Evaluation of the World Health Organisation antibody-testing strategy for the individual patient diagnosis of HIV infection (strategy III)

D. J. Martin, N. K. Blackburn, K. F. O'Connell, E. T. Brant, E. A. Goetsch

Objective. To evaluate the World Health Organisation (WHO) antibody testing strategy for the individual patient diagnosis of HIV infection (strategy III).

Design. Evaluation of a combination of enzyme-linked immunosorbent assays (ELISAs) for the detection of antibodies to HIV-1 and HIV-2 infection. The WHO strategy III calls for a combination of three ELISAs, based on different antigens and/or differing test principles, to be used in a sequential fashion. The first part of the study evaluated various kits as part of a selection process. The second part of the study was an assessment of the three-ELISA testing strategy on routine sera submitted to the National Institute for Virology (NIV) for HIV testing. In all instances, the conventional testing protocol, which utilises a screening ELISA followed by a confirmatory Western blot (WB) on positive specimens, was used as the 'gold standard'.

Setting. The HIV-testing laboratory at the NIV, Johannesburg.

Results. In the first part of the study, all of the ELISA kits evaluated showed high sensitivity and specificity, and a selection of the kits for part two of the study was made in accordance with the WHO recommendation. The kits selected, in order of use, were the Biotest anti-HIV 1/2 (test 1), Pasteur Genelavia Mixt HIV-1/2 (test 2) and Murex Wellcozyme HIV-1 competitive assay (test 3). This combination was evaluated using 7 812 sera submitted to the NIV for serology testing. The sensitivities of the tests were highly satisfactory (99,6 - 100%) as were the specificities (99,2 - 100%). The positive predictive value of

MRC AIDS Virus Research Unit, National Institute for Virology, and Department of Virology, University of the Witwatersrand, Johannesburg

D. J. Martin, M.B. B.CH., M.MED., D.T.M. & H., D.P.H.

N. K. Blackburn, M.PHIL., D.PHIL.

K. F. O'Connell, SPEC. DIP. MED. TECH.

E. T. Brant, N.H.DIP.M.T.

E. A. Goetsch, N.DIP.M.T.

strategy III at various seroprevalences (0,5 - 25,5%) was ⩾ 99,6%. The rate of WB usage when compared with the previous HIV testing protocol was low (4,6%).

Conclusions. **The results of this study suggest that this testing protocol could be introduced in South Africa with considerable cost-saving and no reduction in specificity.**

S Afr Med J 1995; **85:** 877-880.

The National Institute for Virology (NIV)'s conventional algorithm for testing serum for antibodies to HIV has been to use an enzyme-linked immunosorbent assay (ELISA) as a screening test; all negative results were accepted, but sera found to be repeatedly reactive on ELISA were retested by a confirmatory test, usually Western blot (WB). WB has limitations which include high costs, difficulties in interpretation and indeterminate reactions.[1] Recent technological advances have produced tests which, either alone or in combination, provide results as accurate as those obtained with WB.[2] To maximise accuracy and minimise cost, the Global Programme on AIDS (GPA) of the World Health Organisation has recommended strategies for the laboratory testing of HIV infection.[3] The selection of the most appropriate combination of laboratory tests depends on the following: (*i*) the objective of HIV testing in the population concerned (safety of blood or organ donation, surveillance, individual patient diagnosis); (*ii*) the sensitivity and specificity of the laboratory tests used; (*iii*) the prevalence of HIV infections in the population tested; and (*iv*) the operational characteristics of the tests, including the cost.

The NIV decided to evaluate the strategy applied to individual patient diagnosis as outlined in the WHO's strategy III[3] and compare the results to those of the conventional testing protocol; confirmatory WB test was used as the 'gold' standard.

Materials and methods

Selection of antibody tests for strategy III

Strategy III calls for the use of three ELISAs. The tests should be based on differing test principles, e.g. indirect and competitive ELISAs, and/or have different sources of antigen, e.g. recombinant antigens and synthetic peptides. The first test should have a high sensitivity, whereas the subsequent tests should have high specificities. Although the sensitivity and specificity values for individual manufacturers' kits were available and had been recorded in previous evaluations,[4] we felt it important to evaluate certain kits in our laboratory.

