Evaluation of Adaltis Detect–HIV™ v.4

Johanna Curtis, Keith Perry
Microbiological Diagnostics Assessment Service
Evaluations and Standards Laboratory
Health Protection Agency - Centre for Infections
61 Colindale Avenue
London
NW9 5EQ

Report PER06004

July 2006

Tables of Contents

List of Figures and Tables

Assay Description

Detect-HIV™ v.4 is a 'fourth generation' or combination immunoassay, which refers to the ability of such assays to detect antibodies directed against HIV-1 (including rare subtypes such as group O) and HIV-2, as well as HIV-1 p24 antigen. Generally speaking combined antigen/antibody assays allow a significant reduction of the time between initial infection and the laboratory diagnosis (diagnostic window).

Antigens representing immunodominant epitopes of HIV-1 gp41 and HIV-2 gp36 as well as antibodies against the antigen p24 are coated onto wells of a microplate. Antigen and antibodies have been carefully selected to ensure the screening of antibodies to all HIV-1 subtypes, including subtype O and HIV-2, and the detection of p24 antigens. Serum or plasma samples are added to these wells and stable complexes between the bound antibodies / antigen are formed with specific antibodies / antigen present in the sample.

Antibody-antigen complexes are then identified through the successive addition of conjugates 1 and 2. Substrate is then added to the wells and a blue colour will develop in proportion to the amount of anti-HIV 1&2 antibodies and/or antigen bound to the well thus establishing their presence in the sample. A stop solution is added to each well and the resulting yellow colour is read on a microplate reader at 450nm/ 630nm.

The assay includes Sample and Reagent Addition Monitoring. 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

General	
Assay name	Detect-HIV™ v.4
Manufacturer / UK Agent	Adaltis
Product number	RHD302A/305A/300A
Number of tests per pack	192 / 480 / 960
Specimen volume	100 µL

Presentation	
Assay type	Combined Antigen/Antibody
Solid phase	12 x 8 microtitre plates
Coating	Antigens representing immunodominant epitopes of HIV-1 gp41 and HIV-2 gp36, and antibodies against the p24 antigen
Conjugate 1	Biotinylated antigens and antibodies
Conjugate 2	Horseradish peroxidase Streptavidin conjugate
Substrate	Stabilized mixture of 3,3',5,5' tetra-methylbenzidine substrate and hydrogen peroxide

Controls per plate	8
Negative control	3
HIV-1 Calibrator (antibodies against HIV-1 antigens) [Pos 1]	3
HIV-2 positive control (antibodies against HIV-2 antigens) [Pos 2]	2
Reading wavelength	450 nm/630 nm
Cut-off computation	((Mean[Neg]) + ((Mean[Pos1])/6))
Equivocal zone	N/A

Stages	
Preparation / sample well loading	30 minutes
Incubation status	Static
Sample incubation	60 minutes 37°C
Conjugate 1 incubation	30 minutes at 37˚C
Conjugate 2 incubation	30 minutes at 37˚C
Number of washes	5
Substrate incubation (time/temp)	30 minutes 18-25°C
Reading	within 30 minutes of stopping reaction
Total incubation time	150 minutes
Approximate time to completion	180 minutes
Number of optional procedures	N/A

Additional equipment required
Dry Incubator, type not specified
Microplate spectrophotometer (EL 808)
Micropipettes: 40 - 200µL
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, 126 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 insert was followed strictly. Briefly, 100 µL of sample diluent and 100 µL 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. 200 µL of conjugate 1 was added to all wells, which were then incubated for 30 minutes at 37˚C. The plate was washed as before, and 200 µL of conjugate 2 was added to the wells. Then the plate was incubated at 37˚C for 30 minutes. The wells were washed five times and 100 µL of substrate was added to each well. The wells were incubated for 30 minutes in the dark at room temperature. To this 100 µL of stop solution was added to each well; the plate was read at 450 nm / 630 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 Adaltis Detect-HIV™ v4 (batch 1 [6160042])

