HIV DIAGNOSIS: A GUIDE FOR SELECTING RAPID DIAGNOSTIC TEST (RDT) KITS

1) Overview

There are nearly 33 million people infected with the human immunodeficiency virus (HIV) world wide. Sub-Saharan Africa continues to bear the brunt of the global epidemic. Two thirds (68%) of all adults and children with HIV globally live in sub-Saharan Africa, with its epicentre in southern Africa. One third (32%) of all people with HIV globally live in southern Africa and 34% of all deaths due to AIDS in 2006 occurred there[1].

Declines in national HIV prevalence are being observed in some sub-Saharan African countries, but such trends are currently neither strong nor widespread enough to diminish the epidemics' overall impact in this region[1]. Yet while the pandemic's impact is increasingly felt, surveys in these regions have shown that a median of just 12% of men and 10% of women had been tested for HIV and received the results.

Greater knowledge of HIV status is critical to expanding access to HIV treatment, care and support in a timely manner. It offers people living with HIV an opportunity to receive information and tools to prevent HIV transmission to others. Even as ART treatment becomes increasingly available, initial uptake has been slow in large part because many individuals who might qualify for treatment simply do not know their HIV status.

Knowledge of serostatus via antibody testing is the entry point for most HIV prevention and care interventions. Testing is necessary to protect blood supplies, to identify HIV-positive pregnant women for prevention of mother-to-child transmission (PMTCT), to monitor disease trends in populations, and for clinical diagnostic purposes. The availability of rapid HIV-antibody tests has made field diagnosis of HIV inexpensive and technically feasible in low-resource areas. Increased access to HIV testing and counselling is essential in working towards universal access to HIV prevention, treatment, care and support as endorsed by G8 leaders in 2005 and the UN General Assembly in 2006[2, 3].

Numerous studies have shown rapid tests to perform comparably to standard enzyme-linked immunosorbent assay (ELISA) and western blot testing of patients with established infection and as well as in cohorts of newly infected patients tested at regular intervals during the seroconversion period, which refers to the period of time it usually takes to develop detectable antibodies to HIV following infection with HIV. In 75% of persons, antibodies are produced in 4 to 8 weeks. In almost all persons, antibodies are produced within 14 weeks.

Sensitivities and specificities(see details in paragraph on accuracy) approaching 100% are common. Yet, despite the very high performance characteristics of most rapid tests, incorrect results do occur. It is therefore imperative to identify rapid testing algorithms that conserve finances by reducing the number of test kits and support equipment used, while at the same time providing optimal performance. In order to reduce the number of incorrect results, it is possible to use individual tests in combination algorithms that perform better than single tests alone.

Algorithms

Algorithms may consist of one screening test with a second test to confirm initial positive results, or two in parallel with a third test as a tiebreaker for discordant samples. These strategies are commonly called serial and parallel algorithms, respectively.

• A single-test algorithm, which tests all specimens with a single rapid assay.
• A serial algorithm, which tests all specimens with a single rapid assay and retests those found to be positive with a second rapid assay. In the serial algorithm, discordant results are considered indeterminate.
• A parallel algorithm, which tests all specimens with two rapid tests and resolves discordant results by retesting with a third, tiebreaker test.

Body fluids used for HIV rapid testing could be serum, plasma, whole blood, oral fluids or urines[2].

Serological diagnosis of HIV through antibodies testing is part of good clinical practice and should always be part of HIV case management. However, the exception below apply:

- Infants under 18 months, because maternal HIV antibodies cross the placenta, causing positive serologic tests in HIV-exposed infants for the first several months of life. Early definitive diagnosis of HIV requires virologic testing such as polymerase chain reaction (PCR).
- The ability of some tests to detect early infections is sub-optimal.

2. Objective

This guide is meant to help in the decision making-process for procurement of rapid diagnostic tests. It also provides information that is useful for supply chain planning, including product shelf-life and storage requirements. Some product-specific information on RDTs currently available through UNICEF Supply Division is also included. These are all RDTs that have been assessed by the WHO Bulk Procurement Scheme.

3. What is a HIV rapid diagnostic test (RDT)?

Rapid HIV testing differs from conventional HIV testing in that it allows:

- results of the test to be ready in 5 to 30 minutes;
- it allows testing, counselling, and referrals to be done in one visit.

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 or using adequate rapid test allowing such discrimination between the two viruses.

