ANNEX 7. Diagnostics for HIV diagnosis

Acknowledgements: Sands, A

7.1. Detailed description of HIV in vitro diagnostic formats

Rapid diagnostic tests
Rapid diagnostic tests (RDTs) are so called as they produce a test result quickly, usually in less than 30 minutes. The broad class of HIV-1/2 RDTs includes lateral-flow (immunochromatographic) and vertical-flow (immunofiltration) assay formats, which detect the presence of HIV-1/2 antibodies and/or HIV p24 antigen. RDTs are not only quick but also are easier to perform than assays that require a laboratory. With training, both health-care professionals and lay providers can perform HIV testing with high accuracy and reliability using RDTs (1, 2). Most RDTs and their accompanying reagents can be stored between 2 and 30 °C. They do not require any additional equipment and, thus, do not need to necessarily be performed in a laboratory. This means that RDTs are suited for use in both community- and facility-based settings, including sites with limited infrastructure that process low numbers of specimens daily.

Most RDTs can be performed with capillary whole blood collected by a finger-stick procedure using a lancet. This means that phlebotomy by venepuncture is not necessarily required. Always refer to the manufacturer’s instructions for use, however, for specific recommendations on specimen collection. Certain other RDTs are validated for use with oral fluid specimens. Results for RDTs are visually read and interpreted by the operator. The appearance of a control line and test line(s) signifies a reactive result, while the appearance of only a control line signifies a non-reactive result. As with any visually read assay, a second reader should independently read and interpret the test results to mitigate any reading errors.

RDTs are designed for qualitative (yes or no) detection of antibodies and/or antigen, rather than quantitative detection of the amount of antibodies, irrespective of the intensity of the test line(s). However, RDTs may show faint test lines, and so users may have difficulty interpreting these types of test results. It is critical to follow the manufacturer’s instructions for use, which usually say to read any band, irrespective of its intensity, as reactive. Some studies have observed that faint lines can be associated with false reactivity, but this is not always the case. Any visually read assay is prone to inter-reader variability and potential differences in interpretation. Therefore, it is suggested that the relative intensity of the test line(s) be scored on a scale of 0 (no line), +/− (possible line), 1+ (weak line), 2+ (strong line). When there is a

1 World Health Organization, Department of Essential Medicines and Health Products, Geneva
difference in the two readers’ results within the reading time recommended by the manufacturer, the test should be repeated. Automated, electronic RDT readers are increasingly available that can accommodate one or many brands of RDTs. Many of these RDT readers can connect with 3G or 4G wireless networks.

In instances where many tests are being carried out each day and those populations undergoing testing are well-retained, such as inpatients, people in prisons and other closed settings and patients with frequent follow-up visits, testing by laboratory-based techniques, such as EIA, may be more cost-effective than RDTs. RDTs are not well-suited to sites with high throughput (more than 40 specimens per day) unless adequate numbers of staff are trained in the test procedure. For busy testing sites, RDTs may be batched into runs. For immunochromatographic RDTs, no more than 10 tests should be batched into one run. For immunofiltration RDTs, the timing of adding the specimen and the reagents is critical.

The following aspects of HIV testing using RDTs deserve particular attention, especially in community-based testing (level 0) and testing in primary level facilities (level 1):

- **Storage temperature**: Ensure that the lower and upper temperature limits stated in instructions for use are respected, for example, 2 to 30 °C or 2 to 8 °C. If test kits are stored outside these temperature ranges, the quality of testing results cannot be assured.
- **Assay robustness**: Ensure that the exact volume (amount) of specimen and buffer are added to the test device according to the instructions for use. When provided, use the specimen transfer device supplied in the test kit; this device will have been designed and validated for a specific volume. Do not shake buffer/reagents bottles, which causes foaming and, therefore, inconsistent drop volume.
- **Incubation (and reading) times**: Ensure that the minimum and maximum reading times are observed as indicated in the instructions for use. For example, read the test result between 15 to 30 minutes after the specimen and/or buffer was added, but not after 30 minutes. These reading times should be strictly observed, even if a test line appears before the minimum reading time.
- **Interpretation of test results**: Ensure that the manufacturer’s instructions for use are observed, including whether to read faint test lines as reactive and how to read certain test results as invalid, for example, lack of a control line or high background colouring that obscures the reading window.

