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GLOBAL  
PROGRAMME  
ON  
**AIDS**

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

REPORT 5

GENEVA  
OCTOBER 1992



WORLD  
HEALTH  
ORGANIZATION

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## 1. Introduction

This report, the fifth 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 ten assays carried out between January and May 1991.

### Enzyme-linked immunosorbent assays

#### For the detection of antibody to HIV-2

- Clonatec HIV-2 Ab (Clonatec)

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

- Peptide HIV ELISA (Cal-Tech Diagnostics)
- Genelavia Mixt (Diagnostics Pasteur)
- Biotest Anti-HIV-1/-2 recombinant (Biotest)

### Rapid/Simple assays

#### For the detection of antibody to HIV-1

- SimpliRed HIV-1 Ab (Agen Biomedical)
- SUDS Murex HIV-1 Ab test (Murex)

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

- Clonatec Rapid HIV1-HIV2 Ab (Clonatec)

### Immunoblot and immunofluorescence assays

#### For the detection of antibody to HIV-1

- IFA anti-HIV-1 (Waldheim Pharmazeutika)
- New LAV-Blot-I (Diagnostics Pasteur)

#### For the detection of antibody to HIV-2

- IFA anti-HIV-2 (Waldheim Pharmazeutika)

This report differs slightly in format from earlier reports in the series. Section 2 provides background information on the series. Sections 3 and 4 provide a revised 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, 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 and control 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 the enzyme-linked immunosorbent assays (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 using a single initial assay 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 on 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 with 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 with one ELISA or rapid/simple assay. Any serum found reactive on the first assay is retested with 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 on both tests is considered HIV antibody positive. Serum that is non-reactive on the first test is considered HIV antibody negative. Any serum that is reactive on the first test but non-reactive on the second test is also considered antibody negative.

#### **Strategy III**

As in strategy II, all serum is first tested with 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 on the second assay. The three tests in this strategy should be based on different antigen preparations and/or different test principles. Serum reactive on all three tests is considered HIV antibody positive. Serum that is non-reactive on the first test is considered HIV antibody negative, as is serum that is reactive in the first test but non-reactive in the second. Serum that is reactive in the first and second tests but non-reactive in 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, it is most important that quality assurance procedures be stringently complied with 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 regarding HIV testing, infection and disease are available from WHO.

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 ELISA 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 after 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 for surveillance should be reported and analysed separately.

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

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

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

- <sup>1</sup> Strategy I: All samples are tested with one ELISA or rapid/simple test (hereafter referred to as test).
- Strategy II: All samples are first tested with 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 with one test. Any reactive samples are retested with a different 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.

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 a poor prognostic sign.

The polymerase chain reaction (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 genome can be detected; in practice, the technique can be troubled by problems of specificity, is time-consuming, labour intensive, expensive and largely remains a research tool. At this time, it would be unwise to interpret a single positive PCR test result, in the absence of any other detectable marker, as indicative of infection with HIV.

**Note:** The testing of serum or plasma specimens should be performed in such a manner as to minimize the risk of infection. 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 reagent
- 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. 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 ten 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 or because the panel had not yet been completed. 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 (Dupont de Nemours), for the detection of antibodies to HIV-1 and HIV-2 WB (New LAV Blot II, 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 precursor, external and transmembrane glycoproteins) with or without gag and/or pol bands were present (see *Wkly Epidem. Rec.*, 65, 1990, 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

	Peptide HIV ELISA	Genelavia Mixt	Clonatec HIV-2 Ab	Biotest Anti-HIV-1/-2 Recombinant
1. <u>Characterization</u>				
1.1 Manufacture*	Cal-Tech Diagnostics	Diagnostics Pasteur	Clonatec	Biotest
1.2 Antigen type*	synthetic peptides	recombinant proteins gp160 (HIV-1) synthetic peptides env (HIV-1 and HIV-2)	synthetic peptides	recombinant proteins p24, gp41 (HIV-1) gp35 (HIV-2)
1.3 Assay type	indirect ELISA HIV-1+2	indirect ELISA HIV-1+2	indirect ELISA HIV-2	indirect ELISA HIV-1+2
2. <u>Comparison of the results with reference WB</u>				
2.1 HIV-1 and HIV-2 combined				
(a) IR sensitivity (%)	72.6 (69.4-77.6)	100.0 (98.7-100.0)	-	100.0 (98.6-100.0)
(b) RR specificity (%)	95.4 (91.3-97.9)	98.5 (95.6-99.8)	-	97.9 (94.9-99.4)
(c) % indeterminates in assay/in WB	0.2/10.2	0/10.5	-	0/10.6
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 (95.4-100.0)	-
(b) RR specificity (%)	-	-	99.5 (97.4-100.0)	-
(c) % indeterminates in assay/in WB	-	-	0/11.3	-

Table 1 continued

	Peptide HIV ELISA	Genclavia Mixt	Clonatec HIV-2 Ab	Biotest Anti-HIV-1/2 Recombinant
3. <u>Operational aspects</u>				
3.1 Number of tests per kit*	96	96/480	96	96/480
3.2 Dimension (cm) of kit (w-l-h)	6.6-13.3-9.2	16.0-23.0-14.5	14.5-21.5-11.5	13.5-14.5-10.5 (96) 21.8-18.2-12.0 (480)
3.3 Storage conditions (°C)*	2-8	2-8	2-8	2-8
3.4 Preparatory work*				
(a) Reagents	wash buffer	wash buffer, substrate	wash buffer conjugate, substrate	wash buffer, substrate
(b) Predilution of sera	-	1/5	1/25	1/20
(c) Dilution of sera	1/4	1/5	-	1/20
3.5 Vol. of serum (µl)*	50	20	5-40	10-25
3.6 Incubation temperature (°C)*	room temperature	40 and 18-25	18-22	37 or 40
3.7 Ease of performance	easy	less easy	less easy	less easy
3.8 Estimated (h. min) time to perform				
- 90 sera	2.15	2.10	1.25	(a) 2.15, (b) 2.45 (c) 3.15
- 1 serum	0.10	1.40	0.45	(a) 1.25, (b) 1.55 (c) 2.25
3.9 Shelf-life (at °C)*	1 year (2-8)	1 year (2-8)	1 year (2-8)	10 mth (2-8)
3.10 Reading*	visually	spectrophotometer	spectrophotometer	spectrophotometer

