Target product profile for HIV drug resistance tests in low- and middle-income countries: Africa

World Health Organization

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Acronyms

3TC	lamivudine
ABC	abacavir
ART	antiretroviral therapy
ARV	antiretroviral
AZT	zidovudine
d4T	stavudine
DBS	dried blood spot
DTG	dolutegravir
EFV	efavirenz
FTC	emtricitabine
IN	integrase
INSTI	integrase strand-transfer inhibitor
LPV	lopinavir
NNRTI	non-nucleoside reverse-transcriptase inhibitor
NRTI	nucleoside reverse-transcriptase inhibitor
PI	protease inhibitor
PI/r	ritonavir-boosted PI
PR	protease
RAL	raltegravir
RT	reverse transcriptase
TDF	tenofovir disoproxil fumarate
TLD	tenofovir, lamivudine and dolutegravir (co-formulated)

Background

HIV drug resistance surveillance in low- and middle-income countries

Laboratory tests that detect or measure resistance to antiretroviral (ARV) drugs have been used in resource-rich countries to help guide regimen selection for patients since the late 1990s. In contrast, in low- and middle-income countries, drug resistance tests are not widely used for individual patient management decision-making for several reasons, including limited availability, cost constraints and lack of clear evidence of utility in the context of a public health approach to HIV care and treatment. Instead, WHO recommends a public health approach to HIV drug resistance surveillance to generate information about the prevalence and patterns of HIV drug resistance in various populations, which can be used to inform current and future HIV care and treatment guidelines. The resulting survey data is intended to be used to help guide the selection of antiretroviral therapy (ART) regimens at the national, regional, and global levels. Large-scale global surveillance efforts have led to several important observations.

1. More than 10% of adults initiating (or reinitiating) first-line ART have resistance to non-nucleoside reverse transcriptase (RT) inhibitors (NNRTIs) (pretreatment drug resistance) in several countries, and this is generally increasing over time *(1–6)*.
2. The prevalence of NNRTI resistance among infants younger than 18 months is high (34–69%), and the prevalence of nucleoside RT inhibitor (NRTI) resistance is moderate (2–26%) *(4)*.
3. The prevalence of pretreatment drug resistance to integrase strand-transfer inhibitors (INSTIs) and protease inhibitors (PIs) is negligible.
4. People for whom NNRTI-based ART is failing have a high prevalence of acquired resistance to both NNRTIs and NRTIs *(4)*; resistance to PIs among people for whom ritonavir-boosted PI (PI/r)-based regimens are failing is less common *(7–9)*.

As dolutegravir (DTG)-based ART for the treatment of people living with HIV expands, estimating the extent to which acquired DTG drug resistance emerges in populations receiving DTG-containing ART is important from a public health and ART programme perspective. DTG is a well-tolerated and highly effective ARV drug and is recommended by WHO in first- and second-line ART *(10)*. An important advantage of DTG is its high genetic barrier to the selection of drug resistance *(11)*. DTG resistance did not emerge among ART-naive participants in clinical trials *(12,13)* and, to date, has only been described in a few ART-naive people for whom DTG-based ART has failed as their first-line treatment *(14)*. However, DTG resistance can emerge, especially among people with previous exposure to first-generation integrase inhibitors with comparatively lower genetic barriers to the selection of drug resistance or when DTG is used as monotherapy *(15)*. WHO therefore recommends ongoing, cyclical implementation of HIV drug resistance surveys in low- and middle-income countries as part of the overall HIV drug resistance strategy *(16,17)*.

Use of drug resistance tests in low- and middle-income countries for individual patient management

As sequencing capacity in low- and middle-income countries has increased following the scale-up of HIV drug resistance surveillance and other efforts, drug resistance testing has become possible in a limited number of locations for the purpose of informing ART regimen selection for individual people. For example, drug resistance testing is used for algorithm-guided ART optimization among people for whom second-line (usually PI/r-based) ART is failing in South Africa *(18)*. The results are reviewed by a committee of expert clinicians and virologists who make treatment recommendations for each person. The additional expense of drug resistance testing is considered worthwhile in these situations given the still limited availability, increased cost and toxicity of ARV drugs such as etravirine and darunavir that are incorporated into third-line regimens. While limited (about 10 000 tests per year in sub-Saharan Africa) and not part of any official WHO guidance, drug resistance testing for clinical use among individual people is becoming more common, and an increasing number of laboratories in some countries are implementing drug resistance testing as a result.

Types of drug resistance tests

Several methods can be used to measure the susceptibility of HIV to ARV drugs, and these have been extensively reviewed elsewhere *(19–24)*. Drug resistance tests fall into one of the following categories:

- phenotypic: based on replication of a virus (often a recombinant or pseudotyped virus, containing a patient-derived sequence, that facilitates measurement of viral replication) or enzymatic activity in the presence of ARV drugs;
- genotypic: based on analysis of the nucleotide sequence of relevant regions of virus genomes in a patient-derived specimen, with interpretation relying on an algorithm based on existing knowledge about the association between drug resistance mutations, phenotypic susceptibility and clinical response; genotypic tests can be further subdivided:

- nucleotide sequence determination based on the Sanger dye-terminator method or dye-primer method;
- nucleotide sequence determination based on next-generation sequencing methods; and
- detection of sequence changes in specific codons (also referred to as point mutation assays).

Sanger sequencing has been the method used most frequently for HIV drug resistance surveillance in low- and middle-income countries over the last two decades. Equipment, reagents and training are available in a limited (but expanding) number of laboratories, for example in many countries' national HIV reference laboratory. Nucleic acid extraction and Sanger sequencing methods for HIV drug resistance have been established as both commercially available kits and protocols developed in house. For the purpose of surveillance, WHO has developed and implemented an international laboratory designation process that helps to validate these methods and verify that they are being performed in an appropriate environment to limit molecular contamination and ensure high-quality sequence data generation for national surveys of HIV drug resistance *(25)*.

Next-generation sequencing and, to a lesser degree, point mutation assays are also being used in low- and middle-income countries, primarily for surveillance and research purposes. Next-generation sequencing capacity has increased significantly in recent years as part of the global response to the COVID-19 pandemic and the desire to sequence emerging variants of SARS-CoV-2. Next-generation sequencing-based methods for HIV drug resistance testing have some advantages as well as special considerations compared with Sanger-based sequencing *(24,26–29)*.

Phenotypic tests play an important role in characterizing the effects of mutations in the HIV-1 genome on ARV drug susceptibility, especially for new drugs. However, in clinical practice they are less often used than genotypic tests due to their increased complexity, turnaround time and cost.

