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GLOBAL
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OPERATIONAL CHARACTERISTICS OF
COMMERCIALY AVAILABLE ASSAYS TO DETECT
ANTIBODIES TO HIV-1 AND/OR
HIV-2 IN HUMAN SERA

REPORT 8

GENEVA
MARCH 1994



WORLD
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**OPERATIONAL CHARACTERISTICS OF COMMERCIALY AVAILABLE ASSAYS
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REPORT 8**

Geneva, March 1994

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1. Introduction

This report, the eighth in a series dealing with the evaluation of the major operational characteristics of commercially available assays to detect antibodies to HIV, presents assessments of the following 5 assays carried out between February 1993 and June 1993.

Enzyme-linked immunosorbent assays (ELISAs)

For the detection of antibody to HIV-2

- Enzygnost Anti-HIV-2 (Behringwerke)

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

- HIV 1+2 *env* Peptide EIA (Labsystems)
- VIDAS HIV-1+2 (BioMérieux)

Rapid/Simple assays

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

- Serodia HIV-1/2 (Fujirebio)
- SPAN COMBAIDS visual (Span Diagnostics)

Section 2 of this report provides background information on the series. Sections 3 and 4 provide an overview of the laboratory diagnosis of HIV and comments on assay selection. Section 5 outlines how the assessments were carried out. Details of the assay evaluations themselves are contained in the tables in section 6. Cumulative lists of the assays already assessed under the programme and the addresses of manufacturers are given in Annexes 1 and 2.

2. Background information

In 1988, the World Health Organization (WHO) Global Programme on AIDS (GPA), conscious of the need to advise Member States on the laboratory diagnosis of HIV, initiated a programme to provide objective assessments of commercially available assays for detecting antibody to both types of HIV, HIV-1 and HIV-2. This continuing programme is coordinated by the WHO Collaborating Centre on AIDS in the Department of Microbiology, Institute of Tropical Medicine, Antwerp, Belgium.

The assessments focus on the operational characteristics of these assays, such as ease of performance and their sensitivity and specificity on a small panel of well-characterized sera of diverse geographical origins, and indicate their suitability for use in small blood-collection centres.

The assessments are published in the form of reports which are intended for use by health policy-makers, directors of blood banks, and managers of national AIDS prevention programmes, i.e., **for public sector use only**. They may be used in conjunction with consideration of other factors, such as experience with a given test, availability, cost, service and trouble-shooting provided locally by manufacturers, etc., to help select HIV antibody assays appropriate to local needs.

The first report was issued in March 1989, and subsequent reports have been issued on a regular basis; details are given in Annex 1. Further copies of this and earlier reports are available on request from the Global Programme on AIDS, World Health Organization, 1211 Geneva 27, Switzerland.

3. Laboratory diagnosis of HIV infection - a brief overview

The diagnosis of HIV infection is usually made on the basis of the detection of antibodies to HIV. Serological tests for detecting antibodies to HIV are generally classified as **initial tests** (sometimes referred to as screening tests) or **supplemental tests** (sometimes referred to as confirmatory tests). Initial tests provide the presumptive identification of antibody-positive specimens, and supplemental tests are used to determine whether specimens found reactive by an initial test contain antibodies specific to HIV.

The most widely used initial tests are ELISAs and particle agglutination assays. The earliest assays used purified HIV lysates, and deficiencies in sensitivity and specificity were identified and rapidly corrected. The sensitivity and specificity of initial assays have since improved dramatically as a result of new methods of virus purification, different test formats and the greater use of recombinant and synthetic peptide antigens.

A number of rapid/simple initial tests are now available. Most of them use an immunodot format in which specimen and reagents are added by means of a dropper to an absorbent membrane. A positive result is indicated by the appearance of a coloured dot or line. These tests require no instrumentation, can generally be performed in less than 10 minutes, and the results are read visually. These tests are most suitable for use in laboratories that have limited resources and low numbers of specimens.

When a single initial assay is used for testing in a population with a very low prevalence of HIV infection, the probability that a person is infected when a positive test result is obtained (i.e., the positive predictive value) is very low, since the majority of people with positive results are not infected. This problem occurs even when a test with high specificity is used. Accuracy can be improved if a second supplemental test is used to retest all those samples found positive by the first test. Those found negative by the second test are considered negative for antibodies to HIV.

The most commonly used supplemental test is the Western blot (WB). However, its use has proved to be excessively expensive and can, under some conditions, produce a relatively large number of results of uncertain diagnostic significance. Studies have shown that combinations of ELISAs or rapid/simple assays can provide a positive predictive value similar to that of the WB at a much lower cost. WHO therefore recommends that countries consider testing strategies that maximize the use of ELISAs and rapid/simple assays as an alternative to the WB.

WHO recommends three testing strategies to maximize accuracy while minimizing cost. Which strategy is most appropriate will depend on the objective of the test and the prevalence of HIV in the population, as shown in Table A.

- **Strategy I**

All serum is tested using one ELISA or rapid/simple assay. Serum that is reactive is considered HIV antibody positive. Serum that is non-reactive is considered HIV antibody negative.

- **Strategy II**

All serum is first tested using one ELISA or rapid/simple assay. Any serum found reactive by the first assay is retested using a second ELISA or rapid/simple assay based on a different antigen preparation and/or different test principle (e.g., indirect versus competitive). Serum that is reactive by both tests is considered HIV antibody positive. Serum that is non-reactive by the first test is considered HIV antibody negative. Any

serum that is reactive by the first test but non-reactive by the second test is also considered antibody negative.

- **Strategy III**

As in strategy II, all serum is first tested using one ELISA or rapid/simple assay, and any reactive samples are retested using a different assay. Strategy III, however, requires a third test if serum is found reactive using the second assay. The three tests in this strategy should be based on different antigen preparations and/or different test principles. Serum reactive by all three tests is considered HIV antibody positive. Serum that is non-reactive by the first is considered HIV antibody negative, as is serum that is reactive by the first test but non-reactive by the second. Serum that is reactive by the first and second tests but non-reactive by the third test is considered to be equivocal (see "Equivocal (borderline) test results" below for further details).

In the selection of HIV antibody tests for use in strategies II and III, the first test should have the highest sensitivity, whereas the second and third tests should have higher specificities than the first.

When diagnosis is the objective, an additional blood sample should be obtained and tested from all people newly diagnosed as seropositive on the basis of their first sample. This will help eliminate any possible laboratory or clerical error.

For all three strategies, stringent compliance with quality assurance procedures is most important so as to maximize the accuracy of the laboratory results. Procedures for detecting both laboratory and clerical errors must be included in all protocols. For example, procedures that guarantee the correct identification of initially reactive units of donated blood, which must be discarded, are essential to the maintenance of a safe blood supply.

Any positive test results obtained with testing strategy I must not be used for purposes of diagnosis of HIV infection in an individual. If a blood or tissue donor is to be notified of test results, the testing strategies for diagnosis must be used (Table A). Guidelines for counselling people about HIV testing, infection and disease are available from WHO/GPA.

Users should note that differentiation between HIV-1 and HIV-2 infections cannot always be achieved with the currently available antibody tests, even when the two types (HIV-1 and HIV-2) of WB are used. WHO is currently undertaking studies aimed at the development and evaluation of testing strategies for differentiation using ELISAs and/or rapid/simple assays.

Equivocal (borderline) test results

Serum from people being tested for the purpose of diagnosis should be retested if the results are equivocal, that is, neither clearly positive nor clearly negative. If the serum again produces equivocal results, testing with WB may be considered, especially for people from low-prevalence (<1%) populations. A second blood sample should be obtained a minimum of two weeks following the first sample and both should be retested using the appropriate strategy. If the second serum sample also produces an equivocal result, the person is considered to be HIV antibody negative. Equivocal results obtained during HIV surveillance studies should be reported and analysed separately.

Units of donated blood yielding equivocal test results must be discarded, as must units found to be reactive.

Table A. WHO recommendations for HIV testing strategies according to test objective and prevalence of HIV infection in the population

Objective of testing	Prevalence of HIV infection	Testing strategy ¹	
Transfusion/donation safety	all prevalences	I	
Surveillance	> 10%	I	
	≤ 10%	II	
Diagnosis	clinical signs/ symptoms of HIV infection/AIDS	all prevalences	
	asymptomatic	> 10%	II
		≤ 10%	III

¹ Strategy I: All samples are tested using one ELISA or rapid/simple test (hereafter referred to as test).

