

Serological diagnosis of human immunodeficiency virus in Burkina Faso: reliable, practical strategies using less expensive commercial test kits

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Reported are the results of a cross-sectional survey in Burkina Faso to identify reliable, practical strategies for the serological diagnosis of HIV-1 and/or HIV-2 infections, using less-expensive commercial test kits in various combinations, as an alternative to the conventional Western blot (WB) test, which costs US\$ 60. Serum samples, collected from blood donors, patients with acquired immunodeficiency syndrome (AIDS) and pregnant women, were tested between December 1995 and January 1997. Twelve commercial test kits were available: five Mixt enzyme-linked immunosorbent assays (ELISA), three Mixt rapid tests, and four additional tests including monospecific HIV-1 and HIV-2 ELISA. The reference strategy utilized a combination of one ELISA or one rapid test with WB, and was conducted following WHO criteria.

A total of 768 serum samples were tested; 35 were indeterminate and excluded from the analysis. Seroprevalence of HIV in the remaining 733 sera was found to be 37.5% (95% confidence interval: 34.0–41.1). All the ELISA tests showed 100% sensitivity, but their specificities ranged from 81.4% to 100%. GLA (Genelavia Mixt[®]) had the highest positive δ value, while ICE HIV-1.O.2TM (ICE) produced the most distinct negative results. Among the rapid tests, COM (CombAIDS-RS[®]) achieved 100% sensitivity and SPO (HIV Spot[®]) 100% specificity.

Various combinations of commercial tests, according to recommended WHO strategies I, II, III, gave excellent results when ICE was included in the sequence. The best combination of tests for strategy II, which achieved 100% sensitivity and specificity, was to use ICE and COM, the cost of which was US\$ 2.10, compared with US\$ 55.60 for the corresponding conventional strategy. For strategy III, the best combination, which achieved 100% sensitivity and specificity, was to use ICE, ZYG (Enzygnost Anti HIV-1/HIV-2 Plus[®]) and COM, the cost of which was US\$ 2.90 (19.2 times lower than the corresponding strategy requiring WB). No rapid test combination showed 100% sensitivity and specificity. Our results indicate that the serodiagnosis of HIV in Burkina Faso is possible by using reliable, less-expensive strategies which do not require Western blot testing. Moreover, there is a choice of strategies for laboratories working with or without an ELISA chain.

Voir page 738 le résumé en français. En la página 738 figura un resumen en español.

Introduction

The conventional strategy for diagnosis of human immunodeficiency virus (HIV) infection utilizes one enzyme-linked immunosorbent assay (ELISA) as a

first-line test, while the Western blot (WB) is frequently used as a second-line test to confirm

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reactive sera (1). Because this strategy is time-consuming, expensive, and technically not easy to apply in developing countries (2, 3), access to screening and management of HIV infection is not available for the vast majority of these populations (4).

Several evaluations have previously reported that it is feasible to simplify the serological diagnosis of HIV infection and to reduce markedly the cost by combining several ELISA and/or rapid tests without using WB (5–9). However, the application of a simplified serological algorithm to various settings may not be successful for two reasons. First, the geographical variability of HIV can render certain diagnostic strategies unreliable in some regions (10–12). Second, the particular working conditions in each laboratory and the availability of both the technical equipment and skills needed for serological diagnosis of HIV will influence the results (6, 9). An evaluation of alternative strategies for the serological diagnosis of HIV, which takes into account the context and environment where they will be applied routinely, was therefore crucially important.

Our evaluation was performed in Burkina Faso, where the prevalence of HIV infection among urban pregnant women was 8% in 1994 (13). Three laboratories collaborated in this task: the Centre MURAZ/Organisation de Coordination et de Coopération pour la lutte contre les Grandes Endémies (OCCGE), Bobo-Dioulasso; the Blood Transfusion Centre of Sourô Sanou Hospital, Bobo-Dioulasso; and the National HIV Reference Laboratory of the National AIDS Control Committee, Burkina Faso (hosted in the Yalgado Ouedraogo Hospital in Ouagadougou). Ouagadougou, situated in the middle of the country, and Bobo-Dioulasso, in the southwest, are the two largest urban centres in Burkina Faso. The aim of the study was to identify, in the local context, the most reliable, valid and cost-effective commercial tests and to combine them in a simple, easy and low-cost strategy for HIV diagnosis.

Materials and methods

Sera

Sera were collected in Bobo-Dioulasso from blood donors, hospitalized patients, and pregnant women, and in Ouagadougou from hospitalized patients. The panel of serum samples was constituted in order to obtain about 50% prevalence of HIV infection. The required sample size was estimated to be 750 in order to measure $\geq 99\%$ sensitivity with a precision of 1%, at an α -risk of 0.05. Seven aliquots of each serum (100–300 μ l) were separated, stored at $-20\text{ }^{\circ}\text{C}$, and thawed only once before testing.

