68	2.54	4.65	0.36	9.32	1.45	5.86	4.03	6.41	3.17
VITROS Eci HBsAg	843 5307	S/CO	0.62	10.7	4.16	1.28	4.12	11.0	2.47	5.35	0.10	10.5	3.24	2.66	5.96	14.4	11.2
PCR Detection*			N/K	N/K	N/K	N/K	N/K	N/K	N/K	N/K	N/K	N/K	N/K	N/K	N/K	N/K	N/K
HBsAg concentration*		ng/mL	0.6	0.9	1.5	0.3	0.8	1.2	0.5	0.8	0	1.7	0.3	0.8	0.6	1.2	0.8

Notes:

Results are expressed as specimen absorbance to cut-off ratios or equivalent. Ratios equal to or greater than 1.0 are considered reactive

* Data taken from BBI data sheet

N/K = Not Known.

** Cut off for panel member is also 0.108 but from a different plate.

¹ Lot no F107710. ² Lot no F107810