

Medicines and Healthcare products
Regulatory Agency

Evaluation Report NUMBER
MHRA 03031

DIA.PRO HBsAg One-Step EIA

MHRA Evaluation Report
MHRA03031

Health Protection Agency
Specialist and Reference Microbiology
Division



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**DIA.PRO HBsAg One - Step
(Product code: SAG1.CE)**

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Background

The DIA.PRO HBsAg One-Step enzyme immunoassay was evaluated to determine its ability to detect hepatitis B surface antigen (HBsAg) in human serum and plasma specimens.

Evaluation panel

The evaluation panel consisted of 1,225 specimens, including 408 freshly collected HBsAg negative serum specimens from blood donors, 422 randomly selected HBsAg positive specimens and six weakly reactive specimens from long term carriers clearing their antigenaemia ('tail enders'). Also included in the evaluation panel were 345 specimens from 31 commercial seroconversion panels, 15 specimens from one commercial low titre panel, six dilution series and four quality control samples.

Specificity findings

Of the 408 HBsAg negative specimens tested, 12 were initially reactive of which ten were found to be repeatedly reactive. This gave a specificity of 97.5% (a repeat reactive rate of 2.5% ; 95% confidence interval 1.2 – 4.5%).

Sensitivity findings (randomly selected positives)

All of the 422 randomly selected HBsAg positive specimens were found to be reactive in the DIA.PRO HBsAg One-Step assay, giving an observed sensitivity of 100% (95% confidence interval 99.1 – 100%). Of four 'tail-end' weakly positive specimens that had been tested in 27 other assays, 11 of the kits detected all four, while the DIA.PRO HBsAg One-Step kit detected three (as did four other kits). DIA.PRO HBsAg One-Step detected one of two further 'tail-end' specimens.

Sensitivity findings (seroconversion panels)

A scoring system was employed to allow the ability of the DIA.PRO HBsAg One-Step to detect HBsAg in seroconversion panels to be compared with that of 27 other kits. Overall, DIA.PRO HBsAg One-Step was ranked joint twenty-second with a score of 43. The highest scoring assay was PRISM™ with a score of 90. Analysis of the seroconversion data to compare the timing of detection of early seroconversion showed that, compared with the earliest detection of HBsAg, DIA.PRO HBsAg One-Step was 14th most sensitive, with mean and median detection delays of 17.4 and 12 days, respectively. The most sensitive HBsAg detecting kit (PRISM™ HBsAg) had mean and median seroconversion detection delays of 2.3 and 0 days, respectively.

When the aggregate scores achieved for the detection of HBsAg in dilutions of commercial seroconversion panel specimens for twenty-eight kits were compared, DIA.PRO HBsAg One-Step scored eight and was ranked equal twenty-third with four other kits. The highest scoring assay was Murex HBsAg Version 3 with a score of 17.

Comparison of batches

A second batch of the kit was evaluated to investigate any inter-batch variability. A subset of 174 specimens from the main panel was employed, comprising 40 HBsAg negative specimens, 39 randomly selected HBsAg positive specimens, the six weakly reactive specimens from long

term carriers clearing their antigenaemia ('tail enders'), specimens from six commercial seroconversion panels and 15 specimens from one commercial low titre panel. In the comparison of batches, batch 0202 was more sensitive than batch 0302 with scores of 65 and 60 respectively.

Technical appraisal

The DIA.PRO HBsAg One-Step is a microplate-based enzyme-immunoassay and was simple to perform.

Conclusions

The manufacturer claims a specificity of 99.9% for the DIA.PRO HBsAg One-Step assay. However, the specificity of 97.5% and low seroconversion sensitivity found in this evaluation precludes its use in blood centres as there are many alternatives with better specificity and sensitivity. It may have a rôle in clinical settings when it is used alongside other HBsAg kits as part of a testing algorithm.

Introduction

The hepatitis B surface antigen (HBsAg) comprises the outer coat of the 42nm diameter infectious virion (Dane particle)¹. HBsAg is usually produced in large excess, forming non-infectious particles which are normally the target of screening tests to identify potentially infectious blood, organ and tissues. HBsAg elicits antibody responses (anti-HBs) directed against several antigenic domains. A common immunological variant, *a*, is present in all wild strains of HBV. Two sets of mutually exclusive subtype epitopes, *d* and *y*, and *w* and *r*, are also to be found^{2,3}. Thus the most prominent strains of HBV are *adw*, *adr*, and *ayr*. Other subtype alleles⁴ and ‘vaccine escape’ mutants which lack the *a* determinant⁵ have been described.

The development of modified sandwich radioimmunoassays⁶ for the detection of HBsAg allowed much improved sensitivity and specificity compared to previous methods, *eg.* agar gel-diffusion and counter-current electrophoresis. The format still forms the basis of current screening assays but the radioisotopic marker has been replaced by enzyme conjugate/substrate end-points. Despite the high performance of the current screening assays for HBsAg, transfusion-associated HBV infection still occurs^{7,8}. Aside from technical error, there are several explanations for this. One is the rare occurrence of HBV infectious blood donations with HBsAg below detectable limits. A second explanation is donation by one of the rare cases of infection with a HBsAg mutant which may be undetectable by the screening assay applied^{9,10}. Tests utilising monoclonal antibodies have been associated with some failures to detect such mutants, but conclusive data on this, and on the frequency and significance of HBsAg mutants, are not yet available. Epidemiological monitoring of HBV surface mutants employing test reagents which have been validated for detection of the predominant mutants and the introduction of routine screening of blood donors and organ donors by nucleic acid based technology have been proposed¹¹.

Meanwhile, it is essential that data from independent sensitivity and specificity performance assessments of commercially available HBsAg assays, and from the manufacturers of such products, are made available and utilised by those responsible for selecting assays for both HBsAg screening and the confirmation of its presence.

This report describes results obtained from an evaluation of the DIA.PRO One-Step assay. The evaluation focussed on the sensitivity and specificity of the assay and results were compared with those obtained from the evaluation of other screening assays for the detection of hepatitis B surface antigen, the results of which have been published in previous MHRA evaluation reports.¹⁵⁻²⁷

Description of the assay

A summary of the characteristics of the DIA.PRO HBsAg One-Step assay is given in [Table 1a](#). The table includes specific details relating to the kit such as the product number, volume of specimen required, the antigens / antibodies used in the assay and controls required. Also presented are details of the assay stages and necessary equipment. Quotations from the instructions supplied with each kit are shown in [Table 1b](#). These include the claims made by the manufacturer regarding the performance of the assay and its limitations.

The DIA.PRO HBsAg One-Step assay is based on the direct or ‘antigen sandwich’ principle ([Figure 1](#)). The assay uses a 96 well microtitre plate solid phase coated with anti HBsAg specific affinity purified antibody. A run of 91 specimens may be carried out in approximately three hours including time for sample addition and kit temperature equilibrium.

Prior to the addition of controls and samples, the microplate is given a single wash cycle to hydrate the wells. With the exception of well A1, which is left empty for the operation of blanking, a specimen or control (150µL) plus the conjugate (50µL) are added to designated wells. Upon addition of the conjugate, a change in the colour of the samples from yellowish to red should be observed. The microwell plate is then incubated at 37°C (± 0.1°C) for 120 (± 5) minutes, during which time, if HBsAg is present, a solid phase phase specific immunocomplex is formed. Following a wash procedure of 4 or 5 wash cycles, and incubation with chromogen tetramethylbenzidine (TMB) substrate, at room temperature (18 - 24°C) for 20 (± 1) minutes and protected from the light, a colour change is observed. The enzyme - substrate reaction is stopped by the addition of 0.3 M sulphuric acid. If the sample is free of HBsAg, no colour develops. The reactivities are read photometrically at 450 nm with a 620 - 630 nm reference wavelength.

For each run of specimens a cut-off calculation (mean of the negative controls at 450nm plus 0.050) is used to determine the status of the specimens. An optical density / cut off ratio may also be calculated. According to the kit instructions specimens with an OD/CO of less than 1 are deemed negative, those with an OD/CO between 1.0 and 1.2 equivocal and those with an OD/CO greater than 1.2 are deemed reactive.

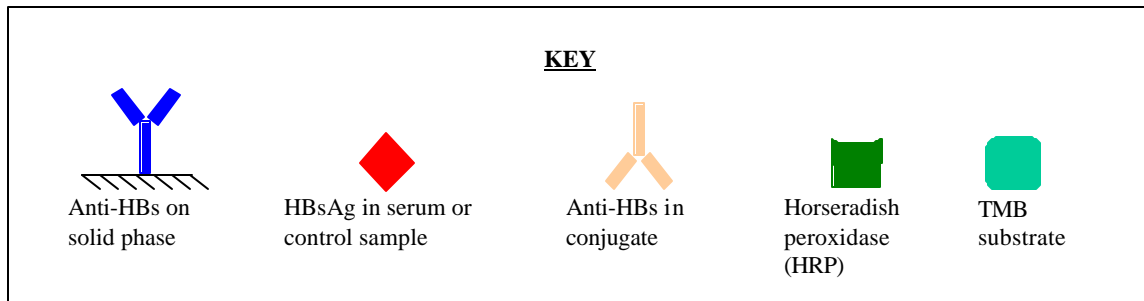
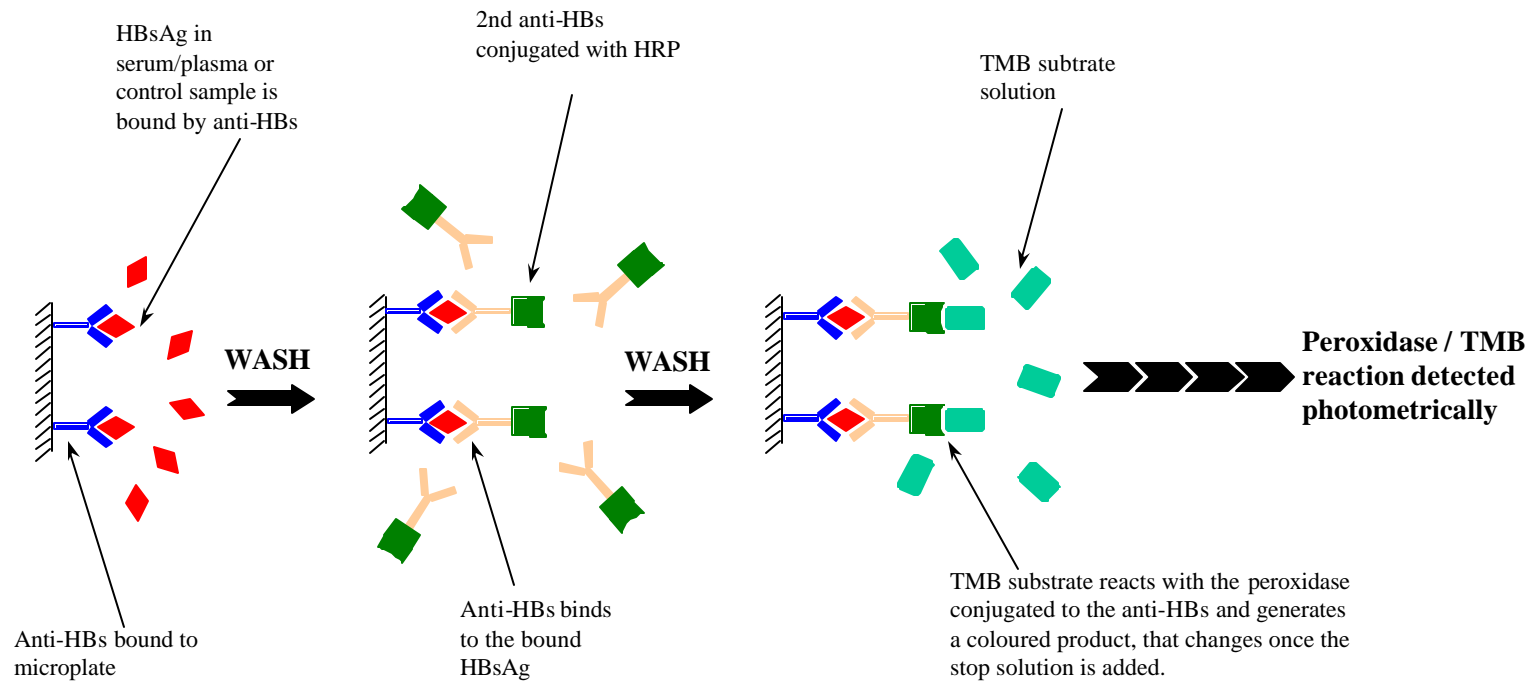
Table 1a: Assay information

General	
Assay name	DIA.PRO HBsAg One-Step
Manufacturer / UK agent	DIA.PRO Diagnostic Bioprobes Srl, Italy / Quest Biomedical
Product number	SAG1.CE
Number of tests in one pack	2 x 96 tests
Test volume	150µl
ISO9000 series certification	UNI EN ISO 9002-UNI CEI EN 46002
TÜV registered	501001146
FDA registered	No
PEI registered	No
AFSSAPS registered	No
CE mark	No
UK launch date	2002
Presentation	
Assay type	direct sandwich enzyme immunoassay
Coating of solid phase	monoclonal anti-HBs
Conjugate	monoclonal anti-HBs / horseradish peroxidase
Substrate	Chromogen tetramethylbenzidine (TMB) / hydrogen peroxide
Controls per run	
HBsAg negative control	3 replicates
HBsAg positive control	1 replicate
Cut-off computation	mean of negative control values + 0.050 (S/CO > 1.2 = reactive)
Equivocal zone	1.0 - 1.2
Stages	
Pre-washing	1
Preparation/sample well loading	approx 30 minutes / 90 specimens
Incubation status	static
Sample/Conjugate incubation (time/temp)	120 ± 5 minutes (37 ± 0.1°C)
Number of washes	4 or 5
Recommended soak time	30 seconds
Substrate incubation (time/temp)	20 ± 1 minutes (18 - 24°C)
Reading	within 30 minutes at 450 nm (630 nm reference filter)
Total incubation times	140 minutes
Number of optional procedures	None
Additional equipment required	
Incubator	
Microplate spectrophotometer	
Micropipettes: 200 – 1000µL; 40 - 200µl	
Multichannel pipette: 50 - 300µl	
Measuring cylinder	
Reagent troughs and bottles	
Disposable tips	
Distilled water	
Notes:	
TÜV = Rheinland Product Safety GmbH, Germany; FDA = Food and Drug Administration, USA;	
PEI = Paul-Ehrlich-Institut, Germany; AFSSAPS = Agence Française de Sécurité Sanitaire des Produits de Santé, France	

Table 1b: Claims for the assay and its limitations

Claims for the assay (from the kit insert)
<p>The limit of detection of the assay has been calculated by means of the following international preparations:</p> <p>Paul Ehrlich Institute, Germany, standards for HBsAg - ay and ad subtypes NIBSC second British working standard code 99/640-005-WI WHO international standard, NIBSC code 80/549.</p> <p>Clinical sensitivity has been tested on panels of samples classified as positive by a kit US FDA approved. Positive samples were collected from different geographical origin (including both ay and ad subtypes) and from different HBV pathologies (acute, asymptomatic and chronic). An overall value of 100% has been found in the study conducted on a total number of more than 200 samples.</p> <p>Boston Biomedica Inc HBsAg sensitivity panel PHA 805 and low titre performance panel PHA 104 have also been studied.</p> <p>All the HBsAg known subtypes, "ay" and "ad", and isoforms "w" and "r" supplied by CNTS, France, were tested in the assay and determined positive by the device as expected.</p> <p>Potentially interfering samples derived from patients with different viral (HCV, HDV, HAV, HEV) and non-viral pathologies of the liver were tested. No crossreaction was observed.</p> <p>Clinical specificity has been determined on panels of negative samples from normal individuals and blood donors, classified negative with a kit registered in Italy by the Ministry of Health. Both plasma, derived with different standard techniques of preparation (citrate, EDTA and EGTA), and sera have been used to determine specificity. On a collection of more than 2000 specimens a value of 99.9% has been found.</p> <p>False positivity was assessed in less than 0.1% of the normal population, mostly due to high titers of Heterophilic Anti Mouse Antibodies (HAMA).</p> <p>Frozen specimens have been tested, as well, to check for interferences due to collection and storage. No interference was observed.</p>
Limitations of the assay (from the kit insert)
<p>Avoid any addition of preservatives; in particular do not use sodium azide as this would affect the enzymatic activity of the conjugate, generating false negative results.</p> <p>Haemolysed and visibly hyperlipemic samples have to be discarded as should samples containing residues of fibrin or heavy particles or microbial filaments and bodies.</p> <p>Sera and plasma can be stored at +2° to +8° C for up to five days after collection. For longer storage periods, up to several months, specimens should be stored frozen at -20° C. Avoid in this case more than a single freeze/thaw cycle.</p> <p>In case of presence of particles, centrifugation at 2000 rpm for 20 min or filtration on 0.2 - 0.8 µ filters is possible to clean up the sample for testing.</p> <p>The thermostatic incubator should be set and maintained at 37°C (± 0.1°)</p>

Figure 1: HBsAg detection by the DIA.PRO HBsAg One-Step assay



Methods

The evaluation was performed in accordance with the [protocol](#) that was agreed by Quest Biomedical. All equipment was already available at MiDAS. Quest Biomedical elected not to provide training in the use of the kit.

The DIA.PRO HBsAg One-Step assay was evaluated against a panel of sera containing 408 freshly collected HBsAg negative blood donors' samples, 422 HBsAg positive sera and forty-two commercial specimen panels with low levels of HBsAg such as are present at seroconversion. Four quality control sera, six HBsAg dilution series and six weakly reactive specimens were also included ([Table 2a](#)). When the residual volume permitted, specimens that gave discordant results were retested in duplicate.

Twenty-five seroconversion panels and one low titre panel were obtained from Boston Biomedica Inc (BBI), USA and 16 seroconversion panels from Impath BioClinical Partners Inc (BCP), USA. Each seroconversion panel comprised a longitudinal series of undiluted plasma samples collected from a single donor close to the time of HBsAg seroconversion. The time at which each infection was contracted was not known. All specimens included in the HBsAg low titre panel PHA105 were undiluted samples obtained from asymptomatic donors. The criterion for inclusion in this panel was a HBsAg concentration < 1IU/mL. As far as possible the commercial panels were selected for inclusion in the evaluation panel so that comparisons could be made with recent evaluations of other kits. Each of the commercial panels obtained had previously been characterised by BBI or BCP and their reference laboratories.