Evaluation of commercial ELISA kits

Four commercial ELISAs were initially selected for the evaluation: (*i*) Wellcozyme HIV 1 + 2 enzyme immunoassay (EIA) (Murex Diagnostics Ltd, England) based on recombinant HIV-1 antigens and HIV-2 synthetic peptide; (*ii*) Abbott recombinant HIV-1/HIV-2 third-generation EIA (Abbott Diagnostic Division, Germany); (*iii*) Genelavia Mixt HIV-1 and HIV-2 EIA (Sanofi Diagnostics Pasteur, France) incorporating recombinant and synthetic peptide HIV-1 antigens and an HIV-2 synthetic peptide antigen; and (*iv*) Biotest anti-HIV-1/2 EIA (Biotest AG, Germany) based on recombinant antigens.

Antigen detection

Abbott HIV AG-1 Monoclonal Kit (Abbott Diagnostic Division, Germany) was used for the detection of HIV-1 p24 antigen. This was used as a supplementary test for sera which gave discrepant results (reactive with at least one ELISA and negative with at least one ELISA).

Western blot

All reactive sera were tested by means of the NEW LAV-BLOT 1 Kit (Sanofi Diagnostics Pasteur, France), which was used as a confirmatory test. The WHO criteria for positivity of the WB were adopted (2 of 3 envelope bands gp41, gp120, gp160). In addition, for the purposes of the study, the presence of a single envelope band, gp160, was considered positive as we felt that it was probably evidence of early seroconversion.

Serum specimens

These were sera that were submitted to the NIV for routine HIV-antibody testing.

Results

The results of this preliminary evaluation, using sera of unknown HIV-antibody status, are detailed in Table I. They suggest that all the ELISAs had high sensitivity and specificity; the selection of the three ELISAs for the use in strategy III was determined by the need for tests with differing test principles (Table II). This decision was made in accordance with the WHO recommendations. The Biotest assay, whose sensitivity in our study confirmed previously published results,[4] was selected as the first-line screening test, followed by the Pasteur Genelavia Mixt EIA. The third test selected, which was not included in the initial panel, was the Murex Wellcozyme HIV-1 competitive assay. The Wellcozyme HIV-1 assay was specifically selected to detect HIV-1 and was pretested against a well-characterised 'in-house' HIV-1 antibody panel which showed a 100% correlation. The Abbott HIV-1/HIV-2 third-generation test gave results equivalent to those of the Wellcozyme HIV-1 and 2 EIA tests, but was not considered for the study because of the relatively large quantity of specimens required (150 µl) in the test protocol. The sensitivities of the three selected ELISAs were further assessed with a seroconversion panel (Boston Biomedica Inc. panel-G) with satisfactory results. The Biotest EIA was found to be the most sensitive (detailed results not shown).

Evaluation of strategy III

The WHO recommendations state that serum that is non-reactive in the first test is to be considered HIV antibody-negative, as is serum that is reactive in the first test but non-reactive in the second. Serum reactive in all three tests is considered HIV antibody-positive. Serum that is reactive in the first and second tests, but non-reactive in the third test, is considered discrepant and should undergo further testing.

Table I. Results of a series of two EIA systems tested in parallel against routine serum specimens

	Abbott EIA (A) and Murex HIV 1/2 EIA (M) tested against 704 sera			True positive 113	True negative 591	
	A + M	A only	M only		A	M
ELISA +ve	116	0	0	Sensitivity	100	100
WB +ve	110			Specificity	99,5	99,5
gp160 band only	3			+ve PV*	97,4	97,4
Not confirmed	3			–ve PV	100	100
	Pasteur EIA (P) and Murex HIV 1/2 EIA (M) tested against 1 483 sera			True positive 200	True negative 1 283	
	P + M	P only	M only		P	M
ELISA +ve	199	6	4	Sensitivity	100	99,5
WB +ve	194	0	0	Specificity	99,5	99,7
gp160 band	5	1	0	+ve PV	97,6	98
Not confirmed	0	5	4	–ve PV	100	99,9
	Pasteur EIA (P) and Biotest EIA (B) tested against 1 041 sera			True positive 190	True negative 851	
	P + B	P only	B only		P	B
ELISA +ve	190	4	4	Sensitivity	99,5	100
WB +ve	187	0	1(p24 Ag)	Specificity	99,4	99,5
gp160 band	1	0	0	+ve PV	97,4	97,9
p24 Ag	0	0	1	–ve PV	99,9	100
HIV-2	1	0	0			
Not confirmed	1	4	3			

PV = Predictive value.

