



## **RAPID HIV TESTS:**

GUIDELINES FOR USE IN HIV TESTING  
AND COUNSELLING SERVICES IN  
RESOURCE-CONSTRAINED SETTINGS



WORLD HEALTH ORGANIZATION  
DEPARTMENT OF HIV/AIDS





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Geneva 2004



WORLD HEALTH ORGANIZATION



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# Abbreviations

<b>AIDS</b>	acquired immunodeficiency syndrome
<b>ANC</b>	antenatal clinic
<b>ARV</b>	antiretroviral
<b>DBS</b>	dried blood spot
<b>EIA</b>	enzyme immunoassay
<b>ELISA</b>	enzyme-linked immunosorbent assay
<b>EQA</b>	external quality assessment
<b>HBV</b>	hepatitis B virus
<b>HCV</b>	hepatitis C virus
<b>HIV</b>	human immunodeficiency virus
<b>MCH</b>	maternal and child health
<b>MTCT</b>	mother-to-child transmission
<b>NGO</b>	nongovernmental organization
<b>PLHA</b>	person living with HIV/AIDS
<b>PMTCT</b>	prevention of mother-to-child transmission
<b>PT</b>	proficiency testing
<b>QA</b>	quality assurance
<b>QC</b>	quality control
<b>SOP</b>	standard operating procedure
<b>STI</b>	sexually transmitted infection
<b>TB</b>	tuberculosis

## Acknowledgements

This document is the result of collaboration between WHO, the Centers for Disease Control and Prevention and many other contributors. Austin Demby and Peter Crippen of the Centers for Disease Control and Prevention, and David Miller and Gaby Vercauteren of WHO, were primarily responsible for the preparation and completion of the document. Other key contributors included Isabelle De Zoysa, Jos Perriens, Anindya Chatterjee, Scott McGill, Kathleen Casey, Rachel Baggaley and Matthew Chersich.

# Preface

HIV testing and counselling is now recognized as a priority in national HIV programmes because it forms the gateway to HIV/AIDS prevention, care, treatment and support interventions. In order to ensure access to HIV testing for large populations and to facilitate access to ARV treatment in the context of the WHO “3 by 5” initiative, radical scaling up of HIV testing and counselling services is urgently required. The use of rapid HIV tests<sup>1</sup> will facilitate this in many settings, particularly in services where the those most likely to benefit from knowledge of their HIV status can be reached, e.g. for the diagnosis and treatment of tuberculosis (TB) and sexually transmitted infections (STIs), in services providing and linked to the prevention of mother-to-child transmission (MTCT), and in general medical settings.

Among the practical advantages of the introduction of rapid tests for HIV testing and counselling are the following: increased numbers of people benefit from knowing their HIV status; there is an increased uptake of results by people being tested; test results are obtained quickly; and less reliance is placed on laboratory services for obtaining the results.

This document reviews the characteristics of rapid HIV tests which make them suitable for HIV testing and counselling services and discusses practical aspects of their use. Consideration is given to counselling issues, the advantages of rapid tests and the precautions necessary in using them. Testing algorithms for the use of rapid tests and current WHO recommendations are presented. Although rapid HIV tests have been developed which use saliva and urine, this document concentrates on tests involving the use of whole blood, serum or plasma.

These guidelines are aimed at testing and counselling services in resource-constrained settings. Rapid tests are also recognized as an important component of efforts to increase the number of people who know their HIV status in resource-rich countries (7).

The document is aimed at policy-makers, managers of HIV testing and counselling services, and planners of HIV prevention, treatment and care programmes. It may also be useful for clinicians, laboratory staff and HIV counsellors.

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<sup>1</sup> The term **rapid test** is used throughout this document. It is synonymous with the term simple/rapid test that has been used in previous documents (excluding those simple tests taking longer than 30 minutes to perform).

# 1. INTRODUCTION

## 1.1 EVOLUTION OF APPROACHES TO HIV TESTING AND COUNSELLING

HIV testing and counselling have been recognized as necessarily linked since the first HIV enzyme-linked immunosorbent assay (ELISA) tests became available for the identification of HIV infection in the mid-1980s. Pre-test and post-test counselling were seen as crucial for the testing process because of the seriousness of the news of HIV infection for people receiving a positive result. Additionally, the process of pre-test counselling was designed to ensure that those tested were sufficiently informed about the testing process and the potential consequences: counselling made informed consent possible and ensured that people were not tested in a coercive manner. People with HIV needed the support of post-test counselling in order to manage disclosure and cope with living with HIV. This counselling included the provision of information on preventing the infection of partners and families in the future and on decision-making about pregnancy.

In this context, protocols were developed for pre-test counselling for people considering testing and for people found to be negative or positive (post-test counselling). These protocols form the basis of today's pre-test counselling and education and post-test counselling (Appendix 1, 2 and 3).

Given the need to ensure informed consent for everyone being tested, WHO recommends that all clients be provided with sufficient information to enable them to decide whether they want to undergo testing (Appendix 1). This may involve providing pre-test education in an individual or group setting, or, where possible, individual pre-test counselling, and the use of a variety of posters or leaflets. WHO also recommends that ALL people be informed of their test results and that people found to be HIV-positive receive post-test counselling and referral for continuing support. Those found to be negative should be counselled on how to remain so. Results should not be given to groups of people but only to individuals or couples.

## **Guiding principles of expanded HIV testing and counselling**

WHO recommends that the following guiding principles be observed in the provision of all HIV testing and counselling services<sup>2</sup>:

### **a. Testing and Counselling must now be scaled up**

Offering HIV testing and counselling should become standard practice wherever they are likely to enhance the health and well-being of the individual. The objective is to enable the greatest possible number of people to benefit from the ever-improving treatment, care and prevention options and realise their right to the highest attainable standard of health care.

### **b. HIV testing should be voluntary**

Mandatory HIV testing is neither effective for public health purposes nor ethical, because it denies individuals choice and violates principles such as the right to health, including the right to privacy and the ethical duties to obtain informed consent and maintain confidentiality.

