National strategy for serological diagnosis of HIV infection

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The initial tests for serum antibodies to the human immunodeficiency virus type 1 (HIV-1), introduced in 1985, were enzyme-linked immunosorbent assays (ELISAs) for which viral lysates were the source of antigens. These tests were not 100% sensitive and, because of the presence of antigenic proteins derived from the cells in which the viruses were cultured, biological false-positive results were sometimes obtained, especially with sera from blacks from malaria- endemic areas, sera from individuals with human leucocyte antigen antibodies, or stored sera.[1,2] This necessitated confirmatory testing of all ELISA-positive sera by means of Western blot (WB), radio-immunoprecipitation assay (RIPA) or indirect immunofluorescent antibody assay.

Soon after the release of the first-generation tests, second-generation tests were developed in which the antigens were either (*i*) *recombinant*, i.e. a portion of a viral genome is inserted into a vehicle, e.g. a bacterium or yeast, which is easily cultured and produces large quantities of specific viral antigens; or (*ii*) *synthetic*, i.e. they form peptide chains of 10 - 40 amino acids homologous to a portion of a viral antigen. Tests based on either recombinant or synthetic peptide antigens have sensitivities and specificities higher than first-generation tests; however, recombinant antigen tests may yield some false-positives (lower specificity) due to contaminants from the vehicle, whereas the synthetic antigen tests may lack some sensitivity. The techniques of second-generation ELISA tests have been varied (e.g. antigen and antibody capture, competitive, immunoglobulin class-specific, and substrate amplification assays) in order to improve sensitivity and specificity. Other tests have used agglutination (gelatin particles, red cells, autologous red cells or latex particles), particle adherence or dot-blot techniques, and are technically simple, instrument-free and yield rapid results. Combination assays have been designed to screen for HIV-1, HIV-2, human T-cell lymphotropic virus type I (HTLV-I) and HTLV-II simultaneously. The generally excellent specificity and sensitivity of commercially available second-generation test kits have been evaluated and reviewed several times; sensitivity should approach 100% and specificity should be above 97% with sera from both blacks and whites.[3,4]

In third-generation tests now available, recombinant or synthetic peptide antigens have been incorporated into antigen sandwich ELISAs, with further gains in sensitivity and specificity.[1] All classes of immunoglobulin antibodies are detected, thereby allowing earlier recognition of infections; the 'window' period between infection and seropositivity is shortened by an average of 5 days,[5] the median length of the window having been estimated previously to be 2,1 months.[6]

The conventional method of anti-HIV testing has been to apply a screening test of which all negative results were accepted but positive sera were retested by means of a confirmatory test, usually WB. This procedure was dictated by the low specificity of first-generation tests, and the WB has the disadvantages of being expensive, technically complicated and difficult to interpret, especially in sub-Saharan Africa.[1] It is no longer the gold standard, being less sensitive for detection of early infections than third-generation ELISAs.[5] Early experience in Zambia and the UK showed that HIV testing could be simplified through the application of a first ELISA for screening followed by a second different ELISA for confirmation,[7-10] the latter replacing WB. The two tests should be based on different principles (e.g. indirect and competitive ELISAs) and have different sources of antigen (e.g. viral-lysate and synthetic peptide). The first test should have high sensitivity, be technically simple and inexpensive; the second test should have high specificity. Only the few sera giving discrepant or indeterminate results need to be referred for WB or RIPA, resulting in very considerable savings in costs. It has been shown consistently in 14 published series of over 35 000 sera tested, that algorithms based on many pairs of second-generation tests yield results identical or nearly identical to those obtained from the conventional algorithm, but at only one-third to one-tenth the cost for reagents.[11-22]

Based on this large body of data, the Global Programme on AIDS of the World Health Organisation has recommended that three testing strategies be applied according to the objective of testing (the safety of blood or organ donation, surveillance or diagnosis) and the prevalence of infection in the population.[23] The AIDS Advisory Group of the Department of National Health and Population Development formed a subcommittee in February 1992 to recommend national strategies for HIV testing of sera in South Africa.

Objectives of HIV antibody testing

1. **Safety of blood transfusion/organ donation**. This requires the screening of blood and blood products, and of sera from donors of tissues, organs, sperm ova and breast milk.

2. **Surveillance**. This requires unlinked and anonymous testing of sera for the purpose of monitoring the prevalence of, and trends in, HIV infection over time in a given population.

3. **Diagnosis of HIV infection**. This entails voluntary testing of sera from (*i*) asymptomatic persons; (*ii*) persons with clinical signs and symptoms suggestive of HIV infection or AIDS; and (*iii*) persons in whom the HIV antibody status is required for insurance, travel, immigration or employment purposes. In these situations the requirements for these tests are not necessarily endorsed; however it is recognised that requests for testing will be made and testing will be carried out.

