

Strategies for laboratory HIV testing: an examination of alternative approaches not requiring Western blot

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Advances in laboratory tests for antibodies to human immunodeficiency virus (HIV) have permitted the development of alternative HIV testing strategies that do not require use of the Western blot approach. Three strategies are proposed. In strategy I, sera are tested for HIV antibody using an enzyme-linked immunosorbent assay (ELISA)/rapid/simple (ERS) test; in strategy II, sera reactive in an initial ERS test are retested using a second ERS test; strategy III involves retesting with a third ERS test all sera reactive in two previous ERS tests. Where the objective is identification of asymptomatic HIV-infected individuals, strategy III is proposed where HIV prevalences in the study population are $\leq 10\%$, and strategy II at prevalences $>10\%$. Strategy II is recommended where the diagnosis of HIV-related disease requires HIV testing. For serosurveillance, strategy II is recommended if the prevalence is $\leq 10\%$, and strategy I if the prevalences are $>10\%$. Use of strategy I is recommended for transfusion and transplantation safety, at any prevalence. Lower-cost laboratory HIV testing will permit such testing to become more widely available.

Introduction

In many laboratories testing for antibodies to human immunodeficiency virus (HIV) (both HIV-1 and HIV-2) currently implies carrying out Western blot (WB) testing of sera found reactive in initial screening by simpler tests, such as enzyme-linked immunosorbent assays (ELISA). WB testing is relatively expensive and technically demanding. However, recent technological advances have produced tests which, alone or in combination, provide results equivalent in accuracy to those obtained with WB tests (1, 2). These include the newer ELISA tests, as well as "simple" and "rapid" tests. "Simple" tests are those that require no additional equipment or instrumentation, and whose use is easily learned; HIV tests that produce results in 30 minutes or less are referred to as "rapid" tests. Based on these newer ELISA, rapid, and simple (ERS) tests, we propose for routine public health use "alternative" laboratory HIV testing strategies that do not require use of WB.

Materials and methods

Selection of laboratory HIV testing strategies

The most appropriate combination of laboratory tests depends on the following:

- the *objective* of HIV testing in the population concerned;
- the *sensitivity and specificity* of the laboratory tests used;
- the *prevalence* of HIV infections in the population tested; and
- the *operational characteristics* of the tests, including the cost.

These are discussed in detail below.

Objectives of HIV testing. In general, there are five main objectives for HIV testing, the test requirements for which are summarized in Table 1.

Sensitivity and specificity of tests. The sensitivity and specificity of an HIV test (3) are qualitative measures of its ability to distinguish accurately between HIV-infected and HIV-uninfected individuals. A high-sensitivity test produces low rates of false-negative results; in contrast, a high-specificity test produces low rates of false-positive results. Values for the sensitivity and specificity of HIV tests, based on the result obtained with commercially available assays at the Institute of Tropical Medicine, Antwerp, Belgium, a Collaborating Centre on AIDS (4), are shown in Table 2.

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Table 1: Comparison of the requirements for different HIV testing objectives

	Method of collecting blood ^a	Test results	
		False positive	False negative
Identification of asymptomatic HIV-infected persons	Voluntary	Minimal ^b	Very low
Diagnosis of HIV-related disease	Voluntary	Minimal	Very low
Sero-surveillance of HIV infection	Unlinked anonymous	Low	Low
Transfusion safety	Mandatory	Low	Minimal
Research	Voluntary	Low or minimal	Low or minimal

^a Methods recommended by WHO for the testing objective.

^b On the relative scale: low > very low > minimal.

Prevalence of HIV infection in the population. For a given sensitivity and specificity, the probability that the HIV antibody status of a sample is correctly identified varies with the prevalence of HIV infection in the population being tested. As the HIV prevalence increases, the proportion of false-positive results decreases, while the proportion of false-negative test results increases.

The predictive value of a positive test (PPV) is the probability that positive results with a given test or combination of tests correctly represents the presence of HIV antibody, i.e., a test with a high PPV has a low rate of false-positive results. The predictive value of a negative test (NPV) is the probability that a negative test result represents the absence of HIV antibody from the sample; thus, a test with a high NPV has a low rate of false-negative results. PPV and NPV therefore measure the variation in test accuracy with the prevalence of HIV (5).

