

# **HIV ASSAYS: OPERATIONAL CHARACTERISTICS**

*(PHASE I)*

**REPORT 13**  
**URINE SPECIMENS**  
**ORAL FLUID (SALIVA) SPECIMENS**



**World Health  
Organization**





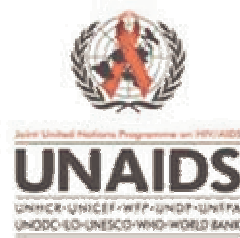
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**World Health Organization**  
**Department of Essential Health Technologies**



## WHO Library Cataloguing-in-Publication Data

World Health Organization.

HIV assays : operational characteristics (Phase I). Report 13: urine specimens, oral fluid (saliva) specimens.

1.AIDS serodiagnosis - methods 2.Urine - virology 3.Saliva - virology 4.Reagent kits, Diagnostic-utilization 5.Clinical trials, Phase I 6.Enzyme-linked immunosorbent assay 7.Blotting, Western I.Title.

ISBN 92 4 159236 2

(NLM classification: WC 503.1)

This publication is a reprint of material originally distributed as [WHO/BCT/02.08](#)

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Printed in France

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**HIV ASSAYS: OPERATIONAL CHARACTERISTICS (PHASE I)**  
**REPORT 13**  
**URINE AND SALIVA SPECIMENS**

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## 1. INTRODUCTION

In 1988, WHO implemented a programme for the evaluation of performance and major operational characteristics of commercially available assays for detection of antibodies to HIV. This report presents the findings of the Phase I evaluations of six HIV assays, three for the detection of HIV antibodies in urine specimens and three for the detection of HIV antibodies in oral fluid, further referred to as saliva. The work was conducted in the years 2000 and 2001. The HIV assays evaluated were:

### Group 1 Urine assays:

- Calypte™ HIV-1 Urine EIA, Fc (Calypte Biomedical Corporation)
- Calypte™ HIV-1 Urine EIA (Recombinant), (Calypte Biomedical Corporation)
- Cambridge Biotech HIV-1 Urine Western Blot Kit (Calypte Biomedical Corporation)

### Group 2 Saliva assays:

- OraScreen™ HIV Rapid Test (Beacon Diagnostics)
- Salivax™-HIV (ImmunoScience Inc)
- SMLX Technologies Diagnostics Test (SMLX Technologies)

### Saliva reference assay

- Wellcozyme HIV 1+2 GACELISA (Murex Biotech Ltd)

Section 2 of this report provides background information on the evaluations. Sections 3 and 4 present the laboratory aspects of HIV testing and describe the way in which the evaluations were conducted and the results analysed. The results and outcomes of the analysis of the assay evaluations are contained in the tables in Section 5. Annexes 1, 2 and 3 show, respectively, the algorithm for characterization of the WHO HIV matched serum panels, the testing algorithm for the urine specimens and the testing algorithm for the saliva specimens. Annex 4 shows the addresses of manufacturers/distributors of the assays evaluated.

This report contains Phase I assessments of two enzyme linked immunosorbent assays, Calypte™ HIV-1 Urine EIA, Fc and Calypte™ HIV-1 Urine EIA (Recombinant) and one Western blot assay, Cambridge Biotech HIV-1 Urine Western Blot Kit, and three simple/rapid tests for saliva specimens, OraScreen™ HIV Rapid Test, Salivax™-HIV and SMLX Technologies Diagnostics Test. Copies of this report are available on request from the Department of Blood Safety and Clinical Technology, World Health Organization, 1211 Geneva 27, Switzerland and also from the WHO website [www.who.int/bct](http://www.who.int/bct)

## 2. BACKGROUND INFORMATION

Testing for HIV is an essential component in the diagnosis and treatment of persons infected with the virus, in screening of blood for transfusion, in surveillance and in HIV/AIDS related research. Thus accurate and cost-effective testing is of great importance in combating the spread of HIV. It is imperative that tests for the diagnosis of HIV infection be as accurate as possible, given the serious ethical, legal and social issues that accompany HIV infection.

The use of body fluids other than blood as specimens for detecting antibodies to HIV has been reported to have potential as an alternative medium for HIV testing. The use of urine samples and also samples derived from the oral cavity (saliva) may be attractive due to the ease of sample collection, possible cost savings, better safety (against needlestick injuries) and higher compliance rates. Assays for these types of specimens can be a useful alternative when it is difficult or impossible to test for HIV in blood samples. It may be that blood cannot be drawn for religious reasons or difficulties may be experienced in collecting blood samples in hard to reach places where it is, nevertheless, important to have epidemiological surveillance.

Today new HIV tests have been developed for use with urine specimens or saliva. Urine contains very low levels of IgG, frequently around 1 mg/L, and therefore very sensitive techniques are required to detect specific antibody. Thus, to date, the tests for urine specimens have been based on ELISA and Western blot techniques, however efforts are being made to develop simple/rapid tests for the detection of antibody to HIV in urine specimens.

Oral fluid specimens consist mostly of saliva, which predominantly contains IgA class antibody, and oral mucosal transudates, which mostly contain IgG, and therefore also have much lower levels of IgG than serum. The levels of IgG normally found in oral fluid specimens (approximately 15 mg/L) are, however, higher than in urine specimens and innovative simple/rapid technology that has been shown to be effective for whole blood, serum and plasma, e.g. lateral flow through a chromatographic membrane, has been developed for use with these specimens. The specifics for the formats for both specimen types have been adapted to account for the lower level of HIV antibody present in these specimens. Although in principle these techniques hold great promise, their accuracy and reliability must be validated.

Since 1988, the World Health Organization (WHO) and UNAIDS have been providing member states with objective assessments of the operational characteristics of commercially available HIV tests. This continuing program is carried out by the collaborating centre at the Department of Microbiology, Institute of Tropical Medicine, Antwerp, Belgium and is coordinated by the Department of Blood Safety and Clinical Technology, WHO, Geneva in conjunction with UNAIDS.

There is a need for testing centres, whether central laboratories or small health centres, to identify the most appropriate assay for their own circumstances. The WHO evaluation program can be invaluable in this respect by making available the information that is needed by decision-makers to allow them to make the most suitable choices. A great deal of information is given in the evaluation reports, not just the obvious requirements of sensitivity and specificity but also such matters as storage and incubation temperatures, shelf-life and ease of use, among others.

