

# Preventing HIV misdiagnosis

## Implementation guide



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# Background

The accuracy of HIV testing is critical to prevent misdiagnosis. The consequences of providing an incorrect test result can be serious for clients, HIV testing services (HTS), HIV programmes and public health. A false-positive diagnosis can lead to unnecessary lifelong antiretroviral therapy (ART) and social and emotional consequences for individuals and their families. A false-negative diagnosis means that someone living with HIV will not benefit from ART and could unknowingly transmit HIV to partners and, in the case of pregnant and breastfeeding women, to their infants.

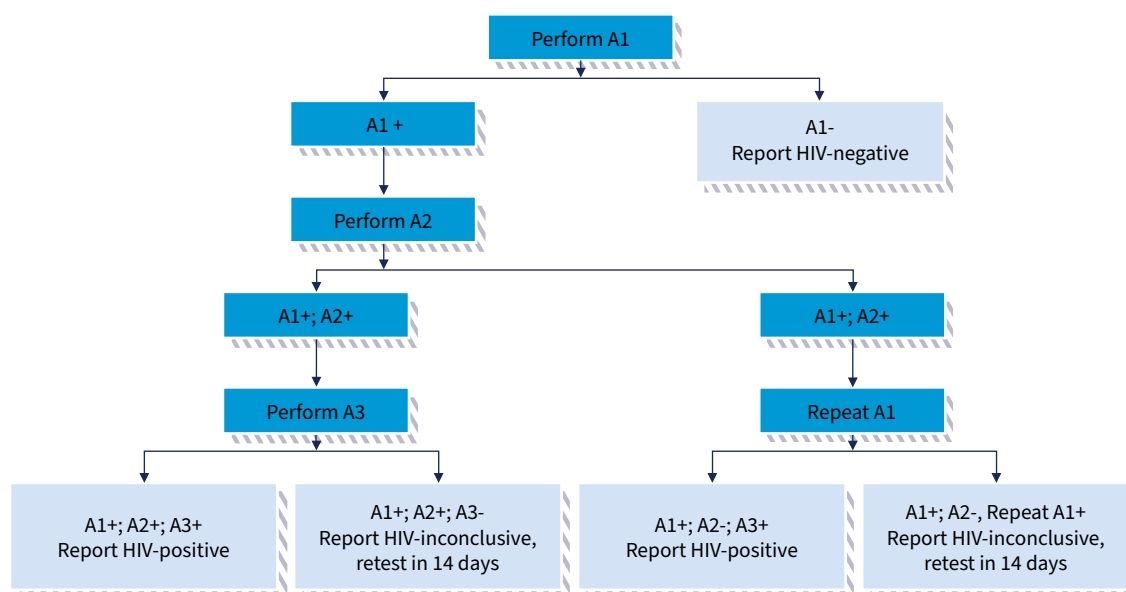
With the evolution of global HIV epidemiology, HIV testing approaches must also evolve to maintain accuracy and efficiency in population-level diagnosis. While significant progress has been made toward achieving global HIV diagnosis goals – with an estimated 86% of individuals living with HIV diagnosed at the end of 2022 (1) – it is essential that HIV testing is accurate, and misdiagnoses are minimized. It also remains critical that HIV testing adheres to the World Health Organization (WHO) 5 Cs of HIV testing: Consent, Confidentiality, Counselling, Correct test results and Connection (linkage to prevention, care and treatment services) (2).

Prior to 2019, WHO recommended that countries with an HIV prevalence greater than 5%, in the testing population, use a **two-test strategy** (that is, two consecutive reactive tests for a positive diagnosis) and that countries with an HIV prevalence less than 5%, in the testing population, use a **three-test strategy** (that is, three consecutive reactive tests for a positive diagnosis, as shown in Fig. 1).

**In 2019 WHO recommended that all countries use a three-test strategy** – regardless of national HIV prevalence. This recommendation stands. In most testing services, the HTS positivity, or “yield” (percent HIV-positive among those undergoing testing), has fallen below 5%. Using a three-test strategy as a standard testing practice maintains the accuracy of diagnosis in HTS programmes and prevents misdiagnosis.

The three-test strategy maintains accuracy and prevents misdiagnosis.

**Fig. 1. WHO-recommended testing strategy using three consecutive tests for a positive diagnosis**



A1: Assay 1 (first test); A2: Assay 2 (second test); A3: Assay 3 (third test).

Source: WHO Consolidated guidelines on HIV testing services, 2019

Note: Following inconclusive results, the full three-test strategy, beginning with A1, should be repeated at 14 days.