Operational characteristics for HIV tests. There is an important price difference between WB and ERS tests. Thus, the adoption of testing strategies that do not require WB testing may result in a considerable cost saving (Table 2). Particularly for developing countries, examples of other important operational criteria for tests may include the following:

- whether testing for HIV-1, HIV-2, or both is required;
- suitability for laboratories where few (or many) tests are run daily;
- ancillary equipment and supply requirements (e.g., ELISA readers, pipettes, reliable electrical power supply);

- after-sales support by the local distributor of tests and availability of ancillary equipment/supplies;
- complexity and robustness of the test in a routine public health setting;
- rapidity with which test results are obtained;
- ease of use; and
- storage shelf-life.

Strategies for HIV antibody testing

Based on the sensitivity and specificity of currently available ERS tests, the PPV and NPV for a range of HIV prevalences were estimated for the following testing strategies not requiring WB testing (Fig. 1):

- testing with a single ERS test (strategy I);
- supplemental ERS testing of all samples reactive in an initial (first) ERS test (strategy II); and
- supplemental ERS testing of all samples previously testing reactive in each of two sequential ERS tests (strategy III).

These strategies were compared against the following strategies using WB:

- supplemental WB testing of all samples reactive in an initial ERS test (strategy WB-I); and
- supplemental WB testing of all samples previously reactive in each of two sequential ERS tests (strategy WB-II).

Assuming an overall cost per test (i.e., including the costs of support and of ancillary equipment) of about US\$ 1.50 for ELISA, US\$ 1.00 for a simple test, US\$ 3.00 for a rapid test, and US\$ 25.00 for WB (Table 2), we estimated the cost per serum sample tested using the different strategies. Not all test combinations were allowed; rather, the cheaper tests were used for initial testing, and the more expensive as supplemental tests.

Table 2: HIV test specifications used in the study

Test type	Sensitivity (%) ^a	Specificity (%) ^a	Estimated cost per assay (US \$) ^b
ELISA	99.0	99.0	1.50 ^{c, d}
Rapid test	99.0	99.0	3.00 ^e
Simple test	99.0	99.0	1.00 ^e
Western blot	99.0	99.99	25.00 ^{c, f}

^a Values obtained by an international reference laboratory.

^b Inclusive of related costs, such as staff and ancillary equipment.

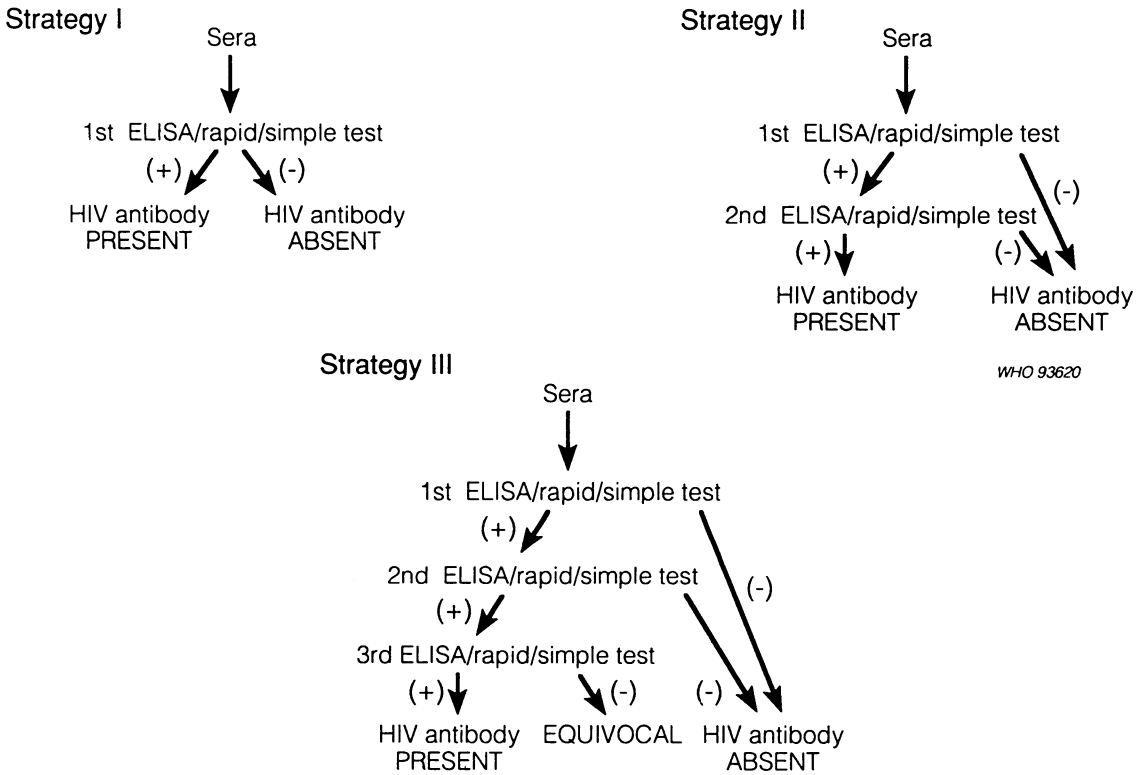
^c Suitable for laboratories with a high HIV-testing volume.

