HIV testing methods

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UNAIDS *Best Practice* Collection

At a Glance

- The three main objectives for HIV tests are (1) screening of donated blood, (2) epidemiological surveillance of HIV prevalence or trends and (3) diagnosis of infection in individuals.
- No single test suits all local conditions or objectives. Different objectives require specific strategies (combination and sequence of tests), while local conditions such as daily volume of tests, staff training levels and comparative costs will influence the type of test chosen.
- The most commonly used screening tests, ELISAs, are appropriate for blood banks doing over 100 samples per day or batch testing for surveillance. For other settings, simple/rapid tests which do not need special equipment or highly trained staff are more appropriate. Both types of test are equally reliable, provided they are used correctly.
- Initial positive results cannot be regarded as conclusive, and must therefore always be confirmed using the appropriate supplemental test(s) before individuals are notified of their HIV serostatus.
- The majority of tests are based on detection of antibodies to HIV in serum or plasma. However, tests are also available that use whole blood, dried bloodspots, saliva and urine. Since collection procedures for these alternative specimens are "user-friendly" and can be done almost anywhere, they are useful for testing hard-to-reach population groups such as sex workers and injecting drug users.
- The recent development known as "home testing" actually includes two different systems. Home collection tests allow users to collect their own sample at home, which they send by mail to a testing facility. Home self-tests are true do-it-yourself products which can be used at home without advice or assistance from anyone else.
- In late 1996, the first sales of a home collection test were authorized on a limited basis in the United States of America. To date, no HIV self-test has been approved by national regulatory authorities in any country.
- HIV test kits account for a substantial portion of spending on HIV/AIDS control. Since 1990, WHO has helped national governments and agencies to obtain high-quality kits at low cost through international tendering for bulk purchases.

UNAIDS *Best Practice* materials

The Joint United Nations Programme on HIV/AIDS (UNAIDS) is preparing materials on subjects of relevance to HIV infection and AIDS, the causes and consequences of the epidemic, and best practices in AIDS prevention, care and support. A *Best Practice* Collection on any one subject typically includes a short publication for journalists and community leaders (Point of View); a technical summary of the issues, challenges and solutions (Technical Update); case studies from around the world (*Best Practice* Case Studies); a set of presentation graphics; and a listing of key materials (reports, articles, books, audiovisuals, etc.) on the subject. These documents are updated as necessary.

Technical Updates and Points of View are being published in English, French, Russian and Spanish. Single copies of Best Practice materials are available free from UNAIDS Information Centres. To find the closest one, visit UNAIDS on the Internet (http://www.unaids.org), contact UNAIDS by email (unaids@unaids.org) or telephone (+41 22 791 4651), or write to the UNAIDS Information Centre, 20 Avenue Appia, 1211 Geneva 27, Switzerland.

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1. Acquired immunodeficiency syndrome—diagnosis
2. AIDS serodiagnosis

WC 503.1

Background

Since 1985, HIV testing has been essential in securing the safety of blood supplies, monitoring the progress of the epidemic and diagnosing individuals infected with the virus. Various assays are now available, allowing testing strategies to be tailored to the epidemiological conditions and budgets of national health systems. New techniques—including simple tests giving instant results—hold great promise, but also raise some serious issues for governments and for individuals.

HIV infection is most frequently diagnosed by detecting antibodies which the body produces as it tries to resist the virus. These antibodies usually begin to be produced within 3 to 8 weeks after the time of infection. The period following infection but before the antibodies become detectable is known as the "window period."

Antibodies are much easier to detect than the virus itself. It is sometimes possible to detect HIV antigen during the window period if, by coincidence, an individual is tested during the short peak of high levels of circulating virus particles. After this peak, the level of p24 antigen steeply declines to the point where it is no longer detectable. It fluctuates or rises steeply again, usually years later, when the clinical situation of the patient starts to deteriorate with the onset of AIDS.