**Simple assays**

This type of HIV assay typically requires 30 minutes to 2 hours to produce results. Like RDTs, simple assays are read visually by the operator. This class of assays includes agglutination assays and indirect solid-phase EIAs. Agglutination assays allow antigen-coated particles to react with antibodies and form a visible clump, while indirect solid-phase EIAs allow antigen-coated combs to react and form a visible colour change. These types of assays are more suited to laboratory or facility-based testing than to use in or the community, as cold-chain storage of test kits and the use of precision pipettes are usually required. Generally, simple assays are designed for use with a serum/plasma specimen and, thus, require phlebotomy to collect an appropriate specimen.

**Immunoassays, including EIA, CLIA, ECL**

Enzyme immunoassays (EIA), chemiluminescence immunoassays (CLIA), and electrochemiluminescence immunoassays (ECLIA) for HIV are laboratory-based techniques to detect the presence of HIV-1/2 antibodies and/or HIV-1 p24 antigen. Generally, these assays are the most cost-effective to perform in laboratory settings with high specimen throughput (>40 per day). Immunoassays require multiple reagents, which typically necessitate cold chain storage and either specialised or general laboratory equipment, as well as an experienced and proficient technician.

A frequent feature of all types of immunoassays is the use of enzyme conjugates that attach to HIV antibodies (monoclonal/polyclonal) and colour-producing and light-producing substrates that then attach to the enzyme conjugates. Serum or plasma (potentially containing HIV-1/2 antibodies and/or HIV p24 antigen) is added to the solid
phase (for example, microtitre plate or plastic beads) and coated with HIV antigens, either recombinant proteins and/or synthetic peptides and, if fourth generation, also monoclonal/polyclonal antibodies. The reaction is allowed to incubate, followed by a wash step to remove unbound particles before the enzyme conjugate is added.

For indirect immunoassays, the antibody portion of the enzyme conjugate is usually anti-human immunoglobulin (Ig). This anti-human Ig will bind to anti-HIV antibodies in the specimen that have been captured by the antigen-coated solid phase. As a result, colour development in these assays is inversely proportional to HIV antibody concentration in the specimen. Colour development can be measured using a spectrophotometer. A cut-off value, usually determined by the manufacturer of the assay, takes into account the point where the results would be considered to be non-reactive; thus, EIA results are generally reported as optical density/cut-off (OD/CO) values.

Assays to detect p24 antigen only are usually immunoassay in format. They detect HIV p24 (core) antigen by employing monoclonal antibodies that target p24 antigen. These assays may be used as a supplemental assay to assist in diagnosis where fourth generation combined detection assays are utilised as part of the testing algorithm. Any antigen reactivity observed should be neutralizable upon repeat testing with the same specimen.

**Nucleic acid testing**

Nucleic acid testing (NAT) utilizes molecular techniques that may be used qualitatively to assist the diagnosis of HIV infection and quantitatively to monitor the progression of HIV infection and the response to ART. They include NAT technologies that detect the presence of HIV viral nucleic acid (RNA, DNA) via techniques based on amplification of viral nucleic acids, such as polymerase chain reaction (PCR) and nucleic acid sequence-based amplification (NASBA), or on amplification of the bound probe signal, as in branched-DNA (bDNA) assays. NAT has the ability to detect very low quantities of viral nucleic acid (high analytical sensitivity); a typical limit of detection for most NAT technologies is at about 50 copies/ml.

Qualitative nucleic acid testing is commonly used for early infant diagnosis (under 18 months of age), given the interference of passively transferred maternal antibody with serological methods. It may also be used to assist in the qualitative diagnosis of adults, including acute infection, when it is validated by the manufacturer as part of the intended use. Quantitative NAT is typically not recommended for diagnosis; as these assays are usually only validated for monitoring the level of virus in HIV-positive individuals, not as a diagnostic assay.

Laboratory-based technologies for NAT require sophisticated equipment, rigorous laboratory conditions and specimen collection, and highly trained staff who can perform precision steps and avoid contamination. Not all NAT technologies detect all HIV-1 subtypes equally well, and certain NAT technologies may not detect HIV-2 well unless they are optimised to do so. Newly developed NAT technologies that are simpler and more robust are intended for use at the point of care and may avoid some of the logistical and technical disadvantages of laboratory-based NAT technologies.