^d Require the availability of an ELISA reader, ELISA washer, an AC power supply, and laboratory consumables.

^e Suitable for laboratories with either high or low HIV-testing volume.

^f Requires the availability of a rocking device, an incubation tray, an AC power supply, a vacuum source, and laboratory consumables.

Fig. 1. Schematic representation of the alternative HIV testing strategies.



The sensitivity and specificity values for WB tests were also based on those obtained by the Institute of Tropical Medicine, Antwerp (4).

Results and conclusions

For the HIV prevalences examined, PPVs virtually equivalent to those for WB-based strategies were obtained with strategy III. At an HIV prevalence of 10%, a high PPV was attainable with all testing strategies: 91.7% with strategy I; 99.9% with strategy II; and >99.99% with strategy III, as well as with the WB-based testing strategies (Fig. 2).

At all HIV prevalences, the highest NPV was obtained with strategy I (Fig. 3).

The estimated median direct costs (including directly related costs, such as staff costs and the cost of ancillary equipment) of the different HIV testing strategies were compared at various HIV prevalences (Fig. 4). At very low HIV prevalences, where only a small proportion of all sera are initially reactive (either true- or false-positives), the cost of the initial ERS testing is decisive, and little savings are

achieved by avoiding WB testing: at an HIV prevalence of 0.1%, the cost saving was about 1%. The proportion of the overall cost of testing due to the cost of supplemental testing increased with the prevalence of HIV, and when the prevalence reach-

Fig. 2. Variation in the positive predictive value (PPV) with HIV prevalence and testing strategy.

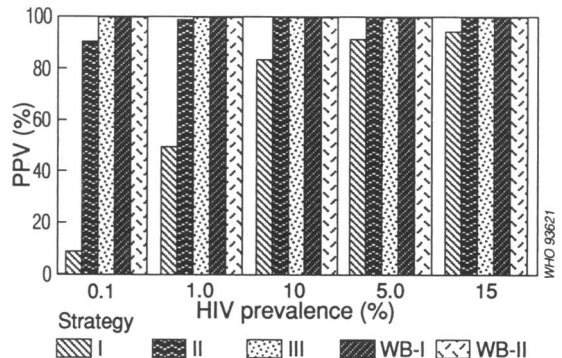
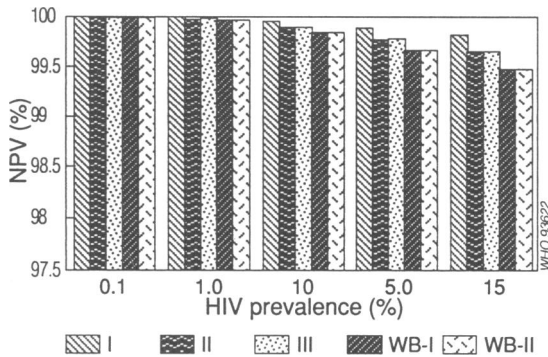


Fig. 3. Variation in negative predictive value (NPV) with HIV prevalence and testing strategy.



ed 10% the cost saving of strategy III was about 50% relative to a WB-based strategy.

Based on the PPV and NPV estimates obtained, alternative laboratory testing strategies that do not require WB testing are outlined below, based on testing objective and HIV prevalence (Table 3),

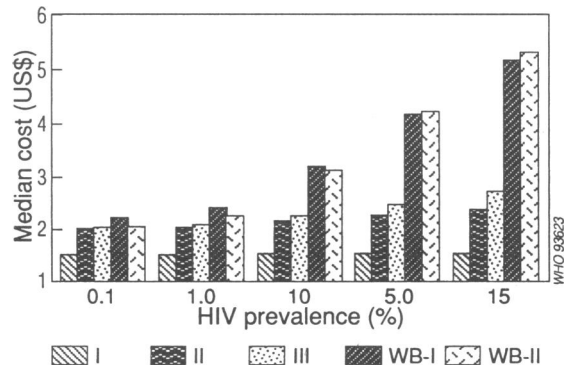
- Where the objective of HIV testing is *identification* of asymptomatic HIV-infected individuals, strategy II can be recommended when the HIV prevalence is >10%; at prevalences ≤10%, strategy III is proposed.
- Where serological confirmation of HIV antibody status is required for the *diagnosis* of HIV-related disease, strategy II is proposed.
- For *serosurveillance* purposes, strategy I can be used when the HIV prevalence is >10%; at prevalences ≤10%, strategy II can be recommended. Equivocal (borderline/indeterminate) results should be reported and analysed separately from definitively positive and negative results.

