

Use of rapid HIV tests in HIV testing strategies



**World Health
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Regional Office for the Eastern Mediterranean

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Contents

Acknowledgements	4
Summary of the guidance	5
Introduction	7
HIV testing methods.....	8
Screening by EIA.....	8
Confirmatory testing by WB.....	9
Rapid tests	11
Fourth generation combined HIV antigen/antibody detection assays	13
Alternative methods to screen for and confirm HIV positivity.....	13
Comparison of characteristics of HIV EIA and rapid HIV tests.....	14
Considerations when choosing HIV tests for use in HIV testing strategies and algorithms.....	16
HIV testing strategies.....	19
General considerations.....	19
Diagnostic HIV testing strategies	20
Conclusion	24
References.....	25
Annex I. Calculations using the recommended test strategies with HIV prevalence ratios.....	27

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Summary of the guidance

HIV testing and counselling (HTC) is recognized as an entry point to HIV/AIDS prevention, treatment, care and support. The purpose of this document is to present the characteristics of rapid HIV tests and their role among the wider range of HIV testing methodologies in facilitating efficient HIV testing on a larger scale in the Eastern Mediterranean Region.

The introduction of rapid HIV testing should be welcomed as these tests offer many advantages for the diverse settings in the Region. The use of rapid testing supports the expansion of HTC services beyond health facilities and is highly beneficial in interventions such as prevention of mother-to-child transmission (PMTCT), and HTC for key populations at higher risk of HIV infection.

Rapid diagnostic HIV tests (RDT) provide an alternative to the conventional testing strategy involving the use of enzyme immunoassay (EIA) and western blot (WB). They are especially suitable for resource-limited settings with low prevalence of HIV and ideal for low specimen throughput. They are accurate, quick, simple to perform and can be cost-saving. They may be performed with little laboratory equipment and few laboratory consumables, and are technically less demanding. A well-trained and competent health worker can perform rapid HIV testing. The main advantage of the use of rapid HIV tests is that they allow return of the test result on the same visit to the persons tested, thus increasing the opportunity for people to know their HIV status and receive the necessary post-test counselling and referrals.

Diagnosis of HIV-seropositivity can be established after conducting three tests in a sequence, after taking into consideration HIV prevalence and the sensitivity of the tests and their specificity. This is known as the three-test serial testing strategy. The exact selection of assays and their sequential use combined with the selection of an appropriate testing strategy is known as a testing algorithm. The accuracy of testing algorithms using rapid tests has been proven to be at least as high as algorithms using EIA and WB combination. Each testing algorithm that is introduced in a specific country or population group must be assessed and validated at country level.

There are some special considerations to keep in mind when selecting assays for use in a country, depending on the country or HTC site capacity and resources. These may include the use of whole blood versus serum/plasma specimen type, the need for refrigeration (and electricity), the need for other equipment and consumables, the capacity of staff, and the shelf-life of assays.

Regardless of whether a chosen algorithm contains EIA and/or rapid tests in any combination, it is crucial that: a) all tests have a sensitivity of at least 99% and a specificity of at least 99%; b) the final positive predictive value (PPV) for any testing algorithm is at least 99%; c) the first test has the highest sensitivity; d) the tests should be used in a sequence that respects increasing specificity; e) all tests in one testing algorithm do not share the same false negative and false positive results (i.e. all tests in one testing algorithm must use different antigen preparations and test kit components based on different manufacturers' preparations to reduce the potential for shared false non-reactivity or false reactivity); and f) the number of algorithms used in a country is limited.

As with EIA and WB, it is very important that countries should put in place quality control and quality assurance measures, including training of personnel who perform rapid HIV tests, testing their proficiency in testing and periodically checking the accuracy of the results.

Introduction

HIV testing and counselling (HTC) is recognized as an entry point to HIV/AIDS prevention, treatment and care, and deserves high priority in national HIV responses. Most people living with HIV (PLHIV) in the Eastern Mediterranean Region do not know that they are infected and therefore do not access HIV treatment and care or psychosocial support services, including antiretroviral therapy (ART). Access to, and uptake of, HTC must be increased dramatically in order to prevent HIV transmission and increase access to ART.

A broad range of HIV testing techniques are available, with increased reliability of rapid testing techniques. The evaluation of the operational characteristics of rapid HIV tests conducted by WHO, recognizes that many rapid tests have a sensitivity and specificity comparable to that of EIA (1). The use of rapid HIV tests provides an excellent opportunity for countries in the Eastern Mediterranean Region to increase the accuracy and efficiency of HIV testing strategies, and creates an opportunity to fulfil their commitment to achieve universal access to HIV prevention, treatment, care and support.

The purpose of this document is to discuss the operational characteristics of rapid HIV tests and their role, among the wider range of HIV testing methodologies, in facilitating efficient HIV testing in the epidemiological, cultural and socioeconomic context of the Eastern Mediterranean Region.

HIV testing methods

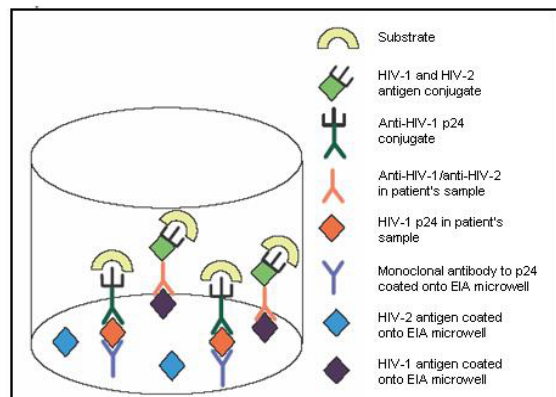
Traditionally, HIV status has been determined through a combination of two EIA assays for screening and a WB for confirmation of the HIV test result. Both assay techniques detect the presence of HIV antibodies, i.e. antibodies to HIV-1 and/or HIV-2 and/or HIV antigen (1) (Fig. 1). With the introduction of instrument-free rapid tests in the market, different combinations of test are now used for screening and confirmation. The different testing techniques used are described below.