This implementation guide provides background for the rationale behind this WHO guidelines on HIV testing (2). It also offers practical advice on switching to a three-test strategy and instituting other measures that can help national HIV programmes to **deliver high quality, accurate HIV testing services and to assure that misdiagnosis is minimized**. The WHO

Global HIV, Hepatitis and STI Programmes, along with WHO regional and country offices, developed this note in response to questions from Member States and other partners about delivering HIV testing services. This guide includes references to other published WHO information relevant to using three serial tests to diagnose HIV, selecting HIV tests and using them in the right order, retesting people prior to starting ART and supporting quality management systems (QMS). Table 1 defines key terms used in this information note.

**Table 1. Key terms**

Terminology	Definition
Assay	A synonym of test kit; in the case of HIV, all the components of a test kit used to identify HIV p24 antigen or HIV-1/2 antibodies.
Testing strategy	A sequence of tests conducted on assays to achieve a specific objective, such as screening for infection or diagnosis of infection.
Testing algorithm	When specific products are populated into a testing strategy, it is a testing algorithm. A specific product is defined with a product name, product code(s), a manufacturing site and a regulatory version. The testing algorithm is likely to change depending on which specific products are verified for use together and are procured.
HTS positivity	The proportion of HIV-positive results among those undergoing HIV testing in a national programme.
Retesting	When an individual is tested again using the same testing algorithm during another testing event – for example, retesting 14 days after an HIV-inconclusive status, periodic retesting for people taking pre-exposure prophylaxis (PrEP), maternal retesting, or retesting to verify an HIV-positive diagnosis prior to ART initiation.
Confirmatory testing	Confirmation of an HIV-positive result needs to be done using the WHO standard three-test strategy (using rapid diagnostic tests (RDT) and/or immunoassays (IA)).  Confirmatory testing refers to an additional testing event providing an HIV-positive result. For example, after a reactive HIV self-test (HIVST) or other A0 test.
Recency testing	An HIV recency assay classifies an HIV infection as recent or long-standing. It is either a serological laboratory-based assay or a rapid test for recent infection (RTRI) conducted at a testing site. Recency assays use one or more biomarkers to determine the longevity of infection, typically by measuring the evolution of the immune response following initial infection. The estimated time since infection depends on the assay, but a “recent” infection is generally considered to be within the preceding 12 months.  WHO does not recommend the use of recency testing in routine HIV testing services.
Positive predictive value	The probability that a person with a positive test result is infected with HIV, that is, that they are truly HIV-positive.
Negative predictive value	The probability that a person with a negative test result is not infected with HIV, that is, that they are truly negative.



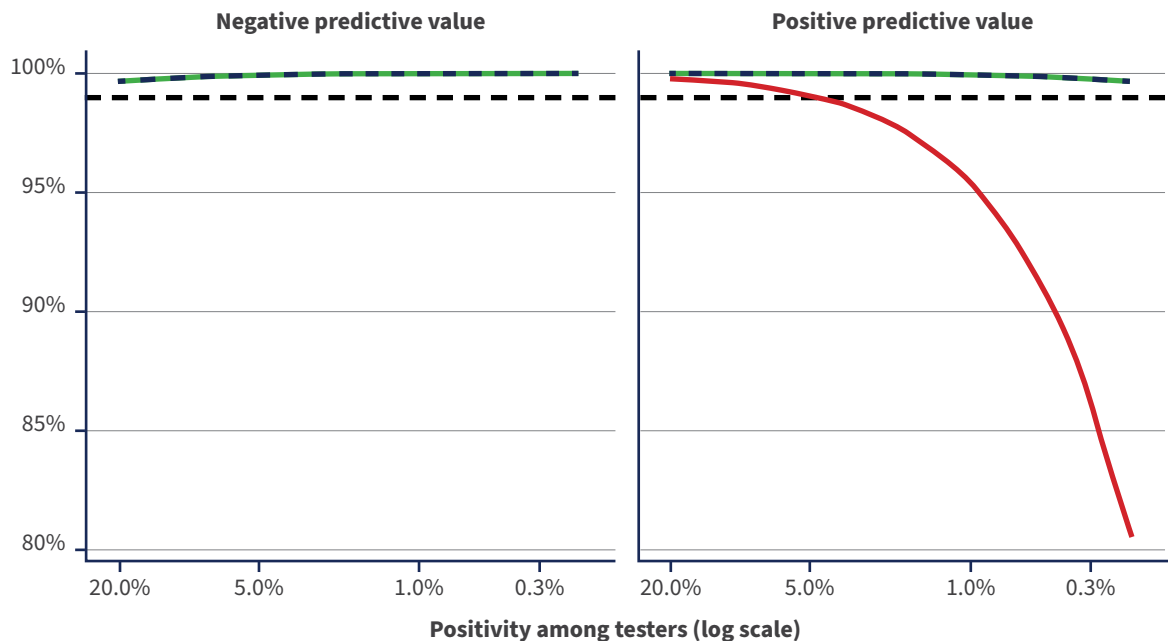
Terminology	Definition
<b>Two-test strategy</b>	An HIV testing strategy in which a person is diagnosed with HIV after two consecutive reactive tests. Positive predictive value of a two-test strategy drops considerably when the test positivity drops below 5%.
<b>Three-test strategy</b>	An HIV testing strategy in which a person is diagnosed with HIV after three consecutive reactive tests. Positive predictive value of a three-test strategy remains high when the test positivity drops below 5%.
<b>Tiebreaker strategy</b>	An HIV testing strategy in which a third test is used to determine the diagnosis in cases of discrepant results between the first and second tests. WHO does not recommend tiebreaker strategies because studies have shown that these increase the likelihood of false-positive results.

