Technical Specifications Series for submission to WHO Prequalification – Diagnostic Assessment

TSS-12

In vitro diagnostic (IVD) medical devices used for the qualitative detection of HIV-1 and HIV-2 nucleic acid (DRAFT)

DRAFT FOR COMMENT: This is a draft intended for review by Member States and all interested parties for the purpose of consultation on the draft text. The content of this document is not final, and the text may be subject to revisions before publication. The document may not be reviewed, abstracted, quoted, reproduced, transmitted, distributed, translated or adapted, in part or in whole, in any form or by any means without the permission of the World Health Organization.
Table of Contents

Acknowledgements ................................................................................................................................ iv
List of contributors ................................................................................................................................. iv
Abbreviations .......................................................................................................................................... 1
A. Introduction .................................................................................................................................... 1
B. How to apply these specifications .................................................................................................. 2
C. Other guidance documents ............................................................................................................ 2
D. Performance principles for WHO Prequalification ........................................................................ 3
   D.1 Intended use ................................................................................................................................. 3
   D.2 Diversity of specimen types, users and testing environments and impact on required studies . 3
   D.3 Applicability of supporting evidence to IVD under review ....................................................... 4
E. Table of Requirements .................................................................................................................... 6
   Part 1: Analytical performance and other evidence ........................................................................ 7
   Part 2: Establishing clinical evidence (clinical performance characteristics) .................................... 19
   Part 3: Qualification of usability for POC testing .............................................................................. 21
F. References .................................................................................................................................... 22
Acknowledgements

The document “Technical Specifications Series for submission to WHO Prequalification – Diagnostic Assessment: In vitro diagnostic (IVD) medical devices used for the qualitative detection of HIV-1 and HIV-2 nucleic acid” was developed with support from the Bill & Melinda Gates Foundation and UNITAID. The document was prepared in collaboration with J. Saldanha; California, United States of America (USA); R. Luo, California, USA; S. Best, Melbourne, Australia; D. Healy, U. Ströher, Prequalification Team – Diagnostic Assessment, WHO and L Vojnov, HIV department, WHO. This document was produced under the coordination and supervision of U. Ströher and I. Prat, Prequalification Team – Diagnostic Assessment, WHO, Geneva, Switzerland

List of contributors

A technical consultation on WHO prequalification requirements for hepatitis C and HIV nucleic acid detection tests was held in Geneva, Switzerland from 27 to 29 May 2019.

Meeting participants: P.N. Akolkar, Office of Blood Research and Review, Center for Biologics Evaluation and Research (CBER), Food and Drug Administration (FDA) Silver Spring, Maryland, USA; H.P.W. Bayer, Weinheim, Germany; S. Best, Melbourne, Australia; S. Carmona, Department of Molecular Medicine and Haematology, University of the Witwatersrand, Johannesburg, South Africa; C.I. Chime, Institute of Human Virology, Abuja, Nigeria; E. Ivanova, Foundation for Innovative New Diagnostics (FIND), Geneva, Switzerland; S. Kamili, Division of Viral Hepatitis, Centers for Disease Control and Prevention (CDC), Atlanta, USA; J. Kress, Paul-Ehrlich-Institut, Langen, Germany; W. Leelawiwat, Thailand Ministry of Public Health-U.S. Centers for Disease Control and Prevention, Nonthaburi, Thailand; P. Musasa Ncube, National Microbiology Reference Laboratory, Harare, Zimbabwe; J. Parry, Middlesex, United Kingdom; J. Saldanha, Oakland, USA; S.M. Samiee, Reference Health Laboratory, Ministry of Health & Medical Education, Tehran, Iran; A. Tanuri, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brasil; C. Zeh, Division of Global HIV & TB, Centers for Disease Control and Prevention (CDC), Atlanta, USA.

WHO Secretariat: M. Lanigan; A.L. Page; I. Prat; U. Ströher, Prequalification Team – Diagnostic Assessment Group, Regulation of Medicines and other Health Products; R. Baggaley and L. Vojnov, HIV Programme.

1 Participated via conference call
A. Introduction

The purpose of this document is to provide technical guidance to in vitro diagnostic (IVD) medical device manufacturers that intend to seek WHO prequalification of qualitative, multiplex, nucleic acid tests (NAT) for the detection of HIV-1 and HIV-2. This document covers both IVDs that detect but do not differentiate HIV-1/HIV-2 and those who detect and differentiate HIV-1 and HIV-2.

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, 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. A full list of the individual studies is provided in chapter E (parts 1-3).
• Part 1 lists the analytical studies that are required to assess the ability of the IVD to measure the relevant analyte(s).
• Part 2 lists the clinical studies that are required to support the clinical performance of an IVD, and 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.
• Part 3 lists the usability studies that are only required for IVDS with the intended use for POC (including near POC) testing by non-lab professionals.

Clinical utility studies, 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 each population or healthcare setting, do not fall under the scope of WHO Prequalification and are not included in this document. Clinical utility studies usually inform programmatic strategy and are thus the responsibility of programme managers, ministries of health and other relevant bodies in individual WHO Member States.

NOTE: For assays that use different combinations of extraction methods/kits and amplification/detection kits, each combination shall be completely validated as described in this document.

B. How to apply these specifications

For purposes of WHO prequalification HIV-1 & HIV-2 assays with the claim to detect HIV-1 and HIV-2 nucleic acid (qualitative tests) shall comply with the specifications in Part 1 and Part 2 of this document. Part 3 only applies if, according to the IFU, testing is performed by non-lab professionals in POC or near POC settings. Semi-quantitative assays shall be validated using the HIV-1 analytical studies described in Part 1 of this document. Depending on the intended use and claims of the semi-quantitative assay, the clinical studies described in this document (TSS 12) or TSS 11: “In vitro diagnostic (IVDs) medical devices used for the quantitative detection of HIV-1 nucleic acid” might apply.

C. Other guidance documents

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

WHO Prequalification documents:

• 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.

