



Evaluation of the Roche HIV combi assay

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Background and description of the assay

HIV combi is a fourth generation sandwich immunoassay manufactured by Roche Diagnostics for the detection of HIV-1 p24 antigen and total antibodies to HIV-1 (including Group O) and HIV-2. The assay characteristics are summarised in Table 1. The HIV combi kit comprises of a single compact unit containing three reagent wells (M, R1 and R2); once any residue in the chamber lids is removed, the unit is loaded onto an automated analyser without the requirement for any further manipulations. The assay can be run on one of two automated analysers which incorporate electrochemiluminescence (ECL) detection: a Roche MODULAR ANALYTICS E170 automated immunoassay analyser (E-module), or a Roche Elecsys 2010. Briefly, the sample is mixed with antigen and antibody conjugates labelled with biotin or a ruthenium complex. The resulting Ab/Ag complexes are captured using streptavidin-coated magnetic microparticles, washed and following application of a voltage in the measuring cell a chemiluminescent signal is produced and measured using a photomultiplier.

This study evaluated the use of HIV combi on the E-module, an instrument which has been widely used within the field of clinical chemistry, but whose potential for the detection of infectious disease markers is less well established. The HIV combi assay was evaluated using a panel of specimens consisting of anti-HIV-1 and -2 positives (including HIV-1 Group O), anti-HIV negatives, quality control specimens and seroconversion panels.



HIV combi kit (above) and Modular Analytics E170-module

The Roche combi assay carries a CE mark and therefore has undergone testing described in the Common Technical Specification for Annex IIa related products and in accordance to the European Union In Vitro Diagnostic Medical Device Directive. This evaluation builds on the work already completed for CE marking by providing comparative performance information on a range of specimens with a particular focus on seroconversion timing. The panel is moderately sized, recognising that a large number of specimens have already been tested as part of the CE Marking process.

This report specifically relates to the kit version and lot numbers supplied for this evaluation. We cannot guarantee that these will reflect the performance of other lot numbers or subsequent versions. Laboratories should always validate and monitor assay performance as part of an ongoing quality control program.

Table 1: Assay information

General	
Assay name	HIV combi
Manufacturer / UK agent	Roche Diagnostics
Product number	04860446
Number of tests per pack	100
Specimen volume	150µL (30µL + 120µL 'dead' volume)

Presentation	
Assay type	Combined Antigen/Antibody
Solid phase	Streptavidin-coated microparticles (M)
Conjugate	Biotinylated monoclonal anti-p24 antibodies/HIV-specific recombinant antigens/HIV-specific peptides (R1), and monoclonal anti-p24 antibodies/HIV-specific recombinant antigens/ HIV-specific peptides labelled with ruthenium complex (R2)
Substrate	None – electrochemiluminescent detection following voltage application
Controls (supplied separately)	PreciControl HIV combi, product number 03599612
Negative control	PC HIVCOM1
Positive controls	PC HIVCOM2, PC HIVCOM3
Calibrators	Cal 1 (HIV-1/2 negative human serum) Cal 2 (inactivated anti-HIV-1 positive human serum)
Reading wavelength	620 nm
Cut-off computation	Performed by E-module based on calibration values
Equivocal zone	Signal/cut-off values ≥ 0.90 to < 1.0

Stages	
Preparation/sample well loading (per five samples)	10 minutes (some laboratories may be able to load primary specimen vessels)
In E-170:	
- Sample and conjugates mixed - Resulting complexes bind to streptavidin-coated microparticles - In measuring cell, microparticles are magnetically captured and washed - Voltage applied, resulting in chemiluminescence captured by a photomultiplier	
Approximate time to completion*	
-1 sample	- 20 min
-10 samples	- 25 min
-100 samples	- 50 min

*Only one photocell was used in this evaluation due to its small scale – it is anticipated that using both cells would decrease these times

Additional equipment required	
Micropipettes	
Centrifuge	

Evaluation panel and methods

The evaluation panel totalled 583 serum or plasma specimens (Table 2). Of these, 200 were anti-HIV negative specimens from blood donors and 223 were anti-HIV positive, including representatives of various risk groups and subtypes, two HIV-1 Group O and 21 anti-HIV-2 positives. Positive and negative specimens were interspersed where possible. A further 127 specimens from 21 seroconversion panels and 11 quality control samples were tested. A subset of this panel was used to assess a second assay lot (Table 3).