# Changing epidemiology

As HTS and ART have been scaled up globally, gaps in diagnosis have narrowed, and in 2022, fewer people with HIV – about 14% – remain undiagnosed (1). Consequently, HTS positivity has also declined globally and is no longer consistently above 5% in all national programmes.


The positive predictive value (PPV) of a test measures the proportion of people who are truly positive among all who test positive. WHO guidance specifies a PPV of at least 99% to maintain diagnostic accuracy in HIV testing strategies. PPV declines when the HTS positivity rate declines (Fig. 2).

**Fig. 2.** Negative and positive predictive values of two-test (red line) and three-test (blue line) strategies according to the positivity rate among testers



Source: adapted from Eaton et al. 2021 (3).





With national test positivity under 5% globally, PPVs of 97–98%, and lower, have been observed where a two-test strategy is used. A drop in PPV of 1–2% results in an increase in misdiagnoses – that is, increased numbers of individuals diagnosed HIV-positive when they are not truly HIV-positive (Fig. 2). With a two-test strategy, PPV drops substantially as positivity falls below 5% but is maintained when a three-test strategy is used. Therefore, in 2019 WHO recommended three consecutive reactive tests for an HIV-positive diagnosis to maintain a PPV of at least 99%. Without changing to a three-test strategy, the PPV in many parts of the world will drop unacceptably, resulting in an increasing

proportion of false-positive diagnoses. A false-positive diagnosis has important consequences for individuals (the psychosocial impact of an HIV diagnosis, the health implications of unnecessary ART, public health consequences (the substantial costs of lifelong ART and related services for misdiagnosed people) and damage to the reputation of and trust in the HIV programme.

Negative predictive value (NPV) measures the proportion of people who are truly negative among all people who are diagnosed negative. The NPV of both two-test and three-test strategies is similar at low and high positivity rates among testers.

# The evidence behind a three-test strategy for HIV diagnosis

Studies have demonstrated the decreased accuracy of using two reactive tests to diagnose HIV (the two-test strategy) in low-prevalence settings and in settings with low test positivity, and improved PPV with the three-test strategy. A mathematical modelling study evaluated changes to diagnostic accuracy associated with switching from a two-test strategy to using three consecutive reactive tests (a three-test strategy) to diagnose HIV. The study found that using a two-test strategy in a setting with 1% HTS positivity produced a PPV of 95.4%, meaning that 4.6% of positive diagnoses would be incorrect (3). A separate modelling study calculated an improved PPV of 99.8% using a three-test strategy in a setting with a prevalence of 1% (4).

These modelling results were confirmed by a retrospective study in Nigeria using household survey data. It found that the performance of the two-test strategy in a low-prevalence setting of 1.4% was below the minimum standards established in WHO guidance, with a PPV of 94% and false-positive misdiagnoses of 5.5% (5). Experience in small-scale implementation of the three-test strategy in Ghana demonstrated that it is feasible to transition from a two-test to a three-test strategy. This experience also identified good practices for quantification,

procurement and development of supportive tools, including standard operating procedures (SOP), bench aides and training materials (6).