4. **Research.** Voluntary testing of serum from subjects for epidemiological, clinical, virological or other HIV-related studies.

Definitions

Sensitivity and specificity of antibody tests

A test with a high sensitivity will have few false-negative results. Therefore, only tests of highest possible sensiti-

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vity should be used when there is a need to minimise the rate of false-negative results (e.g. in testing of blood/organ donations). A test with a high specificity will have few false-positive results and should be used when there is a need to minimise the rate of false-positive results (e.g. in diagnosis of HIV infection).

Positive and negative predictive values

The probability that a test will accurately determine the true infection status of an individual varies with the prevalence of HIV infection in the population from which the person comes. In general, the higher the prevalence of HIV infection, the greater the probability that a person who tests positive is truly infected. The proportion of samples testing false-positive therefore decreases with increasing prevalence (i.e. the greater the positive predictive value [PPV]). Conversely, the likelihood that a person with negative test results is truly uninfected decreases as prevalence increases. Therefore, as prevalence increases, so does the proportion of samples testing false-negative (i.e. the negative predictive value [NPV]).

Equivocal/borderline/indeterminate/doubtful results are test results that are difficult to interpret.

Discrepant/discordant results refer to a situation when a definite positive and negative result are obtained from the same individual on different tests.

Strategies for HIV antibody testing

The WHO recommends three testing strategies to maximise accuracy while minimising cost. Which strategy is most appropriate will depend on the objective of the test and the prevalence of HIV in the population (Table I).

TABLE I.
Recommendations for HIV testing strategies according to test objective and prevalence of infection in the population

Objective of testing	Prevalence of infection	Testing strategy*
Safety of blood transfusions/ organ donations	All prevalences	I
Surveillance	≤ 10%	II
	> 10%	I
Diagnosis		
Clinical signs/symptoms of HIV infection/AIDS	All prevalences	II
Asymptomatic	≤ 10%	III
	> 10%	II

*See text

Strategy I. All sera are tested with one ELISA or rapid/simple assay. Serum that is reactive is considered HIV antibody-positive. Serum that is non-reactive is considered HIV antibody-negative.

Strategy II. All sera are tested first with one ELISA or rapid/simple assay. Serum that is non-reactive on the first test is considered HIV antibody-negative. Any serum found reactive on the first assay is retested (or a second sample from the same subject is tested) with a second ELISA or rapid/simple assay based on a different antigen preparation and/or different test principle (e.g. indirect versus competitive ELISA). Serum that is reactive on both tests is considered HIV antibody-positive. Any serum that is reactive on the first test but non-reactive on the second test is also considered antibody-negative, but in some circumstances will be subject to further tests.

Strategy III. As in strategy II, all sera are tested first with one ELISA or rapid/simple assay and any reactive samples are retested with a different assay. Strategy III, however, requires a third test if serum is found reactive on the second assay. The three tests in this strategy should be based on different antigen preparations and/or different test principles. Serum that is non-reactive on the first test is considered HIV antibody-negative as is serum that is reactive on the first test but non-reactive on the second. Serum reactive on all three tests is considered HIV antibody-positive. Serum that is reactive on the first and second tests but non-reactive on the third test is considered discrepant and should be referred to a reference laboratory.

Design of testing protocols

It is important that quality assurance procedures be stringently complied with so as to maximise the accuracy of the laboratory results. Procedures for detecting both laboratory and clerical errors must be included in all protocols. The selection of appropriate tests will depend on the testing site. At a primary health care level tests not requiring specialised equipment (instrument-free tests) are endorsed. More sophisticated testing systems will be available at regional level, and highly specialised facilities at the reference laboratories.

Donations of blood, organs and tissues

The purpose of HIV antibody testing is to ensure a safe supply of blood/organs/tissues to the recipient. One test, highly sensitive for antibodies to both HIV-1 and HIV-2, is the only requirement. Strategy I should be followed (Table I; Fig. 1). In the event of a patient requiring a transfusion of blood in an emergency situation, before other tests can be carried out, the use of a rapid test is endorsed.