The method outlined in the kit insert was strictly followed. Kit calibration specimens were tested at the start of the evaluation and following introduction of a new reagent lot. The calibration was repeated if a kit remained loaded on the machine for seven days. The three PreciControl specimens were tested as a minimum at the beginning of each day, at the start of each new kit and after calibration. Primary specimen tubes were not used for this evaluation and instead 150 μ L aliquots of plasma or serum were dispensed into barcode-labelled assay cups (Hitachi Standard) and loaded onto the E-module.

The analyser calculated the cut-off based on the measurements of the calibrators Cal1 and Cal2. Specimens were interpreted using the criteria below as outlined in the kit insert.

Specimens with a cut-off index (signal/cut-off value) <0.90 were classed as non-reactive, and considered negative for HIV-1 Ag and HIV-1/-2 specific antibodies. These specimens did not require further testing.

The kit insert states that specimens with a cut off index ≥ 0.90 and <1.0 are considered borderline, and should be retested in duplicate; values of ≥ 0.90 in either of the retested specimens are considered to be repeat reactive. In this evaluation, any borderline results obtained from seroconversion panel specimens were considered to be negative for the analysis.

Specimens with a cut-off index ≥ 1.0 were considered reactive and therefore positive. The kit insert recommended retesting all positives in duplicate but this was not performed in this evaluation since the specimen status was known.

Table 2: Evaluation panel (Lot 1, 17934801)

Specimen category	Number
1. HIV-1 positive (including 2 Group O specimens)	202
2. HIV-2 positive	21
3. HIV negative blood donor specimens	200
4. HIV seroconversion panels (21 Panels, 127 specimens)	
BBI – PRB916	6
BBI – PRB917M	6
BBI – PRB919	3
BBI – PRB922	4
BBI – PRB924	8
BBI – PRB925	6
BBI – PRB927	5
BBI – PRB929	7
BBI – PRB930	4
BBI – PRB932	9
BBI – PRB937	6
BBI – PRB938	3
BBI – PRB939E	9
BBI – PRB940	8
BBI – PRB941	6
BBI – PRB943	7
BBI – PRB944	6
BBI – PRB945	6
BBI – PRB946	4
BBI – PRB948	4
BCP 6245	10
5. Quality Control samples	
HPA HIV1 QC1	1 (3x)
HPA HIV1 QC2	1 (3x)
HPA HIV1 QC3	1 (3x)
HPA HIV1 QC5	1 (3x)
HPA HIV2 QC2	1 (3x)
HPA HIV2 QC3	1 (3x)
HPA p24 Ag QC1	1 (3x)
HPA p24 Ag QC2	1 (3x)
NIBSC HIV1 British working standard	1 (3x)
NIBSC HIV1 British working standard, 1 in 5	1 (3x)
NIBSC HIV2 Monitor sample	1 (3x)
Total number of specimens tested	583

BBI: Boston Biomedica Inc; BCP: Zeptomatrix (formerly BioClinical Partners Inc); HPA: Health Protection Agency; NIBSC: National Institute for Biological Standards and Control

Table 3: Evaluation panel (Lot 2, 17886001)

Specimen category	Number
1. HIV-1 positive	41
2. HIV-2 positive	5
3. HIV negative	41
4. HIV seroconversion panels (5 Panels, 42 specimens)	
BBI – PRB916	6
BBI – PRB924	8
BBI – PRB932	9
BBI – PRB939E	9
BCP 6245	10
5. Quality Control samples	
HPA HIV1 QC1	1 (3x)
HPA HIV1 QC2	1 (3x)
HPA HIV2 QC2	1 (3x)
HPA HIV2 QC3	1 (3x)
HPA p24 Ag QC1	1 (3x)
HPA p24 Ag QC2	1 (3x)
NIBSC HIV1 British working standard	1 (3x)
NIBSC HIV1 British working standard, 1 in 5	1 (3x)
NIBSC HIV2 Monitor sample	1 (3x)
Total number of specimens (tests)	156

BBI: Boston Biomedica Inc; BCP: Zeptometrix (formerly BioClinical Partners Inc); HPA: Health Protection Agency; NIBSC: National Institute for Biological Standards and Control

Specificity findings

Of 197 HIV negative blood donor specimens, one was initially reactive (Table 4), corresponding to an initial reactive rate for the HIV combi kit of 0.5% (95% confidence interval: 0.02 to 2.8%). This specimen was also reactive following retests.

