



*Blood and Transplant*



# **Evaluation of Abbott Architect HIV Ag/Ab Combo Assay Product code 4J27**

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## Introduction

The Microbiological Diagnostics Assessment Service (HPA-MiDAS) in conjunction with the National Blood Service carried out six evaluations of assays for the Abbott Architect i200SR analyser. The object of the evaluations was to assess the ability of the Architect HBsAg, HIV Ag/Ab combo, Anti-HCV, Rubella IgG, anti-CMV and Syphilis TP assays to detect serological evidence of each respective marker in human serum and plasma specimens.

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

Abbott Diagnostics provided all equipment, reagents and consumables required for this evaluation. They were responsible for the training of the operators in the use of the Architect i200SR analyser and for the installation and ongoing maintenance and repair/replacement of any faulty equipment. The Architect analyser was installed in the National Transfusion Microbiology Reference Laboratory (NTMRL), North London Blood Centre, where all testing took place.

HPA-MiDAS was responsible for the testing of the evaluation panel which consisted of anti-HIV positive samples, seroconversion panels and quality control samples. NTMRL was responsible for the specificity testing of a panel of HIV negative ante-natal samples (this category of samples, rather than blood donors' specimens, was chosen as potentially the Architect system would be used on this type of specimen).

## Description of the assay

The Abbott Architect HIV Ag/Ab Combi assay is a two-step sandwich chemiluminescent microparticle immunoassay (CMIA) for the detection of HIV p24 antigen and antibody to HIV in human serum or plasma. HIV p24 present in the sample binds to a mouse monoclonal p24 antibody, and anti-HIV present in the sample binds to recombinant HIV 1 and HIV 2 antigens coated onto the paramagnetic particles. After a wash step, acridinium-labelled HIV 1 recombinant antigens, acridinium-labelled HIV 1 and HIV 2 synthetic peptides and acridinium-labelled mouse monoclonal HIV p24 antibody conjugates are added. Following a further wash step, pre-trigger solution (hydrogen peroxide) and trigger solution (sodium hydroxide) are added. The resulting chemiluminescent reaction is measured in relative light units (RLUs) which are directly proportional to the amount of HIV 1 & 2 antibodies and/or HIV p24 present in the sample.

**Table 1: Assay information**

<b>General</b>	
Assay name	Architect HIV Ag/Ab Combo
Manufacturer/UK agent	Abbott Diagnostics
Product number	4J27
Number of tests per pack	100 / 500
Sample volume (including 'dead volume')	150µL
<b>Presentation</b>	
Assay type	Two-step chemiluminescent sandwich immunoassay
Solid phase	Paramagnetic microparticles coated with HIV 1/HIV 2 antigen (recombinant) and HIV p24 antibody (mouse monoclonal)
Conjugate	Acridinium-labelled HIV 1 antigens (recombinant), acridinium-labelled HIV 1/HIV 2 synthetic peptides and acridinium-labelled HIV p24 antibody (mouse monoclonal)
Substrate	Pre-trigger - hydrogen peroxide solution Trigger - sodium hydroxide solution
Negative control	1
Positive control	
1 - Anti-HIV 1	1
2 - Anti-HIV 2	1
3 - HIV antigen	1
Reading wavelength	n/a - chemiluminescent
Cut-off computation	Mean calibrator RLU value x 0.40
Equivocal zone	None
<b>Stages</b>	
Preparation/sample well loading	5 minutes
Specimen volume (excluding 'dead volume')	100µL
Incubation status	37°C
Sampling time - 1 sample	1 minute
Total time to completion (from initial loading of first sample)*	
- 1 sample	30 minutes
- 10 samples	34 minutes
- 100 samples	60 minutes
<b>Additional equipment requirement</b>	
Centrifuge	
Latex/nitrile gloves & personal protective equipment	
<b>Note:</b> * These data were observed timings by the evaluator. Information provided by Abbott Laboratories: Throughput 100 tests per hour for the first hour and 200 tests per hour after the first result is generated.	

## Evaluation panel and method

A total of 1375 samples were included in the evaluation panel, Table 2.

The main specificity study was carried out by the NTMRL, for which 1001 ante-natal patients' samples previously screened and found negative for anti-HIV were tested. Additionally, the NTMRL also tested a panel of 17 samples which comprised the NBS Lot Release Testing panel. Nine of the 17 samples were positive for anti-HIV 1, five were positive for anti-HIV 2, two were positive for HIV antigen and one was negative.

The evaluation panel used by the HPA-MiDAS totalled 357 specimens, Table 2. Two hundred specimens were from anti-HIV positive samples representing various risk groups and geographical locations, two of which were HIV subgroup 'O' and 20 were from

individuals with HIV 2 infection. Twenty samples were from anti-HIV negative ante-natal patients. Twenty seroconversion panels from commercial sources were also included. It was agreed with the NBS, Abbott Diagnostics and HPA-MiDAS that those panels for which Abbott Diagnostics did not have data would be included in the panel. One panel was chosen to be also tested by HPA-MiDAS to allow for a limited comparison with the Abbott data for that panel. In addition, 13 quality control samples, from the HPA and NIBSC, were included in the panel.

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

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

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

Prior to running a new batch of an assay, a calibration must first be performed. The calibrators are provided in dropper bottles and an appropriate volume is placed into sample cups and loaded onto the analyser. The calibration is valid for all subsequent tests using that particular lot number; it is not time limited. For the HIV Ag/Ab Combo assay, one calibrator is supplied which is run in triplicate. The mean of the triplicate RLU values x 0.40 is calculated to provide the cut-off for the reagent lot.

Four HIV kit controls are provided by Abbott Diagnostics. It is recommended that these are run at least once within every 24 hours that the test is in use. The controls consist of a Negative Control (S/CO  $\leq 0.50$ ), Positive Control 1 (HIV 1, S/CO 1.20-11.50), Positive Control 2 (HIV 2, S/CO 1.52-6.50) and Positive Control 3 (purified HIV viral lysate, S/CO 1.82-4.46)

Samples may be loaded in their primary tubes, if suitable for the analyser, or aliquots made into sample cups. Once ordered and the analyser is put into 'Running' mode, sample processing is initiated by the loading of the samples onto the analyser. The reactions occur in the following processing sequence: -

- A reaction mixture is formed combining sample, sample diluent and microparticles in the reaction vessel.
- After the first incubation is complete, the reaction mixture undergoes a wash step. A magnetic field is applied to retain the paramagnetic microparticles within the reaction vessel during the wash procedure.
- The anti-HIV/acridinium and HIV ag/acridinium conjugates are then added and a further incubation takes place.
- Following a second wash step, pre-trigger (hydrogen peroxide) and trigger (sodium hydroxide) solutions are added to the reaction vessel

- The resultant chemiluminescent signal is measured and expressed as Relative Light Units.