$$\text{Sensitivity} = \frac{\text{True positive}}{\text{True positive + false negative}}$$

$$\text{Specificity} = \frac{\text{True negative}}{\text{True negative + false positive}}$$

$$\text{Positive predictive value} = \frac{\text{True positive}}{\text{True positive + false positive}}$$

$$\text{Negative predictive value} = \frac{\text{True negative}}{\text{True negative + false negative}}$$

Table II. Characteristics of selected tests

ELISA	Manufacturer	Antigens	Test principle
1	Biotest	Recombinant HIV-1/HIV-2	Indirect assay
2	Pasteur	Recombinant HIV-1 + synthetic peptides HIV-1/HIV-2	Indirect assay
3	Murex Wellcozyme	Recombinant HIV-1	Competitive assay

The samples tested by means of strategy III were 7 812 sera routinely submitted for HIV serology testing. In the event of a discrepant result being obtained (reactive with at least one ELISA and negative with at least one), the serum was further tested by WB and for p24 antigen. Sera that were non-reactive on the third ELISA were tested for HIV-2 using the LAV-BLOT 2 (Sanofi Diagnostics Pasteur, France).

Results

The results of the study are detailed in Table III.

Table III. Results of 7 812 sera tested using the WHO strategy III EIA system

	Biotest screen	Biotest + Pasteur	Biotest + Pasteur + Murex recombinant
Number positive	1 237	1 189	1 180
Discrepant positive		48	9
p24 Ag-positive		0	0
WB-positive		1	4
WB-negative		47	5
True positives		1 185	
True negatives		6 627	

Sensitivity and specificity of the 3 ELISAs when tested sequentially

	Biotest	Pasteur	Murex
Sensitivity	100%	99,9%	99,6%
Specificity	99,2%	99,9%	100%

Discussion

The WHO strategy for HIV testing provides an opportunity for developing countries to adopt HIV testing protocols that maximise accuracy and minimise cost. Inherent in this strategy is the replacement of the WB with a combination of ELISAs that utilise different antigens and have differing test principles. Although combinations of immunoassays have been evaluated before,[4-6] it was deemed essential to evaluate the strategy in the local situation, with sera obtained from the local population. This is of major importance in Africa where serological assays have demonstrated lower sensitivity and specificity.[7,8]

The sensitivity and specificity of the tests used in strategy III showed values that were highly satisfactory (Table III). Although the prevalence of HIV infection in the sera tested was relatively high — 1 185 positive out of 7 812 (15,2%) — the sera included cohorts whose seroprevalence varied from 0,5% to 25,5%. The positive predictive value (PPV) of strategy III at various seroprevalences within this range was ⩾ 99,6% and is in accordance with previously published work.[9] The high sensitivity of the Biotest screening test, demonstrated in previously published results,[4] was confirmed in our study. This test, however, had a high rate of false positives (0,6%).

In the WHO strategy III, which is recommended for the identification of asymptomatic HIV-infected individuals, sera that produce equivocal ELISA results should be retested. If they are repeatedly equivocal, WB testing may be considered, especially in populations that have a low HIV prevalence (< 1%). In addition, a second specimen of serum should be obtained and retested after a period of 2 weeks. Similarly, discrepant results from the first and second ELISAs should not be reported as negative, as is recommended by the WHO strategy, but should be reported as probably negative. These may be cases of early seroconversion. A second serum sample should be requested and retested after a period of 2 weeks. One case of seroconversion in our series was identified by means of a follow-up blood specimen. Discrepant results obtained from the third ELISA in our protocol may indicate infection with HIV-2, and are the reason why an ELISA to detect infection with HIV-1 only was included. Clearly, further testing with appropriate WB for HIV-2 and HIV-1 will help identification.

It is clear from the results of our study that the replacement of the WB with WHO strategy III is to be welcomed. WB tests can produce relatively high rates of false-positive or indeterminate results.[10] The added disadvantages of WB tests are its technical demands and the cost. In this study, the rate of WB usage (4,6%) was low compared with our laboratory's previous protocol.

Quite clearly, there is a considerable cost-saving in employing strategy III. It has previously been shown that at very low prevalences of HIV, where only a relatively small number of all sera are positive (both true positives and false positives), there is little saving in cost. With increasing prevalences there is increased cost-saving[9] which was confirmed in the present study. Estimation of costs related to the use of the individual kits showed that, when dealing with reactive specimens, the 3 ELISA-based strategies would result in an 80% saving in cost, compared with an ELISA/WB strategy. Quite clearly, this figure will vary from laboratory to laboratory according to the choice of individual kits, the particular setting of the laboratory and the throughput of specimens in the system. Nonetheless, a considerable saving in cost will be achieved. Worldwide, laboratory testing accounts for approximately 65% of expenditure of national AIDS programmes.[11] In the developing world, where resources are limited, this saving in cost can be utilised to strengthen other facets of national AIDS programmes. In addition, the saving in 'emotional' costs associated with indeterminate WB results will be obviated. Indeterminate WB results frequently necessitate repeated follow-up testing.