Table 3: Proposed HIV testing strategies, by testing objective and HIV prevalence

Objective of testing	HIV prevalence (%)	Laboratory testing strategy ^a
Identification of asymptomatic HIV-infected persons	≤10	III
	>10	II
Diagnosis of HIV-related disease	All	II
Serosurveillance	≤10	II
	>10	I
Transfusion safety	All	I

^a See text for details.

Fig. 4. Estimated median cost per serum sample tested (including related costs, e.g., staff), by HIV prevalence and testing strategy.



- Strategy I is the minimum recommended HIV testing strategy when the objective is *transfusion safety* or the *safety of transplantation*. Donated blood, tissues, organs, or sperm with equivocal results should be discarded, and not used further for transfusion or transplantation.
- Where strategy III is used, sera reactive in the first two ERS tests but negative in the third test should be considered equivocal, since such results may represent sera from recent seroconverters (1).

Discussion

The WB is a technically demanding test that has a relatively high rate of equivocal results (6). Until recently, its use was mandatory, since most other HIV tests lacked adequate specificity, particularly where the risk of false-positive results had to be minimized (e.g., the identification of asymptomatic HIV-infected individuals). Today, improvements in the accuracy, rapidity, and ease of use of tests have made technically viable laboratory strategies that dispense with WB testing.

These alternative strategies are particularly appropriate if financial or other resources are limited. At higher HIV prevalences, the cost saving over WB-based strategies may be considerable (Fig. 4). Additional strategies to lower the cost of ERS tests, such as bulk purchase of tests and microassay techniques, are being implemented or are under evaluation by WHO (7).^a

^a Steering Committees of the Global Programme on AIDS. *Priorities for research*. Unpublished document, WHO GPA Office of Research, November 1992.

The HIV prevalence estimates used in the selection of testing strategies should be the local HIV prevalence estimates previously obtained in the target population. For example, previous HIV prevalences found among those attending antenatal clinics will help to determine the best testing strategy for the future. If previous HIV prevalence estimates are not available, a conservative approach may be recommended, and a more rigorous testing strategy adopted, at least for the "start-up" period. For example, a voluntary HIV testing centre (for the identification of asymptomatic HIV-infected individuals) could adopt strategy III for the start-up period, in the absence of any data suggesting that the HIV prevalence in the target population is greater than 10%.

In developing these alternative HIV testing strategies, we assumed that the supplemental (second or third) ERS test had a different antigen preparation or different test principle (e.g., indirect versus competitive) from the first or preceding test. This is generally considered good laboratory practice, since it increases the independence of the first result from subsequent ones (8). To increase the (apparent) HIV prevalence "seen" by supplemental tests, the highest-sensitivity ERS test should be used first and the subsequent tests should be those with higher specificity.

It has been assumed that the strategies for each testing objective would be selected separately, even if samples are obtained at the same site. This is important because in general, the most appropriate strategy depends on the testing objective. For example, if blood donors are to be identified for diagnosis, counselling, and medical/social follow-up, the appropriate laboratory testing strategy for identification should be followed (i.e., strategy III if the estimated HIV prevalence among blood donors is $\leq 10\%$; strategy II if the prevalence is $>10\%$). Otherwise, the risk that a person is falsely informed that he or she has a life-threatening infection would be excessively high (i.e., the PPV would be unacceptably low).

Conversely, the rate of false-negative test results is lowest (NPV highest) if strategy I is followed. Therefore, for transfusion safety, samples of donated blood should be discarded if the *first* ERS test is positive, even if serum from the donor concerned is (voluntarily) subjected to further ERS testing to determine the HIV antibody status. HIV testing for transfusion safety should be mandatory, to protect transfusion recipients from HIV infection; in contrast, HIV testing to confirm the blood donor's HIV infection status must be *voluntary*.

