



# Evaluation of Genscreen ULTRA HIV Ag-AB

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## Assay Description

The Genscreen ULTRA HIV Ag-Ab kit is a microplate enzyme immunoassay for the detection of HIV antigen or antibodies in human sera and plasma. The kit can be used for screening and simultaneously detects HIV antigen and antibodies against HIV 1 and HIV 2 using the principle of the sandwich technique.

This is an updated version of the Genscreen PLUS HIV Ag-Ab kit. The changes were made to improve sensitivity and specificity. The sensitivity of HIV antigen detection has been improved to approximately 13 pg/mL by changing the monoclonal antibody to p24. Specificity was improved by changing the cut off calculation which is now based on the negative control sera.

HIV p24 antibodies and recombinant HIV protein gp160 on the solid phase bind to any p24 antigen or gp160 antibodies present in the sample. The samples are incubated with conjugate 1 which binds to any p24 bound to the solid phase antibodies. A second conjugate is added. This labels any antibodies present. A coloured signal is achieved by the addition of TMB (tetramethyl benzidine) substrate. Any positive samples will change colour and are read at a wavelength of 450/ 620 – 700 nm.

The assay includes Sample and Reagent Addition Monitoring; thus the addition of samples and reagents can be measured colorimetrically. The kit has a CE Mark and further assay information is shown in Table 1. The assay was evaluated to determine its ability to detect HIV.

**Table 1. Assay Information**

<b>General</b>	
Assay name	Genscreen ULTRA HIV Ag-Ab
Manufacturer / UK Agent	BioRad
Product number	72388
Number of tests per pack	96 / 480
Specimen volume	75µL
<b>Presentation</b>	
Assay type	Sandwich enzyme immunoassay
Solid phase	12 x 8 microtitre plates
Coating	monoclonal antibodies against p24 antigen and purified gp160 recombinant protein (a synthetic peptide that mimics a artificial HIV-1 group O specific epitope and a peptide mimicking the immunodominant epitope of HIV 2 envelope protein
Conjugate 1	Biotinylated polyclonal antibodies to p24 HIV 1
Conjugate 2	Lyophilised peroxidase labelled streptavidin and purified HIV 1 and HIV 2 antigens

Substrate	TMB containing solution diluted in a Peroxidase substrate buffer (sodium citrate and sodium acetate solution containing Hydrogen peroxide)
Controls per plate	5
Negative control	3
Antibody positive control [Pos 1]	1
Antigen positive control [Pos 2]	1
Reading wavelength	450 / 620 - 700nm
Cut-off computation	Mean [Neg results] + 0.200
Equivocal zone	N/A

<b>Stages</b>	
Preparation / sample well loading	30 minutes
Incubation status	Static
Sample + Conjugate 1 incubation	60 minutes 37°C
Conjugate 2 incubation	30 minutes 18-25°C
Number of washes	5
Substrate incubation (time/temp)	30 minutes 18-25°C
Stop solution incubation	2 minutes
Reading	within 30 minutes of stopping reaction
Total incubation time	122 minutes
Approximate time to completion	152 minutes
Number of optional procedures	N/A

<b>Additional equipment required</b>
Dry Incubator, type not specified
Microplate spectrophotometer (EL 808)
Micropipettes: 40 - 200µL, 200 - 1000µL & 2 - 10 mL
Multichannel pipettes: 50 - 300µL
Disposable tips
Reagent troughs and bottles
Measuring cylinder
Distilled Water
Timer

## Evaluation panel and methods

The evaluation panel consisted of 543 specimens (Table 2). Of these, 200 specimens were from HIV negative blood donors, 200 from HIV positive subjects, 131 from 21 commercial seroconversion panels and twelve quality control samples. One hundred and thirty - seven specimens were tested against a second kit batch (Table 3).

The method in the kit inset was followed strictly. Briefly, 25  $\mu\text{L}$  of conjugate 1 and 75  $\mu\text{L}$  of specimen or controls were added to each of the microplate wells. The wells were incubated at 37°C for 60 minutes then washed five times. 100 $\mu\text{L}$  of conjugate 2 was added to all wells, which were then incubated for a further 30 minutes at room temperature (between 18 and 30°C). The wells were washed five times and then 80  $\mu\text{L}$  of substrate was added to each well. The wells were incubated for 30 minutes in the dark at room temperature. To this 100  $\mu\text{L}$  of stop solution was added to each well; the plate was left to stand for 2 minutes and then read at 450/620 – 700 nm.

Results were interpreted using plate reading software and the OD/COs calculated. An OD/CO of more than 1.0 was considered positive.

**Table 2. Specimen panel for the evaluation of Genscreen® ULTRA HIV Ag-Ab (batch 1 [05D0510])**

Sample Category	Number of specimens
1. <i>Anti-HIV negative blood donor sera</i>	200
2. <i>Anti-HIV positive (n=201)</i> Anti-HIV 1 (various risk groups and geographical locations) Anti-HIV 1 subgroup O Anti-HIV 2 positive	175 2 23
3. <i>HIV seroconversion panels (n=131; 21 panels)</i> BBI – PRB 916 BBI – PRB 917 BBI – PRB 919 BBI – PRB 922 BBI – PRB 924 BBI – PRB 925 BBI – PRB 927 BBI – PRB 929 BBI – PRB 930 BBI – PRB 932 BBI – PRB 937 BBI – PRB 938 BBI – PRB 939 BBI – PRB 940 BBI – PRB 941 BBI – PRB 943 BBI – PRB 944 BBI – PRB 945 BBI – PRB 946 BBI – PRB 948 BCP 6240	6 7 3 4 8 6 5 7 4 9 6 3 9 8 6 7 6 6 4 4 13
4. <i>Quality control samples (n=12)</i> HPA anti-HIV 1 QC1 HPA anti-HIV 1 QC2 HPA anti-HIV 1 QC3 HPA anti-HIV 1 QC5 HPA anti-HIV 2 QC2 HPA anti-HIV 2 QC3 HPA HIV 1 p24 QC1 HPA HIV 1 p24 QC2 NIBSC 1 in 5 BWS for anti-HIV 1 NIBSC BWS for anti HIV 1 NIBSC Monitor sample for anti-HIV 2 NIBSC HIV 1 p24 Ag monitor sample	1 (x3) 1 (x3) 1 (x3) 1 (x3) 1 (x3) 1 (x3) 1 (x3) 1 (x3) 1 (x3) 1 (x3) 1 (x3) 1 (x3) 1 (x3) 1 (x3)
<b>Total (number of tests)</b>	<b>543</b>
Notes: BBI = Boston Biomedica Inc; BCP = BioClinical Partners Inc (Zeptometrix); HPA = Health Protection Agency, Colindale UK; NIBSC = National Institute for Biological Standards and Controls	