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

The results are expressed as sample/cut-off (S/CO); S/CO value <1.0 is considered nonreactive and S/CO  $\geq$ 1.0 is considered reactive. The sensitivity of the Abbott Architect HIV Ag/Ab Combo assay for HIV p24 is stated in the kit insert as <50pg/mL.

**Table 2. Evaluation panel**

Specimen Category	Number
<b>NTMRL, NBS</b>	
1. Anti-HIV negative - ante-natal patients' samples	1001
2. HIV lot release testing panel	17
<b>HPA-MiDAS</b>	
1. Anti-HIV negative -	20
2. Anti-HIV positive	
a) Anti-HIV 1 (various risk groups & geographical regions)	178
b) Anti-HIV 1 subgroup O	2
c) Anti-HIV-2 positive	20
3. HIV seroconversion panels	
BBI – PRB916	6
BBI – PRB917	7
BBI – PRB919	3
BBI – PRB922	4
BBI – PRB924	8
BBI – PRB925	6
BBI – PRB929	7
BBI – PRB930	4
BBI – PRB932	9
BBI – PRB937	6
BBI – PRB938	3
BBI – PRB939	9
BBI – PRB940	8
BBI – PRB943	7
BBI – PRB944	6
BBI – PRB945	6
BBI – PRB946	4
BBI - PRB947	4
BBI – PRB948	4
BCP 6240	13
4. Quality Control samples	
HPA-HIV1-QC1	3x 1
HPA-HIV1-QC2	3x 1
HPA-HIV1-QC3	3x 1
HPA-HIV1-QC4	3x 1
HPA-HIV1-QC5	3x 1
HPA-HIV2-QC2	3x 1
HPA-HIV2-QC3	3x 1
HPA-p24 Ag-QC1	3x 1
HPA-p24 Ag-QC2	3x 1
NIBSC-HIV 1 British working standard	3x 1
NIBSC-HIV 1 British working standard, 1 in 5	3x 1
NIBSC-HIV 2 Monitor sample	3x 1
Bio-Rad HIV 1 Ag dilution series	6x 1
<b>TOTAL</b>	<b>1375</b>
BBI = Boston Biomedica Inc; BCP = BioClinical Partners Inc;	
HPA = Health Protection Agency	
NIBSC = National Institute for Biological Standards and Control.	

## Specificity

One thousand and one specimens from ante-natal patients were tested by the Architect HIV Ag/Ab Combo assay at NTMRL. The specimens had been previously screened by the NBS using the bioMérieux Vironostika Uniform II assay, product code: 285047, and found to be HIV unreactive.

Nine hundred and ninety-three of the 1001 specimens were unreactive in the Architect HIV Ag/Ab Combo on initial testing to give an initial specificity of 99.20% (95% confidence interval 98.4-99.7%), Table 3. Eight specimens were initially reactive giving an initial reactive rate of 0.80% (95% confidence interval 0.35-1.57%). Five specimens were again reactive on repeat testing giving a repeat reactive rate 0.5% (95% confidence interval 0.17-1.17%). The S/CO results of these specimens are shown in Table 4, along with the results of further testing where sufficient specimen volume was available.

**Table 3: Specificity**

Architect HIV Ag/Ab Combo result	Number	Number reactive	Reactive rate (95% confidence interval)	Mean S/CO		Median S/CO		Range S/CO	
				Initial	Repeat	Initial	Repeat	Initial	Repeat
Negative	993	NA	NA	0.21	NA	0.19	NA	0.08-0.79	NA
Reactive	8	NA	NA	4.76	2.63	2.79	1.85	1.00-20.73	0.18-8.79
Total	1001	8	0.8% (0.35-1.57%)	0.25	0.23	0.19	0.19	0.08-20.73	0.08-8.79

**Table 4: Results of false positive samples**

Sample number	Architect HIV Ag/Ab Combo			Murex HIV Ag/Ab	Biorad Genscreen Ultra HIV Ag/Ab
	Initial	Repeat			
29	1.92	1.85	1.96	0.293/0.306	0.322/0.300
264	4.51	5.04	4.85	0.344/0.333	0.326/0.282
340	1.25	8.79	3.06	0.290/0.270	0.374
343	3.05	3.28	Insuff	0.286/0.267	0.474/0.366
802	2.53	6.83	3.37	0.410/0.383	0.329/0.362
510	20.73	0.35	0.26	Insuff	Insuff
790	1.02	0.77	0.59	Insuff	Insuff
797	1.00	0.262	0.18	Insuff	Insuff

**Note:** All results given as S/CO

Twenty anti-HIV negative samples from ante-natal patients were interspersed among the anti-HIV positive samples when tested by the HPA-MiDAS. One sample was initially weakly reactive but was below the cut-off when repeat tested, Tables 5a and 5b.

**Table 5a: Initial specificity**

Number tested	Number initially reactive	Mean	Median	Range
		S/CO		
20	1	0.39	0.31	0.13-1.09

**Table 5b. Repeat specificity**

Number tested	Number repeat reactive	Mean	Median	Range
		S/CO		
20	0	0.35	0.31	0.13-0.98

## Sensitivity

Two-hundred specimens positive for HIV were tested by the Architect HIV Ag/Ab Combo assay, lot number 51322HN00. All 200 specimens were detected by the assay giving a sensitivity of 100% (95% confidence interval 98.2-100%). Table 6 shows the number of samples and S/CO values according to risk category.