Commercial assays

We used the following criteria for the selection of commercial kits for the serodiagnosis of HIV antibodies. 1) The test kits should have given satisfactory results in other settings. 2) The tests

should be able to discriminate between HIV-1 and HIV-2, both of which are circulating in Burkina Faso. 3) The test kits should be available in Burkina Faso. 4) The global cost of these tests (including technical equipment, technical assistance and skills) should be reasonable. 5) The test kits should be stable in a tropical climate, despite frequent breaks in the cold chain.

Five Mixt ELISA tests, three rapid tests, and four supplementary tests for discriminating between HIV serotypes (and for confirming reactive sera) were identified and ordered from the different manufacturers. The major operational characteristics of these tests are shown in Table 1. The reference tests were WB I and WB II.

Test procedures

All tests were performed according to the manufacturers' recommendations. The whole panel of serum samples was tested using all the ELISA and rapid tests selected in our study. Sera reactive with at least one ELISA or one rapid test were analysed by supplementary tests for serotyping HIV-1 and HIV-2 and also evaluated by WB I and WB II.

Reference tests

The WB test was chosen as the classic reference test. WB results were interpreted according to WHO criteria (14), whereby positive signals for two out of three *env* bands are considered as *positive*. The WB result was considered to be *negative* if no specific HIV band or a very weak p17 signal was observed, and to be *indeterminate* in any other situation. Serum was taken to have a true HIV-positive status when reactivity was observed with at least one ELISA or rapid test, and also with WB I, WB II or both. A true negative status for HIV was indicated when all the ELISA and/or rapid tests, or WB showed no reactivity. For discriminating between HIV-1 and HIV-2 serotypes, WB showed limited ability because of a large number of cross-reactions. An indirect ELISA test, using synthetic peptides corresponding to the immunodominant HIV-1 and HIV-2 transmembrane glycoprotein, was chosen as a gold standard only for discrimination between HIV serotypes (15).

Among the algorithms for discrimination of HIV-1 and HIV-2, a combination of ELISA tests — namely, ICE HIV-1.O.2TM (ICE) test with Wellcozyme HIV Recombinant[®] (WZY) and ICE HIV-2TM (IC2) — was applied to positive sera, and interpreted as follows:

- when IC2 was negative and WZY was weakly positive (optical density (OD): between the cut-off value (CO) and CO/3), the serum was considered as reactive for a probable “new or recombinant” HIV-1 variant;
- when IC2 was negative and WZY was strongly positive (OD: $< \text{CO}/3$), the serum was considered as reactive for HIV-1;

Table 1. Characteristics of the HIV antibody assays in the study, Burkina Faso (BF), 1995–1997

Assay (abbreviation)	Manufacturer	Type of antigen ^a			Type of assay	Cost per test ^b in Burkina Faso (US\$)
		HIV-1	HIV-2			
Mixt ELISA tests						
Genelavia Mixt [®] (GLA)	Sanofi Diagnostics Pasteur	RPgp160 and RPgp41	SPgp36		Indirect	2.30
Enzygnost Anti HIV-1/HIV-2 Plus [®] (ZYG)	Behring	SPgp41 and SPgp120	SPgp36		Sandwich	2.30
Murex HIV 1+2 [®] (REX)	Murex	RP24 and RPgp41	SPgp36		Sandwich	1.90
Vironostika HIV Uni-form II [®] (VKU)	Organon Teknika	RP24 and RPgp41	SPgp36		Sandwich	2.90
ICE HIV-1.0.2 TM (ICE)	Murex	RP env and core SP group O	SP env		Immunocapture	1.50
Rapid tests						
ComBAIDS-RS [®] (COM)	Span Diagnostics Ltd	SPgp41	SPgp36		DOT	1.50
HIV Spot [®] (SPO)	Genelabs Diagnostics	RPgp120 and RPgp41	SPgp36		DOT	3.50
Multispot HIV-1/HIV-2 [®] (MTT)	Sanofi Diagnostics Pasteur	RPgp41 and SPgp41	SPgp36		DOT	8.20
Supplemental tests (confirmation and serotyping):						
Wellcozyme HIV Recombinant [®] (WZY)	Murex	RPgp24 and RPgp41	– ^c		Competition	1.80
ICE HIV-2 TM (IC2)	Murex	– ^c	SP		Immunocapture	1.80
Pepti-LAV 1-2 [®] (PEP)	Sanofi Diagnostics Pasteur	SPgp41	SPgp36		Line immunoassay	24.60
LIA-TEK HIV1+2 [®] (LIA)	Organon Teknika	SPgp41 & p31, p24, p17	SPgp36		Line immunoassay	38.40
Reference tests						
New Lav Blot I [®] (WB I)	Sanofi Diagnostics Pasteur	HIV lysate	–		BLOT	65.80
New Lav Blot II [®] (WB II)	Sanofi Diagnostics Pasteur	–	HIV lysate		BLOT	78.50

^a Antigens: RP = recombinant proteins; SP = synthetic peptides; gp = glycoprotein.