Strategy II: All samples are first tested using one test. Any reactive samples are subjected to a second test based on a different principle and/or a different antigen preparation.

Strategy III: All samples are first tested using one test. Any reactive samples are retested using a second test. Samples found reactive by the second test are subjected to a third and different test.

A number of other assays have been introduced in recent years which assist in the establishment of the diagnosis of HIV infection and may also be used to monitor the progress of the infection and the response to therapy. These include assays that detect HIV p24 antigen, antibodies to the envelope (gp41) and core (p24) antigens, and the presence of viral nucleic acid by means of the polymerase chain reaction (PCR).

Circulating p24 antigen appears early in the course of HIV infection, is detectable for several weeks, and then disappears or falls to very low levels until the onset of clinical illness. Rising titres of HIV antigen late in the illness are correlated with a poor prognosis. The presence of circulating p24 antigen is also associated with increased levels of infectious virus particles, as the probability of isolating HIV from an infected person is highest when p24 antigen is detected.

Antibodies to envelope (gp41) antigen appear early in the illness and persist for life. By contrast, although antibodies to the core (p24) antigen appear early, they tend to disappear late in the illness, about the time that p24 antigen becomes detectable. A rapid fall in the level of antibodies to p24 is also an indicator of poor prognosis.

PCR is an enzymatic method of amplifying the amount of viral nucleic acid in a specimen until it can be detected by conventional techniques. In theory, as little as a single viral genome can be detected; in practice, the technique can have limited specificity, is time-consuming, labour intensive, expensive, and remains largely a research tool. At this time, it would be unwise to base a diagnosis of HIV infection on a single positive PCR test result, in the absence of any other detectable marker.

Note: The testing of serum or plasma specimens should be performed in such a manner as to minimize occupational risk. Guidelines have been developed that, if followed, will ensure safe laboratory practices and keep laboratory accidents to a minimum. For further details see *Biosafety guidelines for diagnostic and research laboratories working with HIV*, Geneva, World Health Organization, 1991 (WHO AIDS Series 9).

4. Assay selection

In addition to the requirements indicated in section 3, there are various operational factors that influence the selection of assays, including:

- laboratory infrastructure
- access to a reference laboratory
- desired characteristics of the test (antigen, antibody)
- simplicity of test procedure
- equipment necessary to perform the test
- performance time
- shelf-life of the reagents
- price
- storage conditions
- technical skill of available personnel
- support aspects (continuous supply of kits, stability of electrical source, maintenance of equipment, spare parts, availability of service, etc.).

For use in small blood-collection centres and hospitals in developing countries, assays are needed that have the following specific characteristics:

- high level of sensitivity and specificity
- long shelf-life at ambient temperatures
- reasonable cost (generally not exceeding the per-test cost of the most readily available ELISA)
- ease of performance
- rapidity of performance.

The evaluations take these factors into account in assessing suitability for use in small centres. They show that some of the rapid/simple assays now available, which need relatively simple equipment and can be read visually, are more suitable than ELISAs in small centres where there are only a limited number of sera to be screened (< 90 sera at a time). For testing large series of sera, ELISAs are still the most rapid and most appropriate assay type. However, they require expensive equipment which has to be well maintained.

The aim of the assessment programme is to supply managers who will decide which tests to use, and the potential users of the tests, with enough comparative data to apply their own criteria and choose the best tests for particular places. It is clear, for example, that in areas such as West Africa, where HIV-2 is prevalent, a test capable of detecting antibodies to HIV-2 as well as HIV-1 will be required.

5. Materials and methods of assessment

Assay kits

Kits for the 5 commercial assays listed in section 1 were kindly provided free of charge to WHO by manufacturers for these assessments. The manufacturers and distributors were informed that the assessments were to be carried out and that they were free to visit the assessment site and to demonstrate their assays at their own expense.

Sera

The evaluations were carried out using a panel of 530 sera, of which 343 were from Africa, 139 from Europe and 48 from South America. The panel contained 202 sera positive for HIV-1 and 81 positive for HIV-2. For the assessment of assays detecting antibodies only to HIV-1, sera positive for HIV-2 were not used, leaving a total of 449. Similarly, for assays detecting antibodies only to HIV-2, sera positive for HIV-1 were excluded, leaving a total of 328.

Some assays were evaluated on a smaller panel because insufficient tests had been provided by the company. The prevalences (percentage of positive sera in a panel) are indicated in Tables 3 and 4. All samples were stored in aliquots and thawed at least once, at most twice.

Test performance

Usually, one person did all the tests. With the exception of the Western blot assays, the tests on initially reactive samples were repeated. Sera with discrepant results were tested again. Two out of three reading results determined the overall test outcome.

The simple, visually read assays were read independently by three people. Two out of three reading results determined the final outcome.

Reference tests

Results obtained using the test assays were compared with those obtained with two Western blot (WB) assays, HIV-1 WB (Du Pont de Nemours) for the detection of antibodies to HIV-1, and HIV-2 WB (New LAV Blot II, Diagnostics Pasteur) for the detection of antibodies to HIV-2. The PEPTI-LAV 1-2 assay (Diagnostics Pasteur), which is designed to differentiate HIV-1 from HIV-2 infections, was performed for the double reactive sera (9 sera positive in HIV-1 WB and HIV-2 WB); 7 samples were HIV-2 reactive, 1 was HIV-1 reactive, and 1 was double-reactive.

An HIV-1 WB or HIV-2 WB result was considered positive when 2 of 3 *env* bands (*env* precursors, external and transmembrane glycoproteins) with or without *gag* and/or *pol* bands were present (see *Weekly Epidemiological Record* (1990); 65: pp 281-283).

A WB result was considered negative when no HIV-specific band was present and indeterminate when it showed any band pattern not considered positive or negative.

6. Assay evaluations

Tables 1 and 2 summarize the general characteristics of the assays, the results compared with WB analysis, and operational aspects, and present the assessment of suitability for use in small blood-collection centres. Tables 3 and 4 provide further details of operational aspects. Factors taken into account in the calculation of ease of performance and suitability for use in small blood-collection centres are listed in Tables 5 and 6, and 7 and 8, respectively. Explanatory notes are provided following each group of tables.

Table 1. General characteristics and operational aspects: ELISAs

	HIV 1+2 <u>env</u> PEPTIDE EIA	VIDAS HIV 1+2	Enzygnost Anti-HIV-2
1. <u>Characterization</u>			
1.1 Manufacturer*	Labsystems OY	Bio Méricieux s.a.	Behring
1.2 Antigen type*	synthetic peptides (HIV-1 and HIV-2)	synthetic peptides (HIV-1 and HIV-2)	synthetic peptide (HIV-2)
1.3 Assay type	indirect ELISA	automated enzyme assay (indirect ELISA)	indirect ELISA
2. <u>Comparison of the results with reference WB</u>			
2.1 HIV-1 and HIV-2 combined			
(a) IR sensitivity (%)	100.0 (98.6-100.0)	100.0 (98.5-100.0)	
(b) RR specificity (%)	76.2 (70.0-82.4)	97.8 (95.6-100.0)	
(c) % indeterminates in assay/in WB	0.0/11.7	0.3/12.2	
2.2 HIV-1			
(a) IR sensitivity (%)			
(b) RR specificity (%)			
(c) % indeterminates in assay/in WB			
2.3 HIV-2			
(a) IR sensitivity (%)			100.0 (96.7-100.0)
(b) RR specificity (%)			99.5 (98.5-100.0)
(c) % indeterminates in assay/in WB			0.0/12.5

Table 1, continued

	HIV 1+2 <u>env</u> PEPTIDE EIA	VIDAS HIV 1+2	Enzygnost Anti-HIV-2
3.	<u>Operational aspects</u>		
3.1	Number of tests per kit*	60	96
3.2	Dimension (cm) of kit (w-l-h)	30-20-10	18-17.5-12
3.3	Storage conditions (°C)*	2-8	2-8
3.4	Preparatory work*		
(a)	Reagents	none	wash buffer conjugate substrate
(b)	Predilution of sera	none	none
(c)	Dilution of sera	unknown	1/1.25
3.5	Vol. of serum (µl)*	10	100
3.6	Incubation temperature (°C)*	20-25 and 30	20-25 and 37
3.7	Ease of performance	less easy	less easy
3.8	Estimated time to perform (h. min)		
	- 90 sera	2.15	2.30
	- 1 serum	0.40	1.45
3.9	Shelf-life (at °C)*	6 - 9 mth (2-8)	6 mth (2-8)