---

2 Semi-quantitative assays are assays that do not give a result for the concentration of a given analyte in numerical values, such as 50 IU/mL or 2000 genome equivalents/mL, but give an indication of the relative concentration of the analyte by e.g. reporting the Ct value for real-time PCR assays (which is inversely proportional to the concentration of the analyte

3 Available at: http://www.who.int/diagnostics_laboratory/evaluations/en/
WHO HIV programme documents:

- WHO Consolidated guidelines on HIV testing services, 2015

D. Performance principles for WHO Prequalification

D.1 Intended use

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

- what is detected (e.g. to detect nucleic acid from individuals infected with all known subtypes of HIV-1, groups M, including known, circulating recombinant forms (CRFs), such as A/E, A/G, B/G, B/F, HIV-1 groups N and O, and HIV-2, groups A and B);
- the clinical indication and function of the IVD (e.g. detection of HIV-1 and/or HIV-2 in individuals suspected of HIV-1 and/or HIV-2 infections) - the ability of the device to detect HIV-1 and HIV-2 in combination without differentiation or to simultaneously detect and differentiate HIV-1 and HIV-2;
- the testing population and the ages of individuals for which the functions are intended (e.g. adults and paediatric individuals >18 months and infants < 18 months);
- the intended operational setting and the intended user (e.g. for professional use in a laboratory setting, and/or point-of-care (POC));
- the intended specimen type(s) (e.g. whole blood, plasma, serum, dried blood spots (DBS)) and collection device(s) and/or method(s) (e.g. safety lancets for capillary whole blood collection from finger or heel pricks);
- any limitations to the intended use
- The ability of the device to detect acute and early HIV-1 and/or HIV-2 infections (e.g. earlier than the detection of HIV-1/2 serological markers (antibodies, antigens) by tests that been stringently regulated and approved by one of the Regulatory Authorities of the Founding Members of GHTF).

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

For WHO prequalification submission, clinical studies shall be conducted using the specimen types (e.g. plasma, serum, whole blood, DBS) that are claimed in the instructions for use (IFU). For DBS specimens, the brand(s) of filter paper used for the validation studies shall be stated in the IFU.

---

4 http://apps.who.int/iris/bitstream/handle/10665/179870/9789241508926_eng.pdf;jsessionid=FF74509282306289BAA851097F1E99A8?sequence=1

5 Point-of-care in-vitro diagnostic testing (POC) refers to decentralized testing that is performed by a minimally trained healthcare professional near a patient and outside of central laboratory testing facilities. It does not refer just to sample collection procedures.
Technical Specifications for submission to WHO prequalification-Diagnostic Assessment:
IVDs used for the detection of HIV 1 & HIV 2 NA (qualitative)

Prequalified NAT IVDs in low- and middle-income countries are likely to be used by laboratory professionals either in centralised testing laboratories or at POC. The complexity of a test shall be clearly elucidated in the IVD IFU and reflected in the risk analysis. Depending on the intended use of an IVD, analytical and clinical studies shall be designed to consider not only the diversity of knowledge and skills across the population of IVD users, but also the likely operational settings in which testing will occur. It is a manufacturer’s responsibility to ensure that the risk assessment for an IVD reflects the intended operational settings, including laboratory or service delivery complexity, user expertise and test population.

D.3 Applicability of supporting evidence to IVD under review

Analytical and clinical studies shall be undertaken using the specific, final (locked-down design) version of the IVD intended to be submitted for WHO prequalification. For WHO prequalification, design lock-down is the date that final documentation is signed off, including quality control and quality assurance specifications, and the finalized method is stated in the IFU. Where this is not possible, a justification shall be provided; additional supporting evidence may also be required.

This may occur in the case of minor variations to design where no impact on performance has been demonstrated (see WHO document PQDx_121 Reportable Changes to a WHO Prequalified In Vitro Diagnostic Medical Device). If the protocol section of the IFU has been changed in any way, both the protocol provided to laboratory for studies as outlined in Part 2 of this document and that in the final version of the IFU intended for users shall be provided with the submission to WHO prequalification.

The version of the IFU used for performance evaluations submitted to WHO prequalification shall be stated. If the test procedure in the IFU is changed in any way after completing performance verification and validation studies the change shall be reported to WHO, including a rationale for the change, and an explanation of why the study results support the claimed performance.

Specific information is provided in this document for the minimum numbers of lots required for each study where more than one lot are required. Where more than one lot are required, each lot shall comprise different production (or manufacturing, purification, etc.) runs of critical reagents, representative of routine manufacture. 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 considers the variability in performance likely to arise from the interlot diversity of critical components and their formulation or from changes that could occur during the assigned shelf life of the IVD. Differences found between lots during the analytical and clinical performance studies shall be reported. Where the manufacturer supplies the instrumentation required to conduct the assay, (e.g. a closed system), safety and performance data shall be provided in the dossier with this instrumentation. The true HIV nucleic acid status and/or, when applicable, HIV-1 or HIV-2 status, shall be determined using a suitable reference method. For WHO purposes this should be a method that currently is at a developed stage of technical capability based on the relevant consolidated findings of science, technology and experience (commonly referred to as state of the art). Justification for the choice of method, shall be provided such as a laboratory-based assay that has undergone

---

6 Medical technologists, medical laboratory technicians or similar, who have received a formal professional or paraprofessional certification or tertiary education degree.
comprehensive pre-market assessment and has been stringently regulated and approved by one of the Regulatory Authorities of the Founding Members of GHTF.

Estimation (and reporting) of IVD performance shall include the rate of invalid test results and the 95% confidence interval around the estimated values for key performance metrics, as appropriate. The cause of the invalid results should be reported if available (such as sample issues (e.g. age of specimen, storage conditions, inadequate specimen volume, instrument error, operator error). Data should be presented in clear and understandable format.

It is unlikely that clinical specimens will be available in the volumes required for all analytical studies. Therefore, it is acceptable to use contrived specimens, for example, cell culture-derived virus that is characterized and sequenced, or a clinical specimen spiked into the appropriate matrix, i.e. a matrix that has been claimed in the intended use of the IVD (e.g. human plasma, whole blood), and which has been prepared in a validated and standardized manner for such studies. In addition, dilutions of a high-concentration clinical specimen may be used, if they are in an appropriate matrix (e.g. plasma, whole blood) for certain studies, e.g. limit of detection (LOD) studies. The material chosen should use the entire assay system from specimen preparation to interpretation.