Before commencing the evaluation, a performance efficiency check of the EIA, and the equipment to be used was carried out by testing multiple replicates of a strongly positive HBsAg sample interspersed with replicates of a HBsAg negative sample, thereby checking the efficiency of the washer and any potential for carry-over. The practice of systematically alternating expected reactive samples with expected unreactive samples in the microtitre wells as a way of identifying the occurrence of sample carry-over was used as a continuing precaution during the testing of the evaluation panel specimens. On each day of testing the washer was visually checked to ensure it continued to function correctly. Findings were monitored throughout the evaluation so that, if a problem arose, work could be halted temporarily to allow consultation and, if necessary, remedial action. Plugged micropipette tips were used for specimen pipetting to avoid any possibility of cross-contamination from micropipette barrels.¹²

All of the samples included in this evaluation were tested on production lot number 0202 (expiry date May 2003) of the HBsAg One-Step assay. A subset of specimens ([Table 2b](#)) was also tested on an additional production lot (0302, expiry date June 2003) to assess batch - to - batch variation.

Quest Biomedical / DIA.PRO Diagnostic Bioprobes Srl received a draft report of the results on the DIA.PRO HBsAg One-Step assay during April 2003, allowing them the opportunity to comment prior to the preparation of the final report. However, no comments were received prior to publication.

Specimen Panel

Table 2a: Specimen panel (batch 0202)

Sample category	Number	
1. HBsAg negative samples (n=409)		
a) Blood donors'/healthy adults' sera		408
b) HBsAg / anti- HBs negative human plasma*	2x	1
2. HBsAg positive samples (n=428)		
a) Routine HBsAg positive samples		422
b) Long term carriers clearing antigenaemia		6
3. HBsAg seroconversion panels (n=345)		
BBI - PHM903- 904		9
BBI - PHM908 - 912		55
BBI - PHM914 - 931		126
BCP - 6271 - 6282		119
BCP - 6288		9
BCP - 6291 - 6293		27
4. Seroconversion panel dilutions (diluted in NHP*)		
BBI - PHM901-05 (1/20 to 1/1280)		4
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 - PHM921-05 (1/20 to 1/1280)		4
BBI - PHM922-09 (1/20 to 1/1280)		4
5. HBsAg performance panels		
BBI - PHA105 (low titre)		15
6. Quality control samples		
NIBSC British working standard (0.5IU/ml)	6x	1
NIBSC monitor sample (0.125IU/ml)	6x	1
HPA HBsAg quality control serum QC1	6x	1
HPA HBsAg quality control serum QC2	6x	1
TOTAL (number of specimens)		1225

Notes:

* Human plasma negative for HBsAg and anti-HBs, used to prepare dilution series

BBI = Boston Biomedica Inc; BCP = BioClinical Partners Inc;

HPA = Health Protection Agency - Colindale, London;

NIBSC = National Institute for Biological Standards and Control.

Table 2b: Specimen panel (0302)

Sample category	Number	
1. HBsAg negative samples (n=41)		
a) Blood donors'/healthy adults' sera		40
b) HBsAg / anti- HBs negative human plasma*	2x	1
2. HBsAg positive samples (n=45)		
a) Routine HBsAg positive samples		39
b) Long term carriers clearing antigenaemia		6
3. HBsAg seroconversion panels (n=45)		
BBI - PHM903 (subtype undefined)		6
BBI - PHM908 (subtype ay)		8
BBI - PHM914 (subtype ad)		6
BBI - PHM919 (subtype ad)		9
BBI - PHM923 (subtype ay)		4
BCP - 6281		12
7. Seroconversion panel dilutions (diluted in NHP*)		
BBI - PHM901-05 (1/20 to 1/1280)		4
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 - PHM921-06 (1/20 to 1/1280)		4
BBI - PHM922-09 (1/20 to 1/1280)		4
8. HBsAg performance panels		
BBI - PHA105 (low titre)		15
9. Quality control samples		
NIBSC British working standard (0.5IU/ml)	3x	1
NIBSC British working standard (0.125IU/ml)	3x	1
HPA HBsAg quality control serum QC1	3x	1
HPA HBsAg quality control serum QC2	3x	1
TOTAL (number of specimens)		174

Notes:

* Human plasma negative for HBsAg and anti-HBs, used to prepare dilution series
 BBI = Boston Biomedica Inc; BCP = BioClinical Partners Inc;
 HPA = Health Protection Agency - Colindale, London;
 NIBSC = National Institute for Biological Standards and Control.

■ Key to the presentation of results

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

- specificity (Tables 3 - 4)
- sensitivity (Tables 5 - 7)
- distribution of initial reactivities (Figure 2)
- delta values for positive and negative specimens (Table 8 and Figure 3)
- scores for commercial panels tested (Tables 9 - 10)
- detection of low concentrations of HBsAg (Table 11 and Figure 4)
- timing of detection of seroconversion (Table 12 and Figure 5)
- detection of HBsAg in dilution series (Table 13)
- OD/CO ratios and mean values for quality control samples (Tables 14 - 15 and Figure 6)
- manufacturer's kit control results (Table 16 and Figures 7 - 8)
- comparison of two batches (Table 17 and Figures 9 - 10)

■ Specificity

The specificity of the DIA.PRO HBsAg One-Step assay was ascertained by testing 408 freshly collected HBsAg negative specimens from blood donors. Twelve of these negative specimens were initially reactive (OD/CO ranged from 1.01 to 15.54) of which ten were found to be repeatedly reactive. All twelve specimens were non-reactive when tested by the Organon Teknika Uniform II HBsAg assay (Table 4).

Overall, the specificity, was 97.5% (a repeat reactive rate of 2.5%; 95% confidence interval 1.2 – 4.5%). The manufacturer claims a specificity of 99.9% in its instructions for use. Specificity findings for 27 other assays evaluated previously are shown in Table 3. In four cases (PRISM™ automated HBsAg, Monolisa AgHBs Plus, and Murex HBsAg V2 and V3) they include specificity results from joint MHRA / National Blood Authority evaluations and in one case (Bioelisa Colour) the results are taken from the National Blood Authority Monthly Donation Testing Report.

It should be noted that, in the MiDAS evaluation, HBsAg positive and negative specimens were tested alternately in the microtitre plate. The density of HBsAg positive specimens present in this evaluation is unlikely to be encountered in routine clinical practice, even in reference centres. Nevertheless, laboratories should be alert to the problem of cross-contamination, which underlines the importance of confirmatory tests on all reactive specimens, including the collection of a second specimen from the donor or patient concerned. HBsAg positive samples are very rarely encountered in blood centres' assay runs, apart from HBsAg assay controls, therefore the opportunities for carry-over or cross-contamination in blood centres are infrequent.

Table 3: False positive rates for 28 HBsAg screening assays

Assay	Company	Product code	Number tested ^a	Number initially reactive	Number repeatedly reactive	Repeat false positive rate	95% confidence interval	MHRA Reference
Amerlite HBsAg II	Ortho-Clinical Diagnostics	LAN.2212	97*	0	0	0.00	0.00 – 3.73	95/52
Bioelisa HBsAg	Launch Diagnostics / Biokit Ltd	3000-1080	97*	1	0	0.00	0.00 – 3.73	95/52
Enzygnost HBsAg monoclonal II	Dade Behring Ltd	OQC110/11	97*	2	0	0.00	0.00 – 3.73	95/52
Enzymun-Test	Roche Diagnostics Ltd	1288989	97*	2	0	0.00	0.00 – 3.73	95/52
ETI-MAK-3	Incstar Ltd	P3142	97*	0	0	0.00	0.00 – 3.73	95/52
Heprofile HBsAg	Microgen Bioproducts Ltd	M450	97*	0	0	0.00	0.00 – 3.73	95/52
IMx HBsAg	Abbott Diagnostics Ltd	2228-20	97*	0	0	0.00	0.00 – 3.73	95/52
Labsystems HBsAg EIA Plus	Labsystems OY / Quest Biomedical	61 10 800/802	547	1	0	0.00	0.00 – 0.67	01165
MicroTrak II HBsAg	Dade Behring Ltd	8HB29	97*	0	0	0.00	0.00 – 3.73	95/52
Monolisa Ag HBs Plus (method 3)	Sanofi Diagnostics Pasteur Ltd	71314	1285**	2	0	0.00	0.00 – 0.3	2000/16
Monolisa Ag HBs 2nd gen	Sanofi Diagnostics Pasteur Ltd	72204	97*	2	0	0.00	0.00 – 3.73	95/52
Murex HBsAg	Murex Biotech Ltd	GE14/15/16	97*	0	0	0.00	0.00 – 3.73	95/52
Ortho HBsAg Test System 3	Ortho-Clinical Diagnostics	931801	97*	4	0	0.00	0.00 – 3.73	95/52
VIDAS HBsAg	bioMérieux Ltd	30 300	97*	1	0	0.00	0.00 – 3.73	95/52
Vitros <i>ECi</i> HBsAg	Ortho-Clinical Diagnostics	843 5307	368	3	0	0.00	0.00 – 1.00	2000/33
PRISM™ automated HBsAg assay	Abbott Laboratories Ltd	3A4748	9563**	4	1	0.01	0.00 – 0.03	97/52
Murex HBsAg Version 3	Abbott-Murex	GE34/36	3187**	13	3	0.09	0.02 – 0.28	2000/55
Bioelisa colour	Launch Diagnostics / Biokit Ltd	3000-1100	51133***	NA	34	0.10	0.00 – 0.10	99/68
Murex HBsAg (v2)	Murex Biotech Ltd	GE14/15/16	2000**	6	2	0.10	0.01 – 0.36	98/39
Enzygnost HBsAg 5.0	Dade Behring Ltd	OQPW11/21	500	3	1	0.20	0.00 – 1.10	03044
ETI-MAK-4 (Procedure B)	DiaSorin Ltd	N0019	499	6	1	0.20	0.00 – 1.10	01121
Access® HBs Ag	Beckman / Sanofi	34220	500	5	2	0.40	0.04 – 1.43	98/75
AxSYM HBsAg (v2)	Abbott Diagnostics Ltd	7A40-22	500	5	2	0.40	0.00 – 1.40	01125
Bioelisa HBsAg colour plus (MiDAS)	Launch Diagnostics / Biokit Ltd	3000-1155	500	24 ^s	2	0.40	0.00 – 1.40	02096
Bioelisa HBsAg colour plus (NBS)			2262	27	6	0.30	0.10 – 0.60	
Auszyme monoclonal	Abbott Diagnostics Ltd	1980-24	97*	2	1	1.03	0.03 – 5.60	95/52
Hepanostika HBsAg Uni-form II	Organon Teknika Ltd	6019/6023	97*	3	1	1.03	0.03 – 5.60	95/52
COBAS Core HBsAg II	Roche Diagnostics Ltd	07/5350/5	97*	3	2	2.06	0.25 – 7.24	95/52
DIA.PRO HBsAg One-Step	Diagnostic Bioprobes Srl / Quest Biomedical	SAG1.CE	408	12	10	2.50	1.20 – 4.50	03031

Notes:
^aAll specimens expected to be negative *Assays evaluated using the same 97 specimens. ** Includes National Blood Service specificity evaluation data
*** Figures from NBA Monthly Donation Testing Report. \$Includes three specimens with OD/CO values between 0.9 - 1.0 NA = Not Available NBS = National Blood Service
Refer to previous MHRA reports ^{1b-2f} for details of this and other assays listed

Table 4: Further testing of 12 specimens found to be falsely reactive by the DIA.PRO HBsAg One-Step assay

VRD No	DIA.PRO HBsAg One-step				Organon Teknika HBsAg Uniform II
	OD	Cut-off	OD/CO	OD/CO (Mean of 2 repeats)	OD/CO
02-23243	0.063	0.063	1.01	0.98	0.39
02-23334	0.08	0.076	1.05	1.38	0.37
02-26325	0.102	0.069	1.49	1.47	0.35
02-23271	0.127	0.076	1.66	1.91	0.41
02-23272	0.133	0.076	1.74	0.55	0.35
02-23239	0.122	0.063	1.95	2.90	0.35
02-23527	0.197	0.068	2.88	3.92	0.35
02-26322	0.206	0.069	3.00	3.94	0.37
02-23215	0.249	0.063	3.97	1.79	0.38
02-23571	0.368	0.068	5.39	7.78	0.34
02-23228	0.408	0.063	6.51	9.59	0.92
02-23259	0.974	0.063	15.54	13.33	0.51

■ Sensitivity

The overall sensitivity of the DIA.PRO HBsAg One-Step assay was assessed on the basis of initial reactions with 422 randomly selected HBsAg positive specimens.

All 422 specimens were positive in the DIA.PRO HBsAg One-Step assay giving a sensitivity of 100% (95% confidence interval 99.1 – 100%). The overall sensitivity of the assay is shown in [Table 5](#) along with data for 27 other HBsAg assays previously evaluated.

Six of the routine specimens gave OD/CO ratios less than 5 (2.00, 3.98, 4.39, 4.48, 4.74 and 4.94 respectively) in the DIA.PRO HBsAg One-Step assay. These specimens were found to be strongly positive when tested using the Hepanostika UNIFORM II HBsAg screening assay ([Table 6](#)).

Six weakly positive sera obtained from a longitudinal study of four donors who were long-term HBsAg carriers clearing their antigenaemia were tested in duplicate in the DIA.PRO HBsAg One-Step assay. Four of the six specimens had been tested by 27 other HBsAg assays, and comparative data are available ([Table 7](#) and *Appendix Table 19*). Of the four specimens tested by all of the kits, DIA.PRO HBsAg One-Step detected three. In respect of the two extra specimens (which had been tested by only six other kits), DIA.PRO HBsAg One-Step detected one.

Table 5: Sensitivity for 28 HBsAg screening assays

Assay	Company	Product code	Number positive / number tested	Sensitivity % **	95% confidence interval (%)	Range S/CO	Mean S/CO	Median S/CO	MHRA Reference
Auszyme monoclonal	Abbott Diagnostics Ltd	1980-24	150/150	100	97.6 – 100	2.04 – 43.48	37.64	39.22	95/52
AxSYM [®] HBsAg (V2)	Abbott Diagnostics Ltd	7A40-22	491 / 491	100	99.3 – 100	1.20 – 291.55	170.77	196.81	01125
Bioelisa colour	Launch Diagnostics / Biokit Ltd	3000-1100	150/150	100	97.6 – 100	3.07 – 44.02	35.63	37.51	99/68
Bioelisa HBsAg colour plus	Launch Diagnostics / Biokit Ltd	3000-1155	408/408 ^a	100	99.1 – 100	1.13 - 53.36	43.92	45.19	02096
			56/56 ^b	100	93.6 – 100	1.39 - 52.13	6.36	4.24	
COBAS Core HBsAg II	Roche Products Ltd	07/5350/5	150/150	100	97.6 – 100	1.66 – 73.22	71.3	73.22	95/52
DIA.PRO HBsAg One-Step	Diagnostic Bioprobes Srl / Quest Biomedical	SAG1.CE	422/422	100	99.1 – 100	2.00 – 61.28	49.15	55.77	03031
Enzygnost [®] HBsAg 5.0	Dade Behring Ltd	OQPW11/21	483 / 483	100	99.2 – 100	1.52 – 39.30	32.8	35.43	01166
Enzygnost HBsAg monoclonal II	Dade Behring Ltd	OQC110/11	150/150	100	97.6 – 100	1.10 – 54.80	49.62	50.76	95/52
ETI-MAK-3	Sorin Biomedica Diagnostics/Incstar	P3142	150/150	100	97.6 – 100	2.35 – 58.82	56.12	57.69	95/52
ETI-MAK-4 (Procedure B)	DiaSorin Ltd	N0019	285/285	100	98.7 – 100	2.57 – 120	69.98	75.00	01121
Hepanostika HBsAg Uni-Form II	Organon Teknika Ltd	6019/6023	150/150	100	97.6 – 100	1.08 – 35.96	24.18	21.9	95/52
IMx HBsAg	Abbott Diagnostics Ltd	2228-20	150/150	100	97.6 – 100	1.26 – 102.4	68.19	72.35	95/52
Labsystems HBsAg EIA Plus	Labsystems OY / Quest Biomedical	61 10 800/802	445 / 445	100	99.2 – 100	1.17 – 29.13	23.54	26.21	01165
Monolisa [®] Ag HBs 2nd gen	Sanofi Diagnostics Pasteur	72204	150/150	100	97.6 – 100	2.14 – 83.33	72.23	71.43	95/52
Murex HBsAg	Murex Biotech Ltd	GE14/15/16	150/150	100	97.6 – 100	1.56 – 27.27	23.24	25.86	95/52
Murex HBsAg (v2)	Murex Biotech Ltd	GE14/15/16	153/153	100	97.6 – 100	1.45 – 25.00	21.3	23.44	98/39
Murex HBsAg Version 3	Abbott Murex Ltd	GE 34/36	298/298	100	98.8 – 100	5.63 – 24.59	22.98	23.08	2000/55
Ortho HBsAg Test System 3	Ortho Clinical Diagnostics	931801	150/150	100	97.6 – 100	1.60 – 96.77	85.34	88.24	95/52
PRISM [™] HBsAg assay	Abbott Diagnostics Ltd	3A4748	145/145	100	97.5 – 100	4.48 – 813.58	426.35	470.69	97/52
VIDAS HBsAg	bioMérieux Ltd	30 300	150/150	100	97.6 – 100	1.08 – 131.00	70.59	120.73	95/52
Monolisa [®] Ag HBs Plus (method 3)	Sanofi Diagnostics Pasteur	72314	458/459	99.8	98.8 – 100	0.87 – 61.22	55.11	60.00	2000/16
Amerlite HBsAg II	Kodak Ltd	LAN.2212	149/150*	99.3	96.3 – 100	0.32 – 1711.00	1192.66	1254	95/52
Bioelisa HBsAg	Launch Diagnostics / Biokit Ltd	3000-1080	149/150*	99.3	96.3 – 100	0.83 – 61.30	54.97	56.25	95/52
Enzymun-Test	Boehringer Mannheim	1288989	149/150*	99.3	96.3 – 100	0.71 – 114.93	98.84	102.03	95/52
Heprofile HBsAg	Microgen Ltd	M450	149/150*	99.3	96.3 – 100	0.70 – 58.82	27.28	26.55	95/52
MicroTrak II HBsAg	Syva UK Ltd	8HB29	149/150*	99.3	96.3 – 100	0.22 – 25.86	21.61	21.58	95/52
Vitros ECi [®] HBsAg	Ortho Clinical Diagnostics	843 5307	270/272	99.3	97.4 – 99.9	0.24 – 8740	3619.39	3695.00	2000/33
Access [®] HBS Ag	Beckman / Sanofi	34220	201/203	99.0	96.5 – 99.9	1.05 – 1201.41	583.22	579.94	98/75

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.