Screening by EIA

EIAs are highly sensitive and specific and are very efficient for performing large numbers of tests at any one time. They may be used when HIV testing is performed for HIV surveillance surveys, in blood banks where large numbers of blood donations are screened, in health care settings with a high patient throughput and in HIV testing services with large numbers of clients who are retained until the result is given back. HIV testing with EIA requires specialized laboratory equipment (EIA incubator, washer, reader, calibrated pipettes), consumables and durables, and trained personnel (2).

EIAs are performed on special laboratory supplies called well-plates. Each well-plate has 96 wells which can accommodate up to 94 specimens, one negative control and one positive control (trays of 8 wells also exist, called microwells). Each tray is used for one batch of tests only, irrespective of the number of wells filled with specimens. Thus, the cost of EIA becomes high in settings with low specimen throughput as unused wells are wasted (Fig. 2).

In health care facilities and HTC services, where the daily throughput of test requests is low, it might be difficult to have enough samples to fill a well tray. EIA HIV testing requires sending the testing sample to a laboratory located either within the same health facility or elsewhere. At these laboratories, blood specimens are processed and stored, until there are



Source: reproduced with permission from reference (2).

Fig. 1. Principle of generic combined antigen/antibody detection EIA

enough samples to be tested in batches. This is to make best use of resources when performing EIAs. Subsequently, specimens found to be initially reactive must be further tested with one or more different HIV assays to confirm HIV reactivity and to exclude false-positive reactions.

How EIA works:

- by adding an enzyme;
- conjugated HIV antibody/antigen and substrate solution causes a colour change in wells containing HIV-positive serum/plasma;
- a spectrophotometer reads out colour change up to 94 sera/plasma per 96-well plate;
- requires electricity, refrigeration and trained laboratory staff.

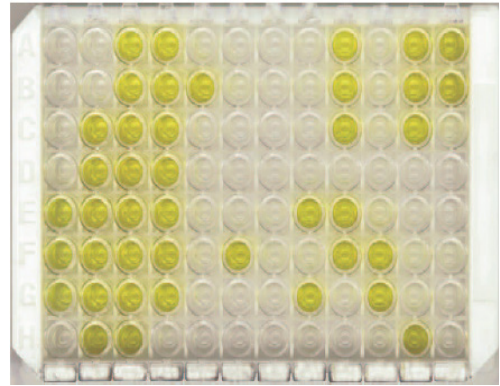
Characteristics of EIA:

- used with serum or plasma;
- time required: 2 to 3 hours;
- capturing HIV antibody/antigen in serum/plasma to form antibody-antigen complexes with HIV antigen/antibody immobilized onto 96-well plate.

Confirmatory testing by WB

WB is a confirmatory assay that is very specific, but very expensive. It is usually used to confirm an initial reactive screening test or to resolve HIV-inconclusive testing results (Fig. 3). It requires specialized laboratory equipment and trained laboratory personnel.

The test identifies antibodies to several of the immunodominant epitopes of the key HIV glycoprotein antigens, which are immobilized on a nitrocellulose strip. Usually a positive WB test result is defined by the detection of a combination of antibodies to two or more specific viral proteins. The intensity of the bands may vary and can be difficult to interpret without experience. Multiple standards for interpretation exist with different combinations of bands confirming HIV positivity. An indeterminate WB result occurs when reactivity is observed to some but not all bands, as defined by the interpretation criteria. This may be caused by seroconversion.



Source: reproduced with permission from reference (1).

Fig. 2. Completed EIA

Characteristics of WB:

- used with serum or plasma;
- requires 2 to 3 hours, although some take longer;
- capturing HIV antibody in serum or plasma to form antibody-antigen complexes with HIV antigen immobilized on nitrocellulose membrane strips;
- adding an enzyme-conjugated HIV antibody and substrate solution causes bands to appear when incubating with HIV-positive plasma;
- HIV glycoproteins are designated “p” or “gp”, followed by their relative molecular mass (x 1000);
- gp160/gp120/gp41 are envelope glycoprotein antigens and p24 is a core antigen.



Source: reproduced with permission from reference (3).

Fig. 3. Completed WB

Depending on the testing strategy and systems used in the laboratories, as well as the specimen throughput, reliance on EIA, WB and immunofluorescence assay (IFA) techniques may take up to two weeks or more to turn around an HIV-positive test result. This incurs long waiting times for clients/patients to get back their result. Long waiting times discourage people who undergo HIV testing from obtaining the HIV test result, with non-return rates as high as 50% (4). The reasons for non-return include the distance and travelling time to the clinic, the difficulty of allocating time for a visit to the testing site, the fear of stigma and discrimination, and the build-up of fear of the result during the waiting time.

Rapid tests

Rapid tests are recommended relative to conventional diagnostics such as EIA since they are faster (under 30 minutes hands-on time) and simpler to perform. This allows faster provision of test results and, consequently, the immediate opportunity for post-test counselling and referral to other related services. They may be performed with little or no laboratory equipment and reagents, and are especially beneficial in resource-limited settings because they are technically less demanding. Evaluation of rapid tests has shown that rapid tests can have sensitivities and specificities of up to 100%, similar to the performance of EIA.

The main advantage of rapid HIV tests is the short time needed to perform testing and hence the possibility of obtaining HIV test results within minutes from specimen collection. This may result in higher rates of people receiving their test results (5) and, subsequently, in higher rates of people entering into post-test counselling, treatment and care programmes. As with other formats of HIV testing, informed consent must be obtained from the individual, and confidentiality of test results must be maintained. In addition, it is important to select reliable rapid tests from the wide range available on the market. Careful selection of rapid tests of proven high quality, combined with selection of suitable testing strategies and algorithms, will achieve accuracy that can be as high as using a combination of EIA and WB (5).

