

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

REPORT 11

**GENEVA
JANUARY 1999**



Joint United Nations Programme on HIV/AIDS
UNAIDS
UNICEF • UNDP • UNFPA • UNDCP
UNESCO • WHO • WORLD BANK



**WORLD HEALTH
ORGANIZATION**

This document is not issued to the general public, and all rights are reserved by the World Health Organization (WHO). The documents may not be reviewed, abstracted, quoted, reproduced or translated, in part or in whole, without the prior written permission of WHO. No part of this document may be stored in a retrieval system or transmitted in any form or by any means – electronic, mechanical or other without the prior written permission of WHO.

The views expressed in documents by named authors are solely the responsibility of those authors.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by WHO/UNAIDS in preference to others of a similar nature that are not mentioned.

**OPERATIONAL CHARACTERISTICS OF COMMERCIALY AVAILABLE ASSAYS
TO DETECT ANTIBODIES TO HIV-1 AND/OR HIV-2 IN HUMAN SERA
REPORT 11**

Geneva, January 1999

CONTENTS

	Page
1. Introduction	1
2. Background information	2
3. Laboratory diagnosis of HIV infection - a brief overview	2
4. Assay selection	7
5. Materials and methods of assessment	8
6. Assay evaluations	12
ELISAs	
Table 1: General characteristics and operational aspects: ELISAs	14
Table 2: Comparison of ELISA results with reference tests	15
Table 3: Detailed operational aspects: ELISAs	16
Table 4: Calculation of ease of performance: ELISAs	18
Table 5: Suitability for use in small laboratories: ELISAs	19
Table 6: Performance on seroconversion panels: ELISAs	20
SIMPLE/RAPID ASSAYS	
Group 1	
Table 7: General characteristics and operational aspects: simple/rapid assays	22
Table 8: Comparison of simple/rapid assay results with reference tests	23
Table 9: Detailed operational aspects: simple/rapid assays	24
Table 10: Calculation of ease of performance: simple/rapid assays	26
Table 11: Suitability for use in small laboratories: simple/rapid assays	27
Table 12: Performance on seroconversion panels: simple/rapid assays	28

Group 2

Table 13: General characteristics and operational aspects: simple/rapid assays	30
Table 14: Comparison of simple/rapid assay results with reference tests.....	31
Table 15: Detailed operational aspects: simple/rapid assays.....	32
Table 16: Calculation of ease of performance: simple/rapid assays	34
Table 17: Suitability for use in small laboratories: simple/rapid assays	35
Table 18: Performance on seroconversion panels: simple/rapid assays.....	36
Table 19: Comparison of assay results with HIV-1 group O specimens	38

Figure 2: Relative performance of the ELISA and simple/rapid assays on early seroconversion panel.....	39
--	----

Explanatory notes for Tables 1 - 19 and Figure 2	40
--	----

7. Annexes

Annex 1: Cumulative list of assays evaluated, which are out of production	45
Annex 2: Cumulative list of assays evaluated which are currently available	50
Annex 3: Cumulative list of assay manufacturers' addresses.....	59

8. Acknowledgments	63
--------------------------	----

1. Introduction

Report 11, dealing with the evaluation of the major operational characteristics of commercially available assays to detect antibodies to HIV, presents assessments of the following 15 assays carried out between May 1994 and June 1998.

Enzyme-linked immunosorbent assays (ELISAs)	page 14
<i>For the detection of antibody to HIV-1 and HIV-2</i>	

- Enzygnost Anti-HIV 1/2 Plus (Behringwerke AG)
- ICE * HIV-1.O.2 (Murex Biotech Ltd.)
- Vironostika HIV Uni-Form II *plus* O (Organon Teknika nv)
- GENSCREEN HIV 1/2 (Sanofi Diagnostics Pasteur)
- HIVA TEST (Lupin Laboratories Ltd.)

Simple/Rapid assays (S/R)

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

Group 1	22
---------------	----

- RED-DOT HIV 1&2 (Cal-Test Diagnostics Inc.)
- HIVCHEK System 3 Test Kit (Ortho Diagnostic Systems)
- HIV TRI-DOT (J. Mitra & Co. Ltd.)
- EasiDot HIV/EasiSpot HIV (Nubenco Diagnostics)
- AccuSpot HIV-1 and 2 (Specialty BioSystems Inc.)

Group 2	30
---------------	----

- IMx HIV-1/HIV-2 3rd generation Plus (Abbott GmbH Diagnostika)
- BIONOR HIV-1&2 (Bionor A/S)
- HIV 1&2 DoubleCheck (Organics)
- SEROCARD HIV (Trinity Biotech plc)
- SERO•STRIP HIV-1/2 (Saliva Diagnostic Systems Pte Ltd.)

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 1-3.

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 carried out by the WHO Collaborating Centre on AIDS in the Department of Microbiology, Institute of Tropical Medicine, Antwerp, Belgium and coordinated by the Blood Safety Unit of WHO in conjunction with UNAIDS.

The assessments focus on the operational characteristics of these assays, such as ease of performance and their sensitivity and specificity on a panel of well-characterized sera of diverse geographical origins, and indicate their suitability for use in small laboratories, i.e. many blood-collection centres in developing countries. Additionally the sensitivity of the assays on 8 seroconversion panels is assessed.

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 Annexes 1 and 2. Further copies of this and earlier reports are available on request from the Blood Safety Unit, Programme on Health Technology, World Health Organization, 1211 Geneva 27, Switzerland. Reports containing information of assays which are currently no longer available are taken out of distribution.

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 **screening tests** (sometimes referred to as **initial tests**) or **confirmatory tests** (sometimes referred to as **supplemental tests**). Initial tests provide the presumptive identification of antibody-positive specimens, and supplemental tests are used to confirm whether specimens found reactive with a particular screening test contain antibodies specific to HIV.

The most widely used screening tests are **ELISAs** as they are the most appropriate for screening large numbers of specimens on a daily basis, e.g. blood donations. The earliest assays used purified HIV lysates (1st generation), and often lacked sensitivity and specificity. Improved assays based on recombinant proteins and/or synthetic peptides, which also enabled the production of combined HIV-1/HIV-2 assays became rapidly available (2nd generation). The so-called 3rd generation or sandwich ELISAs, which use labeled antigen as conjugate, are extremely sensitive and have reduced the window period considerably.

A variety of simple, instrument-free initial tests are now available, including agglutination, immunofiltration (flow through tests) immunochromatographic (lateral flow tests) and dipstick tests. Specimens and reagents are often added by means of a dropper to the test device. A positive result is

indicated by the appearance of a coloured dot or line, or shows an agglutination pattern. Most of these tests can be performed in less than 10 minutes, and are therefore called **simple/rapid (S/R) assays**. Other simple tests are less rapid and their procedures require 30 minutes to 2 hours. The results are read visually. In general, these tests are most suitable for use in laboratories that have limited facilities and process low numbers of specimens daily.

When a single screening 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 test are considered negative for antibodies to HIV.

The most commonly used confirmatory test was the Western blot (WB). However, its use has proven to be very expensive and can, under some conditions, produce a relatively large number of indeterminate results. Similar assays, generically called Line immuno-assays (LIAs), based on recombinant proteins and/or synthetic peptides capable of detecting antibodies to specific HIV-1 and/or HIV-2 proteins, have been developed. Examples of this technology include the INNOLIA, Pepti-Lav, and RIBA assays. In general, these assays produce fewer indeterminate results as compared to WB, but are equally expensive. Studies have shown that combinations of ELISAs or S/R assays can provide results as reliable as the WB at a much lower cost. WHO and UNAIDS therefore recommend that countries consider testing strategies which use ELISAs and S/R assays rather than ELISA/WB for HIV antibody detection.

UNAIDS and WHO recommend three testing strategies, which have been recently updated, 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* and *Figure 1*.

Table A. UNAIDS and WHO recommendations for HIV testing strategies according to test objective and prevalence of infection in the sample population			
Objective of testing	Prevalences of infection	Testing strategy	
<i>Transfusion/transplant safety</i>	All prevalences	I	
<i>Surveillance</i>	>10%	I	
	≤10%	II	
<i>Diagnosis</i>	Clinical signs/ symptoms of HIV infection ¹	>30%	I
		≤30%	II
	Asymptomatic	>10%	II
		≤10%	III

Strategy I

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

¹ World Health Organization. Interim proposal for a WHO staging system for HIV infection and disease. *Weekly Epidemiological Record* 1990, 65:221-228.

Transfusion/transplant safety

When the objective is safeguarding the blood supply, the test selected for this strategy should preferably be a **combined HIV-1/HIV-2 assay** which is **highly sensitive**. Units of donated blood yielding **reactive** or **indeterminate** test results must be considered as probably infected with HIV and should be discarded according to universal safety instructions.² Strategy I is meant for testing the donations, but **must not be used for notifying donors of a positive test result**. If a blood or tissue donor is to be notified of a test result, testing strategies II or III for diagnosis must be applied (*Table 2, Figure 1*). Whatever the final diagnosis, donations which were initially reactive should not be used for transfusion or transplants. Several studies have shown that careful selection of donors is more efficient than HIV antigen testing in minimizing the risk of transfusion related infections.

Surveillance

Sensitivity is less crucial for surveillance purposes; however, for this and the above application the assay chosen should have a specificity of at least 95%. It is recommended that the same assay(s) be used over time to monitor fluctuations in HIV prevalence.

*Diagnosis (see below)***Strategy II**

All serum/plasma is first tested with one ELISA or simple/rapid assay. Any serum found reactive on the first assay is retested with a second ELISA or simple/rapid 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, should be retested with the two assays. Concordant results after repeat testing will indicate a positive or negative result. If the results of the two assays remain discordant the serum is considered indeterminate.

Surveillance

When testing low HIV prevalence populations for surveillance purposes, even if one uses a test of high specificity, the PPV will be very low. Therefore, an additional test is necessary in order not to overestimate the HIV prevalence in such regions. All samples remaining discordant after repeat testing with the two assays are considered indeterminate; unlike for diagnosis, no further testing is needed. The indeterminate results should be reported and analysed separately in the annual surveillance overviews.

*Diagnosis (see below)***Strategy III**

As in strategy II, all serum is first tested with one ELISA or simple/rapid assay, and any reactive samples are retested using a different assay. Serum that is non-reactive on the first test is considered HIV antibody negative. Serum that is reactive in the first test but non-reactive in the second assay, should be repeated with both tests. Strategy III, however, requires a third test if serum is found reactive on the second assay or is reactive on the repeated first 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 remains discordant in the second assay, or is reactive in

² See WHO AIDS SERIES 9, *Biosafety guidelines for diagnostic and research laboratories working with HIV*.

the first and second tests but non-reactive in the third test, is considered to be indeterminate. Serum that is reactive on the first assay and non-reactive on the second and third assays is considered indeterminate for individuals who may have been exposed to HIV in the last three months and negative for those who have not been exposed to any risk for HIV infection.

Diagnosis (Strategies I, II and III)

Newly diagnosed HIV seropositives

An additional blood sample should be obtained and tested from all persons newly diagnosed as seropositive on the basis of their first sample. This will help eliminate any possible technical or clerical error.

Uncertain diagnosis: indeterminate result

Serum from people with clinical signs meeting the WHO criteria³, stages III or IV, may have an indeterminate result due to a decrease in antibodies. In this case serum does not normally need to be retested.

For diagnosis of HIV infection in asymptomatic individuals, with an indeterminate result, a second blood sample should be obtained after a minimum of two weeks following the first sample and should be tested using the appropriate strategy. If the second serum sample also produces an indeterminate result, it should be tested with a confirmatory assay. However, if this result is also indeterminate longer follow-up may be required (3, 6, 12 months). If the results remain indeterminate after 1 year, the person is considered to be HIV antibody negative.

General remarks about Strategies I-II-III

Strategy I can only be used to confirm the clinical diagnosis of individuals meeting the WHO criteria of stage III or IV of HIV infection and when the HIV prevalence in the sample population is above 30% (e.g. a sample of patients from a tuberculosis ward). In lower prevalence populations, strategy II should be used to diagnose persons with the above-mentioned clinical symptoms..

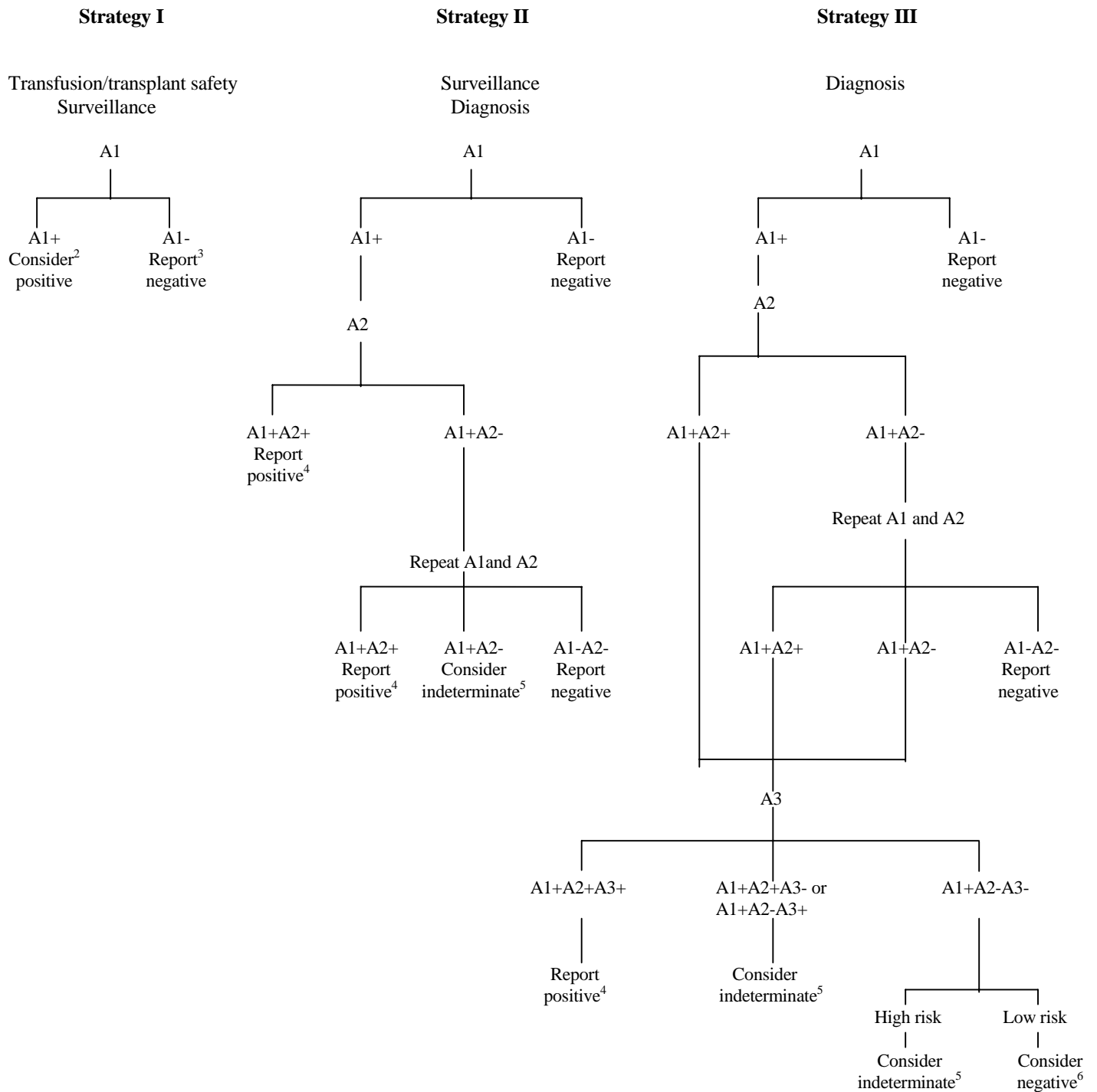
In the selection of HIV antibody tests for use in strategies II and III, the first test should have the highest sensitivity, whereas the second and third tests should have a higher specificity than the first. The number of initial discordant, indeterminate results should not exceed 5%. If it does, quality assurance procedures should be checked and/or a new test combination should be adopted.

Quality assurance

All laboratories carrying out HIV tests, should have a well-functioning quality assurance programme. 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 (technical) 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. It is recommended that laboratories submit to an external quality assessment at least once a year.

³ See footnote ¹ on page 3

Figure 1: Schematic representation of the UNAIDS and WHO HIV testing strategies.



¹ Assay A1, A2, A3 represent 3 different assays.

² Such a result is not adequate for diagnostic purposes; use strategies II or III. Whatever the final diagnosis, donations which were initially reactive should not be used for transfusions or transplants.

³ Report: result may be reported.

⁴ For newly diagnosed individuals, a positive result should be confirmed on a second sample.

⁵ Testing should be repeated on a second sample taken after 14 days.

⁶ Result is considered negative in the absence of any risk of HIV infection.

Follow up after diagnosis

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 virus particles e.g. the HIV p24 antigen ELISA, or the presence of HIV viral nucleic acid sequences (RNA or DNA) by means of nucleic acid amplification techniques. The first assays capable of detecting free circulating HIV particles were the HIV p24 antigen ELISAs.

Circulating p24 antigen appears early in the course of HIV infection, is detectable for 1-2 weeks, and then disappears or falls to very low levels until the onset of clinical illness. Rising titers of HIV p24 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 can be detected.

