Evidence and rationale for using WHO HIV testing strategies and algorithms

Dr Cheryl Johnson, WHO Global HIV, Hepatitis and STIs Programme

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Progress toward global HIV testing targets

- **Initial slow start to steep increase:**
  - In 2005, ~10% PLHIV diagnosed & 700,000 on ART by the end of 2004
  - RDTs & PITC had big impact on scale-up

- **Initial decelerated increase:**
  - High hanging fruits more difficult to reach via traditional strategies

- **Scale-up of successes – but gaps remain:**
  - Costs of additional testing increasing, gaps remain, challenging to effectively focus and rationalize core and additional testing

- **Countries achieving 90-90-90 & ART coverage high:**
  - 84% PLHIV diagnosed & now 27.5 million on ART
  - More HTS options, DSD, decentralization achieving success

Source: WHO forecast 2020; UNAIDS 2022; WHO 2005; CHAI 2015; WHO, UNICEF, PEPFAR, GFTAM 2018
HIV positivity is declining and will continue to decline in sub-Saharan Africa

80-90% of all PLHIV diagnosed

Key HTS trends
The number of people newly diagnosed with HIV declining rapidly due to ART scale-up
Between 2000-2020, HTS positivity declined from 9% to 2.8%; and will continue to decline
No country achieving HTS positivity at or above 5% nationally

In 2020, 3 million people tested 91,000 new positives identified

Example in high HIV burden country: Malawi

- Undiagnosed PLHIV declined from 78% in 2005 to 14% in 2017 and is projected to continue declining to around 6% in 2025.

- By 2025 national HTS positivity is expected to reach 1.5%.
  - Discounting those who already know their status, further reduces HTS positivity to 0.5% in 2025 nationally.

Source: Graphic courtesy MOH Malawi, CROI 2020 Eaton, WHO 2019
Important opportunities to address STIs

Particular focus on syphilis

Source: WHO GHSS 2022; Storey 2019
Important opportunities to address STIs

*Particular focus on syphilis*

Recommends use of dual HIV/syphilis RDTs for key populations

Prioritize for first test in ANC

Recommends use of dual HIV/syphilis RDTs for pregnant women

Annual or bi-annual testing most cost-effective

Source: WHO 2022; WHO 2019

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High quality RDTs

- 23 different WHO PQed RDTs available for procurement

- HIV RDTs
  - All meet WHO’s standards for at least 99% sensitivity and 98% specificity

- Dual HIV/Syphilis RDTs
  - 3 products available for procurement & low cost

Source: [https://extranet.who.int/pqweb/vitro-diagnostics/prequalification-reports/whopr?field_whopr_category_tid=58](https://extranet.who.int/pqweb/vitro-diagnostics/prequalification-reports/whopr?field_whopr_category_tid=58)
Adapting national HIV testing strategies

WHO recommends all countries currently using two consecutive reactive tests for an HIV-positive diagnosis to move toward using three consecutive reactive tests for an HIV-positive diagnosis. This is increasingly important as treatment-adjusted HIV prevalence and national HTS positivity continue to decline over time.

- Ensure that the testing strategy has a positive predictive value ≥99% (PPV)
  - Meaning of the persons classified as HIV+, ≥99% will truly be living with HIV
  - PPV depends on positivity rate among testing population

- Quality assured assays, such as WHO prequalified, should be used:
  - >99% sensitivity: fewer than 1 ‘false negative’ for 100 truly positive
  - >98% specificity: fewer than 2 ‘false positive’ for 100 truly negative
  - Either rapid diagnostic tests (RDTs) or enzyme immunoassay (EIA, CLIA, ECL)

Source: WHO 2019
**Understanding positive predictive value (PPV)**

\[ PPV = \text{probability a person with a reactive HIV positive test result has HIV} \]

- **High HIV prevalence**: HIV testing with more undiagnosed PLHIV = greater likelihood of accurately diagnosing person with fewer tests.

- **Low HIV prevalence**: HIV testing with few undiagnosed PLHIV = lower likelihood of accurately diagnosing person with fewer tests.

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WHO recommended 3-test strategy

- All individuals are tested on Assay 1 (A1). Anyone with a non-reactive test result (A1−) is reported HIV-negative.
- Individuals who are reactive on Assay 1 (A1+) should then be tested on a separate and distinct Assay 2 (A2).
- Individuals who are reactive on both Assay 1 and Assay 2 (A1+; A2+) should then be tested on a separate and distinct Assay 3 (A3).
  - Report HIV-positive if Assay 3 is reactive (A1+; A2+; A3+).
  - Report HIV-inconclusive if Assay 3 is non-reactive (A1+; A2+; A3−). The individual should be asked to return in 14 days for additional testing.
- Individuals who are reactive on Assay 1 but non-reactive on Assay 2 (A1+; A2−) should be repeated on Assay 1.
  - If repeat Assay 1 is non-reactive (A1+; A2−, repeat A1−), the status should be reported as HIV-negative.
  - If repeat Assay 1 is reactive (A1+; A2−, repeat A1+), the status should be reported as HIV-inconclusive, and the individual asked to return in 14 days for additional testing.