For certain analytical studies it may be acceptable to generate evidence of performance using a single HIV-1 group M subtype (e.g. subtype B) and a single HIV-2 group (e.g. HIV-2 group A) as a surrogate for performance of other subtypes claimed in the intended use of the device. However, since HIV-1 group O is quite divergent from group M, a single subtype of HIV-1 group O shall also be tested. A justification for this approach, including a relevant demonstration of the relationship between subtypes, shall be provided.

For part 1 it may be also possible to carefully design a study which will generate useful data for more than one of the required studies, provided the specific criteria for each requirement are met by the study (e.g. number of replicates, concentration of analyte, sample types, etc.). For example, precision testing and whole system failure testing may be combined in a single study. Studies which may fall in this category are indicated in the appropriate sections of part 1.

The performance evaluation of the IVD shall be determined for all specimen types (e.g. blood, plasma, DBS) claimed in the intended use of the device. However, if the validation of specimens (section 1.2) shows equivalency between specimen types, some studies, as indicated in this document, may require validation in one specimen type only. However, if no equivalence between specimen types is shown, validation shall be conducted in all specimen types where indicated in the TSS.

Clinical studies shall be based on testing human specimens only sourced from population cohorts reflective of the intended use.

The use of well-characterised repository specimens and panels may be acceptable if they are relevant to the IVD under assessment, taking into consideration:

- storage conditions (including age of the specimen);
- the stability of the nucleic acid target.
### PART 1: ANALYTICAL PERFORMANCE AND OTHER EVIDENCE

<table>
<thead>
<tr>
<th>Section</th>
<th>Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Stability of sample(s) Specimen collection, processing and stability</td>
</tr>
<tr>
<td>1.2</td>
<td>Validation of specimens Demonstration of validity of all specimen types</td>
</tr>
<tr>
<td>1.3</td>
<td>Metrological traceability of calibrator and control material values Metrological traceability of calibrator and control material values</td>
</tr>
<tr>
<td>1.4</td>
<td>Accuracy of measurement Precision (repeatability &amp; reproducibility)</td>
</tr>
<tr>
<td>1.5</td>
<td>Analytical sensitivity Limit of detection Subtype sensitivity</td>
</tr>
<tr>
<td>1.6</td>
<td>Analytical specificity Potentially interfering substances Endogenous Exogenous Cross-reactivity</td>
</tr>
<tr>
<td>1.7</td>
<td>Validation of assay cut-off Validation of assay cut-off</td>
</tr>
<tr>
<td>1.8</td>
<td>Validation of the assay procedure Validation of the assay procedure Whole system failure</td>
</tr>
<tr>
<td>1.9</td>
<td>Usability/human factors Flex studies/robustness Carry-over contamination Software validation</td>
</tr>
<tr>
<td>1.10</td>
<td>Stability of the IVD Claimed shelf-life (including transport stability) In-use stability (open pack or open vial stability)</td>
</tr>
<tr>
<td>1.11</td>
<td>Performance panels Subtype panels Seroconversion panels</td>
</tr>
</tbody>
</table>

### PART 2: CLINICAL EVIDENCE (CLINICAL PERFORMANCE CHARACTERISTICS)

<table>
<thead>
<tr>
<th>Section</th>
<th>Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Diagnostic sensitivity and specificity General requirements for diagnostic sensitivity and specificity studies Diagnostic sensitivity Diagnostic specificity</td>
</tr>
</tbody>
</table>

### PART 3: QUALIFICATION OF USABILITY FOR POINT OF CARE TESTING

<table>
<thead>
<tr>
<th>Section</th>
<th>Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Qualification of usability for point of care testing Label comprehension (including IFU) Results interpretation</td>
</tr>
</tbody>
</table>
### Part 1: Analytical performance and other evidence

<table>
<thead>
<tr>
<th>Aspect</th>
<th>Testing requirements</th>
<th>Notes on testing requirements</th>
<th>Source Documents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1 Stability of sample(s)</td>
<td>Real time studies shall be conducted for each claimed specimen type taking into account: 1. storage conditions (duration at different temperatures, temperature limits, humidity, freeze/thaw cycles) 2. transport conditions 3. intended use (see note 1) 4. specimen collection and/or transfer devices intended to be used with the IVD 5. using 10 weak positive specimens approx. 3x 95% limit of detection (LOD) at each test point (see note 2) 6. The specimens shall include HIV-1 group M, HIV-1 group O and HIV-2 isolates (see note 3)</td>
<td>1. Evidence shall be provided which validates the maximum allowable time between specimen collection and its processing or addition to the IVD in the setting where testing takes place 2. The 95% LOD is defined as the minimum number of target sequences in a sample volume that can be detected in 95% of tests. (section 1.5.1) 3. Contrived specimens may be used 4. In case the use of archived specimens is considered for Part 2 of this document, evidence of stability in the conditions in which the specimens have been stored shall be demonstrated 5. Acceptance criteria will confirm that claimed specimen types transported, processed and stored under recommended conditions will give expected results. Separated EDTA plasma and centrifuged whole blood in a plasma preparation tube are considered different specimen types in this context. 6. If dried blood spots (DBS) are a claimed specimen type, the details of the filter paper (brand, product code) shall be specified and the use and stability validated 7. Unless all specimens are expected to be processed as fresh samples within a specified time frame, the IVD performance shall be established for each storage condition at the beginning and end of the stated period</td>
<td>TGS 3 (1)</td>
</tr>
<tr>
<td>1.2 Validation of specimens</td>
<td>1. The relationship between IVD performance in claimed specimen types shall be established</td>
<td>6. If multiple specimen types are claimed, (e.g. serum, plasma, venous whole blood, capillary whole blood; DBS), then equivalence shall be demonstrated using paired specimens; in</td>
<td>IMDRF IVD MA TOC (2)</td>
</tr>
<tr>
<td>Aspect</td>
<td>Testing requirements</td>
<td>Notes on testing requirements</td>
<td>Source Documents</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------</td>
<td></td>
</tr>
</tbody>
</table>
| Demonstration of validity of all specimen types | 2. At least 25 positive and 25 negative specimens shall be tested for each claimed specimen type (see notes 1-3)  
3. For assays that detect more than one genotype but do not differentiate the genotype, the justification as to whether to study each genotype shall be risk- and evidence-based  
4. One replicate of each specimen of each specimen type shall be tested  
5. One lot shall be used for testing | other words, demonstrated in each specimen type for all specimens.  
7. Similarly, if equivalence is claimed in specimens collected into multiple anticoagulants, each specimen shall be collected into each of the claimed anticoagulants  
8. Specimens should be chosen that have low to moderate concentrations of the analyte (including levels of analyte at relevant medical decision points)  
9. It is known that IVDs may show different sensitivities between specimen types.  
10. Analytical sensitivity studies (section 1.5) may also contribute to evidence regarding equivalence of specimen types (see chapter D.3).  
11. The established relationship between IVD performance in claimed specimen types (plasma, blood, DBS) shall be considered in the design of subsequent studies. For example, if the studies show that one or more of the claimed specimen types are equivalent, then not all specimen type need to be tested in some of the subsequent studies (where indicated). | WHO (3)  
CLSI MM06-A2 (4)  
European Pharmacopoeia (5) |