New technologies based on the amplification of viral nucleic acids such as PCR and NASBA or amplification of the probe binding signal as in branched-DNA tests have made it possible to detect minute amounts of viral material. In theory, as little as a single viral genome can be detected - the detection limit for most assays is around 300 copies /ml. In practice the technique can have limited specificity. These sensitive procedures are well suited to early diagnosis of mother-to-child transmission and for monitoring the viral load of patients who are taking antiretroviral therapy. However, the tests are very expensive (US\$60-100), need sophisticated equipment, rigorous laboratory conditions and highly trained staff, and are still largely a research tool. Many of these tests need further refinement since not all HIV-1 subtypes are equally well detected, nor is HIV-2. Therefore, 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.

Safety

The testing of serum or plasma specimens should be performed in such a manner as to minimize occupational risk. Guidelines for good laboratory practice have been developed that, if followed, will ensure safety 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 laboratory staff

- Laboratory logistics (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 S/R assays now available, which need no or 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. The choice of the most appropriate HIV tests also depends on the HIV variants present in a particular geographical region (e.g., HIV-1 group O). 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. Therefore, test combinations should always be evaluated in the context in which they will be used before large-scale implementation.

An HIV test kit bulk-purchase programme has been established by WHO in collaboration with UNAIDS in order to provide national AIDS control programmes with tests giving the most accurate results at the lowest possible cost. This list of HIV test kits is updated annually. Tests other than those bulk-purchased through WHO, but meeting the minimum standards in terms of sensitivity and specificity, are also suitable for use with the testing strategies shown in *Table A* and *Figure 1*.

5. Materials and methods of assessment

Assay kits

Kits for the 15 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 reported here were carried out using a panel of 595 sera (as shown in *Table B*), of which 192 were from Africa, 99 from Asia, 206 from Europe and 98 from South America. The panel contained 203 sera positive for HIV-1 and 60 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 535. Similarly, for assays detecting antibodies only to HIV-2, sera positive for HIV-1 were excluded, leaving a total of 392. All samples were stored in aliquots and thawed at least once, at most twice.

Origin	Positive sera		Negative sera	Total Number
	HIV-1	HIV-2		
Africa	54	58	80	192
Asia	40	0	59	99
Europe	64	2	140	206
Latin America	45	0	53	98
Total	203	60	332	595

Seroconversion panels

Additionally eight anti-HIV-1 seroconversion panels: PRB 904, PRB 909, PRB 911, PRB 912, PRB 914, PRB 916, PRB 917 and PRB 918 from Boston Biomedica (BBI) were tested. Western blot and HIV antigen data as provided by BBI are given in Tables 6,12, and 18.

HIV-1 group O sera

Seven HIV-1 group O sera were tested with 6 of the assays evaluated.

The HIV-1 group O sera were diagnosed by the line immuno assay, InnoLia HIV-1 group O (research products, Innogenetics) and further characterised by PCR using group O and M specific primers. (W Janssens et al. Diagnosis of HIV-1 group O infection by polymerase chain reaction, *Lancet*, 1995, 346, pp 451-452). Results are presented in Table 19.

Test performance

The assay were performed according to the manufacturer's instructions. Usually, one person carried out all the tests. With the exception of the Western blot assays, the tests on initially reactive samples were repeated. Sera with discrepant results were repeated twice. Two out of three results determined the overall test outcome. Because of their extreme value, samples belonging to the eight seroconversion panels were also only tested once with each assay under evaluation.

The ELISA results were calculated according to the instructions using the data obtained with the ELISA-reader.

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

Reference tests

The data obtained with the HIV antibody assay were compared to the combined outcome of Western blot HIV-1 (WB HIV-1) (Genelabs Diagnostics, HIV blot (version 1.2)), NEW LAV BLOT II (WB HIV-2) (Sanofi Diagnostic Pasteur) and Pepti-Lav 1+2 (Sanofi Diagnostic Pasteur) - which is designed to differentiate between HIV-1 and/or HIV-2 infections. All samples dually reactive or indeterminate with WB were tested with the Pepti-Lav 1+2. Seven samples were HIV-2 positive, 116 sera were considered to be HIV-1 positive and 109 were HIV negative.

A WB HIV-1 result or WB HIV-2 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 (*WHO Weekly Epidemiological Record* (1990); 65: pp 281-283.) A WB result was considered negative when no HIV specific band was present; indeterminate when it showed any band pattern not considered positive or negative. The results of the Pepti-Lav 1+2 were interpreted according to the instruction manual.

Analysis of the results

Sensitivity, specificity and predictive value of HIV serological tests

		True HIV status		
		+	-	
Results of assay under evaluation	+	a True-positives	b False positives	a+b
	-	c False-negatives	d True-negatives	c+d
		a+c	b+d	

$$\text{Sensitivity} = a/(a+c)$$

$$\text{Specificity} = d/(b+d)$$

$$\text{Positive predictive value} = a/(a+b)$$

$$\text{Negative predictive value} = d/(c+d)$$

Sensitivity : Is the ability of the assay under evaluation to detect correctly sera that contain antibody to HIV (reference assays positive). Thus sensitivity is the number of true positive sera identified by the assay under evaluation as positive (a), divided by the number of sera identified by the reference assays as positive (a+c), expressed as a percentage.

Specificity : Is the ability of the assay under evaluation to detect correctly sera that do not contain antibody to HIV (reference assays negative). Thus specificity is the number of true negative sera identified by the assay under evaluation as negative (d), divided by the number of sera identified by the reference assays as negative (b+d), expressed as a percentage.

NOTE: Indeterminate results, obtained with the assays under evaluation, were included in the calculation of sensitivities and specificities.

Positive Predictive Value (PPV) : The probability that when the test is reactive, the specimen does contain antibody to HIV. This may be calculated in two ways:

1. using the simple formula $a/(a+b)$ which will give an approximate value.
2. using the more precise formula which takes the prevalence of HIV in the population into account

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

Negative Predictive Value (NPV) : The probability that when the test is negative, a specimen does not have antibody to HIV. This may be calculated using:

1. the simple formula $d/(c+d)$ which will give an approximate value.
2. the more precise formula which takes the prevalence of HIV in the population into account:

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

The probability that a test will accurately determine the true infection status of a person being tested varies with the prevalence of HIV infection in the population from which the person comes. In general, the higher the prevalence of HIV infection in the population, the greater the probability that a person testing positive is truly infected (i.e., the greater the positive predictive value [PPV]). Thus, with increasing prevalence, the proportion of serum samples testing false-positive decreases; conversely, the likelihood that a person showing negative test results is truly uninfected (i.e., the negative predictive value [NPV]), decreases as prevalence increases. Therefore, as prevalence increases, so does the proportion of samples testing false-negative.

Confidence limits (CL):

95 % CL of the calculated sensitivity and specificity are given in parenthesis. CL's were calculated using the formula:

$$p \pm 1.96 \sqrt{\frac{P(1 - P)}{n}}$$

where P is the sensitivity or specificity
where N is the number of sera analyzed

95% confidence limits are a means of determining whether observed differences in sensitivity or specificity between assays are significant or not.

Delta value (δ)

This value provides a means of comparing the efficacy of ELISA assays in separating the negative and positive anti-HIV serum populations from the cut-off, as described by Crofts et al. (Journal of Virological Methods, 1988, 22: 51-59) and Maskill et al. (Journal of Virological Methods, 1988, 22: 61-73).

The delta (δ) values for the anti-HIV positive and negative sample populations were calculated by dividing the mean OD ratio (\log_{10}) by the standard deviation of each population. Optical density(OD) ratios were calculated by dividing each reading by the relevant cut-off. In case of overflow (***) in the reader, an OD of 3.000 was given to the serum.

Sample to cut-off OD ratios of the initial test results were taken for the WB positive sera. For the WB negative sera, OD ratios of the repeated test results were taken, in case the ELISA had given a false positive result initially. The higher the positive ($\delta+$) and the negative ($\delta-$) values, the higher the probability that the test will correctly identify antibody positive and negative specimens respectively.

Reproducibility

All initially reactive samples and approximately 10% of the initially non reactive samples are repeated at least once. Reproducibility, expressed as a percentage, is calculated by dividing the number of concordant results by the total number of samples retested.

Inter-reader variability

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 initial test results are differently interpreted by different readers.

Time interval obtained with early seroconversion panels

The time interval for antibody detection tests using early seroconversion panels was determined using the Abbott HIV-1/HIV-2 3rd generation EIA detection time as the reference time 0. The relative performance of the ELISA and simple/rapid assays on seroconversion panels is plotted in Figure 2.

6. Assay evaluations

Tables 1,7, and 13 summarise the general characteristics of the assays. Results of the assays evaluated as compared to the reference tests are given in Tables 2,8, and 14. Tables 3,9, and 15 provide further details of operational aspects. Factors taken into account in the calculation of ease of performance and suitability for use in small laboratories are listed in Tables 4,10, and 16, and Tables 5,11, and 17 respectively. Performance of the assays evaluated on early seroconversion panels is given in Tables 6,12, and 18, and the relative performance of the evaluated assays as compared to the reference test is given in Figure 2. Table 19 shows the results of the 5 assays' performance with HIV-1 group O specimens. Explanatory notes are provided at the end of the assay evaluation tables.

ASSAY EVALUATIONS

Table 1. General characteristics and operational aspects: ELISAs

NAME	Enzygnost Anti-HIV 1/2 Plus	ICE * HIV-1.O.2	Vironostika HIV Uni-Form II plus O	GENSCREEN HIV 1/2	HIVA TEST
Manufacturer	Dade Behring Marburg GmbH, Marburg Germany	Murex Biotech Ltd. Dartford, U.K.	Organon Teknika nv Boxtel, The Netherlands	Sanofi Diagnostics Pasteur Marnes la Coquette, France	Lupin Laboratories Ltd. Mumbai, India
Assay type	sandwich ELISA HIV-1 & HIV-2	IgG & IgM antibody capture ELISA HIV-1 & HIV-2	sandwich ELISA HIV-1 & HIV-2	sandwich ELISA HIV-1 & HIV-2	indirect ELISA HIV-1 & HIV-2
Antigen type	recombinant proteins synthetic peptides	recombinant proteins synthetic peptides	recombinant proteins synthetic peptide	recombinant proteins synthetic peptide	synthetic peptides
Solid phase	microtiterplate, V8	microtiterplate, V8	microtiterplate, V8	microtiterplate, V8	microtiterplate, V8
Number of tests per kit	192/960	96/480	192/576	96/480	96
Lot numbers 1-2 Expiry dates 1-2	27386 - 27671 Sep 95 - Mar 96	K235510-K235310 Mar-Feb 97 K235710-K235610 Mar 97	96031411-96062405 April 97-July 97	6B102F-6D506U-6E507U Aug 96-Dec 96-Jan 97	HV 001 – Oct 98 HV 002 – Jan 99
Shelf life at (°C)	12 months (2-8)	9 months (2-8)	12 months (2-8)	12 months (2-8)	6 months (2-8)
Volume of serum needed (µl) Final dilution of serum	100 1:1.25	50 1:2	50 1:3	75 3:4	10 1:11
Total time to perform the assay (h. min.)	2.05	2.30	2.05	2.05	2.05
Wavelength (nm) single double	450 450/615 -690	450 450/620-690	450 450/620-700	450/620	450
Price/test (US\$)	1.1 (192 tests) 1.0 (960 tests)	0.75 (96 tests) 0.60 (480 tests)	1.5 (192 and 576 tests)	1.5 (96 and 480 tests)	0.60 (96 tests)

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

NAME		Enzygnost Anti-HIV ½ Plus	ICE * HIV-1.O.2	Vironostika HIV Uni-Form II plus O	GENSCREEN HIV ½	HIVA TEST
Sensitivity % (95 CL)**						
Total panel	n = 263	100.0 (99.6 -100.0)	100.0 (99.6 -100.0)*	100.0 (99.6 -100.0)*	100.0 (99.6 -100.0)*	100.0 (99.5 - 100.0)*
HIV-1 sera	n = 203	100.0 (99.6 -100.0)	100.0 (99.6 -100.0)*	100.0 (99.6 -100.0)*	100.0 (99.6 -100.0)	100.0 (99.4 - 100.0)*
African sera	n = 54	100.0 (99.2 -100.0)	100.0 (99.1 -100.0)*	100.0 (99.1 -100.0)*	100.0 (99.2 -100.0)*	100.0 (99.1 - 100.0)*
Asian sera	n = 40	100.0 (99.0 -100.0)	100.0 (99.0 -100.0)*	100.0 (99.0 -100.0)*	100.0 (99.0 -100.0)	100.0 (98.8 - 100.0)*
European sera	n = 64	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 - 100.0)
Latin American sera	n = 45	100.0 (99.1 -100.0)	100.0 (99.1 -100.0)	100.0 (99.1 -100.0)	100.0 (99.1 -100.0)	100.0 (99.0 - 100.0)*
HIV-2 sera	n = 60	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)*	100.0 (99.2 -100.0)	100.0 (99.1 - 100.0)*
Initial Specificity % (95 CL)**						
Total panel	n = 332	99.7 (99.1 -100.0)	99.1 (98.1 -100.0)*	100.0 (99.7 -100.0)*	98.5 (97.2 - 99.8)*	91.5 (88.4 - 94.5)*
African sera	n = 80	98.8 (96.4 -100.0)	97.4 (93.9 -100.0)*	100.0 (99.3 -100.0)	97.5 (94.1 -100.0)	76.6 (67.2 - 86.1)*
Asian sera	n = 59	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)*	96.6 (92.0 -100.0)*	89.1 (80.1 - 98.1)*
European sera	n = 140	100.0 (99.5 -100.0)	99.3 (97.9 -100.0)*	100.0 (99.5 -100.0)*	100.0 (99.5 -100.0)	97.9 (95.5 - 100.0)
Latin American sera	n = 53	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	98.1 (94.4 -100.0)	98.1 (94.4 - 100.0)*
Final Specificity % (95 CL)**						
Total panel		99.7 (99.1 -100.0)	99.4 (98.6 -100.0)*	100.0 (99.7 -100.0)*	98.5 (97.2 - 99.8)*	93.7 (91.0 - 96.4)*
African sera		98.8 (96.4 -100.0)	97.4 (93.9 -100.0)*	100.0 (99.3 -100.0)	97.5 (94.1 -100.0)	80.5 (71.7 - 89.4)*
Asian sera		100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)*	96.6 (92.0 -100.0)*	93.5 (86.3 - 100.0)*
European sera		100.0 (99.5 -100.0)	100.0 (99.5 -100.0)*	100.0 (99.5 -100.0)*	100.0 (99.5 -100.0)	98.6 (96.6 - 100.0)
Latin American sera		100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	98.1 (94.4 -100.0)	100.0 (99.0 - 100.0)*
Reproducibility%		99.7	99.4	99.7	100.0	95.6
Indeterminate results%		0.0	not applicable	not applicable	0.0	1.5
Delta values δ +		19.1	16.8	17.2	22.8	13.3
δ -		-6.6	-4.3	-4.1	-2.7	-1.1
PPV (0.01%)		3.23	1.64	100.00	0.66	0.16
(6.0%)		95.51	91.41	100.00	80.97	50.21
NPV (0.01%)		100.00	100.00	100.00	100.00	100.00
(6.0%)		100.00	100.00	100.00	100.00	100.00

* :Some panel specimens were exhausted, and therefore not included in the evaluation. See explanatory notes, page 41, for details.