Source: WHO 2019
Difference between 2-test and 3-test strategy?

- **2- or 3-test strategy** refer to number of consecutive reactive tests to diagnose HIV
- **both strategies** require 3 assays (A3) & neither uses any tiebreaker approaches
- **3 test strategy** recommended since 1997 & has been used in most settings outside Africa because of lower burden
WHO recommended testing strategy for HIV/syphilis

**Dual test used as the first test in strategy**

1. **A1**: HIV-, TP-
   - Report HIV-negative
   - Report syphilis-negative

2. **A1**: HIV-, TP+
   - Report HIV-negative
   - Report syphilis-positive, indicative of either current or past/resolved infection

3. **A1**: HIV+, TP-
   - Perform A2 (HIV only)
   - A2: HIV+
     - Perform A3 (HIV only)
   - A2: HIV-
     - Repeat A1 (HIV/TP)

4. **A1**: HIV+, TP+
   - Report syphilis-positive, indicative of either current or past/resolved infection

**Treatment for any reactive syphilis result in pregnant women**

- **A1**: Assay 1, **A2**: Assay 2, **A3**: Assay 3, TP: *Treponema pallidum* (syphilis).
- **A1** (Assay 1) is a dual HIV/syphilis rapid diagnostic test (RDT).
- **A2** and **A3** (Assay 2 and Assay 3) are HIV RDTs or enzymelinked immunosorbent assays (ELISAs).
- When resolving discrepant results, all reactive TP (syphilis) results, including **A1**, **TP-** or Repeat **A1**, **TP+**, should be referred for treatment and further testing according to national guidelines.
- When resolving discrepant results, if **A1** and Repeat **A1** are both TP (syphilis) nonreactive results, report syphilis-negative.

**HIV testing strategy is the same as standard of care**

Source: WHO 2022; WHO 2019
PPV and number of tests

Probability of correctly being classified as HIV positive (assuming 99% sensitivity; 98% specificity)

- **After 1 assay**
  - 5,000 HIV+ specimens tested; 5% prevalence
  - 4950 reactive
  - PPV = \( \frac{4950}{4950 + 1900} = 72\% \)

- **After 2 assays**
  - 4901 reactive
  - PPV = \( \frac{4901}{4901 + 38} = 99.2\% \)

- **After 3 assays**
  - 4851 reactive
  - PPV = \( \frac{4851}{4851 + 0.8} > 99.9\% \)

**Simplified algorithm - consecutive reactive HIV tests only**
Why a 3-test strategy for all settings?

No more settings have HTS positivity nationally at 5% or above, thus 99% PPV cannot be maintained. Without the 3-test strategy there will be increasing number of people misdiagnosed with HIV.

Outcomes per 100,000 tested
Assuming 99% sensitivity; 98% specificity; simplified algorithm -- consecutive HIV+ tests only

<table>
<thead>
<tr>
<th>True prevalence</th>
<th>Per 100,000 tested</th>
<th>After 1 assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>10%</td>
<td>10,000 HIV+ 90,000 HIV-</td>
<td>9900 true+ (99%) 1800 false+ (2%) 85% PPV</td>
</tr>
<tr>
<td>5%</td>
<td>5000 HIV+ 95,000 HIV-</td>
<td></td>
</tr>
<tr>
<td>1%</td>
<td>1000 HIV+ 99,000 HIV-</td>
<td></td>
</tr>
<tr>
<td>0.1%</td>
<td>100 HIV+ 99,900 HIV-</td>
<td></td>
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</tbody>
</table>
Conducted modelling with country data to inform WHO the HIV testing guidelines

Modell compared 2-test vs 3-test strategy for varying positivity levels (5% to 0.1%):
- Number of misclassifications.
- PPV and NPV.
- Number of test kits used.
- Total HTS cost.

Source: Eaton et al 2021 https://www.medrxiv.org/content/10.1101/2021.03.31.21254700v1.full
Expected number of false negative, false positive, and inconclusive classifications per 100,000 clients

- Greater false positives diagnoses with 2-test strategy
- Greater number of inconclusives with 3-test strategy (good tradeoff as would have been misdiagnosed HIV positive under 2-test strategy)
Negative predictive value and positive predictive value for 2 vs 3 test strategy

Positive predictive value drops off substantially as HIV positivity in population being tested drops
Test kits used

First test in national algorithm drives costs
Additional third test has limited impact
Cost differences

Additional third test does not increase testing programme costs
WHO retesting considerations