Sample Category	Number of specimens
1. Anti-HIV negative blood donor sera	200
2. Anti-HIV positive (n=200)	
Anti-HIV 1 (various risk groups and geographical locations)	178
Anti-HIV 1 subgroup O	2
Anti-HIV 2 positive	20
3. HIV seroconversion panels (n=126; 21 panels)	
BBI – PRB 916	6
BBI – PRB 917	6
BBI – PRB 919	3
BBI – PRB 922	4
BBI – PRB 924	8
BBI – PRB 925	6
BBI – PRB 927	5
BBI – PRB 929	7
BBI – PRB 930	4
BBI – PRB 932	9
BBI – PRB 937	6
BBI – PRB 938	3
BBI – PRB 939	5
BBI – PRB 940	8
BBI – PRB 941	6
BBI – PRB 943	7
BBI – PRB 944	6
BBI – PRB 945	6
BBI – PRB 946	4
BBI – PRB 948	4
BCP 6240	13
4. Quality control samples (n=12)	
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)
Total (number of specimens)	**538**

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 Adaltis Detect-HIV™ v.4 (batch 2 [6160052])

Sample Category	Number of specimens
1. Anti-HIV negative blood donor sera	40
2. Anti-HIV positive	40
3. HIV seroconversion panels (n=45; 5 panels)	
BBI – PRB 916	6
BBI – PRB 924	8
BBI – PRB 932	9
BBI – PRB 939	5
BCP 6240	13
4. Quality control samples (n=12)	
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)
Total (number of specimens)	**133**

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 to 2.8%). No samples were repeatedly reactive.

Table 4. Specificity of Adaltis Detect-HIV™ v.4 kit

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	0.106 – 1.68	0.30	0.30

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 Adaltis Detect-HIV™ v.4 kit

Subgroup / Risk factor	Number of specimens	Mean OD/CO	Median OD/CO	Range of OD/CO	Sensitivity
Type or Subgroup					
HIV 1 Group O	2	8.63	8.63	8.60 – 8.67	100%
HIV 2	21	11.64	11.81	8.95 – 11.95	100%
HIV 1 or 2	16	10.81	11.61	8.72 – 11.80	100%
Location Risk Factor					
HIV 1 Ivory Coast	2	8.79	8.79	8.71 – 8.88	100%
HIV 1 Zimbabwe	3	8.81	8.80	8.75 – 8.88	100%
HIV 1 Uganda	15	10.93	11.70	8.86 – 11.95	100%
HIV 1 India	3	11.39	11.32	11.07 – 11.78	100%
HIV 1 North America	15	11.23	11.25	10.90 – 11.43	100%
HIV 1 Argentina	2	11.32	11.32	11.29 – 11.36	100%
HIV 1 Mozambique	2	11.22	11.22	11.11 – 11.33	100%
HIV 1 South Africa	11	11.19	11.22	10.96 – 11.36	100%
HIV 1 Ghana	11	11.23	11.27	11.01 – 11.38	100%
Life style Related					
Partner HIV positive	3	10.11	10.07	10.04 – 10.22	100%
Prostitute	4	10.14	10.13	10.08 – 10.23	100%
Bisexual	1	10.23	10.23	10.23	100%
Multiple partners	50	9.53	10.11	7.84 – 10.29	100%
Homosexual	17	8.09	7.95	7.84 – 8.89	100%
IVDU	16	8.07	7.95	7.80 – 8.87	100%
Transfusion received	6	8.05	7.89	7.82 – 8.87	100%
Total	200	10.05	10.20	7.80 – 11.95	100%