Refer to previous MHRA evaluation reports¹⁵⁻²⁷ for details of other assays listed.

Table 6: Routine HBsAg specimens with a DIA.PRO HBsAg One-Step OD/CO < 5.0

Specimen number	DIA.PRO HBsAg One-Step	Hepanostika Uniform II HBsAg	AxSYM HBsAg (V2)	Bioelisa HBsAg colour plus	Enzygnost HBsAg 5.0	Labsystems HBsAg EIA Plus
	OD/CO	OD/CO	OD/CO	OD/CO	OD/CO	OD/CO
95-22260	2.00	9.69	NT	10.99	NT	NT
95-22219	3.98	22.23	NT	2.08 (24.27*)	NT	NT
95-22214	4.39	10.10	NT	13.28	NT	NT
00-24749	4.48	10.31	16.30	8.58	32.19	3.73
95-22266	4.74	7.10	NT	NT	NT	NT
95-22244	4.94	8.74	NT	16.59	NT	NT

Note * mean of two repeats

Table 7 : S/CO ratios for weakly positive HBsAg samples from chronic carriers

Assay	Product number	VRD number					
		93-07060	93-07063	93-07064	93-07065	00-08381	00-08382
AxSYM [®] HBsAg (V2)	7A40-22	1.48	59.01	3.16	4.36	6.15	8.46
Enzygnost [®] HBsAg 5.0	OQPW11/21	1.26	36.14	3.37	8.34	3.27	1.16
ETI-MAK-4	N0019	1.28	61.98	1.91	4.35	8.93	4.42
Murex HBsAg Version 3	GE34/36	2.32	21.74	4.46	9.05	12.25	17.75
Bioelisa HBsAg colour plus	3000-1155	0.95	29.31	1.17	2.64	3.86	1.54
DIA.PRO HBsAg One-Step	SAG1.CE	1.02	17.71	0.73	2.82	1.92	0.27
Labsystems HBsAg EIA Plus	61 10 800/02	0.60	6.03	0.73	1.20	1.23	0.57
Auszyme monoclonal	1980-24	1.30	40.80	2.77	7.24	NT	NT
Monolisa Ag HBs 2nd gen.	72204	1.20	20.91	2.06	3.67	NT	NT
Murex HBsAg	GE14/15/16	1.22	25.66	3.07	4.27	NT	NT
Murex HBsAg (version 2)	GE14/15/16	1.07	19.61	2.04	5.62	NT	NT
Ortho HBsAg Test System 3	931801	1.26	51.25	2.14	3.16	NT	NT
PRISM [™] HBsAg	3A4748	4.41	171.09	5.27	12.40	NT	NT
Vitros [®] ECi HBsAg	843 5307	2.39	115.00	2.25	3.27	NT	NT
Bioelisa HBsAg colour	3000-1100	0.87	29.23	1.10	2.72	NT	NT
ETI-MAK-3	P3142	0.21	28.55	1.15	3.73	NT	NT
Hepanostika HBsAg Uni-form II	6019/6023	0.81	5.46	1.21	1.58	NT	NT
Access HBsAg	34220	0.90	7.72	0.69	1.93	NT	NT
Amerlite HBsAg II	LAN.2212	0.92	103.10	0.40	2.37	NT	NT
Bioelisa HBsAg	3000-1080	0.62	19.20	0.36	1.77	NT	NT
COBAS Core HBsAg II	07/5350/5	0.86	42.40	0.86	7.75	NT	NT
Enzygnost HBsAg monoclonal II	OQC110/11	0.84	10.20	0.73	1.80	NT	NT
Enzymun-Test HBsAg	1288989	-0.02	80.01	0.09	1.09	NT	NT
Heprofile HBsAg	M450	0.88	2.75	0.46	1.01	NT	NT
IMx HBsAg	2228-20	0.69	16.32	0.98	1.06	NT	NT
Monolisa Ag HBs plus (method 3)	72314	0.64	6.13	0.53	1.18	NT	NT
VIDAS HBsAg	30 300	0.77	10.92	0.54	1.69	NT	NT
MicroTrak II HBsAg	8HB29	0.15	3.52	0.19	0.35	NT	NT

Notes:

NT = not tested

Positive reactions are shown in bold type.

93-07064 and 93-07065 are serial bleeds from the same patient taken on 2/12/1991 and 29/1/1993 respectively.

93-07063 and 00-08382 are serial bleeds from the same patient taken on 22/2/1993 and 27/1/2000 respectively.

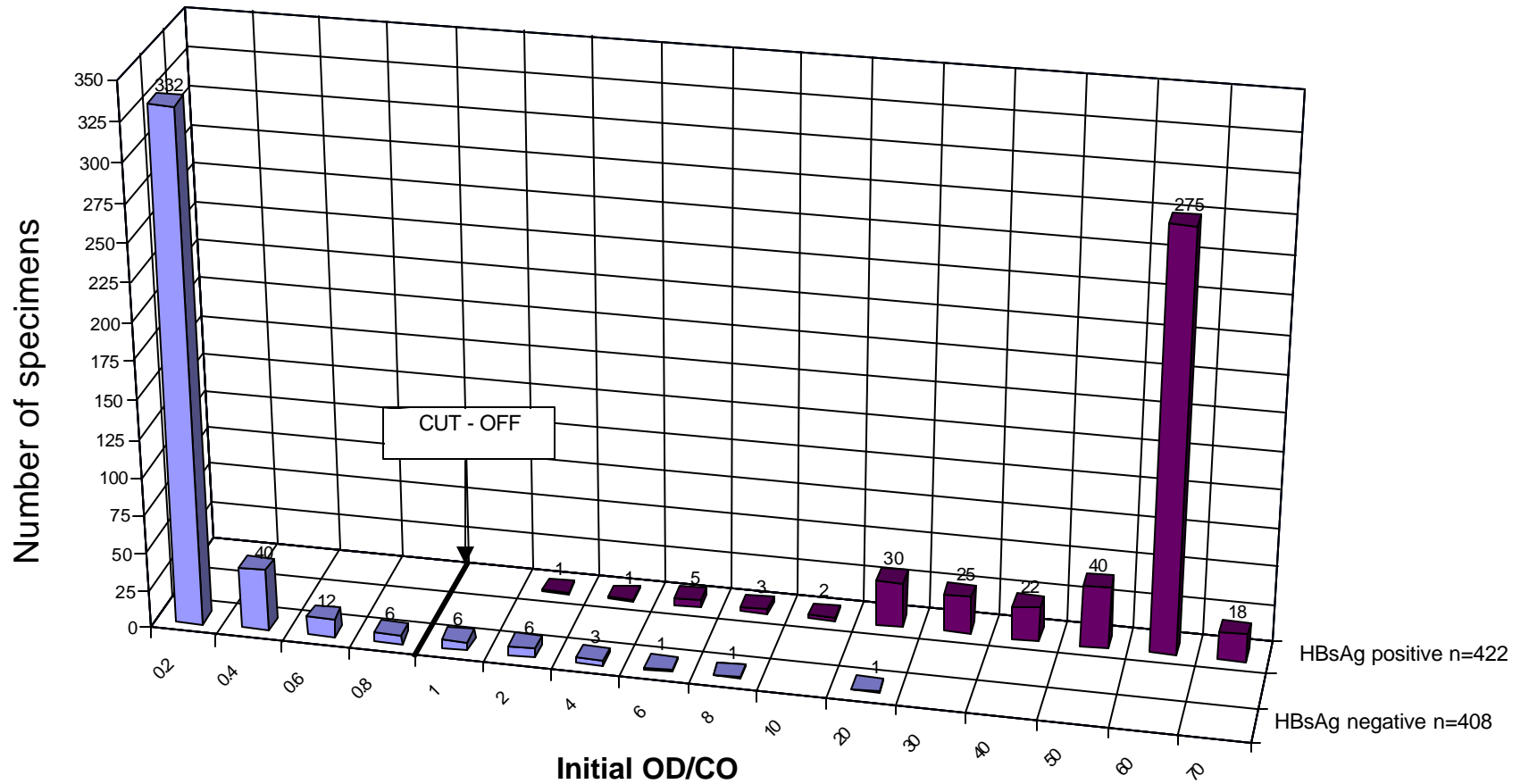
■ Distribution of initial reactivities

The distribution of reactivities for the 408 HBsAg negative, and 422 HBsAg positive sera tested during the evaluation of DIA.PRO HBsAg One-Step are presented in [Figure 2](#). Assays with good discrimination have few, if any, specimens wrongly classified and few reactions in close proximity to the cut-off.

This assay gave rise to no falsely negative results. Twelve known HBsAg negative samples were found to be reactive by this assay, of which 10 were found to be repeatedly reactive when tested in duplicate.

Data from HBsAg seroconversion and performance panels are not included in this histogram.

Figure 2: Distribution of initial reactivities for the DIA.PRO HBsAg One-Step assay



Delta values

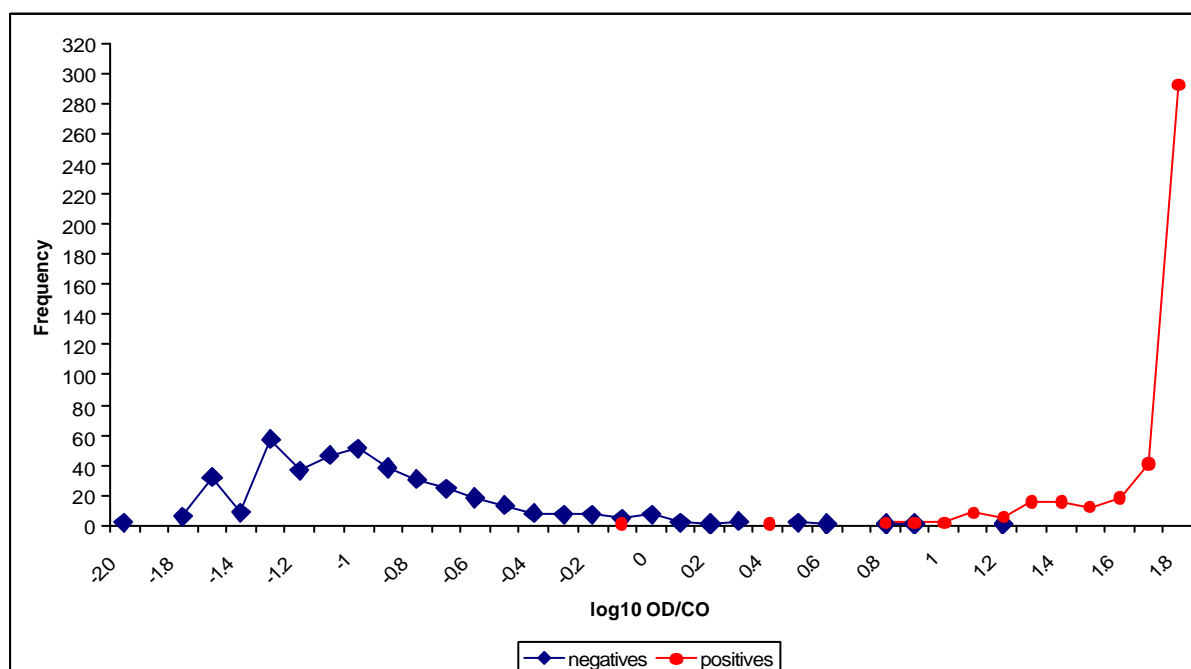
The delta value (δ) provides a means of comparing the efficacy of ELISA assays in separating the negative and positive specimens in serum populations from the cut-off¹³. The δ values for the HBsAg positive and negative samples were calculated by dividing the mean OD/CO ratio (\log_{10}) by the standard deviation of each population. (Table 8 and figure 3). The higher the positive ($\delta+$) and lower the negative ($\delta-$) values, the higher the probability that the assay will correctly identify positive and negative specimens respectively.

Table 8: Delta values of the positive and negative specimens

	Negatives	Positives
Number	408	422
Range \log_{10} OD/CO	-2.0 to 1.2	-0.1 to 1.8
Mean \log_{10} OD/CO	- 1.001*	1.637
Standard Deviation	0.442	0.249
Delta value	-2.264	6.585

Note * two negative samples produced negative OD/COs. The corresponding \log_{10} OD/CO values were scored as -2.0

Figure 3: Plot of the OD/CO(\log_{10}) for the positive and negative specimens



■ Detection of HBsAg in commercial panels

Forty-one seroconversion panels, 25 from Boston Biomedica Inc, USA and 16 from BioClinical Partners Inc, USA, consisting of 345 specimens were included in the sensitivity evaluation. These were included to assess the ability of the DIA.PRO HBsAg One-Step to detect HBsAg at the time of seroconversion. Results of 18 (142 specimens) of the 25 BBI panels are compared with those obtained by 27 previously evaluated HBsAg detection kits which have been available in the UK market for varying periods of time.

For comparison, a total aggregate sensitivity score was obtained for each kit by summing the total number of reactive samples in the 18 seroconversion panels. The most sensitive assay is deemed to be the one that overall detected HBsAg in the panels earlier than other assays, as indicated by attaining the highest aggregate score. DIA.PRO HBsAg One-Step detected HBsAg in 43 specimens and was ranked joint twenty-second (with one other kit) of the 28 kits compared (Table 9). The most sensitive kit (PRISM™ HBsAg) detected HBsAg in 90 specimens.

The remaining seven BBI panels (PHM925 to PHM931) had not been included in the scoring system as they had been tested by too few kits. However, data are available for seven other assays for these panels and the scores obtained are shown along with the scores for six BCP panels (6271 to 6276) in Table 10.

The combined scores for the 31 seroconversion panels are shown in Table 10. The DIA.PRO HBsAg One-Step detected HBsAg in 75 (out of 249 specimens), ranking it seventh of the eight assays compared. AxSYM™ HBsAg version 2 and Murex HBsAg version 3 were the most sensitive, each scoring 134.

The DIA.PRO HBsAg One-Step results for all 41 seroconversion panels tested are detailed in the Appendix, Table 20a - f.

An additional analysis was carried out using the results of 142 specimens from eighteen BBI seroconversion panels for which the concentrations of HBsAg were stated on the data sheets supplied by BBI (Table 11 and Figure 4). DIA.PRO HBsAg One-Step failed to detect any specimen containing concentrations of HBsAg of 0.4 ng/mL or less. Additionally, it detected only two of the eight specimens containing 0.5 ng/mL and 10 of 13 specimens containing concentrations of HBsAg between 0.6 ng/mL and 1.0 ng/mL. Like all of the kits included in this comparison, DIA.PRO HBsAg One-Step detected all 47 specimens with concentrations of HBsAg greater than 1 ng/mL. This suggests that the DIA.PRO HBsAg One-Step does not reliably detect HBsAg below 1 ng/mL.

Details of detection of HBsAg and OD/CO ratios for each panel are appended (Tables 21a–e and 22). Comparative OD/CO ratios and further information for other kits can be obtained in a separate publication, *HBsAg commercial panel data*²⁷.

Table 9: Comparative detection of HBsAg in 18 seroconversion panels

Assay	Product number	Aggregated score* for BBI seroconversion panels PHM903 - 924**	Ranking
		n = 142	
PRISM™ HBsAg	3A4748	90	1
AxSYM® HBsAg (V2)	7A40-22	81	2
Enzygnost® HBsAg 5.0	OQPW11/21	78	3
Murex HBsAg (version 3)	GE34/36	76	4
Vitros Eci HBsAg	843 5307	65	5
ETI-MAK-4	N0019	64	6
Bioelisa HBsAg colour plus	3000-1155	61	7
Monolisa Ag HBs 2nd gen	72204	59	8
Ortho HBsAg Test System 3	931801	53	9=
Bioelisa HBsAg (colour)	3000-1100	53	9=
COBAS Core HBsAg II	07/5350/5	52	11
Auszyme monoclonal	1980-24	51	12=
ETI-MAK-3	P3142	51	12=
Enzygnost HBsAg monoclonal II	OQC110/11	50	14=
Monolisa Ag HBs Plus (method 3)	72314	50	14=
Murex HBsAg	GE14/15/16	49	16
Bioelisa HBsAg	3000-1080	48	17=
Hepanostika HBsAg Uni-form II	6019/6023	48	17=
VIDAS HBsAg	30 300	46	19=
Heprofile HBsAg	M450	46	19=
Labsystems HBsAg EIA Plus	61 10 800/802	45	21
Amerlite HBsAg II	LAN.2212	43	22=
DIA.PRO HBsAg One-Step	SAG1.CE	43	22=
Murex HBsAg (version 2)	GE14/15/16	42	24
Access® HBs Ag	34220	40	25
Enzymun-Test HBsAg	1288989	38	26
IMx HBsAg	2228-20	31	27
MicroTrak II HBsAg	8HB29	21	28

Notes

* The score was calculated by summing the number of positive specimens detected for each panel

A higher score suggests higher sensitivity

** Excluding panels PHM905 - 907 and PHM913

Table 10: Comparative detection of HBsAg[§] in 31 seroconversion panels

Assay	Product number	Score* PHM903–924** n = 142	Score* PHM925–931 n = 48	Score* 6271-6276 n = 59	Aggregated score n = 249
AxSYM® HBsAg (V2)	7A40-22	81	30	23	134
Murex HBsAg (version 3)	GE34/36	76	33	25	134
Enzygnost® HBsAg 5.0	OQPW11/21	78	30	21	129
ETI-MAK-4	N0019	64	30	21	115
Bioelisa HBsAg colour plus	3000-1155	61	29	18	108
Hepanostika HBsAg Uni-form II	6019/6023	48	24	15	87
DIA.PRO HBsAg One-Step	SAG1.CE	43	19	13	75
Murex HBsAg (version 2)	GE14/15/16	42	17	12	71

Notes:

