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Comparative evaluation of eight commercial enzyme linked immunosorbent assays and 14 simple assays for detection of antibodies to HIV

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Abstract

To evaluate the performance of 22 assays for the detection of antibodies to HIV. Twenty-two assays for the combined detection of antibodies to HIV-1 and HIV-2, were evaluated on the same panel of serum specimens of diverse origin. Eight of the assays were ELISAs and the remaining 14 were simple, assays read visually. The specimen panel consisted of anti-HIV positive and negative samples from Africa ($n = 192$), Europe ($n = 206$), Asia ($n = 99$) and Latin America ($n = 98$). In addition to estimations of sensitivity and specificity, the assays were assessed, using a novel scoring system, for their ease of performance and for their suitability for use in small laboratories and clinics. The sensitivities of the assays in terms of seroconversion were assessed using series of specimens collected from nine individuals undergoing seroconversion. Eight ELISAs and eight of 14 simple assays had sensitivities and specificities of > 99 and 95%, respectively. The results of these evaluations will be of assistance to those responsible for the selection of appropriate anti-HIV assays according to laboratory circumstances, the purpose of the testing and the population being tested.

Keywords: HIV-1; HIV-2; ELISA; Sensitivity; Specificity; Evaluation

1. Introduction

The accurate and cost-effective detection of antibodies to human immunodeficiency virus (HIV) is vital to assuring the safety of the global supply of blood and in aiding the control of the spread of HIV infection amongst those individuals at risk. As well as tests needing to be accurate, their formats need to be appropriate for the diverse set of testing environments encountered from those in industrialized cities to those in regional areas of resource-poor countries. Manufacturers of diagnostic test reagents have attempted to provide the wide range of assays necessary to fulfil these diverse needs.

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The screening tests used most widely for detection of antibodies to HIV are enzyme linked immunosorbent assays (ELISAs), their format being most appropriate for screening large numbers of specimens. The earliest (first generation) ELISAs used purified HIV lysates to provide the antigens for the solid phase. Assays based on recombinant proteins and/or synthetic peptides (second generation) became rapidly available, and facilitated the production of combined HIV-1/HIV-2 assays. The third generation or sandwich ELISAs, which use labeled antigen as conjugate are extremely sensitive and have reduced considerably the time known as the window period between infection and appearance of detectable antibodies. Tests based on the simultaneous detection of antigen and antibodies (fourth generation) have recently become available and are purported to reduce the window period even further (Brust et al., 2000; van Binsbergen et al., 1999; Weber et al., 1998).

In addition to ELISAs, a variety of simple instrument-free HIV screening tests is available, including agglutination, immunofiltration (flow through), immunochromatographic (lateral flow), and dipstick tests using a variety of media as the solid phase such as gelatin particles, erythrocytes and immunochromatographic membranes. These tests are most suitable for use in laboratories that have limited facilities and process low numbers of specimens daily.

The present report presents the results of assessments of the major operational characteristics of 22 commercial HIV antibody assays that were evaluated between March 1994 and January 1997. All assays tested are still available commercially. Among the characteristics assessed were the sensitivity of the assay and specificity on a specimen panel containing African, European, Asian and Latin American sera, the variability of result interpretations between different readers in the simple assays, the determination of delta values for ELISAs, the relative performance on seroconversion panels and the ease of performance and suitability for use in small laboratories or clinics.