**Table 6. Sensitivity**

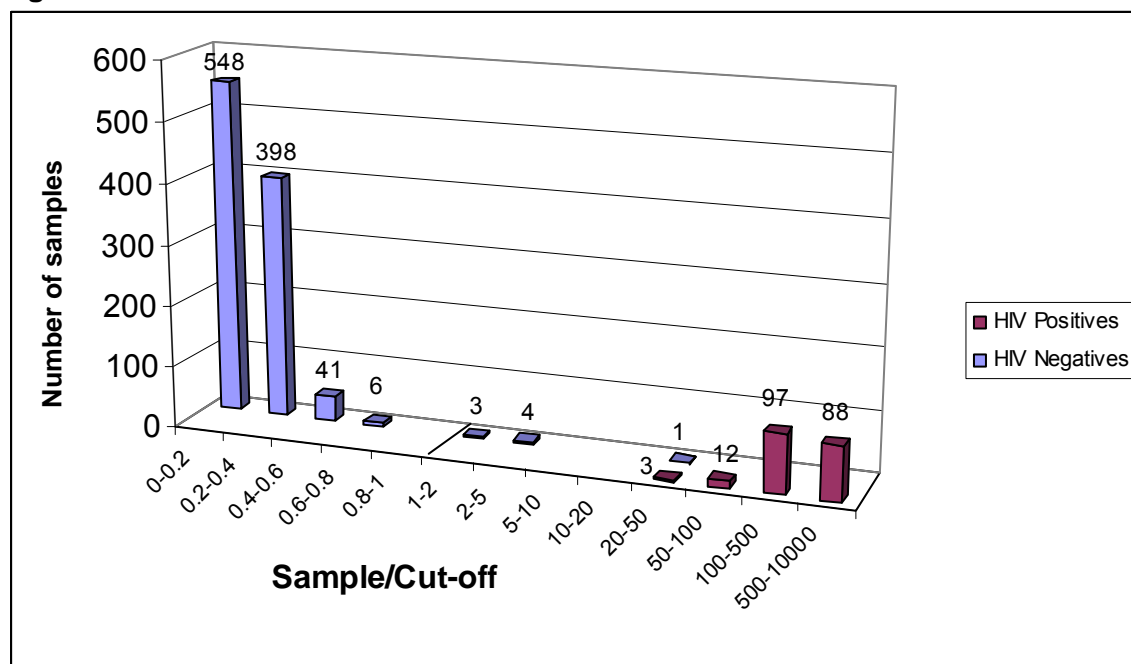
Category	Number tested	Number reactive	Mean	Median	Range
			S/CO		
HIV-1 - Argentina	2	2	461.7	NA	439.2-484.2
HIV-1 - Ghana	11	11	488.4	487.7	256.8-775.7
HIV-1 - India	3	3	435.6	450.4	401.7-454.6
HIV-1 - Ivory Coast	2	2	441.4	NA	373.9-509.0
HIV-1 - Mozambique	2	2	542.3	NA	443.3-641.2
HIV-1 - N. America	15	15	414.5	407.9	43.8-650.0
HIV-1 - S.Africa	11	11	338.2	306.6	110.0-552.4
HIV-1 - Uganda	15	15	406.0	406.6	37.1-665.5
HIV-1 - Zimbabwe	3	3	452.4	465.3	371.6-520.4
HIV-1 Bisexual	1	1	763.4	NA	NA
HIV-1 Homosexual	17	17	587.4	583.3	216.4-802.4
HIV-1 IVDU	15	15	454.0	461.6	119.3-625.7
HIV-1 Multiple partners	51	51	542.3	565.1	190.9-789.2
HIV-1 Partner HIV pos	3	3	430.3	379.0	352.2-559.8
HIV-1 Prostitute	4	4	560.9	546.5	513.4-637.3
HIV-1 Transfusion received	6	6	460.3	404.3	333.9-697.8
HIV-1 Group O	2	2	233.8	NA	22.6-444.9
HIV-1/2	16	16	453.5	452.0	199.8-662.5
HIV-2	21	21	105.3	104.0	59.8-136.4
<b>All</b>	<b>200</b>	<b>200</b>	<b>442.0</b>	<b>477.5</b>	<b>22.6-802.4</b>

## Distribution of initial reactivities

The distribution of initial reactivities for the 1001 HIV negative samples tested by NTMRL and 200 HIV positive samples tested by HPA-MiDAS is shown in Figure 1. Assays with good discrimination have few or no samples wrongly classified and few reactions close to the cut-off.

The Architect HIV Ag/Ab Combo assay gave eight initially false reactive results, of which five were reactive after retesting. No false negative results were observed and all the positive results gave high reactivities.

**Figure 1. Distribution of initial reactivities**



**Note:** The scale for S/CO values is not continuous

## Results of testing of NBS Lot Release Panel

Seventeen specimens that comprise the NBS HIV lot release panel were tested at NTMRL. One specimen was anti-HIV negative, 14 were anti-HIV positive (9 anti-HIV 1 and 5 anti-HIV 2 positive) with reactivities ranging from weak to strong positive, and two were HIV antigen positive.

Fourteen of the 16 positive specimens were reactive in the Architect HIV Ag/Ab Combo assay. Two samples were nonreactive and reactive (S/CO 0.92 / 1.30 and 0.64 / 1.10) when tested in duplicate. The negative specimen was nonreactive in the Architect HIV Ag/Ab Combo assay, Table 7.

Of the two HIV antigen positive samples tested, one was nonreactive and reactive (S/CO 064 and 1.10) when tested in duplicate and one was reactive (S/CO 1.82) in the Architect HIV Ag/Ab Combo assay, Table 7.

**Table 7. NTMRL Lot Release Panel**

Panel/Ctrl Member	Expected Ratios	Expected Result	Architect HIV Ag/Ab Combo	
			S/CO	Result
1	>1.2	Positive	1.09	Reactive
2	>2	Positive	2.22	Reactive
3	>1	Positive	2.02	Reactive
4	>1	Positive	0.92/1.30	Nonreactive/Reactive
5	>1.5	Positive	13.87	Reactive
6	>2.4	Positive	7.16	Reactive
7	>2	Positive	8.42	Reactive
8	>1.4	Positive	7.02	Reactive
9	>2.5	Positive	7.62	Reactive
10	>10	Positive	30.83	Reactive
11	>1.6	Positive	4.98	Reactive
12	>10	Positive	21.88	Reactive
13	<0.5	Negative	0.16/0.14	Nonreactive
14	>1	Positive	4.78	Reactive
15	>1.8	Positive	2.10	Reactive
Ag01	>1.5	Positive	0.64/1.10	Nonreactive/Reactive
Ag02	>1	Positive	1.82	Reactive

Note: Panel members 1-15 are anti-HIV positive. Panel members Ag01 and Ag02 are HIV antigen positive.

## Seroconversion sensitivity

Twenty commercial seroconversion panels were tested by HPA-MiDAS, 19 panels from Boston Biomedica Inc (BBI) and one from Zeptometrix (previously known as BioClinical Partners Inc, BCP).

For 18 of the 19 BBI panels, comparative data were available for 21 other HIV assays. Of the total of 106 samples, 71 were detected by the Architect HIV Ag/Ab Combo assay, which was second most sensitive in the comparison, Table 8. (Panels PRB947 and 6240 were not included as insufficient comparative data were unavailable, however the S/CO values may be seen in *Appendix Table 12*).

Comparative panel data for the 21 kits are shown in *Appendix Tables 11a and 11b* and the S/CO data for the Architect HIV Ag/Ab Combo assay are shown in *Appendix Tables 12 and 13*.