### 3. LABORATORY ASPECTS OF HIV TESTING

#### 3.1 A brief overview

The diagnosis of HIV infection is usually made on the basis of the detection of antibodies to HIV. Similarly to the serological tests for detecting antibodies to HIV, those for other body fluids (including urine and saliva) 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. epidemiological surveillance. The earliest assays used purified HIV lysates (1<sup>st</sup> 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 (2<sup>nd</sup> generation). The so-called 3<sup>rd</sup> generation, or sandwich ELISAs, which use labeled antigen as conjugate, are extremely sensitive and have reduced the window period considerably. An improvement on this test has now been developed, 4<sup>th</sup> generation ELISAs, whereby HIV antigen and antibody may be detected simultaneously which has led to an even earlier detection of markers of HIV infection. For urine specimens, the ELISA assays that have been evaluated in this report have been developed as a variation of the 3<sup>rd</sup> generation ELISA test assay. The antibody to HIV in the urine samples is similarly captured by immobilised HIV antigen but detected using labelled anti-human IgG conjugates. The ELISA test format does require a certain level of laboratory expertise and equipment availability, and is best suited to situations where larger numbers of specimens will be tested at any one time.

A variety of simple, instrument-free initial tests for use with saliva samples are now available, including 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. Most of these tests can be performed in less than 10 minutes, and are therefore called simple/rapid (S/R) assays. The results are read visually. These tests are for use singly and are therefore 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.

Confirmation of the results obtained with alternative specimens may pose some challenges. For serum/plasma specimens 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. An FDA approved WB assay has been developed for use with urine samples and the results of the WHO evaluation of this assay are

reported here. Confirmation of results of specimens other than serum/plasma may also be made by testing of a serum specimen from the individual or, alternatively, by employing a validated testing strategy.

### **3.2 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 applied 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 that the correct results are communicated to the individuals seeking to know their HIV status. External Quality Assessment Schemes (EQAS) are available for HIV serology and it is recommended that laboratories participate in an EQAS, wherever possible, at least once a year to monitor their performance.

### **3.3 Safety**

The testing of urine and saliva specimens should be performed in such a manner as to minimize occupational risk, in the same way as that for serum and plasma specimens. 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 the *Laboratory Biosafety Manual, second edition*, World Health Organization, Geneva, 1993 (ISBN 92 4 154450 3) and the Communicable Diseases Surveillance and Response section of the WHO website, [www.who.int/emc](http://www.who.int/emc), where information on laboratory biosafety and transport of infectious substances may be found.

## **4. MATERIALS AND METHODS**

### **4.1 Assays (test kits) evaluated**

Test kits for these assessments were kindly provided to WHO free of charge by each of the manufacturers of the assays under evaluation. The manufacturers were invited to visit the site at which the assessments were to be conducted in order to provide any required training and to ensure that the assays were performed correctly by the laboratory staff carrying out the evaluation of their assays.

#### **Group 1: Urine assays**

##### **Calypte™ HIV-1 Urine EIA, Fc, (Calypte Biomedical Corporation)**

An indirect enzyme immunoassay which uses the recombinant gp160 envelope protein of HIV-1 to detect the presence of antibodies to HIV-1 in urine. The goat anti-human antibody, which forms part of the conjugate reagent, is raised against the Fc portion of human immunoglobulin.

##### **Calypte™ HIV-1 Urine EIA (Recombinant), (Calypte Biomedical Corporation)**

An indirect enzyme immunoassay which uses the recombinant gp160 envelope protein of HIV-1 to detect the presence of antibodies to HIV-1 in urine.

##### **Cambridge Biotech HIV-1 Urine Western Blot Kit, (Calypte Biomedical Corporation)**

An in-vitro qualitative assay for the detection and identification of antibodies to HIV-1 in urine.

## Group 2: Saliva assays

### **SMLX Technologies Diagnostics Test (SMLX Technologies)**

A rapid immunochromatographic screening test for the detection of antibodies to HIV-1 and HIV-2 in human saliva.

### **OraScreen™ HIV Rapid Test (Beacon Diagnostics)**

An in-vitro rapid flow-through immunochromatographic assay for the qualitative detection of antibodies to HIV in human oral fluid or saliva.

### **Salivax™-HIV (ImmunoScience Inc)**

An immunochromatographic assay for the detection of antibodies to HIV-1 and HIV-2 in human saliva.

## 4.2 Evaluation panels

### 4.2.1 WHO HIV urine evaluation panel

**Table A: WHO HIV Urine matched serum evaluation panel (Phase I)**

<i>HIV Positive</i>	<i>HIV Negative</i>	<i>Total</i>
<b>93</b>	<b>207</b>	<b>300</b>
31%	69%	100%

The phase I evaluations for urine specimens were carried out using a panel of 300 urine specimens (as shown in Table A). The panel contained 93 specimens positive for HIV. Fresh urine samples were collected into a sterile container and aliquoted aseptically into sterile polypropylene screw-capped tubes, without addition of a preservative, and stored at 2-8 °C. Before testing all urine specimens were centrifuged at 200 g for 1 minute and the supernatant transferred into a sterile container (note, this centrifugation step is not stated in the manufacturer's package insert instructions). Matched serum samples for the urine panel were obtained and tested for HIV antibody. The classification of the samples included in the urine panel are based on the results obtained for the serum specimens. All urine specimens that corresponded to the HIV positive matched serum samples were tested by the Cambridge Biotech HIV 1 Urine Western Blot assay. Any urine specimens that were falsely reactive in the Calypte™ HIV-1 Urine EIA, Fc and the Calypte™ HIV-1 Urine EIA (Recombinant) assays were also tested in the Cambridge Biotech HIV 1 Urine Western Blot assay. All serum specimens were stored in aliquots at -30°C and were thawed at least once and not more than twice.

#### 4.2.2 WHO HIV saliva evaluation panel

**Table B: WHO HIV Saliva matched serum evaluation panel (Phase I)**

<i>HIV-1 Positive</i>	<i>HIV Negative</i>	<i>Total</i>
75	147	222
33.8%	66.2%	100 %

The phase I evaluations for saliva specimens were carried out using a panel of 222 saliva specimens (as shown in Table B). The panel contained 75 specimens positive for HIV. Four saliva samples were collected from each patient using the device supplied with the kit for the OraScreen and SMLX assays and by collecting saliva into a plastic cup for the Salivax and the Wellcozyme HIV 1+2 GACELISA assays. The samples collected for the OraScreen HIV Rapid test, Salivax™-HIV and Wellcozyme HIV 1+2 GACELISA assays were stored for up to one week at 4°C and subsequently at –20°C to –25°C prior to testing. The samples collected for the SMLX Technologies Diagnostic Test assay were stored at –20°C to –25°C prior to testing. Matched serum samples for the saliva panel were obtained and tested for HIV (as described below). The classification of the samples included in the saliva panel are based on the results obtained for the serum specimens. The results of the assays under evaluation were also compared with the results obtained for the Wellcozyme HIV 1+2 GACELISA assay. All serum specimens were stored in aliquots at -30°C and were thawed at least once and not more than twice.

#### 4.2.3 WHO HIV matched serum panels

##### **Characterization of the WHO HIV matched serum panels**

The data obtained with the urine specimens and saliva specimens were compared to the combined outcome of the algorithm by which the respective matched serum specimens were categorised, as described in Section 6 Annexes.