The WHO three-test strategy requires three serial tests and should not be confused with a **tiebreaker strategy**: When the first test is reactive and the second test is non-reactive, a third, “tiebreaker”, test is used to decide whether a positive or negative diagnosis should be given. Studies clearly show that use of a tiebreaker strategy to rule in HIV infection increased the likelihood of false-positive results and possible misdiagnosis. In a systematic review on HIV misdiagnosis, 16 of the 30 studies that reported on false positive diagnostic errors reported the use of a tiebreaker testing strategy (7). In one of these studies, 95% (123/129) of false-positive results were specifically due to use of a tiebreaker test (8). Therefore, **WHO does not recommend the use of a tiebreaker strategy.**

In contrast to the recommended three serial tests, a tiebreaker strategy increases the likelihood of false-positive results and should not be used.

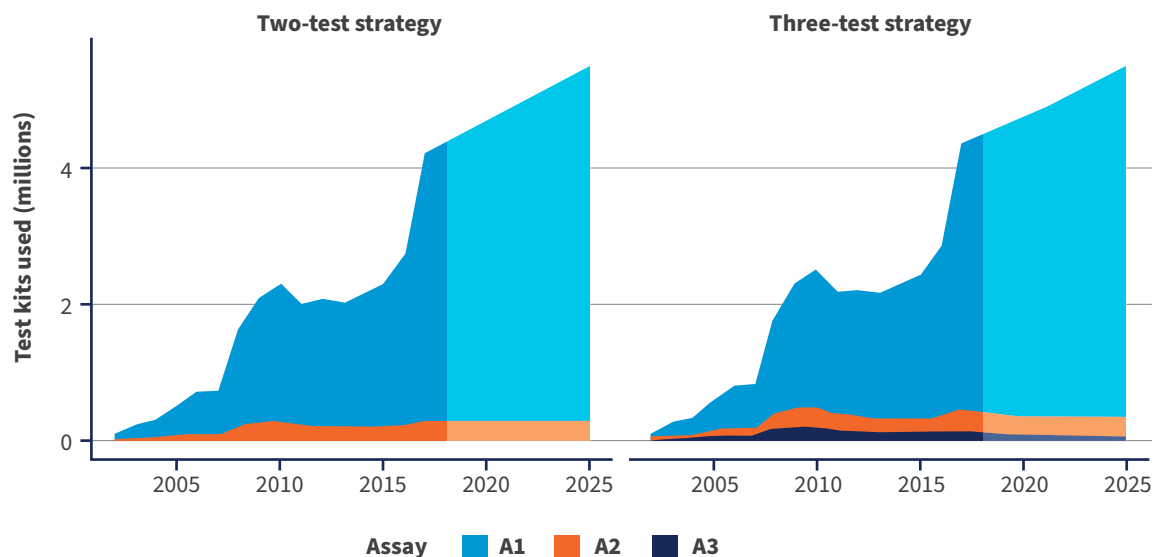
# Cost difference between two- and three-test strategies

HIV testing costs are primarily driven by the volume of clients who receive the first test in the algorithm, A1. Shifting from a two-test to a three-test strategy, therefore, has very little impact on overall testing costs<sup>1</sup> (Fig. 3). Modelling studies have confirmed that cost differences per 100 000 tests conducted are negligible, the total cost of the three-test strategy was only 2.5% greater than that of the two-test strategy at 5% positivity, reflecting the fact that testing cost is primarily determined by the number of A1 assays conducted (Table 2) (3). In contrast, the cost of HIV

misdiagnosis is high, as it includes unnecessary laboratory testing for ongoing monitoring of response to treatment and lifelong treatment costs, as well as personal financial costs and social consequences for individuals and their partners.

Shifting from a two-test to a three-test strategy, therefore, has very little impact on overall testing costs

**Fig. 3. Number of test kits used in Malawi in two- and three-test strategies**



Source: adapted from WHO 2019 (2); presented with projections for the 2018-2025 period

<sup>1</sup> Costs included commodities, staff time, and facility and management overheads.