Table 1 continued

	Peptide HIV ELISA	Geneclavia Mixt	Clonatec HIV-2 Ab	Biotest Anti-HIV-1/2 Recombinant
3.11 Equipment needed* but not provided in the kit				
- wash device	-	±	±	±
- incubator (water-bath)	-	+	-	+
- spectrophotometric reader	-	+	+	+
- refrigerator (storage)	+	+	+	+
- agitator (1), rocker (2)	-	-	-	-
- aspiration device	-	-	-	-
- automatic pipette (µl)	-	+ 20	+ 10/50/100/1000	+ 10/25
- multichannel (µl)	-	± 50/100	± 100/200	± 100/200
- disposable tips	-	+	+	+
- dilution tubes,	-	(a) +	+	(a) +
rack/microtitre plate	-	(b) -	+	(b) -
- distilled water	+	+	+	+
- plate covers	-	-	-	-
- graduated pipette, cylinder (ml)	+	+ 25/100/1000	+ 25/100/1000	+ 12/1000
- sulfuric acid/ sodium hydroxide	-	-	-	+
4. <u>Price/test (US \$)*</u>	0.9 (Jan. 91)	1.5 (April 91)	2.0 (April 91)	1.2 (May 91)
5. <u>Suitability for use in small blood-collection centres</u>	suitable	less suitable	suitable	less suitable

Table 2. General characteristics and operational aspects: rapid/simple, immunoblot and immunofluorescence assays, part A

	SimpliRed HIV-1 Ab	SUDS Murex HIV-1 Ab	Clonatec Rapid HIV-1-HIV-2 Ab
1. <u>Characterization</u>			
1.1 Manufacturer*	Agen Biomedical	Murex Corporation	Clonatec
1.2 Antigen type*	synthetic peptides gp41 (HIV-1)	synthetic peptides (env) HIV lysate (core)	synthetic peptides gp41 (HIV-1) gp36 (HIV-2)
1.3 Assay type	whole blood agglutination assay HIV-1	immunodot assay HIV-1	immunodot assay HIV-1+2
2. <u>Comparison of the results with reference WB</u>			
2.1 HIV-1 and HIV-2 combined			
(a) IR sensitivity (%)	-	-	98.9 (96.8-99.8)
(b) RR specificity (%)	-	-	99.5 (97.2-99.8)
(c) % indeterminates in assay/in WB	-	-	0.4/10.5
2.2 HIV-1			
(a) IR sensitivity (%)	97.5 (94.2-99.2)	100.0 (98.5-100.0)	98.5 (95.6-99.7)
(b) RR specificity (%)	91.2 (86.6-94.7)	75.1 (69.3-80.9)	99.1 (96.7-99.9)
(c) % indeterminates in assay/in WB	0.7/6.5	11.7/6.5	0.4/6.5
2.3 HIV-2			
(a) IR sensitivity (%)	-	-	100.0 (95.1-100.0)
(b) RR specificity (%)	-	-	100.0 (98.3-100.0)
(c) % indeterminates in assay/in WB	-	-	0.0/11.5

Table 2, part A, continued

	SimpliRed HIV-1 Ab	SUDS Murex HIV-1 Ab	Cionatec Rapid HIV-1-HIV-2 Ab
3. <u>Operational aspects</u>			
3.1 Number of tests per kit*	30/60/100	30/90	30
3.2 Dimension (cm) of kit (w-l-h)	17.5-21.0-5.5	32.5-21.0-6.0/ 19.0-17.5-12.5	22.0-30.0-16.0
3.3 Storage conditions (°C)*	2-8	2-8	2-8
3.4 Preparatory work*	-	-	sample diluent
(a) Reagents	-	-	conjugate
(b) Predilution of sera	-	-	1/10
(c) Dilution of sera	-	1/17	-
3.5 Vol. of serum (µl)*	10	30	50
3.6 Incubation temperature (°C)*	room temperature	room temperature	room temperature
3.7 Ease of performance	very easy	very easy	easy
3.8 Estimated (h, min) time to perform			
- 90 sera	2.35	3.05	2.15
- 1 serum	0.04	0.10	0.06
3.9 Shelf life (at °C)*	6 mth (2-8)	1 year (2-8)	1 year (2-8)
3.10 Reading*	visually	visually	visually

Table 2, part A, continued

	SimpliRed HIV-1 Ab	SUDS Murex HIV-1 Ab	Clonatec Rapid HIV-1-HIV-2 Ab
3.11 Equipment needed* but not provided in the kit			
- wash device	-	-	-
- incubator (water-bath)	-	-	-
- spectrophotometric reader	-	-	-
- refrigerator (storage)	+	+	+
- agitator (1), rocker (2)	± (1)	-	-
- aspiration device	-	-	-
- automatic pipette (µl)	(a) - (b) +	-	-
- multichannel (µl)	-	-	-
- disposable tips	(a) - (b) +	-	-
- dilution tubes, rack/microtitre plate	-	-	-
- distilled water	-	-	-
- timer	+	+	+
- plate covers	-	-	-
- graduated pipette, cylinder (ml)	-	-	-
- sulfuric acid/sodium hydroxide	-	-	-
4. Price/test (US \$)*	7.8/4.0/1.5 (March 91)	4.5 (Nov.91)	4.3 (May 91)
5. Suitability for use in small blood-collection centres	suitable	suitable	very suitable

Table 2. General characteristics and operational aspects: rapid/simple, immunoblot and immunofluorescence assays, part B

	IFA anti-HIV-1	IFA anti-HIV-2	New LAV-Blot I
1. <u>Characterization</u>			
1.1 Manufacturer*	Waldheim Pharmazeutika	Waldheim Pharmazeutika	Diagnostics Pasteur
1.2 Antigen type*	HIV-1 infected T-cells	HIV-2 infected T-cells	HIV-1 viral lysate
1.3 Assay type	indirect immunofluorescence assay HIV-1	indirect immunofluorescence assay HIV-2	immunoblot assay HIV-1
2. <u>Comparison of the results with reference WB</u>			
2.1 HIV-1 and HIV-2 combined			
(a) IR sensitivity (%)	-	-	-
(b) RR specificity (%)	-	-	-
(c) % indeterminates in assay/in WB	-	-	-
2.2 HIV-1			
(a) IR sensitivity (%)	99.0 (97.0-99.9)		100.0 (98.1-100.0)
(b) RR specificity (%)	100.0 (98.3-100.0)		100.0 (96.8-100.0)
(c) % indeterminates in assay/in WB	0.7/6.5		30.6/6.5
2.3 HIV-2			
(a) IR sensitivity (%)		98.7 (93.2-100.0)	
(b) RR specificity (%)		100.0 (98.2-100.0)	
(c) % indeterminates in assay/in WB		1.8/11.3	