Rapid tests are based on various immunodiagnostic principles including agglutination, lateral-flow and immunochromatographic technique (also known as lateral-flow) (5) (Fig. 4).

Agglutination device:

- used with serum or plasma;
- requires 10–60 minutes or more;
- cross-linking between HIV antibody in serum/plasma and HIV antigen-coated latex particles leads to agglutination;
- sometimes difficult and subjective to read out, reagents require refrigeration and costs are US\$ 2–4 per test.

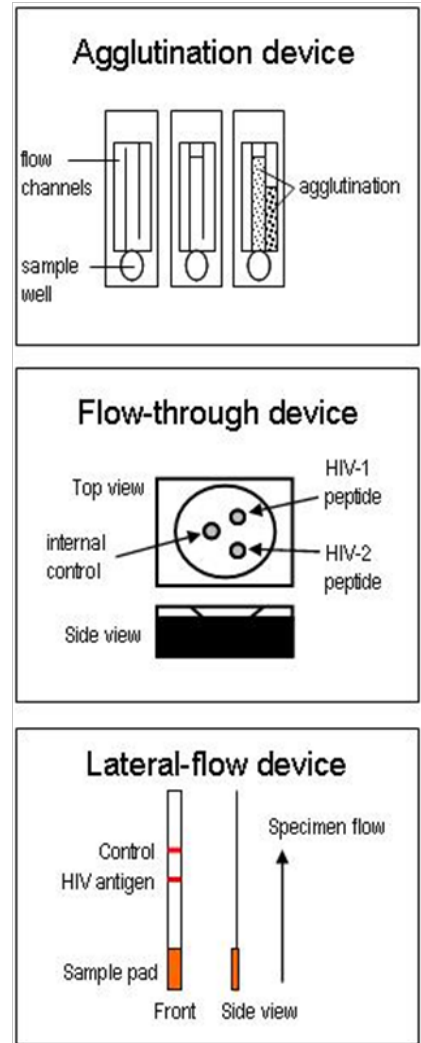
Flow-through device (immunofiltration):

- used with serum, plasma or whole blood;
- requires 5–15 minutes;
- involves several steps;
- HIV antigens are immobilized on a porous membrane and capture HIV antibody in serum/plasma (sometimes whole blood);
- some reagents may require refrigeration;
- costs are US\$ 2–8 per test.

Lateral-flow device

(immunochromatographic):

- potentially only one step because the nitrocellulose strip incorporates both HIV antigen and signal reagent;
- most require little or no additional reagents, no refrigeration and can be performed within 15 minutes;
- used with serum/plasma, whole blood, including finger-stick specimens or oral fluids;
- costs are less than US\$ 2 per test.



Source: reproduced with permission from reference (5).

Fig. 4. Schematic representation of rapid test immunodiagnostic principles

Fourth generation combined HIV antigen/antibody detection assays

The newly-available fourth generation rapid HIV tests are used for the simultaneous or combined detection of HIV-1 p24 antigen and HIV-1/2 antibodies. These assays provide an enhanced level of seroconversion sensitivity in early infection over antibody-only assays by reducing the serological window period. It is therefore recommended to consider them as an A1 assay in diagnostic testing strategies in both high- and low-prevalence settings, wherever possible. However, the HIV-1 antigen detection component of some fourth generation assays may be lacking in sensitivity as recent data has suggested (6).

Alternative methods to screen for and confirm HIV positivity

The results obtained using an HIV testing strategy with EIA followed by confirmation with WB can also be achieved through the use of other combinations of tests. In particular, where test results are needed urgently or within the same visit of the client, testing strategies that require complicated laboratory techniques may not be the most suitable. Moreover, in resource-limited settings the specific economic, infrastructural and human resource constraints may pose specific challenges to the use of HIV testing techniques that require trained laboratory personnel and specialized laboratory equipment. High quality EIA and rapid testing techniques are currently available and, when used in a defined sequence of two or three tests, are equally accurate when used for screening and confirming HIV-positivity (3,5) (see the next section below for more detail). Therefore, for HIV diagnosis purposes, WHO recommends that countries consider testing algorithms using RDTs and/or combinations of RDTs and microtitre plate EIA, rather than EIA/WB combinations. In the following sections, we will discuss further the considerations to be taken into account when choosing testing strategies for the screening and confirmation of HIV test results, with more focus on the inclusion of rapid tests.

Comparison of characteristics of HIV EIA and rapid HIV tests

There are many characteristics that make rapid HIV tests the most suitable for settings with low specimen throughput including those with low HIV prevalence and/or in resource-limited settings (see Table 1 for a comparison between EIA and rapid tests).

In summary:

- the accuracy and reliability of rapid HIV tests is equivalent to that of EIA, provided that both tests are handled and carried out properly, in a quality assured manner;
- training of personnel who perform HIV tests (both EIA and rapid) is extremely important;

Table 1. Comparison between EIA and rapid tests characteristics

EIA	Rapid tests
Highly accurate	Highly accurate
Require testing strategy and algorithm	Require testing strategy and algorithm
Difficult (involving multiple steps)	Easier (require one or few steps)
Slow (time to result 2 to 3 hours)	Quicker (time to result under 30 minutes)
Second appointment required to obtain test result	Same-day result and post-test counselling depending on setting and test result
Only skilled laboratory technician can perform the test	Trained non-laboratory health workers can perform the test in addition to laboratory personnel
Require sophisticated equipment	Require no or minimal equipment
Require refrigeration of reagents	Mostly require no refrigeration
Require multiple reagents	Require no or one reagent
US\$ 0.23–1.20 per test (excluding cost of equipment)	US\$ 0.40–2.00 per test (no special equipment needed)
Stable end-point (reaction is stopped and readings can be printed by EIA plate reader and can still be read at a later time)	Limited end-point stability (test result must be recorded within a specified time limit)
Ideal for large volume testing, especially in health services where a significant number of tests are being conducted and patients retained.	Ideal for low volume testing
Reading of multiple results by EIA plate reader	Reading of individual test results by eye
Standardized interpretation of results	Reader variability in interpretation of results
	More appropriate for settings with low specimen throughput and scale-up of HIV testing services
	Increase access to prevention (e.g. HTC) and interventions (e.g. PMTCT)