** :95% confidence limits

Table 3. Detailed operational aspects: ELISAs

NAME	Enzygnost Anti-HIV 1/2 Plus	ICE * HIV-1.O.2	Vironostika HIV Uni-Form II <i>plus O</i>	GENSCREEN HIV 1/2	HIVA TEST
Dimension (cm) of kit : w-l-h	18-12.5-10 (96 tests) 32-19.5-14 (960 tests) 21.5-16-10.5 (suppl. reagents kit)	base packs: 13-14-10.5 (96 tests) 20-18-10.5 (480 tests) detection packs: 8-14-10.5 (96 tests) 8.5-10.5-10.5 (480 tests)	27 -23.5-7 (192 tests) 27 -23.5-9.5 (576 tests)	20 - 12 - 17 (96 tests) 25.5 - 14 - 23 (480 tests)	20.5 – 16 – 9 (96 tests)
Storage conditions (°C)	2 - 8	2 - 8	2 - 8	2 - 8	2 - 8
Incubation temperature (°C)	37 and 20 - 25	37	37 and 18 - 25	40 and 18 - 30	37 and 18 - 25
Stability after dilution/ reconstitution/opening at (°C)					
- antigen	6 weeks (2-8)	expiry date (2-8)	8 weeks (2-8)	4 weeks (2-8)	expiry date (2-8)
- controls	4 weeks (2-8); 3 mths (<-20)	expiry date (2-8)	expiry date (2-8)	expiry date (2-8)	expiry date (2-8)
- sample diluent	4 weeks (2-8)	expiry date (2-8)	expiry date (2-8)	4 weeks (2-8)	expiry date (2-8)
- conjugate	4 weeks (2-8); 2 days (18-25)	3 days (2-8);10 wks (-15 to -25)	not applicable	4 weeks (2-8)	< 1 hour (18-25)
- substrate	5 days (2-8); 8 hours (18-25)	2 days (2-8) or (15-25)	6 hours (18 -25)	6 hours(18-25)	< 1 hour (18-25)
- wash buffer	1 week(2-8); 1 day (18-25)	4 weeks (15-25)	2 weeks (2-8)	15 days (2-8)	1 week (2-8)
Number of sera per run minimum - maximum	2 - 90	2 - 90	3 - 91	3 - 91	2 - 90
Number of controls per test run	6	6	4 - 5	5	6
- negative	4	3	3	1	2
- cut-off/weak positive	0	0	0	3	0
- positive	2	3	1 - 2	1	3
- blank	0	0	0	0	1
internal control	no	yes	no	yes	no

Table 3 (continued). Detailed operational aspects: ELISAs

NAME	Enzygnost Anti-HIV 1/2 Plus	ICE * HIV-1.O.2	Vironostika HIV Uni-Form II <i>plus O</i>	GENSCREEN HIV 1/2	HIVA TEST
Estimated time to perform: 90 sera (h.min)	2.05	2.30	2.05	2.05	2.05
-preparatory work	0.25	0.15	0.25	0.25	0.25
-incubation	1.30	2.00	1.30	1.30	1.30
-washing	0.05	0.10	0.05	0.05	0.05
-reading, interpretation	0.05	0.05	0.05	0.05	0.05
1 serum (h.min)	1.40	2.10	1.40	1.40	1.40
Equipment needed but not provided in the kit: ¹					
- washer	+	+	+	+	+
- incubator (water-bath)	+	+	+	+	+
- spectrophotometric reader	+	+	+	+	+
- refrigerator (storage)	+	+	+	+	+
- agitator , rocker	-	-	+/-	-	-
- aspiration device	-	-	-	-	-
- automatic pipette (µl)	+100	+50	+50	+75	+10
- multichannel (µl)	+25, 100	+50, 100	+100	+25, 50, 100	+50, 100,200
- disposable tips	+	+	+	+	+
- dilution tubes/rack,microtiterplate	-	+	+	+	+
- distilled or deionised water	+	+	+	+	+
- plate covers	-	-	-	-	-
- graduated pipette; cylinder (ml)	+1000	+1000	+1000	+1000	+1,5; 1000
- sulphuric acid/sodium hydroxide	-	+	+	-	-
- absorbent paper	+	+	+	+	+
- disinfectant	+	+	+	+	+
- gloves	+	+	+	+	+
- reagent trough	+	+	+	+	+
Definition of positive results	N + 0.400	N + 0.200	N + 0.100	A/10	(N + P) / 7
Definition of grey zone	CO - 10% \leq X \leq CO	not defined	not defined	CO - 10% < X < CO	CO – 10% < X < CO

¹ + : not provided in the kit but necessary to perform the test; - : provided in the kit or not necessary to perform the test; +/- : use is optional.

Table 4. Calculation of ease of performance: ELISAs

NAME	Enzygnost Anti-HIV 1/2 Plus	ICE * HIV-1.O.2	Vironostika HIV Uni-Form II <i>plus O</i>	GENSCREEN HIV 1/2	HIVA TEST
Need to prepare:					
1. antigen	1	1	1	1	1
2. substrate	0 ²	0	0	0	0
3. wash solution	0	0	0	0	0
4. conjugate	1	0	1	0	0
5. predilution of serum	1	1	1	1	1
Stability after dilution/opening: (expiry date = 1; less = 0)					
6. -antigen	0	1	0	0	1
7. -controls	0	1	1	1	1
8. -sample diluent	0	1	1	0	1
9. -conjugate	0	0	0	0	0
10. -substrate	0	0	0	0	0
11. -wash buffer	0	0	0	0	0
12. sufficient reagents	1	1	1	0	1
13. wash (yes =0; no = 1)	0	0	0	0	0
Item needed but not provided in the kit:					
14. reagent trough	0	0	0	0	0
15. automatic /multichannel pipette	0	0	0	0	0
16. dilution - tubes, rack/microtiter plate	1	0	0	0	0
17. distilled or deionised water	0	0	0	0	0
18. plate covers	1	1	1	1	1
19. graduated pipette ,cylinder	0	0	0	0	0
20. sulphuric acid/sodium hydroxide	1	0	0	1	1
Total	7/20	7/20	7/20	5/20	8/20
Ease of performance: -less easy ≤ 10 -easy $10 < x \leq 15$ -very easy > 15	less easy	less easy	less easy	less easy	less easy

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

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

Table 5. Suitability for use in small laboratories: ELISAs

NAME	Score	Enzygnost Anti-HIV 1/2 Plus	ICE * HIV-1.O.2	Vironostika HIV Uni-Form II <i>plus</i> O	GENSCREEN HIV 1/2	HIVA TEST
1. Sensitivity						
- 100%	3					
- 98 - 100%	2	3	3	3	3	3
- <98%	1					
2. Specificity						
- >98%	3					
- 95 - 98%	2	3	3	3	3	1
- <95%	1					
3. Incubation temperature						
- room t°	3	1	1	1	1	1
- other than room t°	1					
4. Shelf-life						
- >1 year	3					
- ≥ 6 months ≤ 1 year	2	2	2	2	3	2
- < 6 months	1					
5. Storage at						
- ambient t° possible	3	1	1	1	1	1
- 2-8° C required	1					
6. Price per test US\$						
- ≤ 1.0	3					
- ≤ 2.0	2	3	3	2	2	3
- > 2.0	1					
7. Ease of performance						
- very easy	3					
- easy	2	1	1	1	1	1
- less easy	1					
8. Rapidity of performance:1 serum						
- < 10 min	3					
- 10 - 45 min	2	1	1	1	1	1
- > 45 min	1					
9. Washer/agitator						
- not needed	3	1	1	1	1	1
- needed	1					
10. Reading						
- visual	3	1	1	1	1	1
- reading equipment	1					
Total	30	17/30	17/30	16/30	17/30	15/30
Suitability for use in small laboratories:						
- less suitable < 20		less suitable	less suitable	less suitable	less suitable	less suitable
- suitable 20 ≤ x < 25						
- very suitable ≥ 25						

Table 6. Results on early seroconversion panels: ELISAs

PANEL	SAMPLE	BLEED DATE	HIV AG RATIO ²	ABBOTT 3RD EIA RATIO ³	EIA ₁	EIA ₂	EIA ₃	EIA ₄	EIA ₅	WB RESULT	P 18	P 24	P 31	Western Blot ⁴				GP 120	GP 160
														GP 41	P 51	P 55	P 65		
PANEL D	PRB904-01	29/04/81	0.4	0.3	0.1	0.3	0.4	0.4	0.01	neg									
	-02	20/05/81	0.4	0.4	0.1	0.3	0.4	0.3	0.01	neg									
	-03	17/06/81	0.4	0.4	0.1	0.3	0.4	0.3	0.01	neg									
	-04	30/07/81	0.4	12.5	6.8	10.2	12.4	15.2	6.6	pos	+	+		+	+	+	+-	+	+
	-05	06/08/81	0.4	13.5	6.8	10.2	14.9	15.2	8.7	pos	+	+		+	+	+	+	+	+
PANEL I	PRB909-01	23/01/89	5.5	0.4	0.2	0.7	0.9	0.7	0.2	neg									
	-02	30/01/89	26.5	4.7	6.8	5.4	2.5	12.9	0.2	neg									
	-03	06/02/89	4.5	4.4	6.8	10.2	5.4	15.2	5.0	ind		+							+
	-04	08/02/89	2.2	4.1	6.8	10.2	5.1	15.2	5.8	ind		+							+
	-05	13/02/89	1.7	4.9	6.8	10.2	7.8	15.2	10.0	ind	+	+					+		+
	-06	15/02/89	1.2	5.0	6.8	10.2	8.8	15.2	9.4	ind	+	+			+		+		+
	-07	20/02/89	0.7	6.5	6.8	10.2	12.8	15.2	12.1	pos	+	+			+		+		+
	-08	22/02/89	0.5	8.8	6.8	10.2	12.4	15.2	12.1	pos	+	+			+		+	+-	+
PANEL K	PRB911-01	20/12/89	0.3	0.3	0.2	0.3	0.6	0.3	0.2	neg									
	-02	26/12/89	0.4	0.4	0.3	0.5	0.5	8.0	0.2	neg									
	-03	28/12/89	0.3	0.4	0.3	0.4	0.5	15.2	0.9	neg									
	-04	02/01/90	3.6	0.9	1.1	1.3	0.8	15.2	0.8	ind		+							
	-05	04/01/90	0.4	1.4	2.6	2.7	1.1	15.2	0.8	ind		+							
	-06	09/01/90	0.4	2.1	6.8	5.8	3.1	15.2	3.1	ind		+							+
	-07	11/01/90	0.4	2.1	6.8	6.2	4.2	15.2	3.7	pos		+		+		+-			+
	-08	18/01/90	0.2	2.1	6.8	7.0	5.1	15.2	5.0	pos	+-	+		+		+-		+-	+
	-09	22/01/90	0.4	3.1	6.8	8.8	6.0	15.2	6.7	pos	+	+		+	+	+	+	+-	+
	-10	25/01/90	0.3	4.3	6.8	8.7	6.5	15.2	5.6	pos	+	+		+	+	+	+	+-	+
PANEL L	PRB912-01	14/02/90	10.2	2.0	3.4	0.9	1.1	10.4	0.5	neg									
	-02	23/02/90	24.9	6.8	6.8	10.2	4.0	15.2	4.2	pos	+-	+		+	+	+	+	+-	+
	-03	28/02/90	10.6	7.0	6.8	10.2	5.9	15.2	4.1	pos	+	+		+	+	+	+-	+	
	-04	02/03/90	3.2	6.9	6.8	10.2	7.0	15.2	6.1	pos	+	+		+	+	+	+-	+	
	-05	14/03/90	0.5	8.5	6.8	10.2	11.5	15.2	10.3	pos	+	+		+	+	+	+-	+	
	-06	16/03/90	0.5	9.0	6.8	10.2	11.3	15.2	12.1	pos	+	+		+	+	+	+	+	
PANEL N	PRB914-01	12/01/90	0.4	3.9	6.8	10.2	5.3	15.2	0.7	ind		+-							
	-02	16/01/90	0.5	4.9	6.8	10.2	6.4	15.2	0.9	ind		+							+-
	-03	19/01/90	0.5	6.1	6.8	10.2	7.3	15.2	1.7	ind		+							+-
	-04	06/02/90	0.4	11.8	6.8	10.2	14.9	15.2	6.6	pos	+	+						+-	+
	-05	12/02/90	0.4	10.7	6.8	10.2	14.9	15.2	6.6	pos	+	+						+	+
PANEL P	PRB916-01	10/07/89	0.4	0.5	0.1	0.3	0.4	0.3	0.3	neg									
	-02	14/07/89	0.5	0.4	0.1	0.3	0.4	0.4	0.2	neg									
	-03	19/07/89	0.5	0.3	0.1	0.4	0.5	0.3	0.2	neg									
	-04	25/07/89	11.6	0.3	0.1	0.4	0.5	0.3	0.3	neg									
	-05	09/08/89	0.1	4.9	6.8	10.2	3.3	15.2	5.2	pos		+		+-				+-	+
	-06	14/08/89	2.5	4.8	6.8	10.2	4.8	15.2	6.4	pos		+		+-			+-	+	+

Table 6 (continued) Results on early seroconversion panels: ELISAs

PANEL	SAMPLE	BLEED DATE	HIV AG RATIO ²	ABBOTT 3RD EIA RATIO ³	RATIOS ¹					WB RESULT	Western Blot ⁴								
					EIA ₁	EIA ₂	EIA ₃	EIA ₄	EIA ₅		P 18	P 24	P 31	GP 41	P 51	P 55	P 65	GP 120	GP 160
PANEL Q	PRB917-01	15/10/90	0.4	0.3	0.1	0.3	0.5	0.4	0.8	ind		+-							
	-02	07/12/90	3.9	0.3	0.3	0.3	0.4	0.4	0.4	ind		+-							
	-03	11/12/90	21.6	0.3	0.2	0.3	0.5	0.4	0.7	ind		+-							
	-04	14/12/90	12.7	3.7	5.0	1.9	0.8	15.8	1.3	ind		+-							
	-05	19/12/90	2.4	8.1	6.8	10.2	4.7	15.8	1.1	ind		+-							
	-06	21/12/90	1.6	7.8	6.8	10.2	5.0	15.8	1.5	ind		+-							
	-07	26/12/90	0.4	5.2	6.8	10.2	6.5	15.8	2.4	pos		+		+-					+-
PANEL R	PRB918-01	20/02/91	9.5	0.4	0.4	0.4	0.7	0.8	0.7	neg									
	-02	22/02/91	14.0	1.2	3.0	1.1	2.0	4.1	0.7	neg									
	-03	27/02/91	0.8	6.7	6.8	10.2	6.4	15.8	0.4	ind		+-							
	-04	05/03/91	0.5	3.7	6.8	10.2	6.1	15.8	0.3	ind		+							+
	-05	07/03/91	0.5	3.2	6.8	10.2	5.4	15.8	5.3	ind		+							+
	-06	13/03/91	0.9	4.0	6.8	10.2	6.6	15.8	4.6	ind		+					+-		+

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

² HIV AG Ratio = Abbott HIV Ag (data provided by BBI)

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

⁴ Western Blot = Dupont Western Blot (data provided by BBI)

Legend for Western Blot interpretation: + = protein band present

+- = protein band weakly present

EIA₁ - Enzygnost Anti-HIV 1/2 Plus

EIA₂ - ICE * HIV-1.O.2

EIA₃ - Vironostika HIV Uni-Form II *plus* O

EIA₄ - GENSCREEN HIV 1/2

EIA₅ - HIVA TEST

Table 7. General characteristics and operational aspects: Simple/Rapid assays

NAME	RED-DOT HIV 1 & 2	HIVCHEK System 3 Test Kit	HIV TRI-DOT	EasiDot HIV/ EasiSpot HIV	AccuSpot HIV-1 and 2
Manufacturer	Cal-Test Diagnostics Inc.Chino, USA	Ortho Diagnostic Systems S.A. Cedex, France	J. Mitra & Co. Ltd New Delhi, India	Nubenco Diagnostics New Jersey, USA	Specialty BioSystems, Inc. San Diego USA
Assay type	Flow through membrane HIV-1 & HIV-2	Flow through membrane HIV-1 & HIV-2	Flow through membrane HIV-1 & HIV-2	Flow through membrane HIV-1 & HIV-2	Flow through membrane HIV-1 & HIV-2
Antigen type	recombinant proteins	recombinant protein synthetic peptide	recombinant proteins	viral lysate recombinant proteins	recombinant proteins
Solid phase	membrane	membrane	membrane	membrane	membrane
Number of tests per kit	25/50	100	10/20/50/100	48	50
Lot numbers 1-2-3 Expiry dates 1-2-3	41603-41604-41624 Sept. '95	5FS104-5JS103-5JS104 Feb.'96-June'96-June'96	6903047-6903057 March '97	H3971-H4972 March'98-April'98	ASSW94E-ASSH94E- ASSUE94 Jan. '95
Shelf life at (°C)	14 months (2-8)	at least 6 months (18-25)	12 months (2-8)	12 months (2-8)	15 months (2-8)
Volume of sample needed (µl) Final dilution of sample	25 none	50 none	50 none	120 none	50 none
Total time to perform the assay (h. min.)	0.03	0.03	0.03	0.03	0.03
Reading	visual	visual	visual	visual	visual
Price/test (US\$)	2.9 (50 tests) 3.0 (25 tests)	4.35	2.0	not given	2.5