^b Costs were provided by the distributor of the test during the period of study (June 1995 to February 1997).

^c Test not provided by WHO.

- when IC2 was positive and WZY was weakly positive as described above, the serum was considered as reactive for HIV-2;
- when IC2 was positive and WZY was strongly positive, the serum was considered as dually reactive (HIV-1 and HIV-2).

Data analysis

The prevalence of HIV infection in our panel of serum samples was calculated after excluding indeterminate sera; the sensitivity and specificity were calculated (16); and positive and negative δ -values were calculated, as described by Maskill et al. (18), thereby permitting comparison of the efficiency of ELISA to discriminate non-reactive or reactive populations from the CO values (17, 18). The capacity to identify correctly HIV-1 and HIV-2 types was estimated with reference to the applicable gold standard using the kappa (κ) concordance coefficient (19). Potential strategies for serological diagnosis were largely drawn from WHO recommendations (20, 21). The potential cost of the different tests or combinations of tests was also taken into consideration (4). The costs of the proposed alternative strategies performed in our evaluation were calculated and compared with the costs of conventional strategies.

Results

Sample description

Of the total of 768 sera examined, 334 were non-reactive with all the commercial kits and were considered as HIV-negative. Among the sera tested by WB, 124 were HIV-negative and 35 HIV-indeterminate. Details on these 35 sera are as follows: 17 reacted with only one protein of the HIV-1-specific WB (WB I) (p24, p18, p52 or p68); glycoproteins never appeared except for gp160, which appeared four times, in association with 1, 2, 4 or 6 other proteins; 9 sera reacted with 2, 3, 4, 5 or 7 WB I proteins; and 9 sera did not react with WB I but only with WB II. For HIV-2-specific WB (WB II), 18 sera reacted with only p26 or p16 or both; the others were negative. All 35 sera were tested by the indirect ELISA used to discriminate between HIV serotypes and were found to be negative. These 35 were finally excluded, leaving the results obtained on 733 sera for analysis. From data obtained by WB, the prevalence of HIV infection in this panel was 37.5% (95% confidence interval (CI): 34.0–41.1), or 275 patients infected by HIV. Of these, 246 (90%) were infected by HIV-1, 17 (6%) by HIV-2, and 12 (4%) by both HIV-1 and HIV-2.

Commercial tests performance

Table 2 presents data on the intrinsic validity of the ELISA tests and rapid tests. All ELISA tests had a

Table 2. Sensitivity, specificity and delta (δ) values of the HIV antibody screening assays used in the study, panel of 733 sera, Burkina Faso, 1995–97

Parameter	Antibody screening assays ^a							
	GLA	ZYG	VKU	REX	ICE	MTT	COM	SPO
No. of true positives	275	275	275	275	275	273	275	270
No. of false positives	30	2	16	85	0	6	2	0
No. of true negatives	428	456	442	373	458	452	456	458
No. of false negatives	0	0	0	0	0	2	0	5
Sensitivity (%)	100.0 (98.3–100.0) ^b	100.0 (98.3–100.0)	100.0 (98.3–100.0)	100.0 (98.3–100.0)	100.0 (98.3–100.0)	99.3 (97.1–99.9)	100.0 (98.3–100.0)	98.2 (98.2–99.3)
Specificity (%)	93.4 (90.7–95.5)	99.6 (98.3–99.3)	96.5 (94.3–97.9)	81.4 (77.5–84.8)	100.0 (99.0–100.0)	98.7 (97.0–99.5)	99.6 (98.3–99.9)	100.0 (99.0–100.0)
Delta (δ) values								
WB-positive ^c	+ 24.12	+ 17.40	+ 8.18	+ 17.36	+ 12.10			
WB-negative ^d	– 1.62	– 1.29	– 1.92	– 0.54	– 5.63			

^a GLA, Genelavia Mixt[®]; ZYG, Enzygnost HIV 1/2 Plus[®]; VKU, Vironostika Uni-Form II[®]; REX, Murex HIV 1 + 2[®]; ICE, ICE HIV-1.0.2[™]; MTT, Multispot HIV-1/HIV-2[®]; COM, CombAIDS-RS[®]; SPO, HIV Spot[®].