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

** Excluding panels PHM905–907 and PHM913.

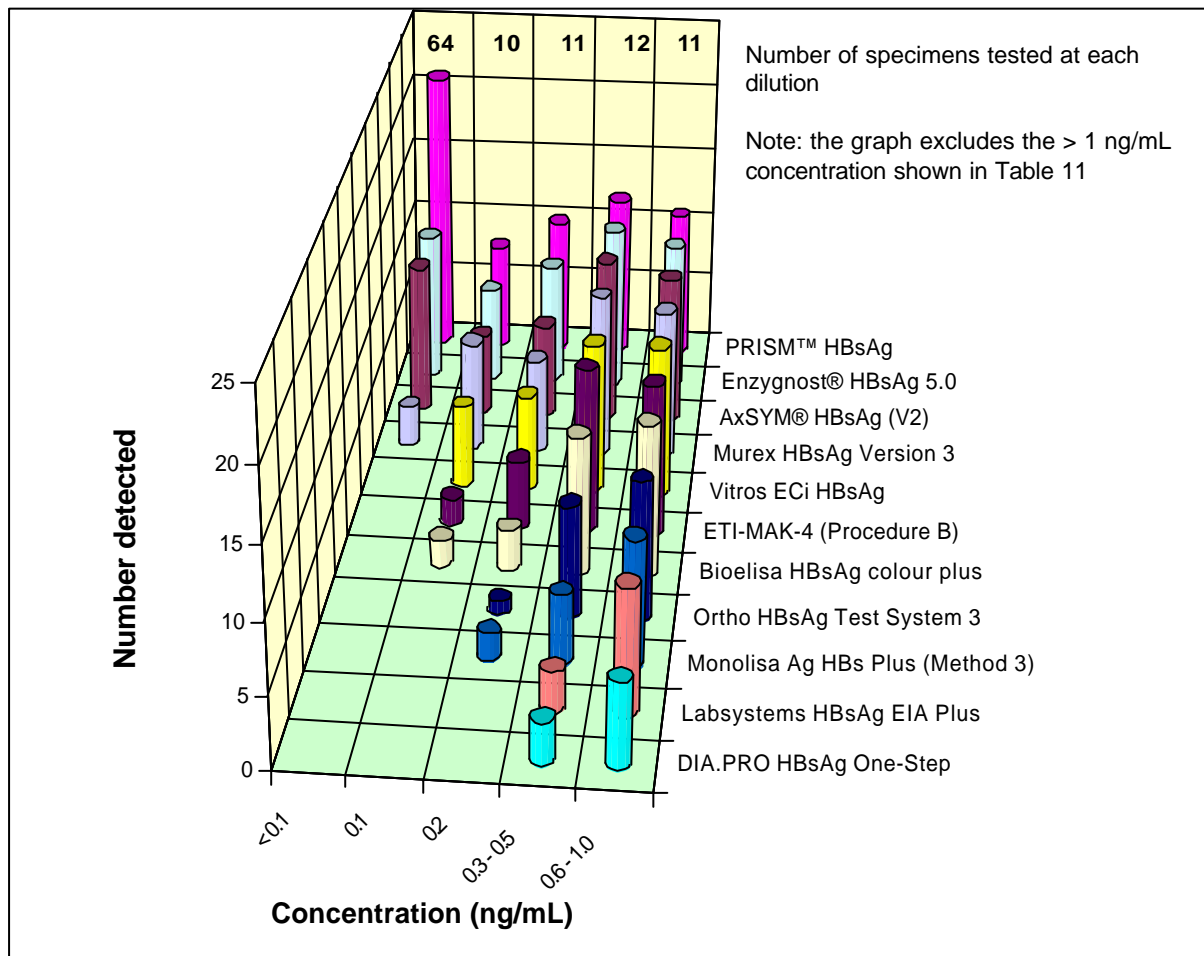
§ Not including data for panels 6277 - 6282, 6288 and 6291 - 6293 which have been tested by too few kits to make a suitable comparison.

Table 11: Estimating the limit of detection of HBsAg: detection of HBsAg by twelve assays for 142 specimens of known concentration of HBsAg

Assay	Number of specimens detected (%)					
	<0.1 ng/mL* n=64	0.1 ng/mL n=10	0.2 ng/mL n=11	0.3–0.5 ng/mL n=12	0.6–1.0 ng/mL n=11	>1 ng/mL n=34
Auszyme monoclonal	0 (0.0)	0 (0.0)	2 (18.2)	7 (58.3)	10 (90.9)	34 (100.0)
AxSYM® HBsAg (V2)	11 (17.2)	6 (60.0)	7 (63.6)	12 (100.0)	11 (100.0)	34 (100.0)
Bioelisa HBsAg colour plus	0 (0.0)	2 (20.0)	3 (27.3)	10 (83.3)	11 (100.0)	34 (100.0)
DIA.PRO HBsAg One-Step	0 (0.0)	0 (0.0)	0 (0.0)	3 (25.0)	6 (54.55)	34 (100.0)
Enzygnost® HBsAg 5.0	11 (17.2)	7 (70.0)	9 (81.2)	12 (100.0)	11 (100.0)	34 (100.0)
ETI-MAK-4 (Procedure B)	0 (0.0)	2 (20.0)	5 (45.5)	12 (100.0)	11 (100.0)	34 (100.0)
Labsystems HBsAg EIA Plus	0 (0.0)	0 (0.0)	0 (0.0)	3 (25.0)	9 (81.8)	34 (100.0)
Monolisa Ag HBs Plus (Method 3)	0 (0.0)	0 (0.0)	2 (18.2)	5 (41.7)	9 (81.8)	34 (100.0)
Murex HBsAg Version 3	3 (4.7)	8 (80.0)	7 (63.6)	12 (100.0)	11 (100.0)	34 (100.0)
Ortho HBsAg Test System 3	0 (0.0)	0 (0.0)	1 (9.1)	8 (66.7)	10 (90.9)	34 (100.0)
PRISM™ HBsAg	21 (32.8)	8 (80.0)	10 (90.9)	12 (100.0)	11 (100)	34 (100.0)
Vitros ECI HBsAg	0 (0.0)	6 (60.0)	7 (63.6)	11 (91.7)	11 (100)	34 (100.0)

Note: Concentration value for each specimen taken from BBI data sheets
 * This category includes specimens collected early in HBsAg seroconversion when some specimens may not contain HBsAg detectable by any assay

Figure 4: Detection of HBsAg by eight assays for 108 specimens of known concentration of HBsAg



■ Comparative timing of detection of seroconversion

The scoring system detailed in the previous section is based on addition of the number of samples positive in each of the 18 seroconversion panels for which there are complete data. To form a better impression of the time difference between detection of seroconversion by the most sensitive assay and its detection by other assays a further analysis was performed. This was based on comparing, for each seroconversion panel, the number of days delay in detection of antigen compared with the most sensitive kit for which data were available. When any kit did not detect seroconversion within the period that the samples were collected (*Appendix Tables 20a –f*) an arbitrary 3 days were added to the maximum calculable delay for that panel so that it was possible to differentiate that kit from any others that detected HBsAg only in the final bleed. Applying this analysis, the mean delay in detection of seroconversion for each kit ranged from 2.3 days to 21.6 days and the median ranged from 0 to 19.0 days.

We believe that the median delay provides the most meaningful indicator of seroconversion sensitivity. The mean 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 (sometimes > 30 days), giving rise to an artefact due to the timing of blood collection.

For the DIA.PRO HBsAg One-Step assay the delay in detection of HBsAg seroconversion compared with its earliest detection ranged from 0 – 45 days, with a mean delay of 17.4 days and a median of 12 days (*Table 12* and *Figure 5*). Two of twenty-eight assays had a median delay of zero days. On the basis of the median delay in detection of seroconversion, this placed the DIA.PRO HBsAg One-Step, equal ninth most sensitive of the twenty-eight assays for which we have complete data, along with five other assays. Fourteen assays had a greater median delay.

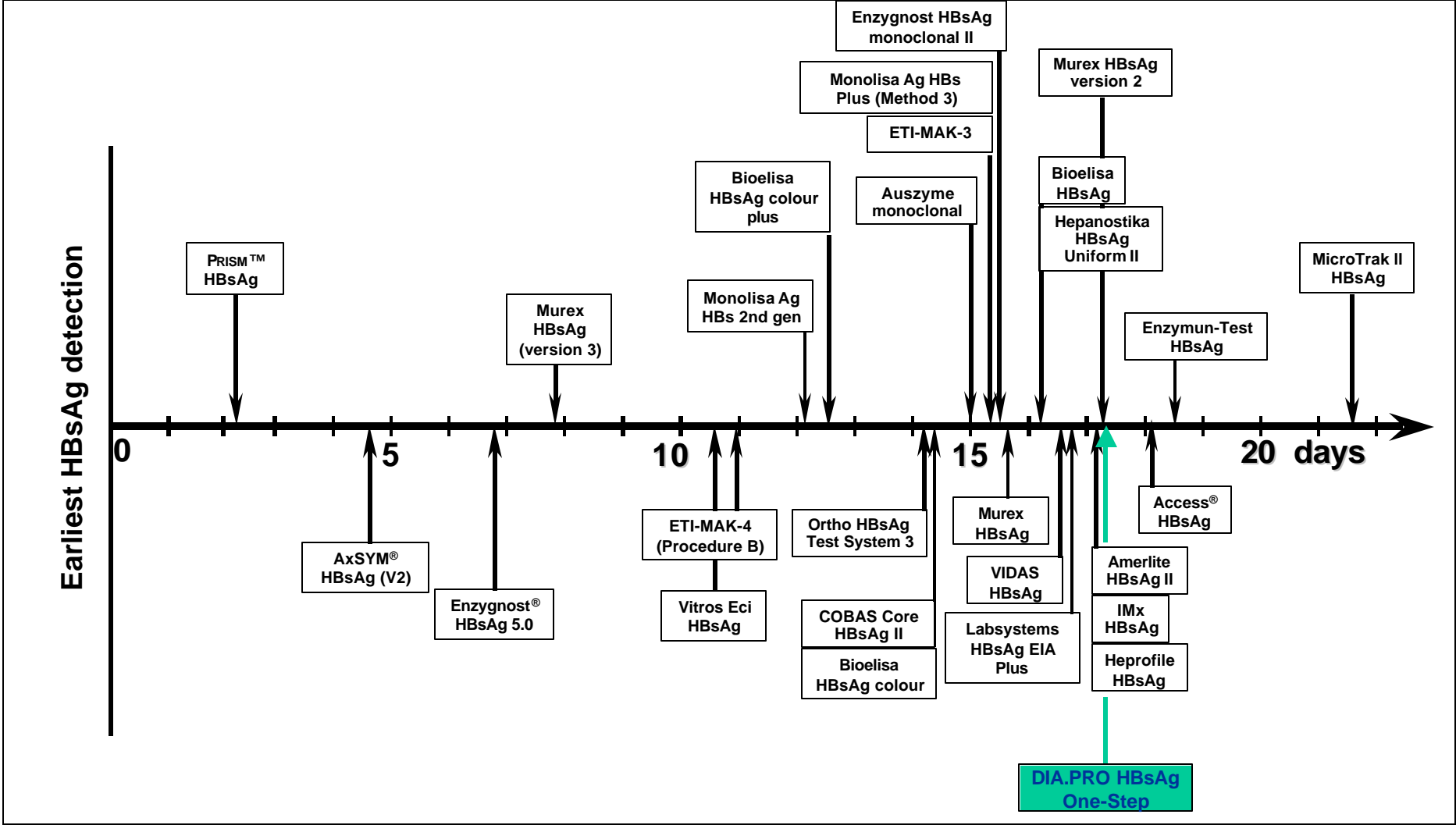
Table 12: Comparison of the timing of detection of HBsAg seroconversion by 28 kits (based on data from 18 seroconversion panels)

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 - 20	2.3	0.0
AxSYM HBsAg (V2)	7A40-22	0 - 35	4.7	0.0
Enzygnost [®] HBsAg 5.0	OQPW11 / 21	0 - 35	6.9	5.0
Murex HBsAg Version 3	GE34/36	0 - 35	7.9	6.5
Vitros HBsAg EIA	843 5307	0 - 42	10.7	7.0
ETI-MAK-4	N0019	0 - 42	11.0	7.0
Monolisa Ag HBs 2nd gen	72204	0 - 42	12.1	7.0
Bioelisa HBsAg colour plus	3000-1155	0 - 42	12.6	7.5
Bioelisa HBsAg Colour	3000-1100	0 - 42	14.4	12.0
Monolisa Ag HBs Plus (short incubation)	72314	0 - 42	15.3	12.0
Enzygnost HBsAg monoclonal II	OQC110/11	0 - 42	15.5	12.0
Bioelisa HBsAg	3000-1080	0 - 42	16.2	12.0
Labsystems HBsAg EIA Plus	61 10 800/802	0 - 42	16.8	12.0
DIA.PRO HBsAg One-Step	SAG1.CE	0 - 45	17.4	12.0
Ortho HBsAg Test System 3	931801	0 - 42	14.3	13.0
COBAS Core HBsAg II	07/5350/5	0 - 42	14.4	13.0
Auszyme monoclonal	1980-24	0 - 42	15.0	13.0
ETI-MAK-3	P3142	0 - 42	15.3	13.0
Murex HBsAg	GE14/15/16	0 - 42	15.7	13.0
Hepanostika HBsAg Uni-form II	6019/6023	0 - 42	16.2	13.0
VIDAS HBsAg	30 300	0 - 42	16.6	13.0
Heprofile HBsAg	M450	0 - 42	17.1	13.0
IMx HBsAg	2228-20	0 - 42	17.1	13.5
Access HBsAg	34220	0 - 45	18.1	14.0
Amerlite HBsAg II	LAN.2212	0 - 42	17.1	14.5
Murex HBsAg (version 2)	GE14/15/16	0 - 42	17.2	15.0
Enzymun-Test HBsAg	1288989	0 - 42	18.6	16.5
MicroTrak II HBsAg	8HB29	7 - 46	21.6	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 detect seroconversion by the last sample available in a panel, an arbitrary extra three days delay was allocated to that kit's result for that panel.

Figure 5: Comparative timing of first detection of HBsAg (mean value) following primary HBV infection



Note: Based on data from 18 BBI seroconversion panels
The origin of the horizontal axis represents the earliest recorded detection of HBsAg and is based upon the mean of the detection by the most sensitive kit for each seroconversion panel. The most sensitive kit was not always the same for each seroconversion panel.

■ Detection of HBsAg in dilution series prepared from six seroconversion specimens

Dilutions of six specimens, one from each of six seroconversion panels (PHM901-05, PHM902-14, PHM907-09, PHM920-06, PHM921-05 and PHM922-09), were made in four-fold steps from 1/20 to 1/1280 in human serum negative for HBsAg and anti-HBs.

The dilutions were tested in the DIA.PRO HBsAg One-Step assay and the results compared with data compiled from previous evaluations (Table 13). A score was assigned to each assay according to the titre at which HBsAg was detected. Each assay has been ranked according to its total score. Sensitive assays that detected HBsAg at high titres in the dilution series had high total scores.

DIA.PRO HBsAg One-Step scored 8 and was ranked joint twenty-third, with four other kits, of the twenty-eight assays included in the comparison. A second production lot scored 4 which would rank the assay equal twenty-eighth. The overall scores for each assay ranged from 4 to 17.

Table 13: Detection of HBsAg in dilution series prepared from six HBsAg seroconversion specimens

HBsAg assay	Product number	Aggregate* score (n = 18)	Rank
Murex HBsAg version 3	GE34/36	17	1
Enzygnost HBsAg 5.0	OQPW11/21	16	2
Auszyme monoclonal	1980-24	15	3=
AxSYM HBsAg (V2)	7A40-22	15	3=
ETI-MAK-4	N0019	15	3=
PRISM™ HBsAg	3A4748	15	3=
Vitros ECi HBsAg	843 5307	15	3=
BioELISA HBsAg	3000-1080	14	8=
COBAS Core HBsAg II	07/5350/5	14	8=
ETI-MAK-3	P3142	14	8=
Monolisa Ag HBs 2nd gen	72204	14	8=
Ortho HBsAg Test System 3	931801	14	8=
Bioelisa HBsAg colour plus	3000-1155	13	13
Enzygnost HBsAG monoclonal II	OQC110/11	12	14=
Hepanostika HBsAg Uni-form II	6019/6023	12	14=
Heprofile HBsAg	M450	12	14=
Murex HBsAg	GE14/15/16	12	14=
Monolisa Ag HBs Plus	72313/72314	11	18=
VIDAS HBsAg	30-300	11	18=
Amerlite HBsAg II	LAN.2212	10	20=
BioELISA HBsAg (colour)	3000-1100	10	20=
Labsystems HBsAg EIA Plus	61 10 800/802	10	20=
BioELISA HBsAg (update)	3000-1130	8	23=
DIA.PRO HBsAg One-Step	SAG1.CE	8	23=
Enzymun-Test HBsAg	1288989	8	23=
IMx HBsAG	2228-20	8	23=
Murex HBsAg version 2	GE14/15/16	8	23=
MikroTrak II HBsAg	8HB29	4	28

Notes:
 * A score was assigned to each assay according to the titre at which HBsAg was detected as follows: neat = 0; 1/20 = 1; 1/80 = 2; >= 1/320 = 3. The aggregate score was calculated by summing the scores obtained for each dilution series. The most sensitive assay had the highest total score and was ranked first.
 For the purposes of this comparison the 1/1280 dilutions have not been scored because some of the assays had not been tested with this dilution.
 The OD/CO values obtained for the DIA.PRO HBsAg one-step are shown in *Appendix Table 29*

■ Quality control samples

Replicates of four quality control (QC) sera were tested in the DIA.PRO HBsAg One-Step assay. The QC sera comprised two controls available from the HPA, HBsAg QC1 and HBsAg QC2, and two controls available from the National Institute for Biological Standards and Control (NIBSC), the 2nd British Working Standard and the 2nd Monitor Sample. All replicates were detected except for one of the HPA QC2 samples (Table 14). A QC specimen / statistical assay control should be chosen to have a reactivity within the linear dynamic range of the assay. The NIBSC BWS and the HPA QC1 were considered the most suitable controls for use in the DIA.PRO HBsAg One-Step assay.

The inter-run reproducibility of the DIA.PRO HBsAg One-Step assay was determined using the HPA QC1 sample selected above. The sample was tested on each plate (replicate 1), immediately after the internal kit controls. Fifteen of the 24 results were determined to be within one standard deviation (SD) of the mean and with the exception of one result that fell within 3 SDs the remaining results were within 2 SDs. As part of this determination, two further replicates were included in the first twelve plates, one in the middle (replicate 2), the other at the end of the plate (replicate 3) to ascertain whether the position on the plate has an effect on performance (Figure 6 and Table 15). It can be seen that there is a general decrease in OD/CO between replicate 1 and the following two replicates. This may be explained by the fact that replicate 1 has a longer period of time to incubate, therefore the antigen in the sample has more time in contact with the antibody bound on the plate. This can also be seen by the slightly higher mean OD/CO in replicate 1. Although it can also be seen that replicates 2 and 3 mirror the general trend of replicate 1 (replicate 3 less so, especially as regards plate 12) it is not possible to comment further since replicates 2 and 3 were not included on plates 13 to 24 inclusive.