ART treatment landscape

When the potential uses of drug resistance testing in low- and middle-income countries are described, it is important to consider the ART treatment landscape over several previous years, since people who are considered eligible for drug resistance testing may have been treated according to the recommendations in place previously. It is also important to consider the future ART treatment landscape to support setting drug resistance testing priorities. Below, treatment recommendations before the widespread availability of dolutegravir (DTG) (pre-DTG era) as well as current preferred regimens that include DTG (DTG era) are summarized.

Pre-DTG era

For many years, recommended ART regimens for adults in low- and middle-income countries largely consisted of an NNRTI, either nevirapine (NVP) or efavirenz (EFV) and two NRTIs, often stavudine (d4T), zidovudine (AZT), abacavir (ABC) or tenofovir disoproxil fumarate (TDF) with lamivudine (3TC) or emtricitabine (FTC) *(30)*. Such regimens are effective and affordable and have led to significant reductions in HIV-related morbidity and mortality among people living with HIV as well as reduced likelihood of HIV-1 transmission. NNRTI-based ART regimens formed the basis of public health recommendations for first-line ART in adults and adolescents until mid-2019, when DTG-based regimens became the preferred approach *(10,31)*.

NNRTIs have also been extensively used for preventing mother-to-child transmission, and although they are effective in reducing transmission to newborn infants, this led to increased transmission of NNRTI-resistant viruses to perinatally infected children *(32,33)* and newly infected adults *(4,5)*. For this reason, the recommended initial ART regimens for young children were based on PI/r *(30)* before the availability and demonstration of efficacy of DTG (in combination with ABC and 3TC) for children *(10,31)*. Surveys of HIV drug resistance among adults and adolescents initiating ART showed that the pretreatment NNRTI resistance prevalence exceeded 10% in several countries *(4)*. Pretreatment drug resistance testing that includes assessment of NNRTI resistance was considered useful in countries where NNRTI pretreatment drug resistance exceeds 10% to guide the choice of initial ART for individual people, before DTG replaced NNRTIs as the basis for preferred initial regimens *(10)*.

Following treatment failure in the pre-DTG era, the recommended second-line regimen for adults and adolescents consisted of a PI/r and two NRTIs. One of the two NRTIs was recommended to be new (3TC or FTC could be maintained). Drug resistance information for people switching to second-line regimens was considered to be of limited utility because pretreatment drug resistance to the PI class is extremely rare, as a result of infrequent selection of PI-resistant variants among people for whom PI/r-based regimens are failing *(34)* and the low frequency of transmission of PI/r-resistant HIV-1 *(4)*. Further, the residual activity of the NRTI component, even if NRTI resistance to both drugs was selected during first-line therapy, may be sufficient in certain circumstances to ensure regimen efficacy and durability *(35)*.

Recommended third-line regimens in the pre-DTG era included NRTIs, INSTIs, ritonavir-boosted darunavir and/or NNRTIs. In the pre-DTG era, the most commonly used INSTI was raltegravir (RAL), which has a low genetic barrier to resistance when it is not accompanied by one or more fully active ARV drugs. As mentioned above, some countries developed an algorithm for optimizing the regimens of individual people that incorporated drug resistance test results *(18)*.

DTG era

More recently, NNRTIs have been replaced by DTG since it is associated with enhanced tolerability, efficacy, lack of circulating pretreatment resistance and a high genetic barrier to the selection of drug resistance *(36,37)*.

Adults starting ART since 2021 are recommended to be prescribed DTG in combination with TDF and 3TC or FTC *(10)*. People receiving an NNRTI-containing regimen may also be switched to DTG with two NRTIs to improve tolerability. An alternative initial regimen would include EFV instead of DTG (for example, if DTG is contraindicated, because of patient choice, or in rare situations in which DTG is not available). Since pretreatment resistance to DTG *(36,37)* and NRTIs *(4)* is uncommon, drug resistance testing before treatment is not needed for people being prescribed TDF + 3TC + DTG (TLD), especially since the priority public health approach is to ensure rapid and immediate ART initiation among people newly diagnosed with HIV. However, if DTG availability is restricted, drug resistance testing could be used to identify people infected with NNRTI-resistant HIV and give them priority to receive DTG.

DTG in combination with two NRTIs is also recommended for people with NNRTI-based first-line treatment failure *(10)*. NRTI resistance selected during previous treatment may not affect the response to DTG-based second-line therapy, and drug resistance testing thus is not likely to be required at this stage *(38,39)*.

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Rationale for a target product profile for a drug resistance test

HIV-1 drug resistance tests are a useful tool for optimizing ART regimens when used in the context of information about viral load, immune and clinical status, adherence practices and available treatment options. Several types of drug resistance tests are available, either as commercial kits or based on published methods for in-house assays. Sanger-based sequencing tests are the predominant type used in high-income countries over the past several decades, where resources enable stringent regulatory oversight and efficient operation. Since the product specifications of some of these commercial kits or in-house assay methods were established in high-income countries many years ago, they may not be ideally suited to the intended use in low- and middle-income countries, where patient treatment histories, ARV drug availability, specimen collection and transport systems, laboratory conditions and climate (such as high temperature and humidity) may differ substantially from the context in which the tests were originally developed. So far, these Sanger-based sequencing tests have been largely implemented in low- and middle-income countries for surveillance applications, where constraints related to turnaround time and cost are less concerning. These older Sanger-based sequencing tests have features (including turnaround time, cost, results interpretation, operator training requirements and challenges in meeting regulatory requirements) that may affect their utility for individual testing in low- and middle-income countries. Although Sanger-based tests are in widespread use, there is potential for developing and implementing newly designed Sanger-based sequencing tests or drug resistance tests based on other types of technologies.

There are several important differences between implementing drug resistance testing for patient management and for surveillance purposes. Table 1 summarizes the experience of WHO HIVResNet in drug resistance testing in low- and middle-income countries.

Table 1. Contrast between implementing drug resistance testing for surveillance and for patient management in low- and middle-income countries

Feature	Surveillance	Patient management
Turnaround time	Months	Days or weeks
Testing model	Few reference laboratories	More widely distributed testing centres
International standardization	Essential	Important
Payers	Governments, nongovernmental organizations, research grants	Governments, nongovernmental organizations, private insurance, public health systems, self-pay
Cost per test	US$ 100s affordable	US$ 100s not affordable
Results reporting	Batched, delays tolerable	Individual, as soon as possible
Quality management systems	In-house and research use only validation	As required by clinical testing regulatory bodies in effect[a]

[a] For example, South African National Accreditation System, United States Food and Drug Administration, WHO Prequalification and European Union In Vitro Diagnostic Medical Devices Regulation.

