

**OPERATIONAL CHARACTERISTICS OF
COMMERCIALLY AVAILABLE ASSAYS
TO DETERMINE ANTIBODIES TO
HIV-1 AND/OR HIV-2
IN HUMAN SERA**

REPORT 9/10

**GENEVA
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1. Introduction

This dual report, numbers 9 and 10, dealing with the evaluation of the major operational characteristics of commercially available assays to detect antibodies to HIV, presents assessments of the following 20 assays carried out between March 1994 and June 1995.

Enzyme-linked immunosorbent assays (ELISAs)

For the detection of antibody to HIV-1 and HIV-2

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- Vironostika HIV Uni-Form II (Organon Teknika nv)
- Biotest Anti-HIV-1/2 recombinant (Biotest AG)
- Innostest HIV-1/HIV-2 Ab.s.p. (Innogenetics nv)
- UBI HIV-1/2 EIA (United Biomedical Inc)
- Enzygnost Anti-HIV 1/-HIV 2 (Behringwerke AG)

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- HIVisual 1 & 2 (Immunodiagnosics Inc.)
- Genelabs Diagnostics HIV-1/HIV-2 ELISA (Genelabs Diagnostics Pte Ltd)
- ETI-AB HIV-1/2 K (Sorin Biomedica Diagnostics S.p.A.)
- HIV EIA (Labsystems OY)
- HIV SCREEN (Labsystems OY)

Simple/Rapid assays (S/R)

For the detection of antibody to HIV-1 and HIV-2

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- Capillus HIV-1/HIV-2 (Cambridge Diagnostics Ireland Ltd)
- SimpliRED HIV-1/HIV-2 Ab test (Agen Biomedical)
- MicroRED HIV-1/HIV-2 Ab test (Agen Biomedical)
- Immunocomb II HIV-1+2 BiSpot (PBS Organics)
- RETROCELL HIV-1 (Abbott GmbH Diagnostica)

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- HIV 1&2 Rapid Sero Test (Diatech Diagnostics Ltd/Healthcare Technologies Ltd)
- ENTEBE HIV Dipstick (Hepatika Laboratories PATH)
- Dipstick-HIV 1+2 (Immuno-Chemical Laboratories)
- SPAN COMBAIDS VISUAL (Span Diagnostics Ltd)
- DIA (Dot Immuno Assay) HIV 1+2 (Wiener Lab.)

ASSAY EVALUATIONS

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Table 1. General characteristics and operational aspects: ELISAs

NAME	VIRONOSTIKA HIV Uni-Form II	BIOTEST Anti-HIV- 1/-2 recombinant	INNOTEST HIV- 1/HIV-2 Ab. s.p.	UBI HIV 1/2 EIA	ENZYGNOST Anti-HIV 1/-HIV 2
Manufacturer	Organon Teknika BV Boxtel, The Netherlands	Biotest AG Dreieich, Germany	Innogenetics n.v. Antwerp, Belgium	United Biomedical Inc. Hauppauge, USA	Behringwerke AGI Marburg, Germany
Assay type	sandwich ELISA HIV-1 & HIV-2	indirect ELISA HIV-1 & HIV-2	indirect ELISA HIV-1 & HIV-2	indirect ELISA HIV-1 & HIV-2	indirect ELISA HIV-1 & HIV-2
Antigen type	recombinant proteins synthetic peptides	recombinant proteins	synthetic peptides	synthetic peptides	synthetic peptides
Solid phase	microtiterplate, H12	microtiterplate, V16	microtiterplate, V8	microtiterplate, V16	microtiterplate, V16
Number of tests per kit	192/576	96/480	96/480	192/960	192
Lot numbers 1-2 Expiry dates 1-2	93121402-94012610 Nov 94 - Dec 94	115014-105123/1 Aug 94	40330827-40227817 April 95 - Jan 95	MR038140-MR030530 June 94	26436 April 95
Shelf life at (°C)	12 months (2-8)	6 months (2-8)	12 months (2-8)	12 months (2-8)	5 months (2-8)
Volume of serum needed (µl) Final dilution of serum	50 1:3	10 1:21	10 1:21	15 1:21	50 1:2
Total time to perform the assay (h. min.)	2.04	1.58	2.08	1.45	2.10
Wavelength (nm) single double	450 450/660 ±40	492/570-650	450 450/620-690	492 ± 2 492/620-690	450 450 /615-690
Price/test (US\$)	1.7	1.2	1.5	1.0	2.6