Although the process of obtaining informed consent will vary according to different settings, all those offered the test should receive sufficient information and should be helped to an adequate understanding of the testing process and possible consequences of being tested. The three crucial elements in obtaining truly informed consent in HIV testing are:

Providing pre-test information on the purpose of testing, and on the treatment and support available once the result is known

Ensuring understanding

Respecting the individual's right to decide if they want to be tested or not.

Only when these elements are in place will individuals be able to make a fully informed decision on whether or not to be tested in light of their own circumstances and values. Once this is assured, the actual process of obtaining informed consent can be adapted to suit the different settings under which expanded HIV testing and counselling services will be implemented.

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<sup>2</sup> World Health Organization. *The right to know: New approaches to HIV testing and counselling*. Geneva: World Health Organization; 2003.

### **c. Post-test support and services are crucial**

The result of HIV testing should always be offered to the person being tested. It is the person's decision to share this result with others. Along with the result, appropriate post-test information, counselling or referral should be offered according to the result. People who receive positive test results should receive counselling and referral to care, support and treatment.

### **d. Confidentiality must be protected**

All medical records, whether or not they involve HIV-related information, should be managed in accordance with appropriate standards of confidentiality. Only health-care professionals with a direct role in the management of patients or clients should have access to such records or the information they contain, and only on a "need to know" basis. In rare circumstances, confidentiality may be breached where there is a clear indication that a third party may be harmed by the actions of the patient<sup>3</sup>. Steps that apply to such a process include:.

The HIV-positive person (source client) has been thoroughly counselled on the need for partner notification/counselling.

The counselling has failed to achieve the appropriate behavioural changes, including the practising of safer sex.

The source client has refused to notify or consent to the counselling of his/her partner(s).

There is a real risk of HIV transmission to the identifiable partner(s).

The health worker gives the source client reasonable advance notice of the intention to counsel.

The identity of the source client is concealed from the partner(s) if this is possible in practice.

Follow-up is provided to ensure support to those involved as necessary and to prevent violence, family disruption, etc.

<sup>3</sup> *Opening the HIV/AIDS epidemic: Guidance on encouraging beneficial disclosure, ethical partner counselling and appropriate use of HIV case-reporting*. Geneva: UNAIDS/WHO; 2000 (UNAIDS/00.42E) (<http://www.who.int/hiv/pub/vct/en/Opening-E%5b1%5d.pdf>).

## 1.2 EVOLUTION OF TESTS FOR HIV

There has been a fast evolution in HIV diagnostic technology since the first HIV antibody tests became commercially available in 1985. Currently a wide range of different HIV antibody tests is available. These include enzyme linked immunosorbant assays (ELISA) and rapid HIV tests, general and operational characteristics of which are shown in Table 1.

Until the development of rapid tests in 1990 the diagnosis of HIV infection was made by using ELISAs to detect antibodies against HIV. The original ELISAs involved the use of viral lysate, and positive specimens were usually confirmed by means of Western Blot technology, which is technically difficult, time-consuming and expensive. Second-generation and third-generation ELISAs were developed on the basis of recombinant proteins and synthetic peptides, which increased sensitivity and specificity and considerably shortened the interval between the time of infection and the ability to detect HIV antibodies, i.e. the window period. This period has been further reduced by means of combined antigen-antibody ELISAs, comprising the fourth generation of such tests .

There are, however, essential requirements for ensuring that ELISAs can be performed reliably. Laboratory equipment and disposables (pipette tips) have to be available, constant supplies of electricity and clean water are necessary, and regular maintenance of equipment is required. The validity of ELISA results depends on skilled technicians who can operate the equipment, prepare the reagents, and pipette accurately. ELISAs require stable incubation steps and the reagents have to be refrigerated at 2–8 °C.

Advances in technology have led to the development of a wide variety of rapid HIV tests, including agglutination assays, dipstick assays, flow-through membrane assays, and lateral flow membrane assays. Many of these tests are presented as strips or cartridges incorporating the reagents and not requiring additional equipment. They are suitable for the performance of single tests, are easy to use and can be carried out by any health care worker who has received appropriate training. Most rapid HIV test kits can be stored at room temperatures of up to +20–30 °C. Furthermore, the diagnostic performance of high-quality rapid tests is comparable to that of traditional ELISAs (2–4). WHO has developed testing algorithms showing that sequential combinations of two or three antibody tests (ELISAs and/or rapid tests) can be reliably used to confirm HIV test results (5, 6).

**TABLE 1. GENERAL AND OPERATIONAL CHARACTERISTICS OF ELISAS AND RAPID TESTS**

	ELISAs	Rapid tests
<b>Detection</b> (sample type/specimen)	HIV antibodies in plasma/serum	Several can detect HIV antibodies in whole blood (finger-prick samples) as well as in serum/plasma.
<b>Accuracy</b> (sensitivity, specificity)	Varies with the test; ELISAs and rapid tests give similar diagnostic performances	
<b>Laboratory equipment</b>	Micropipette, washer, incubator, spectrophotometer	None to minimal (micropipette)
<b>Laboratory personnel</b>	Skilled laboratory technician	Can be performed by any health care worker who has been adequately trained, including counsellors.
<b>Ease of performance*</b>	Level 4	Level 1–3, depending on test type
<b>Time to perform</b>	>2 hours	Mostly 10–30 minutes
<b>Shelf-life</b>	Usually 12 months	Usually 12 months
<b>Storage conditions</b>	2–8 °C	Some 2–8 °C; most 2–30 °C.
<b>Cost per test**</b>	US\$ 0.40–1.20	US\$ 0.47–2.0
<b>Volume of tests</b>	Mostly suitable for medium-volume to large-volume testing, i.e. >40–90 samples per testing tray.	Most kits are suitable for small-volume and large-volume testing, i.e. 1–100 samples per day.

\* Level 1 : little or no laboratory experience required.  
 Level 2 : reagent preparation required; procedure has multiple steps.  
 Level 3 : specific skills required, such as making dilution series or interpretation of agglutination patterns.

Level 4 : trained laboratory technician and complex laboratory equipment required.

\*\* Based on WHO bulk purchase price in 2004, excluding freight and other charges.