Table 8. Comparison of the results of Simple/Rapid assays with reference tests

NAME		RED-DOT HIV 1 & 2	HIVCHEK System 3 Test Kit	HIV TRI-DOT	EasiDot HIV/ EasiSpot HIV (version 1)	AccuSpot HIV-1 and 2
Sensitivity % (95 CL)**						
Total panel	n = 263	100.0 (99.6 -100.0)	99.6 (98.9 -100.0)	99.6 (98.9 -100.0)	95.3 (92.7 - 97.9)*	100.0 (99.6 -100.0)
HIV-1 sera						
HIV-1 sera	n = 203	100.0 (99.6 -100.0)	100.0 (99.6 -100.0)	99.5 (98.5 -100.0)	100.0 (99.6 -100.0)*	100.0 (99.6 -100.0)
African sera	n = 54	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	98.2 (94.6 -100.0)	100.0 (99.1 -100.0)*	100.0 (99.2 -100.0)
Asian sera	n = 40	100.0 (99.0 -100.0)	100.0 (99.0 -100.0)	100.0 (99.0 -100.0)	100.0 (99.0 -100.0)*	100.0 (99.0 -100.0)
European sera	n = 64	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)
Latin American sera	n = 45	100.0 (99.1 -100.0)	100.0 (99.1 -100.0)	100.0 (99.1 -100.0)	100.0 (99.1 -100.0)	100.0 (99.0 -100.0)
HIV-2 sera						
HIV-2 sera	n = 60	100.0 (99.2 -100.0)	98.3 (95.1 -100.0)	100.0 (99.2 -100.0)	78.9 (68.4 - 89.5)*	100.0 (99.2 -100.0)
Initial Specificity % (95 CL)**						
Total panel	n = 332	86.5 (82.8 - 90.1)	96.1 (94.0 - 98.2)	99.1 (98.1 -100.0)	57.6 (52.3 - 63.0)*	78.0 (73.0 - 83.0)
African sera	n = 80	86.3 (78.7 - 93.8)	100.0 (99.3 -100.0)	100.0 (99.3 -100.0)	45.6 (34.6 - 56.6)*	79.2 (70.1 - 88.3)
Asian sera	n = 59	91.5 (84.4 - 98.6)	98.3 (95.0 -100.0)	98.3 (95.0 -100.0)	42.1 (29.3 - 54.9)*	92.7 (84.7 -100.0)
European sera	n = 140	82.9 (76.6 - 89.1)	91.4 (86.8 - 96.1)	99.3 (97.9 -100.0)	71.9 (64.5 - 79.4)*	66.1 (57.2 - 74.9)
Latin American sera	n = 53	90.6 (82.7 - 98.4)	100.0 (99.2 -100.0)	98.1 (94.4 -100.0)	54.7 (41.3 - 68.1)	94.6 (87.3 -100.0)
Final Specificity % (95 CL)**						
Total panel		94.9 (92.5 - 97.3)	99.7 (99.1 -100.0)	99.7 (99.1 -100.0)	71.3 (66.4 - 76.2)*	86.3 (82.5 - 90.1)
African sera		93.8 (88.4 - 99.1)	100.0 (99.3 -100.0)	100.0 (99.3 -100.0)	55.7 (44.7 - 66.7)*	85.0 (77.2 - 92.8)
Asian sera		94.9 (89.3 -100.0)	98.3 (95.0 -100.0)	98.3 (95.0 -100.0)	56.1 (43.3 - 69.0)*	88.7 (80.1 - 97.2)
European sera		95.0 (91.4 - 98.6)	100.0 (99.5 -100.0)	100.0 (99.5 -100.0)	84.9 (78.9 - 90.8)*	81.6 (75.1 - 88.1)
Latin American sera		96.2 (91.1 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	75.5 (63.9 - 87.1)	100.0 (99.2 -100.0)
Reproducibility%		not tested	96.4	98.1	82.6	85.2
Indeterminate results%		1.9	0.2	0.2	12.5	5.0
Inter-reader variability%		9.5	1.0	HIV-1: 1.8; HIV-2 : 4.5	23.7	10.8
PPV (0.01%)		0.20	3.21	3.21	Note: Preliminary data of the modified version (2) are presented on page 41 of the explanatory notes.	0.07
(6.0%)		55.59	95.49	95.49		31.78
NPV (0.01%)		100.00	100.00	100.00		100.00
(6.0%)		100.00	99.97	99.97		100.00

* : Some panel specimens were exhausted, and therefore not included in the evaluation. See explanatory notes, page 41, for details.

** : 95 % confidence limits

Table 9. Detailed operational aspects : Simple/Rapid assays

NAME	RED-DOT HIV 1 & 2	HIVCHEK System 3 Test Kit	HIV TRI-DOT	EasiDot HIV/ EasiSpot HIV	AccuSpot HIV-1 and 2
Dimension (cm) of kit : w-l-h	8 - 7 - 33 (25 tests) 27 - 20.5 - 8.5 (50 tests)	29 - 19 - 11	14.5 - 20.5 - 6 (10 tests) 18.5 - 20.5 - 6 (20 tests) 20.5 - 20.5 - 6.5 (50 tests) 24.5 - 26.5 - 9 (100 tests)	21 - 18 - 10	21 - 10.5 - 11.5
Storage conditions (°C)	2 - 8	RT ¹	4 - 8	reagents (2-8) test devices (4-22)	2 - 8
Incubation temperature (°C)	18 - 25	RT	RT	RT	18 - 25
Stability after dilution/ reconstitution/opening at (°C)					
- antigen	expiry date (2-8)	expiry date (RT)	expiry date (2-8)	expiry date (4-22)	expiry date (2-8)
- controls	expiry date (2-8)	6 months (4), 3 weeks (25)	expiry date (2-8)	expiry date (2-8)	expiry date (2-8)
- sample diluent	expiry date (2-8)	not applicable	not applicable	not applicable	not applicable
- conjugate	expiry date (2-8)	5 days (4)	2 months (2-8), 5 days(RT)	expiry date (2-8)	expiry date (2-8)
- substrate	not applicable	not applicable	not applicable	not applicable	not applicable
- wash buffer	expiry date (2-8)	expiry date (RT)	expiry date (2-8)	expiry date (2-8)	expiry date (2-8)
- buffer		6 months (4)			
Number of sera per run minimum - maximum	1 - 8	1 - 8	1 - 3	1 – 1 ²	1 - 8
Number of controls per test run	2	2-3	2	2	2
- negative	1	1	1	1	1
- cut-off/weak positive	0	0	0	0	0
- positive	1	1-2	1	1	1
-blank	0	0	0	0	0
internal control	no	yes	yes	yes	no

¹RT = room temperature

² According to the technician's report, only 1 assay can be performed at a time

Table 9. (continued). Detailed operational aspects : Simple/Rapid assays

NAME	RED-DOT HIV 1 & 2	HIVCHEK System 3 Test Kit	HIV TRI-DOT	EasiDot HIV/ EasiSpot HIV	AccuSpot HIV-1 and 2
Estimated time to perform: 90 sera (h.min)	1.10	0.45	3.30	1.30	1.10
-preparatory work	0.10	0.10	0.05	0.10	0.10
-incubation	0.40	0.15	0.15	1.00	0.40
-washing	0.10	0.15	3.00		0.10
-reading, interpretation	0.10	0.05	0.10	0.20	0.10
1 serum (h.min)	0.03	0.03	0.03	0.03	0.03
Equipment needed but not provided in the kit: ¹					
- washer	-	-	-	-	-
- incubator (water-bath)	-	-	-	-	-
- spectrophotometer	-	-	-	-	-
- refrigerator (storage)	+	+/-	+	+	+
- agitator, rocker	-	-	-	-	-
- aspiration device	-	-	-	-	-
- automatic pipette (µl)	-	-	-	-	-
- multichannel (µl)	-	-	-	-	-
- disposable tips	-	-	-	-	-
- dilution tubes/rack, microtiterplate	-	-	-	-	-
- distilled or deionised water	-	-	-	-	-
- plate covers	-	-	-	-	-
- graduated pipette, cylinder (ml)	-	-	-	-	-
- sulfuric acid/sodium hydroxide	-	-	-	-	-
- absorbent paper	-	-	-	-	-
- disinfectant	+	+	-	+	+
- gloves	+	+	+	+	+
- reagent trough	-	-	-	-	-
Definition of positive results	a red dot in the inner circle of the device	a red spot in the test area and a small control spot on the edge of the membrane	a red dot in the HIV-1 and/or HIV-2 regions and a red dot in the control region	a pink-rose dot (any intensity) in the test area and a visible control line	a red spot in the centre of the membrane
Definition of grey zone	not defined	not defined	not defined	not defined	not defined

¹ + : not provided in the kit but necessary to perform the test; - : provided in the kit or not necessary to perform the test; +/- : use is optional.

Table 10. Calculation of ease of performance: Simple/Rapid assays

NAME	RED-DOT HIV 1 & 2	HIVCHEK System 3 Test Kit	HIV TRI-DOT	EasiDot HIV/ EasiSpot HIV	AccuSpot HIV-1 and 2
Need to prepare:					
1. antigen	1 ¹	1	1	1	1
2. substrate	1	1	1	1	1
3. wash solution	1	1	1	1	1
4. conjugate	1	0 ²	0	1	1
5. predilution of sample	1	1	1	1	1
Stability after dilution/opening: (expiry date = 1; less = 0)					
6. -antigen	1	1	1	1	1
7. -controls	1	0	1	1	1
8. -sample diluent/buffer	1	0	1	1	1
9. -conjugate	1	0	0	1	1
10. -substrate	1	1	1	1	1
11. -wash buffer	1	1	1	1	1
12. sufficient reagents	1	1	1	1	1
13. wash (yes =0; no = 1)	0	0	0	0	0
Item needed but not provided in the kit:					
14. reagent trough	1	1	1	1	1
15. automatic/multichannel pipette	1	1	1	1	1
16. dilution- tubes,rack/microtiter plate	1	1	1	1	1
17. distilled water	1	1	1	1	1
18. plate covers	1	1	1	1	1
19. graduated pipette, cylinder	1	1	1	1	1
20. sulphuric acid/sodium hydroxide	1	1	1	1	1
Total	19/20	15/20	17/20	19/20	19/20
Ease of performance: - less easy ≤ 10 - easy 10 < x ≤ 15 - very easy >15	very easy	easy	very easy	very easy	very easy

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

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

Table 11. Suitability for use in small laboratories: Simple/Rapid assays

NAME	Score	RED-DOT HIV 1 & 2	HIVCHEK System 3 Test Kit	HIV TRI-DOT	EasiDot HIV/ EasiSpot HIV	AccuSpot HIV-1 and 2
1. Sensitivity						
- 100%	3					
- 98 - 100%	2	3	2	2	1	3
- <98%	1					
2. Specificity						
- >98%	3					
- 95 - 98%	2	1	3	3	1	1
- <95%	1					
3. Incubation temperature						
- room t°	3	3	3	3	3	3
- other than room t°	1					
4. Shelf-life						
- >1 year	3					
- ≥ 6 months ≤ 1 year	2	3	2	3	3	3
- < 6 months	1					
5. Storage at						
- ambient t° possible	3	1	3	1	1	1
- 2-8° C required	1					
6. Price per test US\$						
- ≤ 1.0	3					
- ≤ 2.0	2	1	1	2	1	1
- > 2.0	1					
7. Ease of performance						
- very easy	3					
- easy	2	3	2	3	3	3
- less easy	1					
8. Rapidity of performance: 1 serum						
- < 10 min	3					
- 10 - 45 min	2	3	3	3	3	3
- > 45 min	1					
9. Washer/agitator						
- not needed	3	3	3	3	3	3
- needed	1					
10. Reading						
- visual	3	3	3	3	3	3
- reading equipment	1					
Total	30	24/30	25/30	26/30	22/30	24/30
Suitability for use in small laboratories:						
- less suitable < 20						
- suitable 20 ≤ x < 25		suitable	very suitable	very suitable	suitable	suitable
- very suitable ≥ 25						

Table 12. Results on early seroconversion panels : Simple/Rapid assays

PANEL	SAMPLE	BLEED DATE	HIV AG RATIO ^{1,2}	ABBOTT 3RD EIA RATIO ³	S/R ₁	S/R ₂	S/R ₃	S/R ₄	S/R ₅	WB RESULT	Western Blot ⁴								
											P 18	P 24	P 31	GP 41	P 51	P 55	P 65	GP 120	GP 160
PANEL D	PRB904-01	29/04/81	0.4	0.3	neg	neg	neg	neg	neg	neg									
	-02	20/05/81	0.4	0.4	neg	neg	neg	neg	neg	neg									
	-03	17/06/81	0.4	0.4	neg	neg	neg	ind	neg	neg									
	-04	30/07/81	0.4	12.5	pos	pos	pos	pos	pos	pos	+	+		+-	+	+	+-	+	+
	-05	06/08/81	0.4	13.5	pos	pos	pos	pos	pos	pos	+	+		+	+	+	+	+	+
PANEL I	PRB909-01	23/01/89	5.5	0.4	pos	neg	neg	ind	neg	neg									
	-02	30/01/89	26.5	4.7	pos	neg	ind	ind	neg	neg									
	-03	06/02/89	4.5	4.4	pos	neg	pos	pos	pos	pos		+							+
	-04	08/02/89	2.2	4.1	pos	neg	pos	pos	pos	pos		+							+
	-05	13/02/89	1.7	4.9	pos	neg	pos	pos	pos	pos	+	+					+		+
	-06	15/02/89	1.2	5.0	pos	ind	pos	pos	pos	pos	+	+		+			+		+
	-07	20/02/89	0.7	6.5	pos	pos	pos	pos	pos	pos	+	+		+			+	+-	+
	-08	22/02/89	0.5	8.8	pos	pos	pos	pos	pos	pos	+	+		+			+	+-	+
PANEL K	PRB911-01	20/12/89	0.3	0.3	ind	neg	neg	neg	neg	neg									
	-02	26/12/89	0.4	0.4	neg	neg	neg	neg	neg	neg									
	-03	28/12/89	0.3	0.4	pos	neg	neg	neg	neg	neg									
	-04	02/01/90	3.6	0.9	pos	neg	pos	neg	neg	ind		+							
	-05	04/01/90	0.4	1.4	pos	neg	pos	neg	neg	ind		+							
	-06	09/01/90	0.4	2.1	pos	pos	pos	pos	pos	ind		+							+
	-07	11/01/90	0.4	2.1	pos	pos	pos	pos	pos	pos		+		+-		+-			+
	-08	18/01/90	0.2	2.1	pos	ind	pos	pos	pos	pos	+-	+		+-		+-		+-	+
	-09	22/01/90	0.4	3.1	pos	ind	pos	pos	pos	pos	+	+		+	+	+	+	+-	+
	-10	25/01/90	0.3	4.3	pos	ind	pos	pos	pos	pos	+	+		+	+	+	+	+-	+
PANEL L	PRB912-01	14/02/90	10.2	2.0	neg	neg	neg	neg	neg	neg									
	-02	23/02/90	24.9	6.8	pos	neg	pos	pos	pos	pos	+-	+		+	+	+	+-	+	
	-03	28/02/90	10.6	7.0	pos	neg	pos	pos	pos	pos	+	+		+	+	+	+-	+	
	-04	02/03/90	3.2	6.9	pos	pos	pos	pos	pos	pos	+	+		+	+	+	+-	+	
	-05	14/03/90	0.5	8.5	pos	pos	pos	pos	pos	pos	+	+		+	+	+	+-	+	
	-06	16/03/90	0.5	9.0	pos	pos	pos	pos	pos	pos	+	+		+	+	+	+	+	
PANEL N	PRB914-01	12/01/90	0.4	3.9	pos	neg	pos	ind	neg	ind		+-							
	-02	16/01/90	0.5	4.9	pos	neg	pos	neg	neg	ind		+							+-
	-03	19/01/90	0.5	6.1	pos	neg	pos	neg	neg	ind		+							+-
	-04	06/02/90	0.4	11.8	pos	neg	pos	pos	pos	pos	+	+					+-	+	
	-05	12/02/90	0.4	10.7	pos	neg	pos	pos	pos	pos	+	+					+	+	
PANEL P	PRB916-01	10/07/89	0.4	0.5	neg	neg	neg	ind	neg	neg									
	-02	14/07/89	0.5	0.4	neg	neg	neg	ind	neg	neg									
	-03	19/07/89	0.5	0.3	neg	neg	neg	ind	neg	neg									
	-04	25/07/89	11.6	0.3	neg	neg	neg	neg	neg	neg									
	-05	09/08/89	0.1	4.9	pos	neg	pos	neg	pos	pos		+		+-				+-	+
	-06	14/08/89	2.5	4.8	pos	neg	pos	neg	pos	pos		+		+-		+	+	+	+

Table 12 (continued) Results on early seroconversion panels: Simple/Rapid assays

PANEL	SAMPLE	BLEED DATE	HIV AG RATIO ^{1,2}	ABBOTT 3RD EIA RATIO ³	S/R ₁	S/R ₂	S/R ₃	S/R ₄	S/R ₅	WB RESULT	Western Blot ⁴								
											P 18	P 24	P 31	GP 41	P 51	P 55	P 65	GP 120	GP 160
PANEL Q	PRB917-01	15/10/90	0.4	0.3	neg	neg	neg	ind	neg	ind		+-							
	-02	07/12/90	3.9	0.3	neg	neg	neg	neg	neg	ind		+							
	-03	11/12/90	21.6	0.3	neg	neg	neg	neg	neg	ind		+							
	-04	14/12/90	12.7	3.7	neg	neg	neg	neg	neg	ind		+							
	-05	19/12/90	2.4	8.1	pos	neg	pos	ind	neg	ind		+							
	-06	21/12/90	1.6	7.8	pos	ind	pos	ind	pos	ind		+-							
	-07	26/12/90	0.4	5.2	pos	pos	pos	ind	pos	pos		+		+-					+-
PANEL R	PRB918-01	20/02/91	9.5	0.4	neg	neg	neg	ind	neg	neg									
	-02	22/02/91	14.0	1.2	neg	neg	neg	ind	neg	neg									
	-03	27/02/91	0.8	6.7	pos	neg	pos	pos	neg	ind		+-							
	-04	05/03/91	0.5	3.7	pos	pos	pos	pos	pos	ind		+							+
	-05	07/03/91	0.5	3.2	pos	pos	pos	pos	pos	ind		+							+
	-06	13/03/91	0.9	4.0	pos	pos	pos	pos	pos	ind		+					+-		+

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

² HIV AG Ratio = Abbott HIV Ag (data provided by BBI)

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

⁴ Western Blot = Dupont Western Blot (data provided by BBI)