Table 1, continued

	HIV 1+2 env PEPTIDE EIA	VIDAS HIV 1+2	Enzygnost Anti-HIV-2
3.10	Reading*	spectrophotometer	spectrophotometer
3.11	Extra equipment needed* because it is not provided in the kit		
	- wash device	±	±
	- incubator (water-bath)	+	+
	- spectrophotometric reader	+	+
	- refrigerator (storage)	+	+
	- agitator or rocker	+	-
	- aspiration device	-	-
	- automatic pipette (µl)	+ 10	+ 100
	- multichannel (µl)	+ 90/100	+ 25/100
	- disposable tips	+	+
	- dilution tubes, rack/microtitre plate	+	-
	- distilled water	+	+
	- plate covers	-	-
	- graduated pipette, cylinder (ml)	+ 1000 µl/1/10/1000	+ 1/10/20/400
	- sulphuric acid/sodium hydroxide	+	-
4.	Price/test (US \$)*	2.8 (a) 0.8 (b)	6.2
5.	Suitability for use in small blood-collection centres	less suitable	less suitable
		3.6	3.6
		suitable	less suitable

Table 2. General characteristics and operational aspects: rapid/simple assays

	Serodia HIV-1/2	SPAN COMBAIDS visual
1. <u>Characterization</u>		
1.1 Manufacturer*	Fujirebio	Span Diagnostics
1.2 Antigen type*	lysate (HIV-1 and HIV-2)	synthetic peptides (HIV-1 and HIV-2)
1.3 Assay type	agglutination	immunodot
2. <u>Comparison of the results with reference WB</u>		
2.1 HIV-1 and HIV-2 combined		
(a) IR sensitivity (%)	100.0 (98.5-100.0)	96.5 (93.5-99.5)
(b) RR specificity (%)	100.0 (98.5-100.0)	100.0 (98.3-100.0)
(c) % indeterminates in assay/in WB	0/12.6	0/12.1
2.2 HIV-1		
(a) IR sensitivity (%)		
(b) RR specificity (%)		
(c) % indeterminates in assay/in WB		
2.3 HIV-2		
(a) IR sensitivity (%)		
(b) RR specificity (%)		
(c) % indeterminates in assay/in WB		

Table 2, continued

Serodia HIV-1/2 SPAN COMBAIDS visual

3.	<u>Operational aspects</u>		
3.1	Number of tests per kit*	220	96-192
3.2	Dimension (cm) of kit (w-h)	17-8.5-9	16-16.5-12.5
3.3	Storage conditions (°C)*	2-8	2-8
3.4	Preparatory work*		
(a)	Reagents	HIV-1 sensitized particles HIV-2 sensitized particles unsensitized particles	wash buffer
(b)	Predilution of sera	none	1/2
(c)	Dilution of sera	1/8 unsensitized 1/16 HIV-2 1/32 HIV-2	none
3.5	Vol. of serum (µl)*	25	100
3.6	Incubation temperature (°C)*	20-25	20-25
3.7	Ease of performance	less easy	easy

Table 2, continued

Serodia HIV-1/2

SPAN COMBAIDS visual

4.	Price/test (US\$)*	2.8	0.4
5.	Suitability for use in small blood-collection centres	suitable	very suitable

Explanatory notes for Tables 1 and 2

* Information obtained from the manual provided in the kit or orally from the company. These items were not evaluated.

2. HIV-1 results are compared with HIV-1 WB (DuPont de Nemours); HIV-2 results are compared with HIV-2 WB (New LAV Blot II, Pasteur).

IR : initially reactive

RR : repeatedly reactive. Sera yielding initially positive results are retested; if the 2 results are discrepant, a second repetition of the test is done. The overall outcome is determined by which 2 out of the 3 results match.

Sensitivity and specificity are defined as follows:

Sensitivity: The percentage of sera that have antibody to HIV, that are positive on the test.

Specificity: The percentage of sera that have no antibody to HIV, that are negative on the test.

Indeterminate results are not considered in the calculation of sensitivity and specificity.

95% confidence limits (CL) on the calculated sensitivity and specificity are given in parentheses. CLs are calculated using Documenta Geigy, *Scientific Tables*, Ed. K. Diem, pp. 85-103, p. 185, 1964.

The number of indeterminate results obtained with the test is compared with the number of indeterminate WB results, both expressed as a percentage of the total number of sera being tested.

Example: VIDAS HIV 1 + 2 0.3%, HIV-1 and HIV-2 combined WB 12.2%. This means that of 384 sera tested by VIDAS HIV 1+2, 1 (0.3%) were indeterminate, whereas 47 (12.2%) were indeterminate in HIV-1 and HIV-2 combined WB.

2.1 For the combined HIV-1 and HIV-2 assays and the WB assays for HIV-1 or HIV-2, a serum is regarded as:

positive when it is positive for HIV-1 or HIV-2;

negative when it is negative for both HIV-1 and HIV-2;

indeterminate when it is indeterminate for both HIV-1 and HIV-2, indeterminate for HIV-1 and negative for HIV-2 or indeterminate for HIV-2 and negative for HIV-1.

2.2 The results of the assay under evaluation are compared with those obtained with HIV-1 WB. If the assay distinguishes between HIV-1 and HIV-2, HIV-2 positive sera are omitted from the calculations; of the remaining sera only the HIV-1 results are considered. WB double-reactive sera that show an HIV-2 positive and HIV-1 negative result on the PEPTI-LAV 1-2 assay are also omitted.

2.3 The results of the assay under evaluation are compared with the HIV-2 WB results. If the assay distinguishes between HIV-1 and HIV-2, HIV-1 positive sera are omitted from the calculations; of the remaining sera only HIV-2 results are considered. WB double-reactive sera that show an HIV-2 negative and HIV-1 positive result on the PEPTI-LAV 1-2 assay are also omitted.

Explanatory notes for Tables 1 and 2 (continued)

- 3.4 (a) The reagents indicated require preparatory work.
(b) Predilution is indicated when serum dilutions have to be prepared in dilution tubes or in plates different from the "test plates" or "test tubes".
The dilution factor is given.
(c) For the VIDAS HIV 1+2, the serum dilutions are automatically performed by the VIDAS.
- 3.6 For the HIV 1+2 env PEPTIDE EIA, we used special equipment supplied by the company: Labsystems IEMS Incubator/shaker ELISA, but the assay can also be performed with an incubator/shaker $\pm 30^{\circ}\text{C} \pm 6^{\circ}\text{C}/650\text{-}900$ rpm.
- 3.8 For the Serodia HIV-1/2, four microliter plates are tested consecutively; each plate contains 24 samples at the most.
- 3.10 Visually: visual reading
Spectrophotometer: a spectrophotometer is necessary for reading
- 3.11 + : Not provided in the kit, but necessary to perform the test.
- : Not needed and not provided in the kit.
 \pm : Useful but not absolutely necessary; not provided in the kit.
For the VIDAS HIV 1+2, the assay can only be performed using VIDAS (automated analyzer).
4. Prices provided by the distributor of the test in Belgium during the indicated period. Prices vary with the number of tests ordered and between countries. For the HIV 1+2 env PEPTIDE EIA, the price of the 960 test kit is US\$ 0.8 per test and the 96 test kit US\$ 2.8 per test.
5. The calculation of the suitability for field use of the different assays is given in Tables 7 and 8.