Therefore, a target product profile is desirable (1) to guide the development of new drug resistance tests and (2) to facilitate the evaluation of the suitability of currently available drug resistance tests for specific applications and the identification of areas in which current drug resistance testing are lacking. The target product profile described here primarily focuses on the near-term future for likely ART regimens and their use, in anticipation of an innovative test that will meet the projected demand.

Diagnostic manufacturers require target product profiles at an early stage of the development process so that they can be informed of a test's intended use, desired test performance targets and technical specifications. These parameters are defined by a consensus of stakeholders, considering the intended use, feasibility and utility to the end user. The target product profile has targets for specific characteristics that refer to the measurable requirement or specification (such as assay sensitivity and specificity, cost, turnaround time, biosafety, data interpretation and storage).

Development of priority use case definitions

Target product profiles depend on use cases

For any product to be considered useful, it should meet a set of criteria related to different aspects of its performance in a specific context. This context, or intended use, can be described in one or more potential use cases. For HIV-1 drug resistance testing in low- and middle-income countries, use cases were defined as a combination of variables that describe a clinical situation, as outlined below. In these descriptions, treatment failure is defined according to WHO treatment and monitoring guidelines *(10)*.

1. Patient population:
    a. Adults and adolescents: individuals infected with HIV-1 via exposure other than perinatal.
    b. Children: for simplicity, "children" is intended to comprise all individuals infected perinatally by their HIV-infected mothers, in utero, at delivery or postpartum through breastfeeding.
    c. Pregnant women: special consideration is warranted for this vulnerable population because of the desire to prevent transmission to the infant.
2. The patient's previous ART experience: none, failure of specific regimens (such as NNRTI-, PI/r- or INSTI-based) or pre-exposure prophylaxis breakthrough infection. Certain clinical scenarios, such as optimizing ART for people with history of failure on multiple previous regimens, are more likely than others to be influenced by information about susceptibility to ARV drugs in certain drug classes.
3. The ART drug class(es) (NRTI, NNRTI, PI or INSTI) to be included in the drug resistance test, because the information is likely to influence optimal ART management decisions. The most relevant drug classes are those related to the ART regimen being prescribed at the time of treatment failure and to the recommended (and available) ARV drugs likely to be included in the next regimen.

To simplify the potential combinations of these variables and provide globally realistic scenarios, the 2021 WHO consolidated guidelines on HIV prevention, testing, treatment, service delivery and monitoring *(10)* were used. These guidelines describe the recommended regimens for use in low- and middle-income countries, as well as the recommended regimen to be used after treatment failure; this permits a focus on which drug classes, and hence which regions of the virus, a drug resistance test must provide information about.

Intended use: regimen optimization versus determination of need to switch ART regimen

The intended use of drug resistance testing can be divided into two main categories:

a. To optimize an ART regimen by avoiding prescribing ARV drugs to which the person's virus has reduced susceptibility. Historically, this group of use cases has been the main reason why drug resistance testing is requested in resource-rich countries. When a new ART regimen can be formulated that relies on drugs in classes to which the person living with HIV has had no previous experience and if population-level prevalence of pretreatment HIV drug resistance is known to be low, drug resistance tests are not likely to provide sufficient useful information to justify their cost. For example, this is the most likely situation for adults and adolescents for whom an NNRTI- or INSTI-based regimen is failing being switched to a PI/r-based regimen, or those for whom an NNRTI-based regimen is failing being switched to an INSTI-based regimen. However, with increased cumulative drug exposure histories, selecting a regimen with minimum likelihood of reduced susceptibility becomes more challenging, and drug resistance tests may have clinical utility. In addition, it could be valuable to establish the pattern of drug resistance–associated mutations present after the failure of an initial ART regimen for future reference, since drug resistance variants may become undetectable in circulating virus at the time of the second regimen failure and yet be present in latent T-cell reservoirs. Since the archived resistant virus could re-emerge under appropriate selective pressure, knowledge about its presence could influence the optimal composition of a third regimen. However, this would also require an effective system for storing and retrieving past results from each person.

b. To determine the need to switch ART (distinguish between non-adherence and drug resistance as the reason for treatment failure). Since selecting drug-resistant HIV requires replication in the presence of one or more ARV drugs, if drug resistance is not detected in a treated person with unsuppressed viral load, the person is probably non-adherent and a regimen switch would therefore not be needed. Unnecessary switches are undesirable because of the increased cost, pill count and decreased tolerability of most second- and third-line ART regimens. Adherence issues should be addressed and the current regimen continued with appropriate follow-up. Conversely, if drug resistance is detected (especially if known to be newly selected based on previous drug resistance test results) the person must have been at least partly adherent, and a regimen switch is more likely to be indicated.

Adherence can be assessed by several approaches, such as pill counts, prescription fulfilment rates (medical possession ratio) or some form of drug level measurement in a clinical specimen. A comprehensive evaluation of these interventions is beyond the scope of this target product profile for drug resistance tests, but the application of drug resistance test results in this regard is an important aspect of the priority use cases described below.

After careful deliberation by the target product profile development group (Annex 1), the following use cases were selected to form the basis of the target product profile.

Use case 1: following confirmed failure of initial INSTI-based ART

In recent years, the availability of DTG in low- and middle-income countries has brought about a major change in the ART regimen landscape. DTG-based regimens are now a preferred option for people living with HIV starting ART following initial diagnosis or re-entry into care, for people receiving NNRTI-based regimens to improve tolerability, and for people for whom NNRTI-based regimens are failing. Based on clinical trial data, the vast majority of individuals with no previous INSTI exposure who are initiating ART with DTG + two NRTIs are expected to achieve viral suppression and maintain it for several years *(12,13,40,41)*. Of those who do not achieve viral suppression, a relatively small proportion of patient-derived viruses have been shown to have resistance-related amino acid substitutions in the HIV-1 integrase (IN) coding region. Thus, empirically switching everyone with treatment failure on a first-line DTG-containing regimen may miss an opportunity for adherence counselling and result in unnecessary switching to less well-tolerated, more expensive second-line treatments in a high proportion of the population.

Population: adults (including pregnant women), adolescents and children living with HIV-1 with confirmed treatment failure as defined by WHO.

Children often have unique ARV drug exposure histories because of NNRTI-based prevention strategies and the transmission of drug-resistant HIV to the child. Because of the increased likelihood of having been infected by NNRTI- and NRTI-resistant viruses and the contraindication to using tenofovir prodrugs, infants are more likely to be treated with ABC, ritonavir-boosted lopinavir (LPV/r) and RAL. Drug regimens available for infants are limited, and drug resistance testing can play a role in optimizing ART.

Regarding specimen type, dried blood spots are often used for infant HIV PCR testing, making compatibility with drug resistance tests important for this population.