**1.3 Metrological traceability of calibrators and control material values**

| 1.3.1 Metrological traceability of calibrators and control material values | 1. As applicable, the traceability of an assay-specific quality control specimen to a validated reference material shall be demonstrated (e.g. WHO International Standards (IS) for HIV-1 and HIV-2) or a secondary standard calibrated from it.  
2. Material with well characterized copy number should only be used in cases where material with an assigned value in International Units (IU) is not available. However, this copy number shall have been determined with a method that was originally validated using appropriate reference materials with assigned values in IU. | 1. The IVD should provide accurate external positive and negative controls that are processed as specimens through all steps of testing. External controls are provided either by the manufacturer or are obtained from an independent source. Such controls are processed as normal test samples. Appropriate to the IFU, the positive control contains a defined amount of target (approx. 3 x LOD) in a suitable matrix. The target should cover HIV-1 group M, HIV-1 group O and HIV-2. A negative control is a sample of a suitable matrix, for example plasma, shown to be negative for the target. | WHO (3)  
CLSI MM06-A2 (4)  
European Pharmacopoeia (5) |
<table>
<thead>
<tr>
<th>Aspects</th>
<th>Testing requirements</th>
<th>Notes on testing requirements</th>
<th>Source Documents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.4 Accuracy of measurement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.4.1 Precision (Repeatability &amp; reproducibility)</td>
<td>1. Both repeatability (see note 1) and reproducibility (see note 2) shall be estimated using panels with defined analyte levels. 2. The members of the repeatability and reproducibility panel shall include (see note 3): • 1 x negative specimen • 2 x low positive specimens with a concentration of analyte (approx. 3 x LOD) • 2 x moderately positive specimens with a concentration of analyte (approx. 5-7 x LOD) 3. Each panel member shall be tested: • in 5 replicates • using 3 different lots (see notes 5 &amp; 10) • over 5 days (not necessarily consecutive) with 1 run in that day (alternating morning/afternoon) • at each of 3 different testing sites (see note 10) • using 1 operator/site (see note 6) • by personnel representative of intended users • unassisted • using only those materials provided with the IVD (e.g. IFU, labels and other instructional material)</td>
<td>1. Within run 2. Between -run, -lot, -day, -site, operator • Note: A run will be defined depending on the IVD’s throughput: if the platform can accommodate all specimens in a single run, i.e. in the same test plate, the specimens will be run together. If the assay can only accommodate a smaller set or a single specimen(s), a run will be defined as a testing session carried out on the same instrument/module on the same day. 3. Precision should be determined for a single subtype of HIV-1 group M 4. The testing panel should be the same for all operators, lots and sites. 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 5. Each lot shall comprise different production (or manufacturing, purification, etc.) runs of critical reagents, representative of routine manufacture. 6. If operators are considered a significant source of test result variation (for example, with tests that have a significant proportion of manual manipulations), then at least 2 operators/site shall be used 7. The number of invalid tests shall be reported.</td>
<td>TGS 3 (1) CLSI EP05-A3 (6) CLSI EP12-A2 (7)</td>
</tr>
</tbody>
</table>

3. If a reference material is not available (e.g. for HIV-1 groups O and N), the calibrators and control materials shall be calibrated using commercial NAT quantitative assays that have been authorized for use by a regulatory authority of the founding members of GHTF.
### Analytical Performance

**Technical Specifications for submission to WHO prequalification-Diagnostic Assessment:**

**IVDs used for the detection of HIV 1 & HIV 2 NA**

<table>
<thead>
<tr>
<th>Aspect</th>
<th>Testing requirements</th>
<th>Notes on testing requirements</th>
<th>Source Documents</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.</td>
<td>Where relevant, multiple instruments may be used for the testing</td>
<td>8. Results shall be statistically analyzed by ANOVA or other method, to identify and isolate the sources and extent of any variance.</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>All claimed specimen type shall be tested</td>
<td>9. In addition, the percentage of correctly-identified, incorrectly-identified and invalid results shall be tabulated for each specimen and be separately stratified according to each of site, lot, etc.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10. To understand irregularities in results obtained, at least 2 lots should be tested at each of the 3 testing sites.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>11. Alternative methods used to establish repeatability and reproducibility performance of the assay shall be discussed with WHO in advance of dossier submission.</td>
<td></td>
</tr>
</tbody>
</table>

#### 1.5 Analytical sensitivity

**1.5.1 Limit of detection**

1. Analytical sensitivity as expressed by 95% LOD, shall be determined as follows:
   - testing 20-24 replicates of at least 8 serial 0.5log₁₀ dilutions of suitable biological reference materials (see note 1 & 2) e.g. WHO IS for HIV-1 group M and HIV-2) or a secondary standard calibrated against the appropriate IS (see note 1). The serial dilutions shall be chosen so that the viral loads span the LOD of the IVD
   - The replicate testing shall be conducted on three different days (see note 2)
   - using 2 lots
   - at least 2 dilution series shall be tested
2. The LOD shall be determined for each HIV-1 and HIV-2 group claimed in the intended use (see notes 1 & 2)
3. The LOD shall be determined for each specimen type claimed in the intended use (e.g. plasma, whole blood, DBS)

1. The WHO IS (available for HIV-1 group M and HIV-2 group A) or a secondary standard calibrated against the appropriate IS shall be used.