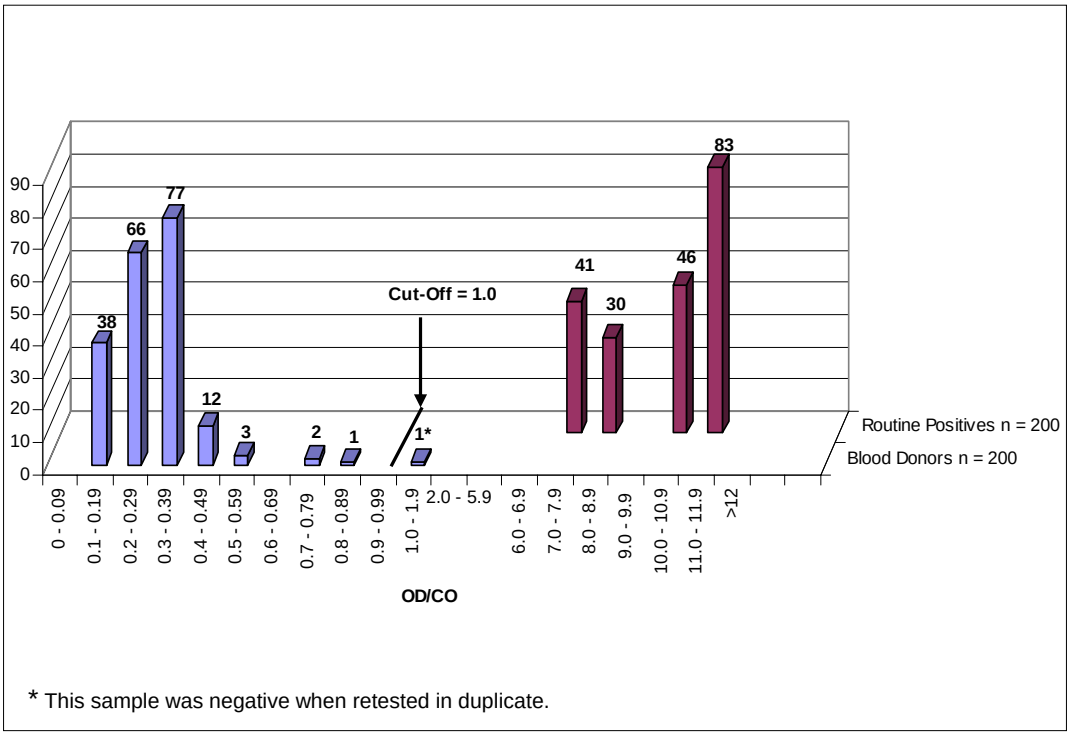


Figure 1. Distribution of initial reactivities.

Seroconversion Sensitivity: Aggregate scores

The ability of the Adaltis Detect-HIV™ v.4 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 80 out of 125 and was shown to be the third most sensitive kit (on a par with the Prism HIV Ag/Ab Combo and the Murex HIV Ag/Ab Combination which also achieved a score of 80). See Table 6. The full details of the seroconversion scores are shown in the Appendix (Tables A1 and A4).

Table 6. Combined Seroconversion Scores (21 Panels)

HIV assay	Product number	Cumulative score * (PRB916-6240) n=126	Rank
AxSYM HIV Ag/Ab Combo	2G83-20	85	1
GENSCREEN Ultra HIV Ag-Ab	72388/72386	82	2
Detect-HIV™ v.4	**RHD302**	**80**	**=3**
Murex HIV Ag/Ab Combination	GE41/42	80	=3
Prism HIV Ag/Ab Combo	7G46-48	80	=3
VIDAS HIV DUO	30114	76	6
GENSCREEN PLUS HIV Ag-Ab	72375/72376	75	7
Enzygnost HIV Integral	31843	73	8
Vironostika HIV Uni-Form II Ag/Ab UPDATE	285047	N/A**	N/A**
Vironostika HIV Uni-Form II Ag/Ab	6029/30/31	63	9
Genscreen HIV1/2 EIA (v2)	72279	58	10
Murex HIV 1.2.O	GE94/95	56	11
Biotest Anti-HIV TETRA ELISA	807 008	55	=12
Access HIV 1/2 NEW	34020	55	=12
Vitros ECi Anti-HIV 1+2	124-1850	55	=12
Abbott HIV1/2 3rd Generation Plus EIA	7A84-24	55	=12
AxSYM HIV 1/2 gO	3D41-20	54	=16
Murex HIV 1+2	VK84/85	54	=16
Imx HIV-1/HIV-2 III Plus	8C98	53	=18
Ortho HIV-1/HIV-2 Ab-capture ELISA Test System	932380	53	=18
Enzygnost Anti-HIV1/2 Plus	OQFK 12/13	51	=20
Vironostika HIV Uni-Form II plus O	84018	51	=20
ICE HIV-1.0.2	100A	49	=22
Wellcozyme HIV 1+2 EIA	VK55	49	=22
Biotest anti-HIV1/2 recombinant	807005	47	24
Clonesystems (IAF Biochem) Detect HIV	851403	30	25
Innotest HIV-1/HIV-2	M422	29	26

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 kit “time zero” and any kit 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 Adaltis Detect-HIV™ v.4 detected HIV infection approximately two days earlier than the next best kit, and only 1.5 days after the Axsym HIV Ag/Ab Combo assay which was ranked first.