**Table 3. Specimen panel for the evaluation of Genscreen® ULTRA HIV Ag-Ab (batch 2 [05K0511])**

<b>Sample Category</b>	<b>Number of specimens</b>
<i>1. Anti-HIV negative blood donor sera</i>	40
<i>2. Anti-HIV positive</i>	40
<i>3. HIV seroconversion panels (n=45; 5 panels)</i>	
BBI – PRB 916	6
BBI – PRB 924	8
BBI – PRB 932	9
BBI – PRB 939	9
BCP 6240	13
<i>4. Quality control samples (n=12)</i>	
HPA anti-HIV 1 QC1	1 (x3)
HPA anti-HIV 1 QC2	1 (x3)
HPA anti-HIV 1 QC3	1 (x3)
HPA anti-HIV 1 QC5	1 (x3)
HPA anti-HIV 2 QC2	1 (x3)
HPA anti-HIV 2 QC3	1 (x3)
HPA HIV 1 p24 QC1	1 (x3)
HPA HIV 1 p24 QC2	1 (x3)
NIBSC 1 in 5 BWS for anti-HIV 1	1 (x3)
NIBSC BWS for anti HIV 1	1 (x3)
NIBSC Monitor sample for anti-HIV 2	1 (x3)
NIBSC HIV 1 p24 Ag monitor sample	1 (x3)
<b>Total (number of tests)</b>	<b>137</b>
Notes: BBI = Boston Biomedica Inc; BCP = BioClinical Partners Inc (Zeptometrix); HPA = Health Protection Agency, Colindale UK; NIBSC = National Institute for Biological Standards and Controls	

## Specificity findings

Of the 200 HIV negative blood donor specimens, there was one initially reactive specimen to give an initial reactive rate of 0.5% (95% confidence intervals 0.000183 to 0.0275%). No samples were repeatedly reactive.

**Table 4. Specificity of Genscreen ULTRA HIV Ag-Ab kit**

Anti – HIV negative blood donors	Number tested	Number initially reactive	Number repeatedly reactive	Initial range OD/CO	Initial mean OD/CO	Initial median OD/CO
Stored for less than 6 months	200	1	0	0.14 – 1.00	0.29	0.27

## Sensitivity findings

All 200 randomly selected HIV positive specimens were reactive to give the assay a sensitivity of 100% (95% CI: 98.2 - 100%).

**Table 5. Sensitivity of Genscreen ULTRA HIV Ag-Ab kit**

Subgroup / Risk factor	Number of specimens	Mean OD/CO	Median OD/CO	Range of OD/CO	Sensitivity
<i>Type or Subgroup</i>					
HIV 1 Group O	2	11.11	11.11	11.10 - 11.11	100%
HIV 2	23	11.99	12.02	10.47 - 13.11	100%
HIV 1 or 2	13	12.97	13.03	12.74 - 13.13	100%
<i>Location Risk Factor</i>					
HIV 1 Ivory Coast	2	11.05	11.05	11.04 - 11.05	100%
HIV 1 Zimbabwe	3	11.80	11.21	11.19 - 13.00	100%
HIV 1 Uganda	15	12.29	12.69	10.85 - 13.07	100%
HIV 1 India	3	12.57	12.55	12.54 - 12.62	100%
HIV 1 North America	16	12.64	12.67	12.00 - 13.14	100%
HIV 1 Argentina	2	12.50	12.50	12.47 - 12.53	100%
HIV 1 Mozambique	2	12.47	12.47	12.46 - 12.47	100%
HIV 1 South Africa	11	12.54	12.57	12.38 - 12.69	100%
HIV 1 Ghana	10	12.67	12.71	12.45 - 12.85	100%
<i>Life style Related</i>					
Partner HIV positive	3	13.07	13.09	12.93 - 13.20	100%
Prostitute	4	12.99	13.02	12.87 - 13.06	100%
Bisexual	1	13.26	13.26	13.26	100%
Multiple partners	49	13.27	13.15	12.83 - 13.99	100%
Unprotected sex	2	13.81	13.81	13.66 - 13.96	100%
Homosexual	18	13.40	13.60	10.85 - 13.83	100%
IVDU	16	13.13	13.59	10.96 - 13.83	100%
Transfusion received	6	12.98	13.31	11.15 - 13.44	100%
<b>Total</b>	200	12.81	12.97	10.47 – 13.99	100%

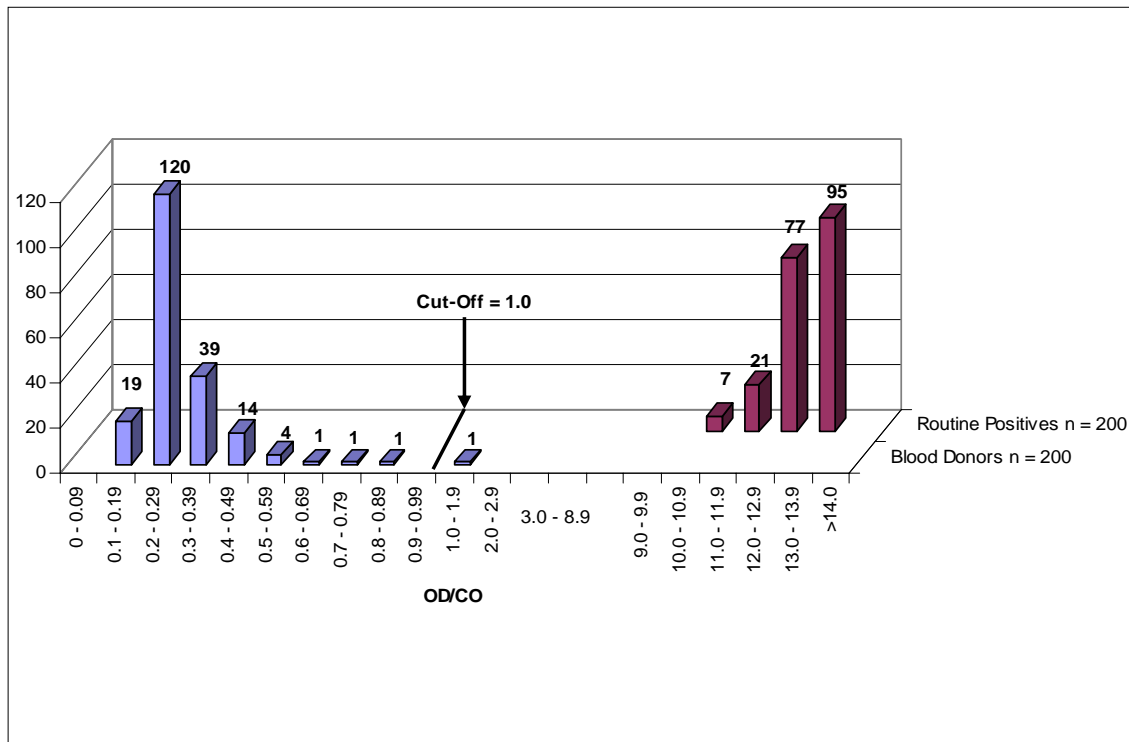


Figure 1. Distribution of initial reactivities.