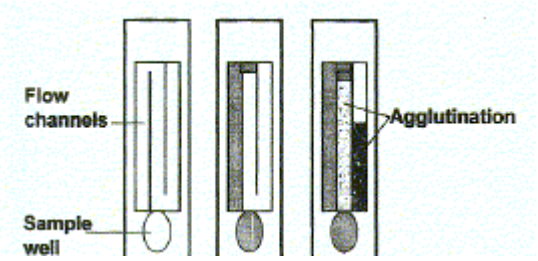
4. Type of HIV rapid diagnostic test

The principles of rapid test is enzyme immunoassay-based with a solid phase/particle coated with synthetic/recombinant HIV I & HIV II antigens.The product should be able to detect antibodies to all of HIV I and HIV II during early sero-conversion period. The

product should have positive and negative controls. A reactive rapid HIV test result must be confirmed before a diagnosis of infection can be given.

Rapid HIV test formats include[5]:

a. Agglutination tests: they use different types of particles to produce clumping or settling patterns of the particles when a specimen is positive. An autologous red cell agglutination method detects HIV antibodies with a hybrid antigen-antibody reagent which agglutinates the patient's red blood cells. A latex particle agglutination detects HIV antibodies by the agglutination of minute latex particles when mixed with the patient's blood. A newer method uses fluid capillary action to enhance and quicken particle agglutination. Particle adherence detects HIV antibodies when the settling pattern of small gelatin particles is altered. (Examples: Capillus, Serodia)

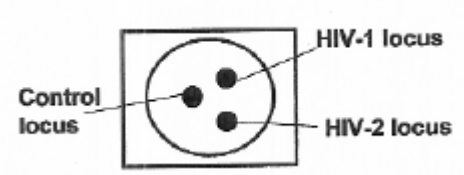


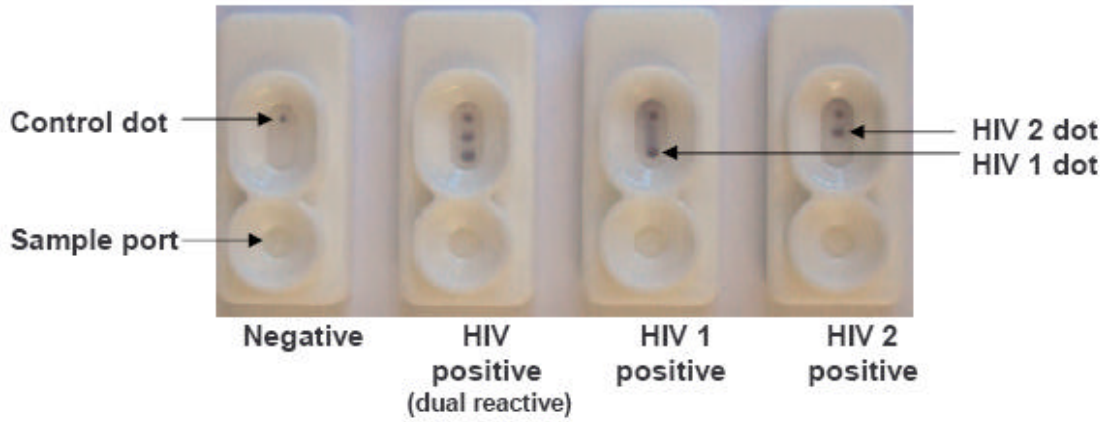
Results

Reactive Latex Aggregation – white clumping

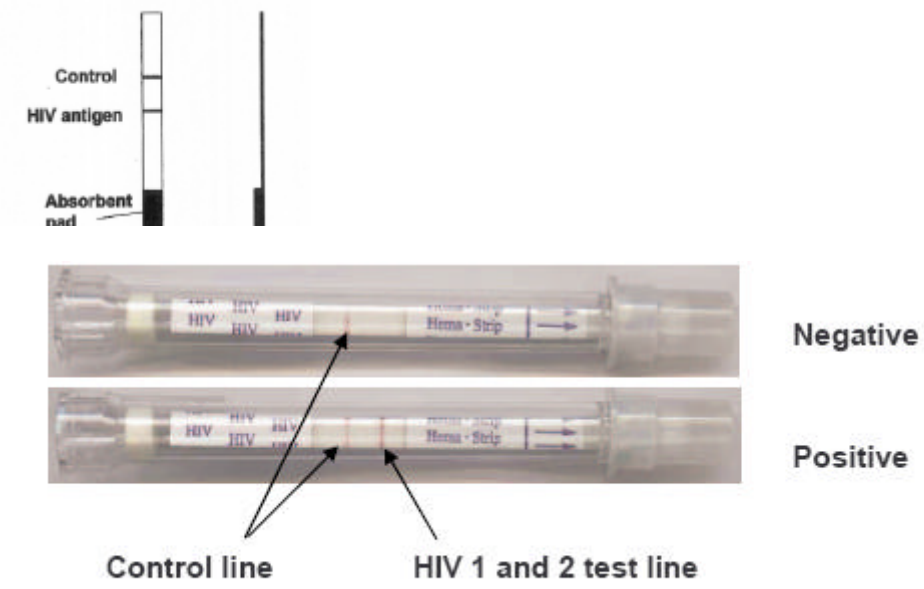
Non-reactive No Latex Aggregation – no white clumping

b. Flow through cassettes, or membrane immunoconcentration devices: they capture and detect HIV antibody in a specimen flowing through a porous membrane. A visible dot or line forms on the membrane when HIV antibodies are present. (Examples: Multi-spot, Genie II). **Solid phase tests** include the dipstick "comb" assay. This assay uses a solid plastic matrix to which an HIV antigen is fixed. When HIV antibodies are present, a spot or dot will be visible when processed with a signal reagent.