Table 4. Specificity of Roche HIV combi

No. of blood donor specimens tested	No. initially reactive	No. repeatedly reactive	Initial signal/CO	Repeat signal/CO values	Initial Reactive rate	95% Confidence Interval
197	1	1	1.03	1.66, 1.65	0.5%	0.02 to 2.8

The HIV negative status of this specimen was confirmed by the Virus Reference Department (VRD) using further assays, namely Enzygnost HIV Integral II (Ag/Ab EIA, Dade-Behring/Siemens; product number OPAA); Murex HIV Ag/Ab Combination EIA (Abbott Diagnostics; 7G79); Genetic Systems HIV-1 Ag EIA (Biorad; 71120); and Genelabs Western Blot (11030) (Appendix A).

A further three blood donor specimens which were assigned as HIV-negative by the National Blood Service (using bioMérieux Vironostika HIV Uni-Form II Ag/Ab test; 6029/30/31) were reactive in the HIV combi. These three were found by confirmatory testing to be HIV-indeterminate (suspected to be non-specific reactivities; details in Appendix A). These results were therefore excluded from the specificity analysis.

Sensitivity findings

All 223 HIV positive specimens were reactive by the HIV combi assay to give a sensitivity of 100% (95% CI: 98.4 -100%). All the specimens were highly positive with signal/CO values greater than 40 (Table 6).

Table 6. Sensitivity of Roche HIV combi

Subgroup / Risk factor	No. of specimens	Signal/CO			Sensitivity
		Mean	Median	Range	
HIV-1 Multiple partners	61	232.7	193.2	54.1 - 1211.0	100%
HIV-1 Homosexual	28	251.2	197.7	53.9 - 609.3	100%
HIV-1 IVDU	16	199.7	146.1	40.0 - 505.1	100%
HIV-1 Transfusion received	6	407.7	454.6	105.8 - 621.9	100%
HIV-1 Prostitute	4	229.1	201.8	134.0 - 378.9	100%
HIV-1 Partner HIV positive	3	247.8	215.4	167.1 - 361.0	100%
HIV-1 Bisexual	1	156.0	156.0	-	100%
HIV-1 North America	17	252.8	233.2	62.9 - 593.6	100%
HIV-1 Argentina	2	264.9	264.9	163.1 - 366.7	100%
HIV-1 India	3	592.4	239.4	93.8 - 1444.0	100%
HIV-1 Uganda	15	203.1	165.3	70.9 - 350.8	100%
HIV-1 South Africa	11	377.1	320.9	210.4 - 914.6	100%
HIV-1 Ghana	11	267.5	276.2	124.5 - 481.6	100%
HIV-1 Zimbabwe	3	136.9	160.9	48.8 - 200.9	100%
HIV-1 Ivory Coast	2	200.9	200.9	103.8 - 297.9	100%
HIV-1 Mozambique	2	168.8	168.8	106.0 - 231.6	100%
HIV-1 Group O	2	100.8	100.8	62.9 - 138.7	100%
HIV-1 or -2	15	247.5	201.8	81.3 - 401.0	100%
HIV-2	21	366.2	252.7	119.1 - 1347.0	100%
TOTAL	223	260.9	215.4	40.0 - 1444.0	100%

Distribution of initial reactivities

The distribution of the initial reactivities for the 197 HIV negative and 223 HIV positive specimens is shown in Figure 1. Assays with good discrimination have few or no samples wrongly classified and few reactivities close to the cut-off.

The HIV combi assay had no false negatives and one false positive result with a reaction just above the cut-off. The HIV positive specimens all gave high reactivities (>40).