Table 3. Detailed operational aspects: ELISAs

	HIV 1+2 <u>env</u> PEPTIDE EIA	VIDAS HIV 1+2	Enzygnost Anti-HIV-2
1. Lot numbers expiry date*	93HA1/93MB1 30 Sept '93/31 Oct '93	930729-0/930811-0 29 July '93/11 Aug '93	24734/25029 19 Aug '93/20 Oct '93
2. Solid surface*	<u>U</u> -microtitreplate, V8	tips	U-microtitreplate, V16
3. n (% preval. HIV-1) (% preval. HIV-2)	419 (36.5) (10.3)	384 (31.8) (9.6)	255 (-) (13.7)
4. PPV (0.01%) PPV (6%)	80.78 86.31	97.85 98.55	99.50 99.67
5. NPV (0.01%) NPV (6%)	100.0 100.0	100.0 100.0	100.0 100.0
6. Reader variability (%)	not applicable	not applicable	not applicable
7. Number of controls per test run*			
- negative	2	1	4
- cut-off	0	0	0
- positive	3	1	2
- reagent	2	0	0

Table 3, continued

	HIV 1+2 <u>env</u> PEPTIDE EIA	VIDAS HIV 1+2	Enzygnost Anti-HIV-2
8. Stability after dilution/ reconstitution/ opening (at °C)			
- antigen	not mentioned	expiry date (2-8)	6 weeks (2-8)
- controls	1 month (2-8)	expiry date (2-8)	4 weeks (2-8) or 3 months <-20°C
- sample diluent	not mentioned	expiry date (2-8)	4 weeks (2-8)
- conjugate	immediately	expiry date (2-8)	4 weeks (2-8) not diluted
- substrate	immediately	expiry date (2-8)	1 week (2-8) or 8 hours (18-25) diluted
- wash buffer	1 week (20-25) 1 month (2-8)	expiry date (2-8)	5 days (2-8)
9. Quantity of reagents	sufficient	sufficient	sufficient
10. Wash cycles*	2 x 4	automatically	1 x 2 and 2 x 5
11. Reading*			
- visually	-	-	-
- spectrophotometrically	+ 450	+ 450	+ 450-630
12. Definition of positive result*			
(a) for HIV-1	$x \geq 0.3$ (P-RB) + RB	$x \geq 0.33$	$x \geq N + 0.100$
(b) for HIV-2	$x \geq 0.3$ (P-RB) + RB	$x \geq 0.33$	$x \geq N + 0.100$

Table 3, continued

	HIV 1+2 env PEPTIDE EIA	VIDAS HIV 1+2	Enzygnost Anti-HIV-2
13.	Definition of grey zone*	$x \geq 0.27$ $x < 0.33$	$co - 10 \% \leq x \leq co$
14.	% results in the 10 % range above or below the co	0.3	
15.	Number of sera per test run - minimum - maximum	1 30	1 90
16.	Time to perform maximum number of sera (h. min) - preparatory work - incubation - washing - reading, interpretation	2.42 0.30 0.30 (for all three)	2.30 0.25 1.30 0.25 0.10

Table 4. Detailed operational aspects: rapid/simple and immunoblot assays

	Serodia HIV-1/2	SPAN COMBAIDS visual
1. Lot numbers expiry date*	TP 30302/TP30504 Dec. 93/May 94	K 2701/K2702 Mar. 94/Mar. 94
2. Solid surface*	gelatin particles	teeth of a plastic comb
3. n (% preval HIV-1) (% preval HIV-2)	397 (34.5) (9.3)	364 (31.6) (9.3)
4. PPV (0.01%) PPV (6%)	100.0 100.0	100.0 100.0
5. NPV (0.01%) NPV (6%)	100.0 100.0	100.0 95.0
6. Reader variability (%)	5.5 (HIV-1) 7.0 (HIV-2)	0.8
7. Number of controls per test run*		
- negative	0	1
- cut-off	0	0
- positive	2	1
- reagent	1	0

Table 4, continued

	Serodia HIV-1/2	SPAN COMBAIDS visual
8. Stability* after dilution/ reconstitution/ opening (at °C)		
- antigen	7 days (2-8)	expiry date (2-8)
- controls	expiry date (2-8)	expiry date (2-8)
- sample diluent	expiry date (2-8)	expiry date (2-8)
- conjugate	not applicable	expiry date (2-8)
- substrate	not applicable	not applicable
- wash buffer	not applicable	1 week (2-8)
9. Quantity of reagents	sufficient	sufficient
10. Wash cycles*	0	2
11. Reading*		
- visually	+	+
- spectrophotometrically	-	-
- single nm		
- double nm		
12. Definition of positive result*		
(a) for HIV-1	definite large ring with a rough multiform outer margin and peripheral agglutination or agglutinated particles spread out covering the bottom of the well uniformly	presence of red colour in the area of the peptides spot

Table 4, continued

Serodia HIV-1/2

SPAN COMBAIDS visual

(b)	for HIV-2	definite large ring with a rough multiform outer margin and peripheral agglutination or agglutinated particles spread out covering the bottom of the well uniformly	presence of red colour in the area of the peptides spot
13.	Definition of grey zone*	Particles concentrated in the shape of a compact ring with a smooth round outer margin	not defined
14.	% results in the 10 % range above or below the co	not applicable	not applicable
15.	Number of sera per test run - minimum - maximum	1 24	1 6
16.	Time to perform maximum number of sera (h.min) - preparatory work - incubation - washing - drying time - reading, interpretation	2.20 0.15 2.00 - - 0.05	0.33 0.05 0.20 0.02 0.05 0.01
17.	For remarks on each of the tests, see explanatory notes		

Explanatory notes for Tables 3 and 4

* Information obtained from the written manual in the kit or orally from the manufacturer. These items were not evaluated.

2. U-microtitreplate: microtitreplate with U-bottomed wells
 U-microtitreplate: microtitreplate with flat-bottomed wells
 h12: horizontal strips composed of 12 wells
 v8 : vertical strips composed of 8 wells

3. For an explanation of the reference panel and reference tests, see section 5 of the report. The figures given in brackets indicate the percentage of positive sera in the panel.

4-5. Predictive value of a positive test (PPV) and predictive value of a negative test (NPV) are defined as follows :

PPV: The probability that the serum does contain antibody to HIV when the test is positive.

$$PPV = \frac{(\text{prevalence})(\text{sensitivity})}{(\text{prevalence})(\text{sensitivity}) + (1 - \text{prevalence})(1 - \text{specificity})}$$

e.g., PPV (0.01%) = 76%: In a population with a prevalence of 0.01%, 76 of a total of 100 positive test results represent true-positive sera.

NPV: The probability that a serum does not have antibody to HIV when the test is negative.

$$NPV = \frac{(1 - \text{prevalence})(\text{specificity})}{(1 - \text{prevalence})(\text{specificity}) + (\text{prevalence})(1 - \text{sensitivity})}$$

e.g., NPV (6%) = 99%: In a population with a prevalence of 6%, 99 of a total of 100 negative test results represent true-negative sera.

For the calculation of PPV and NPV, the sensitivity and specificity are calculated without considering indeterminate results (see Tables 1 and 2, items 2.2 (a) and 2.2 (b)) for assays that detect only HIV-1 antibodies; for assays that detect both HIV-1 and HIV-2 antibodies, sensitivity and specificity results as indicated in 2.1(a) and 2.1(b) (Tables 1 and 2) are used.

6. The reader variability is indicated in the table when readings are performed without any equipment. Three persons independently interpret each test result. The reader variability is expressed as the percentage of sera for which test results are differently interpreted by different readers.

Explanatory notes for Tables 3 and 4 (continued)

7. For the VIDAS HIV 1+2, after receipt of a new lot of reagents and every 14 days the standard should be assayed in duplicate. The controls should be checked periodically.
8. No period indication means that reagents are not required.
For the Enzygnost Anti-HIV-2, there is a table included in the manual with details on the stability and storage of all reagents.
9. For the VIDAS HIV 1+2, all reagents are present in the test strip.
10. For example, 2 x 6 means that 2 wash steps of 6 washings have to be done.
For the VIDAS HIV 1+2, the washing is done automatically.
11. +: Method described in the manual (visually and/or spectrophotometrically).
-: Method not mentioned in the manual.
12. N: Mean optical density (OD) of the negative control serum
P: Mean OD of the positive control serum
RB: OD of the reagent blank
x: OD of a tested serum
co: Mean OD of the cut-off control serum
x <co: A sample is defined as negative when its optical density is smaller than the cut-off value
x ≥co: A sample is defined as positive when its optical density equals or exceeds the cut-off value
14. The percentage of sera yielding an OD value lying in the 10% range above or below the cut-off OD value. The lower this percentage is, the better the assay is able to differentiate between positive and negative sera.
15. The prescribed number of control samples is indicated for each assay. The maximum number of sera for each test run depends on the experience of the person reading the test. For an ELISA, the number corresponds to one complete microtitre plate. For the rapid and the agglutination assays, the number is variable.
For the VIDAS HIV 1+2, due to the periodical check of the positive and negative controls the maximum number of sera per test run is 30 (28 + 2 controls).
16. For the VIDAS HIV 1+2, the whole procedure is automatic.