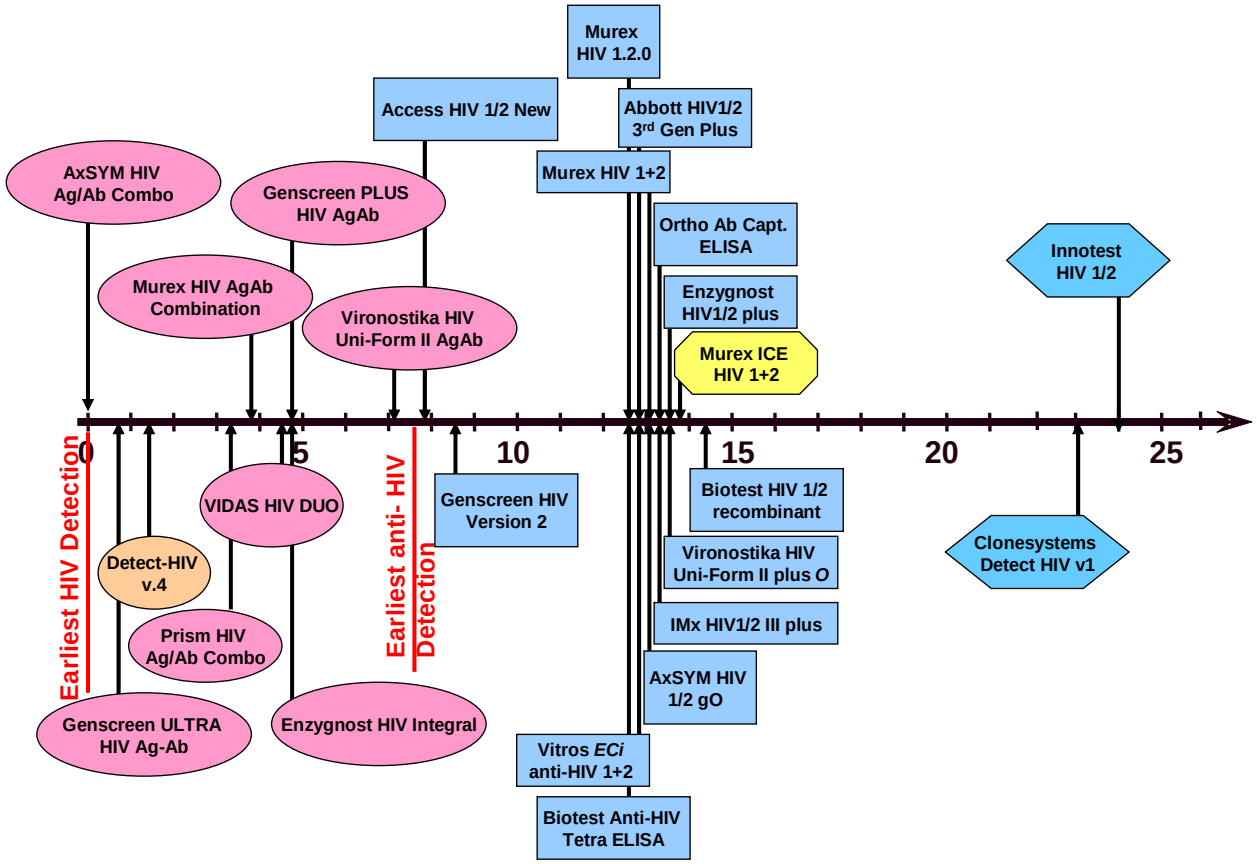
The median detection time for Adaltis Detect-HIV™ v.4 kit was 0 days. The median delay is not affected in the same way as the mean delay which can be strongly influenced by outlying results from seroconversion panels for which the interval between the last negative and the first positive specimen is long. This can give rise to an artefact due to the timing of blood collection.