Compared with using drug resistance tests for individuals who are not pregnant, additional emphasis is placed on rapid suppression of viral loads in pregnant women to reduce the risk of mother-to-child transmission. This is most relevant to women who are INSTI-experienced or if INSTIs are contraindicated. The ideal turnaround time requirement is shorter given the more limited time window for preventing the child from being infected.

Previous ART experience: regimens including an INSTI, such as TLD or ABC + 3TC + DTG. This includes people who may have had previous treatment experience with NNRTI-based regimens and children who may have initiated ART with a PI/r-based regimen but switched to ABC + 3TC + DTG for reasons other than treatment failure. As the use of NNRTI-based regimens among young children or their mothers is replaced by DTG-based regimens, the number of people falling into this category of ART experience is expected to decrease.

Drug classes to be included:

- INSTI: to inform about the possibility that failure is due to resistance and to guide future INSTI selection.
- NRTI: to inform possible NRTI backbone switch (especially for children). Exposure to AZT or ABC (often used in children) can select for multiple, unpredictable patterns of resistance-associated mutations that have varying effects on cross-resistance to other NRTIs. The effect of partial NRTI resistance on ART efficacy among children is less well understood than for adults, making optimizing the NRTI backbone based on susceptibility predictions more important. In adults, NRTI resistance data may not be needed for optimizing regimens that rely on PI/r, since susceptibility to PIs is safe to assume, and NRTI resistance that may be selected by previous ART is not likely to affect the efficacy of treatment with PI/r and two NRTIs *(35,38,39,42)*.
- PI: for children who may have initiated ART with a PI/r-based regimen but switched to ABC + 3TC + DTG for reasons other than treatment failure, but without verification of suppressed viral load at the time of switch, there is a small possibility that resistance to the PI component may have been selected. For these children, PI resistance data could guide the selection of the optimal PI to include in the next regimen.

Testing for NNRTIs is not included, since using NNRTIs is not a preferred WHO treatment option following treatment failure. However, the presence of NNRTI drug resistance–associated mutations can be informative with regard to previous treatment experience and are often captured at the same time as NRTI drug resistance–associated mutations, for example in drug resistance tests that rely on nucleotide sequencing of the RT region.

Use case 2: after failure of multiple regimens, including PI/r-based ART

People with complex ART histories are likely to have more drug resistance–associated mutations relevant to multiple drug classes and an increased likelihood of within-class cross-resistance between drugs.

Population: adults (including pregnant women), adolescents and children living with HIV-1, with confirmed treatment failure as defined by WHO. See use case 1 for a description of special considerations for children and pregnant women.

Previous ART experience: history of treatment failure of regimens including an INSTI or an NNRTI and a regimen including a PI/r. Historically, this corresponds to "second-line" failures, with drug resistance testing results being used to optimize third-line ART. For children, prevention of mother-to-child transmission can be considered as previous ARV drug exposure in the NNRTI drug class; thus, a child with treatment failure on their initial PI/r-based regimen meets the definition for this use case. This is likely to change as ABC + 3TC + DTG or TLD replace NNRTI-based regimens among young children or their mothers, respectively.

Drug classes to be included:

- PI: to confirm that treatment failure resulted from resistance (if not, ART switch may not be indicated) and to inform optimal dosing of darunavir/ritonavir and the possible need for including an INSTI in the optimized third-line regimen.
- NRTI: to inform selection of an optimal NRTI backbone (especially for children, see above) and possible need for including an INSTI.
- NNRTI: to decide whether to include second-generation NNRTIs such as etravirine or doravirine. In countries where these are not available or not recommended, NNRTI resistance data may be of limited value.
- INSTI: for people with history of INSTI use, to guide the selection of optimal INSTIs. For example, INSTI exposure may include the use of RAL among neonates who received ART before being eligible for treatment with DTG, or adults with history of RAL use as part of salvage therapy but later switched to DTG when it became available.

Other use cases

The following use cases were considered but assigned lower priority, although they may be relevant in some special situations.

Adults and children before starting ART

Population: adults (including pregnant women), adolescents and children living with HIV-1, in areas where DTG is not available for all and the prevalence of NNRTI drug resistance is above 10%.

Previous ART experience: none.

Drug classes that could be included:

- NNRTIs: pretreatment drug resistance to NNRTIs is prevalent (>10%) in many countries (especially among women). TLD is the preferred initial regimen, but TDF + 3TC + EFV is an alternative in areas where access to DTG is limited or where other factors such as patient choice may influence regimen selection. Individuals without NNRTI resistance could be treated with EFV-based regimens.
- NRTI resistance is a concern for oral pre-exposure prophylaxis (PrEP, with TDF + FTC) breakthrough infections. Otherwise, testing for NRTIs may not be necessary for people starting ART, since pretreatment drug resistance to these drug classes is less common.

PI and INSTI resistance are less of a concern since PI and INSTI pretreatment drug resistance prevalence is low, and PI-based regimens are not recommended for initial ART for adults and adolescents.

This use case is not considered a priority because (1) TLD is strongly preferred and coverage is high in most countries with a high burden of HIV infection *(43)*; (2) if access to DTG is not limited, there is little value in documenting the absence of NNRTI resistance (see above); (3) PrEP usage in sub-Saharan Africa is still low *(44,45)* and cases of breakthrough infection among people adherent to PrEP are rare *(46)*. Therefore, this potential use case has not been considered in developing the target product profile.

If pretreatment drug resistance testing is performed, initiation of ART should not be delayed while waiting for drug resistance testing results. ART can be modified if the results indicate resistance to one or more components of the regimen that was initially chosen.

In the future, if the ART landscape changes significantly (including the widespread availability of capsid inhibitors, long-acting ARV drug formulations, entry inhibitors or monoclonal antibody therapies) or the prevalence of breakthrough infections on oral PrEP changes significantly, the relative importance of this use case could be reassessed and expanded to include other targets in the HIV genome (gag or env).

New use cases associated with future therapies and PrEP

New ARV drugs for treatment and prevention are being developed and may soon be accessible in low- and middle-income countries. Some of these new therapies and prevention strategies could result in new, currently undefined treatment recommendations and use cases for drug resistance testing. For example, there is a risk of resistance to INSTIs in cases of breakthrough infection during PrEP with long-acting cabotegravir *(47)*. Drug resistance testing following HIV-1 diagnosis among cabotegravir PrEP users may be required to optimize ART, since cabotegravir resistance–associated mutations can also contribute to DTG resistance. Therefore, additional use cases may be needed in the future as the ART and prevention landscapes evolve. These new use cases may influence the targets defined in the target product profile, which will be updated as needed in consultation with the target product profile development group.