Table 5. Calculation of ease of performance: ELISAs

Factor	HIV 1+2 env Peptide EIA	VIDAS HIV-1+2	Enzygnost Anti-HIV-2
1. Need to prepare:			
2. antigen	1	1	1
3. substrate	0	1	0
4. wash	0	1	0
5. conjugate	0	1	0
6. Preadilution of serum	1	1	1
7. Dilution of serum	0	0	0
8. Volume of serum needed (<2.5µl = 1; ≥2.5µl = 0)	1	0	0
9. Incubation temperature (ambient t. = 1; other than ambient t. = 0)	0	0	0
10. Stability after dilution (expiry date = 1; less = 0)			
11. antigen	1	1	0
12. controls	0	1	0
13. sample diluent	0	1	0
14. conjugate	0	1	0
15. substrate	0	1	0
16. wash buffer	0	1	0
17. Sufficient reagents	1	1	1
18. Wash (yes = 0; no = 1)	0	1	0
19. Equipment needed but not provided in the test			
20. wash device	0	1	0
21. incubator (water-bath)	0	1	0
22. spectrophotometric reader	0	1	0
23. refrigerator (storage)	0	0	0
24. agitator	0	1	1
25. aspiration device	1	1	1
26. automatic pipette	0	0	0

Table 5, continued

Factor	HIV 1+2 env Peptide EIA	VIDAS HIV-1+2	Enzygnost Anti-HIV-2
24. multichannel	0	1	0
25. dilution tubes, rack	0	1	1
26. microtitre plate	1	1	1
27. distilled water	0	1	0
28. plate covers	1	1	1
29. graduated pipette	0	1	0
30. sulphuric acid/sodium hydroxide	0	1	0
Total	8/30	25/30	8/30
Ease of performance	less easy	very easy	less easy

Table 6. Calculation of ease of performance: Rapid/simple assays

Factor	Serodia HIV-1/2	SPAN HIV-1+2 Dipstick Test
Need to prepare:		
1. antigen	0	1
2. substrate	1	1
3. wash	1	0
4. conjugate	1	1
5. Predilution of serum	0	0
6. Dilution of serum	0	1
7. Volume of serum needed (<25µl = 1; ≥25µl = 0)	0	0
8. Incubation temperature (ambient t. = 1; other than ambient t. = 0)	1	1
Stability after dilution (expiry date = 1; less = 0)		
9. antigen	0	1
10. controls	1	1
11. sample diluent	1	1
12. conjugate	1	1
13. substrate	1	1
14. wash buffer	1	0
15. Sufficient reagents	1	1
16. Wash (yes = 0; no = 1)	1	0
Equipment needed but not provided in the test		
17. wash device	1	1
18. incubator (water-bath)	1	1
19. spectrophotometric reader	1	1
20. refrigerator (storage)	0	0

Table 6, continued

Factor	Serodia HIV-1/2	SPAN HIV-1+2 Dipstick Test
21. agitator	0	1
22. aspiration device	1	1
23. automatic pipette	0	0
24. multichannel	1	1
25. dilution tubes, rack	1	1
26. microtitre plate	0	1
27. distilled water	1	0
28. plate covers	0	1
29. graduated pipette	0	0
30. sulphuric acid/sodium hydroxide	1	1
Total	19/30	21/30
Ease of performance	less easy	easy

Explanatory notes for Tables 5 and 6

Rating

1 means positive rating of a factor; for example: Item 1, HIV 1+2 ery Peptide EIA; it is not necessary to prepare antigen: 1

0 means negative rating of a factor; for example: Item 3, HIV 1+2 ery Peptide EIA; it is necessary to prepare a washing solution, but this is not good: 0

A test is rated as follows:

- very easy to perform when 25 to 30 of the above-mentioned factors are positively rated;
- easy when 20 to 24 factors are positively rated;
- less easy when ≤ 19 factors are positively rated.

Equipment needed but not provided in the test (items 17-30):

For the VIDAS HIV-1+2, the assay can only be performed using the VIDAS (automated analyzer) available from the same manufacturer.

For the Serodia HIV-1/2, the contents of the wells can also be mixed by tapping gently with the fingers on the side of the microtitre plate.

Table 7. Suitability for field use: ELISAs

Factor	Score	HIV 1+2 env Peptide EIA	VIDAS HIV-1+2	Enzygnost Anti-HIV-2
1. PPV (6%) 90-100%	3	2	3	3
2. 80-90%	2			
3. <80%	1			
4. NPV (6%) 100%	3	3	3	3
5. 99.9%	2			
6. ≤99.8%	1			
Incubation temperature				
7. - ambient t.	3	1	1	1
8. - other than ambient t.	1			
9. Shelf-life				
- ≥ 6 months at ambient t.	2	1	1	1
- or ≥ 1 year at 2-8 °C				
- less	1			
10. Storage at - ambient t. possible	2	1	1	1
11. - 2-8 °C required	1			
12. Price per test - < US\$ 1.50	2	a)1 b)2	1	1
13. - ≥ US\$ 1.50	1			
14. Ease of performance - very easy	3	1	3	1
15. - easy	2			
16. - less easy	1			
17. Rapidity of performance				
18. 1 serum - < 30 min	3	1	2	1
19. - 30-60 min	2			
20. - > 60 min	1			
21. 90 sera - < 120 min	3	2	2	2
22. - 120-180 min	2			
23. - > 180 min	1			

Table 7, continued

Factor	Score	HIV 1+2 env Peptide EIA	VIDAS HIV-1+2	Enzygnost Anti-HIV-2
24. Reading - visual	3	1	1	1
25. - spectrophotometer	1			
Total		a) 14/27 b) 15/27	18/27	15/27
Suitability for use in small blood-collection centres		less suitable	suitable	less suitable

Table 8. Suitability for field use: rapid/simple assays

Factor	Score	Serodia HIV-1/2	SPAN COMBAIDS visual
1. PPV (6%) 90-100%	3	3	3
2. 80-90%	2		
3. <80%	1		
4. NPV (6%) 100%	3	3	1
5. 99.9%	2		
6. $\leq 99.8\%$	1		
Incubation temperature			
7. - ambient t.	3	3	3
8. - other than ambient t.	1		
9. Shelf-life			
- ≥ 6 months at ambient t.	2	2	2
or ≥ 1 year at 2-8 °C			
10. - less	1		
11. Storage at - ambient t. possible	2	1	1
12. - 2-8 °C required	1		
13. Price per test - < US\$ 1.50	2	1	2
14. - \geq US\$ 1.50	1		
15. Ease of performance - very easy	3	1	2
16. - easy	2		
17. - less easy	1		
Rapidity of performance			
18. 1 serum - < 30 min	3	1	3
19. - 30-60 min	2		
20. - > 60 min	1		
21. 90 sera - < 120 min	3	2	2
22. - 120-180 min	2		
23. - > 180 min	1		

Table 8, continued

Factor	Score	Serodia HIV-1/2	SPAN COMBAIDS visual
24. Reading - visual	3	3	3
25. - fluorescence microscope	2		
26. - spectrophotometer	1		
Total		20/27	22/27

Suitability for use in small blood-collection centres

suitable

very suitable

Explanatory notes for Tables 7 and 8

Score

When each item in Tables 7 and 8 is given a value ranging from 3 to 1, a maximum score of 27 can be obtained. A test is then rated as follows:

- very suitable when the total score is ≥ 21 ;
- suitable when it is 17 to 20;
- less suitable when it is ≤ 16 .

9, 13. When the manufacturer does not state price and shelf-life, a score is not possible and therefore the rating is less good.

Annex 1

Cumulative list of assays evaluated

The names (and manufacturers) of the assays evaluated to date under the WHO programme are listed in the table below. The number of the report in which each assay is covered is given, as well as sensitivity and specificity with 95% confidence intervals, δ values for HIV antibody-positive and antibody-negative serum populations, price per test, ease of performance, and suitability for use in small blood-collection centres.