### 1.3 HIV TESTING AND COUNSELLING AS AN ENTRY POINT FOR PREVENTION, CARE, TREATMENT AND SUPPORT

The linkages between the testing and counselling service and the health care facility are extremely important for further prevention and care of people living with HIV/AIDS and their families. In addition, testing and counselling services associated with interventions for the prevention of MTCT are being promoted and expanded, particularly in countries where there is a high prevalence of HIV. Because many women present for antenatal care late in pregnancy, same-day testing can be of great advantage. Women who

attend after 36 weeks of pregnancy can gain access to interventions such as short- course ARV regimens. Single-dose nevirapine (maternal and infant) can be given if testing around the time of labour gives a positive result (7). Confirmatory testing should be done after delivery. If a rapid test is performed shortly after delivery, ARV prophylaxis can be given to the infant. Rapid testing in antenatal care settings has been acceptable for both clients/patients and health care providers and has greatly increased the numbers of pregnant women who learn their test results (8).

Rapid tests also play a critical role in the management of occupational and non-occupational exposures to HIV.

In the context of the strategy for putting three million people on antiretroviral (ARV) treatments by the end of 2005 (the “3 by 5” initiative), WHO recommends that the offer of HIV testing and counselling become commonplace in settings where the people most likely to benefit from knowledge of their HIV status can be reached, e.g. services for tuberculosis, sexually transmitted infections, injecting drug use, acute medical care and antenatal care. At the same time, people who want to know their HIV status should have better access to voluntary counselling and testing in a variety of venues. Against a background of community mobilization in relation to the importance of people knowing their HIV status, HIV testing and counselling should be offered whenever a patient shows signs or symptoms of HIV infection or AIDS. This should also be offered if it can be expected to aid clinical diagnosis and the management of the patient. In these circumstances the offer of testing and counselling should be considered the standard of care.

## 2. ADVANTAGES OF USING RAPID TESTS

### 2.1 FEASIBILITY

In countries with a limited laboratory infrastructure the use of HIV rapid testing algorithms has been more feasible and as effective as ELISA/Western Blot algorithms (9–12).

General information on HIV testing and the operational characteristics of different types of rapid tests can be obtained from: [http://www.who.int/EHT/Main\\_areas\\_of\\_work/DIL/Test\\_Kit\\_Evaluations/HIV.htm](http://www.who.int/EHT/Main_areas_of_work/DIL/Test_Kit_Evaluations/HIV.htm)

Box 1 contains a summary of the characteristics of rapid HIV tests recommended for use in HIV testing and counselling programmes. These characteristics are explained further below.

#### **BOX 1. SUMMARY OF CHARACTERISTICS OF RAPID HIV TESTS FOR TESTING AND COUNSELLING PROGRAMMES**

**Accuracy**

High sensitivity >99%

High specificity >99%

High reproducibility\* >98%

**Specimen type**

Preferably for use on whole blood (finger-prick samples) for ease of collection and to avoid the need for centrifugation

**Little laboratory equipment required****No constant electricity or water supply required****Easy to perform**

Little technical training required

Few steps

**Easy to interpret**

Visual interpretation of results, usually without equipment

Stable end-reading point

**Rapid <30 minutes****Storage temperature**

Storage at room temperature for several weeks (provided there are no significant temperature fluctuations)

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**Shelf-life** 12 months or longer

**Number of tests performed**

Suitable for individual and small volume testing, e.g. 1–40 samples per day

**Minimal waste and waste disposal**

**Low cost** Mostly <US\$ 1.0 for the initial screening

\* Reproducibility, expressed as a percentage, is calculated by dividing the number of concordant results by the total number of samples retested.

## 2.2 RAPID TESTS ENABLE DECENTRALIZATION OF HIV TESTING AND COUNSELLING

A key advantage of using WHO prequalified rapid tests is that the reliance on laboratory services for obtaining test results is dramatically reduced if the minimum standards for ensuring the quality of test procedures and record-keeping are observed. This is of major importance because it allows HIV testing and counselling to be decentralized to community services away from major urban centres. Rapid tests are especially suited for use in rural settings. The available data show that there is a high degree of acceptance of decentralized services by clients. Moreover, the speed of obtaining the test results has led to their greatly increased uptake.

## 2.3 ACCEPTABILITY OF HIV TESTING AND COUNSELLING

### 2.3.1 ACCEPTANCE BY CLIENTS AND INCREASED RETURN RATE OF CLIENTS TO COLLECT TEST RESULTS

In developed countries a large proportion of people who are tested for HIV in clinical settings or at voluntary counselling and testing sites do not return for their test results (13–16). This wastes both financial and human resources and means that some people who test positive do not benefit from treatment, care and prevention options.

Many testing and counselling sites have reported an increased demand after rapid testing was introduced, suggesting that many people prefer services where they can receive their test result without delay (17). This is consistent with several studies reporting that a majority of tested people preferred to receive their results on the day of testing (8). In several studies at HIV testing and counselling sites in resource-constrained settings the proportion of patients who received post-test counselling increased significantly after the introduction of rapid testing. A trial in Kenya in which women were randomly

assigned to receive either rapid tests or ELISAs showed that the former significantly increased the proportion of clients receiving test results (18).

### **2.3.2 ACCEPTANCE BY COUNSELLORS**

In some areas, counsellors were initially concerned about the accuracy of rapid testing and the possibility that same-day testing would be more stressful for them. Counsellors often have to prepare themselves emotionally before informing clients about positive test results. Nevertheless, several reports (4, 8) and case studies (19) have indicated that rapid tests improve the acceptability of HIV testing to both providers and clients.

## **2.4 SHORT TIME TO OBTAIN TEST RESULT**

Most rapid tests provide results within 10–30 minutes. Same-day testing is convenient for people attending HIV testing and counselling sites because it reduces travelling times and expenses.

## **2.5 REDUCED COST**

The cost of both ELISAs and rapid tests has decreased substantially. Prices in the WHO bulk procurement scheme range between US\$ 0.40 and \$2.00 per test. In general, rapid tests are slightly more expensive than ELISAs but do not require the initial investment in equipment and ongoing operational expenses. In practice the reagent cost per test result is considerably higher with ELISAs than with rapid tests, unless all 40–90 reagent wells of the former are used.