Table 14: OD/CO ratios and mean values for quality control samples

Quality control sample	DIA.PRO HBsAg One-Step assay replicate results						Mean
	OD/CO 1	OD/CO 2	OD/CO 3	OD/CO 4	OD/CO 5	OD/CO 6	
HPA HBsAg QC1 (00/B214-04)	4.93	4.57	4.63	4.32	4.54	4.72	4.62
HPA HBsAg QC2 (00/B215-03)	0.99	1.28	1.14	1.19	1.08	1.20	1.15
NIBSC 2nd BWS (99/640/005, 0.5 IU/ml)	2.31	2.42	2.30	2.45	2.71	2.42	2.44
NIBSC/UKBTS 2nd MS (99/646/003, 0.125 IU/ml)	1.32	1.17	1.57	1.52	1.43	1.71	1.45

Figure 6: Inter-run reproducibility of the HPA HBsAg QC1 sample

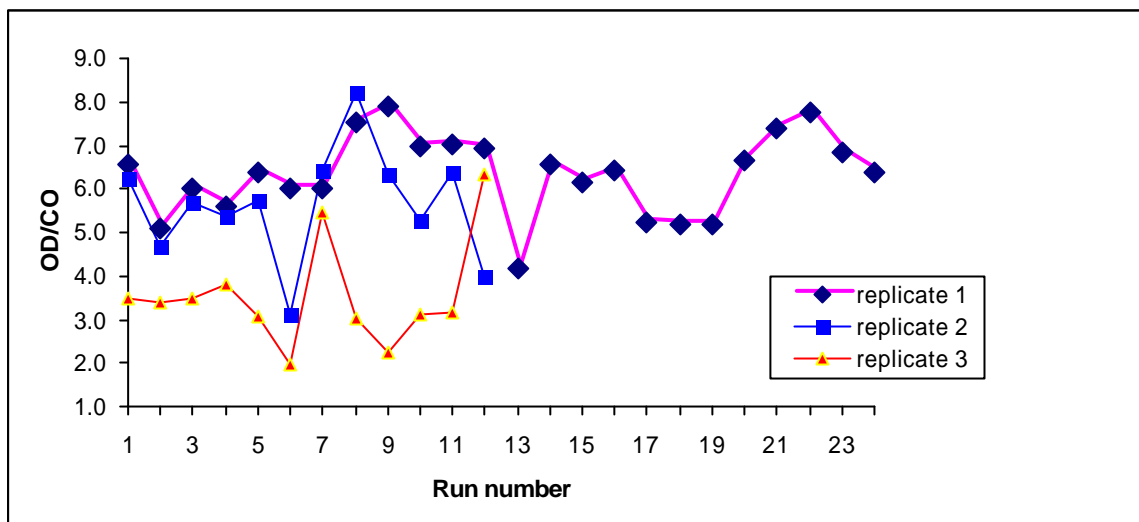


Table 15: Inter-run reproducibility of the HPA HBsAg QC1 sample

	HPA HBsAg QC1 (00/B214-04)			
	Replicate 1 OD/CO n = 12 (n = 24)*	Replicate 2 OD/CO n = 12	Replicate 3 OD/CO n = 12	All replicates OD/CO n = 48
Range	5.08 - 7.91 (4.18 - 7.91)	3.13 - 8.21	1.98 - 6.34	1.98 - 8.21
Mean	6.51 (6.33)	5.62	3.55	5.46
Median	6.47 (6.40)	5.70	3.29	5.87
Standard Deviation	0.82 (0.93)	1.31	1.23	1.58
% coefficient of variation	12.59 (14.67)	23.21	34.58	28.97
Note: *Figures in brackets include the data from all plates.				

■ Manufacturer's kit control results

The results for the two control samples provided by the manufacturer as an integral component of the DIA.PRO HBsAg One-Step assay are shown in [Table 16](#) and [Figures 7](#) and [8](#). The results shown are for batch 0202. The negative control is replicated three times on each plate, while the positive control is tested only once. All results were within the limits specified in the kit insert.

The negative control showed poor inter-run reproducibility with a large range of OD values. As a consequence the percentage co-efficient of variation in respect of the three replicates was high, 25.86, 32.34 and 29.59 respectively.

In contrast, the positive control showed good inter-run reproducibility with a small range of OD values and a good percentage co-efficient of variation (1.31).

Table 16: Inter-run reproducibility of the manufacturer's control samples

	Negative			Positive
	Replicate 1	Replicate 2	Replicate 3	
	OD	OD	OD	
Range	0.005 to 0.025	0.005 to 0.028	0.005 to 0.026	3.504 to 3.676
Mean	0.015	0.015	0.014	3.622
Median	0.015	0.014	0.014	3.634
Standard deviation	0.0040	0.0048	0.0042	0.047
% Coefficient of variation	25.86	32.34	29.59	1.31
Total number of test runs	27	27	27	27
Cut-off calculation: Mean of negative control values + 0.050				

Figure 7: Manufacturer's negative kit control

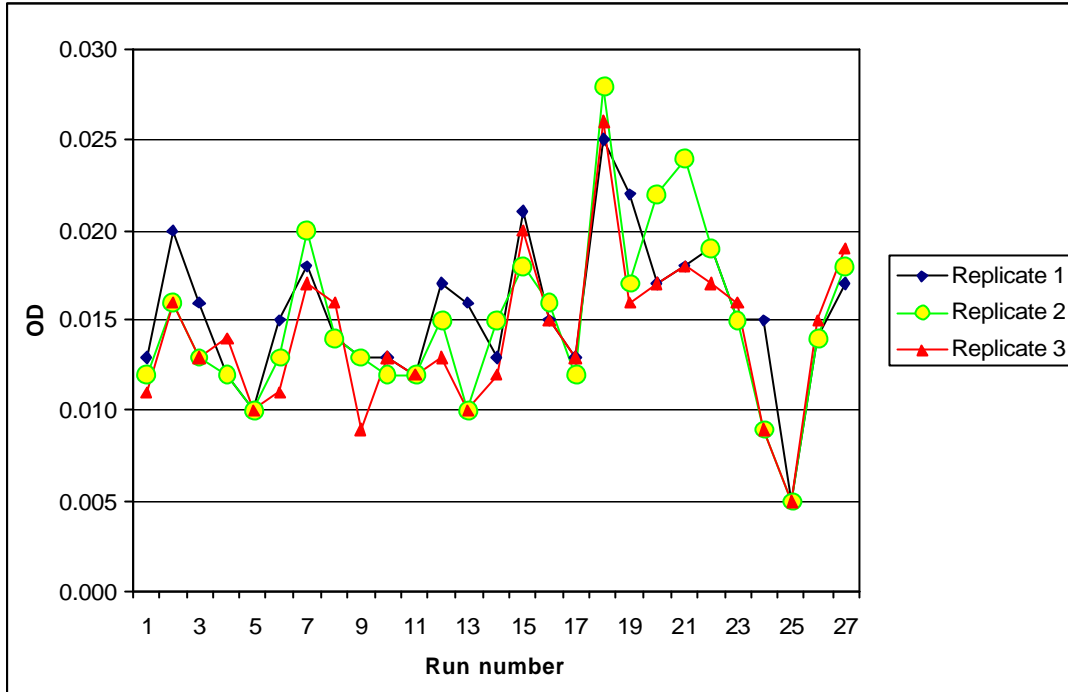
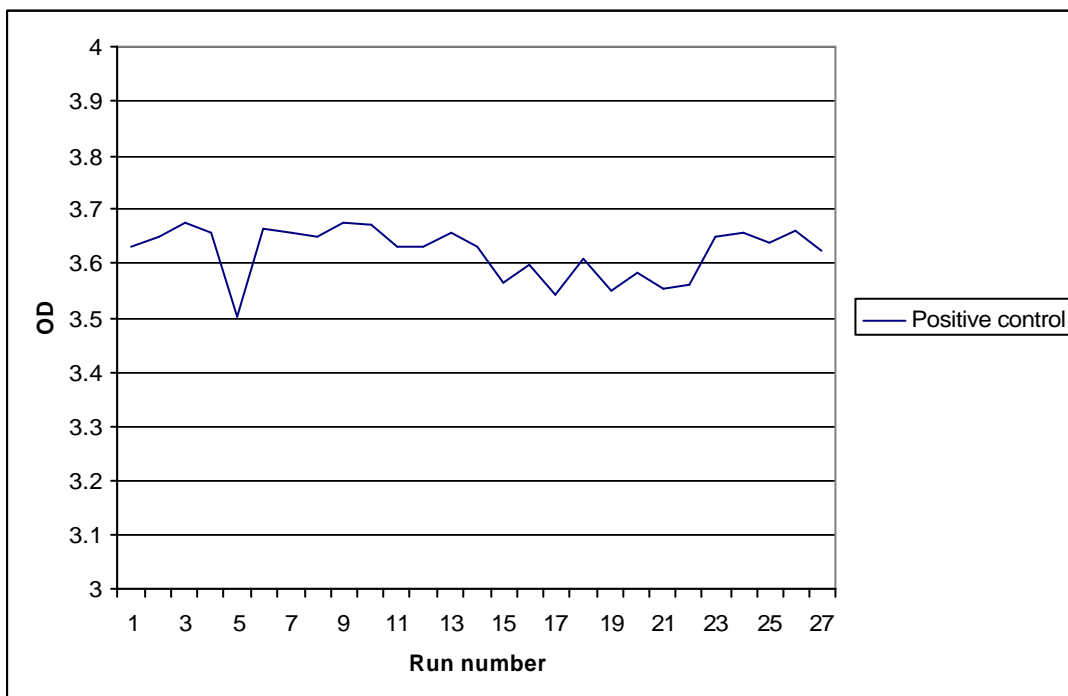


Figure 8: Manufacturer's positive kit control



■ Comparison of reactivities observed with two batches of the DIA.PRO HBsAg One-Step assay

The full specimen panel (Table 2a) was used to evaluate production batch 0202, expiry date March 2003. A subset of the specimen panel (Table 2 b) was used to evaluate a second batch, 0302, expiry date June 2003.

For the comparison of HBsAg negative specimens, all of the forty samples were negative when tested by both batches (Table 17 and Appendix Table 24).

When compared overall for detection of HBsAg, batch 0202 was found to be more sensitive than batch 0302, with scores of 65 and 60 respectively (Table 17). The DIA.PRO HBsAg One-Step detected HBsAg in all 39 positive specimens in both batches of the assay (Table 17 and Appendix Table 24). Although batch 0302 gave slightly higher OD/CO values overall, the difference is not regarded as significant. When six seroconversion panels were compared, both batches detected 15 (out of 45) positive specimens. (It should be noted batch 0302 detected HBsAg in members 1, 2, 3, 4 and 6 of panel PHM908, however, these results are probably erroneous and have been disregarded for the additions in Table 17). There was a significant difference between the two batches in respect of their ability to detect HBsAg in the low titre performance panel (PHA105), where lot 0202 detected HBsAg in seven out of 14 positive members, while lot 0302 detected HBsAg in only three (Appendix Table 26). The full OD/CO ratios for both batches can be found in the Appendix Table 29.

The comparative OD/CO results for the HBsAg positive specimens are presented in Figure 9. This figure illustrates the variation in the OD/CO values for each specimen in each assay batch. It is apparent that there is quite close agreement for specimens with an OD/CO <10. The differences in respect of each sample tested were compared further by plotting the difference in the OD/CO ratios between the two batches against the mean OD/CO ratio for each sample (Figure 10)¹⁴. This analysis shows that there were two samples with a difference greater than three standard deviations from the mean.

Four quality control samples were also included in the comparison (Appendix Table 27). In addition to the one HPA HBsAg QC2 sample that was not detected by batch 0202, batch 0302 failed to detect HBsAg in two of the NIBSC Monitor samples.

Any differences in OD/CO ratios between the two batches are likely to be significant only at lower values. There appears to be good correlation between the batches at both the lower and higher values, with pronounced inter-batch variation only appearing in the middle range of values. This indicates that the observed variation probably has no diagnostic significance if the assay is used in the clinical setting as part of an overall testing strategy.

Table 17 Comparison of two batches of DIA.PRO HBsAg One-Step

Category	Number of specimens	Number of reactive specimens	
		0202	0302
Negative human plasma	2	0	0
HBsAg negative	40	0	0
PHA105 member 11 (negative)	1	0	0
Total HBsAg negative samples reactive:		0	0
HBsAg weak positive	6	4	3
HBsAg positive	39	39	39
PHM903	6	2	2
PHM908	8	2	2
PHM914	6	2	2
PHM919	9	4	3
PHM923	4	1	2
6281	12	4	4
PHA105 (positive)	14	7	3
Total HBsAg positive samples detected:		65	60
Note: Differences in the number of reactive samples detected by the two production lots are highlighted and bold.			

Figure 9: Comparison of results of 66 HBsAg positive specimens tested by two batches of DIA.PRO HBsAg One-Step

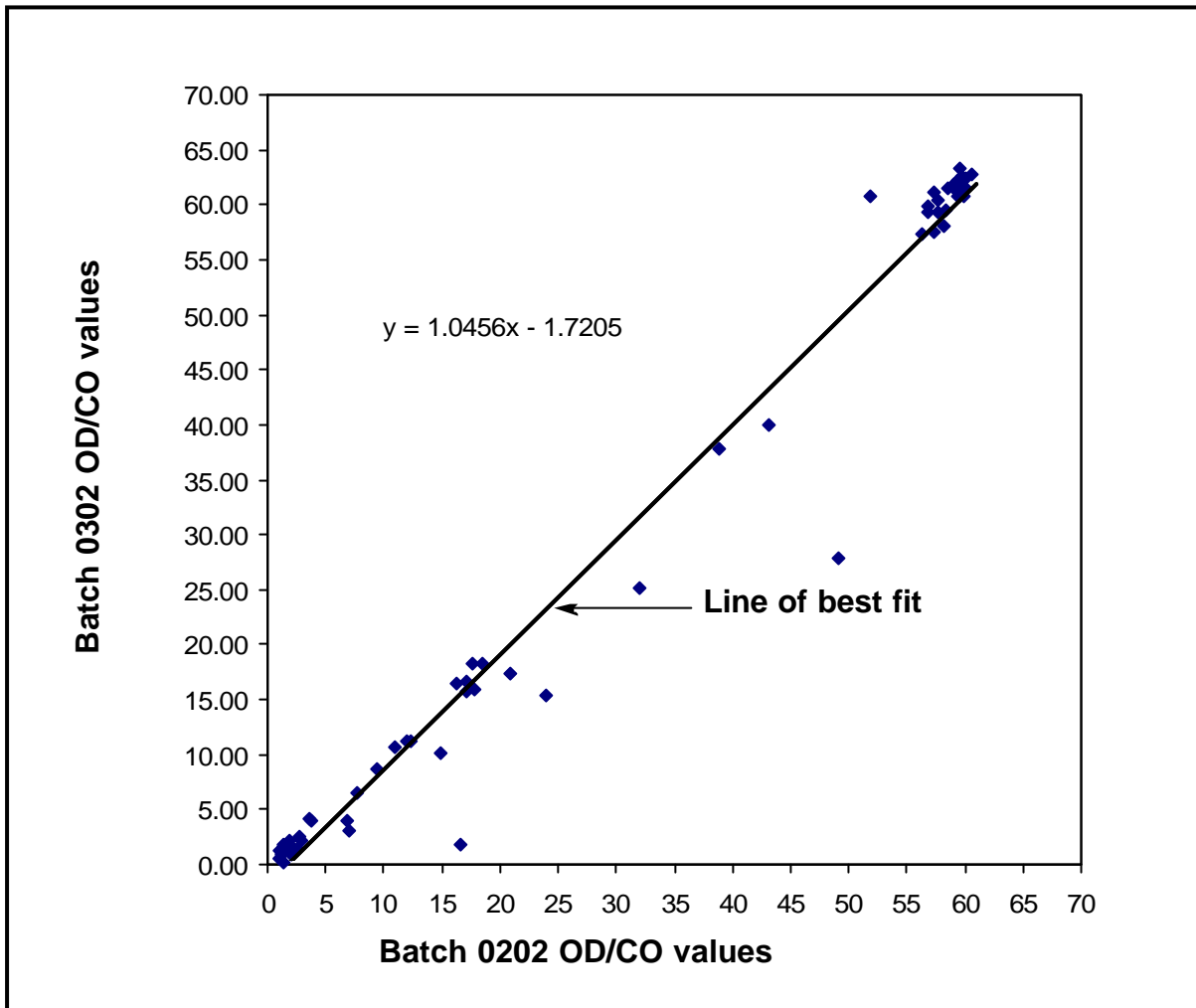
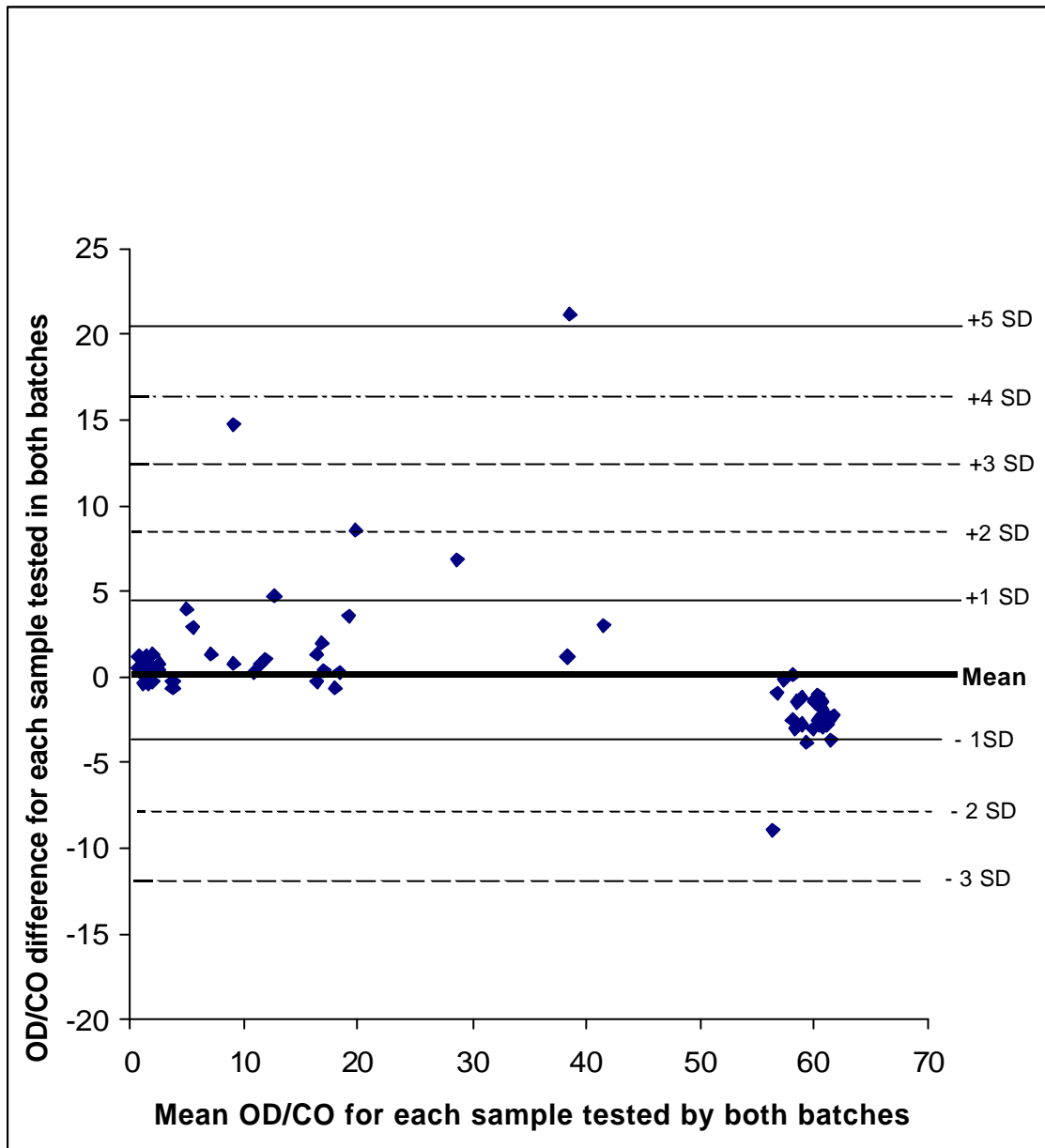


Figure 10: Comparison of reactivity of 66 HBsAg positive specimens tested by both batches of DIA.PRO HBsAg One-Step



Technical appraisal

The DIA.PRO HBsAg One-Step assay kit included 2 microtitre plates, each formatted as 12 x 8 well strips. Full details of the reagents supplied can be found in [Table 18](#).