Table 2, part B, continued

	IFA anti-HIV-1	IFA anti-HIV-2	New LAV-Blot I
3. <u>Operational aspects</u>			
3.1 Number of tests per kit*	25/100	25/100	18
3.2 Dimension (cm) of kit (w-h)	11.5-19.0-13.0	11.5-19.0-13.0	16.0-23.0-14.5
3.3 Storage conditions (°C)*	2-8	2-8	2-8
3.4 Preparatory work*			
(a) Reagents	PBS buffer, conjugate, controls	PBS buffer, conjugate, controls	wash buffer
(b) Predilution of sera	1/30	1/30	1/100
(c) Dilution of sera	5	5	20
3.5 Vol. of serum (µl)*	5	5	20
3.6 Incubation temperature (°C)*	37	37	room temperature
3.7 Ease of performance	less easy	less easy	easy
3.8 Estimated (h. min) time to perform			
- 90 sera	3.15	3.15	6.05
- 1 serum	2.10	2.10	3.45
3.9 Shelf life (at °C)*			
8 mth (2-8)	8 mth (2-8)	8 mth (2-8)	10 mth (2-8)
1 year (-20)	1 year (-20)	1 year (-20)	
3.10 Reading*	immunofluorescence microscope	immunofluorescence microscope	visually

Table 2, part B, continued

	IFA anti-HIV-1	IFA anti-HIV-2	New LAV-Blot I
3.11 Equipment needed* but not provided in the kit			
- wash device	-	-	-
- incubator(water-bath)	+	+	-
- spectrophotometric reader	-	-	-
- refrigerator (storage)	+	+	+
- agitator (1), rocker (2)	± (1)	± (1)	± (2)
- aspiration device	-	-	+
- automatic pipette (µl)	+	+	+
- multichannel (µl)	+	+	+
- disposable tips	-	-	-
- dilution tubes, rack/microtitre plate	+	+	+
- distilled water	+	+	+
- timer	+	+	+
- slide holder	+	+	-
- plate covers	±	±	-
- graduated pipette, cylinder (ml)	+	+	+
- sulfuric acid/sodium hydroxide	-	-	-
4. <u>Price/test (US \$)*</u>	5.6 (Feb.91)	6.0 (Feb.91)	11.6 (April 91)
5. <u>Suitability for use in small blood-collection centres</u>	less suitable	less suitable	suitable

## Explanatory notes for Tables 1 and 2

- \* Information obtained from the manual provided in the kit or orally from the company. These subjects 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 were not considered in the calculation of sensitivity and specificity.  
 95% confidence limits (CL) of the calculated sensitivity and specificity are given in parentheses. CLs were calculated using Documenta Geigy, Scientific Tables, Ed. K. Diem, pp. 85-103, p. 185, 1964.
- The number of indeterminate results obtained with the test was compared with the number of indeterminate WB results, both expressed as a percentage of the total number of sera being tested.  
 Example: IFA anti-HIV-1 0.7%/HIV-1 WB 6.5%. This means that of 450 sera tested by IFA anti-HIV-1, 3 (0.7%) were indeterminate, whereas 29 (6.5%) were indeterminate in HIV-1 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.
- (c) For the IFA anti-HIV-1 and IFA anti-HIV-2, if a definite staining is seen in both infected and uninfected cells, the result is considered questionable. If components with high unspecific protein binding capacity are present in the specimen, both infected and uninfected cells resemble bright, unstructured balls; the result is considered uninterpretable. Questionable and uninterpretable sera were considered "indeterminate" in the evaluation.

**Explanatory notes for Tables 1 and 2 (continued)**

- 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-positive and HIV-1-negative result on the Pepsi-LAV 1-2 assay are also omitted.
- 3.1 For the SimpliRed HIV-1, the 30-test kits contain test cards, 3 wells each, with lid; the 60- and 100-test kits contain 15-well test cards without lid.
- 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 plate" or "test tubes". The dilution factor is given.
- (c) Dilution is indicated when serum dilutions have to be prepared immediately in the "test plates" or "test tubes". The dilution factor is given.
- 3.5 For the Clenatec HIV-2 Ab, the volume of serum needed can be 5 µl up to 40 µl, depending on whether serum is diluted in a microtitre plate or in test tubes.  
For the Biotest Anti-HIV-1/2 Recombinant, the volume of serum needed depends on the dilution method used, as indicated in the instructions:  
(a) 10 µl of serum can be diluted into 200 µl of sample diluent directly into the test plate; or (b) 25 µl of sample can be diluted into 500 µl of diluent in tubes. The latter predilution is not possible though, since not enough sample diluent is supplied in the kits.  
For the evaluation, 11 µl of sample was prediluted into 220 µl of diluent in microtitre plates, then 200 µl of the prediluted samples were added to the testplate, using a multichannel pipette.
- 3.6 For the Biotest Anti-HIV-1/2 Recombinant, incubation is performed at 37 °C when using a heating block or incubator, at 40 °C when using a water-bath.
- 3.7 The calculation of the ease of performance for the different assays is given in Tables 5 and 6.
- 3.8 For the Biotest Anti-HIV-1/2 Recombinant, incubation time is (a) 1 hour 15 min when using a water-bath at 40 °C, (b) 1 hour 45 min when using an incubator at 37 °C, and (c) 2 hours 15 min when using a heating block at 37 °C.  
For the New LAV-Blot J, the time needed to test 90 sera was calculated supposing that one run of 36 tests was followed by a run of 54 tests, the latter starting as soon as the first incubation of the first run had begun.

## Explanatory notes for Tables 1 and 2 (continued)

- 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.  
±: Useful but not absolutely necessary; not provided in the kit.  
For the Genelavia Mixt and the Biotest Anti-HIV-1/-2 Recombinant, sera can be used prediluted (a), or diluted directly into the test plate (b).  
For the SimpliRed HIV-1 Ab, a 10- $\mu$ l pipette is supplied in the 30-test kit with 30 pipette tips, enough to perform the assay on whole blood samples (a); when the assay is performed on serum samples (b), tips are needed to add 10  $\mu$ l of serum, and 10  $\mu$ l of blood type O negative and HIV antibody negative whole blood: therefore, not enough tips are supplied. In the 60- and 100-test kit, no pipette is supplied (b).
4. Prices provided by the distributor of the test in Belgium during the indicated period. Prices vary with the number of tests ordered and with countries.  
For the SimpliRed HIV-1 Ab, the price of the 30-test kit is 7.8 US \$, of the 60-test kit 4.0 US \$, and of the 100-test kit 1.5 US \$.
5. The calculation of the suitability for field use for the different assays is given in Tables 7 and 8.