Legend for Western Blot interpretation: + = protein band present

+- = protein band weakly present

S/R₁ - RED-DOT HIV 1 & 2

S/R₂ - HIVCHEK System 3 Test Kit

S/R₃ - HIV TRI-DOT

S/R₄ - EasiDot HIV/ EasiSpot HIV

S/R₅ - AccuSpot HIV-1 and 2

Table 13. General characteristics and operational aspects: Simple/Rapid assays

NAME	IMx HIV-1/HIV-2 3rd generation Plus	BIONOR HIV-1&2	HIV 1 & 2 DoubleCheck	SEROCARD HIV	SERO•STRIP HIV-1/2
Manufacturer	Abbott GmbH Diagnostika, Wiesbaden- Delkenheim, Germany	Bionor A/S Skien, Norway	Organics Yavne, Israel	Trinity Biotech PLC Bray, Co. Wicklow, Ireland	Saliva Diagnostic Systems Pte Ltd. Singapore
Assay type	microparticle ELISA HIV-1 & HIV-2	rapid EIA HIV-1 & HIV-2	lateral flow membrane	lateral flow membrane	capillary flow membrane
Antigen type	recombinant proteins synthetic peptides	synthetic peptides	recombinant proteins synthetic peptides	synthetic peptides	synthetic peptides
Solid phase	microparticles	magnetic particles	membrane	membrane	chromatographic strip
Number of tests per kit	100	40/200	40	40	30
Lot numbers 1-2-3 Expiry dates 1-2-3	05318HP00 - 05318HP01 Oct '95	950502-155 - 950930-183 Nov '95 - Dec '95	960609 - 960610 May '97	B3282 - C0174 Oct '95 - Dec '95	525426101 - 525426102 Feb '97
Shelf life at (°C)	3 months (2-8)	6 months (2-8)	12 months (2-8)	15 months (2-8)	18 months (2-25)
Volume of sample needed (µl) Final dilution of sample	150 none	30 1:4	150 3:4	50 none	1 1:200
Total time to perform the assay (h. min.)	3.20	0.30	0.11	0.09	0.03
Reading	IMx system	visual	visual	visual	visual
Price/test (US\$)	3 - 4	2.5	2.0	4.0	1.5

Table 14. Comparison of the results of Simple/Rapid assays with reference tests

NAME		IMx HIV-1/HIV-2 3rd generation Plus	BIONOR HIV-1&2	HIV 1 & 2 DoubleCheck	SEROCARD HIV	SERO•STRIP HIV-1/2
Sensitivity % (95 CL)**						
Total panel	n = 263	99.6 (98.9 -100.0)	100.0 (99.6 -100.0)	100.0 (99.6 -100.0)*	100.0 (99.6 -100.0)	98.9 (97.6 -100.0)
HIV-1 sera	n = 203	99.5 (98.5 -100.0)	100.0 (99.6 -100.0)	100.0 (99.6 -100.0)*	100.0 (99.6 -100.0)	99.0 (97.6 -100.0)
African sera	n = 54	98.1 (94.5 -100.0)	100.0 (99.2 -100.0)	100.0 (99.1 -100.0)*	100.0 (99.2 -100.0)	96.3 (91.2 -100.0)
Asian sera	n = 40	100.0 (99.0 -100.0)	100.0 (99.0 -100.0)	100.0 (99.0 -100.0)	100.0 (99.0 -100.0)	100.0 (99.0 -100.0)
European sera	n = 64	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)
Latin American sera	n = 45	100.0 (99.1 -100.0)	100.0 (99.1 -100.0)	100.0 (99.1 -100.0)	100.0 (99.1 -100.0)	100.0 (99.1 -100.0)
HIV-2 sera	n = 60	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)*	100.0 (99.2 -100.0)	98.3 (95.1 -100.0)
Initial Specificity % (95 CL)**						
Total panel	n = 332	94.3 (91.8 - 96.8)	97.6 (95.9 - 99.2)	99.4 (98.6 -100.0)*	95.5 (93.2 - 97.7)	99.1 (98.1 -100.0)
African sera	n = 80	95.0 (90.2 - 99.8)	96.3 (92.1 -100.0)	100.0 (99.3 -100.0)	87.5 (80.3 - 94.7)	98.8 (96.3 -100.0)
Asian sera	n = 59	94.9 (89.3 -100.0)	98.3 (95.0 -100.0)	96.6 (92.0 -100.0)	94.9 (89.3 -100.0)	100.0 (99.2 -100.0)
European sera	n = 140	97.1 (94.3 - 99.9)	97.1 (94.4 - 99.9)	100.0 (99.5 -100.0)*	99.3 (97.9 -100.0)	100.0 (99.5 -100.0)
Latin American sera	n = 53	84.9 (75.3 - 94.5)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	98.1 (94.4 -100.0)	96.2 (91.1 -100.0)
Final Specificity % (95 CL)**						
Total panel		97.9 (96.4 - 99.4)	98.8 (97.6 -100.0)	99.4 (98.6 -100.0)*	97.9 (96.4 - 99.4)	100.0 (99.7 -100.0)
African sera		98.8 (96.4 -100.0)	97.5 (94.1 -100.0)	100.0 (99.3 -100.0)	93.8 (88.5 - 99.1)	100.0 (99.3 -100.0)
Asian sera		94.9 (89.3 -100.0)	98.3 (95.0 -100.0)	96.6 (92.0 -100.0)	98.3 (95.0 -100.0)	100.0 (99.2 -100.0)
European sera		99.3 (97.9 -100.0)	99.3 (97.9 -100.0)	100.0 (99.5 -100.0)*	100.0 (99.5 -100.0)	100.0 (99.5 -100.0)
Latin American sera		96.2 (91.1 -100.0)	100.0 (99.2 -100.0)	100.0 (99.2 -100.0)	98.1 (94.4 -100.0)	100.0 (99.2 -100.0)
Reproducibility%		96.7	98.4	99.7	97.5	98.6
Indeterminate results%		0.3	0.2	0.2	0.2	0.0
Inter-reader variability%			1.0	0.8	1.5	1.5
		(δ +): 9.1; (δ -): -2.1				
PPV (0.01%)		0.47	0.83	1.64	0.47	100.00
(6.0%)		75.17	84.18	91.41	75.24	100.00
NPV (0.01%)		100.00	100.00	100.00	100.00	100.00
(6.0%)		99.97	100.00	100.00	100.00	99.93

* :Some panel specimens were exhausted, and therefore not included in the panel. See explanatory notes, page 41, for details.

** : 95% confidence limits

Table 15. Detailed operational aspects : Simple/Rapid assays

NAME	IMx HIV-1/HIV-2 3rd generation Plus	BIONOR HIV-1&2	HIV 1 & 2 DoubleCheck	SEROCARD HIV	SERO•STRIP HIV-1/2
Dimension (cm) of kit : w-l-h	15.0-6.5-7. (reagent pack) 15.0-6.5-7 (control kit) 16.5-16.5-12 (reaction cells) 16.5-16.5-12 (blank cells) 24-14-19 (wash buffer)	21 - 14 - 13.5 (40 tests)	25.5 - 30.5 - 10.5	14 - 20 - 9.5	18.5 - 18 - 4.5
Storage conditions (°C)	(2-8): reagent pack; control kit (15-30): reaction cells; blank cells; wash buffer	2 - 8	2 - 8	2 - 8	2 - 25
Incubation temperature (°C)	as per IMx system	RT ¹	22 - 26	RT	RT
Stability after dilution/ reconstitution/opening at (°C)					
- antigen	expiry date (2-8)	expiry date (2-8)	expiry date (2-8)	expiry date (2-8)	expiry date (2-25)
- controls	expiry date (2-8)	expiry date (2-8)	expiry date (2-8)	expiry date (2-8)	expiry date (2-25)
- sample diluent	not applicable	not applicable	expiry date (2-8)	not applicable	expiry date (2-25)
- conjugate	expiry date (2-8)	expiry date (2-8)	expiry date (2-8)	expiry date (2-8)	expiry date (2-25)
- substrate	expiry date (2-8)	expiry date (2-8)	expiry date (2-8)	expiry date (2-8)	not applicable
- wash buffer	expiry date (2-8)	expiry date (2-8)	not applicable	expiry date (2-8)	not applicable
Number of sera per run minimum - maximum	1 - 20	1 - 37	1 - 5	1 - 3	1 - 8
Number of controls per test run	4	3	2	2	2
- negative	1	1	1	1	1
- cut-off/weak positive	0	0	0	0	0
- positive	2	2	1	1	1
-blank	1	0	0	0	0
internal control	no	no	yes	yes	yes

¹ RT = room temperature

Table 15. (continued).

Detailed operational aspects : Simple/Rapid assays

NAME	IMx HIV-1/HIV-2 3rd generation Plus	BIONOR HIV-1&2	HIV 1 & 2 DoubleCheck	SEROCARD HIV	SERO•STRIP HIV-1/2
Estimated time to perform:					
90 sera (h.min)	3.20	3.00	4.00	4.00	1.30
-preparatory work	0.20	0.45	0.30	0.25	0.50
-incubation		1.09	3.20	2.20	0.45
-washing	3.00	1.00		1.00	
-reading, interpretation		0.06	0.10	0.15	0.05
1 serum	0.45	0.30	0.11	0.09	0.03
Equipment needed but not provided in the kit: ¹					
- washer	+	-	-	-	-
- incubator (water-bath)	+	-	-	-	-
- spectrophotometer	+	-	-	-	-
- refrigerator (storage)	+	+	+	+	-
- agitator, rocker	-	+	-	-	-
- aspiration device	-	+	-	-	-
- automatic pipette (µl)	+ 150	-	-	-	-
- multichannel (µl)	-	-	-	-	-
- disposable tips	+	-	-	-	-
- dilution tubes/rack,microtiterplate	-	-	-	-	+
- distilled or deionised water	-	-	-	-	-
- plate covers	-	-	-	-	-
- graduated pipette, cylinder (ml)	-	-	-	-	-
- sulfuric acid/sodium hydroxide	-	-	-	-	-
- absorbent paper	-	-	-	-	+
- disinfectant	+	+	+	+	+
- gloves	+	+	+	+	+
- reagent trough	-	-	-	-	-
Definition of positive results	$X \geq \text{mean mode 1 calibrator} \times 25$	a pale pink to red coloured solution	2 gray-blue spots: the test spot and a smaller control spot	blue colour in the test port of greater intensity than control port	2 red lines: the test line and an upper control line
Definition of grey zone	$CO - 10\% \leq X < CO$	not defined	not defined	not defined	not defined

¹ + : not provided in the kit but necessary to perform the test; - :provided in the kit or not necessary to perform the test; +/- : use is optional.

Table 16. Calculation of ease of performance: Simple/Rapid assays

NAME	IMx HIV-1/HIV-2 3rd generation Plus	BIONOR HIV-1&2	HIV 1 & 2 DoubleCheck	SEROCARD HIV	SERO•STRIP HIV-1/2
Need to prepare:					
1. antigen	1 ¹	1 ¹	1	1	1
2. substrate	1	1	1	1	1
3. wash solution	1	1	1	1	1
4. conjugate	1	1	1	1	1
5. predilution of sample	1	1	0 ²	1	1
Stability after dilution/opening: (expiry date = 1; less = 0)					
6. -antigen	1	1	1	1	1
7. -controls	1	1	1	1	1
8. -sample diluent	1	1	1	1	1
9. -conjugate	1	1	1	1	1
10. -substrate	1	1	1	1	1
11. -wash buffer	1	1	1	1	1
12. sufficient reagents	1	1	1	1	1
13. wash (yes =0; no = 1)	0	0	1	0	1
Item needed but not provided in the kit:					
14. reagent trough	1	1	1	1	1
15. automatic/multichannel pipette	0	1	1	1	1
16. dilution- tubes,rack/microtiter plate	1	1	1	1	0
17. distilled water	1	1	1	1	1
18. plate covers	1	1	1	1	1
19. graduated pipette, cylinder	1	1	1	1	1
20. sulphuric acid/sodium hydroxide	1	1	1	1	1
Total	18/20	19/20	19/20	19/20	19/20
Ease of performance:					
- less easy ≤ 10	very easy	very easy	very easy	very easy	very easy
- easy $10 < x \leq 15$					
- very easy > 15					

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

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

Table 17. Suitability for use in small laboratories: Simple/Rapid assays

NAME	Score	IMx HIV-1/HIV-2 3rd generation Plus	BIONOR HIV-1&2	HIV 1 & 2 DoubleCheck	SEROCARD HIV	SERO•STRIP HIV-1/2
1. Sensitivity						
- 100%	3					
- 98 - 100%	2	2	3	3	3	2
- <98%	1					
2. Specificity						
- >98%	3					
- 95 - 98%	2	2	3	3	2	3
- <95%	1					
3. Incubation temperature						
- room t°	3	3	3	3	3	3
- other than room t°	1					
4. Shelf-life						
- >1 year	3					
- ≥ 6 months ≤ 1 year	2	1	2	3	3	3
- < 6 months	1					
5. Storage at						
- ambient t° possible	3	1	1	1	1	3
- 2-8° C required	1					
6. Price per test US\$						
- ≤ 1.0	3					
- ≤ 2.0	2	1	1	2	1	2
- > 2.0	1					
7. Ease of performance						
- very easy	3					
- easy	2	3	3	3	3	3
- less easy	1					
8. Rapidity of performance:1 serum						
- < 10 min	3					
- 10 - 45 min	2	2	2	2	3	2
- > 45 min	1					
9. Washer/agitator						
- not needed	3	3	3	3	3	3
- needed	1					
10. Reading						
- visual	3	3	3	3	3	3
- reading equipment	1					
Total	30	21/30	24/30	26/30	25/30	27/30
Suitability for use in small laboratories:						
- less suitable < 20		suitable	suitable	very suitable	very suitable	very suitable
- suitable 20 ≤ x < 25						
- very suitable ≥ 25						

Table18. Results on early seroconversion panels : Simple/Rapid assays

PANEL	SAMPLE	BLEED DATE	HIV AG RATIO ^{1,2}	ABBOTT 3RDEIA RATIO ³	S/R ₆ RATIO	S/R ₇	S/R ₈	S/R ₉	S/R ₁₀	WB RESULT	Western Blot ⁴								
											P 18	P 24	P 31	GP 41	P 51	P 55	P 65	GP 120	GP 160
PANEL D	PRB904-01	29/04/81	0.4	0.3	1.2	neg	neg	neg	neg	neg									
	-02	20/05/81	0.4	0.4	0.4	neg	neg	neg	neg	neg									
	-03	17/06/81	0.4	0.4	0.9	neg	neg	neg	neg	neg									
	-04	30/07/81	0.4	12.5	11.9	pos	pos	pos	pos	pos	+	+		+-	+	+	+-	+	+
	-05	06/08/81	0.4	13.5	12.0	pos	pos	pos	pos	pos	+	+		+	+	+	+	+	+
PANEL I	PRB909-01	23/01/89	5.5	0.4	0.7	neg	neg	neg	neg	neg									
	-02	30/01/89	26.5	4.7	5.8	pos	pos	neg	neg	neg									
	-03	06/02/89	4.5	4.4	13.3	pos	pos	pos	pos	ind		+							+
	-04	08/02/89	2.2	4.1	10.6	pos	pos	pos	pos	ind		+							+
	-05	13/02/89	1.7	4.9	12.7	pos	pos	pos	pos	ind	+	+					+		+
	-06	15/02/89	1.2	5.0	11.4	pos	pos	pos	pos	ind	+	+		+			+		+
	-07	20/02/89	0.7	6.5	12.1	pos	pos	pos	pos	pos	+	+		+			+	+-	+
	-08	22/02/89	0.5	8.8	13.1	pos	pos	pos	pos	pos	+	+		+			+	+-	+
	PANEL K	PRB911-01	20/12/89	0.3	0.3	0.5	neg	neg	neg	neg	neg								
-02		26/12/89	0.4	0.4	0.4	neg	neg	neg	neg	neg									
-03		28/12/89	0.3	0.4	1.1	neg	pos	neg	neg	neg									
-04		02/01/90	3.6	0.9	1.6	neg	pos	neg	neg	ind		+							
-05		04/01/90	0.4	1.4	2.8	neg	pos	neg	pos	ind		+							
-06		09/01/90	0.4	2.1	6.6	pos	pos	pos	pos	ind		+							+
-07		11/01/90	0.4	2.1	8.3	pos	pos	pos	pos	pos		+		+-		+-			+
-08		18/01/90	0.2	2.1	7.9	pos	pos	pos	pos	pos	+-	+		+-		+-		+-	+
-09		22/01/90	0.4	3.1	7.0	pos	pos	pos	pos	pos	+	+		+	+	+	+	+-	+
-10		25/01/90	0.3	4.3	10.2	pos	pos	pos	pos	pos	+	+		+	+	+	+	+-	+
PANEL L		PRB912-01	14/02/90	10.2	2.0	4.3	neg	neg	neg	neg	neg								
	-02	23/02/90	24.9	6.8	12.5	pos	pos	pos	pos	pos	+-	+		+	+	+	+	+-	+
	-03	28/02/90	10.6	7.0	11.9	pos	pos	pos	pos	pos	+	+		+	+	+	+	+-	+
	-04	02/03/90	3.2	6.9	8.1	pos	pos	pos	pos	pos	+	+		+	+	+	+	+-	+
	-05	14/03/90	0.5	8.5	9.8	pos	pos	pos	pos	pos	+	+		+	+	+	+	+-	+
	-06	16/03/90	0.5	9.0	15.6	pos	pos	pos	pos	pos	+	+		+	+	+	+	+	+
PANEL N	PRB914-01	12/01/90	0.4	3.9	4.7	pos	pos	neg	neg	ind		+-							
	-02	16/01/90	0.5	4.9	8.1	pos	pos	pos	neg	ind		+							+-
	-03	19/01/90	0.5	6.1	n.v.*	pos	pos	pos	neg	ind		+							+-
	-04	06/02/90	0.4	11.8	8.6	pos	pos	pos	neg	pos	+	+						+-	+
	-05	12/02/90	0.4	10.7	12.6	pos	pos	pos	neg	pos	+	+						+	+
PANEL P	PRB916-01	10/07/89	0.4	0.5	0.6	neg	neg	neg	ind	neg									
	-02	14/07/89	0.5	0.4	0.5	neg	neg	neg	neg	neg									
	-03	19/07/89	0.5	0.3	0.5	neg	neg	neg	neg	neg									
	-04	25/07/89	11.6	0.3	0.5	neg	neg	neg	pos	neg									
	-05	09/08/89	0.1	4.9	10.6	pos	pos	pos	pos	pos		+		+-				+-	+
	-06	14/08/89	2.5	4.8	9.2	pos	pos	pos	pos	pos		+		+-			+-	+	+