Assay (manufacturer)	Report No. ^a	Sensitivity ^b (%) ^c	Specificity ^d (%) ^e	δ -values ^f		Price/test ^g (US\$)	Ease of perform. ^h	Suitability ^a
				WB pos. sera	WB neg. sera			
Enzyme-linked immunosorbent assays								
<u>For the detection of antibody to HIV-1</u>								
Du Pont HIV-1 Recombinant ELISA (Du Pont de Nemours)	1	100.0 (98.7-100.0)	97.0 (92.7-98.8)			0.9	LE	LS
Enzygnost Anti-HIV Micro (Behringwerke)	1	100.0 (97.8-100.0)	100.0 (98.1-100.0)			1.8	LE	LS
HIV-TEK G (Sorin Biomedica)	1	100.0 (96.0-100.0)	86.5 (79.5-91.8)			1.0	LE	LS
Ortho HIV ELISA System (Ortho Diagnostic Systems)	1	100.0 (97.8-100.0)	98.0 (95.0-99.4)			1.8	LE	LS
Vironostika Anti-HIV Uni-Form (Organon Teknika)	1	100.0 (97.6-100.0)	99.5 (97.3-100.0)			2.2	LE	LS
HIV-1 env Peptide EIA (Labsystems)	2	96.0 (90.8-98.7)	97.0 (93.5-98.9)			3.9	LE	LS

Annex 1, continued

Assay (manufacturer)	Report No. ^a	Sensitivity ^b (%) ^c	Specificity ^d (%) ^e	δ -values ^f		Price/test ^f (US\$)	Ease of perform. ^g	Suitability ^h
				WB pos. sera	WB neg. sera			
Wellcozyme HIV Recombinant (Wellcome Diagnostics)	2	100.0 (98.2-100.0)	99.1 (96.8-99.9)			1.5	LE	LS
Genetic Systems LAV EIA (Genetic Systems) REC VIH-KCO1 (Heber Biotec)	3	100.0 (98.2-100.0)	96.3 (92.9-98.4)	9.2	-2.13	1.0	LE	LS
	3	97.0 (93.5-98.9)	100.0 (98.3-100.0)	2.1	-4.14	?	LE	LS
UBI HIV-1 EIA (United Biomedical)	6	100.0 (99.9-100.0)	88.2 (87.1-89.3)	7.5	-1.12	1.0	LE	S
Peptide HIV-1 ELISA Test System (Sero-Immuno Diagnostics)	6	82.1 (76.5-87.6)	94.1 (91.0-97.2)			0.6	E	VS
Enzygnost Anti-HIV-1 (Behringwerke)	7	100.0 (98.1-100.0)	100.0 (98.8-100.0)	7.4	-3.3	?	LE	LS
<u>For the detection of antibody to HIV-2</u>								
Genetic Systems HIV-2 EIA (Genetic Systems)	3	100.0 (94.0-100.0)	98.6 (95.9-99.7)	17.4	-3.06	1.7	LE	LS
Clonatec HIV-2 Ab (Clonatec)	5	100.0 (95.4-100.0)	99.5 (97.4-99.9)	6.7	-1.99	2.0	LE	S
Peptide HIV-2 ELISA Test (Sero-Immuno Diagnostics)	6	97.1 (93.0-100.0)	98.1 (96.3-99.9)			0.6	E	VS

Annex 1, continued

Assay (manufacturer)	Report No. ^a	Sensitivity ^b (%) ^c	Specificity ^d (%) ^c	δ -values ^e		Price/test ^f (US\$)	Ease of perform. ^g	Suitability ^h
				WB pos. sera	WB neg. sera			
UBI HIV-2 EIA (United Biomedical)	7	100.0 (97.4-100.0)	96.1 (93.4-98.8)	10.5	-1.7	1.2	LE	S
Enzygnost Anti-HIV-2 (Behringwerke)	8	100.0 (96.7-100.0)	99.5 (98.5-100.0)	23.8	-3.5	6.2	IE	LS
<u>For the detection of antibody to HIV-1 and HIV-2</u>								
Enzygnost Anti HIV-1+2 (Behringwerke)	2	100.0 (98.4-100.0)	97.4 (94.0-99.2)	11.30	-2.15	2.3	IE	LS
Recombinant HIV-1/HIV-2 EIA (Abbot)	2	100.0 (98.5-100.0)	97.4 (94.0-99.2)	3.78	-1.50	1.8	LE	LS
Detect-HIV™ (IAF Biochem)	3	100.0 (98.6-100.0)	97.4 (94.0-99.2)	12.65	-2.21	2.5	IE	LS
Biochrom HIV-1/HIV-2 ELISA Modul-test (Biochrom)	3	100.0 (98.6-100.0)	96.3 (92.5-98.5)	6.20	-1.69	0.9	LE	LS
Du Pont HIV-1/HIV-2 ELISA (Du Pont de Nemours)	3	100.0 (98.7-100.0)	85.6 (79.8-90.2)	9.34	-0.96	1.3	LE	LS
Viroastika HIV MIXT (Organon Teknika)	3	100.0 (98.7-100.0)	100.0 (98.1-100.0)	10.10	-2.94	1.8	IE	LS
Anti-HIV-1/HIV-2 EIA <Roche> (Hoffman-La Roche)	4	100.0 (98.7-100.0)	96.9 (93.4-98.9)	11.30	-2.37	1.7	IE	LS
Elavia Mixt (Diagnostics Pasteur)	4	100.0 (98.7-100.0)	95.1 (91.3-97.8)	54.33	-2.31	2.1	IE	LS

Annex 1, continued

Assay (manufacturer)	Report No. ^a	Sensitivity ^b (%) ^c	Specificity ^d (%) ^c	δ -values ^e		Price/test ^f (US\$)	Ease of perform. ^g	Suitability ^h
				WB pos. sera	WB neg. sera			
Wellcozyme HIV-1 + 2 (Wellcome Diagnostics)	4	100.0 (98.7-100.0)	96.9 (93.3-98.9)	38.51	-1.99	1.5	LE	LS
Peptide HIV ELISA (Cal-Tech Diagnostics)	5	72.6 (69.4-77.6)	95.4 (91.3-97.9)			0.9	E	S
GeneLavia Mixt (Diagnostics Pasteur)	5	100.0 (98.6-100.0)	98.5 (95.6-99.8)	16.77	-2.10	1.5	LE	LS
Biotest Anti-HIV-1/2 Recombinant (Biotest)	5	100.0 (98.6-100.0)	97.9 (94.9-99.4)	50.47	-3.08	1.2	LE	LS
Enzygnum-Test Anti-HIV-1+2 (Boehringer Mannheim)	6	100.0 (98.7-100.0)	100.0 (98.6-100.0)	5.50	-2.48	3.0	LE	S
Innotest HIV-1/HIV-2 Ab (Innogenetics)	6	100.0 (98.8-100.0)	97.9 (95.9-99.9)	7.22	-2.30	1.9	LE	LS
Clonatec HIV (1+2) Ab EIA (Clonatec)	6	99.6 (98.8-100.0)	95.9 (93.1-98.7)	7.47	-1.68	2.7	LE	S
Enzygnost Anti-HIV-1/HIV-2 (Behringwerke)	6	100.0 (99.9-100.0)	99.5 (98.5-100.0)	26.53	-3.50	2.6	LE	LS
UBI HIV-1/2 EIA (United Biomedical)	6	100.0 (99.9-100.0)	88.7 (84.2-93.1)	7.18	-1.24	1.2	LE	S
Peptide HIV-1 and HIV-2 ELISA Test (Sero-immuno Diagnostics)	6	97.6 (95.7-99.5)	98.5 (96.7-100.0)			0.6	E	VS

Annex 1, continued

Assay (manufacturer)	Report No. ^a	Sensitivity ^b (%) ^c	Specificity ^d (%) ^e	δ -values ^f		Price/test ^g (US\$)	Ease of perform. ^h	Suitability ^h
				WB pos. sera	WB neg. sera			
UBI HIV-1/2 EIA 2nd (United Biomedical)	7	99.5 (98.6-100.0)	92.4 (88.6-96.2)	4.8	-1.5	1.2	LE	S
Cobas Core Anti-HIV-1/HIV-2 EIA <Roche> (Hoffmann-La Roche)	7	100.0 (98.6-100.0)	89.2 (84.6-93.8)	10.8	-1.0	2.2	LE	LS
Biochrom HIV-1/2-ELISA Version 2 (Biochrom)	7	99.5 (99.0-100.0)	100.0 (98.6-100.0)	7.5	-7.3	1.0	LE	LS
Abbott Recombinant HIV-1/HIV-2 3rd Generation (Abbott)	7	100.0 (98.5-100.0)	100.0 (98.5-100.0)	11.5	-4.3	1.7/1.8	LE	LS
HIV-1 and/or HIV-2 Recombigen EIA (Cambridge Biotech)	7	100.0 (98.6-100.0)	100.0 (98.6-100.0)	10.4	-5.0	1.65	LE	LS
HIV 1+2 <u>env</u> Peptide EIA (Labsystems)	8	100.0 (98.6-100.0)	76.2 (70.0-82.4)			0.8/2.8	LE	LS
VIDAS HIV-1+2 (BioMérieux)	8	100.0 (98.5-100.0)	97.8 (95.6-100.0)			3.6	VE	S
Rapid/simple assays								
<u>For the detection of antibody to HIV-1</u>								
Recombigen HIV-LA (Cambridge BioScience)	1	95.2 (88.3-98.7)	96.1 (92.6-98.2)			3.0	VE	S
Serodia-HIV (Fujirebio)	1	100.0 (97.6-100.0)	96.9 (93.4-99.0)			1.1	E	S

