Expert Committee on Biological Standardization
Geneva, 17 to 20 October 2017

Technical Specifications Series for submission to WHO Prequalification – Diagnostic Assessment

Human immunodeficiency virus (HIV) rapid diagnostic tests for professional use and/or self-testing – TSS-1

Technical Specifications Series for submission to WHO Prequalification – Diagnostic Assessment: Human immunodeficiency virus (HIV) rapid diagnostic tests for professional use and/or self-testing

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Adopted by the Sixty-eighth Meeting of the World Health Organization Expert Committee on Biological Standardization, 17-20 October 2017. A definitive version of this document, which will differ from this version in editorial but not scientific details, will be published in the WHO Technical Report Series.
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Acknowledgements

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The draft technical specifications document was posted on the WHO website for public consultation on 15 September 2016. Various stakeholders, including manufacturers submitting to WHO Prequalification of IVDs, IVD manufacturing industry associations, various national and international regulatory bodies, and IVD standards organizations were informed of the consultation in order to solicit feedback. A two month response period was provided.

Second round public comments were received from the following:
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The second round of public comments was then incorporated into the document. A revised draft was published on the WHO Biologicals website for a final round of public consultation between 18 June and 18 September 2017. The comments received were incorporated to
produce the document WHO/BS/2017.2305. The document was adopted by the WHO Expert Committee on Biological Standardization as a written standard on 20 October 2017.
Introduction

The purpose of this document is to provide technical guidance to in vitro diagnostic medical device (IVD) manufacturers that intend to seek WHO prequalification of rapid diagnostic tests (RDTs) for the detection of human immunodeficiency virus (HIV).

Minimum performance requirements for prequalification are summarized in this document, and apply equally to RDTs intended solely for HIV detection, and to those tests where HIV detection comprises one component of a multi-detection assay (e.g. a HIV/syphilis dual-detection RDT). This document applies to RDTs intended to be used as an aid to diagnosis of HIV infection. The current version of this document does not address IVDs that discriminate between the detection of HIV-1 and HIV-2 infection, IVDs intended as confirmatory tests, or the requirements for accompanying quality control material.

For the purpose of this document, the verbal forms used follow the usage described below:

- “shall” indicates that the manufacturer is required to comply with the technical specifications.
- “should” indicates that the manufacturer is recommended to comply with the technical specifications but it is not a requirement.
- “may” indicates that the technical specifications are a suggested method to undertake the testing but it is not a requirement.

A documented justification and rationale shall be provided by the manufacturer when the WHO prequalification submission does not comply with the required technical specifications outlined in this document.

Minimum performance requirements for WHO prequalification are summarized in this document, and where possible, WHO performance conditions are aligned with published guidance, standards and/or regulatory documents. Although references to source documents are provided, in some cases WHO prequalification has additional requirements.

For WHO prequalification purposes, manufacturers shall provide evidence in support of the clinical performance of an IVD to demonstrate that reasonable steps have been taken to ensure that a properly manufactured IVD, being correctly operated in the hands of the intended user, will detect the target analyte and fulfil its indications for use.

WHO prequalification requirements summarized in this document do not extend to the demonstration of clinical utility, i.e. the effectiveness and/or benefits of an IVD, relative to and/or in combination with other measures, as a tool to inform clinical intervention in a given population or healthcare setting. To demonstrate clinical utility, a separate set of studies is required. Clinical utility studies usually inform programmatic strategy and are thus the responsibility of programme managers, ministries of health and other related bodies in individual WHO Member States. Such studies do not fall under the scope of WHO prequalification.
A. How to apply these specifications

For the purposes of WHO prequalification, an IVD intended for professional use only (by a laboratory professional, healthcare worker or trained lay provider) shall be supported by studies outlined in Parts 1 and 2 of this document.

An IVD intended both for professional use and for self-testing shall be supported by the studies outlined in Parts 1 and 2 of this document. In addition, the claim for self-testing shall be supported by studies that qualify the usability of the IVD among a broad range of self-testing users, as outlined in Part 3.

An IVD intended for self-testing only, shall be supported by studies outlined in Parts 1, 2 and 3.