The manufacturer's recommendations were rigorously applied to avoid cross-contamination of reagents and other potential procedural errors.

All components were brought to room temperature before use. The assay required 50µL of conjugate and 150µL of sample per well. No sample diluent was required.

Adequate volumes of preserved HBsAg negative control serum and HBsAg positive control serum were each provided in ready to use (liquid) form, one bottle of each per kit. Three negative control wells and one positive control well are required for each run. In addition, cell A1 is left empty for blanking purposes.

The conjugate and chromogen/substrate buffer diluent were both provided in ready to use (liquid) form. The washing solution was provided in 20 x concentrated form and had to be diluted with EIA grade water up to 1200 mL distilled water before use. The diluted solution is stable for one month at +2° to +8°C or one week at room temperature.

Once opened, any unused HBsAg specific purified antibody coated microstrips have to be placed back into the aluminium pouch, in the presence of the desiccant supplied, firmly zipped and stored between +2° and +8°C. The residual strips remain stable until the indicator of humidity inside the desiccant bag turns from yellow to green.

Tests were processed manually for the evaluation and each 96 well assay run took approximately three hours to complete, including loading time.

Table 18: Reagents supplied in the DIA.PRO HBsAg One-Step assay kit

Reagent	Number/volume
Coated microwells	2 x (8 x 12 well strips) (192 tests)
Negative control	4mL
HBsAg positive control	4mL
Concentrated wash solution (x20)	2 x 60mL
Enzyme conjugate	1 x 20mL
Chromogen/Substrate	2 x 20mL
Blocking reagent	1 x 32mL
Plate sealing foils	sufficient
Aluminium storage pouch	one

Conclusions

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

Specificity

- The DIA.PRO HBsAg One-Step assay was found, in testing 408 freshly collected HBsAg negative blood donor specimens, to have a specificity of 97.5%. This equates to a repeat reactive rate of 2.5% (95% confidence interval, 1.2 - 4.5%).

Sensitivity

- All 422 HBsAg positive specimens tested were reactive, giving an observed sensitivity of 100%.
- The total seroconversion score and calculation of timing of detection of primary infection, indicate that although the DIA.PRO HBsAg One-Step was within the acceptable range of sensitivity it performed less well than the majority of HBsAg assays previously evaluated.

Quality control and kit batch variation

- Two independently produced HBsAg quality control materials were identified as suitable for statistical assay control. Statistical assay controls need to be chosen so that they give reactivities in the linear part of the dynamic range of each assay (neither too weak nor too strong), and thereby permit sensitive monitoring of changes in the performance of each run.
- A comparison of two kit production batches was undertaken for randomly selected HBsAg negative and positive specimens, seroconversion panels and a performance panel. The more critical anti-HBsAg positive samples that had low OD/CO ratios showed good agreement. Strongly positive samples had a substantial difference in OD/CO values, although this difference may not be consequential in the clinical setting.

Presentation

- Including specimen addition, 90 specimens and controls could be tested manually within 3 hours.

Applications

- The low specificity and the low seroconversion sensitivity of the DIA.PRO HBsAg One-Step assay precludes its use in blood centres as there are many alternatives with better specificity and sensitivity. However, it may have a rôle in clinical settings when it is used alongside other HBsAg kits as part of a testing algorithm.

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- Colleagues in the Sexually Transmitted and Blood Borne Virus Reference Laboratory.

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■ Evaluation protocol

Evaluation protocol for Diapro HBsAg

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

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

It is anticipated that, assuming satisfactory performance, the laboratory work for the evaluation of Diapro HBsAg will be completed within 4-6 weeks of date of commencement and a further 12-16 weeks are needed to produce an MHRA evaluation report. Before evaluation starts, the manufacturer will be invited to train the evaluator in the use of the kits and equipment and to satisfy themselves that the evaluator is properly trained.

Conduct of the evaluation

The product will be used in exactly the manner laid down in the manufacturer's instructions. Any modifications to the instructions provided with the kit described during the training period or any subsequent changes must be confirmed in writing. All microtitre plates will be read on a plate reader eg Bio-Tek EL808 linked to a computer with KC4 software. All data will be stored on the HBsAg kit database for the duration of the evaluation. The data may subsequently be down-loaded to tape or other long-term back-up system. In addition the evaluators will keep clear records of the practical work. All original printouts from the plate reader will be retained, together with any reader printouts from confirmatory assays.

Content of the evaluation

The object of this evaluation is to assess the ability of the Diapro HBsAg assay to detect, with a high degree of sensitivity and specificity, HBsAg in human serum and plasma. To do this, the kits will be tested against a panel of sera/plasma comprising material found to be either reactive or unreactive by HBsAg screening assays currently in use in the UK. In addition, this evaluation will incorporate several sequential blood collections from individuals undergoing seroconversion for HBsAg and as a further measure of sensitivity, dilution series prepared from several HBsAg positive samples. Both the seroconversion panels and dilution series will include representatives of both major HBsAg subtypes (ad and ay).

Sera sent to PHLS Virus Reference Division because of false positive screening test reactions will not be included in this study because of the potential bias that could be introduced against particular assays.

Storage of samples

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

Other aspects of the evaluation

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

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

Discordant results

A discrepancy will arise when a result by the kit under evaluation disagrees with the result obtained by investigation for the presence of hepatitis markers at the Virus Reference Division. If this occurs tests will be repeated in duplicate on the same aliquot of the serum. Any specimen giving a result, which differs from the observed consensus, will be tested by an HBsAg kit from a different manufacturer and also tested for the presence of anti-HBc.

Where these tests are unreactive the specimen will be regarded as a true HBsAg negative. Should these tests be reactive, further investigations will be undertaken to establish the HBV status of the specimen. If justified HBV DNA assays will be undertaken.

Analysis of results and evaluation report

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

Diapro Diagnostic Bioprobe and Quest Biomedical will be given the opportunity to comment on the results of the evaluation of their product before the MHRA evaluation report is published. Results obtained in other manufacturers' tests undergoing evaluation will not be disclosed at this time. Manufacturers' written comments, where relevant, will be appended to the report.

■ Confirmatory tests and other hepatitis B markers for HBsAg weakly positive sera

The HBsAg weakly positive specimens from long term carriers clearing their antigenaemia comprise six specimens from four blood donors. Each donor has been monitored by the North London Blood Centre for a minimum of five years and may be in the process of losing detectable HBsAg. The strong anti-HBc and anti-HBe reactions imply that the positive HBsAg results are specific.

Table 19: HBsAg weakly positive sera; confirmatory tests and other hepatitis B markers

Specimen number	Neutralisation results*			Anti-HBs (IU/mL)	Total anti-HBc % inhibition	Anti-HBe	HBeAg
	1	2	3				
93-07060	NT	—	+	<10	98.0 / 97.8	Positive	Negative
93-07063 ^a	NT	+	+	<10	94.1 / 95.5	Positive	Negative
93-07064 ^b	+	+	+	<10	97.0 / 98.2	Positive	Negative
93-07065 ^b	+	+	+	<10	96.7 / 97.2	Positive	Negative
00-08381	NT	+	NT	<10	95.7	Positive	Negative
00-08382 ^a	NT	+	NT	<10	98.3	Positive	Negative

Notes:

*Sites at which neutralisation tests were carried out: 1 = University College and Middlesex School of Medicine; 2 = VRD (Heapanostika HBsAg Uniform II Confirmatory EIA); 3 = VRD (Monolisa HBs Ag Confirmation).
NT = not tested.

^aSpecimens 93-07063 and 00-08382 are separate bleeds from the same patient taken on 22.2.93 and 27.1.00, respectively

^bSpecimens 93-07064 and 93-07065 are separate bleeds from the same patient taken on 2.12.91 and 29.1.93, respectively

■ Assessment of sensitivity and OD/CO ratios for commercial HBsAg panels

Forty-one seroconversion panels and one low titre panel were included in the evaluation to assess the sensitivity of the DIA.PRO HBsAg One-Step assay at the time of seroconversion (Tables 20a – f).

Details of OD/CO ratios for DIA.PRO HBsAg One-Step and further information on the panels can be found in Tables 21a – e. Comparative data on S/CO ratios for all other assays previously evaluated and used for comparisons in this evaluation can be found in a separate report entitled *HBsAg commercial panel data (update 1)*²⁷. Supplementary information relating to the panels, supplied by Boston Biomedica Inc, USA and by BioClinical Partners Inc, USA, is also included.

PHA105 is a low titre panel comprised of fifteen specimens that are undiluted aliquots from serum or plasma units collected from asymptomatic blood donors. BBI selected fourteen HBsAg positive specimens that had reactivities near the sensitivity limits of five HBsAg screening tests licensed by the FDA. The samples contain HBsAg concentrations of 0.3 – 0.8ng/mL. One further specimen, that was negative by all five screening methods, was included as an internal control.

Results for this low titre performance panel are given in Table 22. The main batch of the DIA.PRO HBsAg One-Step assay detected only seven of the fourteen HBsAg positive samples (the second batch only detected three). Only three other assays have been tested against this low titre panel and they detected all fourteen HBsAg positive samples (Table 23) .

Table 20a: Detection of HBsAg in BBI seroconversion panels PHM903 - 912

Assay	Product number	No. of positive samples and the number of days from initial bleed to first positive sample (shown in parentheses)							Score* n = 64
		PHM903 (n=6)	PHM904 (n=3)	PHM908 (n=8)	PHM909 (n=7)	PHM910 (n=6)	PHM911 (n=25)	PHM912 (n=9)	
Abbott PRISM™ HBsAg	3A4748	4 (6)	3 (0)	4 (15)	6 (4)	6 (0)	6 (77)	4 (20)	33
AxSYM® HBsAg (V2)	7A40-22	4 (6)	2 (7)	5 (13)	4 (9)	4 (35)	9 (65)	4 (20)#	32
Enzygnost® HBsAg 5.0	OQPW11/21	4 (6)	2 (7)	3 (20)	4 (9)	4 (35)	6 (77)	3 (24)	26
Murex HBsAg (version 3)	GE34/36	4 (6)	2 (7)	3 (20)	5 (7)	4 (35)	6 (77)	3 (24)	27
Vitros ECi HBsAg	843 5307	3 (10)	2 (7)	3 (20)	3 (14)	4 (35)	6 (77)	2 (42)	23
ETI-MAK-4	N0019	3 (10)	1 (18)	3 (20)	4 (9)	4 (35)	5 (79)	2 (42)	22
Bioelisa HBsAg colour plus	3000-1155	2 (14)	1 (18)	2 (33)	3 (14)	4 (35)	5 (79)	2 (42)	19
Monolisa Ag HBs 2nd gen	72204	3 (10)	2 (7)	3 (20)	3 (14)	4 (35)	5 (79)	2 (42)	22
Ortho HBsAg Test System 3	931801	2 (14)	1 (18)	2 (33)	3 (14)	4 (35)	5 (79)	2 (42)	19
Bioelisa HBsAg (colour)	3000-1100	2 (14)	1 (18)	2 (33)	3 (14)	4 (35)	4 (84)	2 (42)	18
COBAS Core HBsAg II	07/5350/5	2 (14)	1 (18)	2 (33)	3 (14)	4 (35)	5 (79)	2 (42)	19
Auszyme monoclonal	1980-24	2 (14)	1 (18)	2 (33)	3 (14)	4 (35)	4 (84)	2 (42)	18
ETI-MAK-3	P3142	3 (10)	1 (18)	2 (33)	3 (14)	4 (35)	4 (84)	2 (42)	19
Enzygnost HBsAg monoclonal II	OQC110/11	2 (14)	1 (18)	2 (33)	3 (14)	4 (35)	4 (84)	2 (42)	18
Monolisa Ag HBs Plus	72314	2 (14)	1 (18)	2 (33)	3 (14)	4 (35)	3 (86)	2 (42)	17
Murex HBsAg	GE14/15/16	2 (14)	1 (18)	2 (33)	3 (14)	4 (35)	4 (84)	2 (42)	18
Bioelisa HBsAg	3000-1080	3 (10)	1 (18)	2 (33)	3 (14)	4 (35)	4 (84)	2 (42)	19
Hepanostika HBsAg Uni-form II	6019/6023	2 (14)	1 (18)	2 (33)	3 (14)	3 (42)	4 (84)	2 (42)	17
VIDAS HBsAg	30 300	2 (14)	1 (18)	2 (33)	3 (14)	4 (35)	3 (86)	2 (42)	17
Heprofile HBsAg	M450	2 (14)	1 (18)	2 (33)	3 (14)	3 (42)	3 (86)	2 (42)	16
Labsystems HBsAg EIA Plus	61 10 800/802	2 (14)	1 (18)	2(33)	3 (14)	3 (42)	4 (84)	2 (42)	17
DIA.PRO HBsAg One-Step	SAG1.CE	2 (14)	1 (18)	2 (33)	3 (14)	3 (42)	3 (86)	2 (42)	16
Amerlite HBsAg II	LAN.2212	2 (14)	1 (18)	2 (33)	3 (14)	3 (42)	3 (86)	2 (42)	16
Murex HBsAg (version 2)[F107710]	GE14/15/16	0 (>17)	1 (18)	2 (33)	3 (14)	4 (35)	3 (86)	2 (42)	15
Access® HBs Ag	34220	2 (14)	1 (18)	2 (33)	3 (14)	3 (42)	3 (86)	2 (42)	16
Enzymun-Test HBsAg	1288989	1 (17)	1 (18)	2 (33)	3 (14)	3 (42)	2 (91)	2 (42)	16
IMx HBsAg	2228-20	2 (14)	1 (18)	2 (33)	3 (14)	3 (42)	3 (86)	2 (42)	14
MicroTrak II HBsAg	8HB29	0 (>17)	0 (>18)	1 (36)	1 (21)	2 (46)	1 (93)	2 (42)	7

Notes:
* The score was calculated by summing the number of positive specimens detected for each panel. A higher score suggests higher sensitivity.
Panel PHM912 was persistently reactive from the first bleed in the AxSYM® HBsAg (V2) assay, and it is possible that the positive reactions in early bleeds are non-specific. This assay has therefore been assigned the same score as the highest scoring assay, PRISM™ HBsAg.