The Rapid test algorithm leads to a greater proportion of clients receiving their test results, reduced wastage of test kits and increased efficiency. In comparison with other testing strategies, testing algorithms based on rapid tests have a lower cost per patient receiving results (20). Clients' transportation costs and travel times are decreased, as rapid tests provide same-day results, and the costs to health services are decreased because fewer return visits to clinics are required.

## **2.6 EASE OF PERFORMANCE AND EASE OF INTERPRETATION OF TEST RESULTS**

Non-laboratory health care workers can perform most of the rapid tests after basic training (Section 4.5). This training should cover correct client identification, the performance and interpretation of the test within the specified reading time, assuring the quality of results, record-keeping, the

maintenance of client confidentiality, and biosafety, including safe waste disposal.

## **2.7** MINIMAL FACILITIES FOR STORAGE AND SHELF-LIFE

Most rapid tests require no laboratory equipment and can be performed in settings with limited facilities. Many such tests do not require refrigeration and are therefore particularly suitable for remote and rural areas and other sites without a constant electricity supply. However, the temperature should not fall below 2 °C or rise above 20–30 °C, depending on the test kit used. Extreme low or high temperatures affect the quality and shelf-life of diagnostic tests. Consequently, it is advisable to monitor temperature fluctuations in storage rooms. In practice, a refrigerator or an air-conditioned room may be required in tropical climates. Central storage facilities should include adequate cold storage space for all rapid tests in stock. As results are read visually there should be sufficient light to allow for correct interpretation.

Stock management procedures should ensure that remote areas and sites performing comparatively small numbers of tests receive regular supplies with appropriate kit size and a longer shelf-life if required.

## **2.8** FLEXIBILITY IN NUMBERS OF TESTS PERFORMED

Several rapid test kits allow the testing of single specimens whereas the design of ELISAs makes them most suitable for batch testing, i.e. at least 40–90 specimens per run. Depending on the set-up, ELISAs may be suitable for settings in which a large number of tests are performed. However, at many testing and counselling sites the ability to perform single or small numbers of tests is a key advantage.

## **2.9** REDUCTION IN OCCUPATIONAL EXPOSURE RISK

Most occupational exposure occurs during venepuncture. The risk of such exposure is substantially reduced with finger-prick blood collection.

# 3. TESTING STRATEGIES FOR TESTING AND COUNSELLING SERVICES

## 3.1 CALCULATING THE ACCURACY OF HIV TESTS USED IN HIV TESTING AND COUNSELLING

In all testing in HIV testing and counselling services it is essential that the results given to individuals be reliable. The rapid tests that can be obtained through the WHO bulk procurement scheme have been evaluated (21) and have met pre-set criteria. The levels of sensitivity and specificity of these rapid tests are greater than or equal to 99% (Box 2). It should be remembered that no test is 100% sensitive and 100% specific. Numerous studies in many countries have shown that the sensitivity and specificity of rapid tests are similar to those of the standard ELISAs.

### BOX 2: CALCULATING THE ACCURACY OF HIV TESTS

Test result	Actual HIV status		
	HIV-infected	HIV-uninfected	Total
Positive	A	B	A + B
Negative	C	D	C + D
Total	A + C	B + D	

A = people with HIV who test positive (**true positive**)      B = people without HIV who test positive (**false positive**)  
 C = people with HIV who test negative (**false negative**)      D = people without HIV who test negative (**true negative**)  
 A + C = all people who are truly infected with HIV      B + D = all people who are truly uninfected with HIV

- **Sensitivity**

Probability of a positive test in people infected with HIV, expressed as a percentage  
 $A/A+C$

- **Specificity**

Probability of a negative test in people uninfected, expressed as a percentage  
 $D/B+D$

- **Positive predictive value**

Probability that the person is HIV-infected when the test is positive, expressed as a percentage  
 $A/A+B$

- **Negative predictive value**

Probability that the person is uninfected when the test is negative, expressed as a percentage  
 $D/C+D$

The accuracy of the test can be described in terms of the degree to which people with and without HIV infection are correctly categorized. The **sensitivity** of a test is its capacity to correctly identify individuals who are not infected with HIV. Thus a very sensitive test gives few false-negative results. The **specificity** of a test is its capacity to correctly identify individuals that are infected with HIV. Thus a very specific test gives few false-positive results.

Alternatively, the accuracy of a test can be expressed as the extent to which being categorized as positive predicts the presence HIV-infection (**positive predictive value**). Similarly, the **negative predictive value** of a test is the proportion of people with a negative test result who are uninfected. The predictive values are the factors most relevant to the decision as to whether a given test or testing algorithm be employed.

The determinants of predictive values are the specificity and sensitivity of the test and the prevalence of HIV in the population concerned. Even with a very accurate test (high sensitivity and high specificity), in settings with a low HIV prevalence (e.g. <1%) the positive predictive value of a test may not be sufficiently reliable (Table 2). In general, the higher the prevalence of HIV infection in the population, the greater is the probability that a person testing positive is truly infected. With increasing HIV prevalence the proportion of false-positives decreases. Conversely, the probability that a person with a negative test result is uninfected declines slightly as HIV prevalence increases. It is necessary to conduct a second or supplemental test if the first test is reactive, as this markedly increases the positive predictive value (Table 2). In settings with a low-level HIV epidemic<sup>4</sup>, tests with a sensitivity or specificity greater than 99% should be used in order to achieve satisfactory positive predictive values.

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<sup>4</sup> The epidemic state in which HIV has never spread to significant levels in any subpopulation, although HIV infection may have existed for many years. (HIV prevalence has not consistently exceeded 5% in any defined subpopulation.)

**TABLE 2. POSITIVE AND NEGATIVE PREDICTIVE VALUES\* AT VARIOUS HIV PREVALENCES**

HIV prevalence	0.1%	1%	5%	10%	30%
<b>NPV with one non-reactive test</b>	100.0%	100.0%	99.9%	99.9%	99.6%
<b>PPV with one reactive test</b>	9.0%	50%	83.9%	91.7%	98.5%
<b>PPV with two reactive tests</b>	90.8%	99.0%	99.8%	99.9%	100.0%

\* A sensitivity of 99% and a specificity of 99% have been used in these calculations. Predictive values have been rounded to one decimal place.

NPV = negative predictive value.

PPV = positive predictive value.