c. Immunochromatographic strip (ICS) tests utilize a one-step method in which the patient's blood specimen is combined with a signal reagent and migrates through a special membrane. A positive reaction is seen as development of a line on the membrane. Most tests take less than 15 minutes. (Examples: Determine, Hemastrip, Oraquick, Unigold)



HIV serology is characterized in large part by the immune response to viral proteins (antigens), particularly those comprising the *gag* and *env* regions. For the majority of commercial diagnostic tests, the main serological target for the detection of HIV infections is based on antibody reactivity to the envelope transmembrane protein: gp41 for HIV-1 and gp36 for HIV-2. The transmembrane protein is highly immunogenic and elicits a strong and sustained antibody response in individuals

infected with HIV. Antibodies to this protein are among the first to appear at seroconversion, and the antibody response remains persistent throughout the course of the disease

5) Appropiate use of RDTs

HIV testing occurs in a variety of settings outside of the laboratory. The settings where testing will likely to occur during an era of expansion of services include: Testing & Counselling Centers (T & C), Antenatal Clinics (ANC), Blood Banks, Surveillance programs, tuberculosis (TB) clinics, hospitals, and Sexually Transmitted Infections (STI) Clinics

While all settings where testing occurs can triage persons to treatment and care, TB clinics and hospitals will be the primary venues for providing anti-retroviral treatment to HIV infected persons, and for providing care to HIV affected persons. T&C, ANC, Blood Banks, and surveillance are the primary venues for providing prevention programs[2, 4].

Advantages of Rapid tests	**Disadvantages of Rapid tests**
HIV rapid tests have the following advantages: • Increases access to prevention (VCT) and interventions (PMTCT) • **Number of tests performed** Suitable for individual and small volume testing, e.g. 1- 40 samples per day: Supports increased number of testing sites • **Minimal or no equipment required** Most require no refrigeration **Shelf-life** 12 months or longer , None or one reagent (a substance used in a chemical reaction to detect or produce other substances **, Minimal waste and waste disposal** • **Minimum technical skill, Easy to interpret** Visual interpretation of results, usually without equipment, Stable end-reading point, Same-day diagnosis and counseling, Test time under 30 minutes, Robust and easy to use ? **Accuracy** High sensitivity >99%, High specificity >99% and High reproducibility* >98% ? **Specimen type** Preferably for use on whole blood (finger-prick samples) for ease of collection ? **Low cost Mostly**	*HIV rapid tests also have a few disadvantages:* ✍ Small numbers for each test run • Quality Assurance/Quality Control at multiple sites • Test performance varies by product • Refrigeration required by some products, e.g., Capillus • Reader variability in interpretation of results • Limited end point stability of the results, i.e., reading should be done in a short time window

5. a. Accuracy (sensitivity and specificity)

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. The **specificity** of a test is its capacity to correctly identify individuals that are infected with HIV. 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 high (Table below). 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. 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 below).

In settings with a low-level HIV epidemic[4], tests with a sensitivity or specificity greater than 99% should be used in order to achieve satisfactory positive predictive values. In all HIV testing 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 they have been evaluated and have met pre set criteria. The levels of sensitivity and specificity of these rapid tests are greater than or equal to 99%. It should be remembered that no test 100% sensitive and 100% specific.

Test result	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

5. b. Choosing a HIV rapid tests

Some practical considerations need to be taken into account such as:

- The choice of specimens to be used in testing (whole blood, serum, plasma, oral fluid...);
- Parallel testing versus serial testing;

Parallel testing involves testing all blood samples with two HIV tests simultaneously, i.e. in parallel.

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.

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. 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.

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. If a result is inconclusive the person tested should be advised accordingly.

Post-test counselling should focus on the possibility of the test having been performed during the window period, i.e. when antibodies have not yet formed after exposure to HIV, or the inconclusive result arising from a non-specific reaction. 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. Alternatively, there is the possibility of sending a sample to a referral laboratory for confirmatory testing

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 this information can be used to select suitable candidates for national algorithms.
The evaluations are available at:
http://www.who.int/bct/Main_areas_of_work/BTS/HIV_Diagnostics/HIV_Test_Kit_Evaluation.htm

5. c. Shelf life stability and Storage

Many 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 ºC or rise above 20- 30 ºC, 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 which is at least 12 months.