Testing objectives

There are three main objectives for HIV tests:

- *screening* of donated blood to reduce transmission of the virus through transfusions;
- *surveillance* of HIV prevalence or trends over time in a given population, through "unlinked" testing of serum (anonymous testing for statistical purposes only);
- *diagnosis* of infection in individuals. The accuracy or reliability of different HIV tests is measured according to their sensitivity and specificity. A test with high *sensitivity* is one that can detect even minute amounts of antibodies. A test with high *specificity* is one which identifies all negatives correctly (i.e. produces no false positives).

Tests with high sensitivity should be used when the objective is to minimize the number of false-negative results, such as in screening of donated blood. When the objective is to minimize false-positives, such as in confirming whether an individual is HIV-infected, tests with high specificity should be used. In areas where HIV prevalence is low, most positive results in initial screening tests are in fact false-positives, so supplemental tests should always be performed if the objective is diagnosis. Today's standards require HIV tests to have a sensitivity of >99% and a specificity of >98%. (Note that the specificity of test kits may vary according to the geographical origin of the serum samples.)

There is no single test suitable for all objectives in all settings. For this reason, different types of tests based on different technologies are often used in combination, depending on the testing objective and the assays' cost-effectiveness under local conditions (number of samples taken per day, size and quality of laboratories, skills of staff, etc.). The choice and sequence of tests can have major cost implications (see Key Materials, Tamashiro *et al.*).

ELISAs

The most commonly used type of test for screening is the ELISA (enzyme-linked immunosorbent assay). ELISAs are probably the most efficient tests for testing large numbers of samples per day, as in large blood banks or for surveillance studies. Since ELISAs require skilled technical staff, equipment maintenance and a steady power supply, they are less suitable for smaller or more isolated hospitals, clinics or laboratories. Regular maintenance of the ELISA equipment is crucial to obtaining reliable results.

Simple/rapid tests

Several tests for antibodies do not need special equipment or highly trained staff, although they can equal the performance of ELISAs. These tests are called rapid if they take less than 10 minutes and simple if they take longer. There are four types: agglutination assays, comb/dipstick assays, flow-through membrane assays and lateral flow membrane assays. In most formats, a positive result is indicated by the appearance of a clearly visible dot or line. Many of these tests have an internal sample addition control that validates each test run.

These tests are appropriate for use in small laboratories and for emergency testing in both developing and industrialized countries. Currently, however, the test kits are relatively expensive and most require refrigeration.

Tests not using plasma or serum

Tests are available that can use whole blood, dried bloodspots, saliva or urine. The collection of these specimens is more client-friendly than traditional blood sampling by venepuncture, and can be done in informal situations such as in a bar or on the street. In particular, saliva and urine tests have proven useful for anonymous testing of hard-to-reach population groups such as sex workers and injecting drug users, and for people who are opposed for religious reasons to giving blood.

The level of antibodies in these alternative specimens is much lower than in serum or plasma, and therefore specific testing procedures must be followed rigorously. While these tests are sufficiently sensitive for surveillance, confirmation of a positive result for diagnosis still requires a serum or plasma sample.

Confirmatory tests

While screening tests are adequate to protect the blood supply, more specific supplemental tests are required for diagnosis, i.e. to confirm that an initial positive result correctly indicates an HIV infection. These tests, which detect antibodies to specific HIV-1 and/or HIV-2 proteins, are more expensive than those used for screening. The most common confirmatory tests are the Western blot and line immunoassays. The indirect fluorescent antibody assay (IFA) is still used in some countries, although it is less sensitive than more recent generations of screening tests. Alternative testing strategies using combinations of screening tests can also be used to confirm initial positive results (see Key Materials, Andersson *et al*).

Tests to detect the virus itself

The first assays capable of detecting free circulating HIV particles were the HIV p24 antigen ELISAs. Since the levels of virus particles and those of the antibodies to p24 fluctuate according to the stage of infection, however, the usefulness of this test is limited (see Key Materials, Bush & Alter).

New technologies based on the amplification of viral nucleic acids, such as PCR and NASBA, or the amplification of the probe binding signal as in branched-DNA tests, have made it possible to detect minute amounts of viral material. These sensitive procedures are well-suited to early diagnosis of mother-to-child transmission and to monitoring the viral load of patients who are taking antiretroviral therapy. However, the tests are very expensive (US$ 60–100), need complex equipment, rigorous laboratory conditions and highly trained staff, and are still largely a research tool. Many of these tests need further refinement since not all HIV-1 subtypes are equally well detected, nor is HIV-2.