## Seroconversion Sensitivity: Aggregate scores

The ability of the Genscreen ULTRA HIV Ag-Ab assay to detect early antigen or antibody in 21 seroconversion panels was compared with previous evaluation results. With a threshold of 1.0, the kit gave a score of 82 out of 125 and was shown to be the second most sensitive kit, with only the AxSym® HIV Ag/Ab Combo scoring a greater aggregate score (Table 6). The full details of the seroconversion score are shown in the Appendix.

When this updated version of the Genscreen combined antigen and antibody was compared with the original version, the Genscreen ULTRA HIV Ag-Ab assay performed better. The original version of the kit (Genscreen PLUS HIV Ag-Ab) had a combined seroconversion aggregate score of 75 and was ranked 6<sup>th</sup>. This shows that the manufacturer's claim of increased sensitivity and specificity is a fair claim.

**Table 6. Combined Seroconversion Scores (21 Panels)**

HIV assay	Product number	Cumulative score * (PRB943-6240) n=125	Rank
AxSYM® HIV Ag/Ab Combo	2G83-20	85	1
<b>GENSCREEN® Ultra HIV Ag-Ab</b>	<b>72388/72386</b>	<b>82</b>	<b>2</b>
Prism HIV Ag/Ab Combo	7G46-48	80	=3
Murex HIV Ag/Ab Combination	GE41/42	80	=3
VIDAS HIV DUO	30114	76	5
GENSCREEN® PLUS HIV Ag-Ab	72375/72376	75	6
Enzygnost® HIV Integral	31843	73	7
Vironostika® HIV Uni-Form II Ag/Ab UPDATE	285047	N/A**	N/A**
Vironostika® HIV Uni-Form II Ag/Ab	6029/30/31	63	8
Genscreen® HIV1/2 EIA (v2)	72279	58	9
Murex HIV 1.2.O	GE94/95	56	10
Biotest Anti-HIV TETRA ELISA	807 008	55	=11
Access® HIV 1/2 NEW	34020	55	=11
Vitros ECi Anti-HIV 1+2	124-1850	55	=11
Abbott HIV1/2 3rd Generation Plus EIA	7A84-24	55	=11
AxSYM® HIV 1/2 gO	3D41-20	54	=15
Murex HIV 1+2	VK84/85	54	=15
IMx® HIV-1/HIV-2 III Plus	8C98	53	=17
Ortho® HIV-1/HIV-2 Ab-capture ELISA Test System	932380	53	=17
Enzygnost® Anti-HIV1/2 Plus	OQFK 12/13	51	=19
Vironostika® HIV Uni-Form II plus O	84018	51	=19
ICE HIV-1.0.2	100A	49	=21
Wellcozyme HIV 1+2 EIA	VK55	49	=21
Biotest anti-HIV1/2 recombinant	807005	47	23
Clonesystems (IAF Biochem) Detect HIV	851403	30	24
Innotest HIV-1/HIV-2	M422	29	25
Notes:			
* The score was calculated by summing the number of positive samples for each of the seroconversion panels. A higher score suggests higher sensitivity.			
** Vironostika® HIV Uni-Form II Ag/Ab UPDATE cannot be given a cumulative score because it was only tested on 19 out of 21 seroconversion panels. The position in this table is an approximate rank based on 19 seroconversion panels.			

## Seroconversion Sensitivity: Comparative timing of detection

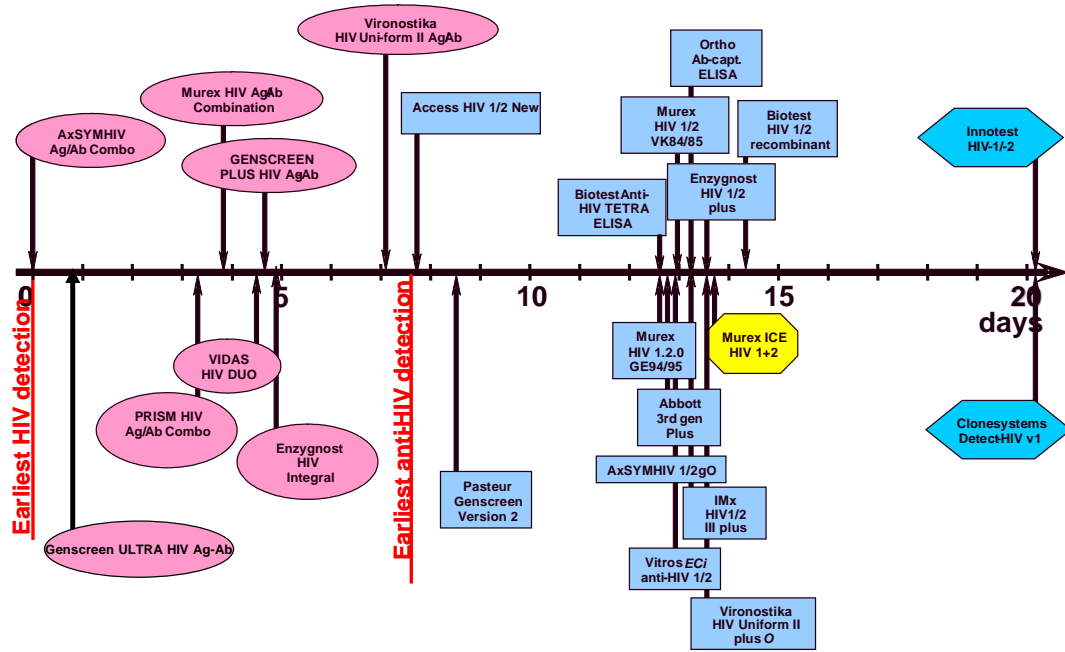
Timing of detection was determined using a method that assigns for each seroconversion panel the most sensitive test “time zero” and any test less sensitive, a positive value (based on the number of days after the most sensitive kit has detected infection). An overall mean and median delay is then calculated for all seroconversion panels tested. This method showed that Genscreen ULTRA HIV Ag-Ab detected HIV infection approximately 2.5 days earlier than the next best kit, and only one day after the AxSYM HIV Ag/Ab Combo assay which was ranked first. Genscreen ULTRA HIV Ag-Ab detected infection approximately 3.8 days earlier than the previous version of the kit.