### 3.2 SELECTION OF TEST KITS AND TESTING ALGORITHMS

Some rapid tests may not have adequate sensitivity or specificity profiles and should not be used. WHO provides reports on evaluations of performance and major operational characteristics of commercially available rapid tests (21). This information can be used to select suitable candidates for national algorithms. The evaluations are available at: [http://www.who.int/EHT/Main\\_areas\\_of\\_work/DIL/Test\\_Kit\\_Evaluations/HIV.htm](http://www.who.int/EHT/Main_areas_of_work/DIL/Test_Kit_Evaluations/HIV.htm)

The selection of the rapid HIV tests and test algorithms to be used in testing and counselling services is a responsibility of national governments and is predominantly performed by health ministries and national AIDS control programmes. This task should not be delegated, either expressly or by neglect, to commercial enterprises, donor agencies or external aid programmes. The decision on which tests to use should be made following country-level technical assessments and the evaluation of other relevant factors, such as cost, current and continued availability, shelf-life and storage requirements. In each country these assessments and follow-up support to the testing and counselling services should preferably be the responsibility of the national HIV reference laboratory and referral laboratories. Before selecting rapid test kits, many countries evaluate tests by using local specimens to confirm sensitivity and specificity in their particular settings. Guidelines are available on the development of country-specific protocols for conducting evaluations of rapid HIV tests and on issues related to the planning of evaluations and to quality assurance (QA), evaluation materials, phases of evaluation, and laboratory safety (22).

Many new rapid tests are being developed and promoted by their manufacturers, while some older tests are no longer available. It is important

that the rapid tests and testing algorithms chosen for use in HIV testing and counselling have been thoroughly evaluated on samples from the geographical region where they are to be used.

The reliability of a result given to an individual depends on the accuracy of the test employed and the testing algorithm, as well as on the overall performance of the testing and counselling service, which is influenced by:

- the quality of the kit and batch (lot number) provided by the manufacturer;

- the transportation and storage conditions of the kits;

- the correct collection and quality of the specimens;

- the skill of the staff who do the testing;

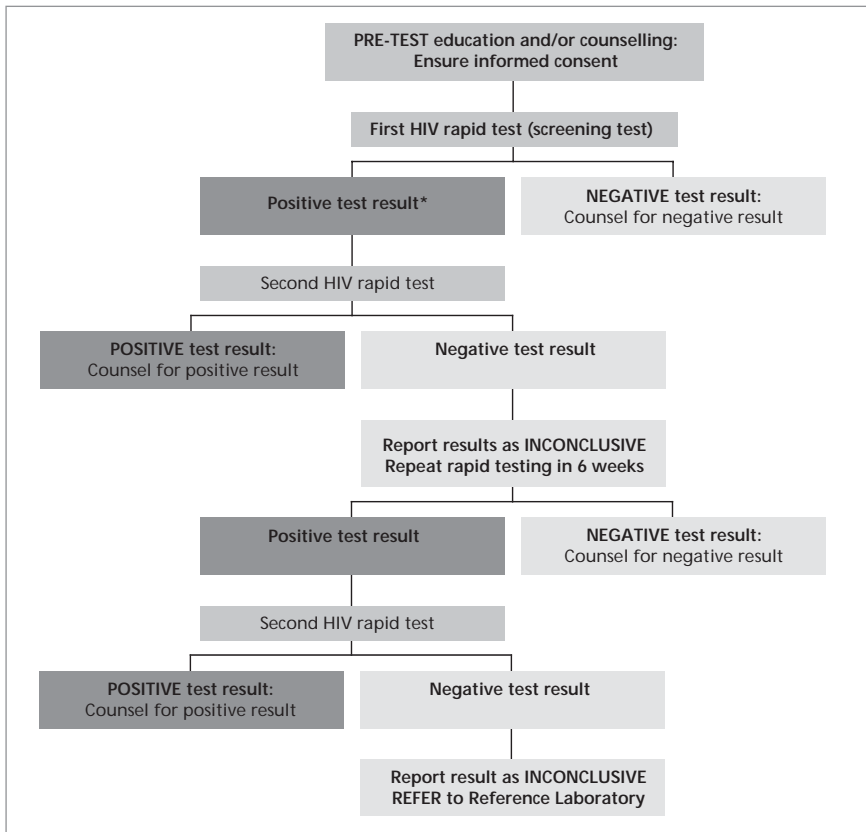
- the presence of a QA system, including standard operating procedures (SOPs) for the complete process, wherever the testing is conducted, whether in a rural testing and counselling service or in a national reference laboratory in a capital city;

- ongoing training and follow-up of testing problems that arise;

- the prevalence of HIV in the population tested, i.e. the proportion of people with HIV infection in the population.

Choosing the most appropriate screening tests and the combination of tests for confirmation is essential to ensure an accurate diagnosis of HIV. The algorithm in Flow Chart 1 is adapted from previously published WHO algorithms for HIV diagnosis and takes into account the increased specificity of current rapid tests, resulting in a higher positive predictive value. Tests can be used in sequence or in parallel (Flow Chart 2).

# FLOW CHART 1. ALGORITHM FOR USE OF RAPID HIV TESTS IN TESTING AND COUNSELLING SERVICES



\* In the context of labour in an MTCT-prevention setting, it is advised to give a single dose of nevirapine on the basis of a single positive rapid test. This should then be confirmed after delivery. In late pregnancy in MTCT-prevention settings

the use of a third rapid test as a tiebreaker may exceptionally be considered after inconclusive results where the need to start short-course ARV prophylaxis must be decided without delay.

When choosing a second test it is important to select one that involves the use of different antigens and/or a different platform and demonstrates appropriate levels of specificity and sensitivity.

If a first positive (reactive) and a second negative (non-reactive) test result occur in more than 5% of cases the testing process should be reviewed.

Suggestions for the content of counselling in the event of negative and positive test results are given in Appendix 1.

If a result is inconclusive the person tested should be advised accordingly. Post-test counselling should focus on the possibility of the test being performed during the window period, i.e. when antibodies have not yet formed after exposure to HIV. All persons with inconclusive results should be encouraged to avoid the possibility of future risk behaviour and should be offered retesting at the same facility after an interval of six weeks in order to allow the window period to have elapsed. Support based in the community or at health centres should be offered during the waiting period. If the same results are given by retesting after six weeks then the person concerned, or a suitable specimen, should be sent to a referral laboratory for further HIV testing.