Recently a HIV variant, the so-called subtype O, was described in West Africa[12,13] and France.[14] The genetic composition of this variant differs significantly from the previously described HIV-1 and HIV-2 subtypes. Certain HIV ELISA testing kits failed to detect antibodies in individuals infected with subtype O.[11] The prevalence of this variant in South Africa is unknown. However, after scrutiny of the testing panels,[11] it was noteworthy that the combination of tests chosen in our protocol would not have missed antibodies to subtype O.

Implementation of the WHO strategy III testing protocol for individual patient diagnosis will result in large cost and labour savings, which would substantially improve the efficiency of our national AIDS programme.

It is not the intention of the authors to recommend or endorse any particular manufacturers' kits. The kits chosen fulfilled the basic criteria of strategy III.

We would like to thank Dr S. Lyons for the p24 antigen assays, Mrs J. Winfield for technical assistance and Dr B. van Niekerk for helpful advice. In addition, our sincere thanks to Mrs J. Perks for the preparation of the manuscript.

REFERENCES

1. Constantine HT. Serologic tests for retroviruses: approaching a decade of evolution. *AIDS* 1993; **7:** 1-13.
2. Van der Groen G, Van Kerckhoven I, Vercauteren G, *et al.* Simplified and less expensive confirmatory HIV testing. *Bull World Health Organ* 1991; **69:** 747-752.
3. Global Programme on AIDS. Recommendations for the selection and use of HIV antibody tests. *Wkly Epidemiol Rec* 1992; **67:** 145-149.
4. McAlpine L, Gandji J, Parry JV, Mortimer RP. Thirteen current anti-HIV-1/HIV-2 enzyme immunoassays. How accurate are they? *J Med Virol* 1994; **42:** 115-118.
5. Urassa W, Matunda S, Bredberg-Raden V, Mhalu F, Biberfeld G. Evaluation of the WHO human immunodeficiency virus (HIV) antibody testing strategy for the diagnosis of HIV infection. *Clinical and Diagnostic Virology* 1994; **2:** 1-6.
6. Van Kerkhoven I, Vercauteren G, Piot P, Van der Groen G. Comparative evaluation of 36 commercial assays for detecting antibodies to HIV. *Bull World Health Organ* 1991; **69:** 753-760.
7. Bredberg-Raden V, Kiango J, Mhalu FS, Biberfeld G. Evaluation of commercial enzyme immunoassays for anti-HIV-1 using East African sera. *AIDS* 1988; **2:** 2281-2285.
8. Constantine NT, Fox E, Abbatte EA, Woody JN. Diagnostic usefulness of five screening assays for HIV in an East African city where prevalence of infection is low. *AIDS* 1989; **3:** 313-317.
9. Sato PA, Maskill WJ, Tamashiro H, Heyman DL. Strategies for laboratory HIV testing: an examination of alternative approaches not requiring Western blot. *Bull World Health Organ* 1994; **72:** 129-134.
10. Mortimer PM. The fallibility of HIV Western blot. *Lancet* 1991; **337:** 286-287.
11. Brasseur O. National AIDS Programmes: an analysis of direct costs (Abstract PO-D28-4233). IXth International Conference on AIDS, Berlin, 6-11 June 1993.
12. De Heys R, Vanderborght B, van den Haesevelde M, *et al.* Isolation and partial characterization of an unusual human immunodeficiency retrovirus from two persons of West-Central African origin. *J Virol* 1990; **64:** 1207-1216.
13. Gurtler LG, Hauser PH, Eberle J, *et al.* A new subtype of human immunodeficiency virus type 1 (MVP-5180) from Cameroon. *J Virol* 1994; **68:** 1581-1585.
14. Ly TD, Loussert-Ajaka I, Chaix ML, *et al.* HIV-1/HIV-2 seronegativity in HIV-1 subtype O infected patients in France (Abstract PO-PB0437). IXth International Conference on AIDS, Yokohama, 7-12 August 1994.

Accepted 8 May 1995.