**Table 2. HIV testing strategy outcomes per 100 000 persons tested for 10%, 5%, 1% and 0.5% true positivity among persons presenting for HIV testing**

	10% positivity		5% positivity		1% positivity		0.5% positivity	
	2-test	3-test	2-test	3-test	2-test	3-test	2-test	3-test
HIV-negative classifications	90 022	90 049	94 968	94 985	98 924	98 934	99 419	99 427
HIV-positive classifications	9922	9781	4985	4891	1035	979	542	490
HIV-inconclusive	55.3	170.0	47.2	124.1	40.7	87.4	39.9	82.8
Observed positivity	9.93%	9.80%	4.99%	4.90%	1.04%	0.98%	0.54%	0.49%
False HIV-positive	43.1	0.86	45.4	0.91	47.4	0.95	47.60	0.95
False HIV-negative	100	120	50.2	59.9	10.0	12.0	5.0	6.0
PPV of entire testing strategy	99.6%	>99.9%	99.1%	>99.9%	95.4%	99.9%	91.2%	99.8%
NPV of entire testing strategy	99.9%	99.9%	99.9%	99.9%	>99.9%	>99.9%	>99.9%	>99.9%
Assay 1 used	101 863	101 863	101 912	101 912	101 950	101 950	101 955	101 955
Assay 2 used	13 563	13 563	8762	8762	4920	4920	4440	4440
Assay 3 used	365	9922	375	4985	382	1035	383	542
Cost (US\$)	384 903	408 796	373 956	385 482	365 198	366 830	364 103	364 499

PPV: positive predictive value; NPV: negative predictive value

Note: 2-test and 3-test denotes the number of consecutive tests needed to provide an HIV-positive diagnosis, not the number of tests used or in a given strategy or algorithm. Note that specimens with repeated discrepant test results under the 2-test strategy proceed to a third test. Under a 3-test strategy, specimens with repeat discrepant test results on the first two tests are ruled negative.

Source: adapted from Eaton et al. 2020 (9).



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# How to select tests for the three-test algorithm: the right tests in the right order

A standardized testing strategy and quality-assured products (such as WHO-prequalified products) are critical for accurate diagnosis, but poorly chosen testing algorithms also can lead to misdiagnosis. Verifying testing algorithms provides objective evidence, before widespread implementation, that a specific combination of three products will accurately diagnose HIV infection. To do this, WHO recommends that, prior to making procurement decisions, countries conduct a verification study, which assesses the level of shared false-reactivity among products. The objective is to construct a three-test algorithm with tests that share the least, or no, common cross-reactivity. Guidance on conducting algorithm verification studies is available in *Optimizing HIV testing algorithms: a generic verification protocol for selecting appropriate HIV serology assays and assessing the level of shared false-reactivity* (9).

Verification studies help facilitate updating and alignment of current HIV testing algorithms with the latest WHO recommendations, ensuring that testing algorithms that minimize the risk of misdiagnosis are selected prior to wider use. Furthermore, these studies support the selection of specific products that suit country-specific operational needs.

Following the completion of a verification study but before national scale-up of the newly defined three-test algorithm, WHO recommends conducting a small-scale implementation pilot study to facilitate the update of all supporting tools (training materials, registers, SOPs, etc.) and to test them along with the new algorithm to make sure that all systems are verified and ready for national scale-up. During that phase countries should also review their entire QMS.

# Retesting prior to starting treatment



The quality of testing services greatly affects the accuracy of diagnosis. In addition to lot-specific quality problems with the tests themselves, human errors in conducting, labelling or interpreting tests and sample mix-ups can result in misdiagnoses. As a result, WHO recommends that programmes retest people diagnosed with HIV prior to ART initiation. Retesting should, ideally, be conducted in a different setting, such as an ART initiation site, and must be conducted by a different health care worker. Retesting to verify an HIV-positive diagnosis is intended to catch individual diagnostic errors before the person begins treatment for life. Retesting prior to ART initiation should be seen as one part of QMS. It does not replace the three-test strategy.

WHO recommends that programmes retest people diagnosed with HIV prior to ART initiation

Misdiagnosis – and especially false-positive diagnosis – can be difficult to resolve once a person starts ART. When retesting in cases of suspected misdiagnosis, the process requires counselling of the client, treatment

interruption, retesting and follow-up. Misdiagnosis can cause emotional and other consequences for the client, damage to the reputation of the programme and large costs for both “re-diagnosing” the client and unnecessary use of ART resources. These costs far likely outweigh the financial costs of additional testing to confirm the status (accurate diagnosis) of all who initially test positive.

Where retesting identifies misdiagnosis, this should be reported to the manufacturer of each of the products used. This systematic process, known as **post-market surveillance**, is conducted by the manufacturer to collect and analyse experience with products that have been placed on the market. Receiving user feedback about false negatives, false positives and other problems related to the quality of the product, including high invalid rates, defective components or damaged packaging, enables the manufacturer to investigate.

If the investigation indicates that the risk–benefit profile has changed, the manufacturer may conduct a field safety corrective action, such as an order to dispose of remaining tests or modification to the product, including modification to its instructions for use if needed.