*n.v: not valid

Table 18 (continued) Results on early seroconversion panels: Simple/Rapid assays

PANEL	SAMPLE	BLEED DATE	HIV AG RATIO ^{1,2}	ABBOTT 3RD EIA RATIO ³	S/R ₆	S/R ₇	S/R ₈	S/R ₉	S/R ₁₀	WB RESULT	Western Blot ⁴								
											P 18	P 24	P 31	GP 41	P 51	P 55	P 65	GP 120	GP 160
PANEL Q	PRB917-01	15/10/90	0.4	0.3	0.8	neg	neg	neg	neg	ind		+-							
	-02	07/12/90	3.9	0.3	0.6	neg	neg	neg	neg	ind		+-							
	-03	11/12/90	21.6	0.3	0.8	neg	neg	neg	neg	ind		+-							
	-04	14/12/90	12.7	3.7	2.7	neg	neg	neg	neg	ind		+-							
	-05	19/12/90	2.4	8.1	11.7	pos	pos	pos	neg	ind		+-							
	-06	21/12/90	1.6	7.8	11.1	pos	pos	pos	pos	ind		+-							
	-07	26/12/90	0.4	5.2	11.6	pos	pos	pos	pos	pos		+		+-					+-
PANEL R	PRB918-01	20/02/91	9.5	0.4	0.6	neg	neg	neg	neg	neg									
	-02	22/02/91	14.0	1.2	3.0	neg	neg	neg	neg	neg									
	-03	27/02/91	0.8	6.7	8.0	pos	pos	pos	neg	ind		+-							
	-04	05/03/91	0.5	3.7	10.3	pos	pos	pos	pos	ind		+							+
	-05	07/03/91	0.5	3.2	8.5	pos	pos	pos	pos	ind		+							+
	-06	13/03/91	0.9	4.0	8.7	pos	pos	pos	pos	ind		+					+-		+

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

² HIV AG Ratio = Abbott HIV Ag (data provided by BBI)

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

⁴ Western Blot = Dupont Western Blot (data provided by BBI)

Legend for Western Blot interpretation: + = protein band present

+- = protein band weakly present

S/R₆ - IMx HIV-1/HIV-2 3rd generation Plus

S/R₇ - BIONOR HIV-1&2

S/R₈ - HIV 1 & 2 DoubleCheck

S/R₉ - SEROCARD HIV

S/R₁₀ - SERO•STRIP HIV-1/2

Table 19. Comparison of assay results with HIV-1 group O specimens

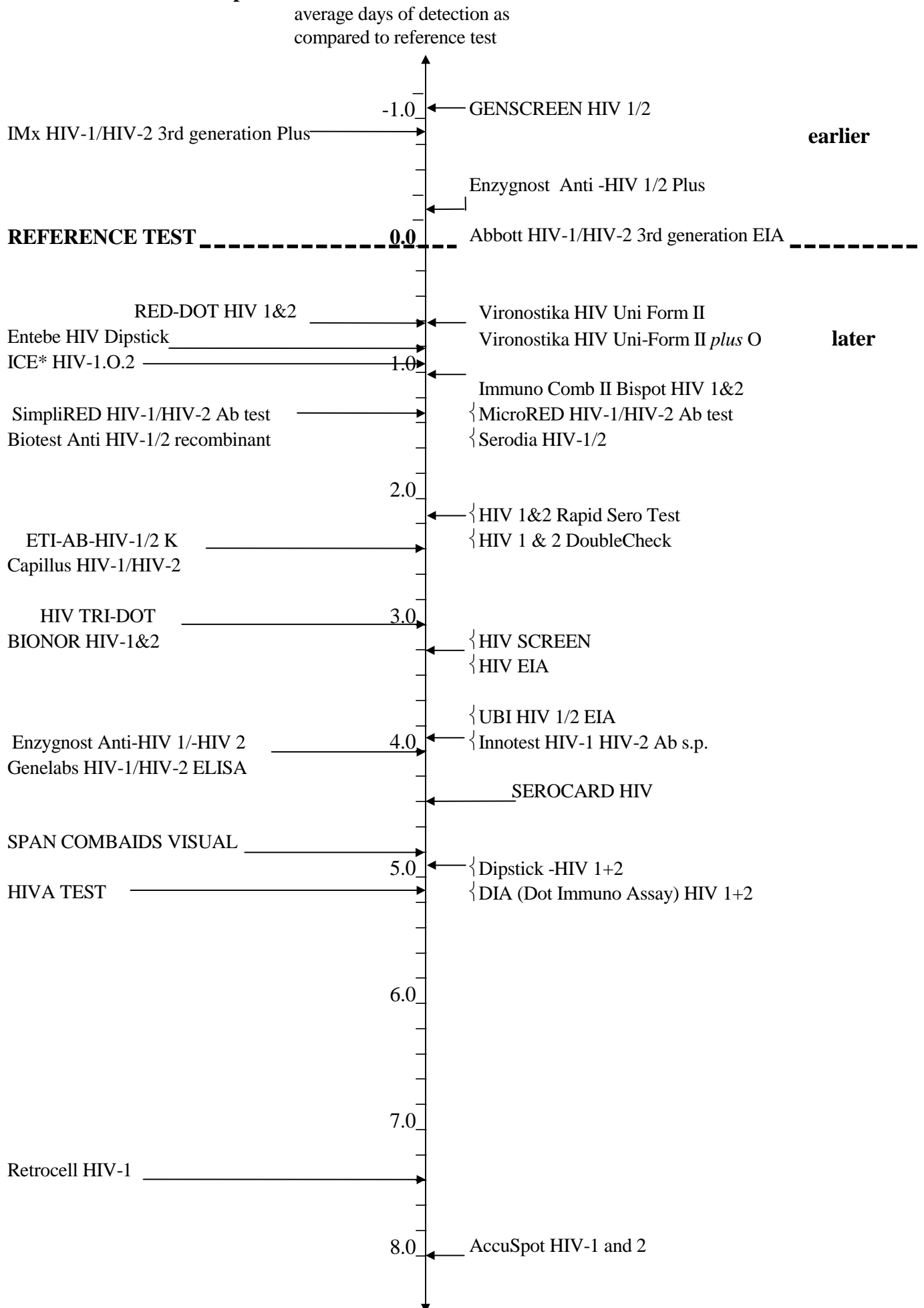
WHO N°	InnoLia HIV-1 group O ¹								RNA PCR ²	Assay Results					
	V3 con	V3 Mal	V3 MPV-5180	V3 ANT-70	V3 V1 686	gp41 ANT-70	gp41 B	gp41 D		ICE * HIV-1.O.2 ratio ³	Vironostika HIV UniForm II plus O ratio	GENSCREEN HIV 1/2 ratio	HIVA TEST ratio	EasiDot HIV/ EasiSpot / HIV	HIV 1&2 Double Check
2001	-	-	+	+	+	+	+	+	HIV-1, O	9.5	4.7	14.6	17.3	pos	pos
2002	-	-	+/-	+	+	+	+	+	HIV-1, O	9.5	12.8	14.6	18.1	pos	pos
2003	-	-	+/-	+	+	+	+	-	HIV-1, O	8.6	9.8	14.6	16.8	pos	pos
2004	-	-	-	+	-	+	+	+	HIV-1, O	9.5	14.3	14.6	16.4	pos	pos
2005	-	-	-	+	+	+	+	+	HIV-1, O	9.5	14.3	14.6	17.5	pos	pos
2006	+	+	+	+	+	+	+	+	HIV-1, O	6.2	6.2	14.6	16.1	pos	pos
2007	+	+	+/-	-	-	+	+	+	HIV-1, O	9.5	14.3	14.6	18.1	pos	pos

¹ : Line immuno assay, HIV-1 group O (research products, Innogenetics).

² : RNA polymerase chain reaction - see reference on page 9 of this report.

³ : ratio = optical density divided by the optical density cut-off.

Figure 2: Relative performance of ELISA and Simple/Rapid assays on early seroconversion panels.



Explanatory Notes for Tables 1 -19 and Figure 2.

Tables 1, 7 and 13: General characteristics and operational aspects of the assays

Assay type:	
Note	: Lateral flow and capillary flow assays are chromatographic membrane tests.
Solid phase	: V8- microtiterplate configured as vertical(V) strips of 8 wells.
Final dilution of the serum	: is the dilution of the serum in the test format, e.g. 10µl serum added to 200µl diluent gives a final dilution of 1:21.
Total time to perform the assay	: reflects the time needed to carry out 1 test run, i.e. the most economical use of the technique. - ELISAs, a complete microtiterplate (96 specimens including controls). - dipstick and comb assays, a complete comb (8-12 specimens including controls). - simple/rapid assays designed for individual tests (1specimen).
Note	: SERO•STRIP HIV-1/2 results become interpretable within a few minutes. Nevertheless, the manufacturer recommends that the final reading be taken after 15 minutes.
Price/test	: as given at the time of the evaluation by the manufacturer, or converted to USD using the currency conversion rate at the time.
Note	: The IMx HIV-1/HIV-2 3rd generation PLUS ELISA assay can only be performed using IMx system equipment. The procedure is fully automated. The Bionor HIV-1&2 rapid EIA assay can only be performed using the testing station (supplied by Bionor a/s), incorporating tilting and magnetic platform, lid, aspirator and lamp.

Tables 2, 8 and 14: Comparison of the results of the assays with reference tests

Sensitivity : calculated as described on page 10 of this document.
 Specificity : calculated as described on page 10 of this document.

For several of the assays evaluated, some panel specimens were exhausted. The variation in numbers of panel specimens from that shown in Tables 2,8 and 14 is shown in the following chart:

ASSAY	ICE * HIV-1.O.2	Vironostika Uniform II plus O	GENSCREEN HIV1/2	HIVA TEST	EasiDot HIV/ EasiSpot HIV	HIV 1 & 2 DoubleCheck
HIV positive						
Total Panel (263)	259	256	262	246	254	258
HIV-1 Total (203)	199	198	202	189	197	199
Africa (54)	51	51	53	49	51	50
Asia (40)	39	38	40	34	37	40
Europe (64)	64	64	64	63	64	64
Latin America (45)	45	45	45	43	45	45
HIV-2 (60)	60	58	60	57	57	59
HIV- negative						
Total Panel (332)	329	330	332	316	328	331
Africa (80)	78	80	80	77	79	80
Asia (59)	59	58	58	46	57	59
Europe (140)	139	139	140	140	139	139
Latin America (53)	53	53	53	53	53	53

Note: the modified EasiDot HIV/EasiSpot HIV assay shows a sensitivity on retested HIV-2 specimens of 11/12 = 91.7% and a specificity on retested HIV negative specimens of 84/88 = 95.5%. The new version of the EasiDot HIV/EasiSpot HIV will be evaluated on the full WHO panel in the next series of evaluations.

Number of controls per test run :
Internal control : ELISAs - The ICE* HIV-1.O.2 (Murex Biotech Ltd.) and Genscreen HIV 1/2(Sanofi Pasteur) assays use a sample addition monitor (an indicator in the sample diluent which changes colour when sample is added) and colour-coded reagents to ensure correct addition of sample and reagent.

Simple/rapid - The following assays all have a control spot or line on all their test devices which checks that sample has been added and reagents function correctly: HIVCHEK System 3 Test Kit (Ortho Diagnostics), HIV 1 & 2 DoubleCheck (Organics), SERO•STRIP HIV1/2 (Saliva Diagnostic Systems Pty, Ltd), and HIV TRI-DOT (J.Mitra & Co Ltd).

The EasiDot HIV/EasiSpot HIV (Nubenco Diagnostics) has an internal control spot which checks the correct functioning of reagents.

The HIV TRI-DOT assay has separate test spots for HIV-1 and HIV-2.

The SEROCARD (Trinity Biotech plc.) assay has a control port to assess the degree of colour that may develop due to non-specific binding of proteins present in the sample.

Definition of positive results : ELISAs - a serum is interpreted as positive according to the formula given in the table where:
X = optical density (OD) of a tested serum B = mean OD of the blank wells
N = mean OD of the negative control sera A = mean OD of the cut-off control sera
P = mean OD of the positive control sera CO = calculated cut-off value

$X < CO$: A sample is defined as negative when its optical density is less than the calculated cut-off value.
 $X \geq CO$: A sample is defined as positive when its optical density is greater than or equal to the calculated cut-off value.

Simple/rapid assays - a sample is interpreted as positive according to the criteria set by the manufacturer and summarised in the table.

Definition of grey zone : a serum is interpreted as indeterminate in ELISA assays according to the formula given in the table.

Tables 4, 10 and 16: Calculation of ease of performance of the assay

The criteria for this calculation are given in the respective tables.

Tables 5, 11 and 17: Suitability of the assay for use in small laboratories

The criteria for this calculation are given in the respective tables.

Note : These criteria are primarily technical and while an assay may be regarded as “technically” suitable for use in laboratories with limited facilities or where small numbers of samples are routinely tested, the sensitivity and specificity of the assay are over-riding factors in determining the suitability of an assay for use in any laboratory.

Note : Since the Bionor HIV-1&2 and IMx HIV-1/HIV-2 3rd generation Plus assays are performed on a testing station and fully automated equipment respectively, there is no need for additional incubators, washers or spectrophotometers. For those criteria, these assays attract the highest rating.

Tables 6, 12 and 18: Performance of the assay on early seroconversion panels

An assay’s performance on the seroconversion panels should be viewed against the specificity of the assay. Assays of relatively low specificity may appear to detect antibody to HIV earlier than other assays of higher specificity; the early detection may be due to false positive results rather than the sensitivity of the assay.

Figure 2.

Eight seroconversion panels (BBI), each containing several samples taken at different time intervals early in the infection period (window period), were tested with a variety of ELISA and simple and/or rapid tests. The results obtained with these assays were compared to those of the Abbott 3rd gen. ELISA (at the time the most sensitive test and therefore chosen as the reference test); the difference in days of the first sample of a panel to become positive with test X as compared to the first positive result with Abbott 3rd gen. ELISA. If a test gave a positive result earlier than the Abbott, the number of days difference in detection were rated as negative; if the test became positive later the number of days were rated as positive. The average of the difference in time period for a test to become positive as compared to the reference test was calculated and plotted on a yardstick. These data provide a relative indication of how well a certain test performs on seroconversion specimens. Please note that the BBI panels are all from American individuals and hence are all HIV-1 subtype B. Although generally the current HIV test kits readily detect all subtypes as well as HIV-2, the sensitivity of the test in the window period may be dependent on the subtype.

Note: The mean result for the following assays was: HIVCHEK System 3 Test Kit > 12.6 days, EasiDot HIV/EasiSpot HIV > 8.5 days and SERO•STRIP HIV-1/2 > 6.2 days after detection by the reference test. These results could not be compared to those of other assays and were excluded from Figure 2.

Annex 1

Cumulative list of assays evaluated whose production has been discontinued

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, cost per test, ease of performance and suitability for use in small blood collection centres.