Table 2. Comparison of the results of ELISAs with reference tests

NAME		VIRONOSTIKA HIV Uni-Form II	BIOTEST Anti-HIV- 1/-2 recombinant	INNOTEST HIV- 1/HIV-2 Ab. s.p.	UBI HIV 1/2 EIA	ENZYGNOST Anti-HIV 1/-HIV 2
Sensitivity % (95 CL)*						
Total panel	n = 263	100.0 (99.6 -100.0)	100.0 (99.6 -100.0)	100.0 (99.6 -100.0)	100.0 (99.6 -100.0)	100.0 (99.6 -100.0)
HIV-1 sera	n = 203	100.0 (99.6 -100.0)	100.0 (99.6 -100.0)	100.0 (99.6 -100.0)	100.0 (99.6 -100.0)	100.0 (99.6 -100.0)
African sera	n = 54	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)
Asian sera	n = 40	100.0 (99.0 -100.0)	100.0 (99.0 -100.0)	100.0 (99.0 -100.0)	100.0 (99.0 -100.0)	100.0 (99.0 -100.0)
European sera	n = 64	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)
Latin American sera	n = 45	100.0 (99.1 -100.0)	100.0 (99.1 -100.0)	100.0 (99.1 -100.0)	100.0 (99.1 -100.0)	100.0 (99.1 -100.0)
HIV-2 sera	n = 60	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)
Initial Specificity % (95 CL)*						
Total panel	n = 332	98.5 (97.2 - 99.8)	98.8 (97.6 -100.0)	98.8 (97.6 -100.0)	99.7 (99.4 -100.0)	99.5 (98.7 -100.0)
African sera	n = 80	95.0 (90.2 - 99.8)	97.5 (94.1 -100.0)	97.5 (94.1 -100.0)	98.8 (96.4 -100.0)	100.0 (99.3 -100.0)
Asian sera	n = 59	100.0 (99.2 -100.0)	96.6 (92.0 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)
European sera	n = 140	99.3 (97.9 -100.0)	100.0 (99.5 -100.0)	98.6 (96.6 -100.0)	100.0 (99.5 -100.0)	99.3 (97.9 -100.0)
Latin American sera	n = 53	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)
Final Specificity % (95 CL)*						
Total panel		98.8 (97.6 -100.0)	99.1 (98.1 -100.0)	98.8 (97.6 -100.0)	100.0 (99.7 -100.0)	not tested
African sera		95.0 (90.2 - 99.8)	97.5 (94.1 -100.0)	97.5 (94.1 -100.0)	100.0 (99.3 -100.0)	
Asian sera		100.0 (99.2 -100.0)	98.3 (95.0 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	
European sera		100.0 (99.5 -100.0)	100.0 (99.5 -100.0)	98.6 (96.6 -100.0)	100.0 (99.5 -100.0)	
Latin American sera		100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	
Reproducibility%		not tested	not tested	not tested	not tested	not tested
Indeterminate results%		not applicable	0.0	not applicable	not applicable	0.0
Delta values $\delta +$		7.4	74.9	14.0	10.8	24.8
$\delta -$		-3.0	-3.3	-3.8	-3.2	-2.6
PPV (0.01%)		0.83	1.10	0.83	100.00	1.96
(6.0%)		84.18	87.64	84.18	100.00	92.74
NPV (0.01%)		100.00	100.00	100.00	100.00	100.00
(6.0%)		100.00	100.00	100.00	100.00	100.00

*.95% confidence limits

Table 3. Detailed operational aspects: ELISAs

NAME	VIRONOSTIKA HIV Uni-Form II	BIOTEST Anti-HIV- 1/-2 recombinant	INNOTEST HIV- 1/HIV-2 Ab. s.p.	UBI HIV 1/2 EIA	ENZYGNOST Anti-HIV 1/-HIV 2
Dimension (cm) of kit : w-l-h	24-23.5-7.0 (192 tests) 27-23.5-9.5 (576 tests)	27-23.5-7.0 (96 tests) 22-16.5-10.5 (480 tests)	24-18- 8.0 (96 tests) 24-26-13.5 (480 tests)	25-16.5-14.8 (192 tests)	18 - 17.5 - 11.5 (192 tests)
Storage conditions (°C)	2 - 8	2 - 8	2 - 8	2 - 8	2 - 8
Incubation temperature (°C)	37 ± 2 and 18-25	40 and 20-25	37 and 20-25	37 ± 2	37 and 18-25
Stability* after dilution/ reconstitution/opening at (°C)					
- antigen	8 weeks (2-8)	expiry date (2-8)	8 weeks (2-8)	expiry date (2-8)	6 weeks (2-8)
- controls	expiry date (2-8)	expiry date (2-8)	expiry date (2-8)	expiry date (2-8)	4 weeks (2-8), 3 mths <-20
- sample diluent	expiry date (2-8)	expiry date (2-8)	expiry date (2-8)	expiry date (2-8)	4 weeks (2-8)
- conjugate	not applicable	expiry date (2-8)	4 hours (15-30)	8 hours (15-30)	4 weeks (2-8), 2 days (18-25)
- substrate	<1 hour (18-25)	immediately	1 hour (15-30)	immediately	5 days(2-8), 8 hours (18-25)
- wash buffer	2 weeks (2-8)	1 week (2-8)	2 weeks (2-8)	3 months (2-8)	1 week (2-8), 1 day (18-25)
Number of sera per run minimum - maximum*	7 - 92	10- 90	4 - 92	8 - 88	2 - 90
Number of controls per test run*	4-5	6	4	8	6
- negative	3	2	2	2	4
- cut-off/weak positive	0	0	0	0	0
- positive	1 or 2	2	2	4	2
- blank	0	2	0	2	0
internal control	no	no	no	no	no