To determine the HIV serological status, each parallel serum specimen included in the evaluation panel was first screened with two ELISAs; Vironostika HIV Uniform II plus O (Organon Teknika) and Enzygnost Anti-HIV 1/2 Plus (Dade Behring). Specimens found negative with both ELISAs were considered to be anti-HIV negative. All dual positive and discordant ELISA results were further characterised by INNO-LIA HIV Confirmation (Innogenetics). When results of the ELISAs and the INNO-LIA HIV Confirmation were all positive the specimen was considered anti-HIV positive.

When the initial ELISA results were discordant and the INNO-LIA HIV Confirmation result was negative, the specimen was considered anti-HIV negative. Similarly, when the initial ELISA results were discordant and the INNO-LIA HIV Confirmation result was positive, the

specimen was considered anti-HIV positive. Specimens that gave a result of indeterminate on the INNO-LIA Confirmation test were removed from the evaluation panel.

All reference assays were interpreted according to the instructions given by the manufacturer. The results of the reference assays were interpreted according to the manufacturers' instructions.

#### **4.2.4 Testing of WHO HIV urine and saliva panels**

##### **Testing of WHO HIV urine panel**

Each urine specimen was initially tested singly in both the Calypte™ HIV-1 Urine EIA, Fc and the Calypte™ HIV-1 Urine EIA (Recombinant) assays (Appendix 2). Those specimens that gave a negative result were classified as negative and no further testing of the specimen took place. Those samples that gave a reactive result on initial testing were repeat tested in duplicate in the same assay and those that were reactive in both tests or in one of the duplicate tests were subsequently tested in the Cambridge Biotech HIV 1 Urine Western Blot assay. All urine specimens for which the matched serum was classified as HIV positive, were tested in the Cambridge Biotech HIV 1 Urine Western Blot assay. The final result, ie positive, indeterminate or negative, for the urine ELISA assays and the Western blot assay were compared with the results for the matched serum specimens to determine the sensitivity and specificity values for the assays.

##### **Testing of WHO HIV saliva panel**

Four saliva samples were collected from each individual using a collection device provided, if appropriate, and tested once in each of the three assays under evaluation, OraScreen™ HIV Rapid Test, Salivax™-HIV and SMLX Technologies Diagnostic Test, and the reference assay, Wellcozyme HIV 1+2 GACELISA (Appendix 3). To determine the sensitivity and specificity values for the assays under evaluation, the results of the assays were compared with the results of the matched serum testing algorithm and also with the Wellcozyme HIV 1+2 GACELISA.

### **4.3 Laboratory testing**

All testing was performed according to the manufacturer's instructions. The specimens in the WHO HIV panels were randomized before testing and all assay runs were performed by one operator. Visual interpretations of the results of the urine Western blot and saliva simple/rapid test assays under evaluation were made independently by three technicians. When the three technicians interpreted the results differently from each other, the consensus was recorded as that interpretation which occurred 2 out of 3 times. In cases where all three interpretations were different, the result was recorded as indeterminate.

Specimens in the WHO HIV urine and saliva panels that gave initial results discordant from the reference results were retested in duplicate. Any result that occurred twice out of the three tests was recorded as the final result for that assay.

## 4.4 Analysis

### 4.4.1 Sensitivity, specificity, confidence limits (CL) and predictive values of HIV tests

The formula for calculation of sensitivity, specificity and predictive values is represented diagrammatically in Table C.

**Table C: Calculation of sensitivity, specificity and predictive values**

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

$$\begin{aligned} \text{Sensitivity} &= a/(a+c) & \text{Positive predictive value} &= a/(a+b) \\ \text{Specificity} &= d/(b+d) & \text{Negative predictive value} &= d/(c+d) \end{aligned}$$

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

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

NOTE: Samples that gave indeterminate results with the assays under evaluation were included in the analyses as a false result.

**Confidence limits (CL):** The 95% confidence limits are a means of determining whether observed differences in sensitivity or specificity between assays are significant or not. Exact 95% confidence limits for binomial proportions were calculated from the F-distribution (Armitage P. and Berry G. Statistical Methods in Medical Research, 2<sup>nd</sup> Edition. Blackwell Scientific Publications, Oxford, 1987, page 119).

#### **Predictive Values:**

The **positive predictive value (PPV)** is 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 (see Table C).
2. using the more precise formula which takes the prevalence of HIV in the population into account

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

The **negative predictive value (NPV)** is 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 (see Table C).
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 PPV). Thus, with increasing prevalence, the proportion of samples that are false-positive decreases; conversely, the likelihood that a person showing negative test results is truly uninfected (i.e. the NPV), decreases as prevalence increases. Therefore, as prevalence increases, so does the proportion of samples testing false-negative.

For calculating the positive and negative predictive values recorded in this report, the more precise formula at option 2 was used.

### **Delta Value:**

This value provides a means of comparing the efficacy of ELISA assays in separating the negative and positive anti-HIV sample 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 ( $\delta$ ) 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 specimen.

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

#### **4.4.2 *Inter-reader variability***

The inter-reader variability was calculated as the percentage of specimens for which initial test results were differently interpreted (i.e. positive, negative or indeterminate) by the independent readers.

#### **4.4.3 *Additional analyses***

The technical aspects of the assays under evaluation were assessed by the technician who performed the testing. These assessments, along with other selected assay characteristics, contributed to an overall appraisal of each assay's suitability for use in small laboratories. To enable comparison between assays, an arbitrary scoring system was used to rate specified assay characteristics.

## **5. ASSAY EVALUATIONS**

The results from the 6 test kits evaluated are presented in Tables 1-10. Tables 1 and 6 summarize the general characteristics of the assays. Results of the assays evaluated as compared to the reference tests are given in Tables 2 and 7; Tables 3 and 8 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, 5, 9a, 9b and 10. Explanatory notes are provided at the end of the assay evaluation tables.