The median detection time for Genscreen ULTRA HIV Ag-Ab kit was 0 days which was 3 days earlier than the previous version of the Genscreen kit. The median delay is not affected in the same way as the mean delay which can be strongly influenced by outlying results from seroconversion panels for which the interval between the last negative and the first positive specimen is long. This can give rise to an artefact due to the timing of blood collection.

**Table 7. Comparative timing of detection.**

HIV assay	Product number	Delay in detecting primary HIV infection in seroconversion panels compared with the earliest detection by and screening assay (Time 0)		
		Range (Days)	Mean (Days)	Median (Days)
AxSYM® HIV Ag/Ab Combo	2G83-20	0	0	0
<b>GENSCREEN® Ultra HIV Ag-Ab</b>	<b>72388/72386</b>	<b>0 – 6</b>	<b>0.95</b>	<b>0</b>
Prism HIV Ag/Ab Combo	7G46-48	0 – 53	3.5	0
Murex HIV Ag/Ab Combination	GE41/42	0 – 53	3.8	0
VIDAS HIV DUO	30114	0 – 53	4.5	0
GENSCREEN® PLUS HIV Ag-Ab	72375/72376	0 – 53	4.8	0
Enzygnost® HIV Integral	31843	0 – 53	5.1	0
<b>Earliest possible detection by any anti-HIV only assay</b>		0 – 65	7.6	5
Vironostika® HIV Uni-Form II Ag/Ab	6029/30/31	0 – 57	7.3	5
Access® HIV 1/2 NEW	34020	0 – 53	8.0	7
Genscreen® HIV1/2 EIA (v2)	72279	0 – 65	8.6	6
Murex HIV 1.2.O	GE94/95	0 – 87	12.8	7
Biotest Anti-HIV TETRA ELISA	807 008	0 – 87	12.8	7
Abbott HIV1/2 3rd Generation Plus EIA	7A84-24	0 – 87	12.9	7
Vitros ECi Anti-HIV 1+2	124-1850	0 – 87	13.0	7
Murex HIV 1+2	VK84/85	0 – 87	13.1	7
AxSYM® HIV 1/2 gO	3D41-20	0 – 87	13.2	7
Ortho® HIV-1/HIV-2 Ab-capture ELISA Test System	932380	0 – 87	13.4	7
IMx® HIV-1/HIV-2 III Plus	8C98	0 – 87	13.4	7
Enzygnost® Anti-HIV1/2 Plus	OQFK 12/13	0 – 87	13.6	7
Vironostika® HIV Uni-Form II <i>plus O</i>	84018	0 – 87	13.7	7
Murex ICE HIV-1.0.2	100A	0 – 87	13.9	7
Biotest anti-HIV1/2 recombinant	807005	0 – 87	14.5	9
Clonesystems (IAF Biochem) Detect HIV	851403	0 – 87	23.1	12
Innotest HIV-1/HIV-2	M422	0 – 87	24.1	13
<p><b>Notes:</b> The upper limit of the range is, to some extent, influenced by the intervals between bleeds for any individual panel. The mean and median values provide a better general guide to each assay's ability to detect seroconversion. When an assay failed to detect seroconversion in a panel it was given an arbitrary extra 3 days delay for that panel. Time 0 = earliest detection of HIV infection by any screening assay.</p>				

Figure 2. Comparative timing of detection.



**Note:**

This figure is based on data generated by testing 21 seroconversion panels in each of the HIV screening tests shown.

- = Combined Ag/Ab assays
- = immunometric assay;
- ⬡ = Class specific antibody capture assay;
- ⬡ = antiglobulin assay.

## Quality Control Reagents

Twelve quality control reagents were each tested in triplicate to identify suitable controls and to use one for monitoring the remaining evaluation test runs. A suitable control is one that has an OD reading that is approximately 2 to 3 times higher than the cut off. For the Genscreen ULTRA HIV Ag-Ab assay there were two suitable controls; HPA anti-HIV 2 QC2 and HPA HIV 1 p24 QC2. For this evaluation, HPA HIV 1 p24 QC2 was included at the beginning, middle and end of each test plate.

**Table 8. Quality Control Reagent results.**

QC sample ID	Batch number	OD/CO 1	OD/CO 2	OD/CO 3	Mean
HPA anti-HIV 1 QC1	03/B355-02	12.43	12.83	12.69	12.65
HPA anti-HIV 1 QC2	03/B356-03	5.85	5.85	6.09	5.93
HPA anti-HIV 1 QC3	03/B359-01	1.68	1.59	1.66	1.64
HPA anti-HIV 1 QC5	99/B168-10	11.83	11.88	12.02	11.91
HPA anti-HIV 2 QC2	03/B379-02	3.22	3.26	3.27	3.25
HPA anti-HIV 2 QC3	04/B406-01	0.45	0.45	0.46	0.45
HPA HIV 1 p24 QC1	01-B276-10	11.50	11.32	11.59	11.47
HPA HIV 1 p24 QC2	01/B273-06	3.02	2.96	3.04	3.01
NIBSC 1 in 5 BWS for anti-HIV 1	99/710-007	1.38	1.31	1.30	1.33
NIBSC BWS for anti HIV 1	99/750-007	4.84	5.01	5.04	4.96
NIBSC Monitor sample for anti-HIV 2	99/674-005	1.49	1.48	1.55	1.51
NIBSC HIV 1 p24 Ag monitor sample	02/146-002	10.33	10.56	10.66	10.52

**Table 9. HPA HIV 1 p24 QC2 results**

Number of tests	Number initially reactive	Initial range OD/CO	Initial mean OD/CO	Initial median OD/CO
23	23	2.53 – 4.20	3.18	3.23

## Batch Comparison

Two batches of Genscreen ULTRA HIV Ag-Ab were tested to examine variation. The comparison showed that there was no significant difference in the number of positive specimens detected by the two batches. See appendix tables A1 and A2 for full details of a comparison of OD/CO readings for both batch 05K0511 and 05D0510.