Table 7: Comparative timing of detection of primary HIV detection following seroconversion

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
GENSCREEN Ultra HIV Ag-Ab	72388/72386	0 – 6	0.95	0
Detect-HIV™ v.4	**RHD302A**	**0 – 6**	**1.5**	**0**
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
Vironostika HIV Uni-Form II Ag/Ab	6029/30/31	0 – 57	7.3	5
Earliest possible detection by any anti-HIV only assay		0 – 65	7.6	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 *plus O*	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

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 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.

Figure 2: Comparative timing of detection of primary HIV infection following seroconversion.



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 throughout the 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 Adaltis Detect-HIV™ v.4 assay there were three suitable controls; NIBSC BWS for anti HIV 1, NIBSC Monitor specimen for anti-HIV 2 and HPA HIV 1 QC2. For this evaluation, NIBSC BWS for anti-HIV 1 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	**8.02**	**8.14**	**8.07**	**8.08**
HPA anti-HIV 1 QC2	03/B356-03	**2.35**	**2.31**	**2.10**	**2.25**
HPA anti-HIV 1 QC3	03/B359-01	0.69	0.68	*N/A**	0.68
HPA anti-HIV 1 QC5	99/B168-10	**7.78**	**7.83**	**7.76**	**7.79**
HPA anti-HIV 2 QC2	03/B379-02	**6.07**	**5.74**	**6.10**	**5.97**
HPA anti-HIV 2 QC3	04/B406-01	0.94	0.81	0.80	0.85
HPA HIV 1 p24 QC1	01-B276-10	**8.09**	**8.04**	**8.14**	**8.09**
HPA HIV 1 p24 QC2	01/B273-07	**3.62**	**3.45**	**3.72**	**3.60**
NIBSC 1 in 5 BWS for anti-HIV 1	99/710-007	0.64	0.68	0.70	0.67
NIBSC BWS for anti HIV 1	99/750-007	**2.18**	**2.10**	**1.99**	**2.09**
NIBSC Monitor sample for anti-HIV 2	99/674-005	**2.33**	**2.31**	**2.34**	**2.33**
NIBSC HIV 1 p24 Ag monitor sample	02/146-002	**7.71**	**7.80**	**7.76**	**7.75**

*This replicate was not tested.

Table 9: NIBSC BWS for anti HIV 1 results

Number of tests	Number initially reactive	Initial range OD/CO	Initial mean OD/CO	Initial median OD/CO
23	23	1.991 – 2.960	2.484	2.501

Batch Comparison

Two batches of Adaltis Detect-HIV™ v.4 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 A2 and A3 for full details of a comparison of OD/CO readings for both batch 6160042 and 6160052.

Table 10: Comparison of two batches.

Specimen Category	Number of Specimens	Number of Reactive Specimens	
		Batch Number	
		6160042	6160052
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	3	4*
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)	0	0
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)	0	0
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

* There was no significant difference between the 2 lots for panel PRB939 (sample number 6 (OD/CO was 0.96 vs 1.10)

Technical Appraisal

The Adaltis Detect-HIV™ v.4 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. A pictorial summary would be useful for reference whilst performing the assay.

Conclusions

The Adaltis Detect-HIV™ v.4 kit allows simultaneous detection of HIV p24 antigen and anti-HIV. In terms of seroconversion sensitivity it was the 3rd most sensitive antigen-antibody kit, detecting HIV a mean of 1.5 days after the AxSYM HIV Ag/Ab assay. It also detected HIV a mean of two days earlier than the next most sensitive kit (PRISM HIV Ag/Ab). In addition, there was a 6.1 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