2. Materials and methods

2.1. Specimens

A panel of 595 serum specimens was tested with 18 of the 22 assays under evaluation. As the evaluations proceeded, 13 samples became progressively exhausted so that, in the case of four of the assays evaluated, only 582–594 specimens were tested as shown in Table 3. Thirty-two percentage of the specimens in the panel were collected from African, 17 from Asian, 35 from European and 16% from Latin American individuals. The numbers of anti-HIV positive and negative specimens from each region are shown in Table 1. All specimens were stored frozen (−20 °C) in aliquots of 120 μl and thawed at least once and twice at most. The specimens in the panel were characterized using one of two ELISAs either the Vironostika Mixt (Organon Teknika) or the HIV-1/HIV-2 third generation EIA (Abbott Diagnostics). In addition, all samples were tested by the HIV blot version 1.2

Table 1
Origin of anti-HIV positive and anti-HIV negative specimens tested on each of 22 anti-HIV assays under evaluation

Specimen origin	Anti-HIV positive (*n*)		Anti-HIV negative (*n*)	Total
	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

n, Number.

(Genelabs Diagnostics) and the NEW LAV BLOT II (Sanofi Diagnostic Pasteur). All specimens positive or indeterminate with both western blots (WB) were tested using the Pepti-LAV 1 + 2 (Sanofi Diagnostic Pasteur). The last assay is designed to differentiate between reactivity to HIV-1 and HIV-2. At the completion of the characterization, any specimen having a final indeterminate status was removed from the panel.

Western blot reactivity was interpreted according to WHO criteria (Proposed WHO criteria, 1990). An HIV-1 or HIV-2 Western Blot result was considered positive when two of three *env* bands (*env* precursor, external and transmembrane glycoproteins) with or without *gag* and/or *pol* bands, were present. A Western Blot result was considered negative when no HIV specific bands were present and indeterminate when it showed any band pattern not considered positive or negative. The results of the Pepti-Lav 1 + 2 (Sanofi Diagnostic Pasteur) were interpreted according to the instructions of the manufacturer.

Eight commercially available anti-HIV-1 subtype B seroconversion panels (PRB 904, PRB 909, PRB 911, PRB 912, PRB 914, PRB 916, PRB 917 and PRB 918, Boston Biomedica Incorporated (BBI)) were tested by each of the assays under evaluation. Each seroconversion panel comprised a series of up to ten specimens collected from an individual recently infected with HIV-1 and undergoing seroconversion.

Finally, a limited number (7) of specimens collected from individuals infected with HIV-1 subtype O (Janssens et al., 1995) became available towards the end of the evaluations. These specimens were only tested by six of the assays under evaluation (Table 2).

2.2. Assays

Of the 22 anti-HIV assays evaluated, eight were ELISAs and 14 were simple assays. All were designed to detect antibodies to HIV-1 and HIV-2. The names of the assays, the antigens included and the type of solid phase used for each assay are included in Table 2.

2.3. Laboratory testing

The assays were carried out according to the instructions of the manufacturer. Usually, one person carried out all the testing for a single assay. Tests on specimens reactive initially were repeated once or, if discrepant (i.e. different from the result assigned following characterization) were repeated twice. In the latter cases, two out of three results which agreed determined the overall test outcome. Samples belonging to the eight seroconversion panels were tested once by each assay under evaluation i.e. reactive results were not repeated.

The ELISA results were calculated and interpreted according to the instructions of the manufacturer. For two ELISAs (indicated in Table 4) the instructions specified an optical density (OD) range around the assay cut-off ('grey zone') which required repeat testing of specimens with initial results falling into it. Repeat test results which remained in the grey zone were considered indeterminate.

Test results from simple HIV antibody assays, where results were interpreted visually, were read independently by three different persons. When there was variation between the three readers, the reading which occurred two out of three times was recorded as the consensus reading. In cases where all three readers disagreed, the test result was considered indeterminate.

3. Data analysis

3.1. Sensitivity

Test sensitivity was calculated as the number of confirmed anti-HIV positive specimens (HIV-1, and HIV-2 where applicable) detected as reactive by the HIV antibody assay under evaluation, divided by the total number of confirmed anti-HIV positive specimens tested, multiplied by 100.