Table 20b: Detection of HBsAg in BBI seroconversion panels PHM914 - 920

Assay	Product number	No. of positive samples and the number of days from initial bleed to first positive sample (shown in parentheses)							Score* n = 51	Aggregated score n = 115
		PHM914 (n=6)	PHM915 (n=13)	PHM916 (n=11)	PHM917 (n=3)	PHM918 (n=3)	PHM919 (n=9)	PHM920 (n=6)		
Abbott PRISM™ HBsAg	3A4748	5 (146)	10 (14)	3 (31)	2 (36)	2 (7)	8 (5)	4 (26)	34	67
AxSYM® HBsAg (V2)	7A40-22	4 (151)	7 (26)	3 (31)	2 (36)	3 (0)	6 (12)	4 (26)	29	61
Enzygnost® HBsAg 5.0	OQPW11/21	5 (146)	10 (14)	3 (31)	3 (36)	2 (7)	6 (12)	4 (26)	32	58
Murex HBsAg (version 3)	GE34/36	4 (151)	10 (14)	3 (31)	1 (43)	2 (7)	5 (14)	4 (26)	29	56
Vitros ECi HBsAg	843 5307	3 (153)	7 (26)	3 (31)	1 (43)	2 (7)	4 (19)	4 (26)	24	47
ETI-MAK-4	N0019	4 (151)	7 (26)	2 (34)	1 (43)	2 (7)	4 (19)	4 (26)	24	46
Bioelisa HBsAg colour plus	3000-1155	3 (153)	9 (19)**	2 (34)	1 (43)	2 (7)	4 (19)	4 (26)	25	44
Monolisa Ag HBs 2nd gen	72204	3 (153)	4 (40)	2 (34)	1 (43)	1 (12)	4 (19)	4 (26)	19	41
Ortho HBsAg Test System 3	931801	2 (158)	4 (40)	2 (34)	1 (43)	0 (>12)	4 (19)	4 (26)	17	36
Bioelisa HBsAg (colour)	3000-1100	2 (158)	5 (33)	2 (34)	1 (43)	1 (12)	4 (19)	4 (26)	19	37
COBAS Core HBsAg II	07/5350/5	2 (158)	3 (42)	2 (34)	1 (43)	0 (>12)	4 (19)	4 (26)	16	35
Auszyme monoclonal	1980-24	2 (158)	4 (40)	2 (34)	1 (43)	0 (>12)	4 (19)	4 (26)	17	35
ETI-MAK-3	P3142	3 (153)	2 (54)	2 (34)	1 (43)	0 (>12)	4 (19)	4 (26)	16	35
Enzygnost HBsAg monoclonal II	OQC110/11	2 (158)	2 (54)	2 (34)	1 (43)	1 (12)	4 (19)	4 (26)	16	34
Monolisa Ag HBs Plus	72314	2 (158)	2 (54)	2 (34)	1 (43)	1 (12)	4 (19)	4 (26)	16	33
Murex HBsAg	GE14/15/16	2 (158)	2 (54)	2 (34)	1 (43)	0 (>12)	4 (19)	4 (26)	15	33
Bioelisa HBsAg	3000-1080	2 (158)	2 (54)	1 (38)	1 (43)	1 (12)	4 (19)	3 (35)	14	33
Hepanostika HBsAg Uni-form II	6019/6023	2 (158)	4 (40)	2 (34)	1 (43)	0 (>12)	4 (19)	3 (35)	16	33
VIDAS HBsAg	30 300	2 (158)	2 (54)	2 (34)	1 (43)	0 (>12)	4 (19)	3 (35)	14	31
Heprofile HBsAg	M450	2 (158)	2 (54)	2 (34)	1 (43)	0 (>12)	4 (19)	4 (26)	15	31
Labsystems HBsAg EIA Plus	61 10 800/802	2 (158)	2 (54)	1 (38)	1 (43)	1 (12)	3 (21)	4 (26)	14	30
DIA.PRO HBsAg One-Step	SAG1.CE	2 (158)	0 (>56)	1 (69)	1 (43)	1 (12)	4 (19)	3 (35)	12	28
Amerlite HBsAg II	LAN.2212	1 (160)	2 (54)	2 (34)	1 (43)	0 (>12)	3 (21)	3 (35)	12	28
Murex HBsAg (version 2)[F107710]	GE14/15/16	0 (>160)	2 (54)	2 (34)	1 (43)	1 (12)	3 (21)	3 (35)	12	27
Access® HBs Ag	34220	1 (160)	0 (>56)	1 (38)	1 (43)	1 (12)	4 (19)	3 (35)	11	27
Enzymun-Test HBsAg	1288989	0 (>160)	2 (54)	1 (38)	1 (43)	1 (12)	3 (21)	3 (35)	11	27
IMx HBsAg	2228-20	2 (158)	2 (54)	1 (38)	0 (>43)	0 (>12)	2 (26)	4 (26)	11	25
MicroTrak II HBsAg	8HB29	0 (>160)	0 (>56)	0 (>38)	0 (>43)	0 (>12)	2 (26)	3 (35)	5	12

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

Table 20c: Detection of HBsAg in BBI seroconversion panels PHM921 - 924

Assay	Product number	Number of positive samples and the number of days from initial bleed to first positive sample (shown in parentheses)				Score* n = 27	Aggregated score** n = 142	Rank
		PHM921 (n=6)	PHM922 (n=12)	PHM923 (n=4)	PHM924 (n=5)			
Abbott PRISM™ HBsAg	3A4748	6 (0)	9 (9)	4 (0)	4 (23)	23	90	1
AxSYM® HBsAg (V2)	7A40-22	6 (0)	7 (16)	3 (7)	4 (23)	20	81	2
Enzygnost® HBsAg 5.0	OQPW11/21	6 (0)	8 (14)	2 (15)	4 (23)	20	78	3
Murex HBsAg (version 3)	GE34/36	6 (0)	9 (9)†	2 (15)	3 (29)	20	76	4
Vitros ECI HBsAg	843 5307	6 (0)	7 (16)	2 (15)	3 (29)	18	65	5
ETI-MAK-4	N0019	6 (0)	7 (16)	2 (15)	3 (29)	18	64	6
Bioelisa HBsAg colour plus	3000-1155	6 (0)	7 (16)	1 (22)	3 (29)	17	61	7
Monolisa Ag HBs 2nd gen	72204	6 (0)	7 (16)	2 (15)	3 (29)	18	59	8
Ortho HBsAg Test System 3	931801	6 (0)	7 (16)	2 (15)	2 (35)	17	53	9=
Bioelisa HBsAg (colour)	3000-1100	6 (0)	6 (21)	2 (15)	2 (35)	16	53	9=
COBAS Core HBsAg II	07/5350/5	6 (0)	6 (21)	2 (15)	3 (29)	17	52	11
Auszyme monoclonal	1980-24	6 (0)	6 (21)	2 (15)	2 (35)	16	51	12=
ETI-MAK-3	P3142	6 (0)	7 (16)	1 (22)	2 (35)	16	51	12=
Enzygnost HBsAg monoclonal II	OQC110/11	6 (0)	6 (21)	2 (15)	2 (35)	16	50	14=
Monolisa Ag HBs Plus	72314	6 (0)	6 (21)	2 (15)	3 (29)	17	50	14=
Murex HBsAg	GE14/15/16	6 (0)	6 (21)	2 (15)	2 (35)	16	49	16
Bioelisa HBsAg	3000-1080	6 (0)	6 (21)	0 (>22)	3 (29)	15	48	17=
Hepanostika HBsAg Uni-form II	6019/6023	6 (0)	6 (21)	1 (22)	2 (35)	15	48	17=
VIDAS HBsAg	30 300	6 (0)	6 (21)	1 (22)	2 (35)	15	46	19=
Heprofile HBsAg	M450	6 (0)	6 (21)	1 (22)	2 (35)	15	46	19=
Labsystems HBsAg EIA Plus	61 10 800/802	6 (0)	6 (21)	0 (>22)	2 (35)	14	45	21
DIA.PRO HBsAg One-Step	SAG1.CE	6 (0)	6 (21)	1 (22)	2 (35)	15	43	22=
Amerlite HBsAg II	LAN.2212	6 (0)	6 (21)	0 (>22)	3 (29)	15	43	22 =
Murex HBsAg (version 2)[F107710]	GE14/15/16	6 (0)	6 (21)	1 (22)	2 (35)	15	42	24
Access® HBs Ag	34220	6 (0)	6 (21)	0 (>22)	1 (43)	13	40	25
Enzymun-Test HBsAg	1288989	6 (0)	6 (21)	0 (>22)	1 (43)	13	38	26
IMx HBsAg	2228-20	6 (0)	6 (21)	0 (>22)	2 (35)	14	31	27
MicroTrak II HBsAg	8HB29	4 (7)	5 (24)	0 (>22)	0 (>43)	9	21	28

Notes:

* The score was calculated by summing the number of positive specimens detected for each panel.

** The aggregated score is the sum of scores for panels PHM903 and PHM904 and PHM908 to PHM924 except for panel PHM913. A higher aggregate suggests higher sensitivity.

† Panel PHM922 members 4, 6-12 were positive; panel members 04, 05 and 06 had OD/CO ratios of 1.04, 0.93 and 2.54, respectively.

Table 20d: Detection of HBsAg in BBI seroconversion panels PHM925 - 931

Assay	Product number	Number of positive samples and the number of days from initial bleed to first reactive sample (shown in parentheses)							Score* n = 48	Aggregated score** n = 190
		PHM925 (n=5)	PHM926 (n=8)	PHM927 (n=6)	PHM928 (n=7)	PHM929 (n=9)	PHM930 (n=5)	PHM931 (n=8)		
AxSYM® HBsAg (V2)	7A40-22	3 (8)	5 (13)	5 (4)	4 (9)	5 (14)	4 (3)	4 (19)	30	111
Murex HBsAg (version 3)	GE34/36	4 (4)	6 (9)	5 (4)	5 (7)	5 (14)	4 (3)	4 (19)	33	109
Enzygnost® HBsAg 5.0	OQPW11/21	3 (8)	6 (9)	5 (4)	3 (14)	5 (14)	4 (3)	4 (19)	30	108
ETI-MAK-4	N0019	3 (8)	5 (13)	5 (4)	4 (9)	5 (14)	4 (3)	4 (19)	30	94
Bioelisa HBsAg colour plus	3000-1155	3 (8)	5 (13)	5 (4)	4 (9)	4 (18)	4 (3)	4 (19)	29	90
Hepanostika HBsAg Uni-form II	6019/6023	2 (14)	4 (15)	4 (7)	3 (14)	4 (18)	4 (3)	3 (21)	24	72
DIA.PRO HBsAg One-Step	SAG1.CE	0 (0)	3 (20)	6 (0)	2 (16)	2 (25)	3 (8)	3 (21)	19	62
Murex HBsAg (version 2)	GE14/15/16	0 (0)	3 (20)	3 (11)	3 (14)	3 (21)	3 (8)	2 (26)	17	38

Notes:
* The score was calculated by summing the number of positive specimens detected for each panel. A higher score suggests higher sensitivity.
** The aggregated score is the sum of scores for the panels shown in this table and also panels PHM903, PHM904, PHM 908 – PHM912 and PHM914 – PHM924 (Tables 20a–c). A higher aggregate suggests higher sensitivity.

Table 20e: Detection of HBsAg in BCP seroconversion panels 6271 - 6276

Assay	Product number	Number of positive samples and the number of days from initial bleed to first reactive sample (shown in parenthesis)						Score* 6271-6276 n = 59	Aggregated score BBI:PHM903-924 BCP:6271-6276 Total = 201
		6271 (n=5)	6272 (n=26)	6273 (n=6)	6274 (n=7)	6275 (n=7)	6276 (n=8)		
AxSYM [®] HBsAg	7A40-22	2 (12)	7 (94)	2 (25)	6 (4)	5 (7)	1 (29)	23	104
Murex HBsAg (version 3)	GE34/36	3 (7)	8 (74)	2 (25)	7 (0)	3 (22)	2 (27)	25	101
Enzygnost [®] HBsAg 5.0	OQPW11/21	3 (7)	4 (104)	2 (25)	7 (0)	3 (22)	2 (27)	21	99
ETI-MAK 4	N0019	3 (7)	5(101)	2 (25)	6 (4)	3 (22)	2 (27)	21	85
Vitros <i>ECi</i> HBsAg	843 5307	2 (12)	2 (111)	2 (25)	6 (4)	3 (22)	2 (27)	17	82
Bioelisa HBsAg colour plus	3000-1155	2 (12)	3 (108)	2 (25)	6 (4)	3 (22)	2 (27)	18	79
Hepanostika HBsAg Uni-form II	6019/6023	2 (12)	3 (108)	2 (25)	5 (8)	3 (22)	0 (>29)	15	63
Monolisa Ag HBs Plus	72314	2 (12)	1 (115)	2 (25)	3 (15)	3 (22)	0 (>29)	11	61
DIA.PRO HBsAg One-Step	SAG1.CE	2 (12)	1 (115)	2 (25)	3 (15)	3 (22)	2 (27)	13	56
Labsystems HBsAg EIA Plus	61 10 800/802	2 (12)	0 (>115)	2 (25)	4 (11)	3 (22)	0 (>29)	11	56
Murex HBsAg (version 2)	GE14/15/16	2 (12)	1 (115)	2 (25)	4 (11)	3 (22)	0 (>29)	12	54
Access [®] HBs Ag	34220	2 (12)	0 (>115)	2 (25)	3 (15)	3 (22)	0 (>29)	10	50

Notes:* The score was calculated by summing the number of positive specimens detected for each panel. A higher score suggests higher sensitivity. Only the twelve kits shown had been tested against the six seroconversion panels included in this table.

Table 20f: Detection of HBsAg in BCP seroconversion panels 6277 – 6282, 6288 and 6291 - 6293

Assay	Product number	Number of positive samples and the number of days from initial bleed to first reactive sample (shown in parenthesis)									
		6277 (n=11)	6278 (n=11)	6279 (n=7)	6280 (n=5)	6281 (n=12)	6282 (n=14)	6288 (n=9)	6291 (n=8)	6292 (n=12)	6293 (n=7)
AxSYM [®] HBsAg (V2)	7A40-22	4 (40)	8 (12)	1 (28)	4 (13)	5 (22)	9 (19)	4 (17)	3 (27)	5 (35)	3 (23)
Bioelisa HBsAg colour plus	3000-1155	6 (33)	8 (12)	1 (28)	4 (13)	5 (22)	8 (21)	5 (14)	3 (27)	6 (29)	3 (23)
DIA.PRO HBsAg One-Step	SAG1.CE	3 (42)	11 (0)	0 (>28)	3 (15)	4 (33)	8 (21)	3 (21)	2 (34)	4 (42)	3 (23)
Enzygnost [®] HBsAg 5.0	OQPW11/21	5 (35)	8 (12)	2 (26)	4 (13)	6 (19)	9 (19)	4 (17)	3 (27)	6 (29)	3 (23)
ETI-MAK 4	N0019	6 (33)	8 (12)	2 (26)	4 (13)	5 (22)	9 (19)	5 (14)	3 (27)	6 (29)	4 (15)
Murex HBsAg version 3	GE/34/36	5 (35)	8 (12)	2 (26)	5 (0)	NT	9 (19)	6 (10)	3 (27)	6 (29)	4 (15)
<p>Notes:* The score was calculated by summing the number of positive specimens detected for each panel. A higher score suggests higher sensitivity. Only the six kits kits had been tested against the ten seroconversion panels included in this table</p>											

Table 21a: DIA.PRO HBsAg One-Step OD/CO ratios for BBI seroconversion panels PHM903 – PHM914

Panel	Days since first bleed	ng/mL*	OD/CO
PHM903-01	0	< 0.1	0.11
PHM903-02	3	< 0.1	0.32
PHM903-03	6	< 0.1	0.24
PHM903-04	10	0.1	0.49
PHM903-05	14	1.0	1.59
PHM903-06	17	1.4	3.72

PHM904-01	0	< 0.1	0.15
PHM904-02	7	0.1	0.34
PHM904-03	18	1.5	1.24

PHM908-01	0	< 0.1	0.35
PHM908-02	5	0.1	0.81
PHM908-03	7	< 0.1	0.38
PHM908-04	13	0.1	0.93
PHM908-05	15	< 0.1	0.83
PHM908-06	20	0.2	0.89
PHM908-07	33	1.7	3.52
PHM908-08	36	> 2.7	14.95

PHM909-01	0	< 0.1	0.15
PHM909-02	4	< 0.1	0.11
PHM909-03	7	0.1	0.12
PHM909-04	9	0.3	0.24
PHM909-05	14	1.1	1.24
PHM909-06	18	> 2.7	5.05
PHM909-07	21	> 2.7	12.47

PHM910-01	0	0.2	0.15
PHM910-02	18	0.2	0.20
PHM910-03	35	0.9	0.89
PHM910-04	42	> 2.7	4.24
PHM910-05	46	> 2.7	8.20
PHM910-06	49	> 2.7	12.51

PHM911-01	0	< 0.1	0.15
PHM911-02	7	< 0.1	0.12
PHM911-03	14	< 0.1	0.12
PHM911-04	16	< 0.1	0.15
PHM911-05	21	< 0.1	0.09
PHM911-06	23	< 0.1	0.09
PHM911-07	30	< 0.1	0.11
PHM911-08	35	< 0.1	0.12
PHM911-09	37	< 0.1	0.14
PHM911-10	42	< 0.1	0.12
PHM911-11	44	< 0.1	0.15
PHM911-12	49	< 0.1	0.18
PHM911-13	51	< 0.1	0.08
PHM911-14	56	< 0.1	0.11
PHM911-15	58	< 0.1	0.12
PHM911-16	63	< 0.1	0.09
PHM911-17	65	< 0.1	0.14
PHM911-18	70	< 0.1	0.18
PHM911-19	72	< 0.1	0.20
PHM911-20	77	0.2	0.52
PHM911-21	79	0.4	0.57
PHM911-22	84	0.7	0.83
PHM911-23	86	1.2	1.42
PHM911-24	91	> 2.7	1.91
PHM911-25	93	> 2.7	5.19

PHM912-01	0	< 0.1	0.12
PHM912-02	5	< 0.1	0.14
PHM912-03	7	< 0.1	0.12
PHM912-04	12	< 0.1	0.11
PHM912-05	17	< 0.1	0.12
PHM912-06	20	< 0.1	0.17
PHM912-07	24	0.1	0.21
PHM912-08	42	> 2.6	7.59
PHM912-09	47	> 2.7	23.46

PHM914-01	0	< 0.1	0.29
PHM914-02	146	0.2	0.55
PHM914-03	151	0.3	0.52
PHM914-04	153	0.5	0.73
PHM914-05	158	0.9	1.18
PHM914-06	160	1.5	1.44

Notes:			
OD/CO ratios of less than 1.0 are highlighted			
Batch number = 0202			
Cut-off = 0.065			
* = ng/mL estimated by BBI			

Table 21b: DIA.PRO HBsAg One-Step OD/CO ratios for BBI seroconversion panels PHM915 – PHM923

Panel	Days since first bleed	ng/mL*	OD/CO
PHM915-01	0	< 0.1	0.26
PHM915-02	7	< 0.1	0.37
PHM915-03	12	0.2	0.47
PHM915-04	14	< 0.1	0.51
PHM915-05	19	0.1	0.32
PHM915-06	21	0.1	0.31
PHM915-07	26	0.2	0.34
PHM915-08	28	0.2	0.34
PHM915-09	33	0.3	0.41
PHM915-10	40	0.4	0.46
PHM915-11	42	0.6	0.57
PHM915-12	54	1.0	0.84
PHM915-13	56	0.8	0.92

PHM916-01	0	< 0.1	0.30
PHM916-02	7	< 0.1	0.79
PHM916-03	41	< 0.1	0.20
PHM916-04	43	< 0.1	0.05
PHM916-05	48	< 0.1	0.08
PHM916-06	50	< 0.1	0.07
PHM916-07	55	< 0.1	0.05
PHM916-08	57	< 0.1	0.23
PHM916-09	62	0.2	0.41
PHM916-10	65	0.5	0.98
PHM916-11	69	2.3	4.51