## **ASSAY EVALUATIONS**

**Urine Specimens**  
**Saliva Specimens**

**Group 1: Urine assays**

**Table 1. General characteristics and operational aspects**

NAME	Calypte™ HIV-1 Urine EIA, Fc	Calypte™ HIV-1 Urine EIA (Recombinant)	Cambridge Biotech HIV-1 Urine Western Blot Kit
Company	Calypte™ Biomedical Corporation, California, USA	Calypte™ Biomedical Corporation, California, USA	Calypte™ Biomedical Corporation, California, USA
Assay type	ELISA	ELISA	Western blot
Antigen type	recombinant HIV-1 gp160	recombinant HIV-1 gp160	purified HIV-1 lysate
Solid phase	polystyrene microtitre wells	polystyrene microtitre wells	nitrocellulose strips
Specimen type	urine	urine	urine
Number of tests per kit (product code)	192 (700012) 480 (700013)	192 (700001) 480 (700000)	27 (98078)
Lot numbers evaluated (expiry date)	L25305 (6/02) L25306 (4/02)	L20002 (8/02) L16937 (4/02)	A2040U (31.01.02) A2012U (31.01.02)
Shelf life at ( °C)	18 months (2-8)	18 months (2-8)	15 months (2-8)
Volume of urine needed (µl) Final dilution of urine	200 none	200 none	1000 none
Total time to perform the assay: h. min. (number of specimens)	3.15 (91)	3.15 (91)	21.50 (24)
Wavelength (nm) single dual	405 405/630	405 405/630	visual reading
Price/test US\$	3.00 – 4.25	3.00 – 4.25	26.00

**Table 2. Comparison of the assays with reference tests**

NAME	Calypte™ HIV-1 Urine EIA, Fc	Calypte™ HIV-1 Urine EIA (Recombinant)	Cambridge Biotech HIV-1 Urine Western Blot Kit
Final Sensitivity % (95% CL)* n = 93	97.8 (92.4-99.7)	98.9 (94.2-100)	98.9 (94.2-100)
Initial Specificity % (95% CL)*	99.0 (96.6-99.9)	94.7 (90.7-97.3)	NA
Final Specificity % (95% CL)* n = 207	100 (98.2-100)	98.6 (95.8-99.7)	NA
Indeterminate results %	NA	NA	1.0
Initial inter-reader variability %	NA	NA	0.0
Delta values $\delta +$ $\delta -$	2.4 -4.4	2.28 -2.42	NA NA
PPV 0.01%	100	0.7	NA
6.0%	100	82.9	NA
NPV 0.01%	100	100	NA
6.0%	99.9	99.9	NA

\* 95 % Confidence Limits    NA = not applicable

**Table 3. Detailed operational aspects**

<b>NAME</b>	<b>Calypte™ HIV-1 Urine EIA, Fc</b>	<b>Calypte™ HIV-1 Urine EIA (Recombinant)</b>	<b>Cambridge Biotech HIV-1 Urine Western Blot Kit</b>
Dimension (cm) of kit: w-l-h	33.5-18-22.5 (192) 25.5-18-15 (480)	25 – 18 – 15 (192 & 480)	18.5 – 14 – 12.5
Storage conditions (°C)	2 - 8	2 - 8	2 – 8
Incubation temperature (°C)	37 ± 1	37 ± 1	20 – 28
Reading endpoint stability (h.min)	0.30	0.30	>24.00
Stability after dilution/ reconstitution/ opening at (°C)			
- antigen	expiry date (2-8)	expiry date (2-8)	expiry date (2-8)
- controls	expiry date (2-8)	expiry date (2-8)	expiry date (2-8)
- sample diluent	expiry date (2-8)	expiry date (2-8)	5 days (2-8)
- conjugate	use immediately	use immediately	use immediately
- substrate	use immediately	use immediately	use immediately
- wash buffer	30 days (15-30)	30 days (15-30)	3 months (20-28)
Number of specimens per run minimum – maximum	1 - 91	1 - 91	1-24
Number of controls per test run			
- negative	1	1	1
- cut-off/weak positive	0	0	1
- positive	1	1	1
- blank	0	0	0
internal control : reagent control	0	0	0
: sample addition control	0	0	0

**Table 3. (continued) Detailed operational aspects**

NAME	Calypte™ HIV-1 Urine EIA, Fc	Calypte™ HIV-1 Urine EIA (Recombinant)	Cambridge Biotech HIV-1 Urine Western Blot Kit
Estimated time to perform: 90 urine samples (h.min)	3.15	3.15	21.50 (24 samples)
- preparatory work	0.30	0.30	0.35
- incubation	2.30	2.30	20.00
- washing	0.10	0.10	0.55
- reading, interpretation	0.05	0.05	0.30
1 urine sample	2.50	2.50	21.20
Equipment needed but not provided in the kit: <sup>1</sup>			
- washer	+	+	-
- incubator (water-bath)	+	+	-
- spectrophotometric reader	+	+	-
- refrigerator (storage)	+	+	+
- agitator , rocker	-	-	+
- aspiration device	-	-	+
- automatic pipette (µl)	+	+	+
- multichannel pipette (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	±	±	-
- timer	+	+	+
Definition of positive results	OD of sample $\geq$ OD of cut off value	OD of sample $\geq$ OD of cut off value	Intensity of gp160 band $\geq$ that on low pos control strip
Definition of grey zone or indeterminate result	not applicable	not applicable	Intensity of gp160 band < that on low pos control strip

<sup>1</sup> + : 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**

NAME	Calypte™ HIV-1 Urine EIA, Fc	Calypte™ HIV-1 UrineEIA (Recombinant)	Cambridge Biotech HIV-1 Urine Western Blot Kit
Need to prepare:			
-antigen	1	1	1
-substrate	0	0	0
-wash solution	0	0	0
-conjugate	0	0	0
-predilution of urine	1	1	1
Stability after dilution/opening: (expiry date = 1; less = 0)			
-antigen	1	1	1
-controls	1	1	1
-sample diluent	1	1	0
-conjugate	0	0	0
-substrate	0	0	0
-wash buffer	0	0	0
-sufficient reagents	1	1	1
-wash (yes =0; no = 1)	1	1	1
Item needed but not provided in the kit:			
-reagent trough	1	1	1
-automatic /multichannel pipette	0	0	0
-dilution – tubes, rack/microtiter plate	1	1	1
-distilled or deionised water	0	0	0
-plate covers	1	1	1
-graduated pipette, cylinder	0	0	0
-sulfuric acid/sodium hydroxide	1	1	1
Total (out of possible 20)	11	11	10
Ease of performance:			
-less easy < 10	easy	easy	easy
-easy $10 \leq x \leq 15$			
-very easy > 15			

1 : positive rating: reagent needs no preparation; item provided in the kit. 0 : negative rating: reagent needs preparation; item not provided in the kit