It is essential that laboratory quality assurance procedures be maintained by all HIV testing laboratories. For example, procedures that guarantee the correct identification of HIV-positive units of donated blood for discarding are vital for maintaining a

safe blood supply. Such testing is in addition to that required for the proposed testing strategies alone. If the objective is diagnosis of HIV-related disease or identification of asymptomatic HIV-infected individuals, sera that produce equivocal results should be re-tested. If they are repeatedly equivocal, WB testing may be considered, especially for populations that have a low HIV prevalence ($<1\%$). Alternatively, a second specimen of serum may be obtained a minimum of 2 weeks after the first for repeat laboratory testing.

The proposed testing strategies do not fully take into consideration the complete differentiation of HIV-1 from HIV-2 infections. The importance of such differentiation is principally for the distinction between asymptomatic HIV-1- and asymptomatic HIV-2-infected individuals in situations where the prevalence of HIV-2 infection is significant. Thus, a less precise differentiation between HIV-1 and HIV-2 infections is adequate for surveillance purposes; for transfusion safety, differentiation between HIV-1 and HIV-2 infections is unnecessary.

Since the sensitivity and specificity values that we have used were obtained from an international reference laboratory, they may not reflect the values associated with a routine public health setting. A field trial in selected laboratories in developing countries has therefore been organized to evaluate testing strategies under conditions that more closely mirror those routinely encountered.

Major differences in the sensitivities and specificities obtainable in an international reference laboratory and in laboratories with less rigorous quality assurance standards are as much a problem for current HIV testing strategies as they would be for the alternative strategies, since in such routine settings, WB tests can produce relatively high rates of false-positive or indeterminate results. This arises because (a) the ERS tests used in the proposed alternative testing strategies are those that are already in routine use and (b) WB tests are generally more technically demanding than ERS tests. Thus, adoption of the proposed testing strategies could, by avoiding WB tests, lead to a lower rate of false-negative or false-positive results in routine settings than with WB-based strategies.

The rate of false-positive or false-negative results has also been minimized by adopting relatively high thresholds for changing over to less complex testing strategies (e.g., a threshold of 10% prevalence for changing over from strategy III to strategy II, for the objective of identifying HIV-infected individuals). The higher the HIV prevalence threshold at which the change-over takes place, the smaller the difference in PPV between the two different testing strategies (see Fig. 2).

Our purpose was to develop alternative laboratory HIV testing strategies that do not require WB testing. A major complementary initiative was to evaluate testing strategies that reduce the cost of ERS tests; such a reduction is potentially of equal or greater importance for reducing the overall cost of HIV testing than the non-use of WB tests (see Fig. 4).

Worldwide, laboratory HIV testing accounts for approximately 65% of the expenditure on materials and supplies of national AIDS programmes (9). Therefore, the proposed strategies are recommended for use in settings where resources are limited—recognizing that the lower the cost of testing, the larger the population to which such testing may be made available—thereby strengthening the global prevention and control of AIDS and HIV infections.

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Résumé

Stratégies de dépistage sérologique du VIH: examen des méthodes n'exigeant pas le recours au Western blot

Les progrès récents du dépistage sérologique du VIH permettent l'application de stratégies n'exigeant pas le recours au Western blot. Dans le présent article sont proposées trois de ces stratégies, faisant appel à des tests immuno-enzymatiques (ELISA), des tests rapides ou des tests simples (tests ERS). Dans la stratégie I, les sérums sont testés au moyen d'un test ERS; dans la stratégie II, les sérums trouvés positifs dans un premier test ERS sont à nouveau testés par un deuxième test ERS; dans la stratégie III, tous les sérums trouvés positifs dans les deux tests ERS précédents sont soumis à un troisième test ERS. Lorsque l'objectif du dépistage du VIH

est l'identification de sujets asymptomatiques infectés par le VIH, il faut réduire au minimum l'éventualité d'un test faussement positif: cela est possible en utilisant la stratégie III lorsque la prévalence du VIH dans la population testée est $\leq 10\%$, et la stratégie II lorsqu'elle est $> 10\%$. Pour la sérosurveillance du VIH, le taux de faux positifs doit être relativement faible; la stratégie II est recommandée lorsque la prévalence du VIH est $\leq 10\%$, et la stratégie I lorsqu'elle est $> 10\%$. La stratégie I est recommandée aux fins de sécurité transfusionnelle et de sécurité des transplantations d'organes, car elle comporte un très faible taux de faux négatifs quelle que soit la prévalence du VIH. En abaissant le coût du dépistage du VIH grâce à des stratégies n'exigeant pas le recours au Western blot, il sera possible de le mettre à la portée de plus vastes populations et de consacrer davantage de ressources aux activités de prévention.

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