**Supplemental assays**

Supplemental assays may only be used for follow-up testing of specimens already found to be reactive on another assay; they are not meant to be used alone to determine HIV status. Certain other RDTs (immunochromatographic assay formats with multiple antigens) and simple immunoassays are used to verify reactive test results found by first-line assays. However, additional evaluation is required in the settings where they would be used in this way.

**Confirmatory assays such as Western blot and immunoblot**

Western blotting uses a blotting immunoassay technique to detect HIV-1 antibodies. In brief, HIV viral lysate is run on a polyacrylamide gel so that the viral proteins separate out according to their molecular weight; these proteins are then blotted on a nitrocellulose membrane. When a specimen containing HIV antibodies is added and incubated with the nitrocellulose membrane, antibodies against the specific viral proteins bind at different regions. In general, these assays are less sensitive than other types of assays, and are prone to high rates of indeterminate results. The methodology for
immunoblotting is similar, but, instead of viral lysate, recombinant proteins are blotted onto the nitrocellulose membrane; this improves the specificity of the assay.

Akin to a supplemental assay, a confirmatory assay should only be used to verify reactive test results found by other assays. The use of confirmatory assays should be able to provide a definitive result, although these assays are more expensive than other assays and are not always helpful if a high rate of indeterminate results is prevalent.

### 7.2 Mathematical model used as the rationale for development of HIV testing strategies

The development of testing strategies for HIV diagnosis is guided by the predictive value of a test result(s), which best indicates the proportion of results that represent the actual status. In contrast, the performance of assays is most often described in terms of their sensitivity and specificity.

#### Performance characteristics

**Table 7.1A. Table for determining performance characteristics**

<table>
<thead>
<tr>
<th>Index test result (HIV status reported by assay under evaluation)</th>
<th>Reference result (true HIV status)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a (true positive)</td>
<td>b (false positive)</td>
</tr>
<tr>
<td>c (false negative)</td>
<td>d (true negative)</td>
</tr>
</tbody>
</table>

Where:

\[
\text{Sensitivity} = \frac{a}{a+c} \quad \text{Specificity} = \frac{d}{b+d} \\
\text{PPV} = \frac{a}{a+b} \quad \text{NPV} = \frac{d}{c+d}
\]

**Sensitivity** is the probability of correctly identifying an HIV-infected individual. Thus, sensitivity is the number of true positives (a), divided by all true positives and false negatives (a+c), expressed as a percentage.

**Specificity** is the probability of correctly identifying an HIV-uninfected individual. Thus, specificity is the number of true negatives (d), divided by all true negatives and false positives (b+d), expressed as a percentage.

**Positive predictive value (PPV)** is the probability that, when the assay is reactive, the specimen does contain HIV. Therefore, it indicates the probability that, in the case of a positive test result, the individual really has HIV.

**Negative predictive value (NPV)** is the probability that, when the assay is negative, the specimen does not contain HIV. Therefore, it indicates the probability that, in the case of a negative test result, the individual really does not have HIV.

Positive and negative predictive values are not intrinsic to the assay but rather are heavily affected by prevalence. The probability that a test result will accurately determine the true status (the PPV) varies with the prevalence of HIV infection in the population from which the person comes. In general, the higher the prevalence of HIV infection in the population, the greater the probability that a person testing positive is truly infected (that is, the greater the PPV). Thus, this means that as HIV prevalence increases, the proportion of individuals testing false-positive will decrease. Conversely, the likelihood that a person whose test result is negative is truly uninfected (the NPV) decreases as prevalence increases. Therefore, as prevalence increases, so does the proportion of individuals testing false-negative.
The following example illustrates calculation of a PPV, in this case using a 1-test testing strategy.

\[
PPV = \frac{\text{prevalence} \times \text{sensitivity}}{\text{prevalence} \times \text{sensitivity} + (1 - \text{prevalence}) \times (1 - \text{specificity})}
\]

