WHO PROTOCOL FOR PERFORMANCE LABORATORY EVALUATION OF HIV SEROLOGY ASSAYS
3. Introduction

3.1. Prequalification of in vitro diagnostics

The World Health Organization (WHO) Prequalification of In Vitro Diagnostics is coordinated through the Prequalification-Diagnostics Assessment Team (PQDx). The aim of the WHO Prequalification of In Vitro Diagnostics Assessment is to promote and facilitate access to safe, appropriate and affordable diagnostics of good quality in an equitable manner. Focus is placed on products for high burden diseases and their suitability for use in resource-limited settings.

The WHO prequalification of in vitro diagnostics process includes three main components:

- Review of an pre-submission form and product dossier;
- Performance evaluation of the product;
- Inspection of the manufacturing site(s).

Performance evaluation will be conducted by two different mechanisms as described at: http://www.who.int/diagnostics_laboratory/evaluations/alternative/en/. Performance evaluation in List 1 laboratories will be coordinated and paid for by WHO. Performance evaluation in List 2 laboratories will be coordinated and paid for by the manufacturer.

This protocol provides the details of the procedure for evaluation of HIV serology simple and rapid assays and Enzyme Immunoassays submitted for laboratory evaluation as part of the WHO Prequalification of In-vitro Diagnostic assays on behalf of World Health Organization at the WHO Prequalification Evaluating Laboratory.

This protocol shall not replace more extensive IVD manufacturer’s protocols required for validation and verification studies of their products.

3.2. WHO Performance evaluation of diagnostics for HIV antibody and/or antigen detection

The performance evaluation determines the accuracy of HIV assays in comparison with established performance criteria. These characteristics include: sensitivity, specificity, negative and positive predictive values as well as the accuracy. In addition, a number of operational characteristics are assessed including the suitability for use in laboratories and/or testing settings with limited infrastructure. Assays for the detection of HIV antibody and assays for the detection of both HIV antibody and HIV antigen are covered in this protocol.

All HIV serology assays submitted for performance evaluation are assessed at a WHO Prequalification Evaluating Laboratory which has been assessed and listed using the WHO Alternative Performance evaluation Mechanism upon the instruction of WHO/PQDx (List 1 laboratories) or a manufacturer (List 2 Laboratories) who has submitted an IVD for WHO assessment.

The HIV specimen reference panel shall comprise a minimum of 1200 anti-HIV positive and anti-HIV negative serum/plasma specimens from Europe, Africa, Asia and Latin America plus several well-characterized commercial seroconversion panels and performance panels, a lot-to-lot variation panel, WHO international biological reference preparations and a culture supernatant panel (for products that include detection of HIV antigen).
4. Study objectives

4.1. Overall objectives

The overall objectives are:

1. To evaluate and compare the accuracy of currently available HIV assays (including EIAs, rapid diagnostic tests and other formats) for detection of HIV antibody and/or HIV antigen against established performance criteria.

4.2. Specific objectives

The specific objectives of the performance evaluation are:

1. To determine the sensitivity and specificity of currently available HIV assays (EIAs, rapid diagnostic tests and other formats) for the detection of antibodies to HIV as compared to a reference assays (two EIA’s, HIV antigen neutralization and one line immunoassay).

2. To evaluate the operational characteristics of HIV assays, e.g. ease of performance, utility of specimen type, inter-reader variability, reaction endpoint stability, rate of invalid runs/devices, suitability for use in extreme climates (high/low temperatures, high humidity), and suitability for use in countries with limited infrastructure (no/limited electricity, no/limited clean water, and inadequate means of biosafety disposal).

5. Study Design

5.1. WHO Prequalification Evaluating Laboratory

The WHO Prequalification Evaluating Laboratory shall be one that has undergone assessment using the WHO Alternative Laboratory Evaluation Mechanism which include submission of an EoI, Stage 1 audit (Assessment of EoI and specific quality management system (QMS) documentation), Stage 2 audit which include On-site audit of the laboratory to assess compliance with WHO requirements and lastly listed as WHO Prequalification Evaluating Laboratories. http://www.who.int/diagnostics_laboratory/evaluations/alternative/en/.