**Table 8. Comparative seroconversion sensitivity based on 18 panels**

HIV assay	Product number	Cumulative score * (PRB916-948) n=106	Rank
AxSYM <sup>®</sup> HIV Ag/Ab Combo	2G83-20	<b>72</b>	<b>1</b>
<b>Architect HIV Ag/Ab Combo</b>	<b>4J27-20</b>	<b>71</b>	<b>2</b>
GENSCREEN <sup>®</sup> Ultra HIV Ag-Ab	72388/72386	<b>69</b>	<b>3</b>
Prism HIV Ag/Ab Combo	7G46-48	<b>67</b>	<b>4</b>
Murex HIV Ag/Ab Combination	GE41/42	<b>67</b>	<b>4</b>
Detect HIV v4	RHD302A	<b>67</b>	<b>4</b>
HIV combi	4860446	<b>64</b>	<b>7</b>
VIDAS HIV DUO	30114	<b>64</b>	<b>7</b>
GENSCREEN <sup>®</sup> PLUS HIV Ag-Ab	72375/72376	<b>63</b>	<b>9</b>
Enzygnost <sup>®</sup> HIV Integral	31843	<b>60</b>	<b>10</b>
Vironostika <sup>®</sup> HIV Uni-Form II Ag/Ab	6029/30/31	<b>50</b>	<b>11</b>
Genscreen <sup>®</sup> HIV1/2 EIA (v2)	72279	<b>46</b>	<b>12</b>
Vitros <i>ECi</i> Anti-HIV 1+2	124-1850	<b>44</b>	<b>13</b>
Murex HIV 1.2.O	GE94/95	<b>44</b>	<b>13</b>
Access <sup>®</sup> HIV 1/2 NEW	34020	<b>44</b>	<b>13</b>
Biotest Anti-HIV TETRA ELISA	807 008	<b>43</b>	<b>16</b>
AxSYM <sup>®</sup> HIV 1/2 gO	3D41-20	<b>43</b>	<b>16</b>
Ortho <sup>®</sup> HIV-1/HIV-2 Ab-capture ELISA Test System	932380	<b>42</b>	<b>18</b>
IMx <sup>®</sup> HIV-1/HIV-2 III Plus	8C98	<b>41</b>	<b>19</b>
Wellcozyme HIV 1+2 EIA	VK55	<b>40</b>	<b>20</b>
Vironostika <sup>®</sup> HIV Uni-Form II <i>plus O</i>	84018	<b>40</b>	<b>20</b>
Enzygnost <sup>®</sup> Anti-HIV1/2 Plus	OQFK 12/13	<b>40</b>	<b>20</b>
<b>Notes:</b>			
* The score was calculated by summing the number of positive samples for each of the seroconversion panels. A higher score suggests higher sensitivity.			
The position in this table is based on 18 seroconversion panels.			

## Timing of detection

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

The median detection time for the Architect HIV Ag/Ab Combo kit was 0 days which ranks the assay as joint first with six other assays, Table 9 and Figure 2a. 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.

On the basis of the mean value, the Architect HIV Ag/Ab Combo was ranked the second most sensitive assay for the panels tested. The assay detected HIV approximately 0.3 days later than the kit ranked first, AxSYM HIV Ag/Ab Combo, Table 9 and Figure 2b.

**Table 9. Delay in detection of seroconversion**

Assay	Product code	Delay in detecting seroconversion in each panel compared with the most sensitive assay		
		Range	Median	Mean
AxSYM <sup>®</sup> HIV Ag/Ab Combo	2G83-20	0 - 7	0	0.6
<b>Architect HIV Ag/Ab Combo</b>	<b>4J27-20</b>	<b>0 - 6</b>	<b>0</b>	<b>0.9</b>
GENSCREEN <sup>®</sup> Ultra HIV Ag-Ab	72388/72386	0 - 7	0	1.5
Detect HIV v4	RHD302A	0 - 7	0	2.1
HIV combi	4860446	0 - 7	0	2.4
Murex HIV Ag/Ab Combination	GE41/42	0 - 53	0	4.8
Prism HIV Ag/Ab Combo	7G46-48	0 - 53	0	4.8
VIDAS HIV DUO	30114	0 - 53	3.5	5.5
GENSCREEN <sup>®</sup> PLUS HIV Ag-Ab	72375/72376	0 - 53	3.5	5.7
Enzygnost <sup>®</sup> HIV Integral	31843	0 - 53	4	6.3
Vironostika <sup>®</sup> HIV Uni-Form II Ag/Ab	6029/30/31	0 - 57	6.5	8.6
Genscreen <sup>®</sup> HIV1/2 EIA (v2)	72279	0 - 65	7	10.2
Access <sup>®</sup> HIV 1/2 NEW	34020	0 - 65	7	10.8
Murex HIV 1.2.O	GE94/95	0 - 87	7	15.0
Vitros <i>ECi</i> Anti-HIV 1+2	124-1850	0 - 87	7	15.1
Biotest Anti-HIV TETRA ELISA	807 008	0 - 87	8	15.0
AxSYM <sup>®</sup> HIV 1/2 gO	3D41-20	0 - 87	8	15.3
Ortho <sup>®</sup> HIV-1/HIV-2 Ab-capture ELISA Test System	932380	0 - 87	8	15.5
Vironostika <sup>®</sup> HIV Uni-Form II <i>plus O</i>	84018	0 - 87	8.5	16.0
IMx <sup>®</sup> HIV-1/HIV-2 III Plus	8C98	0 - 87	9	15.7
Enzygnost <sup>®</sup> Anti-HIV1/2 Plus	OQFK 12/13	0 - 87	9	15.8
Wellcozyme HIV 1+2 EIA	VK55	0 - 87	9	16.1

**Notes:** The upper limit of the range is, to some extent, influenced by the intervals between bleeds for any individual panel. The mean Time 0 = earliest detection of HIV infection by any screening assay.