Assay (manufacturer)	Report No ^a	Sensitivity ^b (%) ^c	Specificity ^d (%) ^c	δ Values ^e		Cost/test ^g (US\$) year	nm ^h	Ease of ^d performance	Suitability ^j	Indeterminate ^k results %
				WB pos sera	WB neg sera					
Enzyme linked immunosorbent assays										
<u>For the detection of antibody to HIV-1</u>										
Dupont HIV-1 Recombinant ELISA (Dupont de Nemours)	1	100.0 (98.7-100.0)	97.0 (92.7-98.8)			0.9/'88	450/410	LE	LS	NA
Enzygnost Anti-HIV Micro (Behringwerke)	1	100.0 (97.8-100.0)	100.0 (98.1-100.0)			1.8/'88	450 450/630	LE	LS	0.0
HIV-TEK G (Sorin Biomedica)	1	100.0 (96.0-100.0)	86.5 (79.5-91.8)			1.0/'88	450	LE	LS	NA
Vironostika Anti-HIV Uni-Form (Organon Teknika)	1	100.0 (97.6-100.0)	99.5 (97.3-100.0)			2.2/'88	492 492/630	LE	LS	NA
HIV-1 env Peptide EIA (Labsystems)	2	96.0 (90.8-98.7)	97.0 (93.5-98.9)			3.9/'89	405 405/630	LE	LS	NA
Genetic Systems LAV EIA (Genetic Systems)	3	100.0 (98.2-100.0)	96.3 (92.9-98.4)	9.2	-2.13	1.0/'90	450 450/615-630	LE	LS	NA
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/'92	492/620-690	LE	S	NA

Annex 1 (continued)

Assay (manufacturer)	Report No ^a	Sensitivity ^b (%) ^c	Specificity ^d (%) ^c	δ Values ^e		Cost/test ^g (US\$) year	nm ^h	Ease of ⁱ performance	Suitability ^j	Indeterminate ^k results %
				WB pos sera	WB neg sera					
Enzygnost Anti-HIV-1 (Behringwerke)	7	100.0 (98.1-100.0)	100.0 (98.8-100.0)	7.4	-3.3		450/615-690	LE	LS	0.0
<u>For the detection of antibody to HIV-2</u>										
Genetic Systems HIV-2 EIA (Genetic Systems)	3	100. (94.0-100.0)	98.6 (95.9-99.7)	17.4	-3.06	1.7/'90	450 450/615-630	LE	LS	NA
Clonatec HIV-2 Ab (Clonatec)	5	100.0 (95.4-100.0)	99.5 (97.4-99.9)	6.7	-1.99	2.0/'91	492	LE	S	0.0
Enzygnost Anti-HIV-2 (Behringwerke)	8	100.0 (96.7-100.0)	99.5 (98.5-100.0)	23.8	-3.5	6.2/'93	450/630	LE	LS	0.0
<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/'89	450 450/615-690	LE	LS	0.0
Recombinant HIV-1/HIV-2 EIA (Abbott)	2	100.0 (98.5-100.0)	97.4 (94.0-99.2)	3.78	-1.50	1.8/'89	490	LE	LS	NA
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/'89	405	LE	LS	1.0
DuPont HIV-1/HIV-2 ELISA (DuPont de Nemours)	3	100.0 (98.7-100.0)	85.6 (79.8-90.2)	9.34	-0.96	1.3/'90	405 or 410 405 or 410/ 620 or 630	LE	LS	NA
Vironostika HIV MIXT (Organon Teknika)	3	100.0 (98.7-100.0)	100.0 (98.1-100.0)	10.10	-2.94	1.8/'90	492	LE	LS	NA

Annex 1 (continued)

Assay (manufacturer)	Report No ^a	Sensitivity ^b (%) ^c	Specificity ^d (%) ^c	δ Values ^e		Cost/test ^g (US\$) year	nm ^h	Ease of ⁱ performance	Suitability ^j	Indeterminate ^k results %
				WB pos sera	WB neg sera					
Elavia Mixt (Diagnostics Pasteur)	4	100.0 (98.7-100.0)	95.1 (91.3-97.8)	54.33	-2.31	2.1/90	492 492/620	LE	LS	0.0
Anti-HIV-1/HIV-2 EIA <Roche> (F. Hoffman-LaRoche)	4	100.0 (98.7-100.0)	96.9 (93.4-98.9)	11.30	-2.37	1.7/90	492	LE	LS	NA
Clonatec HIV (1+2) Ab EIA (Clonatec)	6	99.6 (98.8-100.0)	95.9 (93.1-98.7)	7.47	-1.68	2.7/91	492	LE	S	0.0
Enzymun-Test Anti-HIV-1+2 (Boehringer Mannheim)	6	100.0 (98.7-100.0)	100.0 (98.6-100.0)	5.50	-2.48	3.0/92	405	LE	S	0.0
UBI HIV-1/2 EIA (United Biomedical)	6	100.0 (99.9-100.0)	88.7 (84.2-93.1)	7.18	-1.24	1.2/92	492 492/620-690	LE	S	NA
Enzygnost Anti-HIV-1/HIV-2 (Behringwerke)	6	100.0 (99.9-100.0)	99.5 (98.5-100.0)	26.53	-3.50	2.6/92	450 450/615-690	LE	LS	0.0
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	2.2/93	450	LE	LS	0.0
Biochrom HIV-1/HIV-2 ELISA Version 2 (Biochrom)	7	99.5 (99.0-100.0)	100.0 (98.6-100.0)	7.5	-7.3	1.0/93	450	LE	LS	0.0
Enzygnost Anti-HIV 1/-HIV 2 (Behringwerke)	9	100.0 (99.6-100.0)	99.5 (98.7-100.0)	24.8	-2.55	2.6/92	450 450/615-690	LE	LS	0.0
Genelabs Diagnostics HIV-1/HIV-2 ELISA (Genelabs Diagnostics)	10	100 (99.6 -100.0)	97.3 (95.6 - 99.0)	72.2	-2.7	0.9/94	492	LE	LS	NA
HIV SCREEN (Labystems OY)	10	100.0 (99.6 -100.0)	99.7 (99.1 -100.0)	21.51	-4.11	0.6/95	450	LE	LS	NA

Annex 1 (continued)

Assay (manufacturer)	Report No ^a	Sensitivity ^b (%) ^c	Specificity ^d (%) ^c	Initial inter-reader ^f variability %	Cost/test ^g (US\$) year	Ease of ⁱ performance	Suitability ^j	Indeterminate ^k results %
Simple/Rapid assays								
<u>For the detection of antibody to HIV-1</u>								
HIV CHEK/HIVSPOT (Genelabs Diagnostics)	1	94.5 (89.7- 97.4)	99.0 (96.4-99.9)	12.3	2.5/'88	VE	VS	
Recombigen HIV-LA (Cambridge BioScience)	1	95.2 (88.3-98.7)	96.1 (92.6-98.2)	6.0	3.0/'88	VE	S	
Abbott Retrocell HIV 1 (Abbott GmbH)	9	100.0 (99.6 -100.0)	100.0 (99.7-100.0)	2.2	1.45/'94	VE	S	0.6
Genie HIV-1 (Genetic Systems)	4	99.5 (97.4-100.0)	99.1 (96.7-99.9)	1.1	3.5/'90	VE	VS	0.2
Healthtest HIV-1 Assay (Akers Research Corp.)	6	58.7 (49.2-68.2)	89.4 (84.9-93.9)	7.0	1.4/2.3/'92	VE	S	0.2
<u>For the detection of antibody to HIV-1 and HIV-2</u>								
Immunocomb Bi-Spot (PBS Organics)	3	98.5 (96.3-99.6)	100.0 (98.1-100.0)	7.6	4.0/'90	VE	VS	0.9
Recodot (Waldheim Pharmazeutika)	4	98.9 (97.0-99.8)	88.6 (82.2-93.3)	31.7	2.0/'90	LE	LS	12.3
Genie HIV-1 and HIV-2 (Genetic Systems)	4	99.3 (97.5-99.9)	99.5 (97.2-100.0)	11.8	3.5/'90	VE	VS	0.0
Clonatec rapid HIV 1-HIV 2 Ab (Clonatec)	5	98.9 (96.8-99.8)	99.5 (97.2-99.8)	15.9	4.3/'91	E	VS	0.4

Annex 1 (continued)

Assay (manufacturer)	Report No ^a	Sensitivity ^b (%) ^c	Specificity ^d (%) ^c	Initial inter-reader ^f variability %	Cost/test ^g (US\$) year	Ease of ⁱ performance	Suitability ^j	Indeterminate ^k results %
Recobead LA Assay (Waldheim Pharmazeutika)	6	59.8 (53.9 -65.7)	94.8 (91.7 - 97.9)	22.3	1.7/2.2/'91	VE	S	0.4
Supplemental assays								
<u>For the detection of antibody to HIV-1</u>								
Wespage HIV-1 Western blot Kit (Bio Genex)	6	100.0 (99.9-100.0)	100.0 (99.9-100.0)	NA	21.6/'92	LE	VS	12.8
Wespage HIV-1 Western blot Kit II (Bio Genex)	7	100.0 (98.5 -100.0)	100.0 (98.7 -100.0)	NA	17.7/'93	LE	S	12.4

Annex 2

Cumulative list of assays evaluated; currently commercially available

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, cost per test, ease of performance and suitability for use in small blood collection centres.

Assay (manufacturer)	Report No ^a	Sensitivity ^b (%) ^c	Specificity ^d (%) ^c	δ -Values ^e		Cost/test ^g (US\$) year	nm ^h	Ease of ⁱ performance	Suitability ^j	Indeterminate ^k results %
				WB pos sera	WB neg sera					
Enzyme linked immunosorbent assays										
<u>For the detection of antibody to HIV-1</u>										
Ortho HIV ELISA System (Ortho Diagn. Systems)	1	100.0 (97.8-100.0)	98.0 (95.0-99.4)			1.8/'88	490	LE	LS	NA
Wellcozyme HIV Recombinant (Wellcome Diagnostics)	2	100.0 (98.2-100.0)	99.1 (96.8-99.9)			1.5/'89	450	LE	LS	NA
REC VIH-KCOI (Heber Biotec)	3	97.0 (93.5-98.9)	100.0 (98.3-100.0)	2.1	-4.14		492	LE	LS	NA
Peptide HIV-1 ELISA Test System (Sero-Immuno Diagnostics)	6	82.1 (76.5-87.6)	94.1 (91.0-97.2)			0.6/'92	visual	E	VS	0.0
<u>For the detection of antibody to HIV-2</u>										
Peptide HIV-2 ELISA Test (Sero-Immuno Diagnostics)	6	97.1 (93.0-100.0)	98.1 (96.3-99.9)			0.6/'92	visual	E	VS	NA
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/'93	492/620-630	LE	S	NA

Annex 2 (continued)

Assay (manufacturer)	Report No ^a	Sensitivity ^b (%) ^c	Specificity ^d (%) ^c	δ-Values ^e . WB pos sera WB neg sera		Cost/test ^g (US\$) year	nm ^h	Ease of ⁱ performance	Suitability ^j	Indeterminate ^k results %
<u>For the detection of antibody to HIV-1 and HIV-2</u>										
Detect-HIV™ (Biochem Immunosystemes)	3	100.0 (98.6-100.0)	97.4 (94.0-99.2)	12.65	-2.21	2.5/'90	450 450/600-650	LE	LS	NA
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/'90	492	LE	LS	NA
Peptide HIV ELISA (Cal-Tech Diagnostics)	5	72.6 (69.4-77.6)	95.4 (91.3-97.9)			0.9/'91	visual	E	S	0.2
Genelavia Mixt (Sanofi Diagnostics Pasteur)	5	100.0 (98.6-100.0)	98.5 (95.6-99.8)	16.77	-2.10	1.5/'91	492 492/620	LE	LS	0.0
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/'91	492 492/570-650	LE	LS	0.0
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/'91	450 450/620-690	LE	LS	NA
Peptide HIV-1 & HIV-2 ELISA Test (Sero-Immuno Diagnostics)	6	97.6 (95.7-99.5)	98.5 (96.7-100.0)			0.6/'92	visual	E	VS	NA
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/'93	492	LE	LS	NA
HIV-1 and/or HIV-2 Recombigen EIA (Trinity Biotech plc)	7	100.0 (98.6-100.0)	100.0 (98.6-100.0)	10.4	-5.0	1.7/'93	490/630	LE	LS	NA
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/'93	492/620 or 630	LE	S	NA

Annex 2 (continued)

Assay (manufacturer)	Report No ^a	Sensitivity ^b (%) ^c	Specificity ^d (%) ^c	δ-Values ^e		Cost/test ^g (US\$) year	nm ^h	Ease of ⁱ performance	Suitability ^j	Indeterminate ^k results %
				WB pos sera	WB neg sera					
HIV 1+2 <u>env</u> Peptide EIA (Labsystems OY)	8	100.0 (98.6-100.0)	76.2 (70.0-82.4)			08/2.8/'93	450	LE	LS	0.0
VIDAS HIV-1+2 (Bio Merieux)	8	100.0 (98.5-100.0)	97.8 (95.6-100.0)			3.6/'93	450	VE	S	0.3
VIRONOSTIKA HIV Uni-Form II (Organon Teknika)	9	100.0 (99.6-100.0)	98.8 (97.6-100.0)	7.4	-3.0	1.7/'94	450/660 ± 40	LE	LS	NA
BIOTEST Anti-HIV-1/-2 recombinant (Biotest AG)	9	100.0 (99.6-100.0)	99.1 (98.1-100.0)	74.9	-3.3	1.2/'94	492/570-650	LE	LS	0.0
INNOTEST HIV-1/HIV-2 Ab s.p. (Innogenetics n.v.)	9	100.0 (99.6-100.0)	98.8 (97.6-100.0)	14.0	-3.8	1.5/'94	450 450/620-690	LE	LS	NA
UBI HIV 1/2 EIA (United Biomedical Inc.)	9	100.0 (99.6-100.0)	100.0 (99.7-100.0)	10.8	-3.2	1.0/'94	492 492/620-690	LE	LS	NA
HIVisual 1 & 2 (Immuno Diagnostics Inc.)	10	90.9 (87.4 - 94.4)	94.5 (92.5 -97.3)	1.88	-1.15		450	LE	LS	NA
ETI-AB-HIV-1/2 K (Sorin Biomedica)	10	100.0 (99.6-100.0)	98.8 (97.6-100.0)	10.4	-2.5	1.5/'94	450/630	LE	LS	NA
HIV EIA (Labsystems OY)	10	100 (99.6 -100.0)	99.4 (98.6 -100.0)	14.20	-3.85	0.6'95	450	LE	LS	NA
IMx HIV-1/HIV-2 3rd generation Plus (Abbott GmbH Diagnostika)	11	99.6 (98.9 -100.0)	97.9 (96.4- 99.4)	9.1	-2.1	3-4'95	Imx system	VE	S	0.3
Enzygnost Anti-HIV 1/2 Plus (Behringwerke AG)	11	100.0 (99.6 -100.0)	99.7 (99.1 -100.0)	19.1	-6.6	1.0'95	450 450/615-690	LE	LS	0.0

Annex 2 (continued)

Assay (manufacturer)	Report No ^a	Sensitivity ^b (%) ^c	Specificity ^d (%) ^c	δ -Values ^e WB pos sera WB neg sera		Cost/test ^g (US\$) year	nm ^h	Ease of ⁱ performance	Suitability ^j	Indeterminate ^k results %
ICE * HIV-1.O.2 (Murex Biotech Ltd.)	11	100.0 (99.6 -100.0)	99.4 (98.6 -100.0)	16.8	-4.3	0.6'95	450 450/620-690	LE	LS	NA
Vironostika Uni-Form II <i>plus</i> O (Organon Teknika nv)	11	100.0 (99.6 -100.0)	100.0 (99.7 -100.0)	17.2	-4.1	1.5'97	450 450/620-700	LE	LS	NA
GENSCREEN HIV 1/2 (Sanofi Diagnostics Pasteur)	11	100.0 (99.6 -100.0)	98.5 (97.2 - 99.8)	22.8	-2.7	1.5'95	450/620	LE	LS	0.0
HIVA TEST (Lupin Laboratories Ltd)	11	100.0 (99.5 -100.0)	93.7 (91.0 - 96.4)	12.2	-1.1	0.6'98	450	LE	LS	1.5

Assay (manufacturer)	Report No ^a	Sensitivity ^b (%) ^c	Specificity ^d (%) ^c	Initial inter-reader ^f variability %	Cost/test ^g (US\$) /year	Ease of ⁱ performance	Suitability ^j	Indeterminate ^k results %
----------------------	------------------------	---	---	---	-------------------------------------	----------------------------------	--------------------------	--------------------------------------

Simple/rapid assays

For the detection of antibody to HIV-1

Serodia-HIV (Fujirebio)	1	100.0 (97.6-100.0)	96.9 (93.4-99.0)	0.8	1.1/'88	E	S	
Immunocomb (PBS Organics)	1	98.8 (95.7- 99.9)	98.9 (96.0-99.9)	2.8	2.5/'89	VE	VS	
Serion Immuno Tab HIV-1 (Serion Immunodiagnostica)	2	98.9 (96.9- 99.9)	100.0 (98.3-100.0)	7.1	2.5/'90	LE	LS	1.2

Annex 2 (continued)

Assay (manufacturer)	Report No ^a	Sensitivity ^b (%) ^c	Specificity ^d (%) ^c	Initial inter-reader ^f variability %	Cost/test ^g (US\$) /year	Ease of ⁱ performance	Suitability ^j	Indeterminate ^k results %
PATH HIV Dipstick (PATH)	4	99.5 (97.3-100.0)	98.2 (97.1-99.1)	1.3	<1.5'91	E	VS	0.0
SimpliRed HIV-1 Ab (Agen Biomedical)	5	97.5 (94.2-99.2)	91.2 (86.6-94.7)	10.5	7.8/1.5'91	VE	S	0.7
SUDS Murex HIV-1 Ab test (Murex Corporation)	5	100.0 (98.5-100.0)	75.1 (69.3-80.9)	22.9	4.5'91	VE	S	11.7
Entebe HIV Dipstick (Hepatika Laboratories)	6	97.0 (94.4-99.6)	99.1 (97.8-100.0)			E	VS	
<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)	1.4	4.8'89	VE	VS	0.0
Recombigen HIV-1/HIV-2 Rapid Test Device (Trinity Biotech plc)	7	100.0 (98.7-100.0)	94.5 (91.2-97.8)	11.4	4.0'93	E	VS	2.8
Serodia-HIV-1/2 (Fujirebio)	8	100 (98.5-100.0)	100 (98.5-100.0)	6.3	2.8'93	LE	S	0.0
HIV CHEK 1+2/HIVSPOT 1+2 (DuPont de Nemours)(Genelabs Diagnostics)	3	99.3 (97.4-99.9)	100.0 (98.1-100.0)	7.2	4.0'90	E	VS	1.0
SPAN COMBAIDS VISUAL (Span Diagnostics.)	8	96.5 (93.5-99.5)	100.0 (98.3-100.0)	0.8	0.4'93	E	VS	0.0
CAPILLUS HIV-1/HIV-2 (Trinity Biotech plc)	9	100.0 (99.6-100.0)	98.8 (97.6-100.0)	0.0	2.2'94	VE	VS	0.0