- rapid HIV tests are affordable and can be more economical, especially for settings with low specimen throughput;
- rapid HIV tests are easier to use and require no or minimal equipment and reagents;
- because rapid HIV tests are easier to use, they can be performed both by laboratory personnel and non-laboratory personnel (such as health workers, nurses and counsellors) with training;
- rapid tests are suitable for performing smaller numbers of tests at one time.
- all types of test (including EIA, rapid and WB) require attention to training, supervision and quality assurance;
- EIA is preferred over rapid tests in large throughput settings;
- rapid tests have a particular importance to HIV testing services that target “hard-to-reach” populations.

Considerations when choosing HIV tests for use in HIV testing strategies and algorithms

It is essential that HIV test results have the highest possible degree of accuracy. Obviously, accuracy has to be optimal if the HIV test is done on an individual for the purpose of HIV diagnosis.

The accuracy of any HIV test result is determined by the sensitivity and specificity of the HIV test used, as well as by the testing strategy and testing algorithm applied. Test sensitivity refers to the ability of a test to correctly identify that a specimen contains antibodies and/or antigen to HIV. Low sensitivity leads to false negative tests. Test specificity measures the ability of the given test to correctly identify that a specimen does not contain antibodies and/or antigen to HIV. Low specificity leads to false positive test results. A testing strategy describes a generic testing approach, i.e. the types of assay (EIA, rapid test or WB) and the sequence in which these tests need to be performed.

With any given specificity and sensitivity, the probability that a reactive test result correctly identifies an HIV-infected individual depends on the prevalence of HIV in the population to be tested. In low-prevalence populations, the probability is low that a reactive test result represents true HIV seropositivity. The positive predictive value (PPV) is a measure for the probability of an individual being HIV-infected when the test is reactive. Likewise, the negative predictive value (NPV) is a measure for the probability of an individual being uninfected when the test is non-reactive (Table 2).

The use of one HIV assay alone (whether EIA, rapid test or WB) is not recommended, regardless of the HIV prevalence. Instead, combinations of two or more assays must be used in a sequence as defined in HIV testing strategies and algorithms, in order to achieve the highest possible positive and negative predictive values.

A testing strategy defines the number and sequence of assays to be used in order to determine the HIV status, regardless of the sensitivity, specificity and commercial brand of the test.

A testing algorithm describes the combination of specific commercially available HIV assays to be used in a given testing strategy and in the sequence they need to be performed in.

Table 2. Schematic table used for analysis of a test under evaluation

Test results	True HIV status		Total
	HIV-infected	HIV-uninfected	
Positive (reactive)	a (true-positives)	b (false-positives)	a+b
Negative (non-reactive)	c (false-negatives)	d (true-negatives)	c+d
Total	a+c	b+d	

Sensitivity = $a/(a+c)$ PPV = $a/(a+b)$

Specificity = $d/(b+d)$ NPV = $d/(c+d)$

Source: adapted with permission from reference (1).

Each testing algorithm that is introduced in a specific setting should be evaluated and validated (7,8,9). In addition, quality assurance and quality control are important to safeguard accuracy and reliability (10).

HIV prevalence in the population to be tested is an important factor as it influences the PPV and the NPV. More precise formulas of PPV and NPV, which take the prevalence of HIV in the population into account, are:

$$PPV = \frac{(\text{prevalence})(\text{sensitivity})}{(\text{prevalence})(\text{sensitivity}) + (1 - \text{prevalence})(1 - \text{specificity})}$$

$$NPV = \frac{(1 - \text{prevalence})(\text{specificity})}{(1 - \text{prevalence})(\text{specificity}) + (\text{prevalence})(1 - \text{sensitivity})}$$

In low-prevalence populations, the PPV of any one test result will be low, even though its sensitivity and specificity are high (see Annex 1). For this reason, to diagnose HIV seropositivity in a specimen takes more than one HIV test and may need up to three tests. The selection of a testing strategy will be highly dependent on the purpose of HIV testing and on the HIV prevalence in the population to be tested (10,11,12).

For example, assume that an HIV testing service is serving a population with a low HIV prevalence (0.1%). If the service uses an assay with a sensitivity of 99.5% and a specificity of 99.5%, then:

$$\text{PPV} = \frac{(0.1\%)(99.5\%)}{(0.1\%)(99.5\%) + (1 - 0.1\%)(1 - 99.5\%)} = 16.6\%$$

$$\text{NPV} = \frac{(1 - 0.1\%)(99.5\%)}{(1 - 0.1\%)(99.5\%) + (0.1\%)(1 - 99.5\%)} = 99.999\%$$

The low PPV and very high NPV obtained with one assay explain the reason why in low-prevalence settings, a negative test result can be confirmed after the first assay while a seropositive HIV test result can only be confirmed after three serial reactive assays. With every assay where the positive samples are retested, the PPV increases until it reaches 99–100% with the third assay (see Annex 1 for a more detailed description of calculations).