Table 3. Detailed operational aspects: ELISAs

	Peptide HIV ELISA	Genelavia Mixt	Clonatec HIV-2 Ab	Biotest Anti-HIV-1/2 Recombinant
1. Lot numbers expiry date*	001003/001006 15 Oct. 91	IA010Y/1B124 15 June 91/15 Aug. 91	038C5117 Nov. 91	108010/117011 8 Sept. 91/17 Sept. 91
2. Solid surface*	<u>U</u> -microtitre wells	<u>U</u> -microtitre plate	<u>U</u> -microtitre plate	<u>U</u> -microtitre plate
3. n (% preval. HIV-1) (% preval. HIV-2)	530 (38.1) (15.3)	523 (39.2) (14.7)	327 (23.9)	518 (38.0) (14.1)
4. PPV (0.01%) PPV (6%)	0.2 50.2	0.7 98.6	2.0 92.7	0.5 75.2
5. NPV (0.01%) NPV (6%)	98.2 100.0	100.0 100.0	100.0 100.0	100.0 100.0
6. Reader variability (%)	18.5	not applicable	not applicable	not applicable
7. Number of controls per test run*				
- negative	1	1	1	2
- cut-off	-	3	-	-
- positive	1	1	1	2
- reagent	-	-	-	2
8. Stability* after dilution/reconstitution/ opening at (°C)				
- antigen	expiry date (2-8)	4 wk (2-8)	expiry date (2-8)	expiry date (2-8)
- controls	expiry date (2-8)	expiry date (2-8)	expiry date (2-8)	expiry date (2-8)
- sample diluent	expiry date (2-8)	8 wk (2-8)	expiry date (2-8)	expiry date (2-8)
- conjugate	expiry date (2-8)	8 wk (2-8)	immediately	expiry date (2-8)
- substrate	expiry date (2-8)	immediately	30 min	immediately
- wash buffer	1 year (2-8)	15 days (2-8)	48 h (2-8)	1 wk (2-8)

Table 3 continued

	Peptide HIV ELISA	Geneclavia Mixt	Clonatec HIV-2 Ab	Biotest Anti-HIV-1/2 Recombinant
9.	Quantity of reagents	volume of controls and number of OPD tablets limits number of possible test runs (480-test)	sufficient	volume of controls and substrate tablets (480-test), and of sample diluent (96-and 480-test) limit the number of possible runs
10.	Wash cycles*	1 X 3 2 X 4	1 X 5 2 X 4	2 X 5
11.	Reading* - visually - spectrophotometrically - single run - double run	- + 492 492/620	- + 492 -	- + 492 492/570-650
12.	Definition of positive result*			
(a)	for HIV-1	$x \geq co:10$	-	$x \geq N + 0.300$
(b)	for HIV-2	$x \geq co:10$	$x \geq N + (0.4 \times P)$	$x \geq N + 0.300$
13.	Definition of grey zone*	$0.10 \times co < x < co$	not defined	$0.1 \times co < x < co$
14.	% results in the 10 % range above or below the co	1.0	0.3	0.6
15.	Number of sera per test run - minimum - maximum	1 + 2c 4 + 2c 3 + 5c 91 + 5c	14 + 2c 94 + 2c	10 + 6c 90 + 6c

Table 3 continued

	Peptide HIV ELISA	Genelavia Mixt	Clonatec HIV-2 Ab	Biotest Anti-HIV-1/2 Recombinant
16. Time to perform maximum number of sera (h. min)	0.13	2.10	1.25	(a) 2.15, (b) 2.45, (c) 3.15
- preparatory work	0.02	0.20	0.30	0.35
- incubation	0.07	1.30	0.35	(a) 1.15, (b) 1.45, (c) 2.15
- washing	0.02	0.15	0.15	0.20
- reading, interpretation	0.02	0.05	0.05	0.05
17. For remarks on each of the tests, see explanatory notes				

Table 4. Detailed operational aspects: rapid/simple, immunoblot and immunofluorescence assays, part A

	SimpliRed HIV-1 Ab	SUDS Murex HIV-1 Ab	Clonatec Rapid HIV-1-HIV-2 Ab
1. Lot numbers expiry date*	DH014/DH015 June 91	1113/D1175 Aug. 91/July 91	1901040/041C5227 Aug. 91/Dec. 91
2. Solid surface*	monoclonal antibody that binds to the RBC surface	mixture of latex particles	membrane
3. n (% preval. HIV-1) (% preval. HIV-2)	448 (44.6) ( - )	444 (43.9) ( - )	522 (38.3) (14.2)
4. PPV (0.01%) PPV (6%)	0.1 41.4	0.04 20.4	1.9 92.7
5. NPV (0.01%) NPV (6%)	100.0 99.8	75.1 100.0	100.0 99.9
6. Reader variability (%)	10.5	22.9	15.9
7. Number of controls per test run*	1 (per sample)	1	1
- negative	-	-	-
- cut off	1	1	1
- positive	-	-	-
strong HIV-1+2	-	-	-
weak HIV-1	-	-	-
- reagent	-	-	-

Table 4, part A, continued

	SimpliRed HIV-1 Ab	SUDS Murex HIV-1 Ab	Clonatec Rapid HIV-1-HIV-2 Ab
8. Stability* after dilution/reconstitution/opening at (°C)			
- antigen	expiry date (2-8)	expiry date (2-8)	expiry date (2-8)
- controls	expiry date (2-8)	expiry date (2-8)	expiry date (2-8)
- sample diluent	-	expiry date (2-8)	1 mlh (2-8)
- conjugate	expiry date (2-8)	expiry date (2-8)	1 mlh (2-8)
- substrate	-	expiry date (2-8)	expiry date (2-8)
- wash buffer	-	expiry date (2-8)	expiry date (2-8)
9. Quantity of reagents	sufficient	sufficient	sufficient
10. Wash cycles*	-	2 X 1	1 X 1
11. Reading*			
- visually	+	+	+
- spectrophotometrically	-	-	-
. single nm			
. double nm			
12. Definition of positive result*			
(a) for HIV-1	test well shows agglutination	blue spot in the centre circle	blue spot at position 1
(b) for HIV-2	-	-	blue spot at position 2
13. Definition of grey zone*	not defined	defined	not defined
14. % results in the 10% range above or below the co	not applicable	not applicable	not applicable