**Table 5. Suitability for use in small laboratories**

NAME	Score	Calypte™ HIV-1 Urine EIA, Fc	Calypte™ HIV-1 Urine EIA (Recombinant)	Cambridge Biotech HIV-1 Urine Western Blot Kit
Sensitivity				
- 100%	5			
- 98 – 100%	3	1	3	3
- <98%	1			
Specificity				
- >98%	5			
- 95 – 98%	3	5	5	NT
- <95%	1			
Incubation temperature				
- room t°	3	1	1	3
- other than room t°	1			
Shelf-life				
- >1 year	3			
- ≥ 6 months ≤ 1 year	2	3	3	3
- < 6 months	1			
Storage at				
- ambient t° possible (opened kit)	5			
- ambient t° possible (unopened kit)	3	1	1	1
- 2-8 °C required	1			
Price per test (US\$)				
- ≤ 1.0	3			
- ≤ 2.0	2	1	1	1
- > 2.0	1			
Ease of performance				
- very easy	5			
- easy	3	3	3	3
- less easy	1			
Rapidity of performance:1 urine				
- < 10 min	3			
- 10 – 45 min	2	1	1	1
- > 45 min	1			
Washer/agitator				
- not needed	3			
- needed	1	1	1	1
Reading				
- visual: inter-reader variability ≤3%	5			
- visual: inter-reader variability >3%	3	1	1	5
- reading equipment	1			
<b>Total (out of possible 40)</b>		<b>18</b>	<b>20</b>	<b>21</b>
Suitability for use in small laboratories:				
- less suitable < 23		less suitable	less suitable	less suitable
- suitable 23 ≤ x ≤ 30				
- very suitable > 30				

## Group 2: Saliva assays

**Table 6. General characteristics and operational aspects**

NAME	SMLX Technologies Diagnostic test	OraScreen HIV Rapid Test	Salivax™-HIV	Wellcozyme HIV 1+2 GACELISA
Company	SMLX Technologies, Florida, USA	Beacon Diagnostics Inc, California, USA	ImmunoScience Inc, California, USA	Murex Biotech Ltd
Assay type	immunochromato-graphic	immunoline	immunofiltration	ELISA
Antigen type	synthetic peptides	viral lysate	synthetic peptides and recombinant proteins	synthetic peptide and recombinant proteins
Solid phase	membrane	dipstick	membrane	microwells
Specimen type	saliva	saliva	saliva	saliva
Number of tests per kit (product code)	20	50 (10001-50)	not available (41221) not available (41251)	96 (VK61) 480 (VK62)
Lot numbers evaluated (expiry date)	22503 (08/2000)	CK90007-E (1.4.2000) CK90012 (3.9.2000)	SAA01AN	F872810 (28.4.00)
Shelf life at ( °C)	18 months (4-28)	not available	12 months (2-40)	not available
Volume of saliva needed (µl) Final dilution of saliva	40 none	not stated none	2000 none	50 5/6
Total time to perform the assay: h. min. (number of specimens)	0.12-0.17 (1)	0.20 (1)	0.15-0.45 (1)	2.55 (96)
Reading	visual	visual	visual	spectrophotometer wavelength 490/690
Price/test US\$	not available	not available	not available	not available

**Table 7. Comparison of the assays with reference tests**

NAME	SMLX Technologies Diagnostic test		OraScreen HIV Rapid Test		Salivax™-HIV		Wellcozyme HIV 1+2 GACELISA
	Comparison of results with:						
	Matched serum samples	Wellcozyme HIV 1+2 GACELISA	Matched serum samples	Wellcozyme HIV 1+2 GACELISA	Matched serum samples	Wellcozyme HIV 1+2 GACELISA	Matched serum samples
Sensitivity % (95% CL)* Saliva n = 75	62.7 (51 – 74)	62.7 (51 – 74)	56.0 (44 – 68)	56.0 (44 – 68)	79.4 (67 – 89)	79.4 (67 – 89)	100 (95.2 – 100)
Specificity % (95% CL)* Saliva n = 147	74.8 (67 – 82)	75.2 (67 – 82)	98.6 (95 – 100)	98.6 (95 – 99)	96.0 (91 – 99)	96.0 (91 – 99)	99.0 (95 – 100)
Indeterminate results %	7.7		4.1		2.7		0
Initial inter-reader variability %	22.5		11.3		8.5		not applicable
PPV 0.01%	0.03		0.4		0.2		0.99
6.0%	13.7		72.3		55.9		86.5
NPV 0.01%	100		100		100		100
6.0%	96.9		97.2		98.6		100

\* 95 % Confidence Limits

**Table 8. Detailed operational aspects**

<b>NAME</b>	<b>SMLX Technologies Diagnostic test</b>	<b>OraScreen HIV Rapid Test</b>	<b>Salivax™-HIV</b>	<b>Wellcozyme HIV 1+2 GACELISA</b>
Dimension (cm) of kit: w-l-h	16.5 – 13 – 9	34 – 24.5 – 14	NA	18.2 – 21.2 – 11.2
Storage conditions (°C)	4 – 28	2 – 8	2 – 40	2 – 8
Incubation temperature (°C)	room temperature	15 – 25	room temperature	37
Reading endpoint stability (h.min)	0.15	not stated	not stated	0.15
Stability after dilution/ reconstitution/ opening at (°C)  - antigen - controls - sample diluent - conjugate - substrate - wash buffer	expiry date (4-28) not applicable not applicable not applicable not applicable expiry date (4-28)	expiry date (2-8) not applicable not applicable not applicable not applicable not applicable	expiry date (2-40) not applicable not applicable not applicable use immediately expiry date (2-40)	expiry date (2-8) expiry date (2-8) expiry date (2-8) expiry date (2-8) expiry date (2-8) expiry date (18-30)
Number of specimens per run minimum – maximum	1 – 5	1 – 4	1 – 3	1 - 91
Number of controls per test run  - negative - cut-off/weak positive - positive - blank  internal control : reagent control : sample addition control	not supplied	not supplied	not supplied	3  3 0 2 0  0 0

**Table 8. (continued) Detailed operational aspects**

<b>NAME</b>	<b>SMLX Technologies Diagnostic test</b>	<b>OraScreen HIV Rapid Test</b>	<b>Salivax™-HIV</b>	<b>Wellcozyme HIV 1+2 GACELISA</b>
Estimated time to perform one run: h. min (number of specimens)	0.12 – 0.17 (1)	0.20 (1)	0.15 (1)	2.35 (1) 2.55 (91)
Equipment needed but not provided in the kit: <sup>1</sup>				
- washer	-	-	-	+
- incubator (water-bath)	-	-	-	+
- spectrophotometric reader	-	-	-	+
- 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	-	-	-	±
- timer	+	+	+	+
Definition of positive results	Appearance of two dark pink or red spots in test device well	Appearance of a purple + in the HIV 1 and/or HIV 2 in the test area.	Distinct pink dots in test window at positions C and T	Sample OD ≥ 1
Definition of grey zone or indeterminate result	not applicable	not applicable	not applicable	not applicable

<sup>1</sup> + : 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 9a. Technician's appraisal of the test kit

NAME	Score	SMLX Technologies Diagnostic test	OraScreen HIV Rapid Test	Salivax™-HIV	Wellcozyme HIV 1+2 GACELISA
Number of steps in the test procedure:  -1-2 steps -3-5 steps ->5 steps	6 3 1	3	1	3	1
Clarity of kit instructions:  - good - needs improvement	2 1	1	2	2	2
Kit and reagent packaging and labelling:  - good - needs improvement	2 1	1	2	2	2
Total (out of possible 10)	10	5	5	7	5
Comments on the test kit		In a number of cases, insufficient saliva was collected by the device despite following the instructions.	Specimen collection procedure complex. Difficulty encountered with pushing saliva through collection receptacle	none	none