In general, it is crucial that: a) all tests have a sensitivity of at least 99% and a specificity of at least 99%; b) the final PPV for any testing algorithm is at least 99%; c) the first test has the highest sensitivity; d) all tests in one testing algorithm do not share the same false negative and false positive results; e) all tests in one testing algorithm must use different antigen preparations and test kit components based on different manufacturers' preparations to reduce the potential for such shared false non-reactivity or false reactivity; and f) the number of algorithms used in a country is limited.

Independent of whether combinations of different EIA and/or rapid tests are used in any strategy or algorithm, the same conditions and guidelines for PPV, sensitivity and specificity should be taken into account.

Tests should also be selected according to specifications that are relevant to the HIV type, group and subtype predominant in the population in which testing is performed. In the Eastern Mediterranean Region, HIV is mostly likely to be of the HIV-1 type, group M, with either A or G subtypes (13).

HIV testing strategies

General considerations

Selecting the testing strategy that is most appropriate may be based on several factors including the objectives of testing and the prevalence of HIV in the population to be tested. The testing objective may be for ensuring blood safety, surveillance or diagnostic (i.e. for establishing the HIV status of individuals). The scope of this document is limited to diagnostic testing.

As standardized diagnostic testing strategies differ according to the HIV-prevalence level, it is important to estimate the prevalence of HIV in the population being tested. “Population being tested” refers to the population for which an HIV testing service is provided, for example, the general population in a city or province, tuberculosis (TB) patients, antenatal care (ANC) attendees, or people who inject drugs (PWID). For example, the prevalence of HIV in key populations at higher risk of HIV exposure, such as PWID, may be much higher than the prevalence among women attending ANC. If the prevalence in the population tested is unknown (i.e. there is no previous data on ANC attendees, PWID, TB patients) and the prevalence is presumed to be low, then it is recommended to use the diagnostic testing strategy for low-prevalence settings that consists of three reactive tests to confirm HIV seropositivity.

The diagnostic testing strategies described below are based on the prevalence of HIV in the population to be tested. There are two types of HIV-prevalence setting which can be distinguished.

- High-prevalence settings: HIV prevalence is over 5% in the population to be tested.
- Low-prevalence settings: HIV prevalence is below 5% in the population to be tested, which encompasses settings with low-level HIV epidemics and testing of the general population in concentrated HIV epidemics.

The recommended testing strategies are based on achieving a final PPV and NPV of at least 99% with a given testing strategy for diagnosis.

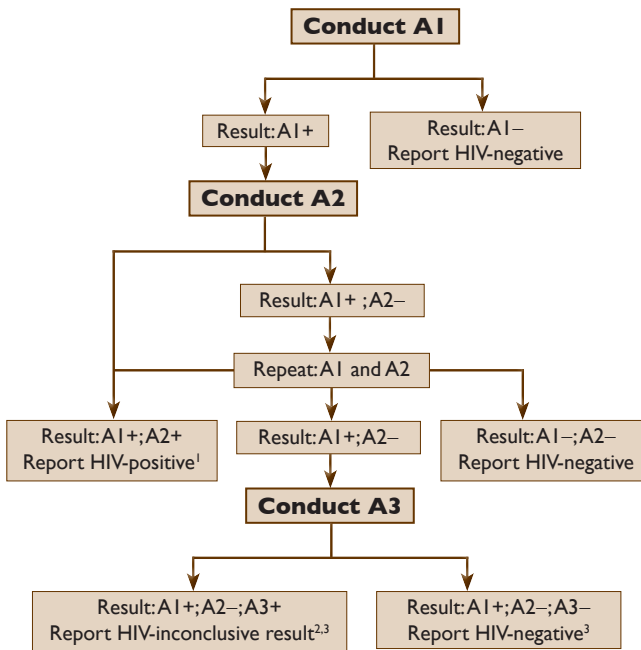
The HIV diagnostic testing strategies described here are based on HIV antibody assays (either EIA and/or rapid) using serum, plasma or whole blood. Additional explanation is provided for the use of HIV antigen-antibody fourth generation assays.

Diagnostic HIV testing strategies (5)

As noted, diagnostic testing strategies vary according to the HIV-prevalence level. These are described below. All testing strategies suggested in this section can be applied equally in both conventional laboratory settings by competent laboratory staff as well as in outreach settings by non-laboratory technicians who have been trained to perform rapid HIV tests.

High-prevalence settings

In the diagnostic testing strategy in high-prevalence settings (Fig. 5), all specimens are first tested with one assay (A1). Specimens that are non-reactive (A1–) on the first assay are considered HIV-negative and reported as such. Specimens that are reactive (A1+) are tested again (A2) using a different assay. Two consecutive tests that are both reactive (A1+; A2+) are sufficient to diagnose HIV seropositivity.



Notes:

Assay A1, A2, A3 represent three different assays (of any test format). Report = result may be reported.

¹ For newly diagnosed individuals, a positive result should be confirmed on a second specimen to rule out laboratory error.

² Re-testing should be performed on a second specimen taken after 14 days to rule out seroconversion.

³ If A1 is an antigen/antibody detection assay and A2 or A3 is an antibody-detection-only assay, re-testing should be performed with a second specimen taken after 14 days.

Fig. 5. HIV testing strategy for diagnosis in high-prevalence settings

Assays with discrepant results in which specimens are reactive on the first assay but non-reactive on the second assay (A1+; A2-) should be repeated with the same two assays and using the same specimen, although a new specimen will have to be obtained if finger-stick whole blood is used. Such repetition of assays is typically best incorporated in testing strategies to exclude potential technical or clerical errors associated with discordant results.

If results remain contradictory (A1+; A2-), discordant specimens should undergo further testing using a third assay (A3). If the third assay is non-reactive (A1+; A2-; A3-), the specimen is considered HIV-negative and accordingly reported as such. However, if the third test is reactive (A1+; A2-; A3+), the specimen remains discordant and, as a result, is reported as HIV-inconclusive. The individual should be counselled and requested to return in 14 days for further analysis and a second specimen may be taken for re-testing. The occurrence of such HIV-inconclusive results should be rare; if not, additional quality assurance measures and different assay selections may be considered.