For an IVD with an intended use that has been amended to include self-testing, and for which performance in professional use is already established, and Parts 1 and 2 of this document have already been satisfied, the additional claim for self-testing shall be supported by studies outlined in Part 3.

These requirements are summarized in Table 1.

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B. Other guidance documents

This document should be read in conjunction with other relevant WHO guidance documentation, including:

- Technical Guidance Series for WHO Prequalification – Diagnostic Assessment
- Sample Product Dossiers for WHO Prequalification – Diagnostic Assessment
- Instructions for Compilation of a Product Dossier, WHO document PQDx_018.

These documents are available at: [http://www.who.int/diagnostics_laboratory/evaluations/en/](http://www.who.int/diagnostics_laboratory/evaluations/en/)

C. Performance principles for WHO prequalification

D.1 Intended use

An IVD intended for prequalification shall be accompanied by a sufficiently detailed intended use statement. This should allow an understanding of at least the following:

- The function of the IVD (e.g. to detect antibodies to HIV-1, HIV-2 and/or HIV p24 antigen, etc.) and whether it is qualitative, semi-quantitative or quantitative;
The testing population for which the functions are intended (e.g. detection of susceptible individuals) and the intended operational setting (e.g. for use in near-patient testing); and

Clinical indication (e.g. aid to diagnosis of HIV infection).

D.2 Diversity of specimen types, users and testing environments and impact on required studies

For WHO prequalification submission, clinical performance studies should be conducted using the specimen types that are most likely to be used in resource-limited WHO Member States (e.g. capillary whole blood and oral fluid) and claimed in the instructions for use. If this is not possible, substantial data shall be presented to show the equivalence between specimen types used in performance studies.

Prequalified RDTs in low- and middle-income countries are likely to be used by laboratory professionals¹ and at point-of-care by healthcare workers, trained lay providers² or by individuals who self-test. Depending on the intended use of an RDT, performance studies shall be designed to take into account not only the diversity of knowledge and skills across the population of RDT users, but also the likely operational settings in which testing will occur. For example, studies that comprise the testing of left-over/repository specimens by research and development staff at a manufacturer’s facility shall not, on their own, be considered sufficient to meet many of the performance requirements summarized in this document.

D.3 Applicability of supporting evidence to IVD under review

Performance studies shall be undertaken using the specific, locked-down version of the IVD intended to be submitted for WHO prequalification. Where this is not possible, a justification shall be provided and additional supporting evidence may also be required. This may occur in the case of minor variations to design where no negative impact on performance has been demonstrated.

Specific information is provided in Parts 1 and 2 of this document for the numbers of lots required for particular studies. Each lot should comprise different batches of critical components. It is a manufacturer’s responsibility to ensure, via risk analysis of its IVD, that the minimum numbers of lots chosen for estimating performance characteristics takes into account the variability in performance likely to arise from the diversity of key components and their formulation.

The true HIV status of a specimen shall be determined using a suitable reference method, for which justification shall be provided. Estimation (and reporting) of IVD performance shall include the rate of invalid test results. For certain analytical studies it may be acceptable to use contrived specimens (e.g. where normal human specimens have been spiked with those containing HIV antibodies). Although all reasonable attempts should be made to use natural specimens, justification should be provided where contrived specimens are used in the submitted studies. Clinical studies should be based on testing in natural specimens only.

For IVDs that include a claim for detection of multiple analytes, evidence of performance shall be provided for each claimed analyte. It should be noted that, depending on the design of an IVD, evidence generated in a similar, related product will usually not be considered sufficient by WHO to support performance claims in an IVD submitted for prequalification.

¹ Medical technologists, medical laboratory technicians or similar, who have received a formal professional or paraprofessional certificate or tertiary education degree.
² Any person who performs functions related to healthcare delivery and has been trained to deliver specific services but has received no formal professional or paraprofessional certification or tertiary education degree.
Example: an IVD designed to detect HIV antibodies only, and the same IVD designed for dual detection of HIV and syphilis. It is unlikely that performance evidence presented for the HIV-only IVD would be acceptable to support performance claims for the dual-detection IVD.