Target product profile description

Assumptions about technology platforms

The description of minimal and optimal targets for the various test performance characteristics is intended to be applicable to tests that rely on any technology. Thus, they should be relevant to tests based on sequence determination by any means (Sanger sequencing or next-generation sequencing), point mutation detection, enzymatic activity or virus (including recombinant viral vector) replication assays or any others developed in the meantime. Nevertheless, in some cases it is challenging to completely separate these descriptions from the large body of knowledge and collective experience that has accumulated through decades of reliance on Sanger-based genotyping assays.

When a manufactured product is used for only a portion of the entire test (such as only the sequencing steps of a Sanger-based assay that also requires nucleic acid extraction and RT-PCR), the requirements in the target product profile apply to the entire process and not only to that product.

Minimal versus optimal targets

This document provides "minimal" and "optimal" targets for each characteristic in the target product profile (Table 2). The minimal requirements are the lowest acceptable output for that characteristic, and the optimal requirements are the ideal, realistically achievable output for that characteristic. These requirements, or targets, have been discussed by the target product profile development group and represent a consensus of the stakeholders represented there. For the best fit between a specific product and the intended use, products should meet all minimal targets and as many of the optimal targets as possible. The optimal characteristics should not be considered as the maximum desirable characteristics; assays that exceed these characteristics are certainly of value.

- Minimal: for a specific characteristic, "minimal" refers to the lowest acceptable output for that characteristic. A test that fails to meet a minimal requirement may still be acceptable in some situations if shortcomings pertain to less rigorously defined targets or if specific rigorously defined targets are missed only marginally.
- Optimal: for a specific characteristic, "optimal" provides an ideal target that is believed to be realistically achievable. Meeting the optimal target will provide the greatest impact for the end-users (clinicians and people living with HIV). Developers would ideally design and develop their solutions to meet the optimal requirements for all characteristics.

The minimal and optimal targets define a range within which tests can be differentiated from each other, which may result in certain tests being more ideally suited for certain use cases or clinical contexts.

Table 2 summarizes the drug resistance testing target product profile. Annex 2 describes each characteristic in more detail.

Table 2. HIV drug resistance tests for low- and middle-income countries: target product profile

Characteristic	Minimal	Optimal	Comment
Scope			
Target user[a]	Clinical laboratory scientist with dedicated training	Medical laboratory technician with minimal dedicated training	Greater use of automation enables test operation by less highly trained personnel. Clinical laboratory scientists have completed a four-year degree training programme. Medical laboratory technicians have completed a two-year training programme
Setting or infrastructure level[a]	Level 3/4 (provincial or national) reference testing laboratories	Level 2 (primary care) testing facilities	Operation in level 1 would require a self-contained, automated point of care or near-point-of-care system *(48)*
Laboratory testing model[a]	Centralized reference laboratory	Point-of-care or near-point-of-care	It is suggested that point-of-care tests also be deployable in centralized or decentralized reference testing laboratories

Characteristic	Minimal	Optimal	Comment
Assay design, performance and functionality			
Region(s) covered (PR, RT and IN)	Priority order: Use case 1: (1) IN, (2) RT, (3) PR Use case 2: (1) PR, (2) RT, (3) IN	(1) *pol* (PR-RT-IN), (2) *gag*, (3) *env*	*gag* and *env* are included under "optimal" in anticipation of future availability of capsid or entry inhibitors. If new data demonstrate a clinically meaningful role for mutations outside of IN in INSTI resistance, these regions should be included in the minimal requirements
Drug or drug class coverage	Priority order: Use case 1: (1) INSTI, (2) NRTI, (3) PI Use case 2: (1) PI, (2) NRTI, (3) NNRTI, (4) INSTI	Add capsid or maturation inhibitors and entry inhibitors	For use case 2, NNRTIs required only if NNRTIs are a treatment option
Mutation coverage	All drug resistance–associated mutations that result in >90% sensitivity in drug resistance detection (see comment)	All drug resistance–associated mutations that result in >99% sensitivity in drug resistance detection (see comment)	Based on drug resistance–associated mutation frequency data within each region (PR, RT, or IN) and in target population. Sensitivity defined as percentage of relevant samples in which drug resistance is detected in comparison to Sanger sequencing
Sensitivity for amplification	>90% for viral load 1000–5000 copies/mL	>90% for viral load between viral load assay limit of detection and 1000 copies/mL	Lower sensitivity (such as requiring viral load ≥ 5000 copies/mL) may be acceptable if there are benefits in cost or turnaround time. Target based on viral load in plasma. The results must also be reproducible, especially at low viral load
Sensitivity for detection of low-abundance drug resistant variants	Same as Sanger sequencing (~20%)	>5% (when viral load ≥1000 copies/mL)	Input copy number and position dependent; desired sensitivity may be <5% if clinical utility demonstrated
HIV-1 subtype coverage (Africa)	In priority order: C, A, CRF02, G, D	A, B, C, D, F, G, CRF01, CRF02, URFs	A subtype C–specific test, for example, could be acceptable in some areas (such as southern African countries[b] and Ethiopia)
Time from specimen receipt to report	Three days	Same day (less than eight hours)	Turnaround time under ideal conditions.Optimal targets preferable for pregnant women
Achievable throughput (tests per day)	10	100	Tests completed per day under ideal conditions per operating unit (such as testing site or equipment installations). Not including controls
Interfering substances	No interference from substances commonly present in the recommended specimen type	Same as minimal requirement	
Quality control	Negative and positive controls included	Same as minimal requirement, with additional positive controls at different levels (such as low and high positive) and multiple steps	Optimally, multiple positive controls are used at discrete steps of the procedure (such as RNA extraction, RT-PCR set-up and sequencing) so that reasons for assay failure can be more easily determined. The copy number of positive controls should be kept to a minimum for the intended purpose, to minimize the risk of sample contamination
Accuracy, precision and reproducibility	Sufficient to support generation of the same end result (detection of drug resistance–associated mutations or drug resistance susceptibility prediction) in ≥90% of replicate tests	Sufficient to support generation of the same end result (detection of drug resistance–associated mutations or drug resistance susceptibility prediction) in ≥99% of replicate tests	Technical specification will be assay dependent. For sequencing assays, minimal requirements should resemble those established for drug resistance test use for surveillance purposes; see Appendix 3 in *WHO HIVResNet HIV drug resistance laboratory operational framework (25)*