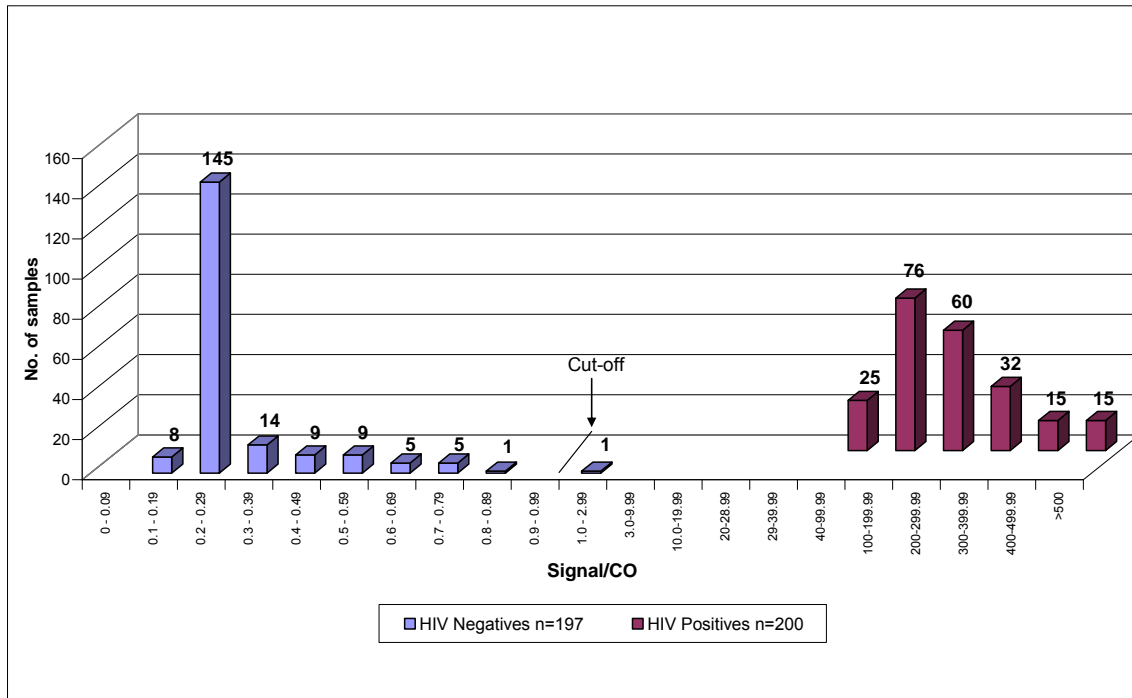


Figure 1. Distribution of initial reactivities

Note: the scale for Signal/CO is not continuous.

Four HIV-negative specimens were initially reactive. Following confirmatory testing, three of these specimens were classed as HIV-indeterminate and are not shown in this figure. The remaining sample was HIV-negative.

Seroconversion sensitivity

Twenty-one seroconversion panels were tested including 20 from Boston Biomedica Inc (BBI) and one from Zeptometrix (formerly BioClinical Partners, Inc). Comparative data from 21 other HIV assays was available for 18 of these panels. HIV combi had a score of 64 out of 106, making it the 7th most sensitive (Table 7). Detailed results of the individual panels are given in Appendix B.

Table 7: Seroconversion assay comparison (18 panels)

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

Comparative timing of detection

Timing of detection was analysed by assigning the most sensitive assay for each seroconversion panel a value of 'time zero', and any less sensitive assay a positive value based on the number of days after the most sensitive assay detected infection. An overall mean and median delay is then calculated for the seroconversion panels tested (Table 8, Figure 2).

The mean delay can be 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. The median delay is not affected in the same way. The median detection time for HIV combi was 0 days, which ranks the assay joint first with seven other assays.

Using mean values, the HIV combi assay was the fifth most sensitive assay for the panels tested, and detected HIV infection approximately 2.4 days earlier than the next best kit, and 1.8 days after the AxSYM HIV Ag/Ab Combo assay which was ranked first (Table 8, Figure 2).