^b Figures in parentheses are 95% confidence intervals.

^c WB-positive: sera confirmed HIV-positive by Western blot.

^d WB-negative: sera confirmed HIV-negative by Western blot.

sensitivity of 100%. Among the rapid tests, CombAIDS-RS[®] (COM) had 100% sensitivity. ICE was the only test with a specificity of 100%. The range of specificities was from 81.4 to 100.0%. Positive δ -values ranged from +8.18 to +24.12, while negative δ -values ranged from –0.54 to –5.63. According to UNAIDS and WHO recommendations, minimal norms for HIV commercial tests used in diagnosis strategies are a sensitivity > 99% and a specificity > 95% (20). Genelavia Mixt[®] (GLA) and Murex HIV-1/HIV-2[®] tests showed a specificity 95%, and HIV Spot[®] (SPO) a sensitivity 99%. ICE reached a sensitivity and a specificity of 100% and was therefore proposed as a first-line test. Because of its excellent sensitivity, COM was considered to be a candidate first-line rapid test.

The cost of using an ELISA to screen for HIV infection and WB to confirm reactive sera averaged US\$ 60.40 if the kits were purchased from the manufacturer (Table 3). Depending on the test used, this cost varied from US\$ 55.60 to US\$ 72.80 if purchased from the manufacturer.

Seven combinations using three ELISA tests — ICE, ZYG (Enzygnost Anti HIV-1/HIV-2 Plus[®]), and VKU (Vironostika HIV Uni-form II[®]) — and the COM rapid test gave satisfactory results with strategy II, i.e. a sensitivity and specificity of 100% (Table 3). In these combinations, the most sensitive test was used first, followed by a proven second test. The two best and lowest-cost combinations responding to these criteria were ICE/COM and ICE/ZYG, corresponding to US\$ 2.10 and US\$ 2.40 per sample, respectively. Only two combinations had a 100% sensitivity and specificity in accord with UNAIDS/WHO recommendations for strategy III (Table 3). These two combinations, with ICE as the first-line test, were ICE/ZYG/COM and ICE/VKU/COM, costing US\$ 2.90 and US\$ 3.10

per sample, respectively. Strategy II only was used with rapid tests because SPO had to be discarded (sensitivity 99%). The combination of COM and MTT (Multispot HIV-1/HIV-2[®]) gave reliable results, with a sensitivity of 99.3% and a specificity of 100%; the cost was US\$ 4.60 per sample. As far as discrimination of HIV-1 and HIV-2 serotypes was concerned, strategies using line immunoassays (Pepti-LAV 1-2[®] (PEP) and Lia-TeK[®] HIV1+2 (LIA)) did not achieve 100% sensitivity or specificity (sensitivity always equal to 99.6% and 99.3% using, respectively, PEP and LIA in strategies II or III, and specificity in the range 98–100%). The most efficient strategy (sensitivity and specificity equal to 100% and κ coefficient equal to 0.99) at a low cost (US\$ 2.80) was to use WZY and IC2 tests in parallel on sera which were positive by ICE (Fig. 1).

Serological diagnosis of HIV infection is needed for three main reasons: safe blood transfusion, serosurveillance, and voluntary testing. Table 4 summarizes the proposed algorithms.

In the case of HIV screening for blood transfusion, it is highly desirable to use the most sensitive test which gives no false-negative results and as few as possible false-positive results. Indeed, unnecessary destruction of a single unit of blood costs on average US\$ 40 (22). Tests such as ICE (US\$ 1.50), ZYG (US\$ 2.30) and VKU (US\$ 2.90) for ELISA tests and COM (US\$ 1.50) as a rapid test can be considered as suitable for screening blood supplies.

Serosurveillance uses unlinked anonymous screening results. According to UNAIDS/WHO recommendations, two situations have to be defined. The first of these concerns serosurveillance in a population with prevalence of HIV infection >10% where strategy I can be used. Selected tests must have the highest positive predictive value (23). ICE and SPO presented a maximal specificity of 100%, but

Table 3. Cost per test for HIV antibody screening using the conventional strategy, with Western blot to confirm all positive screening results^a