For an IVD with an intended use that has been expanded to include self-testing, changes are usually required to improve the usability of the IVD for this new testing population. Such changes may include modification of:

- instructions for use (e.g. simplification of instructions to reflect new intended users)
- buffer vials
- collection procedures
- reading times etc.

It is a manufacturer’s responsibility to verify through testing (as summarised in Parts 1 and 2 of this document) that any changes do not have an adverse impact on critical safety and performance characteristics of an IVD. Usability studies are undertaken to optimize the presentation of an IVD and the understanding of self-testing users. The minimum reporting requirements summarized in Part 3 of this document are not intended to be an exhaustive list or to indicate a particular order in which studies should be undertaken.

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- 3.1 Qualification of usability (self-testing)
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### Part 1 Establishing analytical performance characteristics

#### 1.1 Specimen type

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<td>1.1.1</td>
<td>For each claimed specimen type, testing in at least:</td>
<td>1. The relationship between IVD performance in claimed specimen types and reference materials used for analytical studies shall be established. The design of subsequent studies shall then take that relationship into account.</td>
<td>Technical Guidance Series for WHO Prequalification – Diagnostic Assessment (1)</td>
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<td>- 25 positive specimens</td>
<td>2. If there is no equivalence between claimed specimen types then the impact that this will have on each subsequent performance claim shall be fully understood and described. Where a significant difference in performance exists between specimen types, equivalence may need to be investigated as part of a larger clinical study (See Part 2).</td>
<td>European Commission (2)</td>
</tr>
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<td>- 25 negative specimens</td>
<td>Example: an IVD intended for testing whole blood for which seroconversion sensitivity is estimated using panels of serum/plasma specimens.</td>
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<td>1.1.2</td>
<td>At least 25 positive and 25 negative specimens of each</td>
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<td>claimed anticoagulant. The equivalence of specimen types shall be determined for all claimed analytes (e.g. HIV-1 antibodies, HIV-2 antibodies, p24 Ag, as appropriate) (see comment 3).</td>
<td>3. In some cases it may be acceptable to use diluted or spiked specimens. This approach is acceptable in early development work, but all reasonable attempts should be made to use natural specimens. Justification should be provided if diluted or spiked specimens are used in the submitted studies.</td>
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<td>4. Positive specimens (undiluted) shall be chosen so that the majority are near the IVD cut-off.</td>
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<td>5. Pairs specimens should be used (e.g. if claiming equivalence of four anti-coagulants, then each subject should provide four</td>
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<tr>
<td>Aspect</td>
<td>Testing requirements</td>
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| 1.2 Specimen collection, storage and transport | Real time studies taking into account:  
- storage conditions (duration at different temperatures, temperature limits, freeze/thaw cycles)  
- transport conditions, where applicable  
- intended use (see comment 1)  
- specimen collection and/or transfer devices intended to be used with the IVD. | Evidence shall be provided which validates the maximum allowable time between specimen collection and its addition to the IVD in the setting where testing takes place. | Technical Guidance Series for WHO Prequalification – Diagnostic Assessment (3) |
| 1.3 Precision of measurement | Both repeatability (within-condition – see comment 1) and reproducibility (between-condition – see comment 1) shall be estimated using panels of at least:  
- 1 negative specimen  
- 1 low reactivity positive specimen (near assay cut-off)  
- 1 medium reactivity positive. | 1. E.g. within- or between-run, -lot, -day, -site, etc.  
2. Precision shall be determined for each pathogen and/or analyte for which detection is claimed (e.g. HIV-1 antibody, HIV-2 antibody, HIV-1 p24 antigen (Ag), as appropriate).  
3. The testing panel should be composed of natural (i.e. undiluted) specimens. Where this is not feasible, stock specimens that are to be diluted should represent a range of stages of infection (antibody maturation) in order to take into account the limitations of mimicking low IVD reactivity with a high avidity specimen.  
4. IVDs which include whole blood as a specimen type shall include evidence of precision in, at a minimum, spiked whole blood specimens (negative whole blood spiked with highly-reactive plasma/serum specimens to produce an appropriate range of reactivities in the IVD).  
5. Where possible, the testing panel should be the same for all operators, lots and sites.  
6. Lots shall comprise different batches of critical components.  
7. Results shall be statistically analyzed to identify and isolate the sources and extent of any variance. In addition, the percentage of correctly-identified, incorrectly-identified and invalid results shall be tabulated for each specimen and be separately stratified according to site, lot, etc. This type of analysis is especially important for rapid tests that may not have any | CLSI EP05-A3 (4)  
ISO 13612:2002 (5)  
CLSI EP12-A2 (6) |
### Part 1 Establishing analytical performance characteristics