Table A1. 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)
		PRB916	PRB917M	PRB919	PRB922	PRB924	PRB925	PRB927	
		n=6	n=6	n=3	n=4	n=8	n=6	n=5	n=38
AxSYM® HIV Ag/Ab Combo	2G83-20	4 (9)	6 (0)	3 (0)	4 (0)	4 (26)	2 (44)	4 (28)	**27**
GENSCREEN® Ultra HIV Ag-Ab	72388/72386	3 (15)	6 (0)	3 (0)	4 (0)	4 (26)	2 (44)	4 (28)	26
Detect HIV v4	**RHD302A**	**3 (15)**	**6 (0)**	**3 (0)**	**4 (0)**	**4 (26)**	**2 (44)**	**4 (28)**	**26**
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® PLUS HIV Ag-Ab	72375/72376	3 (15)	5 (53)	3 (0)	4 (0)	4 (26)	2 (44)	4 (28)	**25**
Enzygnost® HIV Integral	31843	3 (15)	5 (53)	3 (0)	4(0)	4 (26)	2 (44)	4 (28)	**25**
Vironostika® HIV Uni-Form II Ag/Ab UPDATE	285047	3 (15)	6 (0)	2 (9)	4 (0)	4 (26)	2 (44)	4 (28)	**25**
Vironostika® 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® 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® 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® HIV 1/2 gO	3D41-20	2 (30)	3 (65)	2 (9)	4 (0)	3 (33)	2 (44)	3(33)	**19**
IMx® HIV-1/HIV-2 III Plus	8C98	2 (30)	3 (65)	2 (9)	3 (4)	3 (33)	2 (44)	4 (28)	**19**
Ortho® 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® Anti-HIV1/2 Plus	OQFK 12/13	2 (30)	3 (65)	2 (9)	3 (4)	3 (33)	2 (44)	4 (28)	**19**
Vironostika® 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**
Clonesystems (IAF Biochem) Detect HIV	851403	2 (30)	3 (65)	2 (9)	2 (7)	2 (35)	1 (49)	3 (33)	**15**
Access® 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® 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 menber PRB917-05 was VOID for PRISM HIV O Plus. This member has been scored as positive so giving this panel the score 3 (65)

Table A1 contd. 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 *	Cumulative score *
		PRB929	PRB930	PRB932	PRB937	PRB938	PRB939	PRB940	PRB941	(PRB929-941)	(PRB916 - 941)
		n=7	n=4	n=9	n=6	n=3	n = 5	n = 8	n=6	n=48	n=86
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**
Detect HIV v4	**RHD302A**	**4 (18)**	**4 (0)**	**6 (27)**	**3 (14)**	**3 (0)**	**3 (7)**	**7 (7)**	**3 (18)**	**33**	**59**
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® HIV1/2 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**
Clonesystems (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	5 (15)	NT	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.

Table A1 contd. 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 *	Cumulative score *
		PRB943	PRB944	PRB945	PRB946	PRB948	6240	(PRB943-6240)	(PRB916-6240)
		n=7	n = 6	n=6	n = 4	n=4	n=13	n = 40	n=126
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**
Detect HIV v4	**RHD302A**	**5 (7)**	**4 (7)**	**3 (13)**	**2 (7)**	**1 (23)**	**6 (23)**	**21**	**80**
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**
Biotest Anti-HIV TETRA ELISA	807 008	2 (19)	2 (14)	3 (13)	0 (>11)	0 (>23)	5 (28)	**12**	**55**
Murex HIV 1.2.O	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**
Biotest 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**
Clonesystems (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 menber 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 A2: Differences in reactivity between two batches of Adaltis Detect-HIV™ v4

Specimen Category	Number of Specimens	Range of OD/CO		Mean OD/CO		Median OD/CO	
		Batch Number		Batch Number		Batch Number	
		6160042	6160052	6160042	6160052	6160042	6160052
1. Anti-HIV negative blood donor sera	40	0.214 – 0.607	0.214 – 0.607	0.296	0.291	0.297	0.278
2. Anti-HIV positive	40	8.09 – 8.45	8.09 – 8.45	8.28	8.28	8.28	8.28

Table A3: Differences in reactivity of quality control specimens between two batches of Adaltis Detect-HIV™ v.4