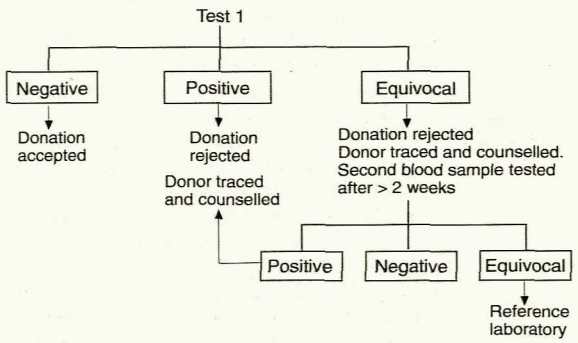
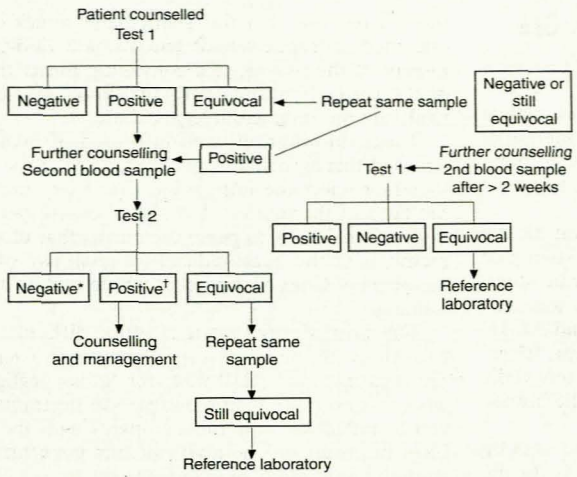


FIG. 1.
Algorithm for donations of blood, organs and tissues.

The donor found to be HIV antibody-positive will be categorised as an asymptomatic or symptomatic patient and will be referred for further diagnostic investigation (Table I; Fig. 2). The blood transfusion service will make a reasonable attempt to inform the donor of an irregular result and will refer the donor for counselling and confirmatory testing. In some instances the blood transfusion service may wish to carry out a confirmatory test for the benefit of the recipients of previous negative donations.

Surveillance

Unlinked and anonymous HIV antibody testing is performed to assess the prevalence of HIV infection in a population at a given time and to monitor trends over time. Strategy II is recommended in populations of low prevalence (≤ 10%) and strategy I in populations of higher prevalence (> 10%) (Table I). At present in South Africa, testing for the purpose of surveillance should follow strategy II. Strategy I will be adopted at a later stage if seroprevalences increase above 10% and, the PPV of a single test will therefore have increased to an acceptable level (Fig. 3).

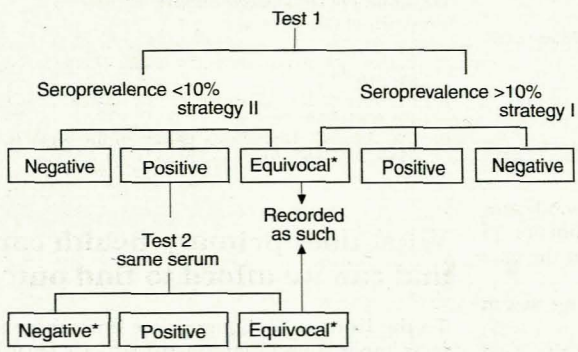


* Discrepant results, i.e. positive on test 1, negative on test 2; the clinician must counsel and retest the patient.

† For the confirmation of seropositivity in an asymptomatic subject from a population where the seroprevalence is ≤ 10%, a third test of high specificity should be applied to the second blood sample (strategy III).

FIG. 2.
Diagnosis: all symptomatic subjects and asymptomatic subjects in populations where seroprevalence is > 10% (strategy II).

Tests designed to detect only anti-HIV-1 may be used for national surveillance for as long as HIV-2 prevalence remains low. It is recommended that tests that detect antibodies to both HIV-1 and HIV-2 be used to monitor their spread in *high-risk sentinel* populations. Whenever HIV-2 is found to be a growing problem, antibodies to this virus should be tested for in national surveillance programmes.



* May be sent to reference laboratories for research purposes.

FIG. 3.
Algorithm for serosurveillance.

Diagnostic

Accurate testing is also crucial in respect of HIV antibody testing for diagnostic purposes. HIV infection is a fatal condition with a considerable impact on the lifestyle of the infected individual, and still carries considerable stigmatisation. Pre-test counselling must be carried out. *A second blood sample must be obtained from all persons who react positively on the first test. The opportunity to test a second sample will help eliminate any possible laboratory or clerical errors. At the time the second sample is obtained the patient should be offered further counselling.* Strategy II is recommended for testing all patients with symptoms suggestive of HIV infection and all asymptomatic patients drawn from populations where seroprevalence is greater than 10% (Fig. 2).

If the patient is not available to give a second sample, the first sample should be subjected to a second test and the results of both tests recorded: if the patient is seen at a later date, the patient should not be informed that he/she is infected until a second sample is collected, tested and found positive. Whenever the first test is positive and the second negative, a specimen error is the likely explanation for the discrepant results. The patient should be counselled again and retested. Should the result of the first test be equivocal, it is recommended that a further sample be collected after a minimum period of 2 weeks. If the result remains equivocal, serum should be submitted to a designated reference laboratory. Strategy III is recommended for testing all asymptomatic patients drawn from populations where seroprevalence is 10% or less (Fig. 2, footnote).