3.2. Specificity

Test specificity was calculated as the number of confirmed anti-HIV negative specimens correctly

Table 2
Characteristics of the 22 commercial HIV antibody assay kits together with those of the reference tests

Number	Test name	Manufacturer	Test type[a]	Antigen type[b]	Solid phase[c]	Cost per test (US$)[d]
ELISAs						
1	INNOTEST HIV-1/HIV-2 Ab.s.p	Innogenetics	i.e.	SP	U v8	1.5
2	UBI HIV 1/2 EIA	United Biomedical Inc	i.e.	SP	U v16	1.0
3	ETI-AB-HIV-1/2 K	Sorin Biomedica	i.e.	RP/SP	U v8	1.5
4	HIV EIA	Labsystems OY	i.e.	SP	U v8	0.6–0.9
5	IMx HIV-1/HIV-2 third generation Plus	Abbott GmbH	m.e.	RP/SP	microparticles	3.0
6	Enzygnost Anti-HIV 1/2 Plus	Behringwerke AG	s.e.	RP/SP	U v8	1.0–1.1
7	Vironostika HIV Uni-Form II *plus*[e]	Organon Teknika	s.e.	RP/SP	U v8	1.5
8	GENSCREEN HIV 1/2[e]	Sanofi Diagnostics Pasteur	s.e.	RP/SP	U v8	1.5
Simple assays						
9	CAPILLUS HIV-1/HIV-2	Cambridge Biotech Ltd	Aggl	RP	Latex beads	2.2–2.8
10	IMMUNO COMB II BISPOT HIV 1 and 2	PBS Orgenics	Dot	SP	Plastic card	1.7
11	HIV 1 and 2 RAPID SERO TEST	Diatech Diagnostica	FT	RP	Membrane	1.9
12	DIPSTICK-HIV 1+2	Immuno Chemical Labs	Dot	SP	Polystyrene comb	0.5
13	SPAN COMBAIDS VISUAL	Span Diagnostics	Dot	RP/SP	Polystyrene comb	0.5
14	HIVCHEK System 3 Test Kit	Ortho Diagnostic Systems	FT	RP/SP	Membrane	4.4
15	HIV TRI DOT	J Mitra and Co Ltd	FT	RP	Membrane	2.0
16	EasiDot HIV/EasiSpot HIV[e]	Nubenco Diagnostics	FT	HL/RP	Membrane	
17	AccuSpot HIV-1 and 2	Specialty BioSystems	FT	RP	Membrane	2.5
18	BIONOR HIV 1 and 2	Bionor A/S	r.e.	SP	Magnetic particles	2.5
19	HIV 1 and 2 DoubleCheck[e]	Orgenics	LFT	RP/SP	Membrane	2.0
20	SEROCARD HIV	Trinity Biotech Plc	LFT	SP	Membrane	4.0
21	SERO°STRIP HIV-1/2	Saliva Diagnostics Systems	CP	SP	Chromatographic strip	1.5
22	HIVSPOT-123[e]	Genelabs Diagnostics	FT	RP	Membrane	3.5
Reference tests						
	Western Blot HIV-1	Genelabs Diagnostics	Blot	HL	Nitrocellulose strip	25.4
	NEW LAV BLOT II	Sanofi Diagnostics Pasteur	Blot	HL	Nitrocellulose strip	23.1
	PEPTI-LAV 1-2	Sanofi Diagnostics Pasteur	Blot	SP	Membrane	31.1

[a] s.e., sandwich ELISA; i.e., indirect ELISA; m.e., microparticle ELISA; c.e., antibody capture ELISA; Aggl., agglutination assay; dot, immunodot assay; FT, flow through; r.e., rapid ELISA; LFT, lateral flow through; CP, capillary flow; Blot, immunoblot assay.

[b] RP, recombinant proteins; SP, synthetic peptides; HL, HIV lysate.

[c] U, microplate with U-bottomed wells; U, microplate with flat bottomed wells; h12, horizontal strips composed of 12 wells; v16, v8, vertical strips composed of 16 and eight wells, respectively.