Table 3 (continued). Detailed operational aspects: ELISAs

NAME	VIRONOSTIKA HIV Uni-Form II	BIOTEST Anti-HIV- 1/-2 recombinant	INNOTEST HIV- 1/HIV-2 Ab. s.p.	UBI HIV 1/2 EIA	ENZYGNOST Anti-HIV 1/-HIV 2
Estimated time to perform:					
90 sera (h.min)	2.04	1.58	2.08	1.45	2.10
-preparatory work	0.25	0.30	0.25	0.30	0.25
-incubation	1.30	1.15	1.30	1.00	1.30
-washing	0.04	0.08	0.08	0.10	0.10
-reading, interpretation	0.05	0.05	0.05	0.05	0.05
serum (h.min)	1.40	1.30	1.45	1.15	45
Equipment needed but not provided in the kit: ¹					
- washer	+	+	+	+	+
- incubator (water-bath)	+	+	+	+	+
- spectrophotometric reader	+	+	+	+	+
- mirror	-	-	-	-	-
- refrigerator (storage)	+	+	+	+	+
- agitator , rocker	+/-	-	-	-	-
- aspiration device	-	-	-	-	-
- automatic pipette (µl)	+ 50	+10	+10	+10 to 300	+50
- multichannel (µl)	+ 100	+100, 200	+50, 100, 200	+50,100, 200	+50,100
- disposable tips	+	+	+	+	+
- dilution tubes/rack,microtiterplate	+	+	+	+	+
- distilled or deionised water	+	+	+	+	+
- plate covers	-	-	-	-	-
- graduated pipette; cylinder (ml)	+ 10;1000	+5; 1000	+10; 1000	+10; 1000	+10,20; 1000
- sulphuric acid/sodium hydroxide	+	+	+	-	+
- absorbent paper	+	+	+	+	+
- disinfectant	+	+	+	+	+
- gloves	+	+	+	+	+
- reagent trough	+	+	+	+	+
Definition of positive results	$x \geq N+0.100$	$x \geq N+0.300$	$x \geq (N+P)/6$	$x \geq (0.1P)+N$	$x \geq (N+0.250)$
Definition of grey zone	not defined	$CO-10\% \leq x < CO$	not defined	not defined	$CO-10\% \leq x < CO$

+ :not provided in the kit but necessary to perform the test; - : provided in the kit or not necessary to perform the test; +/- :use is optional.

Table 4. Calculation of ease of performance: ELISAs

	VIRONOSTIKA HIV Uni-Form II	BIOTEST Anti-HIV- 1/-2 recombinant	INNOTEST HIV- 1/HIV-2 Ab. s.p.	UBI HIV 1/2 EIA	ENZYGNOST Anti-HIV 1/-HIV 2
Need to prepare:					
1. antigen	1 ¹	1	1	1	
2. substrate	0 ²	0	0	0	0
3. wash solution	0	0	0	0	0
4. conjugate	1	0	0	0	0
5. predilution of serum	1	1	1	0	1
Stability after dilution/opening: (expiry date = 1; less = 0)					
6. -antigen	0	1	0	1	0
7. -controls	1	1	1	1	0
8. -sample diluent	1	1	1	1	0
9. -conjugate	0	1	0	0	0
10. -substrate	0	0	0	0	0
11. -wash buffer	0	0	0	0	0
12. sufficient reagents	1	1	1	1	1
13. wash (yes =0; no = 1)	0	0	0	0	0
Item needed but not provided in the kit:					
14. reagent trough	0	0	0	0	0
15. automatic /multichannel pipette	0	0	0	0	0
16. dilution - tubes, rack/microtiter plate	0	0	0	0	1
17. distilled or deionised water	0	0	0	0	0
18. plate covers	1	1	1	1	
19. graduated pipette ,cylinder	0	0	0	0	0
20. sulphuric acid/sodium hydroxide	0	0	0	1	1
Total	7/20	8/20	6/20	7/20	
Ease of performance:					
-less easy ≤ 10	less easy	less easy	less easy	less easy	less easy
-easy 10 < x ≤ 15					
-very easy > 15					

¹ 1 - positive rating: reagent needs no preparation; item provided in the kit.