**Table 10. Comparison of two batches.**

Specimen Category	Number of Specimens	Number of Reactive Specimens	
		Batch Number	
		05D0510	05K0511
1. Anti-HIV negative blood donor sera	40	0	0
2. Anti-HIV positive	40	40	40
3. HIV seroconversion panels			
BBI – PRB 916	6	3	3
BBI – PRB 924	8	4	4
BBI – PRB 932	9	6	6
BBI – PRB 939	9	4	3
BCP 6240	13	6	6
4. Quality control samples (n=12)			
HPA anti-HIV 1 QC1	1 (x3)	3	3
HPA anti-HIV 1 QC2	1 (x3)	3	3
HPA anti-HIV 1 QC3	1 (x3)	3	3
HPA anti-HIV 1 QC5	1 (x3)	3	3
HPA anti-HIV 2 QC2	1 (x3)	3	3
HPA anti-HIV 2 QC3	1 (x3)	0	0
HPA HIV 1 p24 QC1	1 (x3)	3	3
HPA HIV 1 p24 QC2	1 (x3)	3	3
NIBSC 1 in 5 BWS for anti-HIV 1	1 (x3)	3	3
NIBSC BWS for anti HIV 1	1 (x3)	3	3
NIBSC Monitor sample for anti-HIV 2	1 (x3)	3	3
NIBSC HIV 1 p24 Ag monitor sample	1 (x3)	3	3

## Technical Appraisal

The Genscreen ULTRA HIV Ag-AB kit is an enzyme immunoassay. The kit was easy to use with addition monitors at each stage of the assay. The instructions were clear and easy to follow, although a pictorial summary would be useful for reference whilst performing the assay.

The only issue was with the packaging. When unpacking the reagents from the supplied box one of the glass bottles (containing Conjugate 2) was not secure and fell out resulting in the glass breaking. This could be a potential Health and Safety issue, especially if this had been a control bottle.

## Conclusions

The Genscreen Ultra HIV Ag-Ab kit allows simultaneous detection of HIV p24 antigen and anti-HIV. In terms of seroconversion sensitivity it was the 2<sup>nd</sup> most sensitive antigen-antibody kit, detecting HIV a mean of one day after the AxSYM HIV Ag/Ab assay. It also detected HIV a mean of 2.5 days earlier than the next most sensitive kit (PRISM HIV Ag/Ab) and was 3.8 days earlier than the current version of this kit, Genscreen HIV PLUS Ag/Ab. In addition, there was a 6.6 day improvement in detection time when compared to antibody-only kits.

The kit showed excellent sensitivity and specificity when tested against a moderate number of routine positive and negative specimens.

The test was very easy to use and did not require a great deal of hands on time. This kit is suitable for any laboratory considering changing from an antibody-only EIA kit or changing to a more sensitive antigen-antibody kit. It is suitable for HIV screening in diagnostic and blood centre laboratories.

## Appendix 1: Seroconversion Scores

HIV assay	Product number	The number of positive samples and the number of days from initial bleed to first reactive sample (shown in parentheses)										Score* (PRB916-927) n=37	
		PRB916	PRB917M	PRB919	PRB922	PRB924	PRB925	PRB927					
		n=6	n=5	n=3	n=4	n=8	n=6	n=5					
AxSYM <sup>®</sup> HIV Ag/Ab Combo	2G83-20	4 (9)	6 (0)	3 (0)	4 (0)	4 (26)	2 (44)	4 (28)					27
<b>GENSCREEN<sup>®</sup> Ultra HIV Ag-Ab</b>	<b>72388/72386</b>	<b>3 (15)</b>	<b>6 (0)</b>	<b>3 (0)</b>	<b>4 (0)</b>	<b>4 (26)</b>	<b>2 (44)</b>	<b>4 (28)</b>					<b>26</b>
Prism HIV Ag/Ab Combo	7G46-48	3 (15)	5 (53)	3 (0)	4 (0)	4 (26)	2 (44)	4 (28)					25
Murex HIV Ag/Ab Combination	GE41/42	3 (15)	5 (53)	3 (0)	4 (0)	4 (26)	2 (44)	4 (28)					25
VIDAS HIV DUO	30114	3 (15)	5 (53)	3 (0)	4 (0)	4 (26)	2 (44)	4 (28)					25
GENSCREEN <sup>®</sup> PLUS HIV Ag-Ab	72375/72376	3 (15)	5 (53)	3 (0)	4 (0)	4 (26)	2 (44)	4 (28)					25
Enzygnost <sup>®</sup> HIV Integral	31843	3 (15)	5 (53)	3 (0)	4 (0)	4 (26)	2 (44)	4 (28)					25
Vironostika <sup>®</sup> HIV Uni-Form II Ag/Ab UPDATE	285047	3 (15)	6 (0)	2 (9)	4 (0)	4 (26)	2 (44)	4 (28)					25
Vironostika <sup>®</sup> HIV Uni-Form II Ag/Ab	6029/30/31	3 (15)	4 (57)	2 (9)	4 (0)	4 (26)	2 (44)	4 (28)					23
Genscreen <sup>®</sup> HIV1/2 EIA (v2)	72279	2 (30)	3 (65)	3 (0)	4 (0)	3 (33)	2 (44)	4 (28)					21
Biotest Anti-HIV TETRA ELISA	807 008	2 (30)	3 (65)	3 (0)	4 (0)	3 (33)	2 (44)	4 (28)					21
Murex HIV 1.2.O	GE94/95	2 (30)	3 (65)	2 (9)	4 (0)	3 (33)	2 (44)	4 (28)					20
Access <sup>®</sup> HIV 1/2 NEW	34020	2 (30)	3 (65)	2 (9)	4 (0)	3 (33)	2 (44)	4 (28)					20
Vitros ECI Anti-HIV 1+2	124-1850	2 (30)	3 (65)	2 (9)	4 (0)	3 (33)	2 (44)	3 (33)					19
Abbott HIV1/2 3rd Generation Plus EIA	7A84-24	2 (30)	3 (65)	2 (9)	3 (4)	3 (33)	2 (44)	4 (28)					19
AxSYM <sup>®</sup> HIV 1/2 gO	3D41-20	2 (30)	3 (65)	2 (9)	4 (0)	3 (33)	2 (44)	3 (33)					19
IMx <sup>®</sup> HIV-1/HIV-2 III Plus	8C98	2 (30)	3 (65)	2 (9)	3 (4)	3 (33)	2 (44)	4 (28)					19
Ortho <sup>®</sup> HIV-1/HIV-2 Ab-capture ELISA Test System	932380	2 (30)	3 (65)	2 (9)	3 (4)	3 (33)	2 (44)	3 (33)					18
Murex HIV 1+2	VK84/85	2 (30)	3 (65)	2 (9)	4 (0)	3 (33)	2 (44)	3 (33)					19
Enzygnost <sup>®</sup> Anti-HIV1/2 Plus	OQFK 12/13	2 (30)	3 (65)	2 (9)	3 (4)	3 (33)	2 (44)	4 (28)					19
Vironostika <sup>®</sup> HIV Uni-Form II plus O	84018	2 (30)	3 (65)	2 (9)	3 (4)	3 (33)	2 (44)	4 (28)					19
ICE HIV-1.0.2	100A	2 (30)	3 (65)	2 (9)	4 (0)	3 (33)	2 (44)	3 (33)					19
Wellcozyme HIV 1+2 EIA	VK55	2 (30)	3 (65)	2 (9)	4 (0)	3 (33)	2 (44)	3 (33)					19
Biotest anti-HIV1/2 recombinant	807005	2 (30)	3 (65)	2 (9)	4 (0)	3 (33)	2 (44)	3 (33)					19
Innotest HIV-1/HIV-2	M422	2 (30)	3 (65)	2 (9)	3 (4)	1 (40)	2 (44)	3 (33)					16
Clonestems (IAF Biochem) Detect HIV	851403	2 (30)	3 (65)	2 (9)	2 (7)	2 (35)	1 (49)	3 (33)					15
Access <sup>®</sup> HIV-1/2 assay	34000	3 (15)	3 (65)	2 (9)	3 (4)	3 (33)	2 (44)	3 (33)					19
Bioelisa HIV 1+2	3000-1107	2 (30)	2 (67)	2 (9)	1(11)	1 (40)	1 (49)	2 (35)					11
Detect-HIV™ (v2)	RHD-202B	2 (30)	3 (65)	2 (9)	2 (7)	3 (33)	2 (44)	3 (33)					17
Recombigen <sup>®</sup> HIV-1/HIV-2 EIA	96040	2 (30)	3 (65)	2 (9)	3 (4)	3 (33)	2 (44)	3 (33)					18
PRISM anti-HIV 1+2	4A2748	2 (30)	3 (65)	2 (9)	NT	3 (33)	2 (44)	3 (33)					N/A
PRISM HIV O Plus	3D34-48	2 (30)	3 (65)	NT	NT	3 (33)	NT	3 (33)					N/A