Retesting prior to ART initiation recommended by WHO

- Strongly reinforced in 2014 as part of Treat All guidance when clinical assessment requirements were removed
- Highly cost-saving compared to even few cases of misdiagnosis and wrongful initiation of life-long treatment

What does retesting do?
- Provides quality assurance to prevent unnecessary lifelong ART initiation
- Primarily addresses human error that occurs in HIV testing services
- Does not replace need for 3-test strategy, as it has a completely different purpose

Cost of retesting before ART initiation

<table>
<thead>
<tr>
<th></th>
<th>Low Prevalence</th>
<th>High Prevalence</th>
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<tbody>
<tr>
<td>HIV prevalence among</td>
<td>1.0%</td>
<td>10.0%</td>
</tr>
<tr>
<td>testers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serial testing strategy</td>
<td>3-test</td>
<td>2-test</td>
</tr>
<tr>
<td>Total testing cost</td>
<td>$82628</td>
<td>$87020</td>
</tr>
<tr>
<td>Number of HIV–initiated on ART</td>
<td>9.2</td>
<td>38.9</td>
</tr>
<tr>
<td>Expected lifetime ART cost for HIV–</td>
<td>$57832</td>
<td>$243399</td>
</tr>
<tr>
<td>Total retesting cost</td>
<td>$2011</td>
<td>$14020</td>
</tr>
<tr>
<td>HIV–initiated on ART with retesting</td>
<td>0.03</td>
<td>0.6</td>
</tr>
<tr>
<td>Expected lifetime ART cost for HIV–</td>
<td>$186</td>
<td>$3628</td>
</tr>
<tr>
<td>Expected savings from retesting</td>
<td>$55634</td>
<td>$225751</td>
</tr>
<tr>
<td>Time to recover retesting costs by averted ART costs</td>
<td>0.5 y</td>
<td>0.8 y</td>
</tr>
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# Retesting on ARVs

*Potential factors associated with false negative results*

<table>
<thead>
<tr>
<th>Assay-specific</th>
<th>Host-specific</th>
<th>Virus-specific</th>
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</table>
| • Seronegativity associated with assays using only env antigen to detect HIV antibody | • Immunity:  
  o ARVs (including PrEP) blunt antibody response  
  o Antibody titer reduced over time among those virally suppressed | • ARVs induce viral suppression  
  o Low VL associated with nonreactivity |
| • Among individuals diagnosed and treated during acute infection, 4th gen IA was less sensitive than 3rd gen IA regarding Ab seroconversion | • Timing of ART initiation during infection:  
  o In AH1, starting ART in Fiebig stage I-II produced greatest non-reactivity  
  o Children started on ART<6 months of age had greatest non-reactivity  
  o Starting ART based on CD4 count (>350 vs. <350) appears to have no effect | • Clade, sub-type  
  o HIVCRF01_AE developed reactive Oraquick results earlier |
| • Oral fluid assays performed poorly among adults and children infected with HIV | • Genetics:  
  o Children genetically predisposed to seronegativity in presence of ART (HLA alleles) | • ARVs reduce size of viral reservoir |

CONTINUUM (Often difficult to tease out specific causes)
Retesting on ARVs

Key considerations from WHO guidelines

Key guidance for addressing retesting on ARVs

• Most PLHIV who are on ART and who retest will continue to test positive
  • However, there are a few cases that can be missed, sometimes people diagnosed and started on ARV during the acute HIV infection period which is generally rare
  • Oral fluid HIV RDTs (i.e. often used for self-testing) were also slightly more affected when compared to other HIV assays (but remember overall cases were still very few)

• Programmes should not actively seek to retest PLHIV on ART

• PLHIV on ART who retest should be made aware of the possibility of false negative results

• Efforts to accurately establish HIV infection are important among individuals who may have acquired HIV while taking PrEP prior to initiating treatment

Source: https://www.who.int/publications/i/item/978-92-4-155058-1
Conclusions

Testing strategies should reflect changes in epidemiology:

- 3-test strategy substantially reduces false-positive misclassifications to ensure that 99% PPV target is achieved.
- Increases ‘inconclusive’ results (A1+/A2+/A3-), but most will be confirmed negative at day 14 (a good thing).
- Retesting on ARVs among PLHIV can result in false negative results, but is unlikely a key contributor to false negative results

Incremental budgetary impacts are low:

- Cost of 3- vs. 2-test algorithm are similar; switching to 3 test strategy doesn’t substantially increase costs
- Lessons learned are that lowest cost first test has greatest impact
- Retesting prior to ART initiation remains cost-saving

Programmatic implications:

- Finding new ways to organize and restructure HTS is important (test for triage, HIV self-testing).
- Incorporate dual test into updates and roll-out of 3-test strategy
- Retesting prior to ART initiation still advised, but could be prioritized to increase feasibility in certain settings
For more information on HIV testing services

Contact: Cheryl Johnson johnsonc@who.int
Céline Lastrucci lastruccic@who.int