2. For HIV-1 group O, the reference material shall be, quantitated with a suitable HIV-1 quantitative assay, authorized for use by a regulatory authority of the founding members of GHTF. In this case the unitage may vary, for example, results may be reported in copies/mL or genome equivalents/mL
3. For low through-put instruments, the number of testing days may be increased
4. Analytical sensitivity shall be estimated by determining the 95% LOD with 95% confidence intervals (CI) (e.g. by probit analysis)
5. Where an IS is available for a particular analyte, the sensitivity of the IVD for that analyte shall be expressed in both IU and their copies/mL equivalent
6. Each lot shall comprise different production (or manufacturing, purification, etc.) runs of critical reagents

**European Commission decision on CTS**

(8)

**CLSI EP17-A2**

(9)
<table>
<thead>
<tr>
<th>Aspect</th>
<th>Testing requirements</th>
<th>Notes on testing requirements</th>
<th>Source Documents</th>
</tr>
</thead>
</table>
| **1.5.2 Subtype sensitivity** | 1. Subtype sensitivity shall be demonstrated by testing:  
• at least 14 specimens of the predominant subtypes (see note 1)  
• at least 5 specimens of all the other claimed subtypes (if available) (see note 1)  
• 3 concentrations (0.5 x LOD, 1 x LOD and 3 x LOD) per specimen (see notes 2 & 3)  
• 10 replicates/concentration  
• using 1 lot  
• one claimed specimen type | 1. All subtypes and recombinant forms (CRFs) circulating in populations for which claims are made and all other subtypes claimed in the intended use shall be tested  
2. Contrived specimens may be used  
3. For subtypes for which an approved quantitative assay is not available (e.g. HIV-1 group N and HIV-2), a log dilution series of each specimen shall be tested  
4. Statistical analysis shall be used to estimate the %Hit Rate for each subtype | TGS 3 (1) PQDx_018 (10) |
| **1.5.3 Sensitivity for differentiation of dual HIV-1/HIV-2 infection** | For devices that differentiate between HIV-1 and HIV-2, the ability of the device to accurately detect dual HIV-1 and HIV-2 infection without a significant loss of sensitivity for either HIV type shall be demonstrated by testing:  
1. At least 3 specimens with both HIV-1 and HIV-2 at the following dilutions:  
   • approx. 3 x LOD for HIV-1 and approx. 10^5 IU/mL HIV-2  
   • approx. 3 x LOD HIV-2 and approx. 10^6 IU/ml HIV-1  
2. using 1 lot  
3. one claimed specimen type | 1. Contrived specimens may be used  
2. Although HIV-2 viral loads are typically lower than HIV-1 viral loads in individuals infected with both HIV-1 and HIV-2, it is still important to evaluate both test cases (a low HIV-1 level with a higher HIV-2 level and vice versa), as both can occur | |
| **1.6 Analytical specificity** | 1. The potential for false results (false negatives and false positives) arising from interference by the substances/conditions listed below shall be determined by testing confirmed HIV-negative samples, both spiked (spiking one subtype each of HIV-1 group M, HIV-1 group O and HIV-2) and unspiked: | 1. The risk assessment conducted for an IVD shall identify substances at medically relevant levels for which the potential for interference can reasonably be expected for the analyte being detected (HIV-1 group M, HIV-1 group O and HIV-2)  
2. Endogenous substances shall be spiked at abnormally high levels compared with healthy individuals (e.g. triglycerides up to 3186 | European Commission decision on CTS (8) CLSI EP07 (11) |
### Aspect | Testing requirements | Notes on testing requirements | Source Documents
---|---|---|---
**Endogenous** | • testing a minimum of 5–10 specimens per substance/condition  
• spiking with HIV at approx. 3 x LOD  
• using only one claimed specimen type | mg/dL, haemoglobin up to 472 mg/dL, unconjugated bilirubin up to 62 mg/dL, albumin up to 9.6 g/dL or human DNA up to 0.4 mg/dL)  
3. Any observed interference shall be further investigated and performance limitations of the IVD reported in the IFU.  
4. 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.  
5. Prior to spiking, samples should be confirmed to be HIV-negative prior to testing with suitable HIV-1 and HIV-2 qualitative and/or quantitative assays | CLSI EP37 (12)  
FDA (13)