Table 4, part A, continued

	SimpliRed HIV-1 Ab	SUDS Murex HIV-1 Ab	Clonatec Rapid HIV-1-HIV-2 Ab
15. Number of sera per test run			
- minimum	1	1	1
- maximum	5	10	12
16. Time to perform maximum number of sera (h. min)			
- preparatory work	0.09	0.20	0.18
- incubation	0.06	0.05	0.05
- washing	0.02	0.08	0.05
- reading, interpretation	-	0.06	0.07
	0.01	0.01	0.01
17. For remarks on each of the tests, see explanatory notes			

Table 4. Detailed operational aspects: rapid/simple, immunoblot and immunofluorescence assays, part B

	IFA anti-HIV-1	IFA anti-HIV-2	New L.A.V.-Blot I
1. Lot numbers expiry date*	01004/01107 Sept. 91/Oct. 91	00816/01118 Aug. 91/Nov. 91	1B114Y/1B118Y 1 Nov. 91/15 Feb. 92
2. Solid surface*	immunofluorescence slide	immunofluorescence slide	nitrocellulose membrane
3. n (% preval. HIV-1) (% preval. HIV-2)	450 (44.7) ( - )	328 ( - ) (24.1)	447 (44.3) ( - )
4. PPV (0.01%) PPV (6%)	100.0 100.0	100.0 100.0	100.0 100.0
5. NPV (0.01%) NPV (6%)	100.0 99.9	100.0 99.9	100.0 100.0
6. Reader variability (%)	13.8	11.0	not applicable
7. Number of controls per test run*			
- negative	1	1	1 ?
- cut-off	-	-	-
- positive	1	1	1
- reagent	-	-	-
8. Stability* after dilution/ reconstitution/ opening at (°C)			
- antigen	expiry date (2-8)	expiry date (2-8)	expiry date (2-8)
- controls	3 mth (2-8)	3 mth (2-8)	expiry date (2-8)
- sample diluent	-	-	expiry date (2-8)
- conjugate	immediately	immediately	expiry date (2-8)
- substrate	-	-	expiry date (2-8)
- wash buffer	until contamination or salt precipitation (2-8)	until contamination or salt precipitation (2-8)	1 mth (2-8)

Table 4, part B, continued

	IFA anti-HIV-1	IFA anti-HIV-2	New LAV-Blot 1
9. Quantity of reagents	volume of wash buffer limits number of possible test runs	volume of wash buffer limits number of possible test runs	sufficient
10. Wash cycles*	2 X 2	2 X 2	2 X 3
11. Reading*	-	-	+
- visually	+	+	-
- microscopically	-	-	-
- spectrophotometrically			
. single nm			
. double nm			
12. Definition of positive result*			
(a) For HIV-1	fluorescence of infected cells	-	2 of 3 <u>env</u> bands
(b) For HIV-2	-	fluorescence of infected cells	-
13. Definition of grey zone*	not defined	not defined	not defined
14. % results in the 10% range above or below the CO	not applicable	not applicable	not applicable

Table 4, part B, continued

	IFA anti-HIV-1	IFA anti-HIV-2	New LAV-Blot I
15. Number of sera per test run			
- minimum	3	3	1
- maximum	48	48	16
16. Time to perform maximum number of sera (h. min)	2.40	2.40	4.05
- preparatory work	0.25	0.25	0.10
- incubation	1.00	1.00	3.10
- washing	1.00	1.00	0.30
- dry time	-	-	0.05
- reading, interpretation	0.15	0.15	0.10
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 provided orally by the company. These subjects were not evaluated.

- 2. U-microplate: microplate with U-bottomed wells
- U-microplate: microplate 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 result (PPV) and predictive value of a negative test result (NPV) are defined as follows:  
 PPV: The probability that a 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 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 were 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 items 2.1 (a) and 2.1 (b) of Tables 1 and 2) were used.

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

Explanatory notes for Tables 3 and 4 (continued)

7. For the New LAV-Blot I, it is not clear if the negative control should be used for each run or not.
8. No period indication means that reagents are not required.
9. For the Biotest Anti-HIV-1/-2 Recombinant, if predilutions are made in test tubes following the instructions (25 µl of sample + 500 µl of diluent), not enough diluent is supplied in the kits.  
For the SimpliRed HIV-1 Ab, see explanatory notes for Tables 1 and 2, item 3.11.
10. For example, 2 X 6 means that 2 wash steps of 6 washings have to be done.
11. +: Method described in the manual.  
-: 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  
x: OD of a tested serum  
co: Mean OD of the cut-off control serum  
x < co: A sample is defined as positive when its OD is smaller than the cut-off value  
x ≥ co: A sample is defined as positive when its OD equals or exceeds the cut-off value  
For the Peptide HIV ELISA, a serum is interpreted as positive when the well shows a blue colour and negative when the well does not show colour within 3 minutes. A slight bluish tinge, much lighter than the positive reference well, may result from insufficient washing, and should be considered as negative. Results have to be read within 3 minutes.  
In the IFA anti-HIV-1 and IFA anti-HIV-2, HIV-infected cells exhibit strong fluorescein isothiocyanate-specific apple-green or white fluorescence with high contrast. Uninfected reference cells should give an impression similar to the negative control (weak yellowish or pale white shadows with low contrast).

Explanatory notes for Tables 3 and 4 (continued)

13. For the Genelavia Mixt, 0.6% of the sera tested yielded an OD in the grey zone at least once.  
For the Biotest Anti-HIV-1/-2 Recombinant, 0.4% of the sera tested yielded an OD in the grey zone at least once.
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 (c) 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.
16. For the Biotest Anti-HIV-1/-2 Recombinant, see the Explanatory notes for Tables 1 and 2, item 3.8.
17. Biotest Anti-HIV-1/-2 Recombinant, see the Explanatory notes for Tables 1 and 2, item 3.8; in this evaluation, both incubator and heating block were used for incubations.  
Clonatec Rapid HIV-1/HIV-2 Ab.  
The assay can be performed on serum, plasma or whole blood samples. It is recommended that fresh samples are used.  
A blue circle appears on the membrane as a control for correct manipulation and functioning of the reagents; it is not a control for sample addition.  
The assay distinguishes between HIV-1 and HIV-2 antibodies, since a separate antigen spot is present for HIV-1 and for HIV-2. In cases of cross-reactivity, the diagnosis of HIV-1 or HIV-2 infection will be given by the most intense spot. 3.8% of the sera tested showed cross-reactivity. Taking the intensity of the spots into consideration, the diagnosis of HIV-1 or HIV-2 infection corresponded well with the WB HIV-1 and HIV-2 results.  
Indeterminate results could be due to a high background colour, or to the presence of a very faint or not clearly distinguishable spot. The latter possibility is not mentioned in the instructions.  
IFA Anti-HIV-1 and IFA Anti-HIV-2  
No lot number was marked on the conjugate vial. In future, conjugate vials will carry the lot number.  
New LAV-Blot I  
In the instructions it is not clear by which band the gp41 protein is represented; in the photograph supplied this band should be marked by an accolade, since it is a broad band. It is not clear if the negative control should be used for each run or not. These remarks will be corrected in the photograph and in the package insert in future. The numbering of the strips supplied in one kit was not in a consecutive numerical order.