Characteristic	Minimal	Optimal	Comment
Specimen handling			
Specimen type(s)	Plasma, DBS	Plasma, DBS, plasma separation cards, whole blood, peripheral blood mononuclear cells	Use of peripheral blood mononuclear cells may affect viral load requirements (can be performed on samples with undetectable viral load). Use of proviral DNA as the starting material may introduce a requirement for more stringent quality assurance procedures, because of the possibility of templates that have been inactivated by host defence mechanisms (such as APOBEC mutation).
Specimen volume	≤1 mL of plasma or equivalent	≤0.2 mL of plasma or equivalent	Specimen volume determined in conjunction with amplification sensitivity targets above. Requirements for larger specimen quantity would limit utility among children
Specimen preparation at point of collection[a]	Requiring skills and materials similar to plasma separation	None required	Not applicable for point-of-care testing
Specimen preparation in the laboratory[a]	Requiring skills and materials similar to plasma separation	None required	Not applicable for point-of-care testing, assuming that the test is completed at the point of care and not in a separate laboratory
Stability of specimen between collection and arrival at laboratory	24 hours requiring refrigeration (4°C)	48 hours at ambient conditions	Not applicable for point-of-care testing, assuming that the test is completed at the point-of-care and not in a separate laboratory
Specimen shipping conditions	Refrigerated (on wet ice)	Shipping not required	Not applicable for point-of-care testing, assuming that the test is completed at the point-of-care and not in a separate laboratory. The packaging should conform to all transport of dangerous goods regulations.
Specimen storage conditions at laboratory	Stable for at least one year at –20°C	Stable at ambient temperature and humidity	
Report and data handling			
Software requirements	Freely available and user-friendly software only, cross-platform (Mac OS and Windows) and not requiring more computational power than typically found on personal laptop up to five years old	None (included)	Requirement for external software should not incur additional costs and should be included in the assay validation
Reporting formats	PDF or similar for printing or electronic transmission; no requirement for manual re-entry of patient information or test results	For optimal record keeping and enabling of easy access to historical results for a patient over time, the output should be compatible with national or international electronic medical records systems and databases	Consider security to safeguard patient confidentiality
Result interpretation	Binary (susceptible or not) or simplified category of resistance level for each drug that is available for use	Simplified output with indication for regimen change and regimen recommendation based on drug availability, drug resistance test result and patient history	Drug susceptibility assessment should be based on internationally accepted standards, and validated for clinical use, and be presented in as simplified a manner as possible

Characteristic	Minimal	Optimal	Comment
Data capture and transfer	Local information system with flexible output options for data transfer, compatible with national database, no requirement for manual data re-entry	Meets international data transfer format standards	Compatibility with existing databases within or outside the laboratory may be achieved via custom transfer tools or modification of the database. For example, for a sequence-based assay, it should be possible to export the sequence in fasta format
Cost considerations			
Cost per test (laboratory testing portion)	<US$ 40	<US$ 10	Manufacturer's ex works list price plus laboratory-provided consumables, or in-house assay cost of goods
Instrument cost	<US$ 100 000	None (included)	Cost for dedicated instruments needed for test performance. A reagent rental contract may be preferable in some labs or countries. Includes annual maintenance costs.
Extraneous requirements			
Instrument automation	Semiautomated platform, modular	Fully integrated and automated system	Fully automated, self-contained systems may be desirable in high-volume testing environments and can enable less stringent requirements for operator training and hands-on time
External technical support	On-site support available in low- and middle-income countries within one month of request	On-site support available in low- and middle-income countries within one week of request	
Reagent stability and storage	≥12 months	≥24 months	Assuming recommended storage conditions
Reagent storage requirements	–20°C storage required for at least some components; tolerant to freeze-thaw	All components stable at ambient temperature in the defined operating environment (see below)	
Biosafety[a]	Biosafety level 2	None, following specimen loading	Handling of patient specimens must follow universal safety precautions *(49)*
Regulatory status (manufactured kits)	Manufactured under ISO 13485	WHO prequalification or similar	
Regulatory status (laboratory-developed test)	In-house validation and minimum quality management system	Meets or exceeds standards analogous to United States Centers for Medicare & Medicaid Services or European Union In Vitro Diagnostic Medical Devices Regulation	For minimum assay validation standards for HIV drug resistance surveillance work, see Appendix 3 in WHO HIVResNet HIV drug resistance laboratory operational framework *(25)*. See Annex 3 for additional details
Operating environment	Requires temperature and humidity control (such as 18–25°C and 30–70% relative humidity)	Tolerant to high ambient temperature (such as up to 40°C) and humidity (up to 90% relative humidity)	

[a] The minimal target is a more rigorous or stringent requirement because it is a less burdensome performance target for the product.

[b] Botswana, Eswatini, Lesotho, Malawi, Mozambique, Namibia, South Africa, Zambia and Zimbabwe.

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Annex 1. Target product profile development group

Table A1.1. List of participants in the target product profile development group

Name	Institution	City, state	Country
Randy Allen	Clinton Health Access Initiative	Boston, MA	USA
Alisen Ayitewala	Uganda National Health Laboratory Services	Kampala	Uganda
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Rami Kantor	Brown University	Providence, RI	USA
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Maria Santoro	University of Rome "Tor Vergata"	Rome	Italy
Jonathan Schapiro	National Hemophilia Center	Ramat Gan	Israel
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Lara Vojnov	World Health Organization	Geneva	Switzerland
Clement Zeh	United States Centers for Disease Control and Prevention	Atlanta, GA	USA

The members of the Target Product Profile Development Group are listed in alphabetical order by surname.

Annex 2. Description of target product profile characteristics

Note: the characteristics marked with an asterisk (*) have been defined using the specific context for HIV drug resistance testing, and generalizing or applying these to other types of tests may not be appropriate.

Scope

- **Target user:** type of test operator, defined by minimum required training or certification. This is related to the degree of automation inherent in test performance.
- **Setting and infrastructure level:** minimum laboratory infrastructure level in which the test can be performed (as defined in the WHO consolidated guidelines on HIV testing services *(1)*).
- **Laboratory testing model:** requirement for the test to be performed in a central reference laboratory and requiring specimen transport from the collection site, or for a decentralized model.