For prevalence of 5%, using an serological assay with sensitivity of 99% and specificity of 98%

\[
PPV = \frac{0.05 \times 0.99}{(0.05 \times 0.99) + ((1 - 0.05) \times (1 - 0.98))}
\]

\[
= \frac{0.0495}{(0.0495) + (0.95 \times 0.02)}
\]

For prevalence of 1%, using an serological assay with sensitivity of 99% and specificity of 98%

\[
PPV = \frac{0.01 \times 0.99}{(0.01 \times 0.99) + ((1 - 0.01) \times (1 - 0.98))}
\]

\[
= \frac{0.0099}{(0.0099) + (0.99 \times 0.02)}
\]

Thus, an assay with the same sensitivity and specificity will give a different PPV depending on the prevalence.
The following example applies this equation to a high-prevalence setting with up to three serological assays that detect the same analyte.

Population: 10 000; prevalence: 5%; A1, A2 A3 sensitivity: 99%; A1, A2 A3 specificity: 98%

Total true positive individuals: 495
490 individuals with A1+ A2+
5 individuals with A1+ A2+ upon repeat

Total false positive individuals: 8
4 individuals with A1+ A2+
4 individuals with A1- A2 +

Total true negative individuals: 9496
9310 individuals with A1-
178 individuals with A1- A2-
4 individuals with A1+ A2+ A3-

Total false negative individuals: 5
5 individuals with A1-

Source: Mercedes Perez Gonzalez, WHO
The following example applies this equation to a low-prevalence setting with up to three serological assays that detect the same analyte.

Population: 10 000; prevalence: 1%; A1, A2, A3 sensitivity: 99%; A1, A2, A3 specificity: 98%

Total true positive individuals: 98
98 with A1+ A2+ A3+

Total false positive individuals: 0

Total true negative individuals: 9896
9702 with A1-
4 with A1+ A2-
186 with A1- A2-
4 with A1+ A2+ A3-
4 with A1- A2+ upon repeat

Total false negative individuals: 2
1 with A1-
1 with A1+ A2+ A3- (would be reported as HIV inconclusive)

Source: Mercedes Perez Gonzalez, WHO
7.3 Worked example of validation of an HIV testing algorithm comprised of three serological assays

A testing algorithm is the combination and sequence of specific assays (brands) used within a given HIV testing strategy. Validation refers to the “confirmation by examination and provision of objective evidence that the particular requirements for a specific intended use are fulfilled” (3). Therefore, validation in this context does not equate to evaluation but rather is gives evidence that a particular testing algorithm may be used by comparing its performance to that of the reference standard testing algorithm.

Phase 1: Prepare for validation study

a. Select candidate serological assays
   These 6-10 candidate assays that are available commercially on the market in the setting of intended use, from the following lists of quality assured products and in accordance with national requirements:
   - national registration database for approved HIV in vitro diagnostics; and
   - list of products eligible for WHO procurement, including WHO-prequalified in vitro diagnostics2; and/or
   - list of products eligible for procurement under donor arrangements.

   These assays will already have been assessed to determine if the manufacturer has sufficient validation and verification data to support the claims they have made about the performance and operational characteristics for the product, such as clinical sensitivity and clinical specificity.

   Therefore, the aim of the validation study is not to repeat an evaluation of diagnostic accuracy but rather to determine the combination of assays that work best together within a testing algorithm.

If necessary, a preliminary set of specifications (performance and operational characteristics) may be used to rule in candidate assays to be considered for the testing algorithm. See Table 7.5 in the Consolidated Guidelines of HIV testing services (http://www.who.int/hiv/pub/guidelines/hiv-testing-services), Specific considerations for selection of HIV diagnostics, for additional information.

The most sensitive assay available should be selected for use as the first line assay (A1), with more specific assays selected as second line (A2), and third line (A3) assays. Of particular note, many commercially available assays are designed as screening assays, meaning that intended to be used as A1. These assays are less suitable for use as A2 and A3 as they are designed to rule in any potential reactivity rather than to rule out false reactivity. Determining the assays to be used as A2 and A3 is critical, particularly for high prevalence settings where the result of A2 will determine the HIV-status.

Combinations of RDTs can be used to accurately diagnose HIV infection, when validated to ensure that these RDTs do not share false (incorrect) test results for the same specimens.