[d] Prices quoted are those in effect at the time of the evaluation.

[e] Tested on HIV-1 subtype O sera.

identified as negative by the HIV antibody assay under evaluation (following repeat testing of initially reactive specimens where necessary), divided by the total number of confirmed anti-HIV negative specimens tested, multiplied by 100.

3.3. Confidence limits

The 95% confidence limits (CL) of the estimated sensitivity and specificity were calculated using the formula: $P \pm 1.96\sqrt{P(1-P)/N}$, where P is the sensitivity or specificity and N is the number of specimens tested. For sensitivities and specificities of 100% a value corresponding to 99.9% was substituted for P in this equation.

3.4. Delta values

The delta values for the anti-HIV positive and anti-HIV negative sample populations were calculated by dividing the mean of the $\log_{10}$ OD ratios by the standard deviation of the log transformed OD ratios, for each population (Crofts et al., 1988; Maskill et al., 1988). OD ratios were calculated by dividing each reading by the relevant assay cut-off value. Only one OD ratio for each specimen could be included in the antibody positive and antibody negative data sets for delta calculation. The OD ratio derived from the initial test result was included provided that the result was concordant with the reference result. If the result was discrepant, and hence was repeated twice, the OD ratio included for delta calculation was any one of those whose interpretations agreed with each other. While delta values could calculated for results from the ELISAs under evaluation they cannot be calculated for assays with non-quantitative readings.

3.5. Positive predictive values (PPV)

The PPV of each assay was determined for two hypothetical populations where the prevalences of HIV infection were 0.01 and 6%, respectively. PPV was calculated using the formula:

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

(Operational characteristics of commercially available assays to determine antibodies to HIV-1 and/or HIV-2 in human sera, WHO-UNAIDS Report, 1998).

The medians of the PPVs estimated for ELISAs and simple assays for each of the populations were compared using the Mann–Whitney two-sample rank test.

3.6. Inter-reader variability

An estimation of inter reader variability was only relevant to the visually read assays and was calculated as the percentage of specimens for which interpretation of initial test results varied between the three readers.

3.7. Sensitivity in seroconversion panels

The results obtained with early seroconversion panels using the assays under evaluation were compared with those obtained using the Abbott HIV-1/HIV-2 third generation EIA, the assay designated arbitrarily as the reference for determination of relative sensitivity in these panels. For each seroconversion series (panel) the date of collection of the first specimen in the sample sequence to become reactive with Abbott HIV-1/HIV-2 third generation EIA was considered day '0'. The difference in the number of days between day '0' and the specimen collection date of the first specimen which reacted with each of the assays under evaluation was determined. For example, if an assay became reactive 5 days earlier in a series than Abbott HIV-1/HIV-2 third generation EIA, the value assigned for that series in that assay was -5. Similarly, if an assay became reactive 3 days later than Abbott HIV-1/HIV-2 third generation EIA, the value assigned was $+3$. The assigned values over the eight seroconversion series were averaged to determine an assay's relative seroconversion sensitivity.

3.8. Operational characteristics

A novel scoring system was developed to enable the comparison of the assays under evaluation with respect to ease of performance and suitability for use in small laboratories or clinics. For each HIV antibody assay under evaluation, ease of performance was assessed by assigning a score of + 1 for each of the following 20 conditions that was fulfilled.

If preparation of antigen, substrate, wash solution, or conjugate was not required (conditions 1–4); if predilution of serum prior to its addition to the microtitre well or testing device was not required (condition 5); if the antigen, controls, sample diluent, conjugate, substrate and wash solution remained stable after opening, reconstitution or dilution until the kit expiry date (conditions 6–11); if sufficient reagents were available in the kit for all tests (condition 12); if washing procedures were not necessary (condition 13); if reagent troughs, automatic/multichannel pipettes, tubes/racks/microtitre plates for dilution, distilled/demonized water, plate covers, graduated pipettes/cylinder, sulfuric acid/sodium hydroxide were not necessary to perform the test (conditions 14–20). A test was defined as very easy (VE), easy (E), or less easy (LE) to perform if scores of ≥ 15, ≥ 10, or < 10 were obtained, respectively. The appropriateness of the assays under evaluation for use in centres having minimum sample throughput and/or limited facilities or personnel was assessed by applying a further arbitrary score of 1–3 (shown in parenthesis) to specific assay characteristics:

If the sensitivity was 100% (3), 98–100% (2) or < 98% (1); if the specificity was > 98% (3), 95–98% (2) or < 95% (1); if the test could be conducted at ambient temperature (3) or required incubation at a controlled temperature (1); if the test had a shelf-life > 1 year (3), ≥ 6 months but < 1 year (2) or < 6 months (1); if storage of the kit at ambient temperature was possible (3) or a refrigerator (2–8 °C) was required (1); if the price per test was US$ 1.0 (3), US$ 1.0–2.0 (2), or > US$ 2.0 (1); if the assay performance was very easy (3), easy (2), or less easy (1); if the time necessary to test a single serum was < 10 min (3), 10–45 min (2), or > 45 min (1); if a washer/agitator was not needed (3), or needed (1); if visual reading of the test was possible (3) or reading equipment was required (1).

Using the scoring system described, a maximum score of 27 could be obtained; a test was defined very suitable (VS) if it had a total score ≥ 25, suitable (S) if its score was < 25 and ≥ 20, or less suitable (LS) if its score was < 20.

4. Results and discussion

4.1. Sensitivity, specificity and delta values

The sensitivity, specificity and 95% CL for the 22 HIV assays evaluated are summarized in Table 3. Also presented are the delta values calculated for the ELISAs.

Seven ELISAs and eight simple assays were 100% sensitive. Overall the specificity varied from 71.3 to 100.0%. Applying minimum WHO criteria for sensitivity and specificity of HIV antibody tests (> 99, 95%, respectively; Revised recommendations for the selection and use of HIV antibody test. WHO-UNAIDS Revised version, 1998), all eight ELISAs and ten of 14 simple assays fulfil the minimum standards for sensitivity; all ELISAs and 11 of 14 simple assays fulfil the minimum standards for specificity. Eight ELISAs and eight simple assays meet the minimum criteria for both sensitivity and specificity. Two ELISAs, UBI HIV 1/2 EIA (No. 2) and Vironostika HIV Uni-Form II *plus* O (No. 7) were found to be 100% sensitive and 100% specific in this limited evaluation.

Of the five assays (indicated in Table 2) whose evaluation included testing of 7 HIV-1 subtype O specimens, all identified successfully as positive subtype O specimens. These assays included two ELISAs and three simple assays: Vironostika HIV Uni-Form II *plus* O (No. 7), Genscreen HIV 1/2 (No. 8), EasiDot HIV/EasiSpot HIV (No. 16), HIV 1 and 2 DoubleCheck (No. 19) and HIVSPOT-123 (No. 22). More extensive evaluations of assays using HIV-1 subtype O specimens have been reported previously (Thorstensson et al., 1998; Apetrei et al., 1996).

Table 3
The final sensitivity and specificity with 95% confidence limits of the 22 HIV assays together with the delta values of the eight ELISAs