The development of home testing

The combination of simple/rapid tests with easy specimen collection has made HIV home tests a practical reality. Currently, the term "home testing" is somewhat confusing because it is used to refer to two different testing systems:

- *Home collection tests* provide users with a kit to collect their own sample (usually a blood spot from a finger-prick) at home. The users then mail the sample by post to a testing facility, wait a week, then phone the facility to check on their HIV status. If the result is negative, the user is counselled with a recorded message which explains the result and its implications. If the result is positive, a trained counsellor speaks directly with the user. The testing is anonymous since identification is by kit number only.
- *Home self-tests* are true do-it-yourself products which provide an instant result and could be used at home without advice or assistance from anyone else.

In late 1996, the US Food and Drug Administration (FDA) approved the first home collection test kit for limited sales in two states. The results of this initial marketing are being followed with interest. To the knowledge of UNAIDS, no HIV self-test has been approved by national regulatory or control authorities in any country.

The Challenges

The choice and distribution of HIV testing methods pose a variety of technical, financial and ethical challenges to public health decision-makers and health care staff.

Cost-effectiveness decisions

HIV test kits account for a substantial portion of the budget in all national AIDS programmes. Therefore analysis and control of their costs are extremely important.

Prices of tests vary greatly. Recent figures range from US$ 0.45–2.00 for screening tests to over US$ 30.00 for confirmatory tests. Some of the cheapest simple tests can be obtained for less than US$ 1.00. Locally produced tests—currently being manufactured in countries such as Argentina, India and Thailand—can be even less expensive, although purchasers must make sure that they meet today's standards.

Considerable care must be given to choosing the most appropriate and cost-effective products for each particular setting. Besides the cost of the test kits, other factors such as storage, equipment maintenance and training of personnel must be taken into account. Reconciling all of these variables within a given budget can be a difficult challenge.

Ensuring quality of diagnostic products and testing procedures

The ability to generate reliable results depends not only on the quality of the test itself, but also on rigorous standards in the laboratory that processes it. Ensuring that quality is maintained and standard operating procedures are followed is an ongoing challenge to all laboratories.

The international market currently offers a variety of HIV diagnostic products which, if used according to instructions, perform very well. Nevertheless, purchasers should be aware that some test kits (both ELISAs and simple/rapid tests) produced by smaller companies do not meet today's standards. Also, unscrupulous entrepreneurs sometimes make inflated or false claims for their products; in fact, UNAIDS is aware of self-tests currently being marketed in brochures and on the Internet with fraudulent claims of approval by WHO or by the US Food and Drug Administration. (Since neither WHO nor UNAIDS has a mandate to issue approvals or licences for products, any claims of such approval are false.)

The challenges of home testing

Knowledge of one's HIV status allows people to make informed personal choices and decisions about prevention and care. Home testing offers an alternative to people who might otherwise not seek testing in traditional health care facilities. However, it is not clear whether this potential benefit might be accompanied by certain negative impacts on public health.

Only one other technically comparable home test—for pregnancy—is widely available in industrialized countries. While they have potential advantages if they allow women to get an prompt diagnosis and therefore seek earlier prenatal care, home pregnancy tests are, on average, less accurate than those administered by trained staff. As well, users need to understand package instructions and the implications of proper timing, since the latency period poses the same problem as the HIV "window". (See Key Materials, Schopper & Vercauteren, p 1461.)

Governments need to address a number of ethical and technical questions raised by HIV home testing, and carefully balance the dangers and potential benefits before giving approval for licensing. Arrangements for immediate access to counselling and confirmation of results will also be necessary. Finally, it should be borne in mind that home testing carries significant risk for abuse if people are forced to take tests against their will.

Comparing reliability

At country level, simple/rapid HIV tests have often proven to be more reliable than ELISAs.