Tests combination ^b			No. of sera tested	No. of sera to be confirmed	Cost per test (US\$) ^c	Cost ratio ^d
1st	2nd	3rd				
Strategy using WB to confirm first-line assay						
GLA	WB	–	733	305	62.30	
ZYG	WB	–	733	277	56.80	
VKU	WB	–	733	291	60.20	
REX	WB	–	733	360	72.80	
ICE	WB	–	733	275	55.60	
MTT	WB	–	733	279	63.10	
COM	WB	–	733	277	56.00	
SPO	WB	–	733	270	56.60	
Strategy II						
ZYG	ICE	–	733	277	2.90	19.60
ZYG	COM	–	733	277	2.90	19.60
VKU	ICE	–	733	291	3.50	17.20
VKU	COM	–	733	291	3.50	17.20
ICE	ZYG	–	733	275	2.40	23.20
ICE	VKU	–	733	275	2.60	21.40
ICE	COM	–	733	275	2.10	26.50
Strategy III						
ICE	ZYG	COM	733	275+275	2.90	19.20
ICE	VKU	COM	733	275+275	3.10	17.90

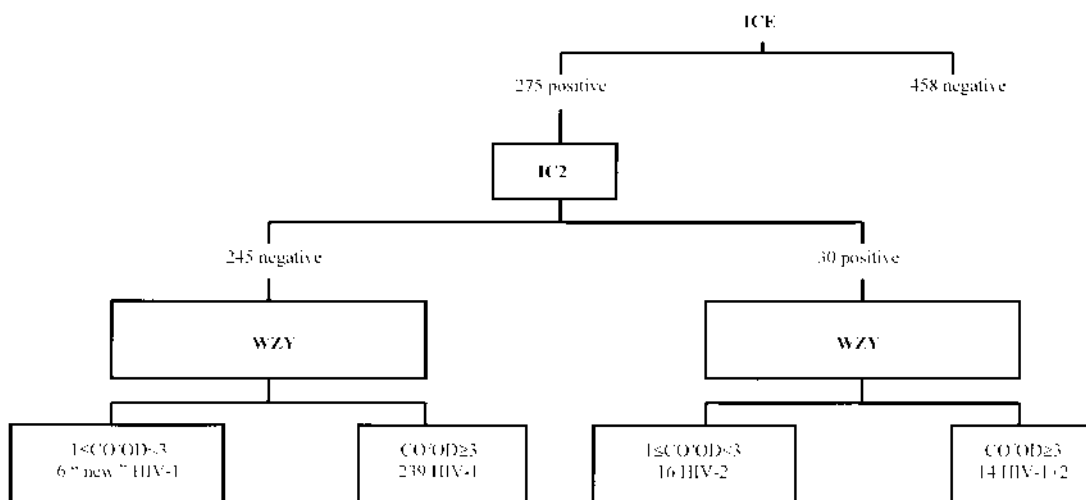
^a Comparison of cost per test for HIV antibody screening with sensitivity and specificity of 100%, using strategy II and strategy III recommended by WHO; evaluation performed in Burkina Faso, panel of 733 sera, 1995–97.

^b GLA: Genelavia Mixt[®]; ZYG: Enzygnost HIV 1/2 Plus[®]; VKU: Vironostika Uni-Form II[®]; REX: Murex HIV 1 + 2[®]; ICE: ICE HIV-1.0.2[™]; MTT: Multispot HIV-1/HIV-2[®]; COM: CombAIDS-RS[®]; SPO: HIV Spot[®].

^c Cost per tested serum was calculated for each combination, taking into account the cost of each assay (see Table 1) and the number of sera tested using each assay.

^d The cost per serum to test *n* sera with the conventional strategy, divided by the cost per serum to test the same number of sera with the alternative algorithm.

Fig. 1. Algorithm for the serodiagnosis of HIV infection with discrimination between HIV serotypes 1 and 2: evaluation for 733 sera, Burkina Faso, 1995–97



ICE: ICE HIV-1.0.2[™]; WZY: Wellcozyme HIV Recombinant[®]; IC2, ICE HIV-2[™]; CO, cut-off; OD, optical density.

Table 4. Serodiagnostic strategies for HIV infection (alternatives to Western blot) responding to several objectives : transfusion safety, serosurveillance, clinical diagnosis, Burkina Faso, 1995–1997

Objectives	Test combinations	Laboratories equipped with an ELISA chain ^a	Cost per test ^b / cost ratio ^c	Laboratories using only rapid test ^a	Cost per test/ cost ratio in Burkina Faso (US\$)
WHO Strategy I					
Blood transfusion	One very sensitive test	ICE	1.50 / 37.10	COM	1.50 / 37.30
Surveillance prevalence >10%	One very specific test	ZYG	2.30 / 24.70		
Diagnosis of symptomatic individuals prevalence >30%	One very specific test	VKU	2.90 / 20.70		
WHO Strategy II					
Surveillance prevalence ≤ 10%	2 tests combination	ICE / COM	2.10 / 26.50	COM / MTT	4.60 / 12.20
Diagnosis of symptomatic individuals prevalence ≤ 30%	2 tests combination	ICE / ZYG	2.40 / 23.20		
Diagnosis of asymptomatic individuals prevalence >10%	2 tests combination	ICE / VKU	2.60 / 21.40		
WHO Strategy III					
Diagnosis of asymptomatic individuals prevalence ≤ 10%	3 tests combination	ICE / ZYG / COM ICE / VKU / COM	2.90 / 19.20 3.10 / 17.90		
Serotyping of HIV-1 / HIV-2	3 tests combination	ICE / WZY + IC2 in parallel	2.80 / 19.80	COM / MTT	4.60 / 12.20