Figure 2a. Timing of detection for combined HIV antigen and antibody detection assays – median values

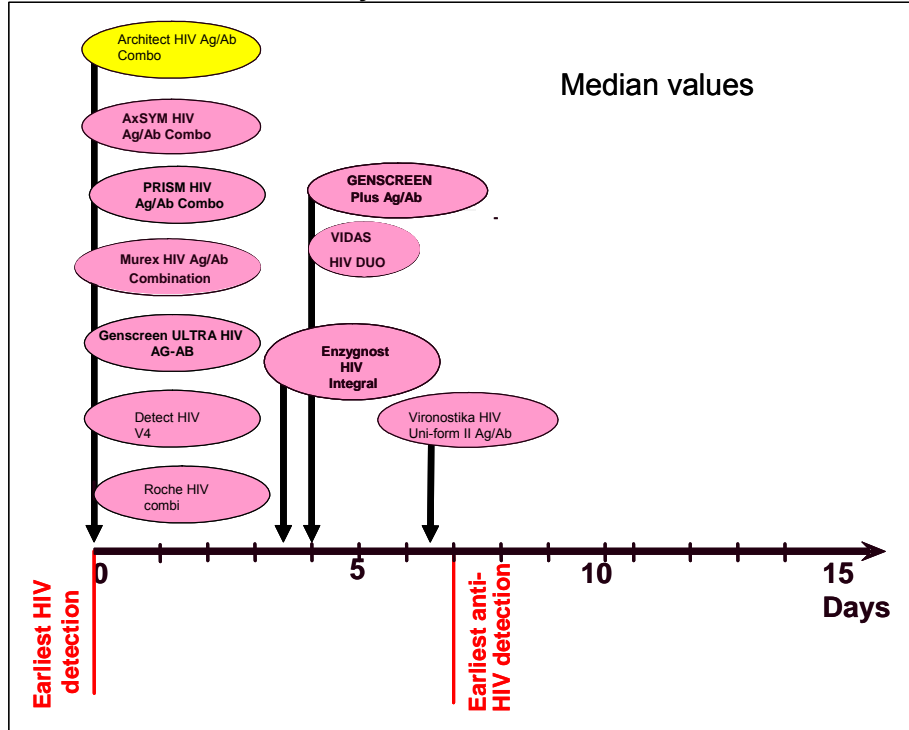
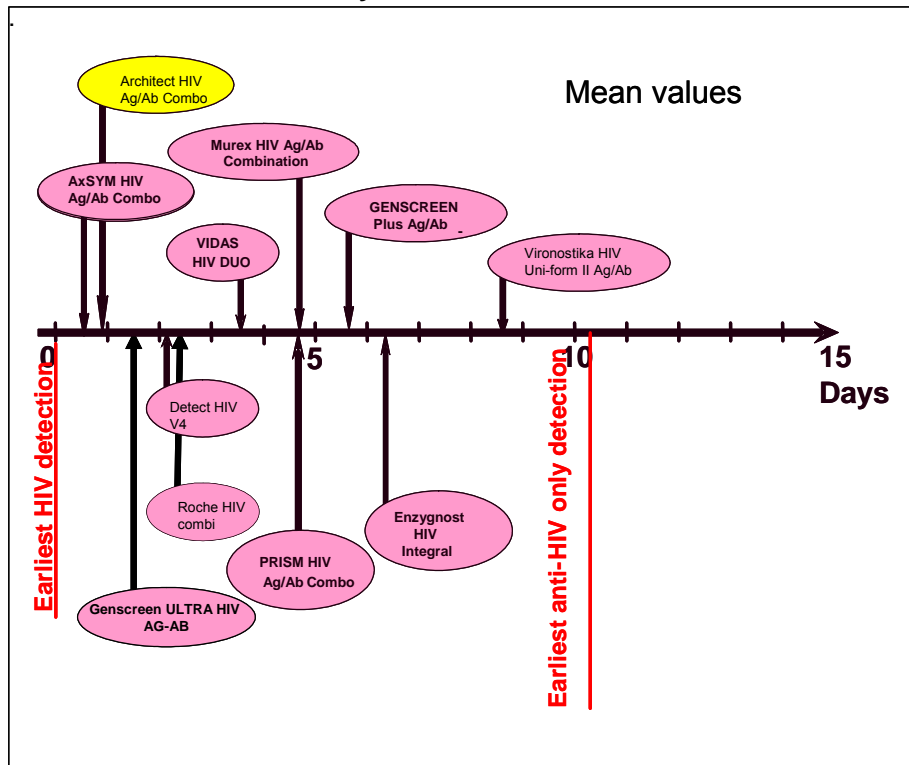


Figure 2b. Timing of detection for combined HIV antigen and antibody detection assays – mean values



## Lot comparison

A subset of the main evaluation panel was tested by a second lot of the assay (Lot number 50482HN00). Forty-nine anti-HIV positive samples, 10 negative samples and five seroconversion panels were compared, Tables 10a, 10b, 10c and 13. The S/CO results obtained from both lots were very similar for the positive specimens; however one negative sample initially gave a low reaction (S/CO 1.26) when tested with Lot 2. For the five seroconversion panels, lot 1 detected 23 samples and lot 2 detected 24 of the total of 43 specimens. The sample which was discordant, 6240-07, had a S/CO value of 0.79 for lot 1 and 1.00 for lot 2.

Additionally, eight HPA quality control samples, three NIBSC samples and a dilution series of the Bio-Rad HIV1 Antigen Standard were tested in both lots of the assay, Tables 10d and 10e.

**Table 10a. Comparison of 49 Anti-HIV positive samples**

Anti-HIV Positive	Lot 1		Lot 2	
	51322HN00		50482HN00	
Number tested	49		49	
Number reactive	49		49	
Mean	420.07		438.17	
Range	22.63-802.38		21.69-748.13	

**Table 10b. Comparison of 10 Anti-HIV negative samples**

Anti-HIV negative	Lot 1		Lot 2	
	51322HN00		50482HN00	
Number tested	10		10	
Number initial reactive	0		1*	
*S/CO 1.26 (not retested)				

**Table 10c. Comparison of 5 seroconversion panels**

Panel (number of samples)	Number reactive	
	Lot 1	Lot 2
	51322HN00	50482HN00
PRB916 (6)	3	3
PRB924 (8)	4	4
PRB932 (9)	6	6
PRB939 (9)	4	4
6240 (11)	6	7
Total (43)	23	24

**Table 10d. Results of quality control samples**

Quality control sample	Lot 1 51322HN00 (S/CO)				Lot 2 50482HN00 (S/CO)			
	Test 1	Test 2	Test 3	Mean	Test 1	Test 2	Test 3	Mean
HPA-HIV1-QC1	116.78	117.04	119.91	117.91	112.90	114.04	114.50	113.82
HPA-HIV1-QC2	6.85	6.94	6.99	6.93	6.68	6.91	6.89	6.83
HPA-HIV1-QC3	1.74	1.79	1.83	1.78	1.87	1.84	1.82	1.84
HPA-HIV1-QC5	54.12	54.12	54.55	54.26	52.92	55.09	52.45	53.49
HPA-HIV2-QC2	18.62	19.43	19.48	19.18	19.29	19.96	20.06	19.77
HPA-HIV2-QC3	4.41	4.54	4.74	4.56	4.41	4.66	4.36	4.48
HPA-p24 QC1	43.51	43.91	44.11	43.84	43.74	40.72	41.07	41.84
HPA-p24 QC2	2.82	2.82	2.83	2.82	2.71	2.61	2.94	2.75
NIBSC-HIV 1 British working standard	7.45	7.53	8.32	7.77	7.81	7.53	7.24	7.53
NIBSC-HIV 1 British working standard, 1 in 5	1.43	1.45	1.51	1.47	1.50	1.44	1.43	1.46
NIBSC-HIV 2 Monitor sample	13.08	13.04	14.45	13.52	14.59	14.30	13.57	14.16

A quality control sample/statistical assay control should be chosen to have a reactivity within the linear dynamic range of the assay. Our findings suggest that HPA-HIV1-QC3, HPA-HIV2-QC3 and HPA-p24-QC2I controls would be suitable for use in the Architect HIV Ag/Ab Combo assay.