**Notes:**  
 \* The score was calculated by summing the number of positive samples for each of the seroconversion panels. A higher score suggests higher sensitivity.  
 Panel member PRB917-05 was VOID for PRISM HIV O Plus. This member has been scored as positive so giving this panel the score 3 (65)

HIV assay	Product number	The number of positive samples and the number of days from initial bleed to first reactive sample (shown in parentheses)												Score * (PRB929-941) n=48	Cumulative score * (PRB916 - 941) n=85
		PRB929	PRB930	PRB932	PRB937	PRB938	PRB939	PRB940	PRB941						
		n=7	n=4	n=9	n=6	n=3	n=5	n=8	n=6						
AXSYM® HIV Ag/Ab Combo	2G83-20	4 (18)	4 (0)	6 (27)	4 (9)	3 (0)	4 (2)	7 (7)	3 (18)					35	62
GENSCREEN® Ultra HIV Ag-Ab	72388/72386	5 (14)	3 (3)	6 (27)	3 (14)	3 (0)	4 (2)	7 (7)	3 (18)					34	60
Prism HIV Ag/Ab Combo	7G46-48	4 (18)	4 (0)	6 (27)	3 (14)	3 (0)	4 (2)	7 (7)	3 (18)					34	59
Murex HIV Ag/Ab Combination	GE41/42	4 (18)	4 (0)	6 (27)	3 (14)	3 (0)	3 (7)	7 (7)	3 (18)					33	58
VIDAS HIV DUO	30114	4 (18)	3 (3)	6 (27)	3 (14)	3 (0)	3 (7)	7 (7)	2 (21)					31	56
GENSCREEN® PLUS HIV Ag-Ab	72375/72376	4 (18)	3 (3)	6 (27)	3 (14)	2 (3)	3 (7)	7 (7)	3 (18)					31	56
Enzygnost® HIV Integral	31843	4 (18)	4 (0)	6 (27)	1 (21)	3 (0)	3 (7)	7 (7)	3 (18)					31	56
Vironostika® HIV Uni-Form II Ag/Ab UPDATE	285047	4 (18)	2 (7)	NT	1 (21)	2 (3)	3 (7)	7 (7)	3 (18)					N/A	N/A
Vironostika® HIV Uni-Form II Ag/Ab	6029/30/31	3 (21)	2 (7)	6 (27)	1 (21)	1 (9)	3 (7)	6 (11)	3 (18)					25	48
Genscreen® HIV 1/2 EIA (v2)	72279	2 (25)	2 (7)	6 (27)	1 (21)	1 (9)	2 (9)	6 (11)	3 (18)					23	44
Biotest Anti-HIV TETRA ELISA	807 008	2 (25)	2 (7)	6 (27)	1 (21)	1 (9)	1 (89)	6 (11)	3 (18)					22	43
Murex HIV 1.2.O	GE94/95	3 (21)	2 (7)	6 (27)	1 (21)	1 (9)	1 (89)	6 (11)	3 (18)					23	43
Access® HIV 1/2 NEW	34020	2 (25)	2 (7)	6 (27)	1 (21)	1 (9)	2 (9)	6 (11)	2 (21)					22	42
Vitros ECI Anti-HIV 1+2	124-1850	3 (21)	2 (7)	6 (27)	1 (21)	1 (9)	1 (89)	7 (7)	3 (18)					24	43
Abbott HIV1/2 3rd Generation Plus EIA	7A84-24	2 (25)	2 (7)	6 (27)	1 (21)	1 (9)	1 (89)	6 (11)	3 (18)					22	41
AXSYM® HIV 1/2 gO	3D41-20	3 (21)	2 (7)	6 (27)	1 (21)	1 (9)	1 (89)	6 (11)	3 (18)					23	42
IMx® HIV-1/HIV-2 III Plus	8C98	2 (25)	2 (7)	6 (27)	1 (21)	1 (9)	1 (89)	6 (11)	3 (18)					22	41
Ortho® HIV-1/HIV-2 Ab-capture ELISA Test System	932380	3 (21)	2 (7)	6 (27)	1 (21)	1 (9)	1 (89)	6 (11)	3 (18)					23	41
Murex HIV 1+2	VK84/85	3 (21)	3 (3)	6 (27)	1 (21)	1 (9)	1 (89)	6 (11)	3 (18)					24	43
Enzygnost® Anti-HIV1/2 Plus	OQFK 12/13	2 (25)	2 (7)	6 (27)	1 (21)	1 (9)	1 (89)	6 (11)	3 (18)					22	41
Vironostika® HIV Uni-Form II plus O	84018	3 (21)	2 (7)	5 (34)	1 (21)	1 (9)	1 (89)	6 (11)	3 (18)					22	41
ICE HIV-1.0.2	100A	2 (25)	2 (7)	5 (34)	1 (21)	1 (9)	1 (89)	6 (11)	3 (18)					21	40
Wellcozyme HIV 1+2 EIA	VK55	2 (25)	2 (7)	5 (34)	0 (>21)	1 (9)	1 (89)	6 (11)	3 (18)					20	39
Biotest anti-HIV1/2 recombinant	807005	2 (25)	1 (10)	5 (34)	0 (>21)	0 (>9)	1 (89)	6 (11)	3 (18)					18	37
Innotest HIV-1/HIV-2	M422	0 (>28)	2 (7)	0 (194)	0 (>21)	0 (>9)	1 (89)	5 (15)	2 (21)					10	26
Clonestest (IAF Biochem) Detect HIV	851403	1 (28)	1 (10)	1 (163)	0 (>21)	0 (>9)	0 (>89)	5 (15)	2 (21)					10	25
Access® HIV-1/2 assay	34000	2 (25)	1 (10)	5 (34)	NT	NT	NT	NT	NT					N/A	N/A
Bioelisa HIV 1+2	3000-1107	0 (>28)	0 (>10)	0 (194)	NT	NT	NT	NT	NT					N/A	N/A
Detect-HIV™ (v2)	RHD-202B	1 (28)	2 (7)	5 (34)	NT	NT	NT	NT	NT					N/A	N/A
Recombigen® HIV-1/HIV-2 EIA	96040	2 (25)	1 (10)	5 (34)	NT	NT	NT	NT	5 (15)					N/A	N/A
PRISM anti-HIV 1+2	4A2748	2 (25)	2 (7)	NT	NT	1 (9)	NT	NT	NT					N/A	N/A
PRISM HIV O Plus	3D34-48	NT	2 (7)	6 (27)	0 (>21)	1 (9)	1 (89)	NT	NT					N/A	N/A