The Responses

The challenges of reducing the costs of testing while maintaining or increasing effectiveness are being met in a variety of ways. National and local choices of testing strategies, and well-conceived purchasing plans all play an important part in this.

Responses to the challenges of home testing are less clear, but the potential impacts of widespread home testing are such that rapid action—particularly in research and policy formation—is urgent. (For more information, see UNAIDS *Policy on HIV testing and counselling*.)

Quality control of HIV diagnostic products

Standards and regulations governing the licencing of HIV tests vary slightly between most countries. Countries which do not have their own regulatory agencies usually adopt standards set elsewhere. As a safety mechanism, these countries should not permit the sale of any diagnostic product which is not approved in the product's country of origin. This helps ensure the quality of the product and makes dumping of lower-quality products to developing countries more difficult.

WHO has been evaluating diagnostic products since 1989. The results are published in the WHO series *Operational Characteristics of Commercially Available Assays to Detect Antibodies to HIV-1 and/or HIV-2 in Human Sera* (see Key Materials).

Quality management of laboratory results

Since samples must be processed correctly and consistently, all laboratories should have quality assurance programmes to ensure that the results forwarded by the laboratory are accurate and reliable. It is also strongly recommended that each country establish a national external quality control assessment scheme (EQAS) that enrols all laboratories performing HIV tests, whether they are public or private sector.

Selection of assays and cost-effective testing strategies

In 1992, WHO issued recommendations for the selection and use of HIV antibody tests using serum or plasma. It proposed three testing strategies aimed at providing maximum accuracy with minimum cost.

Since then, the market for testing products has changed, notably with the development of increasingly sensitive assays to reduce the length of the "window period" and the withdrawal from the market of some less sensitive but highly specific tests. Accordingly, the WHO strategies were updated in March 1997. (See WHO/UNAIDS *Revised recommendations for the selection and use of HIV antibody tests* in the Key Materials.)

Major issues about new HIV diagnostic tests

"... In addition, some minimum requirements that must be fulfilled for any tests proposed should be defined, including (1) any test marketed as a self-test must have an internal control mechanism which validates the test result; (2) no test should be marketed in another country before having been approved by the regulatory body of the country of production, as there is a real danger that tests of low quality are brought on the market in developing countries with weak or no regulatory bodies, and (3) clear guidelines must be provided with the test on how to confirm a positive result. Access to counselling, medical and support services should be guaranteed. A legislative framework should be established to minimize the possibility for abuse."

Schopper & Vercauteren, *AIDS*, 1996; 10

WHO/UNAIDS test kit procurement

Since 1990, WHO has assisted national governments and agencies to obtain high-quality HIV test kits at low cost by negotiating bulk purchase prices from selected companies whose test kits have passed the WHO evaluation process. The number of tests thus purchased over the years rose from 2.8 million in 1992 to 4.5 million in 1995, while the average cost per kit fell from US$ 1.07 to US$ 0.83 over the same period.

In partnership with UNAIDS, the bulk purchase programme continues to negotiate prices and to update its list of evaluated kits annually. This list and programme criteria are available from the blood safety unit (BLS) at WHO headquarters.

Use of new technologies to improve HIV testing

New technologies such as lateral flow tests that are simple (i.e. 1 or 2 steps), can use a wide range of samples and provide instant results are changing the HIV testing scene considerably. At the same time, as do-it-yourself home tests have become a practical reality, potential ethical challenges have come to international attention. These are accompanied by practical concerns such as ensuring that counselling is provided, guaranteeing accuracy of the test and making sure that initial reactive results are confirmed properly.

To date, health authorities in Australia, Austria, France, Germany, Japan, the Netherlands, Switzerland and the UK have taken the position that testing for HIV should be performed by health care workers and accompanied by counselling.

Current voluntary counselling and testing programmes have had only moderate success in increasing access to testing among the general population. For instance, many who agree to take an HIV test do not come back to receive results. Tests with instant results have the potential to change this, not through over-the-counter sales but by incorporating their use in existing health care programmes. This would allow individuals who wish to know their HIV status to visit a general practitioner or nearby health care facility, and receive their results "on the spot." If the initial result turns out to be reactive, a blood sample can be taken immediately for further testing and confirmation.