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<td>numerical values.</td>
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<td>8. The effect of operator-to-operator variation on IVD performance may also be considered as a human factor when designing robustness (flex) studies (see 1.10.1 Flex studies) and may be addressed as part of clinical studies in representative populations (see Part 2).</td>
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<td>9. Users should be selected based on a pre-determined and contextually appropriate level of education, literacy and auxiliary skills that will challenge the usability of the IVD and reflect the diversity of intended users and operational settings.</td>
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### 1.4 Performance panels

#### 1.4.1 Subtype panels

Testing of WHO International Reference Preparations and/or commercial HIV subtype panels shall include:
- all HIV-1 subtypes (e.g. A, B, C, D, G, etc.)
- HIV-2, HIV-1 group O, and common circulating recombinant forms (CRFs)
- at least 10 each of the most common subtypes (Subtype C, Subtype A, Subtype B, CRF02_AG, CRF01_AE, CRF07_BC and Subtype G)
- at least 3 less common subtypes (other CRFs and unique recombinant forms (URFs))

1. Testing should be performed using more than 1 lot of the final design (locked-down).
2. All confirmed subtype-positive specimens shall be detected by the IVD.
3. All reasonable attempts shall be made to test rare subtypes.
4. For IVDs including a claim for detection of HIV Ag, appropriate specimens for the same subtypes shall also be included in the testing panel. Use of panels of viral-like-particles (VLPs) or viral cultures may be considered acceptable, however their use in place of characterized specimens shall be justified.

#### 1.4.2 Mixed titre panels

Testing of panel of specimens with a range of analyte concentrations (e.g. antibody ‘mixed-titre’ panel).

### 1.5 Validation of reading times

#### 1.5.1 Validation of reading times

For IVDs where a reading interval is specified (i.e. time when result can first be read; time beyond which result should not be read), validation of critical time points shall be provided.

Performance studies shall be conducted at each of three temperatures (at the mid-point and two extremes of the

1. The ranges of humidity tested for shall be risk-based, taking into consideration likely operational settings.
2. The intended operating temperature, upon which reading time has been validated, shall be clearly stated in the instructions for use.
3. Some of these aspects could be evaluated within the flex

Health Products and Food Branch, Health Canada (7)