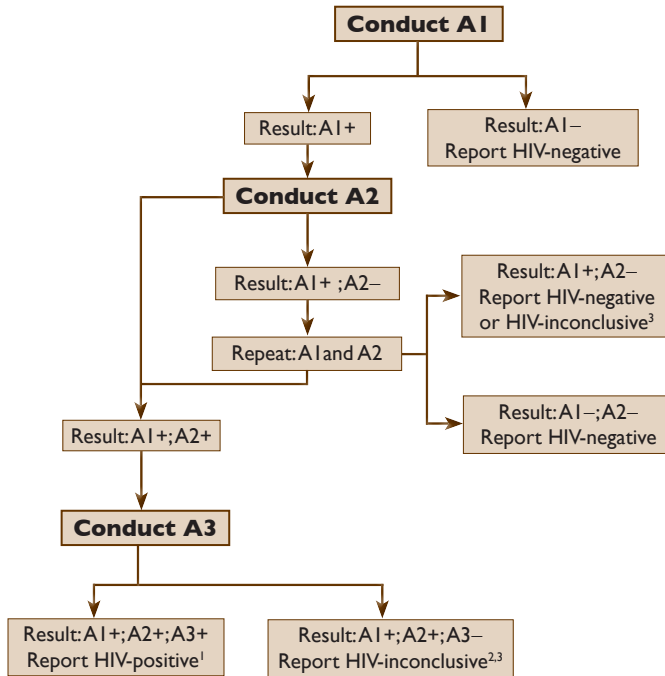
Nevertheless, if, upon repetition, both assay results are in accordance/match one another (either A1-; A2- or A1+; A2+), then they may be reported as HIV-negative or HIV-positive, respectively. Individuals with HIV-positive results should be referred for immediate evaluation of their eligibility for treatment and access to health care services. However, if a fourth generation HIV antigen/antibody identification assay is used as A1, upon a reported HIV-inconclusive result, re-testing should be carried out with a new specimen taken after 14 days.

Low-prevalence settings

The diagnostic testing strategy used in low-prevalence settings (Fig. 6) should be applied when HIV prevalence is below 5% in the population to be tested. This encompasses settings with low-level HIV epidemics and testing of the general population in concentrated HIV epidemics.

In such settings, individuals can only be diagnosed as HIV-positive after three consecutive assays have been performed of which all are reactive (A1+; A2+; A3+). This is because the PPV based on the results of two reactive assays remains too low in a low-prevalence population; hence a third is needed to confirm HIV-reactive specimens. HIV test results are reported as HIV-negative if the first assay is non-reactive (A1-).

If the first assay is reactive and the second is non-reactive (A1+; A2-), both assays have to be repeated to exclude potential technical or clerical errors typically associated with discordant results. Such repetition is performed with the same two assays using the



Notes:

Assay A1, A2, A3 represent three different assays (of any test format). Report = result may be reported.

¹ For newly diagnosed individuals, a positive result should be confirmed on a second specimen to rule out laboratory error.

² Re-testing should be performed on a second specimen taken after 14 days to rule out seroconversion.

³ If A1 is an antigen/antibody detection assay and A2 or A3 is an antibody-detection-only assay, re-testing should be performed with a second specimen taken after 14 days.

Fig. 6. HIV testing strategy for diagnosis in low-prevalence settings

same plasma or serum specimen, although a new specimen will have to be obtained if finger-stick whole blood is used instead. If repeating A1 and A2 yields the same results as before (i.e. A1+; A2-), or both assays turn out non-reactive results (A1-; A2-), the test result can be considered HIV-negative and reported as such.

If the third assay is non-reactive after two repeated reactive assays (A1+; A2+; A3-), then the result is considered HIV-inconclusive, and the individual should be counselled and requested to return in 14 days for further analysis.

If A1 is a combined antigen/antibody-detection assay and is reactive, and A2 or A3 is an antibody-detection-only assay and is non-reactive, a test result should be reported as inconclusive. Hence re-testing should be carried out with a new specimen taken after 14 days. The occurrence of such HIV-inconclusive results should be rare; if not, additional quality assurance measures and alternative assay selections may be considered.

Although the third assay may be necessary for diagnosis in both high- and low-prevalence populations according to the relevant testing strategy, whether it is performed at the initial testing site or not depends on local factors such as specimen-throughput at the testing facility and the experience of technicians. If the third test is not performed at the initial testing site, then the individual or specimen can be referred to another more competent/experienced testing site or laboratory where the third assay can be reliably conducted. Extensive counselling and active referral and follow up are required to motivate the individual to take the third test, to return for the final test result and to take the necessary prevention measures in the meantime.

When the HIV status of a person remains inconclusive, this person should be referred to a reference laboratory for re-testing after 14 days to resolve their HIV status through more advanced technologies if available.

In some cases where the HIV testing quality at the testing site where the individual was diagnosed is doubtful, it might be wise to obtain a second specimen from individuals newly diagnosed with HIV after a time interval (i.e. not the same day) for re-testing in order to re-confirm HIV serostatus. Such re-testing helps eliminate possible technical or clerical errors including specimen mislabeling and transcription errors, and is usually performed in competent quality-assured sites as part of the clinical and laboratory-based evaluation of treatment eligibility and access to care.

Conclusion

Rapid tests are accurate, yet simple and quick to perform, and may be more economical in certain settings. Their use in HIV diagnostic testing is recommended due to their faster provision of test results and the immediate opportunity for post-test counselling and referral. They are less technically demanding and do not need a laboratory set-up. They support expansion of HTC services and are highly beneficial in interventions that are targeted to key populations at higher risk of HIV exposure. They can also be considered useful in low throughput health care settings such as PMTCT, for diagnosis of symptomatic patients and for asymptomatic individuals attending outpatient clinics for drug dependence, TB and sexually transmitted infection (STI) treatment, and in other health care settings.