^a ZYG, Enzygnost HIV 1/2 Plus[®]; VKU, Vironostika Uni-Form II[®]; ICE, ICE HIV-1.O.2[™]; MTT, Multispot HIV-1/HIV-2[®]; COM, CombAIDS-RS[®] WZY, Wellcozyme HIV Recombinant[®]. IC2, ICE HIV-2[™].

^b Cost per tested sera was calculated for each combination, taking into account the cost of each assay (see Table 1) and the number of sera tested using each assay.

^c The cost per serum to test *n* sera with the conventional strategy, divided by the cost per serum to test the same number of sera with the alternative algorithm.

SPO was discarded because of its low sensitivity. COM, which performed to the minimal norm of sensitivity and specificity required by UNAIDS/WHO, can be a candidate for screening for HIV infection. The cost per test of ICE and COM was US\$ 1.50. In the second situation, where prevalence of HIV infection is ≤ 10%, a sequential strategy using two tests is recommended. Considering cost savings and efficiency, we found the combination of ICE and COM to be the most accurate test for laboratories equipped with an ELISA chain: the cost (US\$ 2.10) was 26 times lower than that for the corresponding strategy requiring WB. For laboratories able to perform only rapid tests, the combination of COM and MTT (cost, US\$ 4.60) could be adopted, with a 0.7% level of false-negative results using the 1992 WHO testing strategy (21); these would become indeterminate results if the 1997 WHO strategy is used (20). Finally, identification of HIV-infected persons visiting health services requires the highest level of reliability in three situations, as discussed below.

- Subjects with HIV-related signs and/or symptoms. If the seroprevalence of HIV infection is ≤ 30%, strategy II is recommended, using combinations of ICE and COM (US\$ 2.10) for laboratories equipped with an ELISA chain, or COM and MTT (US\$ 4.60) for laboratories using only rapid tests. If the seroprevalence of HIV infection in the

population is 30%, one very specific test is required: ICE or COM (US\$ 1.50 each).

- Asymptomatic subjects. If the seroprevalence of HIV infection is ≤ 10%, strategy III is recommended by WHO. The combination exhibiting the best cost-efficiency ratio and which is recommended for laboratories with an ELISA chain was ICE/ZYG/COM (US\$ 2.90). For laboratories without an ELISA chain, our study was not able to identify a combination fulfilling all prerequisites using only rapid tests. If the seroprevalence of HIV infection is >10%, strategy II is recommended.
- Reliable discrimination between serotypes 1 and 2 of HIV. This may be necessary in clinical studies that include only HIV-1-positive subjects. For laboratories equipped with an ELISA chain, the only suitable combination we found for strategy II was ICE with WZY and IC2 tested in parallel (cost, US\$ 2.80 per sample). For laboratories without an ELISA chain, when strategy II is suitable, the only combination was COM and MTT. Any combination corresponding to the requirements of strategy III gave reliable results.

Discussion

Our findings demonstrate that serodiagnosis of HIV infection, satisfying the three main objectives of testing, can be performed reliably and at low cost in

Burkina Faso. Similar studies have been performed in other developing countries. For example, in Brazil, a study in a São Paulo hospital, with a very low HIV prevalence, showed that excellent results were obtained with an ELISA test combination using strategy II. On the other hand, addition of a rapid test for applying strategy III reduced the sensitivity and specificity (24). Two studies, carried out in South Africa and Honduras, have evaluated rapid tests (25, 26). In South Africa, Wilkinson et al. showed that a combination of the Capillus HIV-1/HIV-2[®] (Cambridge Biotech, Galway, Ireland) test and the Abbott Test Pack HIV-1/HIV-2[®] (Abbott Laboratories, Delkenheim, Germany) had a sensitivity of 100% and a specificity of 99.6% (25). These workers proposed using only one rapid test, in view of the high HIV seroprevalence in the area. In Honduras, Stetler et al. evaluated seven rapid tests (26), all of which gave excellent results. MTT and SPO, under their former denominations (respectively, Genie 1/2[®] and HIVCHECK 1+2[®]), had a sensitivity and specificity >99%. The use of combinations of three tests was not recommended because of the excellent results given by strategy II under conditions of high and low prevalence. Both these findings and those of the present study suggest that a combination of rapid tests can be used successfully in strategy III. In addition, our study independently confirms the ability of well-selected ELISA tests to discriminate between HIV-1 and HIV-2.