**Table 9b. Calculation of ease of performance**

NAME	SMLX Technologies Diagnostic test	OraScreen HIV Rapid Test	Salivax™-HIV	Wellcozyme HIV 1+2 GACELISA
Need to prepare: -antigen -substrate -wash solution -conjugate -predilution of specimen	1 1 1 1 1	1 1 1 1 1	1 1 1 0 1	1 0 0 0 1
Stability after dilution/opening: (expiry date = 1; less = 0) -antigen -controls -sample diluent -conjugate -substrate -wash buffer -sufficient reagents -wash (yes =0; no = 1)	1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1	1 1 1 0 1 1 1 1	1 1 1 0 0 1 1 1
Item needed but not provided in the kit: -reagent trough -automatic /multichannel pipette -dilution – tubes, rack/microtiter plate -distilled or deionised water -plate covers -graduated pipette, cylinder -sulfuric acid/sodium hydroxide	1 1 1 1 1 1 1	1 1 1 1 1 1 1	1 1 1 1 1 1 1	1 0 1 0 1 0 1
Technician's appraisal of the test kit <sup>3</sup> (rating out of 10)	5	5	7	5
Total (out of possible 30)	25	25	25	17
Ease of performance: -less easy < 20 -easy 20 ≤ x ≤ 25 -very easy > 25	easy	easy	easy	less easy

1 : positive rating: reagent needs no preparation; item provided in the kit. 0 : negative rating: reagent needs preparation; item not provided in the kit <sup>3</sup>see table 9a

**Table 10. Technical suitability for use in small laboratories**

NAME	Score	SMLX Technologies Diagnostic test	OraScreen HIV Rapid Test	Salivax™-HIV	Wellcozyme HIV 1+2 GACELISA
Sensitivity					
- 100%	5				
- 98 – 100%	3	0	0	0	5
- <98%	0				
Specificity					
- >98%	5				
- 95 – 98%	3	0	5	3	5
- <95%	0				
Incubation temperature					
- room t°	3	3	3	3	1
- other than room t°	1				
Shelf-life					
- >1 year	3				
- ≥ 6 months ≤ 1 year	2	3	NA	2	NA
- < 6 months	1				
Storage at					
- ambient t° possible (opened kit)	5				
- ambient t° possible (unopened kit)	2	5	5	5	1
- 2-8 °C required	1				
Price per test (US\$)					
- ≤ 1.0	3				
- ≤ 2.0	2	NA	NA	NA	NA
- > 2.0	1				
Ease of performance					
- very easy	5				
- easy	3	3	3	3	1
- less easy	1				
Rapidity of performance: 1 specimen					
- < 10 min	3				
- 10 – 30 min	2	3	2	2	1
- > 30 min	1				
Washer/agitator					
- not needed	3	3	3	3	1
- needed	1				
Reading					
- visual: inter-reader variability ≤ 3%	5				
: inter-reader variability > 3%	3	3	3	3	1
- reading equipment	1				
Total (out of possible 40)		24 – 26	26 – 30	25 – 27	18 - 22
Suitability for use in small laboratories:					
- less suitable < 23		suitable	suitable	suitable	less suitable
- suitable 23 ≤ x ≤ 30					
- very suitable > 30					

**Explanatory notes for Tables 1-10**

**Tables 1 and 6**

**General characteristics and operational aspects of the assays.**

Specimen type	The Cambridge Biotech Western blot assay may be used with urine or serum/plasma specimens, although the protocol is different for the two specimen types.
Shelf life at (°C)	is the shelf life from manufacture at the storage temperature recommended by the manufacturer
Final dilution of the specimen	is the dilution of the sample in the test format, e.g. 10µl specimen 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. <ul style="list-style-type: none"> <li>- simple/rapid assays designed for individual tests: the number which can be run simultaneously</li> <li>- ELISA tests: the number of test samples that may be run on a whole microtitre plate</li> <li>- line assays: the number of samples that can be run on a complete kit</li> </ul>
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. The prices stated are catalogue prices and therefore indicative.

**Tables 2 and 7**

**Comparison of the results of the assays with reference tests**

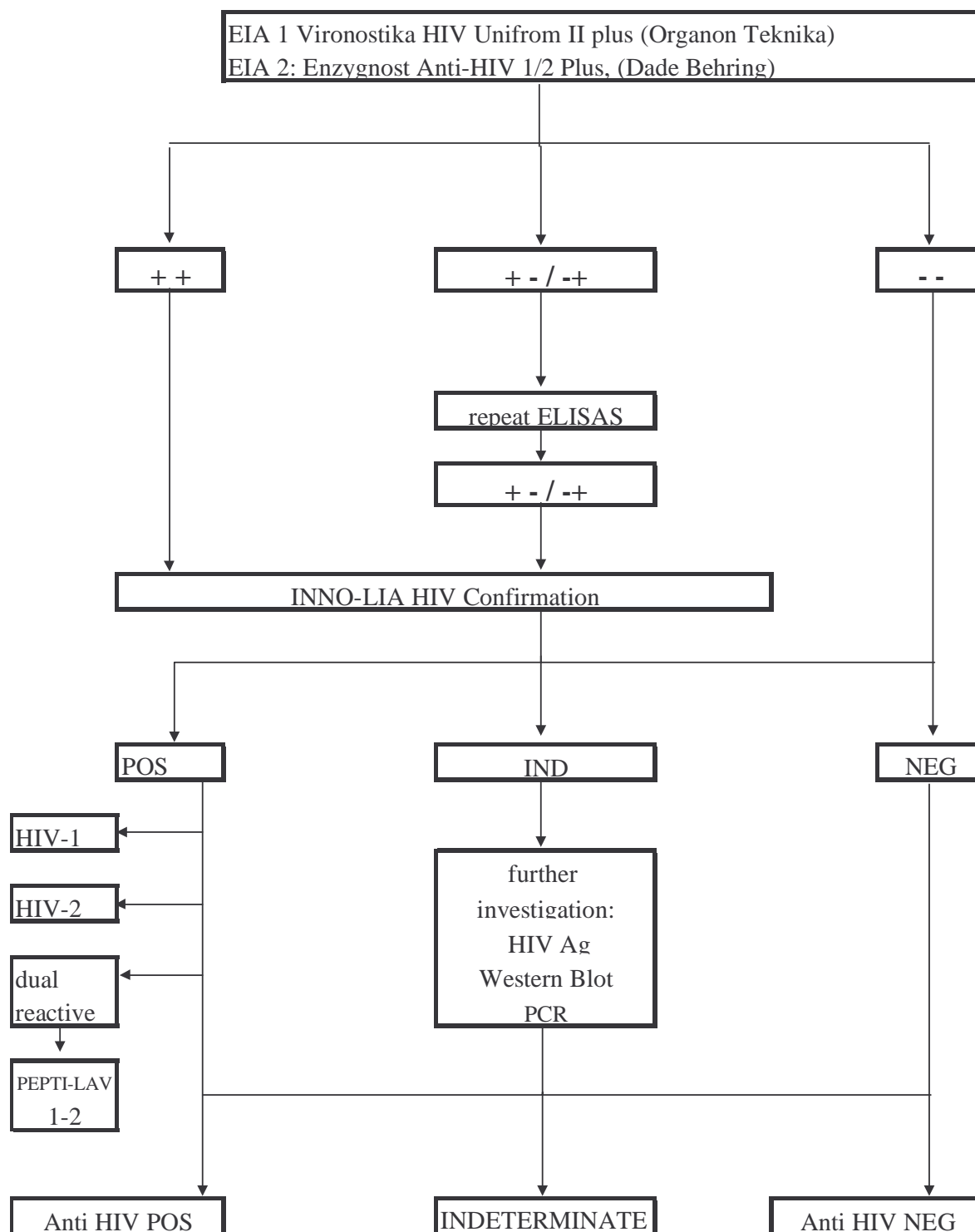
Sensitivity	calculated as described on page 8 of this document.
Specificity	calculated as described on page 8 of this document.
95% Confidence limits (CL)	calculated as described on page 8 of this document
PPV and NPV	calculated as described on pages 8 and 9 of this document
Delta value	calculated as described on page 9 of this document
Indeterminate results	Simple/rapid assays - test results which could not be interpreted as clearly positive or negative were considered indeterminate. ELISA assays - indeterminate results (ie results in the grey zone) are not applicable for the Calypte ELISA Urine assays Western blot - indeterminate test results were interpreted according to the manufacturer's instructions as described in Table 3
Inter-reader variability	calculated as described on page 10 of this document.