WHO Prequalification – Diagnostic Assessment (8)
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<td>claimed operating range; the effect of humidity on reading times shall also be investigated.</td>
<td>studies (1.10.1)</td>
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<td>1.6 Analytical sensitivity</td>
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| 1.6.1 Seroconversion | A minimum of 25 commercial or well-characterized seroconversion panels shall be tested:  
• test at least 40 early seroconversion specimens (see comment 2)  
• all seroconversion specimens shall be reactive (see comment 3)  
• start with a negative bleed(s), and should have narrow bleeding intervals. | 1. Panels should have been collected at short intervals to cover the seroconversion period and should also cover the whole window period.  
2. Early seroconversion:  
   – p24 Ag and/or HIV RNA-positive  
   – Not recognized by all of European Conformity (CE)-marked 3rd generation enzyme immunoassays  
   – Indeterminate or negative by confirmatory assays.  
3. Seroconversion:  
   – p24 Ag and/or HIV RNA-positive  
   – Recognized by all of European Conformity (CE)-marked 3rd generation enzyme immunoassays  
   – Indeterminate or positive by confirmatory assays.  
4. Seroconversion sensitivity shall be reported to the user in the instructions for use.  
5. Optimally, testing should be conducted using more than one lot of the final design (locked-down). | European Commission (2)  
Health Products and Food Branch, Health Canada (7)  
CLSI EP12-A2 (9) |
| 1.6.2 Limit of detection for HIV-1 p24 Ag, where appropriate | Analytical sensitivity estimated as the concentration of HIV-1 p24 Ag at the assay cut-off.  
The determination shall comprise a minimum of 15-20 replicate tests of an 8-member dilution panel of a suitable biological reference material (e.g. WHO International Standard HIV-1 p24 Ag, NIBSC code 90/636). | | |
| 1.7 Prozone/high dose hook effect | | | |
| 1.7.1 Prozone/High dose hook effect | For each claimed analyte, the potential for a prozone/high-dose hook effect shall be determined:  
• using multiple, highly-reactive specimens (minimum of 20)  
• using at least two different concentrations (diluted by at least a factor of 10)  
• by testing of several replicates by the same operator on the same day. | 1. Specimens shall be chosen that have a high analyte concentration, as determined using an IVD method other than the IVD intended to be prequalified e.g. enzyme immunoassay. This second method shall be of a design not subject to prozoning.  
2. An increase in signal upon dilution of a specimen implies a hook effect. | Health Products and Food Branch, Health Canada (7)  
Butch, AW (10) |
| 1.8 Analytical specificity | The potential for false results (false negatives and false positives) arising from interference from at least the substances/conditions listed below shall be determined | 1. The risk assessment conducted for an IVD shall identify substances where the potential for interference can reasonably be expected for the analyte being detected (e.g. HIV-1/2) | Health Products and Food Branch, Health Canada (7) |
## Part 1 Establishing analytical performance characteristics

### Notes on testing requirements

1. The types of interferences tested for shall be risk-based, taking into consideration the operational setting as well as the intended users for the analyte being detected (e.g. HIV-1/2 antibodies and/or HIV-1 p24 Ag).

2. Any observed interference shall be investigated and performance limitations of the IVD reported in the instructions for use. Results shall be reported with respect to each condition and not be reported as an aggregate of the total number of specimens tested in the study.

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| substances | using (See Comment 1):  
- a minimum of 100 specimens (either naturally occurring or spiked to a low reactivity)  
- each substance/condition represented, where possible, by at least 3-5 specimens from different individuals.  
Testing shall be undertaken in both HIV-negative and HIV-positive specimens, unspiked or spiked, with each potentially interfering substance at physiologically relevant dosages.  
1.8.1.1 Endogenous  
- Human antibodies to the expression system (for recombinants), e.g. anti-\textit{Escherichia coli} (anti-\textit{E.coli} positive), human anti-mouse antibody (HAMA)  
- recipients of multiple blood transfusions, pregnant (including multiparous) women  
- haemoglobin, lipids, bilirubin and protein  
- elevated Immunoglobulin G and Immunoglobulin M  
- rheumatoid factor  
- sickle-cell disease  
- other autoimmune conditions including systemic lupus erythematosus (SLE) and anti-nuclear antibodies. (ANA)  
1.8.1.2 Exogenous  
- Relevant medicines, including: antiparasitic, antimalarial, antiretroviral and anti-tuberculosis medications  
- common over-the-counter anti-inflammatory medications (aspirin, paracetamol, ibuprofen)  
- ethanol, caffeine.  
1.8.2 Cross-reactivity | | Canada (7)  
| | | European Commission (2)  
| | | CLSI EP07-A2 (11) |
### Aspect: Testing requirements

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<td>performance limitations of the IVD reported in the instructions for use.</td>
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### Notes on testing requirements

#### Source documents

- Infection, acute hepatitis A infection, cytomegalovirus, acute Epstein–Barr virus, varicella zoster virus, Yellow fever virus post-immunization, measles, influenza A and B, tick borne encephalitis
- Other retroviruses, including: human T-lymphotropic cell virus-1 and -2
- Bacteria/parasites, including: malaria, visceral leishmaniasis, tuberculosis and human African trypanosomiasis
- Influenza vaccine recipient
- Vaccine-induced HIV seropositivity
- Other unrelated conditions known to cause cross-reactivity in HIV IVDs.