### Exogenous

1. **Endogenous** | The interference of endogenous substances in human plasma on the performance of the device shall be investigated, such as  
1. triglycerides, haemoglobin, unconjugated bilirubin, albumin  
2. human DNA (see note 2)  
3. autoimmune diseases/markers:  
   • Anti-Nuclear Antibodies,  
   • Rheumatoid factor  
   • Systemic lupus erythematosus |  
<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
</table>
| 1.6.1.2 | Exogenous substances shall be tested, such as  
1. Therapeutic drugs commonly used by potential end users:  
   • Acetaminophen, acetylsalicylic acid, atorvastatin, fluoxetine, loratadine, nadolol, ascorbic acid, and phenylephrine.  
2. Medicines used in the treatment of malaria (e.g. quinine, primaquine), TB (e.g. rifampicin), HIV (e.g. from each drug class), hepatitis B and C (e.g. adefovir dipivoxil, Peginterferon alfa-2a, direct acting antivirals), herpes simplex virus (e.g. aciclovir)  
3. Medicines used to treat infections common in the region where the IVD will be used  
4. Medicines or biologicals that increase circulating nucleic acid  
5. Presence of nucleic acid-based medicines and metabolites and binding substances |  
|  |  |  |  
|---|---|---|---|
| Aspect                          | Testing requirements                                                                                                                                                                                                 | Notes on testing requirements                                                                                                                                                                                                 | Source Documents |
|--------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|
| 1.6.2 Cross-reactivity         | Determination of the potential for false positive results arising from cross-reactivity with other organisms unrelated to HIV or disease states commonly found in regions of the intended use of the IVD, including, where possible, at least 3-5 each of:  
   1. Potential cross-reacting organisms found in the blood (including viruses, bacteria, parasites & fungi) (see notes 1 & 2)  
      • Plasmodium spp.  
      • Leishmania  
      • Trypanosoma cruzi  
      • Trypanosoma brucei  
      • Chikungunya virus  
      • Hepatitis A, B, C  
      • Cytomegalovirus  
      • Epstein-Barr virus  
      • Herpes simplex 1 & 2  
      • HTLV I & II  
      • Mycobacterium tuberculosis  
      • Staphylococcus aureus  
      • Propionibacterium acnes  
      • Staphylococcus epidermidis  
      • Staphylococcus haemolyticus  
      • Yeast infections  
      • Candida albicans  
      • Pneumocystis  
   2. Using one claimed specimen type | 1. Where clinical specimens from individuals with the disease state to be tested are unavailable, a negative specimen shall be spiked with the organism of interest to a high concentration (approximately $10^4$ to $10^5$ IU or copies/mL if possible)  
   2. Where either the scientific literature and/or risk analysis identifies the potential for false results in co-infected individuals (e.g. decreased sensitivity or specificity), further investigation shall be undertaken using both HIV-negative and HIV-positive specimens.  
   3. Any observed cross-reactivity shall be further investigated and performance limitations of the IVD reported in the IFU.  
   4. Samples should be confirmed to be HIV-negative prior to testing with suitable HIV-1 and HIV-2 qualitative and/or quantitative assays |                  |
<table>
<thead>
<tr>
<th>Aspect</th>
<th>Testing requirements</th>
<th>Notes on testing requirements</th>
<th>Source Documents</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1.7 Validation of assay cut-off</strong></td>
<td>The assay cut-off shall be determined by testing: 1. 100 HIV-1 and 20 HIV-2 positive clinical specimens with low, medium and high viral loads (see notes 1-4) 2. 1000 HIV negative specimens (see notes 1-3) 3. The sensitivity of the assay at the cut-off shall be determined using samples which have been calibrated against reference materials traceable to the WHO IS (see note 1) 4. One claimed specimen types should be tested 5. The manufacturer shall justify the positioning of the cut-off and describe the algorithm/method used to set the cut-off for the test, or in cases where the cut-off is set for each run or set of tests, the manufacturer shall describe the algorithm/method specified in the IFU or used by the instrument to set the cut off</td>
<td>1. Test samples shall be quantitated with at least one suitable HIV quantitative assay, authorized for use by a regulatory authority of the founding members of GHTF 2. The test panel shall include 10 positive and 10 negative samples close to the cut-off 3. Clinical specimens shall be collected from at least 2 different regions to account for different genotypes/subtypes 4. Contrived specimens may be used if clinical specimens are not available</td>
<td></td>
</tr>
<tr>
<td><strong>1.8 Validation of the assay procedure</strong></td>
<td>For each claimed analyte, evidence supporting the choice of critical reagents (primers and probes sequences) shall be provided</td>
<td>1. Rationale for selection of primers and probes including specific sequences used, 2. Justification for alignments made to generate consensus sequences or best-fit modifications made to existent sequences e.g. to permit maximum homology to several strains, and 3. Information on size, GC content, melting temperatures, hairpin or other secondary structures if any, and the nucleotide position on the genome map of the primers and probes 4. For assays designed to detect or quantitate multiple HIV subtypes or variants, data should be provided to demonstrate that the primers and or probes chosen are effective for all of the subtypes or variants identified in the label</td>
<td>IMDRF IVD MA ToC (2) FDA (13)</td>
</tr>
<tr>
<td>Aspect</td>
<td>Testing requirements</td>
<td>Notes on testing requirements</td>
<td>Source Documents</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>1.8.2 Whole system failure</td>
<td>1. Testing shall be done on a panel of (see note 1):  • 10 specimens containing HIV-1 (approx. 3 x LOD)  • 10 specimens containing HIV-2 (approx. 3 x LOD)  2. The panel shall be tested  • on 5 consecutive days (to give a total of 100 test results)  • using 1 lot  • with 1 user  3. The whole system failure shall be determined for the most viscous specimen type claimed (e.g. whole blood)</td>
<td>1. This may be conducted as part of precision studies if the minimum number of replicates are met (see chapter D.3).</td>
<td></td>
</tr>
<tr>
<td>1.9 Usability/human factors studies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.9.1 Flex studies/robustness</td>
<td>1. Evidence is required to demonstrate that the conditions recommended in the IFU are validated and how they were verified.  2. The influence of the following factors on expected results (both reactive and non-reactive) shall be considered as applicable (see note 1):  • Specimen and/or reagent volume (see note 3)  • IVD instrument sturdiness (including the effect of non-level work surface)  • Lighting, humidity and barometric pressure (simulating high altitude),  • Handling contamination (e.g. from latex, powder, hand lotion, sweat, and/or soap, etc. as appropriate)  • Operating temperature  3. Instrumentation (both extraction and amplification) including:  • Ruggedness (including the effect of vibration from other instruments)</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  2. The risk assessment conducted for an IVD shall identify factors which have potential to affect the performance of the assay  3. Studies investigating the impact of specimen volume/specimen adequacy shall be conducted in all specimen types  4. Additional factors may be relevant for point-of-care devices or devices requiring significant manual interventions (e.g. manual extraction). These factors may include errors during sample collection, sample handling and loading, and handling of relevant test components after sample application  5. The factors 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 can be understood. For example, in addition to investigating deviations of temperature within those claimed in the IFU (in the middle and at both lower and upper extremes of a claimed temperature)</td>
<td>PQDx_018 (10)  IEC 62366-1:2015 (14)</td>
</tr>
</tbody>
</table>
### Analytical Performance