Assay number	Sensitivity (%)	Specificity (%)	Delta values	
			WB-positive sera	WB-negative sera
ELISAs				
1	100.0 (99.6–100.0)	98.8 (97.6–100.0)	14.0	3.8
2	100.0 (99.6–100.0)	100.0 (99.7–100.0)	10.8	3.2
3	100.0 (99.6–100.0)	98.8 (97.6–100.0)	10.4	2.5
4	100.0 (99.6–100.0)	99.4 (98.6–100.0)	14.2	3.9
5	99.6 (98.8–100.0)	97.9 (96.4–99.4)	9.1	2.1
6	100.0 (99.6–100.0)	99.7 (99.1–100.0)	19.1	6.6
7[a]	100.0 (99.6–100.0)	100.0 (99.7–100.0)	17.2	4.1
8[b]	100.0 (99.6–100.0)	98.5 (97.2–99.8)	22.8	2.7
Simple assays				
9	100.0 (99.6–100.0)	98.8 (97.6–100.0)	n.a.	n.a.
10	100.0 (99.6–100.0)	99.7 (99.1–100.0)	n.a.	n.a.
11	97.7 (95.9–99.5)	96.7 (94.8–98.6)	n.a.	n.a.
12	100.0 (99.6–100.0)	98.2 (96.8–99.6)	n.a.	n.a.
13	100.0 (99.6–100.0)	88.0 (84.5–91.5)	n.a.	n.a.
14	99.6 (98.8–100.0)	99.7 (99.1–100.0)	n.a.	n.a.
15	99.6 (98.8–100.0)	99.7 (99.1–100.0)	n.a.	n.a.
16[c]	95.3 (92.7–97.9)	71.3 (66.4–76.2)	n.a.	n.a.
17	100.0 (99.6–100.0)	86.3 (82.6–90.0)	n.a.	n.a.
18	100.0 (99.6–100.0)	98.8 (97.6–100.0)	n.a.	n.a.
19[d]	100.0 (99.6–100.0)	99.4 (98.6–100.0)	n.a.	n.a.
20	100.0 (99.6–100.0)	97.9 (96.4–99.4)	n.a.	n.a.
21	98.9 (97.6–100.0)	100.0 (99.7–100.0)	n.a.	n.a.
22	98.9 (97.6–100.0)	99.4 (98.6–100.0)	n.a.	n.a.

Numbers within parenthesis indicate the 95% confidence limits; n.a., not applicable.
[a] Western blot positive specimens tested: 256, Western blot negative specimens tested: 330.
[b] Western blot positive specimens tested: 262.
[c] Western blot positive specimens tested: 254, Western blot negative specimens tested: 328.
[d] Western blot positive specimens tested: 258, Western blot negative specimens tested: 331.

The delta values calculated for the ELISAs under evaluation ranged from + 9.1 to + 22.8 for the antibody positive population and − 2.1 to − 6.6 for the antibody negative population. GENSCREEN HIV 1/2 (No. 8) had the highest positive delta value of + 22.8 while the Enzygnost Anti-HIV1/2 Plus (No. 6) showed the highest negative value of − 6.6. The delta value is a statistical estimate based on the OD ratios given by an ELISA and provides a factor by which ELISAs of similar sensitivity and specificity can be differentiated, the higher the delta value the greater the probability of obtaining the correct result. The delta value gives a measure of how well removed the antibody positive and negative populations are from the assay cut-off and also reflect the spread of the OD ratios within a specimen population. However it is important to consider the parameters that are used in calculation of a delta value and to understand that the values of those parameters influence the outcome of delta calculations. The improvements made to third generation assays can result in every anti-HIV positive specimen identified correctly giving an OD which is at the upper limit of a microtitre plate reader. In calculating a delta value by assigning a maximum OD reading to such specimens a bias is created in the calculation, albeit

that the bias is minimized by the log transformation of the data. However the same is not true of delta calculations for assays with different cut-off values. For a set of 263 specimen ODs, 262 of which are 3.0 (i.e. at the upper limit of the reader), and the last which is 2.163, the delta value will be 73 for an assay with an average cut-off of 0.325 and 25 for an assay with an average cut-off of 0.525. Therefore to suggest that the former assay is superior to the latter based on the respective delta values is not valid. In practice, assays with positive delta values greater than three achieve OD ratios for positive samples which are adequately removed from the assay cut-off.