Annex 2 (continued)

Assay (manufacturer)	Report No ^a	Sensitivity ^b (%) ^c	Specificity ^d (%) ^c	Initial inter-reader ^f variability %	Cost/test ^g (US\$) /year	Ease of ⁱ performance	Suitability ^j	Indeterminate ^k results %
Immunocomb II BiSpot HIV 1&2 (PBS Orgenics)	9	100.0 (99.6-100.0)	99.7 (99.1-100.0)	4.5	1.7/94	VE	VS	0.2
MicroRed HIV-1/HIV-2 Ab Test (Agen Biomedical)	9	98.5 (97.0-100.0)	95.5 (93.2-97.7)	1.5	1.5/1.0/94	VE	S	0.5
SimpliRed HIV-1 /HIV-2Ab Test (Agen Biomedical)	9	99.2 (98.2 -100.0)	87.3 (83.7 -90.9)	9.5	4.0/3.0/94	VE	S	0.3
HIV (Sav) 1&2 Rapid Sero Test (Diatech (Savyon) Diagnostica Ltd.)	10	97.7 (95.9 -99.5)	96.7 (94.8 -98.6)	5.1	1.9/94	VE	S	0.2
ENTEBE HIV Dipstick (Hepatika Laboratories)	10	100.0 (99.6 -100.0)	96.4 (94.4 -98.4)	5.0	0.8/94	VE	VS	1.3
Dipstick-HIV 1 + 2 (Pacific Biotech Co., Ltd.)	10	100.0 (99.6- 100.0)	98.2 (96.8 -99.6)	1.0	0.5/94	E	VS	0.3
SPAN COMBAIDS VISUAL (Span Diagnostics Ltd.)	10	100.0 (99.6-100.0)	88.0 (84.5-91.5)	6.3	0.5/94	E	S	3.2
DIA (Dot Immuno Assay) HIV 1 + 2 (Weiner Lab.)	10	99.6 (98.8-100.0)	99.4 (98.6-100.0)	0.8	<1.0/94	VE	VS	0.2
RED-DOT HIV 1&2 (Cal-Test Diagnostics Inc.)	11	100.0 (99.6 -100.0)	94.9 (92.5 - 97.3)	9.5	2.9/94	VE	S	1.9
HIVCHEK System 3 Test Kit (Ortho Diagnostic Systems)	11	99.6 (98.9 -100.0)	99.7 (99.1 -100.0)	1.0	4.35/95	E	VS	0.2

Annex 2 (continued)

Assay (manufacturer)	Report No ^a	Sensitivity ^b (%) ^c	Specificity ^d (%) ^c	Initial inter-reader ^f variability %	Cost/test ^g (US\$) /year	Ease of ⁱ performance	Suitability ^j	Indeterminate ^k results%
HIV TRI-DOT (J. Mitra & Co. Ltd.)	11	99.6 (98.9 -100.0)	99.7 (99.1 -100.0)	3.2	2.0'96	VE	VS	0.2
AccuSpot HIV-1 and 2 (Specialty BioSystems Inc.)	11	100.0 (99.6 -100.0)	86.3 (82.5 - 90.1)	10.8	2.5'95	VE	S	5.0
BIONOR HIV-1&2 (Bionor A/S)	11	100.0 (99.6 -100.0)	98.8 (97.6 -100.0)	1.0	2.5'95	VE	S	0.2
SEROCARD HIV (Trinity Biotech plc)	11	100.0 (99.6 -100.0)	97.9 (96.4 - 99.1)	1.5	4.0'94	VE	VS	0.2
SERO•STRIP HIV-1/2 (Saliva Diagnostic Systems)	11	98.9 (97.6 -100.0)	100.0 (99.7 -100.0)	1.5	1.5'95	VE	VS	0.0
HIV 1 & 2 DoubleCheck (Orgenics)	11	100 (99.6 -100.0)	99.4 (98.6 -100.0)	0.8	2.0'96	VE	VS	0.2
EasiDot HIV/EasiSpot HIV (Nubenco Diagnostics)	11	95.3 (92.7 - 97.9)	71.3 (66.4 - 76.2)	23.7		VE	S	12.5
Supplemental assays								
<u>For the detection of antibody to HIV-1</u>								
RIBA HIV-1 (Chiron)	1	99.4 (96.6-100.0)	100.0 (97.9-100.0)	NA	27.6/'88	E	S	
Ancoscreen (Ancos)	2	100.0 (97.8-100.0)	90.4 (82.6-95.5)	NA	10.8/21.5/'89	LE	LS	31.4

Annex 2 (continued)

Assay (manufacturer)	Report No ^a	Sensitivity ^b (%) ^c	Specificity ^d (%) ^c	Initial inter-reader ^f variability %	Cost/test ^g (US\$) /year	Ease of ⁱ performance	Suitability ^j	Indeterminate ^k results%
HIV Western Blot Kit (Organon Teknika)	3	100.0 (98.2-100.0)	100.0 (98.0-100.0)	NA	21.0/'90	LE	S	10.5
IFA anti-HIV-1 (Waldheim Pharmazeutika)	5	98.9 (96.9-99.8)	100.0 (98.3-100.0)	13.8	5.6/'91	LE	LS	0.7
New Lav-Blot-I (Sanofi Diagnostice Pasteur)	5	100.0 (98.1-100.0)	100.0 (96.8-100.0)	NA	11.6/'91	E	S	30.6
HIV-1 Western Blot Kit (Open Tray Procedure) (Bio Genex)	7	100.0 (98.5-100.0)	100.0 (98.7-100.0)	NA	17.7/'93	LE	S	6.7
IFA anti-HIV-2 (Waldheim Pharmazeutika)	5	98.7 (93.1-99.7)	100.0 (98.2-100.0)	11.0	6.0/'91	LE	LS	1.8
CBC HIV-2 Western blot kit (Cambridge Biotech)	7	100.0 (97.0-100.0)	100.0 (98.5-100.0)	NA	16/'93	LE	S	13.9
<u>For the detection of antibody to HIV-1 and HIV-2</u>								
INNO-LIA HIV-1/HIV-2 Ab (Innogenetics)	2	100.0 (98.6-100.0)	100.0 (98.0-100.0)	NA	18.4/'89	LE	S	4.3
Speedscreen HIV (British Bio-Technology)	4	100.0 (99.4-100.0)	66.4 (57.9-74.1)	NA	17.0/'90	LE	S	16.9
Pepti-Lav 1-2 (Sanofi Diagnostic Pasteur)	4	99.3 (96.4-99.9)	100.0 (98.1/100.0)	NA	21.5/'90	LE	S	0.7

Legend for Annexes 1 and 2

- 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/RES/BMR/89.4
 - Report 2 - unpublished document GPA/RES/BMR/90.1
 - Report 3 - unpublished document GPA/RES/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/93.6
 - Report 8 - unpublished document GPA/RES/DIA/94.4
 - Report 9/10 - unpublished document WHO/BLS/98.1
 - Report 11 - unpublished document WHO/BTS/99.1
- b,c,d: Sensitivity, specificity and 95% confidence limits were calculated as described on pp 10 - 11 of this document.
- e: δ -values were calculated as described on page 12 of this document.
- f: Inter-reader variability was calculated as described on page 12 of this document.
- g: Prices quoted are those in effect at the time of the evaluation.
- h: The wavelength(s) of the spectrophotometer (single and/or double) is specified by the manufacturer.
- i: Ease of performance is defined on tables 4,10 and 16.
- j: Suitability for use in small laboratories is defined on tables 5,11 and 17.
- k: Indeterminate results were calculated as described in the explanatory notes on page 42.

Annex 3

Cumulative list of assay manufacturers' addresses

Abbott GmbH, Diagnostika, Max-Planck-Ring 2, 65205 Wiesbaden, Germany.
Tel: (49 6122) 58 16 23; Telex: 4182555; Fax: (49 6122) 58 16 12.

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 Laboratories Inc., 201 Grove Road, Thorofare, New Jersey 08086, USA.
Tel: (1 609) 848 8698; Fax: (1 609) 848 0269.

Ancos Denmark ApS., Tengslemarkvej 4, 4573, Højby, Denmark.
Tel: (45 59) 30 65 55; Telex: 42580 ancospk; Fax: (45 59) 30 60 45.

Biochem Immunosystèmes., 10900 rue Hamon, Montréal (Québec), Canada H3M 3A2. Tel: (1 514) 335 9922; Telex: 058-27642 IAF BCM MTL; Fax: (1 514) 335 9919.

Biochrom KG, Leonorenstrasse 2-6, 12247 Berlin, Germany.
Tel: (49 30) 77 99 06 00; Telex: 185 821 bio d; Fax: (49 30) 771 0012.

Bio Genex, 4600 Norris Canyon Road, San Ramon, CA 94583, USA.
Tel: (1 510) 275 0550, Fax: (1-510) 276 0580.

BioMérieux S.A., 69280 Marcy-l'Etoile, France.
Tel: (33 78) 87 20 00; Fax: (33 78) 87 20 90.

BIONOR A/S, P.O. Box 1868, N-3705 Skien, Norway
Tel: (47 35) 53 84 88; Fax: (47 35) 53 71 30

Biotest AG, Landsteiner Str. 5, 63303 Dreieich, Germany.
Tel: (49 6103) 8-0 10; Telex: 4185429; Fax: (49 6103) 8-0 11 30.

Boehringer Mannheim GmbH, Sandhofer Strasse 116, 68298 Mannheim, Germany.
Tel: (49 621) 759 8838; Telex: 463193 bmd/462420 bmd; Fax: (49 621) 759 8842.

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.

Annex 3, continued

CAL-TEST DIAGNOSTICS, 13760 Mountain Avenue, Chino, CA 91710, USA.

Tel: 0001 909 902-0550, Fax: 0001 909 902 0044.

Cambridge Diagnostics Ireland Ltd. (see Trinity Biotech plc)

Catalina Bio-Diagnostic Consulting, Inc. 5595 E. 7th Street, Long Beach, CA 90804, USA.

Tel: (1 310) 983 8111; Fax: (1 310) 987 0670.

Chiron Corporation, 4560 Horton Street, Emeryville, CA 94608-2916, USA. Tel: (1 510) 655

8730; Fax (1 510) 655 9910.

Clonatec Diagnostics S.A., 60 rue de Wattignies, 75580 Paris Cedex 12, France.

Tel: (33 1) 43 42 43 88; Telex: 214044F; Fax: (33 1) 43 40 48 86.

Dade Behring Marburg GmbH, Postfach 1149, 35001 Marburg, Germany.

Tel: (49 6421) 39 4478; Fax: (49 6421) 66064.

Fujirebio Inc., 19th floor, Shinjuku Daiichi Seimei Building, 7-1 Nishi-Shinjuku 2-Chome, Shinjuku-Ku, Tokyo 163-07, Japan.

Tel: (81 3) 3348 0947; Telex: J 28612; Fax: (81 3) 3342 6220.

Fujirebio Europe BV, Takkebijsters 69c, 4817 BL Breda, The Netherlands

Tel: (31 76) 571 0440; Fax: (31 76) 587 2181; E.mail: febv@xs4all.nl

Genelabs Diagnostics, Halle de Frêt, P. O. Box 1015, 1215 Geneva 15 Airport, Switzerland.

Tel: (41 22) 788 1908; Fax (41 22) 788 1986.

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, Yayasan Hati Sehat, Jalan Bung Hatta 3A, Mataram, Lombok, Indonesia, under license from the Concept Foundation Program for Appropriate Technology in Health (PATH), Seattle, WA, USA.

Tel: (62 3) 64 31 662; Fax: (62 3) 64 35642

Hoffmann-La Roche F. AG, Grenzacherstr 124, 4058 Basel, Switzerland.

Tel: (41 61) 688 55 55; Fax: (41 61) 681 98 67.

Immuno-Chemical Laboratories. (see Pacific Biotech Co.Ltd.)

Immuno Diagnostics, Inc., 85 Great Arrow Avenue., Buffalo, New York 14216, USA.

Tel: (1 716) 873 9400; Fax: (1 716) 876 7919.

Annex 3, continued

Innogenetics S.A., Technologiepark 6, 9052 Ghent, Belgium
Tel: (32 9) 329 1329; Fax: (32 9) 329 1911.

J. Mitra & Co. Ltd., A-180, Okhla Industrial Area, Phase-1, New Delhi-110 020, India
Tel: (91 11) 681 8971, (91 11) 681 8973, (91 11) 681 3995, (91 11) 681 3989; Fax: (91 11) 681 0945, (91 11) 681 8970

Johnson & Johnson International, Roissy Pole B.P. 10784, 1, Place de Londres,
F-95727 Roissy CDG Cedex, France.
Tel: (33 1) 48 62 08 75; Fax: (33 1) 48 62 00 54

Labsystems OY, Pulttitie 8, P. O. Box 8, 00881 Helsinki, Finland.
Tel: (358 0) 75821; Telex: 123569 Labsy sf; Fax: (358 0) 7557610.

Lupin Laboratories Ltd., 159, CST Road, Kalina, Santacruz (E), Mumbai 400098, India.
Tel: (91 22) 611 3391; Fax: (91 22) 611 4008.

Murex Biotech Limited, Central Road, Temple Hill, Dartford, Kent DA1 5LR, England.
Tel: (44 1322) 27 77 11; Telex MUREX G 896113; Fax: (44 1322) 27 32 88

Nubenco Enterprises, Inc. One Kalisa Way, Suite 207 Paramus, New Jersey 07652-3508,
USA. Tel: (1 201) 967 9000; Fax +1 201 967 9444; Email: info@nubenco.com

Organon Teknika N.V., Boseind 15, 5280 AB Boxtel, the Netherlands
Tel: (31 411) 654 911; Fax (31 411) 654 115; www.organonteknika.com

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.

Pacific Biotech Co., Ltd. 6 Ladprao 110 (Sonthiwattana 3), Ladprao Road, Bangkok,
Bangkok 10310, Thailand.
Tel: (66 2) 530 4608 or 530 2754; Fax: (66 2) 530 4619.

PBS Organics, 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.
North Industrial Zone, P. O. Box 360, Yavne, 70650 Israel.
Tel (972 8) 43 87 52-2; Fax: (972 8) 43 87 58.

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

Annex 3, continued

Saliva Diagnostic Systems (SDS), SDS International Ltd., 11 Sovereign Close, Sovereign Court, London E1 1HW, UK

Tel: (44 171 415 0550; Fax: (44 171) 415 0553

Saliva Diagnostic Systems, (SDS), 11719 NE 95th Street, Vancouver, WA 98682, USA

Tel: (1 360) 696 4800; Fax: (1 360) 254 7942

Sanofi Diagnostics Pasteur, 3 bd. Raymond Poincaré, B.P. 3, 92430 Marnes-la-Coquette, France.

Tel: (33 1) 47 95 60 00; Telex: 631293F; Fax: (33 1) 47 41 91 33.

Savyon Diagnostics, LTD, Kiryat Minrav, 3 Habosem, Ashdod 77101, Israel.

Tel: (972 8) 562920; Fax (972 8) 563258

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 SpA, Divisione Diagnostici, 13040 Saluggia (Vercelli), Italy.

Tel: (39 161) 487243; 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.

Specialty BioSystems, Inc. 5870 Pacific Center Boulevard, Suite A, San Diego, California 92121 USA. Tel: (1 619) 457 9927; Fax: (1 619) 457 2425

Trinity Biotech plc, IDA Business Park, Bray, Co. Wicklow, Ireland.

Tel: (353 1276) 9800; Fax: (353 1276) 9888.

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: (43 1) 319 1456; Telex: 116487 wamed a; Fax: (43 1) 319 1456-44;
email: 100302.2552@compuserve.com.

Wiener Laboratories, Riobama 2944, 2000 Rosario, Argentina.

Tel: (54 41) 39 01 73/8; Fax: (54 41) 37 13 77

Wellcome Diagnostics, (see Murex Diagnostics Limited)

Acknowledgement

We would like to thank Dr S. Broor, All India Institute of Medical Sciences, New Delhi, India; Dr G.M. Gershy-Damet, Institute Pasteur, Abidjan, Côte d'Ivoire; Dr P. Ghys, Projet Retro-ci, Abidjan, Côte Ivoire; Dr Van der Borcht, Innogenetics, Rio de Janeiro, Brasil; Dr O. Varnier, AIDS Center San Luigi, Italy; Dr C. Wasi, Siriraj Hospital, Bangkok, Thailand; for supplying sera to the WHO serum panel.