In South Africa it is anticipated that initial testing by means of instrument-free and technically simple kits could be performed at the primary health care level, and the second and third tests at a district/regional laboratory. Sera providing discrepant results could be sent to a reference laboratory of which there should be between six and eight designated to act in this capacity.

REFERENCES

1. Constantine NT. Serologic tests for the retroviruses: approaching a decade of evolution. *AIDS* 1993; **7:** 1-13.
2. Hunsmann G, Schneider J, Wendler I, Fleming AF. HTLV positivity in Africans. *Lancet* 1985; **2:** 952-953.
3. Van Kerckhoven 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.
4. World Health Organisation Global Programme on AIDS. *Operational Characteristics of Commercially Available Assays to Detect Antibodies to HIV-1 and/or HIV-2 in Human Sera.* (Report 5. GPA/RES DIA/92). Geneva: WHO, 1992.
5. Zaaijer HL, Van Exel-Oehlers P, Kraaijeveld T, *et al.* Early detection of antibodies to HIV-1 by third-generation assays. *Lancet* 1992; **340:** 770-772.
6. Horsburgh CR, Ou C-Y, Jason J, *et al.* Duration of human immunodeficiency virus infection before detection of antibody. *Lancet* 1989; **2:** 637-640.
7. Fleming AF. AIDS in Africa — an update. *AIDS Forschung* 1988; **3:** 116-138.
8. Simooya OO, Mwendapole RM, Siziya S, Fleming AF. Relation between falciparum malaria and HIV seropositivity in Ndola, Zambia. *BMJ* 1988; **297:** 30-31.
9. Day A, Mortimer PP. Simplified confirmatory HIV testing. *Lancet* 1988; **2:** 509-510.
10. Fleming AF. Simplified confirmatory HIV testing. *Lancet* 1988; **2:** 848.
11. Spielberg F, Kabeya CM, Quinn TC, *et al.* Performance and cost-effectiveness of a dual rapid assay system for screening and confirmation of human immunodeficiency virus type 1 seropositivity. *J Clin Microbiol* 1990; **28:** 303-306.
12. Nick S, Chimfuembe E, Hunsmann G, Fleming AF. Simple and inexpensive detection and confirmation of anti-HIV-1 in sera from Africa. *AIDS* 1991; **5:** 232-233.
13. Laleman G, Kambale M, Van Kerckhoven I, *et al.* A simplified and less expensive strategy for confirming anti-HIV-1 screening results in a diagnostic laboratory in Lubumbashi, Zaire. *Ann Soc Belge Méd Trop* 1991; **71:** 287-294.
14. Mitchell SW, Mboup S, Mingle J, *et al.* Field evaluation of alternative HIV testing strategy with a rapid immunobinding assay and an agglutination assay. *Lancet* 1991; **337:** 1328-1331.
15. Van der Groen G, Van Kerckhoven I, Vercauteren G, Piot P. Simplified and less expensive confirmatory HIV testing. *Bull World Health Organ* 1991; **69:** 747-752.
16. Shaw MA, Turner AC, Blackwell JM, *et al.* Setting up HIV serology for the Karonga leprosy vaccine trial in Malawi. *Lepr Rev* 1991; **62:** 87-104.
17. Nunn AJ, Biryahwaho B, Downing RG, *et al.* HIV-1 test algorithms: results from a rural Ugandan population cohort (Abstract T TR 235). Sixth International Conference on AIDS in Africa, Dakar, Dec 1991.
18. Kouadio J, Maran M, Brattegaard K, *et al.* Evaluation of an alternative testing strategy for HIV-1 and HIV-2 infections when laboratory resources are limited. (Abstract TO 110). Sixth International Conference on AIDS in Africa, Dakar, Dec 1991.
19. Urassa WK, Bredberg-Rådén U, Mbena E, *et al.* Alternative confirmatory strategies in HIV-1 antibody testing. *J Acquir Immune Defic Syndr* 1992; **5:** 170-176.
20. Nkengasong J, Van Kerckhoven I, Carpels G, *et al.* HIV screening and confirmation: a simplified and less expensive testing algorithm. *Ann Soc Belge Méd Trop* 1992; **72:** 129-139.
21. Behets F, Bishagara K, Disasi A, *et al.* Diagnosis of HIV infection with instrument-free assays as an alternative to the ELISA western blot testing strategy: an evaluation in central Africa. *J Acquir Immune Defic Syndr* 1992; **5:** 878-882.
22. Mortimer J. An alternative approach to confirming anti-HIV reactivity: a multi-country collaborative study. *Bull World Health Organ* 1992; **70:** 751-756.
23. World Health Organisation Global Programme on AIDS. Recommendations for the selection and use of HIV antibody tests. *Wkly Epidem Rec* 1992; **67:** 145-149.