Table 8: 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 [®] HIV Ag/Ab Combo	2G83-20	0 - 7	0	0.6
Architect HIV Ag/Ab Combo	4J27-20	0 - 6	0	0.9
GENSCREEN [®] Ultra HIV Ag-Ab	72388/72386	0 - 7	0	1.5
Detect HIV v4	RHD302A	0 - 7	0	2.1
Roche 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 [®] PLUS HIV Ag-Ab	72375/72376	0 - 53	3.5	5.7
Enzygnost [®] HIV Integral	31843	0 - 53	4	6.3
Vironostika [®] HIV Uni-Form II Ag/Ab	6029/30/31	0 - 57	6.5	8.6
Genscreen [®] HIV1/2 EIA (v2)	72279	0 - 65	7	10.2
Access [®] HIV 1/2 NEW	34020	0 - 65	7	10.8
Murex HIV 1.2.O	GE94/95	0 - 87	7	15.0
Biotest Anti-HIV TETRA ELISA	807 008	0 - 87	8	15.0
Vitros <i>ECi</i> Anti-HIV 1+2	124-1850	0 - 87	7	15.1
Murex HIV 1+2	VK84/85	0 - 87	8	15.1
Abbott HIV1/2 3rd Generation Plus EIA	7A84-24	0 - 87	7	15.2
AxSYM [®] HIV 1/2 gO	3D41-20	0 - 87	8	15.3
Ortho [®] HIV-1/HIV-2 Ab-capture ELISA Test System	932380	0 - 87	8	15.5
IMx [®] HIV-1/HIV-2 III Plus	8C98	0 - 87	9	15.7
Enzygnost [®] Anti-HIV1/2 Plus	OQFK 12/13	0 - 87	9	15.8
Vironostika [®] HIV Uni-Form II <i>plus O</i>	84018	0 - 87	8.5	16.0
ICE HIV-1.0.2	100A	0 - 87	9	16.0
Biotest anti-HIV1/2 recombinant	807005	0 - 87	11	16.8
Clonesystems (IAF Biochem) Detect HIV	851403	3 - 136	13.5	25.7
Innotest HIV-1/HIV-2	M422	0 - 170	15	27.6

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.