Annex 1, continued

Assay (manufacturer)	Report No. ^a	Sensitivity ^b (%) ^c	Specificity ^d (%) ^e	δ -values ^c		Price/test ^f (US\$)	Ease of perform. ^g	Suitability ^h
				WB pos. sera	WB neg. sera			
HIV CHEK (Du Pont de Nemours)	1	94.5 (89.79-97.47)	99.0 (96.4-99.9)			2.5	VE	VS
Immunocomb (PBS Organics)	1	98.8 (95.7-99.9)	98.9 (96.0-99.9)			2.5	VE	VS
Serion Immuno Tab HIV-1 (Serion Immunodiagnostica)	2	98.9 (96.9-99.9)	100.0 (98.3-100.0)			2.5	LE	LS
Genie HIV-1 (Genetic Systems)	4	99.5 (97.4-100.0)	99.1 (96.7-99.9)			3.5	VE	VS
PATH HIV Dipstick (Program for Appropriate Technology in Health)	4	99.5 (97.3-100.0)	98.2 (97.1-99.1)			<1.5	E	VS
SimpliRed HIV-1 Ab (Agen Biomedical)	5	97.5 (94.2-99.2)	91.2 (86.6-94.7)			7.8/1.5	VE	S
SUDS Murex HIV-1 Ab test (Murex Diagnostics)	5	100.0 (98.5-100.0)	75.1 (69.3-80.9)			4.5	VE	S
Healthtest HIV-1 Assay (Akers Research)	6	58.7 (49.2-68.2)	89.4 (84.9-93.9)			1.4/2.3	VE	S
Entebe HIV Dipstick (Hepatika Laboratories)	6	97.0 (94.4-99.6)	99.1 (97.8-100.0)			?	E	VS

Annex 1, continued

Assay (manufacturer)	Report No ^a	Sensitivity ^b (%) ^c	Specificity ^d (%) ^e	δ -values ^e		Price/test ^f (US\$)	Ease of perform. ^g	Suitability ^h
				WB pos. sera	WB neg. sera			
<u>For the detection of antibody to HIV-1 and HIV-2</u>								
Test Pack HIV-1/HIV-2 Ab (Abbott)	2	100.0 (98.5-100.0)	95.9 (92.0-98.2)			4.8	VE	VS
HIV CHEK 1+2 (Du Pont de Nemours)	3	99.3 (97.4-99.9)	100.0 (98.1-100.0)			4.0	E	VS
Immunocomb Bi-Spot (PBS Organics)	3	98.5 (96.3-99.6)	100.0 (98.1-100.0)			4.0	VE	VS
Recodot (Waldheim Pharmazeutika)	4	98.9 (97.0-99.8)	88.6 (82.2-93.3)			2.0	LE	LS
Genie HIV-1 and HIV-2 (Genetic Systems)	4	99.3 (97.5-99.9)	99.5 (97.2-100.0)			3.5	VE	VS
Clonatec rapid HIV1-HIV2 Ab (Clonatec) Recobead LA Assay (Waldheim Pharmazeutika)	5	98.9 (96.8-99.8)	99.5 (97.2-99.8)			4.3	E	VS
	6	59.8 (53.9-65.7)	94.8 (91.7-97.9)			1.7/2.2	VE	S
Recombigen HIV-1/HIV-2 Rapid Test Device (Cambridge Biotech)	7	100.0 (98.7-100.0)	94.5 (91.2-97.8)			4.0	E	VS
Serodia HIV-1/2 (Fujirebio)	8	100.0 (98.5-100.0)	100.0 (98.5-100.0)			2.8	LE	S

Annex 1, continued

Assay (manufacturer)	Report No. ^a	Sensitivity ^b (%) ^f	Specificity ^d (%) ^f	δ -values ^e WB pos. sera	WB neg. sera	Price/test ^c (US\$)	Ease of perform. ^g	Suitability ^h
Span COMBAIDS visual (Span Diagnostics)	8	96.5 (93.5-99.5)	100.0 (98.3-100.0)			0.4	E	VS
Supplemental assays								
<u>For the detection of antibody to HIV-1</u>								
RIBA HIV (Chiron)	1	99.4 (96.6-100.0)	100.0 (97.9-100.0)			27.6	E	S
Ancoscreen (Ancos)	2	100.0 (97.8-100.0)	90.4 (82.6-95.5)			10.8/1 21.5/2	LE	LS
HIV Western Blot Kit (Organon Teknika)	3	100.0 (98.2-100.0)	100.0 (98.0-100.0)			21.0	LE	S
IFA anti-HIV-1 (Waldheim Pharmazeutika)	5	98.9 (96.9-99.8)	100.0 (98.3-100.0)			5.6	LE	LS
New LAV-Blot-I (Diagnostics Pasteur)	5	100.0 (98.1-100.0)	100.0 (96.8-100.0)			11.6	E	S
Wespage HIV-1 Western Blot Kit (Bio Genex)	6	100.0 (99.9-100.0)	100.0 (99.9-100.0)			21.6	LE	VS
Wespage HIV-1 Western Blot Kit II (Bio Genex)	7	100.0 (98.5-100.0)	100.0 (98.7-100.0)			17.7	LE	S

Annex 1, continued

Assay (manufacturer)	Report No. ^a	Sensitivity ^b (%) ^c	Specificity ^d (%) ^e	δ -values ^f WB pos. sera	WB neg. sera	Price/test ^g (US\$)	Ease of perform. ^h	Suitability ^b
<u>For the detection of antibody to HIV-2</u>								
IFA anti-HIV-2 (Waldheim Pharmazeutika)	5	98.7 (93.1-99.7)	100.0 (98.2-100.0)			6.0	LE	LS
HIV-1 Western Blot Kit (Open Tray Procedure) (Bio Genex)	7	100.0 (98.5-100.0)	100.0 (98.7-100.0)			17.7	LE	S
CBC HIV-2 Western Blot Kit (Cambridge Biotech)	7	100.0 (97.0-100.0)	100.0 (98.5-100.0)			16.0	LE	S
<u>For the detection of antibody to HIV-1 and HIV-2</u>								
Speedscreen HIV (British Bio-Technology)	4	100.0 (99.4-100.0)	66.4 (57.9-74.1)			17.0	LE	S
INNO-LIA HIV-1/HIV-2 Ab (Innogenetics)	2	100.0 (98.6-100.0)	100.0 (98.0-100.0)			18.4	LE	S
PEPTI-LAV 1-2 (Diagnostics Pasteur)	4	99.3 (96.4-99.9)	100.0 (98.1-100.0)			21.5	LE	S

Legend for Annex 1

a: Operational characteristics of commercially available assays to detect antibodies to HIV-1 and/or HIV-2 in human sera:

- Report 1 - unpublished document GPA/BMR/89.4
- Report 2 - unpublished document GPA/BMR/90.1
- Report 3 - unpublished document GPA/BMR/91.1

- Report 4 - unpublished document GPA/RES/DIA/91.6
- Report 5 - unpublished document GPA/RES/DIA/92.8
- Report 6 - unpublished document GPA/RES/DIA/93.4
- Report 7 - unpublished document GPA/RES/DIA/93.1
- Report 8 - unpublished document GPA/RID/CRD/94.4

- b, c, d: Sensitivity, specificity and 95% confidence limits were calculated as described in paragraph 2 of the explanatory notes for Tables 1 and 2, page 15 of this document.
- e: δ -values for the HIV antibody-positive and antibody-negative samples were calculated by dividing the mean of the \log_{10} sample to cut-off optical density (OD) ratios by the standard deviation of each population. WB = Western blot. The δ -value provides a statistical estimate of the test sensitivity and specificity and permits differentiation between ELISAs of similar sensitivity and specificity. High positive δ -values reflect the ability of an ELISA to consistently produce high sample/cut-off OD ratios for HIV antibody-positive sera, and consequently to have a decreased chance of producing false negatives. A high negative δ -value is correlated with a decreased chance of producing false positives, compared with the other assays in Annex 1, when tested on the same serum panel.
- f: Prices were provided by the distributor of the test in Belgium during the period of the evaluation. The prices may have changed since.
- g: Ease of performance is defined in the explanatory notes for Tables 5 and 6, page 29 of this document.
- h: Suitability for use in small blood collection centres is defined in the explanatory notes for Tables 7 and 8, page 33 of this document.