# QMS for HIV testing

HTS programmes must implement QMS, irrespective of where or how testing takes place – whether through community-based mobile testing, health facilities or laboratories – and irrespective of who conducts testing – whether trained laboratory personnel, other health care workers, lay providers or self-testers.

The basic principles of QMS must apply to all services conducting HIV testing and providing HIV diagnosis. Both facility-based (laboratories and health facilities) and community-based and mobile testing services should assure quality. Site supervisors are responsible for quality activities and should be trained in QMS principles.

HTS programmes must implement QMS, irrespective of where or how testing takes place

All testing services must have a quality policy that specifies the following 12 aspects, as described in WHO's *Laboratory quality stepwise implementation tool (10)* and the *Laboratory quality management system: handbook (11)*:

1. **organization:** ensuring that quality is at the forefront of any testing service;
2. **personnel:** ensuring that competent staff, including lay providers, are employed;
3. **equipment:** ensuring appropriate, fully functional equipment (mostly applicable to laboratory-based testing services);
4. **purchasing and inventory:** ensuring the purchase and management of quality-assured test kits and consumables;
5. **quality control:** ensuring process control of daily testing processes;
6. **information management:** creating and managing documents and records, and keeping records confidential and, preferably, electronically;
7. **documents and records:** ensuring that SOPs are up-to-date and standardized records are maintained;
8. **occurrence management:** recording and following up on complaints;
9. **assessment:** evaluating and following up on results of external quality assessment (EQA) schemes/ proficiency testing and on-site supervision;
10. **process improvement:** ensuring the effectiveness of preventive and corrective actions that are implemented;
11. **client service:** measuring customer satisfaction;
12. **facilities and safety:** ensuring the safety of staff and clients through proper waste disposal and cleaning and decontamination procedures.

These 12 aspects apply to testing services using either laboratory-based methods or RDTs. Additional guidance on how to improve the quality of HIV-related point-of-care testing is available in *Improving the quality of HIV-related point-of-care testing: ensuring the reliability and accuracy of test results (12)*.



# Avoiding use of recency testing in routine HIV testing services

While recency testing is a recommended tool for surveillance activities, recency testing does not improve outcomes for people with HIV, nor does it improve HIV testing services. **WHO does not currently recommend the use of recency testing for the clinical management of individuals or their partners**, as there is insufficient evidence of its clinical utility or its utility in HTS.<sup>2</sup> Since 2015 WHO has recommended that all those who are diagnosed with HIV should be initiated on ART, regardless of when HIV infection was acquired (the “Treat All” approach). **WHO recommends against the use of recency testing in routine HIV testing services.**

Recency testing is costly and complex, involving an additional RDT and viral load testing. Introducing recency assays requires considerable additional training and support. Furthermore, because viral load testing is performed in laboratories, recency testing delays delivering HIV diagnoses to clients and may require the client to return to the testing site. Delayed test results can result in increased loss to follow-up, poor linkage to care and reduced or delayed uptake of treatment, all of which can adversely affect efforts to prevent ongoing transmission through early achievement of viral suppression. Therefore, WHO recommends that resources be prioritized for accurate, quality testing practices at scale and not used for recency testing.

## Key WHO resources

- *Consolidated guidelines on HIV testing services (2019) (2)*
  - *Web Annex D. GRADE table: should western blotting and line immunoassays be used in national testing strategies and algorithms? (2020) (14)*
  - *Web Annex E. HIV testing strategy performance: considerations for global guideline development (abstract) (2020) (9)*
  - *Web Annex I. In vitro diagnostics for HIV diagnosis (2020) (15)*
- *Web Annex J. Ensuring the quality of HIV testing services (2020) (16)*
- *Laboratory quality management system: handbook (2011) (12)*
- *Laboratory quality stepwise implementation tool (2023) (11)*
- *Optimizing HIV testing algorithms: a generic verification protocol for selecting appropriate HIV serology assays and assessing the level of shared false-reactivity (2021) (11)*
- *WHO encourages countries to adapt HIV testing strategies in response to changing epidemic (2019) (17).*

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<sup>2</sup> WHO cautions against the use of recency testing in programmatic settings for routine surveillance due to several challenges; it should be considered only when existing HIV testing coverage of the population being studied is high (for example, in antenatal care services) and when a combination of assays, including viral load, can be incorporated into a recent infection testing algorithm (RITA) to reduce false recent results. Analysis plans should make appropriate statistical adjustments and infer population-specific trends in recent infection.





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