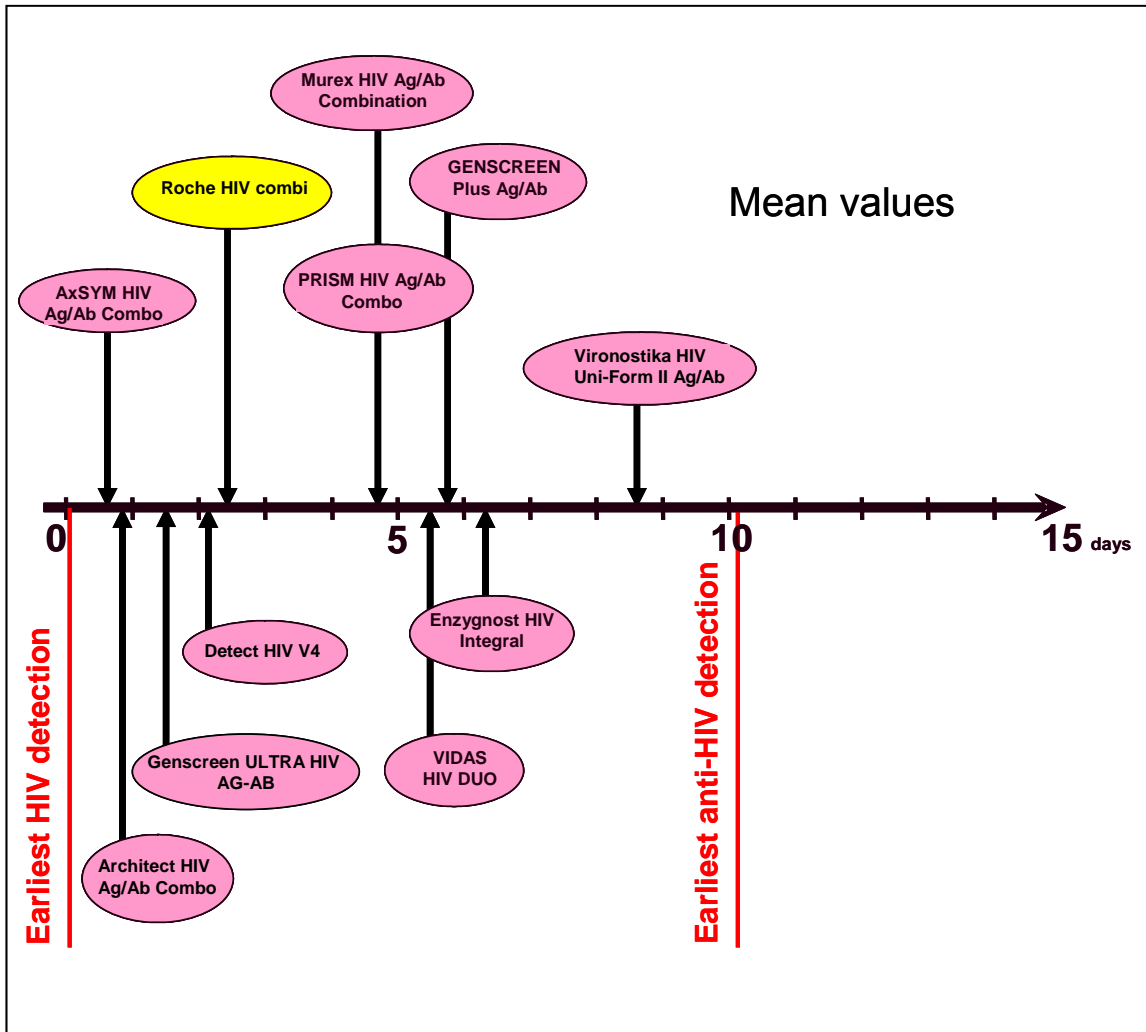


Figure 2. Timing of detection for combined antibody/antigen detection assays

Quality control reagents

Eleven quality control reagents were tested in triplicate (Table 9). An ideal control is one with a dynamic range 2 to 3 times higher than the cut off. Two samples met this criterion, HPA anti-HIV QC2 (hereafter QC2) and NIBSC HIV-1 1 in 5 British Working Standard. QC2 was selected as the run control and was tested at least once per day along with the test samples. The average signal/CO for each of the 20 runs of QC2 was calculated (Table 10).

Table 9: Quality Control Reagent results

QC sample ID	Lot number	Signal/CO			Mean
		1	2	3	
HPA HIV1 QC1	03/B355-05	60.72	59.32	61.59	60.54
HPA HIV1 QC2*	05/B450-02	2.96	3.03	2.98	2.99
HPA HIV1 QC3	07/B488-00	0.95	0.97	1.01	0.98
HPA HIV1 QC5	06/B461-00	26.29	26.68	26.34	26.44
HPA HIV2 QC2	06/B471-02	173.70	179.20	178.70	177.20
HPA HIV2 QC3	04/B06-04	30.41	31.29	30.32	30.67
HPA HIV1 p24 QC1	05/B448-08	12.82	12.80	12.94	12.85
HPA HIV1 p24 QC2	06/B477-05	1.34	1.40	1.38	1.37
NIBSC HIV1 1 in 5 BWS	99/710-009	3.08	3.12	3.19	3.13
NIBSC HIV1 BWS	99/750-009	11.71	11.70	11.95	11.79
NIBSC HIV2 Monitor	99/674-007	127.1	126.10	130.8	128.00

*HPA HIV1 QC2 was selected to be the run control for this evaluation

Table 10: HPA HIV 1 QC 2 results

Number of tests	Signal/CO		
	Range	Mean	Median
20	2.96 - 4.15	3.41	3.33

Lot comparison

A subset of the specimens used for the main part of the evaluation was tested using a second kit lot (lot number 17886001). This comprised of 41 HIV-1 positives, 5 HIV-2 positives, 41 HIV negatives, 5 seroconversion panels (42 specimens) and 27 Quality Control samples.

All of the HIV-positive and negative specimens were correctly assigned by both assay lots. Two specimens which were reactive in the initial assay lot were not detected in the second assay lot (one from seroconversion panel PRB 924 was negative; and one from seroconversion panel BCP 6240 sample was borderline in the second assay).

Table 11: Comparison of two assay Lots

Specimen Category	No. of Specimens	No. of Reactive Specimens	
		Lot Number 1: 17934801	Lot Number 2: 17886001
HIV-1 positive	41	41	41
HIV-2 positive	5	5	5
HIV negative blood donor sera	41	0	0
HIV seroconversion panels (n=5)			
BBI – PRB 916	6	3	3
BBI – PRB 924	8	4	3
BBI – PRB 932	9	6	6
BBI – PRB 939E	9	3	3
BCP 6245	10	5	4(+1)*
Quality control samples (n=9)			
HPA HIV1 QC1	1 (x3)	3	3
HPA HIV1 QC2	1 (x3)	3	3
HPA HIV2 QC2	1 (x3)	3	3
HPA HIV2 QC3	1 (x3)	3	3
HPA HIV1 p24 QC1	1 (x3)	3	3
HPA HIV1 p24 QC2	1 (x3)	3	3
NIBSC HIV1 1 in 5 BWS	1 (x3)	3	3
NIBSC HIV1 BWS	1 (x3)	3	3
NIBSC HIV2 Monitor sample	1 (x3)	3	3

* one specimen was borderline (signal/CO: 0.98)

Technical appraisal

The HIV combi assay instructions were clear and easy to follow, and the kit was very simple to load onto the E-module. The E-module had some problems reading a few of the barcodes, in which case samples were manually assigned to positions within racks. The reagents used for detection by the E-module (ProCell and CleanCell) are only stable for one week once loaded, but this will not be an issue for laboratories with a high-throughput of samples.