Immunoassays such as EIAs may be used, particularly as A1 given their high sensitivity, in settings with high throughput and the necessary infrastructure and laboratory trained staff. Due care should be taken if immunoassays are used as A2 and A3 to ensure that specificity is high and cross-reactivity with A1 is minimal.

Supplemental assays and confirmatory assays may also be selected for use within a given testing algorithm. Confirmatory assays such as Western blots and line immunoassays were traditionally used to give a definitive diagnosis but are relatively resource intensive and generate high rates of indeterminate results. Supplemental

---

2 List of products eligible for WHO procurement http://www.who.int/diagnostics_laboratory/procurement/purchase/en/;
assays such as HIV p24 antigen assays and HIV-2 antibody only or HIV-1 antibody only assays are examples of such assays that might be used to determine acute infection or to type HIV infection, respectively.

b. **Request test kits**

It is the responsibility of the study principle investigator to obtain sufficient number of tests from **one lot of each assay** from the manufacturer (or distributor in the country of study). These test kits should be stored in conditions stated in the manufacturer’s instructions for use. Any additional consumables that are required to perform each of the assays must are available.

c. **Request validation panel**

The validation panel will contains challenging locally-derived specimens, for example, all HIV subtypes, naturally occurring low titre specimens, panels of specimens collected from individuals undergoing seroconversion, specimens that contain commonly occurring interferents and specimens that exhibit false-reactivity on other commonly used assays. The validation panel should be well characterised by the provider of the panel, and this will not need to be repeated. The reference results will be made available along with the panel.

Specimens will be characterised for HIV (antibodies and antigen) using serological assays (RDTs, immunoassays, confirmatory assays, supplemental assays) and possibly for HIV nucleic acid using NAT technologies.

Serum/plasma specimens will be used for their ease and ability to be collected in larger volumes. As the manufacturer will have validated the candidate assay on other specimen types such as capillary whole blood, if it is claimed in the instructions for use, as part of the validation and verification studies. The validation panel should be stored according to the instructions of the panel provider which will usually be -20 °C or -80 °C.

**Phase 2: Conduct validation study**

a. **Study site**

The validation study may be conducted in any setting, as designated by national authorities. Although, it is likely that this study would most easily be conducted within a laboratory given that up to 6-10 candidate assays will need to be tested on the same panel of up to 500 specimens.

b. **Study staff**

All laboratory technicians should follow standard operating procedures, and be trained in the performance of each candidate assay with adequate documentation in the form of standard operating procedures and standardized run worksheets. Accurate recordkeeping is crucial, and it should be emphasized that transcription errors are common. Each operator testing the candidate assays should be blinded to the expected reference result for each of the specimens.

c. **Study panel**

Each specimen of the validation panel should be labelled with specimen ID numbers that do not reveal their expected reference result. All candidate assays should be tested (in single) on the same validation panel, preferably by the same operator. It is critical to be judicious when using the validation panel, aliquots should only be removed when needed. It is preferable to test all assays with the same specimen on the same day, this will reduce inter- aliquot variability.

d. **Results interpretation**

**Results for visually read assays (RDTs, other simple assays)**

---

3 It is anticipated that institutions such as WHO Collaborating Centre will be able to provide validation panels.
Ideally, a second reader should make a blinded rereading of any visually read assay after the first reader (usually the operator) has read and recorded the overall test result. In addition, the intensity of the test line/band should be recorded.

- If the two readers interpret the test results the same way, then the status of the specimen is recorded as is.
- If the two readers do not agree, a third reader should adjudicate on the reading. With the majority reading taken.

If a result cannot be conclusively reached, the result should be recorded as inconclusive and the specimen retested on a new test device from the same lot. These should be included in the data analysis as a misdiagnosed specimen.

**Results for instrument based assays (EIA, CLIA, ECL)**

Certain immunoassays will display an OD/CO ratio that is within the grey zone, according to the manufacturer's instructions for use (usually 0.90 – 1.10). These specimens should be repeated in duplicate on the same lot. These should be included in the data analysis as a misdiagnosed specimen.

**Invalid results**

For RDTs, invalid test results are typically when the control line does not appear, irrespective if the test line appears or not, and when high background colour completely obscures the result window. Other anomalies should also be recorded such as streaking across the membrane, non-migration of specimen, debris on the membrane, etc.