This arrangement would ensure the quality of testing, and ensure that counselling is provided if needed. Using the new technologies in this way would make HIV testing more "client-friendly," and would increase public access to testing without losing reliability.

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Key Materials

General

Schochetman G, George JR (editors). *AIDS testing: a comprehensive guide to technical, medical, social, legal, and management issues*. New York: Springer-Verlag, 1994 edition. New edition includes information on laboratory testing as well as on blood bank and hospital applications, infection in children, pyschosocial and legal issues, and management of testing the workplace and among health care workers.

Quality assurance

Constantine NT, Callahan JD, Watts DM. *HIV testing and quality control: a guide for laboratory personnel*. Durham, NC, USA: Family Health International, 1991. Useful manual covering various aspects of testing including quality control, quality assurance and laboratory techniques.

WHO/UNAIDS/96.5. *Guidelines for organizing national external quality assessment schemes for HIV serological testing*. Document available from WHO headquarters or UNAIDS in Geneva.

HIV antibody tests and testing strategies

WHO. *Operational characte-ristics of commercially available assays to detect antibodies to HIV-1 and/or HIV-2 in human sera*. Series of evaluations of HIV tests performed by WHO/UNAIDS.

WHO/UNAIDS. Revised recommendations for the selection and use of HIV antibody tests. *Weekly Epidemiological Record*, 1997 (March 21): 81–87. Update of WHO-recommended strategies for cost-effective testing programmes.

Tamashiro H; Maskill W; Emmanuel J. Reducing the cost of HIV antibody testing. *Lancet*, 1993; **342**(8863): 87–90. Cost of HIV antibody testing can be reduced by: use of tests appropriate for existing laboratory capabilities; adoption of cost-effective testing strategies; prior pooling of serum samples; and ensuring best possible purchase prices. Cost reduction increases the sustainability of testing programmes, even in settings of limited resources.

Andersson S *et al*. Field evaluation of alternative testing strategies for diagnosis and differentiation of HIV-1 and HIV-2 infections in an HIV-1 and HIV-2 prevalent area. *AIDS*, 1997; **11**:1815–1822. Evaluation using combinations of several anti-HIV screening assays, including simple tests, found that several combinations gave the same diagnostic accuracy as the screening assay followed by Western blot analysis.

Brattegard *et al*. Rapid and simple screening and supplemental testing for HIV-1 and HIV-2 infections in West Africa. *AIDS*, 1993; **7**(6): 883–885. Combination of rapid tests, used as alternative to strategy based on enzyme immunoassay and Western blot, offered comparable performance without requiring running water, electricity or a well-developed laboratory. Limiting factors are costs of tests and training of staff.

Alternative specimens

Bayer R, Stryker J, Smith M. Sounding Board: Testing for HIV infection at home. *New England Journal of Medicine*, 1995 (May 11); **332**(19):1296–1299. A discussion of potential dangers and advantages of home testing in the U.S. context. The article judges that evidence of serious risk is less than that of potential benefits to individual and public health.

Schopper D, Vercauteren G. Testing for HIV at home: what are the issues? *AIDS*, 1996; **10**:1455–1465. Describes available "home tests," and compares their performance with tests using serum or plasma. Discussion of public health issues, including problems of patient compliance.

Antigen testing

Bush M, Alter H. Will human immunodeficiency virus p24 antigen screening increase the safety of the blood supply, and if so at what cost? *Transfusion*, 1995; **35**:536–539. Review of studies investigating costs and benefits of p24 antigen screening. High cost per transmission prevented and workload considerations are among the issues discussed.

Kongsin S, Rerks-Ngarm. *Assessment of HIV testing in blood donations: cost analysis of routine screening with HIV-Ab and HIV-Ag tests in Thailand*. Abstract Mo.C.120, XI International Conference on AIDS, Vancouver, 1996.Donor self-selection method showed cost benefits for routine screening of blood donations, but screening self-declared donations with 3rd generation ELISA is essential for safety. Use of HIV-Ag in screening donated blood proved highly costly even after self-selection and HIV-Ab screening.