Our study was subject to limitations. The first is the consequence of discordant results sometimes obtained with the first two tests used. UNAIDS and WHO, in their recommendations published in March 1997, recommended that discordant sera should be re-tested using these two assays (20). This recommendation was published after our study had been completed. Consequently, we did not repeat tests on sera that gave discordant results in the first two tests (ELISA or rapid tests). Evaluation of serum samples that should have been re-tested in each combination showed that all combinations using ICE as the initial test gave concordant results between the first and second tests. Therefore, the combinations of tests that we recommend for laboratories equipped with an ELISA chain are consistent with the recommendations made by UNAIDS/WHO in 1997. In contrast, the combination of rapid tests recommended to laboratories without the ELISA chain would require re-testing of four discordant sera if the tests used include COM and MTT. This slightly increased the cost and changed to a small extent both sensitivity and specificity; if discordance is maintained after the re-testing, the sera are classed as indeterminate. Strategy II with rapid tests may be of interest for serodiagnosis in settings with sub-optimal laboratory facilities.

The second limitation was the exclusion from our analysis of sera that were indeterminate in WB testing. Classification of sera as indeterminate clearly depends on the criteria used to interpret the WB, some criteria being more sensitive than others. HIV variability (10–12) and early seroconversion can also

impair HIV antibody detection by WB and increase the proportion of indeterminate results (14). All of these indeterminate sera were negative when tested using the highly specific indirect ELISA, which is used to discriminate between HIV-1 and HIV-2 serotypes. Strategies II and III recommend that serum samples with discordant results in two or three repeated ELISA tests be considered as indeterminate. For cases that have seroconverted, the p24 antigenaemia assay could detect early infections (27). However, this method is expensive and not available in most laboratories. A systematic follow-up of subjects whose HIV status is indeterminate may be required (28). Our panel of samples consisted mainly of sera containing high titres of HIV antibodies, as exemplified by the high OD values in ELISA. This panel may not be optimal for evaluation of test kits sensitive enough to detect early seroconversions, but probably responds to the needs of routine HIV testing.

Although each test's intrinsic validity is fundamental to testing, the quality of the results also depends on the available laboratory equipment and technical skills of the staff (6, 7, 9). In this respect, Murex Immuno-Capture assays are supplied with a colour control at each step in order to minimize human error. In order to carry out HIV diagnosis strategies in the field it is therefore essential that laboratory technicians receive proper training and that a functional quality assurance programme be in place (29). The present evaluation is not final and must be continued. For example, the dissemination of certain HIV variants may render some currently reliable strategies invalid in the future. Also, major improvements in test quality are continually being made and require evaluation.

Our results indicate that reliable and cost-effective test combinations can be identified and adapted to specific situations. They could render HIV diagnosis more accessible, even in settings where laboratory equipment and skills are scarce. ■

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Résumé

Le sérodiagnostic de l'infection à virus de l'immunodéficience humaine (VIH) au Burkina Faso : des stratégies fiables et pratiques basées sur des tests du commerce meilleur marché

Une étude transversale a été menée au Burkina Faso afin de trouver des stratégies fiables et pratiques pour le sérodiagnostic des infections à VIH-1 et VIH-2 en combinant divers tests commerciaux meilleur marché que le Western Blot (WB) classique qui coûte US \$60. Des échantillons de sérum ont été prélevés sur des donneurs de sang, des malades du SIDA et des femmes enceintes et ils ont été soumis aux différents tests entre décembre 1995 et janvier 1997. Nous disposons de 12 tests du commerce : cinq tests immunoenzymatiques ELISA mixtes, trois tests rapides mixtes et quatre autres tests, notamment des tests ELISA monospécifiques pour le VIH-1 et le VIH-2. La stratégie de référence consistait à combiner un test ELISA ou un test rapide avec le WB. L'interprétation a été faite d'après les critères de l'OMS.

Au total, on a testé 768 sérums; 35 ont donné un résultat indéterminé et ont été exclus de l'analyse. La séroprévalence du VIH dans les 733 autres était de 37,5% (IC à 95 % : 34,0 — 41,1). Tous les tests ELISA ont fait preuve d'une sensibilité de 100%, mais leur spécificité allait de 81,4 à 100%. En ce qui concerne la valeur de δ , c'est le GLA (Genelavia Mixt[®]) qui présentait la valeur positive la plus élevée, la valeur la plus négative étant obtenue avec le test ICE HIV-1.O.2TM.