#### Explanatory notes for Tables 3 and 4 (continued)

When comparing strips of different kits, the distance between the gp160 and the p18 protein band may differ by almost 1 cm. If different kits are run at the same time, one positive control per kit should therefore be run. A "smile effect" (☺) can be seen on the strips of one blot. The gp41 band of lot number 1B114Y was very weak.

#### Peptide HIV ELISA

The instructions are not clearly described, in particular, the washing procedure is not explained. In future, a pictorial test procedure will be supplied in the kit.

The stability of the wash buffer after dilution is not mentioned in the manual.

The plastic pipettes supplied do not fit small tubes, and the plastic bags containing the coated wells are not well closed.

Since the substrate reaction is not stopped, the result has to be read within a few minutes. 12.1 % of the sera had to be repeated twice because a discrepant result was obtained after the first repetition.

#### SimpliRed HIV-1 Ab

The assay is designed for use with freshly collected capillary or venous whole blood. When performed using serum or plasma samples, an HIV-1 negative whole blood sample from an 0 negative donor is required to provide red cells for the assay. The evaluation was performed on a panel of sera. In the instructions there is no clear description of how to read the results and within what time results should be read (the spots dry). In future, a pictorial guide will be included in each kit. According to the company, results can still be read at 3 minutes. It is not indicated in any of the kits that the rocking can be performed both by hand and by a rotary shaker. It is not indicated in the 30-test kit that it is possible to run only one positive control per 15-well test card. This is explained in the 60- and 100-test kits, which contain the 15-well test cards. Perhaps it should be mentioned more clearly that the test card cover is not to be clipped in place while rocking of the slide, since it makes the reading of the results very difficult because of condensation on the cover. In future, this will be added to the instructions.

Table 5. Calculation of ease of performance: ELISAs

Factor	Peptide HIV ELISA	Genelavia Mixt	Clonatec HIV-2 Ab	Biotest Anti-HIV-1/-2 Recombinant
1. Need to prepare antigen substrate	1	1	1	1
2. wash	1	0	0	0
3. conjugate	0	0	0	0
4. Predilution of serum	1	0	0	1
5. Dilution of serum	1	1 (0)	0	1 (0)
6. Volume of serum needed (<25=1; ≥25=0)	0	0 (1)	0	0 (1)
7. Incubation temperature (room t. = 1; other than room t. = 0)	0	1	1 (a) 0 (b)	1 (a+c) 0 (b)
8. Stability after dilution (expiry date = 1; less = 0)	0	0	1	0
9. antigen	1	0	1	1
10. controls	1	1	1	1
11. sample diluent	1	0	1	1
12. conjugate	1	0	0	1
13. substrate	1	0	0	0
14. wash buffer	0	0	0	0
15. Sufficient reagents	1	1 (a) 0 (b)	1	0
16. Wash (yes = 1; no = 0)	0	0	0	0
17. Equipment needed but not provided in the test				
17. wash device	1	1	1	1
18. incubator (water-bath)	1	0	1	0
19. spectrophotometric reader	1	0	0	0
20. refrigerator (storage)	0	0	0	0
21. agitator	1	1	1	1
22. aspiration device	1	1	1	1
23. automatic pipette	1	0	0	0

Table 5 continued

Factor	Peptide HIV ELISA	GeneLavia Mixt	Clonatec HIV-2 Ab	Biotest Anti-HIV-1/2 Recombinant
24. multichannel	1	1	1	1
25. dilution tubes, rack	1	1	1 (0)	1 (a), 0 (b), 1 (c)
26. microtitre plate	0	1 (c), 0 (d)	0 (f)	1 (a), 1 (b), 0 (c)
27. distilled water	1	0	0	0
28. plate covers	1	1	1	1
29. graduated pipette	0	0	0	0
30. sulfuric acid/sodium hydroxide	1	1	1	0
Total	21/30	11/30 (b+d) 12/30 (a+d,b+c) 13/30 (a+c)	15/30 (b) 16/30 (a)	13/30 (b) 14/30 (c) 15/30 (d)
Ease of performance	easy	less easy	less easy	less easy

Table 6. Calculation of ease of performance: Rapid/simple immunoblot and immunofluorescence assays\*

Factor	A	B	C	D	E	F
1. Need to prepare antigen substrate	1	1	1	1	1	1
2. wash	1	1	1	1	1	1
3. conjugate	1	0	0	0	0	1
4. Predilution of serum	1	0	0	0	1	1
5. Dilution of serum	1	1	1	1	0	1
6. Volume of serum needed (<25 = 1; ≥ 25 = 0)	1	0	1	1	1	0
7. Incubation temperature (room t. = 1; other than room t. = 0)	1	1	0	0	1	1
8. Stability after dilution (expiry date = 1; less = 0)						
9. antigen	1	1	1	1	1	1
10. controls	1	1	0	0	1	1
11. sample diluent	1	0	1	1	1	1
12. conjugate	1	0	0	0	1	1
13. substrate	1	1	1	1	1	1
14. wash buffer	1	1	0	0	0	1
15. Sufficient reagents	1	1	0	0	1	1
16. Wash (yes = 1; no = 0)	1	0	0	0	0	0
Equipment needed but not provided in the test						
17. wash device	1	1	1	1	1	1
18. incubator (water-bath)	1	1	0	0	1	1
19. spectrophotometric reader/microscope	1	1	0	0	1	1
20. refrigerator (storage)	0	0	0	0	0	0
21. agitator	1	1	1	1	0	1
22. aspiration device	1	1	1	1	0	1
23. automatic pipette	1 (a)	1	0	0	0	1
	0 (b)					

Table 6 continued

Factor	A	B	C	D	E	F
24. multichannel	1	1	1	1	1	1
25. dilution tubes, rack	1	1	1	1	1	1
26. microtitre plate	1	1	0	0	0	1
27. distilled water	1	1	1	1	1	1
28. plate covers	1	1	1	1	1	1
29. graduated pipette	1	1	0	0	0	1
30. sulfuric acid/sodium hydroxide	1	1	1	1	1	1
Total	28/30 (b) 29/30 (a)	23/30	15/30	15/30	20/30	26/30
Ease of performance	very easy	easy	less easy	less easy	easy	very easy

- \*A SimpliRed HIV-1 Ab
- B Clonatec Rapid HIV-1-HIV-2 Ab
- C IFA anti-HIV-1
- D IFA anti-HIV-2
- E New LAV-Blot I
- F SUDS Murex HIV-J Ab

## Explanatory notes for Tables 5 and 6

Rating

1 means positive rating of a factor. For example: Item 1, Peptide-HIV ELISA; it is not necessary to prepare antigen: 1

0 means negative rating of a factor. For example: Item 3, Peptide HIV ELISA; 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  $\leq 19$  factors are positively rated.