4.2. Positive predictive values

The positive predictive values of the assays under evaluation for two populations where the hypothetical prevalences of HIV infection are 0.01 and 6% are shown in Table 4. The probability that a test will determine accurately 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 with a reactive test result is truly infected i.e. the greater the positive predictive value (PPV). For the population with the lower hypothetical prevalence the PPVs of the evaluated assays varied between 0.47 and 100% for ELISAs and 0.03 and 100% for simple assays. In the case of the population with the higher prevalence, the PPVs of ELISAs were between 75.17 and 100% while the lowest PPV of 17.49% was calculated for the simple assay EasiDot HIV/EasiSpot HIV. The difference between the two assay types was not significant when the median PPV calculated for ELISAs was compared with that calculated for simple assays at either of the hypothetical infection prevalences ($P = 0.1$).

4.3. Sensitivity in seroconversion panels

The seroconversion sensitivities of the assays under evaluation relative to the Abbott HIV-1/HIV-2 third generation EIA are shown in Table 4. For the ELISAs, the values varied from -1.1 days to 3.9 days, all eight showing a relative seroconversion sensitivity of $\leq +4$. Among the simple assays, seven of fourteen assays showed a relative seroconversion sensitivity of $\leq +4$. Three ELISA assays: IMX HIV-1/HIV-2 third generation Plus (No. 5), Enzygnost Anti-HIV 1/2 Plus (No. 6), and GENSCREEN HIV 1/2 (No. 8) detected reactivity in one of the eight seroconversion panels earlier than the reference test. One other ELISA, the Vironostika HIV Uni-Form II *plus* O (No. 7) was, with the exception of one panel, as sensitive as the reference test. This corresponds with the findings of a previous study (Thorstensson et al., 1998) that third generation sandwich assays appear to have a higher sensitivity for detection of early appearing HIV antibodies. One simple assay, SERO°STRIP HIV-1/2 (No. 21), was not able to detect as reactive any specimen in one seroconversion panel and two other simple assays, HIVCHEK System 3 Test Kit (No. 14) and the EasiDot HIV/EasiSpot HIV (No. 16) were not able to detect as reactive any specimen in two out of the eight seroconverion panels.

4.4. Inter-reader variability

The inter-reader variability expressed as the percentage of specimens whose initial test results were interpreted differently by different readers relative to the total number of sera tested is shown in Table 4. CAPILLUS HIV-1/HIV-2 (assay No. 9) proved to be the assay interpreted most consistently by readers with no variation recorded for any of the specimens (inter-reader variability 0%). In contrast, variability in readings was recorded for 23.7% of specimens using EasiDot HIV/EasiSpot HIV (assay No. 16).

4.5. Operational characteristics

As shown in Table 4, all ELISAs were found to be 'less suitable' for use in small laboratories and clinics and all except one, 'less easy' to perform using the arbitrary scoring systems described. The IMx HIV-1/HIV-2 Microparticle EIA, included in

Table 4
Comparative evaluation of various operational characteristics of the 22 commercially available HIV antibody assays