**Notes:**  
The score was calculated by summing the number of positive samples for each of the seroconversion panels. A higher score suggests higher sensitivity.

HIV assay	Product number	The number of positive samples and the number of days from initial bleed to first reactive sample (shown in parentheses)										Score * (PRB943-6240) n = 40	Cumulative score * (PRB943-6240) n=125
		PRB943 n=7	PRB944 n = 6	PRB945 n=6	PRB946 n = 4	PRB948 n=4	6240 n=13						
AxSYM® HIV Ag/Ab Combo	2G83-20	5 (7)	5 (2)	4 (7)	2 (7)	1 (23)	6 (23)	23	85				
GENSCREEN® Ultra HIV Ag-Ab	72388/72386	5 (7)	5 (2)	3 (13)	2 (7)	1 (23)	6 (23)	22	82				
Prism HIV Ag/Ab Combo	7G46-48	5 (7)	4 (7)	3 (13)	2 (7)	1 (23)	6 (23)	21	80				
Murex HIV Ag/Ab Combination	GE41/42	5 (7)	5 (2)	3 (13)	2 (7)	1 (23)	6 (23)	22	80				
VIDAS HIV DUO	30114	4 (12)	4 (7)	3 (13)	2 (7)	1 (23)	6 (23)	20	76				
GENSCREEN® PLUS HIV Ag-Ab	72375/72376	4 (12)	4 (7)	3 (13)	2 (7)	1 (23)	5 (28)	19	75				
Enzygnost® HIV Integral	31843	4 (12)	2 (14)	3 (13)	1 (11)	1 (23)	6 (23)	7	73				
Vironostika® HIV Uni-Form II Ag/Ab UPDATE	285047	4 (12)	2 (14)	2 (15)	1 (11)	1 (23)	NT	N/A	N/A				
Vironostika® HIV Uni-Form II Ag/Ab	6029/30/31	4 (12)	2 (14)	2 (15)	1 (11)	0 (>23)	5 (28)	15	63				
Genscreen® HIV1/2 EIA (v2)	72279	2 (19)	4 (7)	3 (13)	0 (>11)	0 (>23)	5 (28)	14	58				
Biotech Anti-HIV TETRA ELISA	807 008	2 (19)	2 (14)	3 (13)	0 (>11)	0 (>23)	5 (28)	12	55				
Murex HIV 1.2.0	GE94/95	3 (14)	2 (14)	3 (13)	0 (>11)	0 (>23)	5 (28)	13	56				
Access® HIV 1/2 NEW	34020	2 (19)	3 (9)	3 (13)	0 (>11)	0 (>23)	5 (28)	13	55				
Vitros ECI Anti-HIV 1+2	124-1850	2 (19)	2 (14)	3 (13)	0 (>11)	0 (>23)	5 (28)	12	55				
Abbott HIV1/2 3rd Generation Plus EIA	7A84-24	3 (14)	3 (9)	3 (13)	0 (>11)	0 (>23)	5 (28)	14	55				
AxSYM® HIV 1/2 gO	3D41-20	2 (19)	2 (14)	3 (13)	0 (>11)	0 (>23)	5 (28)	12	54				
IMx® HIV-1/HIV-2 III Plus	8C98	2 (19)	2 (14)	3 (13)	0 (>11)	0 (>23)	5 (28)	12	53				
Ortho® HIV-1/HIV-2 Ab-capture ELISA Test System	932380	2 (19)	2 (14)	3 (13)	0 (>11)	0 (>23)	5 (28)	12	53				
Murex HIV 1+2	VK84/85	2 (19)	2 (14)	3 (13)	0 (>11)	0 (>23)	4 (30)	11	54				
Enzygnost® Anti-HIV1/2 Plus	OQFK 12/13	2 (19)	2 (14)	2 (15)	0 (>11)	0 (>23)	4 (30)	10	51				
Vironostika® HIV Uni-Form II plus O	84018	2 (19)	2 (14)	2 (15)	0 (>11)	0 (>23)	4 (30)	10	51				
ICE HIV-1.0.2	100A	2 (19)	2 (14)	2 (15)	0 (>11)	0 (>23)	3 (36)	9	49				
Wellcozyme HIV 1+2 EIA	VK55	2 (19)	2 (14)	3 (13)	0 (>11)	0 (>23)	3 (36)	10	49				
Biotech anti-HIV1/2 recombinant	807005	2 (19)	2 (14)	1 (20)	0 (>11)	0 (>23)	5 (28)	10	47				
Innotest HIV-1/HIV-2	M422	0 (>21)	0 (>16)	0 (>20)	0 (>11)	0 (>23)	3 (36)	3	29				
Clonestest (IAF Biochem) Detect HIV	851403	0 (>21)	1 (16)	1 (20)	0 (>11)	0 (>23)	3 (36)	5	30				
Access® HIV-1/2 assay	34000	NT	NT	NT	NT	NT	NT	N/A	N/A				
Bioelisa HIV 1+2	3000-1107	NT	NT	NT	NT	NT	NT	N/A	N/A				
Detect-HIV™ (v2)	RHD-202B	NT	NT	NT	NT	NT	NT	N/A	N/A				
Recombigen® HIV-1/HIV-2 EIA	96040	NT	NT	NT	NT	NT	NT	N/A	N/A				
PRISM anti-HIV 1+2	4A2748	NT	NT	NT	NT	NT	NT	N/A	N/A				
PRISM HIV O Plus	3D34-48	2 (19)	3 (9)	3 (13)	NT	NT	4 (74)	N/A	N/A				