### 1.9 Metrological traceability of control material values

#### 1.9.1 Metrological traceability of control material values

| The traceability of an assay-specific quality control specimen to a validated reference material shall be demonstrated (e.g. WHO International reference panel (antibody), WHO International Standard HIV-1 P24 Antigen). |                   |

1. HIV RDT kits may not include external quality control specimens, but the IVD shall have a procedural control. The extent to which a control band corresponds to a valid test (identification of and traceability to a suitable reference) should be demonstrated.

   NOTE 1: The nature of the procedural control (specimen addition or only reagent addition) shall be explained.

   NOTE 2: An external control specimen is one that is run in conjunction with the IVD, but is physically separate from it, for example, an RDT test cassette.

2. In some jurisdictions there is a requirement for use of a ‘National Testing Panel’ for lot release and IVD validation. Such a national requirement does not obviate (or remove) the need for evidence of traceability to a validated reference material as described here.

#### Source documents

- WHO Prequalification – Diagnostic Assessment (8)

### 1.10 Stability

| Replicate testing shall be undertaken using a panel consisting for each claimed pathogen/analyte, of at least: 1 non-reactive specimen 2 low-reactivity specimens, near assay cut-off (see |                  |

1. The testing panel shall include all claimed analytes and include whole blood specimens and/or oral fluid specimens, as appropriate, in accordance with intended use (for example to verify proper flow, no background interference and account for

#### Source documents

- ISO 23640:2011 (12)
- CLSI EP25-A (13)
- WHO Technical
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<td>comment 2)</td>
<td>• 1 medium reactivity specimen. Wherever possible, specimens chosen for the testing panel shall include panel members that reflect the main specimen types intended for use with the IVD (e.g. capillary whole blood/oral fluid, as appropriate).</td>
<td>2. Where detection of multiple genotypes and/or subtypes is claimed, equivalent performance (e.g. sensitivity and specificity) shall have been demonstrated; otherwise evidence of stability in these genotypes/subtypes will need to be provided. 3. Ideally, the stability testing panel shall be composed of natural (i.e. undiluted) specimens. Where this is not feasible, stock specimens to be diluted should represent a range of stages of infection (antibody maturation) so as to take into account the limitations of mimicking low IVD reactivity with a high avidity specimen.</td>
<td>Guidance Series for WHO Prequalification – Diagnostic Assessment (14) ASTM D4169-14 (15)</td>
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<td>1.10.1 Shelf life (including transport stability)</td>
<td>Real time, minimum of 3 lots of final design product transport stressed (simulated) before real time studies are undertaken • IVD in final packaging subjected to drop-shock testing.</td>
<td>4. Lots shall comprise different batches of critical components. 5. Determination of shipping stability shall be performed using simulated extreme stress conditions, ensuring that application of those conditions is consistent and controlled. 6. Claims for stability shall be based on the second-last successful data point from the least stable lot, with, if lots are different, a statistical analysis showing that the bulk of lots will be expected to meet the claimed life. For example: for testing conducted at 3, 6, 9, 12 and 15 months, if stability was observed at 15 months, then the maximum stability claim shall be 12 months. 7. Accelerated studies do not replace the need for real time studies. 8. In-use stability of labile components shall be conducted using components in their final configuration.</td>
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<td>1.10.2 In-use stability</td>
<td>• minimum of 1 lot, using panel(s) compiled as above • testing of all labile components (e.g. buffers vials, sealed cartridges, etc.; see Comment 8).</td>
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<td>1.11 Flex studies</td>
<td>The influence of the following factors on expected positive and negative results shall be considered: • specimen and/or reagent volume • buffer pH (measure of robustness – e.g. due to evaporation of the buffer)</td>
<td>1. Refer to WHO document PQDx_018 “Instructions for compilation of a product dossier” for other flex studies that may be relevant, taking into consideration the broad range of operational and environmental conditions consistent with intended use.</td>
<td>WHO Prequalification – Diagnostic Assessment (8)</td>
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<td>• reading time (i.e. the interval between when the first and last readings can be taken)</td>
<td>2. The factors listed opposite should be investigated in ways that not only reflect, but also exceed, likely operating conditions in lower- and middle-income countries so that the limitations of the device to be understood. For example, in addition to investigating deviations of temperature within those claimed in the instructions for use, temperature ranges should be investigated that exceed those of claimed operating conditions and which cause test failure (incorrect/invalid results).</td>
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<td>• IVD sturdiness including robustness of packaging and labelling lighting and humidity (See Comment 3)</td>
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<td>• operating temperature.</td>
<td>3. The impact of lighting can be twofold – i.e. the impact of lighting on packaging e.g. fading, and the sufficiency of lighting to read the test lines.</td>
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## Part 2 Establishing clinical performance characteristics (professional use and/or self-testing)