Conclusion

The HIV combi assay allows the simultaneous detection of HIV p24 antigen and anti-HIV. By employing various measures of seroconversion sensitivity the Roche HIV Combi was listed as the 7th most sensitive assay (using addition of reactive samples); 5th most sensitive (using the timing of detection: mean method) and equal first with six other Ag/Ab kits (timing of detection: median). By the timing of detection (mean) method it detected HIV a mean of 2.4 days earlier than the next best kit, and 1.8 days after the AxSYM HIV Ag/Ab Combo assay.

When used in combination with the E-module, the HIV combi showed excellent sensitivity when tested against a moderate number of routine positive specimens. In terms of specificity, one false positive was identified among 197 HIV negative blood donor specimens (99.5% specificity; 95% CI 97.2 to 100%). As this is a relatively small number in terms of specificity testing we recommend that further evidence is gathered on this aspect.

The HIV combi assay is easy to use and suitable for laboratories wishing to use the E-module range of assays.

Acknowledgements

We would like to thank members of the Virus Reference Department for testing and specialist advice, and QCRU for the provision of quality control reagents.

Appendix A: Initial reactive HIV-1 negative specimens and details of confirmatory tests

Sample number	Signal/CO	Repeat tests signal /CO	Behring Integral (OD/CO)	Abbott Murex (OD/CO)	HIV-1 p24 antigen (OD/CO); neutralisation	Genelabs WB	Interpretation
07N0153a	1.14	1.21, 1.19	0.085 (negative)	2.033 (reactive)	negative (0.622); negative	trace p24	HIV indeterminate
07N0172a	2.24	2.30, 2.19	0.148 (negative)	1.336 (reactive)	negative (0.684); negative	trace p24	HIV indeterminate
07N0183a	1.01	1.09, 1.11	1.291 (reactive)	0.780 (negative)	negative (0.531); negative	negative	HIV indeterminate
07N0204a	1.03	1.66, 1.65	0.104 (negative)	0.649 (negative)	negative (0.359); negative	negative	HIV negative

Appendix B: Comparative results of seroconversion panels PRB916-937

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)
		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
Architect HIV Ag/Ab Combo	4J27-20	3 (15)	6 (0) ²	3 (0)	4 (0)	4 (26)	2 (44)	5 (14)	4 (0)	6 (27)	3 (14)	40
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)¹	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
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)
¹Panel members PRB917-04 and -07 were not tested but were scored as positive
²Panel member PRB917-04 was not tested but was scored as positive

Appendix B: 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 n=3	PRB939E n=9	PRB940 n=8	PRB943 n=7	PRB944 n=6	PRB945 n=6	PRB946 n=4	PRB948 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
Architect HIV Ag/Ab Combo	4J27-20	3 (0)	4 (16)	8 (0)	5 (7)	5 (2)	3 (13)	2 (7)	1 (23)	31	71
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 <i>ECi</i> 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
Vironostika [®] HIV Uni-Form II <i>plus O</i>	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)	2 (14)	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.