This algorithm may be used for testing of any persons over the age of eighteen months.

# 4. PRACTICAL CONSIDERATIONS WHEN USING RAPID TESTS

## 4.1 CHOICE OF SPECIMENS TO BE USED IN TESTING

### 4.1.1 WHOLE BLOOD FINGER-PRICK SPECIMENS

Rapid test kits that use whole blood from finger-prick samples may be preferred because such tests are easy to obtain, they require minimal equipment and can be performed by appropriately trained counsellors. Used lancets must NOT be reused. They must be disposed of in an appropriate sharps container and the puncture site must be covered. Depending on the algorithm being used, an additional finger-prick sample may be necessary if a positive result is obtained.

### 4.1.2 SERUM SPECIMENS

In contrast to the use of whole blood finger-prick specimens, the use of serum specimens requires venous blood to be drawn by means of syringes and collection tubes (e.g. vacutainers) and the serum to be separated from the blood. This takes much longer and involves staff who are more highly trained. Moreover, collecting venous blood samples is costly. However, there is a wider range of rapid tests that can be used with serum specimens and only one sample has to be collected.

## 4.2 PARALLEL TESTING VERSUS SERIAL TESTING

Parallel testing involves testing all blood samples with two HIV tests simultaneously, i.e. in parallel. The results are given as indicated in Flow Chart 2. For serial testing an initial blood sample is taken and tested. If the result is negative it is given. If the result is positive the blood sample is tested using a second, different HIV test. If a finger-prick sample has been used a further finger-prick sample must be taken for the second test. WHO recommends serial testing in most settings because it is more economic, a second test being required only when the initial test is positive. The decision on whether to use serial testing or parallel testing should be taken after a thorough analysis of the scientific evidence, logistics, test performance and costing/affordability of the alternative algorithms.

### 4.3 SAFETY PRECAUTIONS

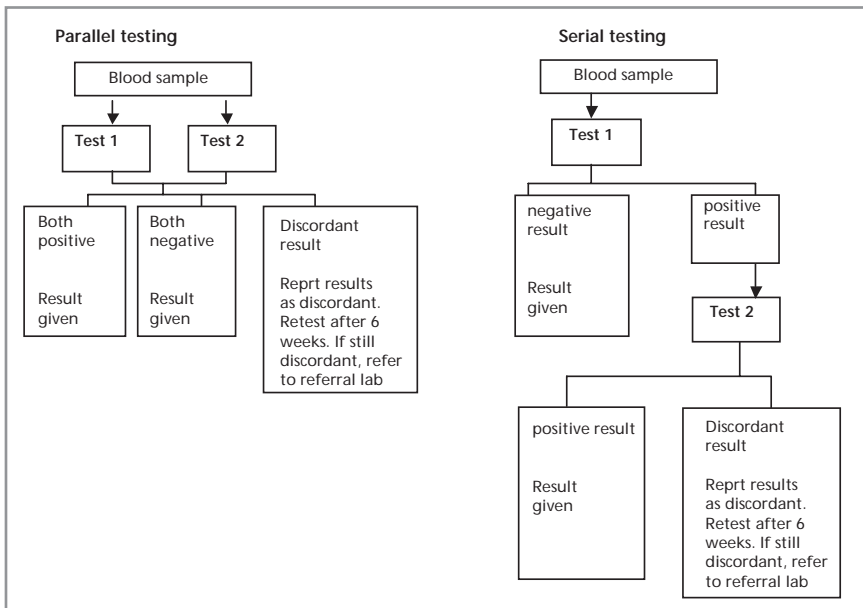
Each laboratory or testing site must follow universal (standard) precautionary measures in order to ensure the safety of health care staff and to prevent the transmission of HIV, hepatitis B virus (HBV), hepatitis C virus (HCV) and other bloodborne pathogens. Under universal precautions, blood and certain body fluids of ALL patients should be considered as potentially infectious for such pathogens. Guidelines for good laboratory practice have been developed which, if followed, can ensure safety and keep accidents to a minimum. Staff should be trained to deal with accidents (e.g. spills) and provided with written copies of safety precautions. For further details see:

*Laboratory biosafety manual* (Second edition). Geneva: World Health Organization; 1993 (ISBN 92 4 154450 3).

*Communicable diseases surveillance and response* section at: <http://www.who.int/emc>

These documents provide information on laboratory biosafety and the transportation of infectious substances. Safety rules for testing areas are given in Box 3.

#### FLOW CHART 2. PARALLEL VERSUS SERIAL TESTING



## 4.4 WHO CAN PERFORM RAPID TESTS?

After appropriate training and with supervision, health care workers with little or no previous laboratory experience can perform most rapid tests. The use of non-laboratory staff facilitates access to testing and counselling in small communities and rural sites where professional laboratory personnel are often unavailable. If non-laboratory personnel are to perform rapid tests, initial training, continuing supervision and periodic assessment of proficiency should be provided in order to ensure that the quality of testing is maintained. In some countries it is necessary to take account of legal restrictions concerning the qualifications of people who perform blood tests: persons lacking the required qualifications should work under the authority of people who possess such qualifications.

### BOX 3. SAFETY RULES FOR TESTING AREAS

The following rules, not necessarily in order of importance, should be observed when work is performed in a laboratory or when laboratory tests are performed at another location.

1. Pipetting by mouth is prohibited.
2. Eating, drinking, smoking, storing food and applying cosmetics is not permitted.
3. Labels must not be licked; materials must not be placed in the mouth.
4. The testing site must be kept neat, clean and free of materials that are not pertinent to the work being done.
5. Work surfaces must be decontaminated after any spill of potentially dangerous material and at the end of each working day.
6. Needles must not be recapped. Sharps such as needles and lancets must not be reused. They must be disposed of in a special waste container.
7. Before taking a finger-prick sample the finger must be decontaminated. After the sample has been obtained the wound must be covered with a plaster.
8. Staff members must wash their hands after handling infectious materials and before leaving the laboratory or testing area.
9. Potentially contaminated and ordinary office waste must be kept in separate and clearly labelled waste containers.
10. All potentially contaminated materials and specimens must be decontaminated before disposal or cleaning for reuse.
11. Only persons who have been advised of the potential hazards are allowed to enter testing areas. Doors must be kept closed when testing is in progress. Children must be excluded from testing areas.