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| **2.1 Diagnostic sensitivity and specificity** | Diagnostic sensitivity and specificity shall be determined for each claimed specimen type. Testing should be conducted:  
- at different geographical settings (minimum of 2 regions)  
- by a variety of intended users  
- using more than 1 lot. | 1. Prequalified HIV RDTs are generally used by lay providers and health care workers. For WHO prequalification purposes, these should be considered as the intended user rather than a trained laboratory professional.  
2. Where an IVD is intended to detect multiple analytes without differentiating which analyte is detected, specimens chosen for the testing panel shall comprise those that are reactive only for each individual analyte (i.e. not dual HIV-1/HIV-2 positive, etc).  
3. A separate specimen shall be collected prior to testing to establish the reference result. The testing algorithm used to determine the reference results shall include a state of the art 4th generation immunoassay (EIA), with all initially reactive specimen reflexed for full characterization of the HIV status.  
4. Problematic specimens, those with unexpected results but which otherwise meet selection criteria for a study, shall not be systematically excluded from analysis.  
5. Consideration shall be given to the influence of antiretroviral medications present in a specimen on the serostatus of such specimens, and how this might affect specimen selection.  
6. Lots (locked-down design) shall comprise different batches of critical components.  
7. Where possible, all discrepant results (between assay under evaluation and the reference results) shall be repeated using the same lot, and then on all available lots and the variability noted. Performance characteristics shall be reported using initial results, only. The results of further testing of specimens with discrepant results shall be reported separately as additional information about IVD performance.  
8. All indeterminate results shall be included in the denominator data for European Commission (2)  
Health Products and Food Branch, Health Canada (7) | |
| 2.1.1 Diagnostic sensitivity | Testing of:  
- at least 400 specimens confirmed HIV-1 antibody positive  
- at least 100 specimens confirmed HIV-2 antibody positive (where HIV-2 detection is claimed; see Comment 2)  
- at least 50 specimens confirmed HIV p24 Ag positive (where Ag detection is claimed; see Comment 2). | | |
| 2.1.2 Diagnostic specificity | Testing of:  
- at least 1000 HIV antibody/antigen negative specimens. | | |
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<td>analysis.</td>
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<td>All invalid test results shall be recorded.</td>
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<td>9.</td>
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<td>Estimates of diagnostic/clinical sensitivity and specificity shall be reported with 95% confidence intervals.</td>
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<td>10.</td>
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<td>Results shall be expressed separately for each specimen type and for each specimen type per intended use (no aggregation of results).</td>
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### Part 3 Qualification of usability (self-testing)

**PURPOSE:** Assessment of product design, instructions for use and usability of RDTs for self-testing by analysis of the following:

- Results of a questionnaire to assess whether the key messages and instructions from packaging and labelling would be understood and easily followed by untrained intended users (i.e. self-testers).
- Results of the interpretation of test-results by untrained users (i.e. self-testers) of simulated RDTs (e.g. pre-made and with contrived results).
- Test results and interpretations when assay is performed by untrained intended users (i.e. self-testers) (16, 17, 18, 19).
- For each of the studies summarized below, the study group shall comprise untrained subjects whose age, gender, level of education, literacy and additional, supplementary skills may challenge the usability of the IVD in intended users and in unfavourable operational settings (e.g. poor lighting).
- These assessment activities will determine the changes needed to optimize the IVD for use by self-testers. Changes may range from minor (simplification of instructions for use) to major. The impact of any change on safety and performance shall be determined.
- Results from any one of the stages summarized below may indicate that assay redesign is necessary. This may in turn result in a need to revalidate the IVD or to perform additional specific performance studies and to update the risk analysis.