<table>
<thead>
<tr>
<th>Aspect</th>
<th>Testing requirements</th>
<th>Notes on testing requirements</th>
<th>Source Documents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.9.2 Carry-over contamination</td>
<td>1. The potential for carry-over contamination or similar shall be investigated by testing a panel of 40 alternating high-positive (≥10^5 IU/mL or copies/mL) and negative specimens (see note 1):</td>
<td>1. Contrived samples prepared by spiking the test matrix with a high-titre cell culture virus may be used for these studies</td>
<td>European Commission decision on CTS (8) Haeckel R (15)</td>
</tr>
<tr>
<td></td>
<td>• at least 5 different runs</td>
<td>2. The specimen type with the highest viscosity (e.g. whole blood versus plasma) shall be chosen as the test specimen type.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• on 3 different days</td>
<td>3. If DBS are a claimed specimen type and require manual punching/excision of the DBS prior to extraction, those manual handling steps shall also be evaluated in the carry-over contamination studies</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• at least 2 users</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• using one lot</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2. For testing platforms that can only accommodate a single specimen, testing shall be conducted on a single instrument:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• at least 4 tests per run</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• using alternating high-positive (≥10^5 IU/mL or copies/mL) and negative specimens</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• a total of 10 runs</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• at least 2 users</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• using one lot</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3. Only one specimen type should be used (see notes 2 &amp; 3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.9.3 Software validation</td>
<td>Software validation (including verification of built-in fail-safe and alert mechanisms)</td>
<td>1. If software is utilized for amplification, detection, and calculation of quantitative or qualitative results, validation of such software for the intended function should be provided.</td>
<td>FDA (13)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

**Technical Specifications for submission to WHO prequalification-Diagnostic Assessment:**

IVDs used for the detection of HIV 1 & HIV 2 NA

---

**Notes on testing requirements**

- Impact of dust and mold on componentry (e.g. optics)
- Impact of power/voltage fluctuation
- One negative and 2 low positive (3 x LOD) specimens
- Using one specimen type (see notes 2 & 3)
- Testing to be performed in one lot

---

**Source Documents**

- European Commission decision on CTS (8)
- Haeckel R (15)

---

**Testing requirements**

- Range, temperature ranges should be investigated that exceed those of claimed operating conditions and which cause test failure (incorrect/invalid results)
- For a closed system, evidence should be provided that the parameters of the assay have been optimized
- Devices should consider including easily understandable quick reference guides and/or IFUs with visuals and terminology that can be understood by the intended users (see part 3)
<table>
<thead>
<tr>
<th>Aspect</th>
<th>Testing requirements</th>
<th>Notes on testing requirements</th>
<th>Source Documents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.10 Stability of the IVD</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| 1.10.1 Shelf-life (including transport stability) | 1. Stability studies shall be evaluated for the shelf life of the test kit. The following conditions shall be investigated:  
   - Conditions to mimic extremes of conditions (temperature, humidity, pressure) exposed to during transport  
   - Storage temperature and humidity range  
   - Operating temperature and humidity range | 1. Each lot shall comprise different production (or manufacturing, purification, etc.) runs of critical reagents, representative of routine manufacture.  
2. The number of invalid tests with each kit lot shall be reported.  
3. 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 majority 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 can be 12 months.  
4. Determination of shipping stability shall be performed using simulated extreme stress conditions, ensuring that application of those conditions is consistent and controlled.  
5. Accelerated studies do not replace the need for real time studies.  
6. In-use stability of labile components shall be conducted using components in their final configuration. | ISO 23640:2011 (16)  
CLSI EP25-A (17)  
TGS 2 (18)  
ASTM D4169-14 (19) |
| | 2. At least 3 lots shall be tested (see notes 1 & 2) | | |
| | 3. The stability panel will consist of the following contrived specimens:  
   - 1 HIV-1 group M specimen (approx. 3 x LOD)  
   - 1 HIV group O specimen (approx. 3 x LOD)  
   - 1 HIV-2 specimen (approx. 3 x LOD)  
   - 3 negative specimens | | |
| | 4. Each panel member shall be tested in triplicate at each time point/condition | | |
| | 5. Each claimed specimen type shall be tested | | |
| | 6. Multiple Instruments may be used to allow simultaneous testing at each time point. | | |

| 1.10.2 In-use stability (open pack or open vial stability) | 1. Minimum of 1 lot shall be tested using a panel composed of:  
   - One negative specimen  
   - One specimen spiked with HIV-1 group M (approx. 3 x LOD)  
   - One specimen spiked with HIV-1 group O (approx. 3 x LOD)  
   - One specimen spiked with HIV-2 (approx. 3 x LOD) | | |
### Aspect Testing requirements

<table>
<thead>
<tr>
<th>Testing requirements</th>
<th>Notes on testing requirements</th>
<th>Source Documents</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Each panel member shall be tested in triplicate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Only one claimed specimen type should be tested</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. All labile components (e.g. buffers vials, sealed cartridges, etc.) shall be evaluated (see note 6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. On-board stability shall be tested for an IVD used with an instrument</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### 1.11 Performance panels

1. **Subtype panels**

   The ability of the device to detect all claimed HIV-1 and HIV-2 subtypes shall be demonstrated by testing well-characterized subtype panels available from commercial companies and Regulatory Authorities (e.g. NIBSC and FDA HIV-1 panels).

   1. Suitable performance panels should consist of members which have been well characterized and quantitated by other recognized HIV molecular assays (see chapter D.3).
   2. The panel may comprise a combination of both clinical and cell culture-derived specimens diluted in plasma.