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12. Gloves appropriate for the work must be worn for all procedures that may involve accidental direct contact with blood and infectious materials. After use, gloves should be removed and disposed with other laboratory waste . The hands must then be washed. Do not wash or disinfect surgical or examination gloves with a view to reusing them.
13. All spills, accidents and potential exposures to infectious materials must be reported immediately to a supervisor. A written record of such accidents and incidents must be maintained.
14. Supervisors must ensure that training in testing area safety is provided. A safety manual or operations manual must be adopted that identifies known and potential hazards and specifies practices and procedures for minimizing or eliminating them.

## 4.5 CORE TRAINING FOR PEOPLE ADMINISTERING RAPID HIV TESTING

Training curricula on core competencies for administering rapid tests and managing data associated with testing and counselling are being developed by WHO and key partners. However, such training is presently being provided in some settings (e.g. CDC-Kenya) and the curricula they use comprise the following core topics as a three-day module included within three weeks of training in testing and in counselling:

the virology and immunology of HIV/AIDS and the principles of test kit operation;

SOPs for sample collection, packaging and transportation in relation to rapid testing, quality control (QC) and QA;

biosafety in testing and counselling settings;

principles of HIV testing with particular reference to rapid HIV testing, criteria for test kit selection, testing principles and procedures, the interpretation of test results, and problem-solving;

principles and concepts of QC and QA, particularly in testing and counselling settings;

practical sessions on sample collection, HIV testing and biosafety;

managing data entry and management in testing and counselling services, particularly in order to avoid transcription errors and to maintain confidentiality.

All topics are covered in language appropriate to the audience and suitable examples to illustrate key topics are given throughout. It is recommended that trainers have practical experience of all types of testing and appropriate data management.

## **4.6 DETECTION OF THE DIFFERENCE BETWEEN HIV-1 AND HIV-2**

In some parts of West Africa a significant proportion of HIV infection is attributable to HIV-2. Most rapid tests detect both HIV-1 and HIV-2 but most of these tests do not differentiate between them. Although this is not an immediate concern for testing and counselling services, it may be significant for ARV treatment programmes in regions where HIV-2 is endemic. In these settings, differentiation between HIV-1 and HIV-2 infections may be appropriate before therapy begins and should be performed in referral laboratories.

## **4.7 ROLES OF NATIONAL REFERENCE AND REFERRAL LABORATORIES FOR CONFIRMATION AND QUALITY ASSURANCE**

### **4.7.1 NATIONAL REFERENCE LABORATORIES**

Technical assessments and the evaluation of test kits should be a responsibility of national HIV reference laboratories. The development and review of test algorithms should be undertaken by these laboratories. QA programmes can be supported from these laboratories or similar institutions with professionally trained laboratory staff. Requirements should be rigorously complied with in order to ensure the accuracy and reliability of the results given. National reference laboratories should also coordinate the training of personnel and the QA procedures of testing and counselling services.

### **4.7.2 REFERRAL LABORATORIES**

A crucial advantage of rapid tests is that they enable HIV testing to be done at a decentralized level and that dependence on referral laboratories is minimized (if minimum standards for ensuring the quality of test procedures and record-keeping are observed).

Written policies and SOPs for each key activity within the entire testing process, – from the time when a client enters a testing and counselling centre until a result is issued – assist in identifying problems and areas needing improvement.

Where testing produces discordant results, i.e. the first test is positive (reactive) and the second is negative (non-reactive), the client should be informed that the result is inconclusive and counselling should be provided (see page 22). If the same discordant result is found when retesting is done after six weeks, samples should be sent to a referral laboratory. Backup provided by a referral laboratory is a prerequisite for all testing and counselling facilities. When necessary, referral laboratories can also assist with more sophisticated testing, such as differentiation between HIV-1 and HIV-2 or early detection in infants born to HIV-positive mothers.

Referral laboratories should also support the training of staff in testing and counselling services and should assist with QA procedures.

# 5. QUALITY ASSURANCE

**Q**uality assurance (QA) is the set of planned and systematic activities providing adequate confidence that requirements for quality will be met.

It is critical that each facility performing HIV testing establish and implement a QA programme to monitor and evaluate all functions and services throughout the total testing process, i.e. the entry of the client into the service, the counselling and testing steps, the provision of the result, and possible referral.

## 5.1 QUALITY CONTROL

Quality control (QC) comprises measures for verifying that a test is working. These measures are taken in order to monitor the validity of the technical aspects of the test procedure, concerning, for example, whether all reagents were at room temperature before the test procedure began and whether the control line of the test was clearly visible. QC includes the testing of samples with known results so as to verify that the testing procedure and materials are working properly. When QC specimens that are analysed daily produce acceptable results, and all other conditions related to a test kit performance have been met, the test results for samples from clients can be accepted as valid.

## 5.2 EXTERNAL QUALITY ASSESSMENT

Every testing facility must be able to demonstrate and document its competence in performing all HIV tests. External quality assessment (EQA) is an integral part of any QA programme. EQA focuses on the identification of laboratories or testing sites that perform below standard so that additional training and/or other measures can be instituted to improve their performance. The three complementary ways in which the quality of testing services can be assessed by an external authority are outlined below.

### a. On-site audit

On-site audit is necessary in order to confirm that all SOPs are adhered to, including QC, record-keeping and observation of staff performance. Additionally, on-site audit provides an opportunity to administer a proficiency test directly to each individual who performs testing. A programme of on-

site auditing should include a standard checklist of testing service indicators. Auditors, inspectors and supervisors should be trained to perform consistent reviews of testing sites. Standard checklists and evaluation methods allow for the collection and comparison of consistent information from multiple sites.

#### **b. Proficiency testing or external quality assessment schemes**

Proficiency testing (PT) or EQA schemes involve the distribution of small panels of well-characterized test samples comprising 6–10 specimens by the EQA scheme organizer (e.g. a national reference laboratory or another organizer) to all testing sites. PT/EQA schemes have the limitation that they are spot checks in time. They represent the upper performance level and usually involve a small number of samples. Moreover, there is a limited number of assessments per year. The test results frequently do not represent the routine test performance.