7. For the Clonatec HIV-2 Ab, depending on whether serum is diluted in a microtitre plate (a), or in test tubes (b), the volume of serum used is  $<25 \mu\text{l}$  or  $\geq 25 \mu\text{l}$ .
15. For the Genelavia Mixt, enough reagents are supplied in the 96-test kit, but not in the 480-test kit. For the Biotest Anti-HIV-1/-2 Recombinant, see Explanatory notes for Tables 1 and 2, item 3.5.
- 21-23. For the SimpliRed HIV-1 Ab, see Explanatory notes for Tables 1 and 2, item 3.11.
- 25-26. For the Biotest Anti-HIV-1/-2 Recombinant, see Explanatory notes for Tables 1 and 2, item 3.5.
26. For the Genelavia Mixt, a dilution can be made directly into the test plate (c), or a predilution can be made into a microtitre plate (d).

Table 7. Suitability for field use: ELISAs

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

Table 7 continued

Factor	Score	Peptide HIV ELISA	Genelavia Mixt	Clonatec HIV-2 Ab	Biotest Anti-HIV-1/-2 Recombinant
24. Reading - visual	3	3	1	1	1
25. - spectrophotometer	1				
<b>Total</b>		<b>18/27</b>	<b>16/27</b>	<b>19/27</b>	<b>14/27 (a, b) 13/27 (c)</b>
<b>Suitability for use in small blood-collection centres</b>		suitable	less suitable	suitable	less suitable

Table 8. Suitability for field use: rapid/simple tests, immunoblot and immunofluorescence assays

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

Table 8 continued

Factor	Score	A	B	C	D	E	F
24. Reading - visual	3	3	3	2	2	3	3
25. - fluorescence microscope	2						
26. - spectrophotometer	1						
Total		19/27	22/27	14/27	14/27	19/27	20/27
Suitability for use in small blood-collection centres		suitable	very suitable	less suitable	less suitable	suitable	suitable

- A SimpliRed HIV-1 Ab
- B Clonatec Rapid HIV-1-HIV-2 Ab
- C IFA anti-HIV-1
- D IFA anti-HIV-2
- E New LAV-Blot I
- F SUDS Murex HIV-1 Ab

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 value is  $\geq 21$ ;
- suitable when it is 17 to 20;
- less suitable when it is  $\leq 16$ .

21- For the Biotest Anti-HIV-1/-2 Recombinant, see Explanatory notes for Tables 1 and 2, item 3.8.  
23.

## 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 in the right hand column. The references for these reports are given below the table.

Assay (manufacturer)	Report No. *
<b>Enzyme-linked immunosorbent assays</b>	
<u>For the detection of antibody to HIV-1</u>	
Dupont HIV-1 Recombinant ELISA (Dupont de Nemours)	1
Enzygnost Anti-HIV Micro (Behringwerke)	1
Genetic Systems LAV EIA (Genetic Systems)	3
HIV-1 <u>env</u> Peptide EIA (Labsystems)	2
HIV-TEK G (Sorin Biomedica)	1
Ortho HIV ELISA System (Ortho Diagnostic Systems)	1
RECVIH-KC01 (Heber Biotec)	3
Vironostika Anti-HIV Uni-Form (Organon Teknika)	1
Wellcozyme HIV Recombinant (Wellcome Diagnostics)	2
<u>For the detection of antibody to HIV-2</u>	
Clonatec HIV-2 Ab (Clonatec)	5
Genetic Systems HIV-2 EIA (Genetic Systems)	3
<u>For the detection of antibody to HIV-1 and HIV-2</u>	
Anti-HIV-1/HIV-2 EIA <Roche> (Hoffmann-La Roche)	4

**Annex 1 (continued)**

Biochrom HIV-1/HIV-2 ELISA Modul-test (Biochrom)	3
Biotest Anti-HIV-1/-2 recombinant (Biotest)	5
DETECT-HIV (IAF Biochem)	3
DuPont HIV-1/HIV-2 ELISA (DuPont de Nemours)	3
Elavia Mixt (Diagnostics Pasteur)	4
Enzygnost Anti HIV-1 + 2 (Behringwerke)	2
Genelavia Mixt (Diagnostics Pasteur)	5
Peptide HIV ELISA (Cal-Tech Diagnostics)	5
Recombinant HIV-1/HIV-2 EIA (Abbott)	2
Vironostika HIV MIXT (Organon Teknika)	3
Wellcozyme HIV-1 + 2 (Wellcome Diagnostics)	4

**Rapid/simple assays****For the detection of antibody to HIV-1**

Genie HIV-1 (Genetic Systems)	4
HIV CHEK (DuPont de Nemours)	1
Immunocomb (PBS Organics)	1
Path HIV Dipstick (Program for Appropriate Technology in Health)	4
Recombigen HIV-LA (Cambridge BioScience)	1
Serion Immuno TAB HIV-1 (Serion)	2
Serodia-HIV (Fujirebio)	1
SimpliRed HIV-1 Ab (Agen Biomedical)	5
SUDS Murex HIV-1 Ab test (Murex)	5

**Annex 1 (continued)**

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

Clonatec Rapid HIV1-HIV2 Ab (Clonatec)	5
Genie HIV-1 and HIV-2 (Genetic Systems)	4
HIV CHEK 1 + 2 (DuPont de Nemours)	3
Immunocomb Bi-Spot (PBS Orgenics)	3
Recodot (Waldheim Pharmazeutika)	4
Test Pack HIV-1/HIV-2 Ab	2

**Supplemental assays**

For the detection of antibody to HIV-1

Ancoscreen (Ancos)	2
HIV Western Blot Kit (Organon Teknika)	3
IFA anti-HIV-1 (Waldheim Pharmazeutika)	5
New Lav-Blot-I (Diagnostics Pasteur)	5
RIBA HIV (Chiron)	1

For the detection of antibody to HIV-2

IFA anti-HIV-2 (Waldheim Pharmazeutika)	5
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For the detection of antibody to HIV-1 and HIV-2

INNO-LIA HIV-1/HIV-2 Ab (Innogenetics)	2
Speedscreen HIV (British Bio-Technology)	4

## Annex 1 (continued)

## Discriminatory assays

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

Pepti-Lav 1-2 (Diagnostics Pasteur)

4

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\* 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

**Annex 2**

**Cumulative list of assay manufacturers**

**Abbott GmbH, Diagnostika**, Max-Plank-Ring 2, 6200 Wiesbaden, Delkenheim, Germany.  
Tel: 06122 501 01; Telex: 4182555; Fax: 4961 22 501 244.