	Ease of[a] performance	Suitability[b]	Time[c]		PPV[d]		Inter-reader[e] variability(%)	Indeterminate[f] results (%)	Early seroconv[g]
			90 sera	1 serum	0.01%	6%			
ELISAs									
INNOTEST HIV-1/HIV-2 Ab.s.p	LE	LS	2.08	1.45	0.83	84.18	n.a.	n.a.	3.9
UBI HIV 1/2 EIA	LE	LS	1.45	1.15	100.00	100.00	n.a.	n.a.	3.9
ETI-AB-HIV-1/2 K	LE	LS	2.04	1.40	0.83	84.18	n.a.	n.a.	2.4
HIV EIA	LE	LS	1.50	1.25	1.64	91.41	n.a.	n.a.	3.2
IMx HIV-1/HIV-2 third generation Plus[h]	VE	LS	3.20	0.45	0.47	75.17	n.a.	0.3	−0.9
Enzygnost Anti-HIV 1/2 Plus)	LE	LS	2.05	1.40	3.23	95.51	n.a.	0.0	−0.3
Vironostika HIV Uni-Form II *plus* O	LE	LS	2.05	1.40	100.00	100.00	n.a.	n.a.	0.6
GENSCREEN HIV 1/2[h]	LE	LS	2.05	1.40	0.66	80.97	n.a.	0.0	−1.1
Rapid assys									
CAPILLUS HIV-1/HIV-2	VE	VS	1.30	0.03	0.83	84.18	0.0	0.0	2.4
IMMUNO COMB II BISPOT HIV 1 and 2	VE	VS	1.25	0.40	3.23	95.51	4.5	0.0	1.0
HIV 1 and 2 RAPID SERO TEST	VE	S	1.10	0.05	0.30	65.39	5.1	0.2	2.1
DIPSTICK-HIV 1+2	E	VS	2.00	0.30	0.55	78.00	1.0	0.3	4.9
SPAN COMBAIDS VISUAL	E	S	2.00	0.30	0.08	34.72	6.3	3.2	4.8
HIVCHEK System 3 Test Kit	E	VS	0.45	0.03	3.21	95.49	1.0	0.2	>12.6
HIV TRI DOT	VE	VS	3.30	0.03	3.21	95.49	3.2	0.2	3.0
EasiDot HIV/EasiSpot HIV	VE	S	1.30	0.03	0.03	17.49	23.7	12.5	>8.5
AccuSpot HIV-1 and 2	VE	S	1.10	0.03	0.07	31.78	10.8	5.0	8.0
BIONOR HIV 1 and 2	VE	S	3.00	0.30	0.83	84.18	1.0	0.2	3.0
HIV 1 and 2 DoubleCheck	VE	VS	8.00	0.11–0.15	1.64	91.41	0.8	0.2	2.1
SEROCARD HIV	VE	VS	4.00	0.09	0.47	75.24	1.5	0.2	4.4
SERO°STRIP HIV-1/2	VE	VS	1.30	0.16	100.00	100.00	1.5	0.0	>6.2
HIVSPOT-123	VE	VS	0.30	0.01	1.62	91.32	0.3	0.0	2.1

N.a., not applicable according to the manufacturers instructions.
[a] LE, less easy; E, easy; VE, very easy.
[b] LS, less suitable; S, suitable; VS, very suitable.
[c] The time (h min) to run 1–90 sera.
[d] Positive predictive values (%) in populations where the prevalence of HIV is 0.01 or 6%.
[e] The percentage of sera whose test results were interpreted differently by different readers, relative to the total number of sera tested.
[f] The percentage of sera that yield an indeterminate result, relative to the total number of sera tested.
[g] The relative sensitivity in seroconversion of a test when compared with Abbott HIV-1/HIV-2 third generation EIA.
[h] Those ELISAs where a grey zone was specified.

Table 4 with the ELISAs, achieved the status of 'very easy' to perform primarily because no reagents required preparation, almost no equipment (apart from the instrument) was required and all the reagents remained stable until the expiry date of the kit. All simple assays were found to be either 'easy' or 'very easy' to perform and 'suitable' or 'very suitable' for use in small settings.

5. Conclusions

The selection of HIV antibody assays appropriate to specific needs and circumstances depends on a variety of factors all of which require consideration during the selection process. No single test will be appropriate for all situations. Our objective was to provide potential users of tests with the comparative data necessary to choose the best test for their particular purpose and environment.

The evaluations reported above were coordinated by the Department of Blood Safety and Clinical Technology, WHO in conjunction with UNAIDS. The results of these, and other evaluations conducted under the same auspices are considered, along with other criteria, when selecting assays for inclusion in WHO's bulk purchase programme for HIV assays. This programme provides national AIDS control coordinators of WHO's Member States with access to high quality HIV tests at reasonable cost.

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