**Table 10e. Results of dilution series of BioRad HIV1 Antigen Standard**

HIV antigen concentration	Lot 1 51322HN00 (S/CO)				Lot 2 50482HN00 (S/CO)			
	Test 1	Test 2	Test 3	Mean	Test 1	Test 2	Test 3	Mean
0.25ng/mL	11.75	12.30	12.07	12.04	12.06	12.76	12.52	12.45
0.125ng/mL	5.48	5.86	5.48	5.61	5.52	5.87	5.56	5.65
0.063ng/mL	2.60	2.59	2.82	2.67	2.41	2.35	2.45	2.40
0.031ng/mL	1.08	1.12	1.11	1.10	1.05	1.25	1.13	1.15
0.016ng/ml	0.42	0.52	0.51	0.48	0.44	0.42	0.43	0.43
0.008ng/mL	0.26	0.29	0.23	0.26	0.26	0.27	0.27	0.27

The BioRad HIV1 Antigen Standard is the French national standard approved by the AFSSAPS. A dilution series of the standard was made and tested by both lots of the Architect HIV Ag/Ab Combo assay. The assay detected HIV antigen at a level of 0.31ng/ml.

## Conclusion

The Architect HIV Ag/Ab Combo assay had an initial reactive rate of 0.80% (95% confidence interval 0.35-1.57%). The eight initially reactive samples had S/CO values between 1.00 and 20.73. Five of the eight specimens were again reactive on repeat testing giving a repeat reactive rate 0.5% (95% confidence interval 0.17-1.17%) and repeat reactive rate of 0.5%, with S/CO values between 1.85 and 8.79. (Note that the specificity rate was established in a

population of ante-natal patients, whereby the specificity may be slightly reduced when compared with that observed in blood donor populations [Personal communication].)

All 200 anti-HIV positive samples tested were positive in the assay to give a sensitivity of 100%. The specimens were strongly positive with all samples giving S/CO values >20.

Twenty seroconversion panels were tested, for 19 of which comparative data are available. The Architect HIV Ag/Ab Combo assay was ranked 2nd most sensitive when compared with a total of 27 assays previously evaluated. When assessed for the delay in seroconversion detection, the Architect HIV Ag/Ab Combo assay was joint first most sensitive with five other assays when based on the median value and second most sensitive when based on the mean value.

A small lot comparison was undertaken in which two lots of the Architect HIV Ag/Ab Combo assay were compared. The S/CO results obtained from both lots were very similar for the positive specimens; however one negative sample initially gave a low reaction (S/CO 1.26) when tested with Lot 2. For the five seroconversion panels, lot 1 detected 23 specimens and lot 2 detected 24 of the total of 43 specimens.

Appendix

Table 11a. Comparative results of seroconversion panels PRB916 – PRB 937

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 * (PRB916-937) n=59
		PRB916	PRB917M	PRB919	PRB922	PRB924	PRB925	PRB929	PRB930	PRB932	PRB937	
		n=6	n=6	n=3	n=4	n=8	n=6	n=7	n=4	n=9	n=6	
AxSYM® HIV Ag/Ab Combo	2G83-20	4 (9)	6 (0)	3 (0)	4 (0)	4 (26)	2 (44)	4 (18)	4 (0)	6 (27)	4 (9)	41
<b>Architect HIV Ag/Ab Combo</b>	<b>4J27-20</b>	<b>3 (15)</b>	<b>6 (0)<sup>2</sup></b>	<b>3 (0)</b>	<b>4 (0)</b>	<b>4 (26)</b>	<b>2 (44)</b>	<b>5 (14)</b>	<b>4 (0)</b>	<b>6 (27)</b>	<b>3 (14)</b>	<b>40</b>
GENSCREEN® Ultra HIV Ag-Ab	72388/72386	3 (15)	6 (0)	3 (0)	4 (0)	4 (26)	2 (44)	5 (14)	3 (3)	6 (27)	3 (14)	39
Detect HIV v4	RHD302A	3 (15)	6 (0)	3 (0)	4 (0)	4 (26)	2 (44)	4 (18)	4 (0)	6 (27)	3 (14)	39
Prism HIV Ag/Ab Combo	7G46-48	3 (15)	5 (53)	3 (0)	4 (0)	4 (26)	2 (44)	4 (18)	4 (0)	6 (27)	3 (14)	38
Murex HIV Ag/Ab Combination	GE41/42	3 (15)	5 (53)	3 (0)	4 (0)	4 (26)	2 (44)	4 (18)	4 (0)	6 (27)	3 (14)	38
Roche HIV combi	4860446	3 (15)	6 (0) <sup>1</sup>	3 (0)	4 (0)	4 (26)	2 (44)	4 (18)	3 (3)	6 (27)	2 (16)	37
VIDAS HIV DUO	30114	3 (15)	5 (53)	3 (0)	4 (0)	4 (26)	2 (44)	4 (18)	3 (3)	6 (27)	3 (14)	37
GENSCREEN® PLUS HIV Ag-Ab	72375/72376	3 (15)	5 (53)	3 (0)	4 (0)	4 (26)	2 (44)	4 (18)	3 (3)	6 (27)	3 (14)	37
Enzygnost® HIV Integral	31843	3 (15)	5 (53)	3 (0)	4 (0)	4 (26)	2 (44)	4 (18)	4 (0)	6 (27)	1 (21)	36
Vironostika® HIV Uni-Form II Ag/Ab	6029/30/31	3 (15)	4 (57)	2 (9)	4 (0)	4 (26)	2 (44)	3 (21)	2 (7)	6 (27)	1 (21)	31
Vitros ECi Anti-HIV 1+2	124-1850	2 (30)	3 (65)	2 (9)	4 (0)	3 (33)	2 (44)	3 (21)	2 (7)	6 (27)	1 (21)	28
Murex HIV 1.2.O	GE94/95	2 (30)	3 (65)	2 (9)	4 (0)	3 (33)	2 (44)	3 (21)	2 (7)	6 (27)	1 (21)	28
Genscreen® HIV1/2 EIA (v2)	72279	2 (30)	3 (65)	3 (0)	4 (0)	3 (33)	2 (44)	2 (25)	2 (7)	6 (27)	1 (21)	28
Biotest Anti-HIV TETRA ELISA	807 008	2 (30)	3 (65)	3 (0)	4 (0)	3 (33)	2 (44)	2 (25)	2 (7)	6 (27)	1 (21)	28
AxSYM® HIV 1/2 gO	3D41-20	2 (30)	3 (65)	2 (9)	4 (0)	3 (33)	2 (44)	3 (21)	2 (7)	6 (27)	1 (21)	28
Ortho® HIV-1/HIV-2 Ab-capture ELISA Test System	932380	2 (30)	3 (65)	2 (9)	3 (4)	3 (33)	2 (44)	3 (21)	2 (7)	6 (27)	1 (21)	27
Access® HIV 1/2 NEW	34020	2 (30)	3 (65)	2 (9)	4 (0)	3 (33)	2 (44)	2 (25)	2 (7)	6 (27)	1 (21)	27
Vironostika® HIV Uni-Form II plus O	84018	2 (30)	3 (65)	2 (9)	3 (4)	3 (33)	2 (44)	3 (21)	2 (7)	5 (34)	1 (21)	26
IMx® HIV-1/HIV-2 III Plus	8C98	2 (30)	3 (65)	2 (9)	3 (4)	3 (33)	2 (44)	2 (25)	2 (7)	6 (27)	1 (21)	26
Enzygnost® Anti-HIV1/2 Plus	OQFK 12/13	2 (30)	3 (65)	2 (9)	3 (4)	3 (33)	2 (44)	2 (25)	2 (7)	6 (27)	1 (21)	26
Weilcozyme HIV 1+2 EIA	VK55	2 (30)	3 (65)	2 (9)	4 (0)	3 (33)	2 (44)	2 (25)	2 (7)	5 (34)	0 (>21)	25
Vironostika® HIV Uni-Form II Ag/Ab UPDATE	285047	3 (15)	6 (0)	2 (9)	4 (0)	4 (26)	2 (44)	4 (18)	2 (7)	NT	1 (21)	N/A
Detect-HIV™ (v2)	RHD-202B	2 (30)	3 (65)	2 (9)	2 (7)	3 (33)	2 (44)	1 (28)	2 (7)	5 (34)	NT	N/A
PRISM HIV O Plus	3D34-48	2 (30)	3 (65)	NT	NT	3 (33)	NT	NT	2 (7)	6 (27)	0 (>21)	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)  
<sup>1</sup>Panel members PRB917-04 and -07 were not tested but were scored as positive  
<sup>2</sup>Panel member PRB917-04 was not tested but was scored as positive