**Agen Biomedical Ltd.**, 11 Durbell Street, Acacia Ridge, 4110 Brisbane, Australia.  
Tel: (07) 173 6266; Fax: (07) 273 62 24.

**Ancos Aps.**, Skovgaardsvej 1, 4560 Vig, Denmark. Tel: 45 53 41 52 55; Telex: 42580 ancoss dk;  
Fax: 45 53 41 53 32.

**Behringwerke AG, Diagnostica**, P.F. 1140, 3550 Marburg, Germany. Tel: 0642 1391;  
Fax: 0642 1391.

**Biochrom KG**, Leonorenstr. 2-6, 1000 West Berlin, Germany. Tel: (030) 779906-0;  
Telex: 185821 bio d; Fax: (030) 7710012.

**Biotest AG**, 5 Landsteinerstrasse, 6072 Dreieich, Germany. Tel: (06103) 8010;  
Telex: 4185429; Fax: (06103) 88279.

**British Bio-Technology Ltd.**, Watlington Road, Cowley, Oxford OX4 5LY, England.  
Tel: (0865) 748747; Telex: 838083 BIOTEC G; Fax: (0865) 717598.

**Cal-Tech Diagnostics**, 1580 A. West San Bernardino Road, Covina, CA 91722, USA.  
Tel: (818) 331-9763, (818) 571-6826, (818) 369-3755; Telex: 9102409630 Cal-Tech UQ;  
Fax: (818) 331-1882, (818) 280-4846.

**Cambridge BioScience Co.**, 365 Plantation Street, Worcester, MA 0165, USA.  
Tel: (800) 637 8376, (508) 797 5777.

**Chiron Co., Peagausus™ Diagnostics Systems**, Emmerlyville, CA, USA. Tel: (415) 655 87 30.

**Clonatec**, 60 Rue de Wattignies, 75580 Paris Cedex 12, France. Tel: (1) 434 238 30;  
Telex: 214044F, Fax: (1) 434 048 86.

**Diagnostics Pasteur**, 3 Blvd. Raymond Poincaré, PB 3, 92430 Marnes-la-Coquette, France.  
Tel: (1) 479 56 000; Telex: 200464F; Fax: (1) 474 19 133.

**Dupont de Nemours (International SA)**, Medical Products Dept., 2 Chemin des Pavillons,  
CH-1218 Grand Saconnex, Geneva, Switzerland. Tel: 022 717 5111; Telex: 845-415777 DUP CH;  
Fax: 22 717 5109.

**Fujirebio Inc.**, Shinjuku Daiichi Seimei Bldg., P.O. Box 5032, Tokyo 160, Japan.  
Tel: 813 (348) 0691; Telex: J 28612; Fax: 03 (342) 6220.

**Annex 2 (continued)**

**Genetic Systems Corporation**, 3005 First Avenue, Seattle, Washington 98121, USA.

Tel: (1) (206) 728-4900; Telex: 532050 Genetic Systems; Fax: (1) (206) 7284950.

**Heber Biotck S.A.**, Calle 8, 306, Miramar, Havana, Cuba. Tel: 291187; Telex: 511269 cimex cu; Fax: 222261.

**Hoffmann-La Roche AG**, 4002 Basle, Switzerland. Tel: (061) 688 5555; Fax: (061) 681 9867.

**Innogenetics nv**, Kronenburgstraat 45, 2000 Antwerpen, Belgium. Tel: 16 48 20;

Telex: 32248 inngen b; Fax: 16 44 97.

**IAF Biochem International Inc.**, 10900 Hamon Street, Montreal, Quebec, Canada H3M 3A2.

Tel: (514) 335 9922; Telex: 058-27642 IAF BCM MTL; Fax: (514) 3359919.

**Labsystems OY**, Pultritie 8, 00880 Helsinki, Finland. Tel: 80 75821; Telex: 1002048 Labsy sf;

Fax: 80 789 732.

**Murex Corporation**, 3075 Northwoods Circle, Norcross, GA 30091, USA. Tel: 404 662 0660;

Fax: 404 447 49 89 or **Dominion Biologicals Ltd.**, 5 Insnor Drive, Dartmouth, Nova Scotia, Canada B3B 1M1.

**Organon Teknika**, Veedijk 58, 2300 Turnhout, Belgium. Tel: 014 404040; Telex 71939 obtel;

Fax: 014 421600.

**Ortho Diagnostic Systems Inc.**, US Route 202 Raritan, NJ 08869, USA. Tel: (201) 218 1300;

Telex: 833 425; Fax: (201) 218 8582.

**PBS Orgenics**, Parc de l'Innovation, B.P. 209, 67405 Illkirch Cedex, Strasbourg, France.

Tel: 88670830; Telex: 890665; Fax: 88673861.

**Program for Appropriate Technology in Health**, 4 Nickerson Street, Seattle, WA 98109, USA.

Tel: (1) (206) 285 3500; Telex: 47 400 49 PATH UI; Fax: (1) (206) 285 6619.

**Serion Immunodiagnostica**, Bronnbachergasse 18a, 8700 Würzburg, Germany. Tel: 0931 14079;

Telex: 68480 virion d; Fax: 0931 52650.

**Sorin Biomedica**, Divisione Diagnostici, 13040 Saluggia (Vercelli), Italy. Tel: 0161 4871;

Telex: 200064 I SORIN.

**Waldheim Pharmazeutika GmbH**, Boltzmanngasse 11, 1091 Vienna, Austria. Tel: (222) 34 66 28;

Telex: 116487 wamed a; Fax: (222) 34 66 28 44.

**Wellcome Diagnostics**, Dartford, Kent DA5 1AH, England. Tel: (0322) 27 77 11; Fax: 0322 27381.

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