Assay design, performance and functionality

- **Region(s) covered*:** PR, RT or IN: indicates the region of HIV-1 that is the target of the ARV drug(s) of concern with respect to previous ART exposure and thus possible drug resistance determinants, or to ARV drugs under consideration for the new regimen (see use case descriptions for details).
- **Drug or drug class coverage*:** similar to region covered, but categorization of RT inhibitors as NRTI and NNRTI. This may simplify assay design by reducing the size of an amplicon in PCR-based assays (such as codons 41–219 for NRTIs versus 98–238 for NNRTIs) or the number of sites targeted by a point-mutation assay. Setting priorities for drug class within the target description is related to distinctions in the use case definition (such as for children or if TLD is not universally available).
- **Mutation coverage*:** the list of mutations that should be included in tests that are designed to detect changes in specific codons (point mutation assays) to detect the proportion of samples with resistance listed in the target product profile. For example, Rhee et al. *(2)* analysed sequence data from people for whom NNRTI-based first-line ART failed to identify six mutations in RT that can provide high (98.8%) sensitivity for detecting NRTI and NNRTI resistance. A similar approach identified six mutations in PR that afforded 91.6% sensitivity for detecting LPV resistance among people for whom an LPV/r-based regimen failed. An analogous analysis of mutations in IN that can provide high sensitivity for detecting DTG resistance among people for whom TLD failed has not yet been published and might be less robust due to the limited amount of publicly available sequence data. Preliminary characterization of viruses from people for whom DTG-containing ART regimens failed indicates that mutations at four sites in HIV-1 IN predominate, suggesting that designing a point mutation assay to detect DTG resistance may be feasible *(3)*. It is assumed that a reduced resistance detection sensitivity or requirement for higher viral loads inherent with this approach could be an acceptable trade-off against an associated reduced cost, turnaround time and potential for point-of-care deployment.
- **Sensitivity for amplification*** (viral load in plasma): the proportion of samples within the indicated viral load range that can be successfully amplified and tested. Sensitivity when using dried blood spots (DBS) is expected to be lower than for plasma, but the targets are defined based on plasma viral load. If sensitivity in DBS does not meet the minimal target, DBS would not be recommended for this application. Amplification sensitivity should be assessed by testing samples with a range in viral load that includes at least 20 samples with viral load that are close (within five-fold) of the minimum stated value and be based on the percentage of successfully completed tests (for an assay involving PCR, not simply assessed by producing enough DNA to be visible on an agarose gel). Consideration must be given to the expectation that, at low viral load, the accurate and reproducible representation of mixed virus species containing low-abundance drug resistance variants will be more challenging *(4)*. This is especially important for assays that claim to be able to detect low-abundance drug-resistant variants at proportions below 20% *(5, 6)*. Amplification sensitivity targets are based on expected viral load for people meeting the criteria described in the priority use cases.
- **Sensitivity for detecting low-abundance drug-resistant variants*:** minimum proportion of a low-abundance drug-resistant variant within a sample required for its detection. Sensitivity for detecting low-abundance drug-resistant variants by PCR-based assays is intimately connected to input copy number, which in turn depends on viral load, sample volume and other factors. For example, a variant present at only 10 copies per mL of plasma would not be reliably detected if only 100 µL of plasma is processed and half of the extracted RNA is used for RT-PCR (input copy number = 0.5). Therefore, sensitivity claims depend on input copy number and must not be overstated if the intended use includes patients with low viral load. In addition, the sensitivity for detection of specific mutations across the region of interest is expected to vary because of localized effects of adjacent sequences; therefore, sensitivity should ideally be determined for each individual mutation.

- **Subtype coverage*:** HIV-1 group M subtypes for which the stated sensitivity target is met. Inclusion of specific subtypes and priority setting within the list of targeted subtypes is related to the predominance of certain subtypes in some countries (such as subtype C in southern Africa) *(7)*. Non–group M HIV-1 or HIV-2 are not included.
- **Turnaround time for specimen receipt to report:** time required from when the specimen is received in the laboratory to the generation of the final report, despite the possible impact of sample testing volume, batch size and the proportion of repeat testing required (such as in samples with low viral load).
- **Achievable throughput** (tests per day): the maximum number of samples (not including controls) that can be tested in a 24-hour period, despite the possible impact of staffing, infrastructure limits and the expected number of patients meeting use case definitions.
- **Interfering substances:** lack of interference by endogenous (biological molecules, etc.) or exogenous (anticoagulants, bacteria, viruses and fungi) substances that are commonly found in the specimen type being used should be demonstrated. For example, for a sequence-based assay using plasma as the specimen type, attention should be focused on how lipids, bilirubin, haemoglobin etc. affect nucleic acid extraction efficiency and how other blood-borne viruses such as hepatitis B or C virus affect RT-PCR efficiency.
- **Quality control*:** control samples should be included that permit expected assay performance to be monitored and errors detected (such as contamination by an exogenous amplifiable template for a test that relies on RT-PCR).
- **Accuracy, precision and reproducibility*:** these are standard components of an assay validation that should be performed either by the test manufacturer (for kits) or the performing laboratory (for laboratory-developed tests). The principle is to ensure that the assay results generated from the same specimen on different replicate tests are sufficiently similar to not affect the clinical interpretation and potential action. Definition of technical specifications depends on the nature of the analyte and test principle. For a sequence-based assay, this would include analysis of the nucleotide sequence that underlies the prediction of drug susceptibility.

Specimen handling

- **Specimen type(s):** type of specimen that is compatible with the test and that supports stated sensitivity requirements. For some specimen types, amplification sensitivity targets may be affected, such as peripheral blood mononuclear cells, since they do not depend on viral load in plasma. Requirements associated with using DBS for drug resistance testing are likely to differ from those of other tests (such as early infant diagnosis) that depend less on sampling of RNA versus DNA *(8)*. The selection of specimen type should not impose requirements that negatively affect other characteristics such as turnaround time and cost.
- **Specimen volume:** minimum volume of specimen needed to support stated sensitivity requirements.
- **Specimen preparation at point of collection:** modifications made to the specimen that are necessary before shipment to the testing site (such as drying).
- **Specimen preparation in the laboratory:** modifications made to the specimen at the testing site that are necessary before testing is started, if not already performed at the collection site.
- **Stability of specimen between collection and arrival at laboratory:** length of time within which test performance is not affected (for example, including temporary storage time at either end and shipping time).
- **Shipping conditions:** temperature required during shipping that supports stability for the time noted above.
- **Specimen storage conditions at laboratory:** conditions required for long-term storage of specimens before and after testing.

Report and data handling

- **Software requirements:** any computer software associated with test performance in the laboratory.
- **Reporting formats:** the format in which a drug resistance test report is transmitted from the testing laboratory to the treating clinician.
- **Result interpretation:** the report provided to the treating clinician.
- **Data capture and transfer:** the format in which drug resistance test results are generated.

Cost considerations

- **Cost per test** (lab testing portion): manufacturer's ex works list price per test for assay kits and reagents, consumables (such as tubes and pipette tips), etc. *(9)*. A test developer should separately consider how test design might affect labour costs, since the test properties can dictate the amount of hands-on time required or a requirement for more highly trained operators. Other costs, including those of waste disposal, common equipment usage and maintenance, utilities, specimen collection and transport and local distributor markups, should be minimized but are not an inherent property of the drug resistance test itself and so are not included here.
- **Instrument and initial set-up cost:** one-time costs for dedicated instruments needed for test performance.