For instrument-based assays (including immunoassays such as EIA, CLIA, ECL), invalid results or invalid runs occur when the internal and/or external test kit controls (HIV negative, HIV positive) are not within the acceptance range specified in the manufacturer's instructions for use.

All invalid results should be recorded in the data analysis.

e. **Study data analysis**

If the initial testing result does not agree with the expected reference result, the specimen in question should be tested again in duplicate on the same lot.

- If the same result is observed, then the result is recorded as not in accordance with the reference result and is included in the data analysis. For example, if a HIV-1 subtype O specimen is found non-reactive by a candidate assay, the specimen would be repeated on the same assay (same lot), if the specimen is still non-reactive, then the assay does not detect that HIV-1 subtype O specimen, i.e. disagreement with the expected reference result.

- If the results remain discrepant from the reference result, the result is recorded as discrepant and the result is included in the data analysis. For example, if a HIV-1 subtype O specimen is found non-reactive by a candidate assay, the specimen would be repeated on the same assay (same lot), if the specimen is then found to be reactive, then results are discrepant for detection of HIV-1 subtype O specimen, i.e. initially false non-reactive, final reactive result.

**Determining seroconversion sensitivity**

The results obtained from seroconversion panels using the candidate assays should be compared against an assay designated as the reference assay for determining relative seroconversion sensitivity.

- For each seroconversion panel tested, the first specimen in the sequential panel to become reactive with reference assay is assigned the value “0”. Results from the candidate assays are compared with
reference assay by determining the difference between the specimen assigned value "0" and the relative position in the sequence of the first specimen showing a reactive result for the candidate assays. For example, if a candidate assay becomes reactive two specimens earlier in a series than the reference assay, the value assigned for that series in that assay would be -2. Similarly, if a candidate assay becomes reactive one specimen later than the reference assay, the value assigned would be +1. The assigned values over the total number of seroconversion panels are averaged to determine a mean relative seroconversion sensitivity index for each candidate assay and the 95% confidence interval is determined.

- Seroconversion panels should be characterized according to the current national validated HIV testing algorithm.
- The results may be presented in the following way whereby any candidate assay with a value that is above 0 detects HIV infection later than the benchmark assay, and any candidate assay with a value below 0 detects HIV infection earlier than the benchmark assay.

Fig 7.2A: Relative seroconversion sensitivity compared to benchmark assay

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower 95%</td>
<td>-0.24</td>
<td>-0.106</td>
<td>-0.842</td>
<td>-0.4206</td>
<td>-0.4206</td>
<td>-0.4206</td>
<td>0.521</td>
</tr>
<tr>
<td>mean</td>
<td>0.5</td>
<td>0.125</td>
<td>0.125</td>
<td>0.125</td>
<td>0.125</td>
<td>-0.125</td>
<td>0.5</td>
</tr>
<tr>
<td>Upper 95%</td>
<td>2.274</td>
<td>0.4206</td>
<td>0.3422</td>
<td>0.1706</td>
<td>0.1706</td>
<td>0.1706</td>
<td>0.5469</td>
</tr>
</tbody>
</table>

**Determining analytical sensitivity**

The number of specimens correctly classified by the candidate assay is compared to the expected reference result for panels that challenge analytical sensitivity such as HIV-1 mixed titre and HIV-1 p24 antigen mixed titre performance panels, and the WHO international reference preparation.

- In certain cases, the format of the assay will limit its ability to detect at certain stage of HIV infection. For example, a fourth generation assay that incorporates both detection of antibodies to HIV-1/2 and HIV antigen will likely detect HIV infection earlier than assays that detect antibodies to HIV-1/2 only.
- Panels for analytical sensitivity should be characterized according to the current national validated HIV testing algorithm.

**Determining analytical specificity**

The number of specimens correctly classified by the candidate assay is compared to the expected reference result for panels that challenge analytical specificity such as cross-reacting and interfering substances.

- Panels for analytical specificity should be characterized according to the current national validated HIV testing algorithm.

f. **Study results**

The results generated on all of the candidate assay should be tabulated and disaggregated between results for candidates for first-line assay and candidates for second-line and third-line assays.
• **One assay with the highest sensitivity** (analytical, seroconversion) should be chosen as first-line, with one back-up first-line assay. These assays should be interchangeable.