#### **c. Blinded rechecking**

Retesting a selected sample of specimens in a reference laboratory may be an option for assessing the quality of testing. This can be accomplished by forwarding all positive and 5–10% of negative specimens for retesting when a serum or plasma specimen is available.

Alternatively, dried blood spots (DBSs) can be used for blinded rechecking in situations where it is impractical to refer specimens for additional testing (e.g. whole blood finger-prick tests). DBSs are collected on filter paper when the testing of patients occurs and are transported to a reference laboratory. This method requires a laboratory that has demonstrated proficiency in eluting specimens and performing standard enzyme immunoassays (EIAs). Additional concerns include the logistics and methods of collecting DBSs in the testing protocol. Although a sample of specimens retested by DBSs may be desirable, this may be difficult to implement while the testing and counselling of patients is proceeding. Additionally, testing a percentage of specimens, e.g. 10%, may be problematic. Countries may consider random sampling of DBSs, e.g. every two months or at a given time or on a given day. Further development of DBS protocols is necessary in order to assist with the expansion of rapid testing for testing and counselling services, especially at remote sites.

# 6. ADDITIONAL CONSIDERATIONS WHEN USING RAPID TESTS

## 6.1 DECISION TIME OR THINKING TIME

Before rapid tests were widely available, people attending voluntary counselling and testing sites received pre-test counselling before a venous blood sample was taken for HIV testing. There was then a period ranging from three days to two weeks before the clients returned to receive the results of their tests and post-test counselling. It was argued that this waiting time afforded an opportunity to reflect on issues raised in pre-test counselling and to involve partners or family members in decision-making. However, for the majority of people this waiting time is a worrying period and they prefer to receive the result on the day of sampling.

The compression of pre-test and post-test counselling sessions into one period with the absence of a three-day to two-week waiting period led to concerns that the HIV counselling associated with rapid testing might not be as effective in promoting HIV risk reduction as a counselling and testing process spread over several days. However, there is little information comparing the effectiveness of same-day testing and counselling with a testing and counselling process spread over a longer time.

Particular care should be taken for people who are attending services for other reasons and not primarily for HIV testing and counselling, e.g. persons attending antenatal clinics, tuberculosis and general hospital services, and injecting drug users attending drug-dependence treatment programmes. Such people may not fully understand the possible consequences of testing. When rapid tests are used at testing and counselling sites, counsellors should ensure that clients understand the implications of HIV testing and that adequate support is available in the event that positive or inconclusive results are obtained (see **Guiding principles of expanded HIV testing and counselling**, section 1.1). If a client is reluctant or uncertain about HIV testing, it should be deferred and a further pre-test counselling session arranged.

















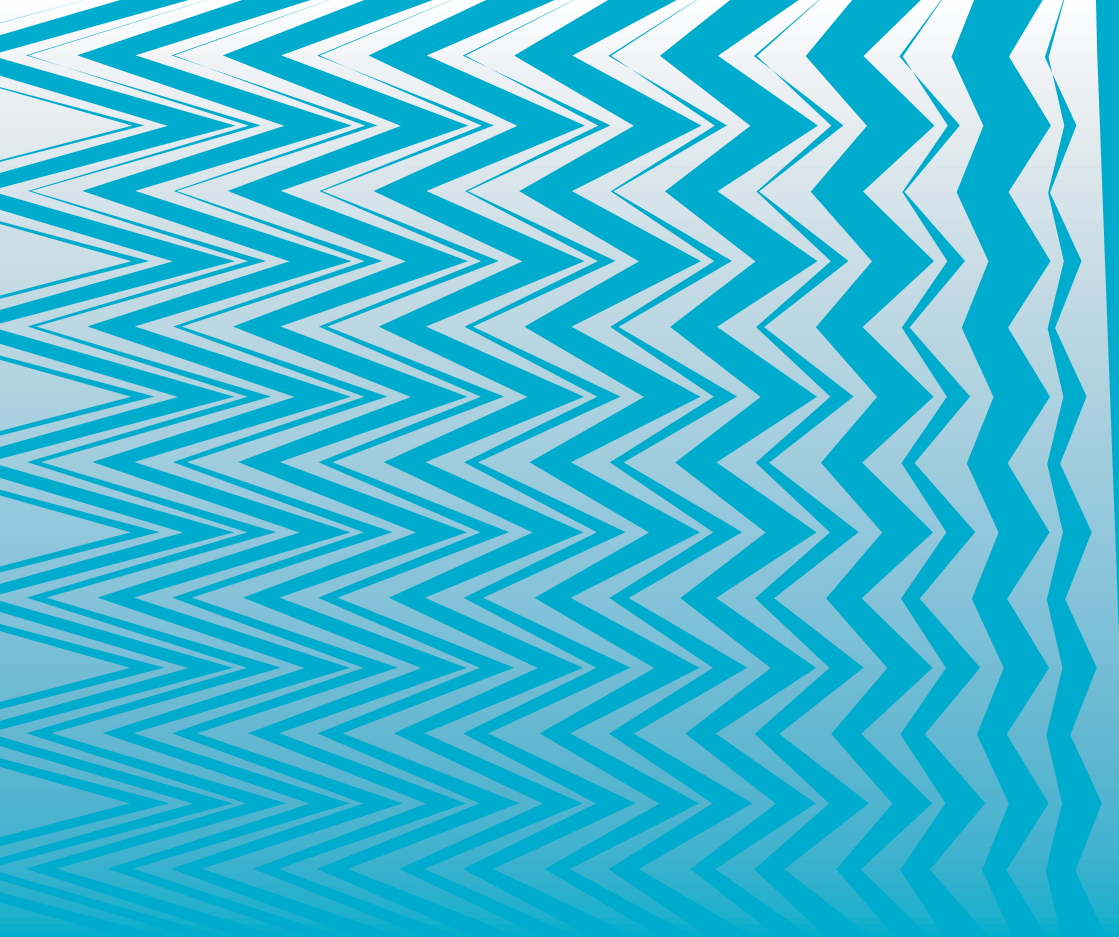












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