Annex 2

Cumulative list of assay manufacturers

Abbott GmbH, Diagnostika, Max-Planck-Ring 2, Postfach 1303, Delkenheim, 6200 Wiesbaden, Germany.

Tel: (49 6122) 501-01; Telex: 4182555; Fax: (49 6122) 50 12 44.

Agen Biomedical Ltd, 11 Durbell Street, P.O. Box 391, Acacia Ridge, Queensland 4110, Australia.

Tel: (61 7) 173 6266; Fax: (61 7) 273 6224.

Akers Research Corp., 201 Grove Road, Suite #1, Thorofare, New Jersey 08086, USA.

Tel: (1 609) 848 2116; Fax: (1 609) 848 0269.

Ancos A/S., Skovgaardsvej 1, 4560 Vig, Denmark.

Tel: (45 53) 41 52 55; Telex: 42580 ancoss dk; Fax: (45 53) 41 53 32.

Behringwerke AG, Diagnostica, Postfach 1140, 3550 Marburg, Germany.

Tel: (49 6421) 39 4464; Fax: (49 6421) 66064.

Biochrom KG, Leonorenstr. 2-6, 1000 West Berlin 46, Germany.

Tel: (49 30) 77 99 06-0; Telex: 185 821 bio d; Fax: (49 30) 77 10 01-2.

Bio Genex, 4600 Norris Canyon Road, San Ramon, CA 94583, USA.

Tel: (1 510) 275 05 50, (Toll Free 1 800 421 41 49).

BioMérieux S.A., 69280 Marcy-l'Etoile, France.

Tel: (33 78) 87 20 00; Fax: (33 78) 87 20 90.

Biotest AG, Landsteiner Str. 5, 6072 Dreieich, Germany.

Tel: (49 6103) 8010; Telex: 4185429; Fax: (49 6103) 88279.

Boehringer Mannheim GmbH, Sandhofer Strasse 116, Postfach 310120, D-6800 Mannheim 31, Germany.

Tel: (49 621) 759 8838; Telex: 463193 bmd/462420 bmd; Fax: (49 621) 759 8798.

British Bio-Technology Ltd, Watlington Road, Cowley, Oxford OX4 5LY, England.

Tel: (44 865) 748747; Telex: 838083 BIOTEC G; Fax: (44 865) 717598.

Cal-Tech Diagnostics, 1580 A. West San Bernardino Road, Covina, CA 91722, USA.

Tel: (1 818) 331 9763, (1 818) 571 6826, (1 818) 369 3755; Fax: (1 818) 331 1882, (1 818) 280 4846; Telex: 9102409630 Cal-Tech UQ.

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Cambridge BioScience Co., 365 Plantation Street, Worcester, MA 01605, USA.
Tel: (1 508) 797 5777; (Toll free 1 800 637 8376).

Cambridge Biotech Corp., Mervue Industrial Estate, Mervue, Galway, Ireland.
Tel: (353 91) 757534; Fax: (353 91) 757096.

Chiron Corporation, Pegasus™ Diagnostic Systems, Emmerlyville, CA 94608, USA.
Tel: (1 415) 655 87 30.

Clonatec, 60 rue de Wattignies, 75580 Paris Cedex 12, France.
Tel: (33 1) 43 42 38 30; Telex: 214044F; Fax: (33 1) 43 40 48 86.

Diagnostics Pasteur, 3 bd. Raymond Poincaré, B.P. 3, 92430 Marnes-la-Coquette, France.
Tel: (33 1) 47 95 60 00; Telex: 200464F; Fax: (33 1) 47 41 91 33.

Du Pont de Nemours International S.A., Medical Products Dept., 2 chemin du Pavillon,
1218 Grand Saconnex, Switzerland.
Tel: (41 22) 717 51 11; Telex: 845-415777 DUP CH; Fax: (41 22) 717 51 09.

Fujirebio Inc., 6th floor, Kourakuen Shinjuku Bldg., 15-7 Nishi-Shinjuku 4-Chome,
Shinjuku-ku, Tokyo 160, Japan.
Tel: (81 3) 348 0691; Telex: J 28612; Fax: (81 3) 342 6220.

Genetic Systems Corporation, 3005 First Avenue, Seattle, WA 98121, USA.
Tel: (1 206) 728 4900; Telex: 532050 Genetic Systems; Fax: (1 206) 728 4950.

Heber Biotec S.A., Calle 8, No. 306, Miramar, Havana, Cuba.
Tel: (537) 291187; Telex: 511269 cimex cu; Fax: (537) 222261.

Hepatika Laboratories, Mataram, Indonesia, under license from the Concept Foundation
Program for Appropriate Technology in Health (PATH), Seattle, WA, USA.

Hoffmann-La Roche AG, 4002 Basel, Switzerland. Tel: (41 61) 688 55 55;
Fax: (41 61) 681 98 67.

IAF Biochem International Inc., 10900 Hamon Street, Montreal, Quebec,
Canada H3M 3A2. Tel: (1 514) 335 9922; Telex: 058-27642 IAF BCM MTL; Fax:
(1 514) 335 9919.

Innogenetics S.A., Canadastraat 21, Haven 1009, 2070 Antwerp, Belgium
Tel: (32 3) 252 3711; Telex: 32248 ingen b; Fax: (32 3) 252 3799

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Labsystems OY, Pultitie 8, P. O. Box 8, 00880 Helsinki, Finland. Tel: (358 0) 72821; Telex: 123569 Labsy sf; Fax: (358 0) 7557524.

Murex Diagnostics Limited, Central Road, Temple Hill, Dartford, Kent, DA1 5LR, England. Tel: (44 322) 277711; Telex: MUREX G 896113; Fax: (44 322) 273288.

Organon Teknika N.V., Veedijk 58, 2300 Turnhout, Belgium. Tel: (32 14) 40 40 40; Telex: 71939 obtel; Fax: (32 14) 42 16 00.

Ortho Diagnostic Systems Inc., US Route 202, Raritan, N.J. 08869, USA. Tel: (1 201) 218 1300; Telex: 833 425; Fax: (1 201) 218 8582.

PBS Orgenics, Parc de l'Innovation, B.P. 209, 67405 Illkwich Cedex, Strasbourg, France. Tel: (33 88) 67 08 30; Telex: 890665; Fax: (33 88) 67 38 61.

Program for Appropriate Technology in Health, 4 Nickerson Street, Seattle, WA 98109, USA. Tel: (1 206) 285 3500; Telex: 47 100 49 PATH UI; Fax: (1 206) 285 6619.

Serion Immunodiagnostica, Bronnbachergasse 18a, 8700 Würzburg, Germany. Tel: (49 931) 14079; Telex: 68480 virion d; Fax: (49 931) 52650.

Sero-Immuno Diagnostics, P.O. Box 616, 2177-J Flintstone Drive, Tucker, GA 30084, USA. Tel: (1 404) 496 1370; Telex: 750747 SERO UD; Fax: (1 404) 938 7189.

Sorin Biomedica, Divisione Diagnostici, 13040 Saluggia (Vercelli), Italy. Tel: (39 161) 4871; Telex: 200064 I SORIN; Fax (39 161) 487672.

Span Diagnostics PVT-Ltd, 173-B New Industrial Estate UDHNA-394210 (SURAT), India. Tel: (91 261) 67 71 43; Telex: 0188284 span in; Fax: (91 261) 66 57 57.

United Biomedical Inc., 25, Davids Drive, Hauppauge, NY 11788, USA. Tel: (1 516) 273 2828; Fax: (1 516) 273 1717.

Waldheim Pharmazeutika GmbH, Boltzmanngasse 11, 1091 Vienna, Austria. Tel: (431 222) 34 66 28; Telex: 116487 warned a; Fax: (431 222) 34 66 28 44.

Wellcome Diagnostics, (Murex Diagnostics Limited) Central Road, Temple Hill, Dartford, Kent, England DA1 5LR. Tel: (44 322) 277711; Telex: MUREX G 896113; Fax: (44 322) 273288.

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