**Table 11b. Comparative results of seroconversion panels PRB938 – 948**

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 * (PRB938-948)	Cumulative score * (PRB916 - 948)
		PRB938	PRB939E	PRB940	PRB943	PRB944	PRB945	PRB946	PRB948		
		n=3	n=9	n=8	n=7	n=6	n=6	n=4	n=4		
AxSYM® HIV Ag/Ab Combo	2G83-20	3 (0)	4 (16)	7 (7)	5 (7)	5 (2)	4 (7)	2 (7)	1 (23)	31	72
<b>Architect HIV Ag/Ab Combo</b>	<b>4J27-20</b>	<b>3 (0)</b>	<b>4 (16)</b>	<b>8 (0)</b>	<b>5 (7)</b>	<b>5 (2)</b>	<b>3 (13)</b>	<b>2 (7)</b>	<b>1 (23)</b>	<b>31</b>	<b>71</b>
GENSCREEN® Ultra HIV Ag-Ab	72388/72386	3 (0)	4 (16)	7 (7)	5 (7)	5 (2)	3 (13)	2 (7)	1 (23)	30	69
Prism HIV Ag/Ab Combo	7G46-48	3 (0)	4 (16)	7 (7)	5 (7)	4 (7)	3 (13)	2 (7)	1 (23)	29	67
Murex HIV Ag/Ab Combination	GE41/42	3 (0)	3 (21)	7 (7)	5 (7)	5 (2)	3 (13)	2 (7)	1 (23)	29	67
Detect HIV v4	RHD302A	3 (0)	3 (21)	7 (7)	5 (7)	4 (7)	3 (13)	2 (7)	1 (23)	28	67
Roche HIV combi	4860446	3 (0)	3 (21)	7 (7)	4 (12)	4 (7)	3 (13)	2 (7)	1 (23)	27	64
VIDAS HIV DUO	30114	3 (0)	3 (21)	7 (7)	4 (12)	4 (7)	3 (13)	2 (7)	1 (23)	27	64
GENSCREEN® PLUS HIV Ag-Ab	72375/72376	2 (3)	3 (21)	7 (7)	4 (12)	4 (7)	3 (13)	2 (7)	1 (23)	26	63
Enzygnost® HIV Integral	31843	3 (0)	3 (21)	7 (7)	4 (12)	2 (14)	3 (13)	1 (11)	1 (23)	24	60
Vironostika® HIV Uni-Form II Ag/Ab	6029/30/31	1 (9)	3 (21)	6 (11)	4 (12)	2 (14)	2 (15)	1 (11)	0 (>23)	19	50
GenScreen® HIV1/2 EIA (v2)	72279	1 (9)	2 (23)	6 (11)	2 (19)	4 (7)	3 (13)	0 (>11)	0 (>23)	18	46
Vitros ECi Anti-HIV 1+2	124-1850	1 (9)	1 (103)	7 (7)	2 (19)	2 (14)	3 (13)	0 (>11)	0 (>23)	16	44
Murex HIV 1.2.O	GE94/95	1 (9)	1 (103)	6 (11)	3 (14)	2 (14)	3 (13)	0 (>11)	0 (>23)	16	44
Access® HIV 1/2 NEW	34020	1 (9)	2 (23)	6 (11)	2 (19)	3 (9)	3 (13)	0 (>11)	0 (>23)	17	44
Biotest Anti-HIV TETRA ELISA	807 008	1 (9)	1 (103)	6 (11)	2 (19)	2 (14)	3 (13)	0 (>11)	0 (>23)	15	43
AxSYM® HIV 1/2 gO	3D41-20	1 (9)	1 (103)	6 (11)	2 (19)	2 (14)	3 (13)	0 (>11)	0 (>23)	15	43
Ortho® HIV-1/HIV-2 Ab-capture ELISA Test System	932380	1 (9)	1 (103)	6 (11)	2 (19)	2 (14)	3 (13)	0 (>11)	0 (>23)	15	42
IMx® HIV-1/HIV-2 III Plus	8C98	1 (9)	1 (103)	6 (11)	2 (19)	2 (14)	3 (13)	0 (>11)	0 (>23)	15	41
Wellcozyme HIV 1+2 EIA	VK55	1 (9)	1 (103)	6 (11)	2 (19)	2 (14)	3 (13)	0 (>11)	0 (>23)	15	40
Vironostika® HIV Uni-Form II plus O	84018	1 (9)	1 (103)	6 (11)	2 (19)	2 (14)	2 (15)	0 (>11)	0 (>23)	14	40
Enzygnost® Anti-HIV1/2 Plus	OQFK 12/13	1 (9)	1 (103)	6 (11)	2 (19)	2 (14)	2 (15)	0 (>11)	0 (>23)	14	40
Vironostika® HIV Uni-Form II Ag/Ab UPDATE	285047	2 (3)	3 (21)	7 (7)	4 (12)	4 (7)	2 (15)	1 (11)	1 (23)	N/A	N/A
PRISM HIV O Plus	3D34-48	1 (9)	1 (103)	NT	2 (19)	3 (9)	3 (13)	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.