### Explanatory notes for Tables 1-10

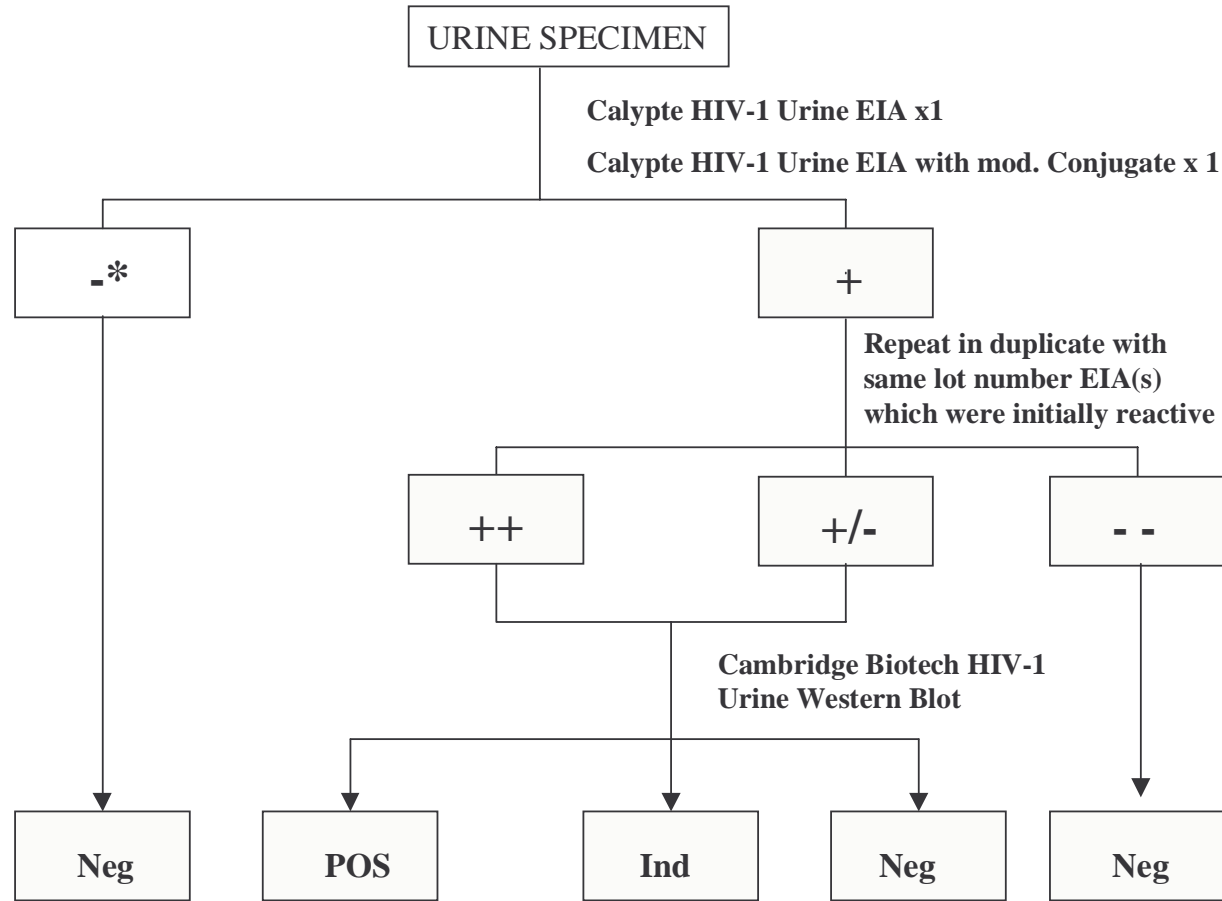
<b>Tables 3 and 8</b>	<b>Detailed operational aspects of the assay</b>
minimum - maximum number of samples	<ul style="list-style-type: none"> <li>- minimum number = 1 sample in addition to the required controls</li> <li>- maximum number = the maximum number of samples in addition to the required controls which can be simultaneously tested within the limits of the assay procedure.</li> </ul>
Number of controls per test run Internal control	<p>The Calypte Urine ELISA assays contain positive and negative controls but do not contain a sample addition colour change control.</p> <p>The Cambridge Biotech Western Blot Kit contains positive, cut-off and negative controls but does not contain a sample or reagent addition control on the nitrocellulose strips.</p> <p>The following assay has control lines on the test device which check that sample has been added, the procedure has been followed and reagents function correctly: OraScreen HIV Rapid Test.</p> <p>The following assays have a control line or spot on the test device which checks that the procedure has been followed and reagents function correctly: SMLX Technologies Diagnostic Test and Salivax™-HIV.</p> <p>The number of controls shows the number of replicates of each control required for each assay run. For the internal controls the reagent control is normally shown by the addition of a coloured reagent and the sample addition control shows a colour change.</p>
Definition of positive results	a sample is interpreted as positive according to the criteria set by the manufacturer and summarized in the table
<b>Tables 4, 9a and 9b</b>	<b>Calculation of ease of performance of the assay</b>
	The criteria for this calculation are given in the respective tables.
<b>Tables 5 and 10</b>	<b>Suitability of the assay for use in small laboratories</b>
	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.