En ce qui concerne les tests rapides, on a obtenu une sensibilité de 100% avec le COM (CombAIDS-RS[®]) et une spécificité de 100% avec le SPO (HIV Spot[®]).

Les diverses combinaisons de tests du commerce selon les stratégies I, II et III recommandées par l'OMS, ont donné d'excellents résultats lorsque le test ICE faisait partie de la série. Pour la stratégie II, la meilleure combinaison, qui a donné une sensibilité et une spécificité de 100 %, consistait à utiliser l'ICE et le COM, qui coûtent US \$2,10, contre US \$55,60 dans le cas de la stratégie habituelle. En ce qui concerne la stratégie III, la meilleure combinaison (sensibilité et spécificité égales à 100 %), consistait à utiliser l'ICE et le ZYG (Enzygnost Anti HIV-1/HIV-2 Plus[®]) avec en outre le COM, pour un coût de US \$2,90 (soit un prix de revient 19,2 fois moins élevé qu'avec la stratégie correspondante basée sur le WB). Aucune des combinaisons basées sur les tests rapides n'a atteint 100% de sensibilité et de spécificité. Nos résultats montrent qu'on peut procéder au sérodiagnostic de l'infection à VIH au Burkina Faso en ayant recours à des stratégies fiables et meilleur marché que celles qui sont basées sur le Western Blot. Par ailleurs, les laboratoires qui sont ou non équipés d'une chaîne ELISA disposent d'un choix de stratégies.

Resumen

Diagnóstico serológico de la infección por el virus de la inmunodeficiencia humana (VIH) en Burkina Faso: estrategias prácticas y fiables con kits comerciales más baratos

En Burkina Faso se realizó un estudio transversal para identificar estrategias prácticas y fiables de diagnóstico serológico de las infecciones por el VIH-1 y/o el VIH-2, usando los kits más baratos hallados en el mercado, solos o combinados, como alternativa a la prueba convencional de Western Blot (WB), que cuesta US\$ 60. Entre diciembre de 1995 y enero de 1997 se analizaron muestras de suero de donantes de sangre, pacientes de SIDA y mujeres embarazadas. Se disponía de 12 kits comerciales: cinco pruebas de inmunosorción enzimática (ELISA) mixtas, tres pruebas rápidas mixtas y cuatro pruebas más, entre ellas pruebas ELISA mono-específicas para el VIH-1 y el VIH-2. Como estrategia de referencia, aplicada siguiendo los criterios de la OMS, se utilizó una combinación del WB y la prueba ELISA o una prueba rápida.

Se analizaron en total 768 muestras de suero; 35 de ellas se excluyeron del análisis por haber dado un resultado indeterminado. La seroprevalencia del VIH en las 733 restantes resultó ser del 37,5% (IC 95%: 34,0-41,1). Todas las pruebas ELISA alcanzaron una sensibilidad del 100%, pero su especificidad se situó entre el 81,4% y el 100%. En cuanto a los valores delta, GLA (Genelavia Mixt[®]) dio el valor delta positivo más alto, mientras que la prueba ICE HIV-1.O.2TM (ICE) dio

los resultados negativos más claros. Entre las pruebas rápidas, COM (CombAIDS-RS[®]) mostró una sensibilidad del 100% y la prueba SPO (HIV Spot[®]) una especificidad del 100%.

Diversas combinaciones de las pruebas comerciales, conformes con las estrategias I, II y III recomendadas por la OMS, daban excelentes resultados cuando incluían en su secuencia la prueba ICE. La mejor combinación para la estrategia II, que consiguió un 100% de sensibilidad y especificidad, fue la de las pruebas ICE y COM, cuyo costo era de US\$ 2,10, frente a los US\$ 55,60 de la estrategia convencional equivalente. Para la estrategia III, la mejor combinación, que consiguió un 100% de sensibilidad y especificidad, fue la de las pruebas ICE, ZYG (Enzygnost Anti HIV-1/HIV-2 Plus[®]) y COM, cuyo costo era de US\$ 2,90 (19,2 veces más bajo que el de la estrategia equivalente que requiere la prueba WB). Ninguna combinación de pruebas rápidas mostró un 100% de sensibilidad y especificidad. Nuestros resultados indican que el serodiagnóstico del VIH en Burkina Faso puede hacerse mediante estrategias fiables y baratas para las que no se requiere el Western Blot. Además, hay distintas estrategias posibles para los laboratorios, usen o no una cadena de pruebas ELISA.

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