Appendix B: Seroconversion panels - raw data

Seroconversion panel name	Signal/CO Lot 1: 17934801	Signal/CO Lot 2: 17886001
BCP6245-01	0.213	0.231
BCP6245-02	0.160	0.194
BCP6245-03	0.191	0.207
BCP6245-04	0.185	0.195
BCP6245-05	0.197	0.172
BCP6245-06	1.150	0.980*
BCP6245-07	4.090	3.300
BCP6245-08	11.110	9.130
BCP6245-09	15.540	12.480
BCP6245-11	436.300	392.200
PRB916-01	0.175	0.218
PRB916-02	0.158	0.172
PRB916-03	0.183	0.222
PRB916-04	24.740	19.710
PRB916-05	169.600	150.400
PRB916-06	193.300	180.700
PRB924-01	0.159	n/t
PRB924-02	0.196	0.226
PRB924-03	0.170	0.165
PRB924-04	0.222	0.168
PRB924-05	8.760	5.420
PRB924-06	126.300	n/t
PRB924-07	212.800	211.400
PRB924-08	234.100	213.900
PRB932-01	0.165	0.201
PRB932-02	0.179	0.191
PRB932-03	0.144	0.212
PRB932-04	1.990	1.960
PRB932-05	83.870	67.430
PRB932-06	60.550	53.170
PRB932-07	112.500	92.950
PRB932-08	149.000	119.400
PRB932-09	178.100	184.600
PRB939E-01	0.143	0.164
PRB939E-02	0.147	0.166
PRB939E-03	0.142	n/t
PRB939E-04	0.122	0.142
PRB939E-05	0.246	0.266
PRB939E-06	0.447	0.374
PRB939E-07	26.910	27.920
PRB939E-08	62.340	54.530
PRB939E-09	305.800	316.500
PRB917M-01	0.377	n/t
PRB917M-02	2.650	n/t
PRB917M-03	22.100	n/t
PRB917M-05	104.000	n/t
PRB917M-06	132.800	n/t
PRB919-01	2.040	n/t
PRB919-02	68.240	n/t
PRB919-03	70.270	n/t
PRB922-01	7.090	n/t
PRB922-02	49.560	n/t
PRB922-03	103.700	n/t
PRB922-04	156.400	n/t
PRB925-01	0.173	n/t
PRB925-02	0.154	n/t
PRB925-03	0.213	n/t
PRB925-04	0.150	n/t
PRB925-05	18.970	n/t
PRB925-06	147.500	n/t
PRB927-01	0.146	n/t
PRB927-02	185.800	n/t
PRB927-03	147.300	n/t
PRB927-04	118.800	n/t
PRB927-05	180.400	n/t

Seroconversion panel name	Signal/CO Lot 1: 17934801	Signal/CO Lot 2: 17886001
PRB929-01	0.147	n/t
PRB929-02	0.141	n/t
PRB929-03	0.255	n/t
PRB929-04	8.570	n/t
PRB929-05	50.180	n/t
PRB929-06	142.800	n/t
PRB929-07	198.500	n/t
PRB930-01	0.440	n/t
PRB930-02	3.810	n/t
PRB930-03	54.370	n/t
PRB930-04	252.500	n/t
PRB937-01	0.165	n/t
PRB937-02	0.150	n/t
PRB937-03	0.170	n/t
PRB937-04	0.808	n/t
PRB937-05	1.610	n/t
PRB937-06	7.440	n/t
PRB938-01	1.170	n/t
PRB938-02	5.710	n/t
PRB938-03	53.390	n/t
PRB940-01	0.448	n/t
PRB940-02	27.960	n/t
PRB940-03	4.460	n/t
PRB940-04	46.930	n/t
PRB940-05	64.610	n/t
PRB940-06	68.330	n/t
PRB940-07	82.190	n/t
PRB940-08	102.100	n/t
PRB941-01	0.154	n/t
PRB941-02	0.160	n/t
PRB941-03	0.278	n/t
PRB941-04	86.590	n/t
PRB941-05	108.000	n/t
PRB941-06	138.500	n/t
PRB943-01	0.163	n/t
PRB943-02	0.128	n/t
PRB943-03	0.653	n/t
PRB943-04	14.680	n/t
PRB943-05	55.650	n/t
PRB943-06	45.830	n/t
PRB943-07	134.600	n/t
PRB944-01	0.197	n/t
PRB944-02	0.191	n/t
PRB944-03	2.190	n/t
PRB944-04	1.580	n/t
PRB944-05	23.670	n/t
PRB944-06	77.390	n/t
PRB945-01	0.157	n/t
PRB945-02	0.152	n/t
PRB945-03	0.322	n/t
PRB945-04	3.030	n/t
PRB945-05	35.130	n/t
PRB945-06	254.900	n/t
PRB946-01	0.137	n/t
PRB946-02	0.188	n/t
PRB946-03	2.080	n/t
PRB946-04	8.120	n/t
PRB948-01	0.151	n/t
PRB948-02	0.170	n/t
PRB948-03	0.240	n/t
PRB948-04	4.340	n/t

Negative values shaded in grey, borderline results indicated with an asterisk

PRB944-04 was initially 0.961 (borderline) and was retested

n/t: not tested

Signature :

Date: 14th November 2007

Signature of Principal Investigator, Dr Keith Perry, to confirm that this report is a fair representation of the work performed.

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