## 6. ANNEXES

### ANNEX 1. Algorithm for characterization of the WHO HIV matched serum panels



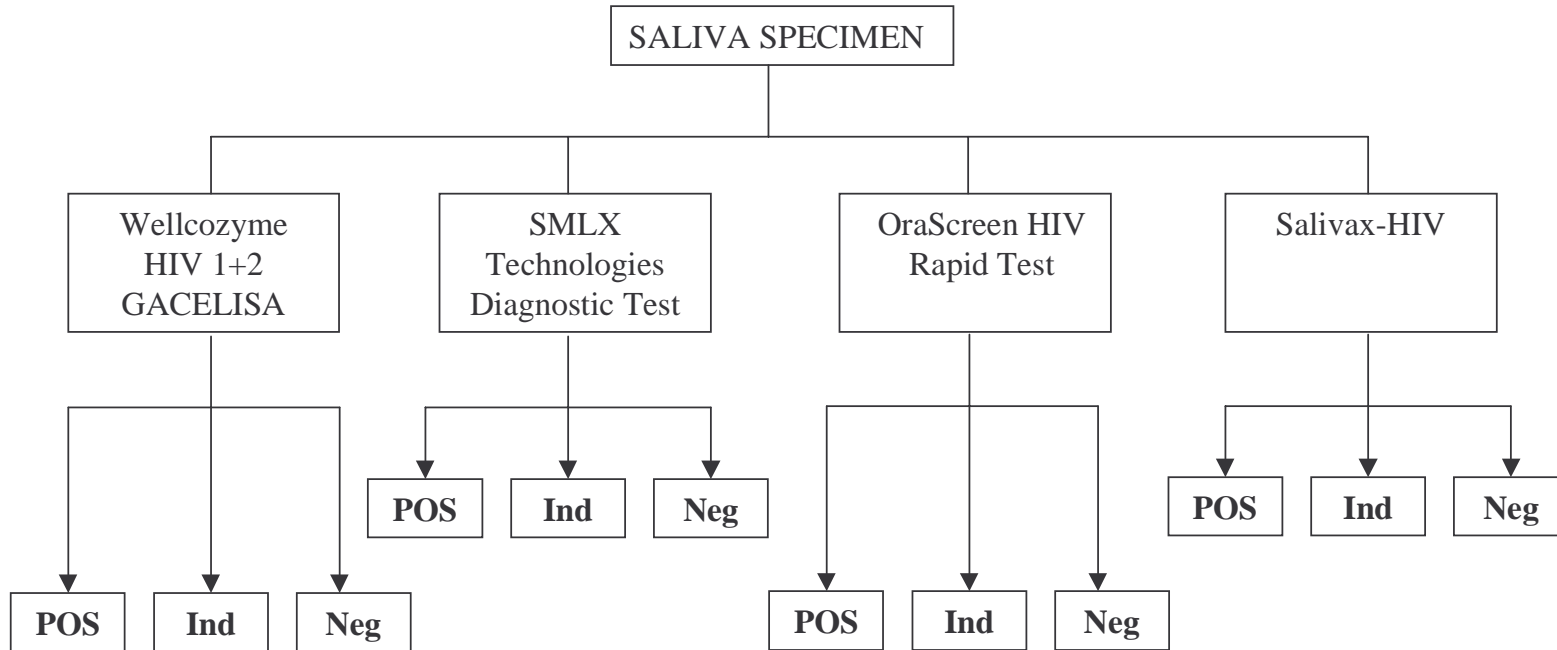
**ANNEX 2. Testing algorithm for evaluation of HIV 1 urine assay**



All discrepant results, ie -ve urine but +ve serum or +ve urine but -ve serum: urine specimen repeated in duplicate with same lot number of urine ELISA. If still discrepant, retested in duplicate with other lot number of urine ELISA.

\*One HIV +ve urine specimen was negative by both ELISA kits. The specimen was indeterminate in the Western blot assay.

**Annex 3: Testing algorithm for evaluation of saliva assays**



Four saliva specimens were collected from each patient and tested once only in each saliva assay. The results obtained for all assays were compared to the results obtained with the serum specimens and the results for the assays under evaluation were also compared with the results of the Wellcozyme HIV 1+2 GACELISA.

#### **ANNEX 4. List of assay manufacturers'/distributors' addresses**

**Calypte™ Biomedical Corporation**, 1265 Harbor Bay Parkway, Alameda, Ca 94502, USA.

Tel: +1 510 749 5153, Fax: +1 510 814 8408; Website: [www.calypte.com](http://www.calypte.com)

**Beacon Diagnostics Inc.**, 21343 Cabot Boulevard, Hayward, Ca 94545, USA.

Tel: +1 510 782 2444; Fax: +1 510 782 1951.

**ImmunoScience Inc.**, 7066-D Commerce Circle, Pleasanton, Ca 94588 USA.

Tel: +1 925 460 8111; Fax +1 925 460 8120; Website: [www.immunoscience.com](http://www.immunoscience.com)

**Simplex Medical Systems Inc.**, 122 Durea Lane, Nanuet, New York 10954, USA.

Tel: +1 914 6624 2520; Fax +1 914 624 0140.

## **7. ADDITIONAL READING**

Additional reading may be obtained by visiting the BCT section of the WHO website at [www.who.int/bct](http://www.who.int/bct) and follow the links to Key Initiatives, HIV Diagnostics. In addition to general information on diagnostics, assay evaluation reports for HIV, HCV and HBV are available as well as details for the WHO HIV Test Kit Bulk Procurement Scheme.

## **8. ACKNOWLEDGEMENTS**

We should like to thank the Institute of Tropical Medicine, Antwerp, Belgium for supplying the saliva, urine and serum samples that constituted the evaluation panels for this evaluation.

We acknowledge the four companies, Calypte™ Biomedical Corporation, Beacon Diagnostics Inc., ImmunoScience Inc. and Simplex Medical Systems, for supplying the test kits free of charge.

## **9. NOTE**

The OraQuick® HIV-1/2 – Rapid HIV-1/2 Antibody Test, OraSure Technologies Inc., has been produced for use with oral fluid, whole blood and serum/plasma specimens, but was not included in this evaluation of HIV kits for saliva specimens. The kit has, however, been evaluated on serum/plasma specimens by WHO and the results will be published in a report at a later date and also posted on the BCT section of the WHO website ([www.who.int/bct](http://www.who.int/bct)).