In addition to simplicity and rapidity, these assays are ideal in resource-limited settings and in populations with low HIV prevalence. A well-trained non-laboratory health worker can perform the tests without sophisticated laboratory equipment. Moreover, these tests allow reading of individual test results and are ideal for low turn-over of testing.

In the Eastern Mediterranean Region, the introduction of rapid HIV testing should be welcomed as these assays offer many advantages for its diverse settings. Most countries in the Region have a limited number of HTC sites. The introduction of rapid tests increases the feasibility of scaling-up HTC and provides better opportunities to reach “hard-to-reach” populations. Furthermore, provider-initiated testing and counselling (PITC) is generally conducted in countries of generalized epidemic (i.e. in high-prevalence settings) or in low and concentrated epidemic areas in ANC, PWID, STI and TB clinic services. In such settings, rapid tests can offer the advantage of same-day diagnosis (in most cases), post-test counselling and immediate referral to treatment, care and support for key populations at higher risk of HIV exposure. Nevertheless, for services where patients are retained and there are already significant numbers of tests being conducted, such as in ANC settings and blood screening services, laboratory-based diagnostics (i.e. EIA) may be more cost-effective and suitable.

References

1. HIV antigen/antibody assays: operational characteristics. Report 16: Rapid assays. Geneva: World Health Organization; 2009 (http://apps.who.int/iris/bitstream/10665/44042/1/9789241597692_eng.pdf, accessed 27 December 2013).
2. HIV assays: operational characteristics (phase 1). Report 15: Antigen/antibody ELISAs. Geneva: World Health Organization; 2004 (<http://apps.who.int/medicinedocs/documents/s15200e/s15200e.pdf>, accessed 27 December 2013).
3. HIV rapid testing: training package. Geneva: World Health Organization; 2005 (WHO/EHT/DIL/05.1; http://www.who.int/diagnostics_laboratory/documents/guidance/hivrtraining_participant/en/index.html, accessed 27 December 2013).
4. Branson BM. Point-of-care rapid tests for HIV antibodies. *Laboratoriums Medizin*. 2003;27:288–295. doi.org/10.1046/j.1439-0477.2003.03049.x.
5. HIV assays: operational characteristics (phase 1). Report 14: Simple/rapid tests. Geneva: World Health Organization; 2004 (http://www.who.int/entity/diagnostics_laboratory/publications/en/hiv_assays_rep_14.pdf, accessed 27 December 2013).
6. Service delivery approaches to HIV testing and counselling (HTC): A strategic HTC policy framework. Geneva: World Health Organization; 2012 (http://apps.who.int/iris/bitstream/10665/75206/1/9789241593877_eng.pdf, accessed 27 December 2013).
7. Scaling-up HIV testing and counselling services: a toolkit for programme managers. Geneva: World Health Organization; 2005 (<http://www.who.int/hiv/pub/vct/counsellingtestingtoolkit.pdf>, accessed 27 December 2013).
8. Department of Health and Human Services, Centers for Disease Control and Prevention, World Health Organization Regional Office for Africa, Association of Public Health Laboratories. Guidelines for appropriate evaluations of HIV testing technologies in Africa. Harare, Centers for Disease Control and Prevention/World Health Organization Regional Office for Africa, 2002 (http://whqlibdoc.who.int/afro/2002/a82959_eng.pdf, accessed 27 December 2013).

9. Global programme on AIDS. Recommendations for the selection and use of HIV antibody tests. *Wkly Epidemiol Rec.* 1992;67:145–14.
10. Joint United Nations Programme on HIV/AIDS (UNAIDS)-WHO. Revised recommendations for the selection and use of HIV antibody tests. *Wkly Epidemiol Rec.* 1997;72:81–87 (http://www.who.int/diagnostics_laboratory/publications/en/Selection_HIV_tests.pdf, accessed 27 December 2013).
11. The importance of simple/rapid assays in HIV testing: WHO/UNAIDS recommendations. *Wkly Epidemiol Rec.* 1998; 73:321–326 (http://www.who.int/diagnostics_laboratory/publications/en/simple_rapid_wer.pdf, accessed 27 December 2013).
12. Branson BM. Rapid tests for HIV antibody. *AIDS Rev.* 2000;2:76–83.
13. Kandathil AJ, Ramalingam S, Kannangai R, David S, Sridharan G. Molecular epidemiology of HIV. *Indian J Med Res.* 2005;121:333–344 (<http://icmr.nic.in/ijmr/2005/april/0410.pdf>, accessed 27 December 2013).

Annex I.

Calculations using the recommended test strategies with HIV prevalence ratios

Testing algorithm based on sensitivities and specificities realistic for resource-limited field settings

This Annex applies to using either EIA, rapid tests or a combination of the two.

HIV prevalence (%)	0.1	1.0	2.0	5.0	10.0	30.0
Final NPV (%) after 2 or 3 tests depending on strategy used	99.998 ^a	99.975 ^a	99.950 ^a	99.921 ^a	99.834 ^a	99.363 ^a
PPV (%) with 1 test	4.744	33.445	50.380	72.364	84.681	95.520
PPV (%) with 2 tests	76.673	97.073	98.530	99.425 ^a	99.727 ^a	99.929 ^a
PPV (%) with 3 tests	99.389 ^a	99.939 ^a	99.970 ^a	–	–	–

^a values higher than 99%.

Scenario	First test	Second test	Third test
Sensitivity (%)	99.5	99.0	99.0
Specificity (%)	98.0	98.5	98.0

All calculations in table A1.1 use the schematic table in Table A1.2.