• **Two different assays with the highest specificity** (analytical) should be chosen as second-line and third-line assays, with one back-up for second-line assay and third-line assay.

g. An appropriate combination of assays should be determined. It is critical to ensure as far as possible that the selected assays did not produce concordant false-reactive or false-non-reactive results in the validation panel.

| The results of the validation study should remain valid for a period of 3-5 years. |

**Phase 3: Monitor implementation of the testing algorithm(s)**

a. Implement the newly proposed testing algorithm in parallel with an existing algorithm for a period of two weeks for high throughput testing sites or four weeks for low throughput testing sites. Data should be collected on the rate of reported HIV-inconclusive status and the rate of invalid test results (no more than 5% is acceptable) and any comments related to test procedures or other operational characteristics should be documented. A discrepancy rate of >1% between new and existing algorithms is significant and requires investigation and possible repeat of the validation study.

b. Monitoring of the testing algorithm(s) should continue in keeping with quality systems principles (see Chapter 8 on the Consolidated Guidelines of HIV testing services [http://www.who.int/hiv/pub/guidelines/hiv-testing-services](http://www.who.int/hiv/pub/guidelines/hiv-testing-services)).
7.4 Additional information on recommendations for retesting of HIV negative individuals

See Chapter 5 on the Consolidated Guidelines of HIV testing services (http://www.who.int/hiv/pub/guidelines/hiv-testing-services) for additional information on messages for people who are HIV-negative and retesting.

**HIV-negative people with ongoing risk:**

1. **People with ongoing risk**, including people from **key populations** and persons with a **known HIV-positive partner**, should be tested for HIV at least annually and provided with appropriate risk-reduction counselling for the respective population.

2. Persons with **known HIV exposure who test HIV negative** at the first HIV test following the incident (for example, sex with a known HIV-positive person or sharing of injecting equipment with a known HIV-positive person) warrant retesting four weeks after the initial test to verify that they are truly HIV-negative.

3. For individuals who have had a **specific incident of possible HIV exposure** in the past 72 hours, baseline HIV testing is recommended for the individual with potential exposure as well as for the source person of the exposure. This will guide appropriate clinical action and inform the exposed individual and, when possible, the source person of their HIV status. Post-exposure prophylaxis (PEP) should not be delayed awaiting the HIV test result for the source person. If HIV testing is not immediately available for the exposed individual, PEP should be offered and HIV testing provided as soon as feasible. PEP is not indicated if the exposed person is already HIV-positive. If an individual considered eligible for PEP is found to be already HIV-positive, he or she should be referred to appropriate services for assessment for ART eligibility according to national guidelines.

All individuals given PEP should be encouraged to undergo HIV testing three months following their exposure. In persons who have been exposed to PEP, the production of HIV-1/2 antibodies may be affected and, in some cases, the window period may be longer. In some case false-negative results may be seen; hence the requirement for retesting three months after exposure.

4. **Pregnant women in generalized epidemics** should be tested for HIV as early in pregnancy as possible, preferably in the first trimester, to start treatment for PMTCT as soon as possible. Women who test HIV-negative in the first trimester should be retested in the third trimester, preferably between the 28th and 36th weeks. If a woman has not been tested during pregnancy, or has been tested during the first trimester but does not return for testing during her third trimester, testing should be done during labour or, if that is not possible, immediately after delivery.

5. Individuals seen for diagnosis or treatment of **STIs**, **TB** patients with a new potential HIV exposure or at higher risk for HIV exposure and outpatients with **clinical conditions indicative of HIV infection** with an HIV-seronegative status should be retested four weeks after they were initially tested. After this first retesting, guidance on further testing varies by population, as described in specific guidance published elsewhere (4).

**References**


---

4 For further information on when people receiving PEP should be retested, see Guidelines on post-exposure prophylaxis for HIV and the use of cotrimoxazole prophylaxis for HIV-related infections among adults, adolescents and children. Recommendations for a public health approach - December 2014 supplement to the 2013 consolidated RV guidelines (http://www.who.int/hiv/pub/guidelines/arv2013/arvs2013supplement_dec2014/en/).