To ensure that a product will retain its quality, it should be stored and transported within the transporting requirements and necessary precautions as outlined under *transport and storage*. The details of these requirements can be found in the package insert which accompanies the RDT. Longer shelf-life reduces the pressure on the

supply chain and the probability of wastage of expired tests. A minimum of 18 months (e.g. at least 15 months after purchase) is recommended in remote, poorly resourced areas.

5. d. Ease of use

The intended conditions of use must be considered when choosing an RDT. If the RDTs are to be used in a remote area without temperature-controlled storage, stability (shelf-life) will be of great importance, compared to storage and use in temperature-controlled laboratories. If RDTs are to be used by isolated volunteer health workers, an easy-to-use format will be of greater importance than in a laboratory setting.

5. f. Cost

RDTs are often more costly than ELISA or Western Blot and this should be borne in mind when deciding purchase quantities and level of use in a health care system.

Cassette format RDTs are usually 10-20 per cent higher priced than dipstick RDTs, although dipsticks sometimes require the procurer to provide wells, resulting in a similar total cost. When used by health workers, cassette RDTs are probably more reliable than dipstick RDTs, and so may provide savings through improved diagnosis.

5. f. Others considerations

- Detection of the difference between HIV-1 and HIV-2

Please refer to page 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 recordkeeping 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. 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.

- Programmatic considerations

Decisions to use HIV rapid tests for HIV testing and counselling should take into account factors such as:

- Cost and availability of laboratory logistics (test kits, reagents and equipment)
- Available staff, resources and infrastructure
- Laboratory expertise and personnel available
- Number of samples to be tested
- Sample collection and transport

- The setting in which testing is proposed
- Convenience
- The ability of individuals to return for results
- High level of sensitivity and specificity
- Long shelf life at ambient temperature (20- 30 ºC)
- Easy of performance
- Rapidity of performance

Definitive diagnosis of HIV infection in children younger than 18 months requires virological tests, as the presence of maternal HIV antibodies may complicate the interpretation of positive results of HIV rapid tests or ELISA tests. Virological testing depends upon complex procedures such as HIV-DNA or HIV-RNA polymerase chain reaction (PCR), is expensive and requires highly trained staff. WHO promotes a centralized virological testing approach where specimens are collected on filter papers which are easily transported to a central laboratory, even in tropical conditions.

5. g. Transport and storage

Exposure to extreme temperatures is a major contributor to poor performance of RDTs, particularly during transfer from the manufacturer, and transport within a country as well as storage. High humidity can rapidly degrade RDTs, including prolonged exposure to humidity after removal from the envelope or if the envelope is damaged.

In order to maintain quality and performance, the following indications should be followed:

- The consignee must be informed well in advance of the shipment.
- Goods should be dispatched in such a manner that no shipment arrives on a Friday afternoon (the day preceding a weekend) or during the weekend and public holidays.
- Consignees must facilitate prompt clearance so that shipments are moved immediately to moderate temperature storage (less than 30ºC if possible) and materials are not left on airport tarmacs, in customs sheds or in vehicles parked in the sun.
- Ground transportation during any stage of delivery must be carried out without delay and with attention to ambient temperature while the vehicle is moving and if parked.
- Storage at central and final field facilities should be within the manufacturer's specifications (usually 30ºC) to maintain a cold chain.

5. h. Quality

UNICEF procurement is currently limited to RDTs included in the WHO Bulk Procurement Scheme (http://www.who.int/diagnostics_laboratory/procurement/en/). The list is based on evidence of quality manufacturing, through provision of evidence of independent certification and a stability testing protocol provided by the companies to WHO. Whilst requiring this evidence from manufacturers as a pre-requisite for listing of products, WHO (and thus UNICEF Supply Division) cannot guarantee the accuracy of the information.

For more information, please contact UNICEF Supply Division in Copenhagen at supply@unicef.org.

References

1- www.unaids.org UNAIDS AIDS Epidemic update 2007

2- David Owen BSc and Nigel O'Farrell: Rapid HIV testing in a clinic setting International Journal of STD & AIDS, Volume 18, Number 6, June 2007 , pp. 418-419(2)

3- Dewsnap CH, McOwan A. A review of HIV point of care tests. Int J STD AIDS 2006;17:357–9

4- www.who.int/hiv/pub/vct/en

5- BASHH Clinical Governance Committee. Guidance on the Appropriate Use of HIV Point of Care Tests. [www.bashh.org/committees/cgc/reports/final_hiv_point_of_care_tests_guidance_rev080606.pdf]

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