Since all newly HIV-positive diagnosed individuals are recommended to undergo a second testing cycle, on a second blood sample, a falsely HIV-positive diagnosed individual is likely to be then detected as being falsely diagnosed HIV-positive. Note that there could be a “window period”, in which individuals who are infected will not yet show a reactive result.

Test result	HIV positive	HIV negative	Total
Reactive	A	B	A + B
Non-reactive	C	D	C + D
Total	A + C	B + D	A + B + C + D

Low-prevalence settings

For all low-prevalence settings, three serial tests should be applied (Fig. 6).

As an example we use 0.1% HIV prevalence and a population of 10 000 people (Fig. A1.1).

First test

10 000 people in a population with 0.1% HIV prevalence means: 10 HIV-positive and 9990 HIV-negative people.

Sensitivity = 99.5%: 99.5% of HIV-positive individuals will show a reactive test result and 0.5% of HIV-positive individuals will show a “false” non-reactive test result.

Specificity = 98%: 98% of HIV-negative individuals will show a non-reactive test result and 2% of HIV-negative individuals will show a “false” reactive test result.

All non-reactive individuals will receive an HIV-negative diagnosis after the first test.

All individuals with a reactive test will be tested again with the second test.

Second test

From the 209.75 people with a reactive first test result, 9.95 are HIV-positive and 199.80 are HIV-negative.

Sensitivity = 99%: 99% HIV-positive individuals will show a reactive test result and 1% of HIV-positive individuals will show a “false” non-reactive test result.

Specificity = 8.5%: 98.5% of HIV-negative individuals will show a non-reactive test result and 1.5% of HIV-negative individuals will show a “false” reactive test result.

All non-reactive individuals will receive an HIV-negative diagnosis after the second test.

All individuals with a reactive test will be tested again with the third test.

This may or may not be performed at the initial testing site.

Third test

Of the 12.85 people with a reactive second test result, there are 9.85 HIV-positive and 3 HIV-negative people.

Sensitivity = 99%: 99% of HIV-positive individuals will show a reactive test result and 1% of HIV-positive individuals will show a “false” non-reactive test result.

Specificity = 98%: 98% of HIV-negative individuals will show a non-reactive test result and 2% of HIV-negative individuals will show a “false” reactive test result.

All reactive individuals will receive an HIV-positive diagnosis after the third test.

All non-reactive individuals receive an indeterminate test result after the third test. These individuals must re-test 14 days later with a new specimen being taken.

First test

$$PPV = A/(A+B) = 0.04744 = 4.744\%$$

$$NPV = D/(C+D) = 0.999995 = 100\%$$

True HIV status			
Test result	HIV positive	HIV negative	Total
Reactive	9.95	199.80	209.75
Non-reactive	0.05	9 790.20	9 790.25
Total	10.00	9 990.00	10 000.00

Second test

$$PPV = 76.673\%$$

$$NPV = 99.999\%$$

True HIV status			
Test result	HIV positive	HIV negative	Total
Reactive	9.85	3.00	12.85
Non-reactive	0.10	196.80	196.90
Total	9.95	199.80	209.75

Third test

$$PPV = 99.389\%$$

$$NPV = 99.998\%$$

True HIV status			
Test result	HIV positive	HIV negative	Total
Reactive	9.75	0.06	9.81
Non-reactive	0.10	2.94	3.04
Total	9.85	3.00	12.85

Fig.A1.1 Example for low-prevalence settings

High-prevalence settings

For high-prevalence settings two serial tests should be applied (Fig. 5).

As an example we use 10% HIV prevalence in key populations at increased risk for HIV exposure and a population of 10 000 people (Fig A1.2).

First test

10 000 people in a population with 10% HIV prevalence means: 1000 HIV-positive and 9000 HIV-negative people.

Sensitivity = 99.5%: 99.5% HIV-positive individuals will show a reactive test result and 0.5% HIV-positive individuals will show a “false” non-reactive test result.

Specificity = 98%: 98% HIV-negative individuals will show a non-reactive test result and 2% HIV-negative individuals will show a “false” reactive test result.

All non-reactive individuals will receive HIV-negative diagnosis after the first test.

All individuals with a reactive test will be tested again with the second test.

Second test

From the 1175 people with a reactive first test result: 995 are HIV-positive and 180 are HIV-negative.

Sensitivity = 99%: 99% HIV-positive individuals will show a reactive test result and 1% HIV-positive individuals will show a “false” non-reactive test result.

Specificity = 98.5%: 98.5% HIV-negative individuals will show a non-reactive test result and 1.5% HIV-negative individuals will show a “false” reactive test result.

All reactive individuals will receive an HIV-positive diagnosis after the second reactive test.

All non-reactive individuals must undergo a risk assessment by an experienced counsellor to examine the likelihood of exposure to HIV.

- If the risk is considered low, then the individual will receive an HIV-negative diagnosis.
- If the risk is considered high, then the individual receives an HIV antibody indeterminate result. These individuals must re-test 14 days later with a new specimen being taken.

First test

PPV = 84.681%

NPV = 99.943%

True HIV status			
Test result	HIV positive	HIV negative	Total
Reactive	995	180	1 175
Non-reactive	5	8 820	8 825
Total	1 000	9 000	10 000

Second test

PPV = 99.726%

NPV = 94.686%

True HIV status			
Test result	HIV positive	HIV negative	Total
Reactive	985.05	2.70	987.75
Non-reactive	9.95	177.30	187.25
Total	995.00	180.00	1 175.00

Fig.A1.2 Example for high-prevalence settings

HIV testing and counselling is recognized as an entry point to HIV/AIDS prevention, treatment, care and support. The purpose of this document is to present the characteristics of rapid HIV tests and their role among the wider range of HIV testing methodologies in facilitating efficient HIV testing on a larger scale in the Eastern Mediterranean Region.