**Notes:**  
 \* The score was calculated by summing the number of positive samples for each of the seroconversion panels. A higher score suggests higher sensitivity. Panel member PRB917-05 was VOID for PRISM HIV O Plus. This member has been scored as positive so giving this panel the score 3 (65)

## Appendix 2: Batch Comparison

**Table A1. Differences in reactivity between two batches of Genscreen ULTRA HIV Ag/Ab.**

Specimen Category	Number of Specimens	Range of OD/CO		Mean OD/CO		Median OD/CO	
		Batch Number		Batch Number		Batch Number	
		05D0510	05K0511	05D0510	05K0511	05D0510	05K0511
1. Anti-HIV negative blood donor sera	40	0.162 – 1.046	0.154 – 0.335	0.287	0.234	0.254	0.222
2. Anti-HIV positive	40	10.995 – 13.756	11.872 – 13.211	12.359	12.636	12.263	12.685

**Table A2. Differences in reactivity of quality control specimens between two batches of Genscreen ULTRA HIV Ag/Ab**

QC Type	Number of tests	Mean OD/CO (Batch 05D0510)	Mean OD/CO (Batch 05K0511)
HPA anti-HIV 1 QC1	3	12.65	12.79
HPA anti-HIV 1 QC2	3	5.93	6.34
HPA anti-HIV 1 QC3	3	1.64	1.66
HPA anti-HIV 1 QC5	3	11.91	12.28
HPA anti-HIV 2 QC2	3	3.25	2.95
HPA anti-HIV 2 QC3	3	0.45	0.47
HPA HIV 1 p24 QC1	3	11.47	11.52
HPA HIV 1 p24 QC2	3	3.01	2.713
NIBSC 1 in 5 BWS for anti-HIV 1	3	1.30	1.44
NIBSC BWS for anti HIV 1	3	5.04	5.51
NIBSC Monitor sample for anti-HIV 2	3	1.55	1.54
NIBSC HIV 1 p24 Ag monitor sample	3	10.66	10.60

**Table A3. Full details of comparative data for seroconversion panels.**

Seroconversion panel name	OD/CO (Batch 05D0510)	OD/CO (Batch 05K0511)
PRB916-01	0.303	0.370
PRB916-02	0.466	0.476
PRB916-03	0.462	0.490
PRB916-04	12.730	12.007
PRB916-05	13.335	12.750
PRB916-06	13.436	12.556
PRB924-01	0.408	0.349
PRB924-02	0.419	0.404
PRB924-03	0.512	0.412
PRB924-04	0.423	0.420
PRB924-05	9.578	7.561
PRB924-06	11.856	10.227
PRB924-07	12.900	12.129
PRB924-08	13.420	13.075
PRB932-01	0.392	0.369
PRB932-02	0.365	0.337
PRB932-03	0.369	0.314
PRB932-04	5.938	4.443
PRB932-05	12.939	12.788
PRB932-06	11.911	12.365
PRB932-07	12.369	12.173
PRB932-08	6.431	4.843
PRB932-09	6.454	4.792
PRB939-01	0.330	0.431
PRB939-02	0.380	0.486
PRB939-03	0.377	0.459
PRB939-04	0.338	0.459
PRB939-05	0.675	0.557
PRB939-06	1.195	0.894
PRB939-07	12.505	11.635
PRB939-08	13.137	12.694
PRB939-09	13.490	13.361
BCP 6240-01	0.333	0.304
BCP 6240-02	0.283	0.300
BCP 6240-03	0.313	0.300
BCP 6240-04	0.313	0.293
BCP 6240-05	0.333	0.296
BCP 6240-06	0.409	0.450
BCP 6240-07	0.631	0.600
BCP 6240-08	2.637	2.762
BCP 6240-09	12.833	12.413
BCP 6240-10	13.185	12.556
BCP 6240-11	13.383	12.830
BCP 6240-12	13.261	12.552
BCP 6240-13	13.066	12.505

Red Values indicate negative values / specimens

## Manufacturer's Comments

**From Jean-François Delagneau, Research Manager, BioRad:**

*First of all, I would like to thank you for the courtesy in allowing us to comment on the results obtained from Genscreen ULTRA HIV Ag-Ab. Your evaluation and associated report presentation of the results are reproducibly outstanding. We are very pleased that you have confirmed Genscreen ULTRA HIV Ag-Ab as one of the two currently available most sensitive assays for HIV immunodiagnosis and screening. You showed evidence of a substantial sensitivity increase in early seroconversion detection with regard to our previous Genscreen Plus Ag-Ab assay. Indeed, your results reflect and extend our experience. We also collected data (which were published during the 2004 ISBT Congress In Edingurg) demonstrating that the new assay may rank higher than the Axsym one when a 85 seroconversion panels was tested.*

*The quality control reagent results and the batch comparison data meet our expectations. We do know that reproducibility data (within and between batches) are even better when Genscreen Plus Ag-Ab assay is integrated into our fully automated ELISA platform.*

*Finally, we are keen to mention that the reported issue with the glass vial breaking is not a Genscreen Ultra specific issue. We are sorry for this sporadic incident. We confirm that our kits including Genscreen Ultra are in compliance with strict CE requirement, especially regarding Health and Safety issues.*

*We are convinced that kit users and those who read your technical report will benefit as usual from your positive and professional comments and conclusions.*

*I remain yours sincerely.*

*Jean-François Delagneau  
Research Manager*