QC Type	Number of tests	Mean OD/CO (Batch 6160042)	Mean OD/CO (Batch 6160052)
HPA anti-HIV 1 QC1	3	8.08	8.30
HPA anti-HIV 1 QC2	3	2.25	1.96
HPA anti-HIV 1 QC3	3	0.68	0.59
HPA anti-HIV 1 QC5	3	7.79	8.03
HPA anti-HIV 2 QC2	3	5.97	5.47
HPA anti-HIV 2 QC3	3	0.85	0.72
HPA HIV 1 p24 QC1	3	8.09	8.41
HPA HIV 1 p24 QC2	3	3.60	2.95
NIBSC 1 in 5 BWS for anti-HIV 1	3	0.67	0.74
NIBSC BWS for anti HIV 1	3	2.09	2.48
NIBSC Monitor sample for anti-HIV 2	3	2.33	2.18
NIBSC HIV 1 p24 Ag monitor sample	3	7.75	8.18

Table A4: Differences in reactivities of two batches of Adaltis Detect-HIV™ v.4 when tested against seroconversion panels

Seroconversion panel name	OD/CO (Batch 6160042)	OD/CO (Batch 6160052)
PRB916-01	***0.312***	***0.252***
PRB916-02	***0.281***	***0.270***
PRB916-03	***0.295***	***0.360***
PRB916-04	8.398	8.392
PRB916-05	8.443	8.240
PRB916-06	8.646	8.337
PRB924-01	***0.301***	***0.321***
PRB924-02	***0.294***	***0.298***
PRB924-03	***0.321***	***0.321***
PRB924-04	***0.294***	***0.314***
PRB924-05	7.885	8.233
PRB924-06	8.284	8.115
PRB924-07	8.208	7.817
PRB924-08	8.370	8.166
PRB932-01	***0.305***	***0.282***
PRB932-02	***0.319***	***0.275***
PRB932-03	***0.294***	***0.275***
PRB932-04	8.038	7.963
PRB932-05	8.420	8.477
PRB932-06	8.133	7.916
PRB932-07	7.081	6.352
PRB932-08	8.443	8.353
PRB932-09	8.459	8.325
PRB939-05	***0.380***	***0.388***
PRB939-06	***0.964***	1.099
PRB939-07	8.297	8.480
PRB939-08	8.139	8.410
PRB939-09	8.251	8.450
BCP 6240-01	***0.271***	***0.252***
BCP 6240-02	***0.304***	***0.249***
BCP 6240-03	***0.313***	***0.282***
BCP 6240-04	***0.322***	***0.224***
BCP 6240-05	***0.391***	***0.219***
BCP 6240-06	***0.284***	***0.224***
BCP 6240-07	***0.529***	***0.741***
BCP 6240-08	3.056	3.090
BCP 6240-09	8.348	8.521
BCP 6240-10	8.326	8.595
BCP 6240-11	8.066	8.240
BCP 6240-12	8.082	8.316
BCP 6240-13	8.124	8.364

Red Values indicate negative values / specimens

Manufacturer's Comments

ADALTIS

July 14th 2006

Dr. Keith Perry
Microbiological Diagnostics Assessment Service
61, Colindale Avenue
London NW9 5HT

Re: **Draft Report, Evaluation of Adaltis Detect-HIV (v. 4)**

Dear Dr. Perry,

First and foremost, we would like to thank you for giving us the opportunity to comment on this draft report. We would also like to mark our appreciation to Ms. Curtis and Ms. Dean for the quality of their work and their availability throughout this evaluation.

We are very pleased with the results that you obtained, particularly on the score obtained with the 21 seroconversion panels as well as the mean timing of detection of 1,5 days; these two parameters make Detect-HIV (v. 4) one of the three best HIV assays on the market.

The excellent specificity you observed and the results obtained with the Quality Control Reagents confirm our internal results and our other external evaluations. Finally, your conclusion about the user-friendliness and the suitability of our kit is also well appreciated.

Yours sincerely,

Michel Houde Ph.D.

Head of Research
Adaltis Development inc.
Laval, Quebec, Canada

Adaltis Development Inc.
500 Carter West
Suite 134
Laval, Quebec
Canada H7V 5B7
r_d@adaltis.com www.adaltis.com