PHM917-01	0	< 0.1	0.21
PHM917-02	36	< 0.1	0.35
PHM917-03	43	1.0	1.62

PHM918-01	0	< 0.1	0.29
PHM918-02	7	0.1	0.49
PHM918-03	12	0.9	1.59

Panel	Days since first bleed	ng/mL*	OD/CO
PHM919-01	0	< 0.1	0.07
PHM919-02	5	< 0.1	0.05
PHM919-03	7	< 0.1	0.05
PHM919-04	12	< 0.1	0.20
PHM919-05	14	0.1	0.28
PHM919-06	19	0.5	1.13
PHM919-07	21	0.9	1.84
PHM919-08	26	> 2.7	7.74
PHM919-09	28	> 2.7	9.39

PHM920-01	0	< 0.1	-0.03
PHM920-02	5	< 0.1	0.02
PHM920-03	26	0.5	0.89
PHM920-04	35	> 2.7	18.57
PHM920-05	37	> 2.7	36.77
PHM920-06	42	> 2.7	58.75

PHM921-01	0	0.7	1.64
PHM921-02	5	> 2.7	4.64
PHM921-03	7	> 2.7	7.46
PHM921-04	12	> 2.7	25.75
PHM921-05	14	> 2.7	21.67
PHM921-06	19	> 2.7	59.43

PHM922-01	0	< 0.1	0.00
PHM922-02	2	< 0.1	0.02
PHM922-03	7	< 0.1	0.03
PHM922-04	9	< 0.1	0.08
PHM922-05	14	< 0.1	0.18
PHM922-06	16	0.3	0.41
PHM922-07	21	1.1	4.28
PHM922-08	24	> 2.7	14.71
PHM922-09	28	> 2.7	35.34
PHM922-10	31	> 2.7	56.43
PHM922-11	35	> 2.7	60.16
PHM922-12	38	> 2.7	61.64

PHM923-01	0	< 0.1	0.34
PHM923-02	7	< 0.1	0.20
PHM923-03	15	0.2	0.95
PHM923-04	22	0.5	1.43

Notes:			
OD/CO ratios of less than 1.0 are highlighted			
Batch number = 0202			
Cut-off =0.061(except for PHM915, PHM917 and PHM918 where it is 0.065)			
* = ng/mL estimated by BBI			

Table 21c: DIA.PRO HBsAg One-Step OD/CO ratios for BBI seroconversion panels PHM924 – PHM931

Panel	Days since first bleed	ng/mL*	OD/CO
PHM924-01	0	< 0.1	0.02
PHM924-02	23	< 0.1	0.23
PHM924-03	29	0.2	0.39
PHM924-04	35	0.5	1.57
PHM924-05	43	2.1	4.61
PHM925-01	0	Not available	0.08
PHM925-02	4	Not available	0.18
PHM925-03	8	Not available	0.41
PHM925-04	14	Not available	0.82
PHM925-05	17	Not available	0.61
PHM926-01	0	Not available	0.05
PHM926-02	2	Not available	0.00
PHM926-03	9	Not available	0.15
PHM926-04	13	Not available	0.44
PHM926-05	15	Not available	0.61
PHM926-06	20	Not available	1.33
PHM926-07	23	Not available	1.87
PHM926-08	27	Not available	4.20
PHM927-01	0	Not available	2.30
PHM927-02	4	Not available	2.59
PHM927-03	7	Not available	2.28
PHM927-04	11	Not available	6.34
PHM927-05	14	Not available	7.36
PHM927-06	18	Not available	34.41
PHM928-01	0	Not available	0.03
PHM928-02	2	Not available	0.07
PHM928-03	7	Not available	0.05
PHM928-04	9	Not available	0.23
PHM928-05	14	Not available	0.77
PHM928-06	16	Not available	1.02
PHM928-07	21	Not available	12.21
PHM929-01	0	Not available	0.02
PHM929-02	4	Not available	0.05
PHM929-03	7	Not available	0.08
PHM929-04	11	Not available	0.10
PHM929-05	14	Not available	0.15
PHM929-06	18	Not available	0.46
PHM929-07	21	Not available	0.67
PHM929-08	25	Not available	2.07
PHM929-09	29	Not available	6.07
PHM930-01	0	Not available	0.33
PHM930-02	3	Not available	0.80
PHM930-03	8	Not available	4.87
PHM930-04	12	Not available	12.62
PHM930-05	15	Not available	39.89
PHM931-01	0	Not available	-0.07
PHM931-02	5	Not available	-0.05
PHM931-03	12	Not available	0.13
PHM931-04	14	Not available	0.18
PHM931-05	19	Not available	0.53
PHM931-06	21	Not available	1.27
PHM931-07	26	Not available	5.47
PHM931-08	28	Not available	8.40

Notes:

OD/CO ratios of less than 1.0 are highlighted

Batch number = 0202

Cut-off = 0.061 (except for PHM930 and PHM931 where it is 0.055)

* = ng/mL estimated by BBI

Table 21d: DIA.PRO HBsAg One-Step OD/CO ratios for BCP seroconversion panels 6271 – 6277

Panel	Days since first bleed	OD/CO
6271-01	0	-0.05
6271-02	2	0.00
6271-03	7	0.29
6271-04	12	2.51
6271-05	18	19.58

Panel	Days since first bleed	OD/CO
6274-01	0	0.09
6274-02	4	0.36
6274-03	8	0.80
6274-04	11	0.96
6274-05	15	5.49
6274-06	18	15.60
6274-07	22	48.22

6272-01	0	0.15
6272-02	2	0.22
6272-03	7	0.16
6272-04	23	0.11
6272-05	25	0.11
6272-06	30	0.25
6272-07	32	0.20
6272-08	37	0.22
6272-09	39	0.22
6272-10	44	0.20
6272-11	46	0.07
6272-12	51	0.09
6272-13	53	0.13
6272-14	58	0.22
6272-15	60	0.16
6272-16	65	0.20
6272-17	67	0.13
6272-18	72	0.51
6272-19	74	0.36
6272-20	94	0.11
6272-21	97	0.18
6272-22	101	0.20
6272-23	104	0.35
6272-24	108	0.60
6272-25	111	0.98
6272-26	115	1.98

6275-01	0	0.02
6275-02	2	-0.07
6275-03	7	0.09
6275-04	9	0.11
6275-05	22	1.85
6275-06	27	5.75
6275-07	29	7.45

6276-01	0	0.38
6276-02	3	0.38
6276-03	7	0.29
6276-04	10	0.33
6276-05	14	0.69
6276-06	17	0.44
6276-07	27	1.56
6276-08	29	2.65

6277-01	0	-0.04
6277-02	4	0.00
6277-03	21	-0.05
6277-04	26	0.02
6277-05	28	0.07
6277-06	33	0.16
6277-07	35	0.45
6277-08	40	0.91
6277-09	42	1.33
6277-10	47	5.25
6277-11	49	5.71

6273-01	0	-0.07
6273-02	3	-0.11
6273-03	7	-0.09
6273-04	14	0.07
6273-05	25	3.82
6273-06	30	24.13

Notes:
 OD/CO ratios of less than 1.0 are highlighted
 Batch number = 0202
 Cut-off = 0.055

Table 21e: DIA.PRO HBsAg One-Step OD/CO ratios for BCP seroconversion panels 6278 – 6282, 6288 and 6291 – 6293

Panel	Days since first bleed	OD/CO
6278-01	0	1.83
6278-02	4	2.10
6278-03	8	1.07
6278-04	12	1.49
6278-05	16	7.35
6278-06	19	30.72
6278-07	23	56.10
6278-08	26	57.62
6278-09	33	57.78
6278-10	37	55.77
6278-11	41	53.91

6279-01	0	0.06
6279-02	12	0.06
6279-03	14	0.08
6279-04	19	0.09
6279-05	21	0.23
6279-06	26	0.42
6279-07	28	0.92

6280-01	0	0.08
6280-02	13	0.78
6280-03	15	1.38
6280-04	21	6.70
6280-05	27	25.06

6281-01	0	0.14
6281-02	5	0.11
6281-03	7	0.16
6281-04	13	0.19
6281-05	19	0.26
6281-06	22	0.92
6281-07	33	2.21
6281-08	36	2.91
6281-09	41	6.99
6281-10	43	2.21
6281-11	50	0.16
6281-12	54	0.23

Notes:

OD/CO ratios of less than 1.0 are highlighted
 Batch number = 0202
 Cut-off = 0.064

Panel	Days since first bleed	OD/CO
6282-01	0	0.11
6282-02	2	0.09
6282-03	7	0.14
6282-04	12	0.14
6282-05	14	0.23
6282-06	19	0.72
6282-07	21	1.23
6282-08	26	6.61
6282-09	28	8.41
6282-10	33	21.86
6282-11	35	45.20
6282-12	40	56.61
6282-13	43	57.33
6282-14	47	57.50

6288-01	0	0.11
6288-02	3	0.08
6288-03	7	0.14
6288-04	10	0.25
6288-05	14	0.47
6288-06	17	0.76
6288-07	21	1.26
6288-08	24	1.52
6288-09	28	2.27

6291-01	0	0.09
6291-02	2	0.09
6291-03	8	0.09
6291-04	10	0.12
6291-05	15	0.11
6291-06	27	0.70
6291-07	34	3.20
6291-08	36	4.63

6292-01	0	0.09
6292-02	2	0.08
6292-03	9	0.09
6292-04	11	0.09
6292-05	21	0.14
6292-06	23	0.12
6292-07	29	0.34
6292-08	35	0.64
6292-09	42	6.23
6292-10	44	9.87
6292-11	49	36.64
6292-12	52	48.87

6293-01	0	0.11
6293-02	6	0.16
6293-03	9	0.14
6293-04	15	0.30
6293-05	23	2.13
6293-06	29	11.91
6293-07	35	48.50

Table 22: DIA.PRO HBsAg One-Step OD/CO ratios for BBI low titre performance panel PHA105

Panel	IU/mL	OD/CO
PHA105-01	0.3	1.01
PHA105-02	0.8	1.93
PHA105-03	0.3	0.94
PHA105-04	0.3	0.63
PHA105-05	0.3	0.82
PHA105-06	0.6	2.10
PHA105-07	0.1	0.66
PHA105-08	0.2	0.88
PHA105-09	0.2	1.41
PHA105-10	0.3	16.53
PHA105-11	negative	0.29
PHA105-12	0.3	1.31
PHA105-13	0.6	2.65
PHA105-14	0.2	0.85
PHA105-15	0.2	0.85

Notes: OD/CO ratios of less than 1.0 are highlighted.
Batch number = 0202 Cut off = 0.068

Table 23: Detection of HBsAg in BBI low titre performance panel PHA105

Assay	Product number	Positive (n=14)	Negative (n=1)
AxSYM [®] HBsAg (V2)	7A44-20	14	0
Bioelisa HBsAg colour plus	3000-1155	14	0
Enzygnost HBsAg 5.0	OQPW11/21	14	0
DIA.PRO HBs Ag One-Step	SAG1.CE	7/3*	0

Notes: In each case the number of positive samples is shown
* Results are given for two lot numbers tested.
Only the four kits shown had been tested against this low titre performance panel

■ Comparison of reactions obtained with two batches of DIA.PRO HBsAg One-Step

Table 24: Comparison of reactions (OD/CO) obtained with 40 HBsAg negative and 39 positive HBsAg specimens

Specimen type	Batch number	Range	Mean	Median
HBsAg negative n = 40	0202	-0.06 – 0.70	0.13	0.09
	0302	0.03 – 0.80	0.19	0.10
HBsAg positive n = 39	0202	6.90 – 60.53	44.59	57.34
	0302	3.98 – 63.22	44.67	59.39

Table 25: Comparison of reactions (OD/CO) obtained with six weakly HBsAg positive sera from chronic carriers

Sample number	93-07060	93-07063	93-07064	93-07065	00-08381	00-08382
Batch: 0202	1.02	17.71	0.73	2.82	1.92	0.27
Batch: 0302	0.51 / 0.52	19.05 / 17.60	0.94 / 0.94	2.54 / 2.42	2.09 / 2.14	0.55 / 0.62

Table 26: Comparison of two batches of DIA.PRO HBsAg One-Step to detect primary infection in six seroconversion panels and one performance panel

Batch number	Number of positive samples and the number of days from initial bleed to first reactive sample (shown in parenthesis)						Number of positive samples detected	Score*
	PHM903 n=6	PHM908 n=8	PHM914 n=6	PHM919 n=9	PHM923 n=4	6281 n=12	PHA105 n=14	
0202	2 (14)	2 (33)	2 (158)	4 (19)	1 (22)	4 (33)	7	22
0302	2 (14)	2 (33) ^a	2 (158)	3 (21)	2 (15)	4 (33)	3	18

Note:
 * The score was calculated by summing the number of positive specimens detected for each panel.
^a Lot 0203 additionally found members 1 to 4 of PHM908 positive, however by reference to the performance of other kits, these positive readings were considered to be erroneous and have been disregarded

Table 27: Comparison of reactions obtained with four quality control samples when tested by two batches of DIA.PRO HBsAg One-Step

Quality control sample	Batch 0302				Batch 0202
	OD/CO 1	OD/CO 2	OD/CO 3	Mean	Mean
PHLS HBsAg QC1 (98/B113-04)	7.41	3.18	7.19	5.93	4.62
PHLS HBsAg QC2 (98/B115-05)	1.54	1.38	1.31	1.41	1.15
NIBSC Working Standard (99/640/002)	2.06	2.39	2.27	2.24	2.44
NIBSC/UKBTS Monitor Sample (99/646-001)	0.94	1.01	0.96	0.97	1.45

Table 28: Comparison of reactions obtained by two batches of DIA.PRO HBsAg One-Step from six dilution series

Seroconversion panel member	Dilution	Batch 0202	Batch 0302
		OD/CO	OD/CO
PHM901-05	1:20	6.01	3.77
	1:80	1.66	0.70
	1:320	0.44	0.17
	1:1280	0.22	0.12
PHM902-14	1:20	0.54	0.24
	1:80	0.22	0.09
	1:320	0.13	0.07
	1:1280	0.12	0.09
PHM907-09	1:20	10.97	5.03
	1:80	2.50	1.16
	1:320	0.79	0.29
	1:1280	0.29	0.14
PHM920-06	1:20	9.88	3.95
	1:80	1.81	0.66
	1:320	0.56	0.24
	1:1280	0.22	0.14
PHM921-05	1:20	2.35	0.65
	1:80	0.62	0.31
	1:320	0.22	0.12
	1:1280	0.37	0.14
PHM922-09	1:20	1.97	0.48
	1:80	0.71	0.22
	1:320	0.41	0.12
	1:1280	0.31	0.12
Total number of positive reactions		8	4

Table 29: Comparison of commercial panel OD/CO ratios for two batches of DIA.PRO HBsAg One-Step

Panel	Days since first bleed	ng/mL	OD/CO	
			0202	0302
PHM903-01	0	< 0.1	0.11	0.17
PHM903-02	3	< 0.1	0.32	0.20
PHM903-03	6	< 0.1	0.24	0.03
PHM903-04	10	0.1	0.49	0.56
PHM903-05	14	1.0	1.59	1.55
PHM903-06	17	1.4	3.72	3.95

PHM908-01	0	< 0.1	0.35	3.53 / 1.88
PHM908-02	5	0.1	0.81	1.96 / 2.35
PHM908-03	7	< 0.1	0.38	0.88 / 1.46
PHM908-04	13	0.1	0.93	1.50 / 1.20
PHM908-05	15	< 0.1	0.83	0.94 / 0.78
PHM908-06	20	0.2	0.89	1.36 / 0.84
PHM908-07	33	1.7	3.52	5.37 / 4.12
PHM908-08	36	> 2.7	14.95	12.89 / 10.19

PHM914-01	0	< 0.1	0.29	0.17
PHM914-02	146	0.2	0.55	0.48
PHM914-03	151	0.3	0.52	0.49
PHM914-04	153	0.5	0.73	0.63
PHM914-05	158	0.9	1.18	1.07
PHM914-06	160	1.5	1.44	1.84

PHM919-01	0	< 0.1	0.07	0.10
PHM919-02	5	< 0.1	0.05	0.14
PHM919-03	7	< 0.1	0.05	0.09
PHM919-04	12	< 0.1	0.20	0.20
PHM919-05	14	0.1	0.28	0.24
PHM919-06	19	0.5	1.13	0.90
PHM919-07	21	0.9	1.84	1.23
PHM919-08	26	> 2.7	7.74	6.44
PHM919-09	28	> 2.7	9.39	8.66

PHM923-01	0	< 0.1	0.34	0.87
PHM923-02	7	< 0.1	0.20	0.31
PHM923-03	15	0.2	0.95	1.33
PHM923-04	22	0.5	1.43	1.60

Panel	Days since first bleed	ng/mL	OD/CO	
			0202	0302
6281-01	0	NK	0.14	0.03
6281-02	5	NK	0.11	0.00
6281-03	7	NK	0.16	0.12
6281-04	13	NK	0.19	0.17
6281-05	19	NK	0.26	0.19
6281-06	22	NK	0.92	0.49
6281-07	33	NK	2.21	1.50
6281-08	36	NK	2.91	2.18
6281-09	41	NK	6.99	3.09
6281-10	43	NK	2.21	1.11
6281-11	50	NK	0.16	0.17
6281-12	54	NK	0.23	0.05

Panel	HBsAg status	ng/mL	OD/CO	
			0202	0302
PHA105-01	POS	0.3	1.01	0.48
PHA105-02	POS	0.8	1.93	1.13
PHA105-03	POS	0.3	0.94	0.53
PHA105-04	POS	0.3	0.63	0.41
PHA105-05	POS	0.3	0.82	0.36
PHA105-06	POS	0.6	2.10	0.85
PHA105-07	POS	0.1	0.66	0.20
PHA105-08	POS	0.2	0.88	0.34
PHA105-09	POS	0.2	1.41	0.26
PHA105-10	POS	0.3	16.53	1.79
PHA105-11	neg	negative	0.29	0.27
PHA105-12	POS	0.3	1.31	0.63
PHA105-13	POS	0.6	2.65	1.36
PHA105-14	POS	0.2	0.85	0.39
PHA105-15	POS	0.2	0.85	0.53

Notes:

OD/CO ratios less than 1.0 are highlighted

Cut-off values for batch 0202:

PHM903, PHM908 and PHM914 = 0.065

PHM919 and PHM923 = 0.061

6281 = 0.064 and PHA105 = 0.068

Cut-off values for batch 0302 = 0.059

N/K = not known

■ Contact details for participating manufacturer and UK agent

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Product code: SAG1.CE

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