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| 3.1.1 Label comprehension study | Questionnaire-based testing of subjects, representative of end users, to assess ability of intended users to correctly comprehend key messages from packaging and labelling:  
- proper self-selection (whether or not users understand if it is appropriate for them to undertake testing)  
- understanding key warnings, limitations and/or restrictions  
- proper test procedure  
- test result interpretation.  
Questionnaire shall be administered to at least 200 subjects, representative of end users, in order to demonstrate comprehension of key messages. | 1. Instructions for use and labelling shall be clear and easy to understand; use of pictorial instructional material is encouraged. | ISO 18113:2009 (20)  
ISO 15197:2013 (21)  
IEC 62366-1:2015 (22)  
MHRA (23)  
Poffenberger, K (24)  
FDA (25)  
European Commission (2)  
European Parliament and European Council (26)  
Center for Devices |
<p>| 3.1.2 | A minimum of 400 subjects to interpret the results of | 1. The study group may include subjects recruited as part of the | |</p>
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| Results interpretation study         | Contrived IVDs (e.g. static/pre-made tests) to assess their ability to correctly interpret pre-determined test results. Contrived tests shall be made to demonstrate the following potential test results:  
  - non-reactive  
  - range of invalid results  
  - reactive  
  - weak reactive.  
  Testing subjects to consist of at least 200 self-testers from two high-prevalence (>5%), geographically diverse populations and at least 200 self-testers from a low-prevalence (<5%) population to demonstrate correct interpretation of simulated test results. | Label comprehension study.                                                                      | and Radiological Health, FDA (27)  
WHO (28)  
USAID and WHO (29)  
Center for Devices and Radiological Health, FDA (30) |
| 3.1.3 Observed untrained user study  | Testing by at least 900 self-testing subjects comprising: at least 200 self-testers in each of two high-prevalence (>5%), geographically diverse population and at least 500 self-testers from a low-prevalence (<5%) population.  
  - Each subject to self-collect test specimen and perform test according to only those materials provided with the IVD (e.g. instructions for use, labels and other instructional materials).  
  - Each such test to be observed by trained laboratory or healthcare professional. The observing professional does not tutor or interact with subject conducting test, but notes errors and other observations about the self-tester. Observation may also be conducted by way of video recording of self-testing.  
  - Observing professional also interprets the test result, in a blinded fashion and within the validated reading time stated in the instructions for use. | 1. A separate venous whole blood specimen shall be collected prior to testing to establish the reference results for HIV-1 status (and HIV-2 where detection is claimed). The testing algorithm used to determine the reference results shall include use of a state of the art 4th generation immunoassay, with all initially reactive specimens reflexed for confirmation of the HIV status.  
2. For WHO purposes, the term ‘professional use’ encompasses a diversity of skills, training and experience and does not necessarily imply ‘highest standard of skills, training and experience’. It may be a useful step in development of usability to compare performance between self-testers with that of healthcare workers, lay providers, and laboratory technicians. However, concordance observed between types of users may mask poor performance within each user group. Consequently, such comparisons do not replace the need for comparisons to ‘clinical truth’ by establishment of reference results for each subject.  
3. There may be a high likelihood of bias at the community level when simple study population sample methodologies are applied. Efforts shall be made to avoid convenience sampling of people (participants) who already know they are HIV positive. |
References


Note: The above guidance does not apply to HIV test kits which are used for patient management, or HIV test kits intended to be used outside the laboratory i.e. at the point of care and/or for home use. A separate guidance titled “Draft Guidelines for HIV Simple/Rapid Test Kit” is available for manufacturers of near-patient HIV test kits upon request from Health Canada’s Medical Devices Bureau (MDB), e-mail: device_licensing@hc-sc.gc.ca.