Extraneous requirements

- **Instrument automation:** desired level of automated sample processing. While this may vary depending on testing volume and cost, a minimum amount of automation is needed to reduce pipetting errors, the potential for cross-contamination and sample mix-ups.
- **Reagent stability:** approximate time during which reagents remain useable and support other test characteristics, assuming that the required storage conditions are followed.
- **Reagent storage requirements:** temperature of storage needed to maintain useability of reagents for recommended duration. For PCR-based assays, this includes a sequestered storage area and freezer for pre-amplification reagents (RNA extraction and PCR master mix reagents).
- **Biosafety:** requirements for the safety of personnel operating the test. Patient specimen collection and handling before drug resistance testing should follow international guidelines *(10)*.
- **Regulatory status:** the nature of external regulatory oversight or internal quality management systems that a drug resistance test is subjected to before being used for patient management. Because of the potential risks associated with how inaccurate test results affect patient outcomes, performance of drug resistance testing for patient management demands a higher level of regulatory scrutiny than drug resistance testing for surveillance purposes. For example, a single inaccurate result (such as failure to detect a resistant variant or swapping sample identifiers in the laboratory) will have negligible impact on the result of a drug resistance survey at the population level but could have severe undesirable effects at the individual treatment level. Annex 3 more completely considers quality management systems for clinical use of drug resistance test in individual patients.
- **Operating environment:** environmental conditions required in the laboratory or other testing site for test performance.

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Annex 3. Regulatory oversight and quality management systems

Laboratory tests that generate information used to guide the clinical management of patients must be performed under stringent conditions to ensure a high level of quality, accuracy and repeatability of the results. This is especially important with regard to tests that are developed and performed (in whole or only partly) within a single laboratory, in contrast to using kits manufactured by an external entity. The collective set of procedures, policies, internal and external quality control and quality assurance that govern the operation of clinical diagnostic tests is referred to as a quality management system. Appropriate quality management system implementation in each laboratory may be ensured by internal laboratory directorates, national programmes or external regulatory agencies that are specific to the geographical location of the laboratory. Examples of such regulatory agencies include the South African National Accreditation System, European Union In Vitro Diagnostic Medical Devices Regulation and the United States Centers for Medicare & Medicaid Services.

In contrast, laboratory tests that are used for public health surveillance are generally not governed by these agencies. Instead, national and international public health agencies make recommendations that should be applied for test results to be used in surveillance efforts. For drug resistance testing in support of HIV drug resistance surveillance, WHO-recommended standards for quality management system are described in the *WHO HIVResNet HIV drug resistance laboratory operational framework (1)*. In general, the standards applied to tests used for surveillance purposes are less stringent than those for individual patient clinical management, since the consequences of erroneous results are considered to be less severe. For example, switching the identifiers of two samples in the context of a survey of drug resistance prevalence in a defined population will not affect the survey outcome, whereas switching identifiers when the results are used for clinical decision-making could result in inappropriate treatment decisions at the individual level. Table A3.1 summarizes important differences in quality management systems that govern the operation of drug resistance test for surveillance versus clinical purposes.

Table A3.1. Comparison of key elements of quality management systems for genotyping for clinical versus surveillance purposes

Element	Surveillance	Clinical management
Written procedures (standard operating procedures)	Required for core procedures; may sometimes be "research grade"	Required for core procedures, associated procedures and policies. Controlled document management system in place
Test requisition procedures/ specimen and patient information	Anonymous; detailed treatment or clinical history not required (survey eligibility criteria)	Patient information recorded and kept confidential; treatment and clinical history needed to help interpret results
Specimen handling (storage, organization and traceability)	Required (basic)	Required, comprehensive and error-proof
Assay validation	Minimal assay validation and verification required. Reverification recommended for procedure changes. Required performance characteristics moderate	Extensive assay validation and verification required. Revalidation required for procedure changes. Rigorous performance characteristics required. Ongoing verification of all reagents (including new lots) required
Personnel training and experience	Minimum requirements and competency testing and documentation	Extensive certification and annual recertification, documentation and continuing education
Equipment calibration and maintenance	Required (basic)	Required, comprehensive
External quality assurance	Required	Required; more frequent
Data management	Sufficient to support low- to medium-level throughput and privacy protection	High-level privacy protection required
International standardization	Strongly recommended to ensure comparability of results	May not be necessary (though still preferred, for example, if routine data are used for surveillance purposes)
Reporting	In bulk, formats vary; laboratory supervisor review	Individual reports designed for end-user application (clinicians); medical officer review and approval required. Clinically validated interpretation system preferred
Internal quality control	Minimal requirements for in-process controls	Emphasis on trends using quality indicators etc. based on in-process controls and other variables

Consider a laboratory that performs drug resistance testing for surveillance purposes (for example, a laboratory designated by WHO for HIV-1 drug resistance testing) that is also considering testing samples for clinical use for individuals. Assuming that the WHO laboratory designation for surveillance purposes is sufficient to ensure the level of quality required for clinical testing would be inappropriate and potentially dangerous. Nevertheless, the standards for WHO laboratory designation can be used as a framework and starting place, with additional stringency applied as outlined in Table A3.1. In addition, guidance from other institutions (such as Good Clinical Laboratory Practice Guidelines of the Division of AIDS of the United States National Institute of Allergy and Infectious Diseases *(2)* and International Organization for Standardization standards such as ISO 15189) should be consulted *(3–5)*. It should be noted that these are general quality management standards and are not specific to HIV drug resistance testing.

If the country in which the laboratory is located has a national agency in place that is responsible for laboratory quality, it should be verified that expertise in molecular diagnostics is present and that regulators are familiar with special issues relevant to HIV drug resistance genotyping (such as unidirectional workflow; assay design for relevant subtypes; judgement of heterogeneous sequences and mixed base calling; sequence quality assurance including phylogenetic analysis; and sequence interpretation). If not, external experts should be consulted.

WHO HIV drug resistance designated network laboratories, especially specialized drug resistance laboratories, can serve as a valuable resource for developing a rigorous quality management system that encompasses some of the aspects specific to HIV drug resistance testing. These laboratories can assist less experienced laboratories in the following aspects of quality management system as they relate to HIV drug resistance testing:

- identifying the need for enhanced quality management system and assay validation standards;
- establishing a local or regional proficiency testing programme (including both "wet" and "dry" panels where applicable);
- conducting regular parallel testing of clinical specimens between laboratories for accuracy evaluation;
- training; and
- ongoing oversight and quality assurance.

Other in-country or international partners (such as the African Society for Laboratory Medicine) could form a working group of laboratory experts that is familiar with molecular diagnostics and competent to define quality management systems relevant to implementing HIV drug resistance testing in the local context.

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