**Table 12. Results of 15 seroconversion panels tested with Lot 1 51322HN00 only**

Panel	S/CO	Panel	S/CO
PRB917-1	<b>1.02</b>	PRB940-1	<b>1.07</b>
PRB917-2	<b>11.47</b>	PRB940-2	<b>89.73</b>
PRB917-3	<b>92.55</b>	PRB940-3	<b>13.09</b>
PRB917-4	<b>NT</b>	PRB940-4	<b>9.62</b>
PRB917-5	<b>19.48</b>	PRB940-5	<b>17.14</b>
PRB917-6	<b>19.78</b>	PRB940-6	<b>28.15</b>
PRB919-1	<b>8.92</b>	PRB940-7	<b>25.95</b>
PRB919-2	<b>28.86</b>	PRB940-8	<b>16.31</b>
PRB919-3	<b>32.17</b>	PRB943-1	0.12
PRB922-1	<b>38.30</b>	PRB943-2	0.18
PRB922-2	<b>49.34</b>	PRB943-3	<b>3.01</b>
PRB922-3	<b>19.25</b>	PRB943-4	<b>75.73</b>
PRB922-4	<b>9.11</b>	PRB943-5	<b>264.81</b>
PRB925-1	0.09	PRB943-6	<b>65.26</b>
PRB925-2	0.16	PRB943-7	<b>33.72</b>
PRB925-3	0.09	PRB944-1	0.36
PRB925-4	0.14	PRB944-2	<b>1.79</b>
PRB925-5	<b>22.38</b>	PRB944-3	<b>16.72</b>
PRB925-6	<b>32.69</b>	PRB944-4	<b>12.04</b>
PRB929-1	0.14	PRB944-5	<b>15.75</b>
PRB929-2	0.10	PRB944-6	<b>28.28</b>
PRB929-3	<b>1.19</b>	PRB945-1	0.09
PRB929-4	<b>32.61</b>	PRB945-2	0.11
PRB929-5	<b>340.61</b>	PRB945-3	0.85
PRB929-6	<b>354.67</b>	PRB945-4	<b>11.68</b>
PRB929-7	<b>72.18</b>	PRB945-5	<b>29.51</b>
PRB930-1	<b>1.99</b>	PRB945-6	<b>43.93</b>
PRB930-2	<b>8.79</b>	PRB946-1	0.11
PRB930-3	<b>21.96</b>	PRB946-2	0.47
PRB930-4	<b>62.26</b>	PRB946-3	<b>16.60</b>
PRB937-1	0.10	PRB946-4	<b>58.86</b>
PRB937-2	0.10	PRB947-1	0.27
PRB937-3	0.24	PRB947-2	<b>8.80</b>
PRB937-4	<b>6.51</b>	PRB947-3	<b>10.77</b>
PRB937-5	<b>10.17</b>	PRB947-4	<b>7.76</b>
PRB937-6	<b>43.42</b>	PRB948-1	0.09
PRB938-1	<b>4.33</b>	PRB948-2	0.10
PRB938-2	<b>22.38</b>	PRB948-3	0.52
PRB938-3	<b>191.33</b>	PRB948-4	<b>16.26</b>

**Table 13. Results of lot comparison for five seroconversion panels**

Panel	S/CO	
	Lot1: 51322HN00	Lot 2: 50482HN00
PRB916-01	0.15	0.15
PRB916-02	0.12	0.17
PRB916-03	0.19	0.21
PRB916-04	<b>87.78</b>	<b>88.68</b>
PRB916-05	<b>29.86</b>	<b>32.05</b>
PRB916-06	<b>50.64</b>	<b>50.00</b>
PRB924-1	0.12	0.17
PRB924-2	0.18	0.15
PRB924-3	0.16	0.14
PRB924-4	0.17	0.20
PRB924-5	<b>45.48</b>	<b>44.76</b>
PRB924-6	<b>15.26</b>	<b>15.08</b>
PRB924-7	<b>9.75</b>	<b>10.53</b>
PRB924-8	<b>19.04</b>	<b>20.41</b>
PRB932-1	0.18	0.14
PRB932-2	0.18	0.18
PRB932-3	0.12	0.15
PRB932-4	<b>13.24</b>	<b>14.00</b>
PRB932-5	<b>16.18</b>	<b>15.25</b>
PRB932-6	<b>5.21</b>	<b>5.56</b>
PRB932-7	<b>7.62</b>	<b>7.62</b>
PRB932-8	<b>3.74</b>	<b>4.22</b>
PRB932-9	<b>3.37</b>	<b>3.58</b>
PRB939-1	0.13	0.14
PRB939-2	0.13	0.14
PRB939-3	0.14	0.13
PRB939-4	0.14	0.13
PRB939-5	0.40	0.42
PRB939-6	<b>1.68</b>	<b>1.99</b>
PRB939-7	<b>103.22</b>	<b>122.99</b>
PRB939-8	<b>206.84</b>	<b>249.99</b>
PRB939-9	<b>118.77</b>	<b>123.11</b>
6240-1	0.23	0.23
6240-2	0.17	0.17
6240-3	0.13	0.16
6240-4	0.13	0.14
6240-5	0.12	0.13
6240-6	0.09	0.12
6240-7	0.79	<b>1.00</b>
6240-8	<b>4.17</b>	<b>4.95</b>
6240-9	<b>205.93</b>	<b>230.01</b>
6240-10	<b>221.83</b>	<b>281.21</b>
6240-11	<b>46.28</b>	<b>51.92</b>
6040-12	<b>15.70</b>	<b>16.56</b>
6040-13	<b>14.89</b>	<b>16.82</b>