<table>
<thead>
<tr>
<th>Notes on testing requirements</th>
<th>Source Documents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Suitable performance panels should consist of members which have been well characterized and quantitated by other recognized HIV molecular assays (see chapter D.3).</td>
<td>TGS 3 (1)</td>
</tr>
<tr>
<td>2. The panel may comprise a combination of both clinical and cell culture-derived specimens diluted in plasma.</td>
<td></td>
</tr>
</tbody>
</table>
### Part 2: Establishing clinical evidence (clinical performance characteristics)

<table>
<thead>
<tr>
<th>Aspect</th>
<th>Testing requirements</th>
<th>Notes on testing requirements</th>
<th>Source Documents</th>
</tr>
</thead>
</table>
| 2.1 Diagnostic sensitivity and specificity | Testing shall be done:  
1. On specimens from all sections of the population for which claims are made, for example, adults and pediatric individuals  
2. Using specimens from at least 2 different, geographically diverse regions  
3. By a variety of intended users representing relevant intended use settings (e.g. primary, provincial) and at different test settings  
4. Using at least 2 lots (see note 2)  
5. Using specimens from individuals clinically and laboratory confirmed without HIV-1 and/or HIV-2 and individuals clinically and laboratory confirmed with HIV-1 and/or HIV-2  
6. The specimens shall include all the common genotypes/subtypes found in the region as well as any other genotypes/subtypes claimed  
7. All claimed specimen types (see note 6)  
8. The specimens shall be tested with a NAT test (reference method), and/or in cases where appropriate, with serology (see note 7)  
9. Specimens with discrepant results shall be further evaluated. Where possible, follow-up testing shall be done (see note 9)  
10. The procedure for selection of study specimens, how these represent an intended use population and how bias has been addressed shall be clearly described. | 1. Problematic specimens, those with unexpected results but which otherwise meet selection criteria for a study, shall not be systematically excluded from analysis.  
2. Each lot shall comprise different production (or manufacturing, purification, etc.) runs of critical reagents, representative of routine manufacture.  
3. All results that are indeterminate by the IVD shall be included in the denominator data for analysis.  
4. All invalid results shall be recorded and evaluated in comparison to the reference result. Invalid results should be reported as individual categories (e.g. internal control failure, extraction failure, etc.) and not aggregated. Invalid results should be analyzed separately in the final performance calculations.  
5. Estimates of diagnostic sensitivity and specificity shall be reported with 95% confidence intervals.  
6. Clinical sensitivity and specificity shall be calculated for each specimen type and not for the aggregated data  
7. The reference method shall be an assay as described in chapter D.3 of this document. In some cases, comparing to a serology reference standard may also be appropriate, but shall be accompanied by an appropriate rationale.  
8. Clinical sensitivity and specificity shall be calculated for each specimen type and not for the aggregated data  
9. Problematic specimens, and those specimens with initial discrepant results shall not be excluded from the final analysis | European Commission decision on CTS (8)
<table>
<thead>
<tr>
<th>Aspect</th>
<th>Testing requirements</th>
<th>Notes on testing requirements</th>
<th>Source Documents</th>
</tr>
</thead>
</table>
| 2.1.2. Diagnostic sensitivity | **Diagnostic specimens shall be:**  
  1. Specimens with a range of viral loads  
  2. In total 400 HIV-1 and 100 HIV-2 well-characterized, clinically and laboratory confirmed specimens shall be tested (see note 10).  
  3. The HIV-1 specimens shall be from 300 adult individuals and 100 pediatric individuals.  
  4. The HIV-2 specimens shall include at least 10 pediatric individuals.  
  5. At least 25 of the 400 HIV-1 positive specimens shall be from patients on anti-retroviral therapy or HIV pre-exposure prophylaxis (PrEP). | **10.** Up to 25% of the test specimens may be well-characterised archived specimens, assuming that freezing specimens has been validated during analytical studies (section 1.1.1). The status of the archived specimens should be reconfirmed before testing using a test authorised for use by a regulatory authority of the founding members of the GHTF. |                  |
| 2.1.3 Diagnostic specificity | **Diagnostic specimens shall be:**  
  1. At least 500 well-characterized, clinically and laboratory confirmed non-HIV specimens (HIV antibody negative and NAT non-reactive) (see note 10)  
  2. Negative specimens should include at least 25 pediatric individuals. |                                                                                                                                                                                                                                           |                  |
### Part 3: Qualification of usability for POC testing

<table>
<thead>
<tr>
<th>Aspect</th>
<th>Testing requirements</th>
<th>Notes on testing requirements</th>
<th>Source Documents</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>3.1 Qualification of usability for POC testing</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| **3.1.1 Label comprehension (including IFU)** | 1. Testing of subjects to assess ability of intended users to correctly comprehend key messages from packaging and labelling that relate to POC testing:  
   - Understanding key warnings, limitations and/or restrictions, including correct use of self-collection methods and equipment  
   - Proper test procedure  
   - Test result interpretation  
2. Studies shall include at least 15 intended users, users including those whose native language may not be the language of the IFU if necessary, to demonstrate comprehension of key messages in each population described above. | IFU and labelling should be clear and easy to understand. Use of pictorial instructional material is encouraged. | IEC 62366-1:2015 (14)  
European Parliament IVD regulations (20)  
Backinger CL and Kingsley PA (21) |
| **3.1.2 Results interpretation** | 1. For POC tests, intended users to interpret the results of contrived IVDs (e.g. static/pre-made tests, or similar) to assess their ability to correctly interpret pre-determined test results and error messages. Contrived tests should be made to demonstrate the following potential test result interpretations, as appropriate.  
2. Testing subjects to consist of at least 15 intended users including those whose native language may not be the language of the IFU if necessary from at least two geographically diverse populations to demonstrate correct interpretation of simulated test results. | Intended for IVDs which include quantitative outputs (e.g. cycle times, viral load) that must be interpreted by a user in a POC setting.  
2. Study group may include subjects recruited as part of the label comprehension study. | |
References


5. European Pharmacopoeia vol. 20, no. 3 20621 2008. 2.6.21 Nucleic Acid Amplification Techniques.


13. Guidance for Industry In the Manufacture and Clinical Evaluation of In Vitro Tests to Detect Nucleic Acid Sequences of Human Immunodeficiency Viruses Types 1 and 2; FDA, 1999; https://www.fda.gov/media/72268/download