² 0 - negative rating: reagent needs preparation; item not provided in the kit.

Table 5. Suitability for use in small laboratories: ELISAs

NAME	Score	VIRONOSTIKA HIV Uni-Form II	BIOTEST Anti-HIV- 1/-2 recombinant	INNOTEST HIV- 1/HIV-2 Ab. s.p.	UBI HIV 1/2 EIA	ENZYGNOST Anti-HIV 1/-HIV 2
1. Sensitivity						
- 100%	3					
- 98 - 100%	2	3	3	3	3	3
- <98%	1					
2. Specificity						
- >98%	3					
- 95 - 98%	2	3	3	3	3	3
- <95%	1					
3. Incubation temperature						
- room t°	3	1	1	1	1	1
- other than room t°	1					
4. Shelf-life						
- >1 year	3					
- ≥ 6 months ≤ 1 year	2	3	2	3	3	1
- < 6 months	1					
5. Storage at						
- ambient t° possible	3					
- 2-8° C required	1	1	1	1	1	
6. Price per test US\$						
- ≤ 1.0	3					
- ≤ 2.0	2	2	2	2	3	
- > 2.0	1					
7. Ease of performance						
- very easy	3					
- easy	2	1	1	1	1	1
- less easy	1					
8. Rapidity of performance: 1 serum						
- < 10 min	3					
- 10 - 45 min	2	1	1	1	1	1
- > 45 min	1					
9. Washer/agitator						
- not needed	3	1	1	1	1	1
- needed	1					
10. Reading						
- visual	3	1	1	1	1	1
- reading equipment						
Total	30	17/30	16/30	17/30	18 /30	14/30
Suitability for use in small laboratories:						
- less suitable < 20		less suitable	less suitable	less suitable	less suitable	less suitable
- suitable 20 ≤ x < 25						
- very suitable > 25						

Table 6 (continued)

Results on early seroconversion panels: ELISAs

PANEL	SAMPLE	BLEED DATE	HIV AG RATIO ²	ABBOTT 3RD EIA RATIO ³	EIA ₁	EIA ₂	EIA ₃ RATIOS ¹			EIA ₄	EIA ₅	WB RESULT	Western Blot ⁴									
							P	P	P				GP	P	P	P	GP	GP				
													18	24	31	41	51	55	65	120	160	
PANEL Q	PRB917-01	15/10/90	0.4	0.3	0.4	0.0	0.2	0.1	0.3	0.1	0.3	ind										
	-02	07/12/90	3.9	0.3	0.5	0.0	0.2	0.1	0.2	0.1	0.2	ind										
	-03	11/12/90	21.6	0.3	0.5	0.0	0.2	0.1	0.2	0.1	0.2	ind										
	-04	14/12/90	12.7	3.7	0.8	0.8	0.2	0.1	0.3	0.1	0.3	ind										
	-05	19/12/90	2.4	8.1	3.6	5.3	1.3	2.4	2.3	0.1	0.3	ind										
	-06	21/12/90	1.6	7.8	4.0	5.9	1.8	3.8	2.9	0.1	0.3	ind										
	-07	26/12/90	0.4	5.2	4.9	7.8	4.7	11.4	5.3	0.1	0.3	pos										
PANEL R	PRB918-01	20/02/91	9.5	0.40	0.7	0.0	0.2	0.1	0.3	0.1	0.3	neg										
	-02	22/02/91	14.0	1.2	2.2	0.2	0.2	0.1	0.3	0.1	0.3	neg										
	-03	27/02/91	0.8	6.7	4.9	4.6	1.3	1.3	2.1	0.1	0.3	ind										
	-04	05/03/91	0.5	3.7	3.6	8.2	5.8	15.2	7.6	0.1	0.3	ind										
	-05	07/03/91	0.5	3.2	3.9	12.3	6.1	15.8	7.5	0.1	0.3	ind										
	-06	13/03/91	0.9	4.0	4.1	12.5	6.4	14.1	7.7	0.1	0.3	ind										

¹ Ratio = Optical density of serum divided by optical density cut-off

² HIV AG Ratio = Abbott HIV Ag

³ Abbott 3rd EIA Ratio = HIV-1/HIV-2 3rd generation ELISA, Abbott.

⁴ Western Blot = Dupont Western Blot

Legend for Western Blot interpretation : + = protein band present

+ = protein band weakly present

EIA₁ - Vironostika HIV Uni-Form II
 EIA₂ - Biotest Anti-HIV-1/-2 recombinant
 EIA₃ - Innostest HIV-1/ HIV-2 Ab. s.p.
 EIA₄ - UBI HIV 1/2 EIA
 EIA₅ - Enzygnost Anti-HIV 1/-HIV 2