The laboratory shall hold the following certification for quality management within the laboratory: ISO17025 (General requirements for the competence of testing and calibration laboratories), ISO15189 (Medical laboratories: Particular requirements for quality and competence) or equivalent.

The person(s) listed in the EoI letter to WHO will act as the Principal Investigator (PI) for the work performed by the WHO Prequalification Evaluating Laboratory.

5.2. Training, performance evaluation and supervision

The following issues are key to minimizing error and maximizing the value of this performance evaluation:

- The PI will be responsible for training the laboratory technicians on the details of the evaluation protocol and on the performance of each assay undergoing evaluation;
- Only those personnel who have received specific training for this evaluation will be employed in the evaluation;
- Accurate record keeping is crucial to the success of the evaluation and the PI will be responsible for ensuring that all data required for the evaluation are recorded on the agreed data collection sheets, and are accurate and up to date;
It is important to plan work in advance and follow standard operating procedures as prepared and controlled by the WHO Prequalification Evaluating Laboratory;

- To reduce the risk of adding an incorrect specimen to a test device/well, before starting the test run, the operator will prepare worksheets and label all tubes, dilution vessels, test devices or plates with the specimen’s unique number;
- Because objective, machine-generated, permanent results for simple/rapid diagnostic tests are not feasible, it is essential that the PI emphasizes to the operator performing the tests the need for accurate recording of results and recordkeeping;
- To minimize the risk of error, it is recommended that the results are read and recorded independently by three trained staff members;
- To allow immediate correction of erroneous recording of results (rather than differences in visual interpretation), the PI or designee should assess the results as soon as possible to allow her/him to return to the original test device to investigate apparently discordant readings;
- For the performance evaluations performed at the WHO Prequalification Evaluating Laboratory, at least one representative result from both HIV positive and negative specimens will also be recorded by taking electronic images. Unexpected test results will also be digitally recorded as well as an image of the instructions for use.

5.3. Safety

HIV, hepatitis B and hepatitis C and other viruses are transmissible by blood and body fluids. Therefore, all types of specimens (including venous and capillary whole blood, serum/plasma, oral fluid, etc.) must be handled as potentially infectious. Appropriate precautions to minimize infectious hazards must be taken at all stages from the collection of specimens to the disposal of used materials from the laboratory. The WHO Guidelines on HIV Safety Precautions, and Guidelines for the Safe Transport of Specimens (WHO/EMC/97.3) and the WHO Prequalification Evaluating Laboratory guidelines on laboratory safety should be followed carefully by the laboratory staff.

5.4. Storage of assays

All reagents must be stored as indicated in the instructions for use. Some assays may not need refrigeration. If refrigerated storage space is inadequate to store the entire test kit, they may be divided so that labile reagents can be refrigerated separately from the non-labile supplies. Calibrated thermometers are placed at each location where reagents and specimens are stored, i.e. ambient, refrigerator and freezer. Temperatures are recorded daily on the WHO Prequalification Evaluating Laboratory temperature logs. The lot numbers of the test kits received/used and their expiry dates are recorded on the individual run worksheets.

Two separate production lots (with different lot numbers and different expiry dates) will be requested for evaluation, according to the following definition\(^1\) of a lot: “The amount of material that is uniform in its properties and has been produced in one process or series of processes. The material can be either starting material, intermediate material or finished product.” Furthermore, lots must be sourced from a representative production run and not produced especially for the purpose of this evaluation. WHO will verify this information before the product assessment has been finalized.

\(^1\)ISO 18113-1:2009 In vitro diagnostic medical devices -Information supplied by the manufacturer (labelling) – Part 1: Terms, definitions and general requirements
6. Specimens

6.1. HIV specimen reference panel

6.1.1. Collection of specimens for the HIV specimen reference panel

Specimens are collected as serum or plasma (with EDTA as an anti-coagulant). Panel specimens are assigned a unique identification number at the collection site and sent as large volumes to the WHO Prequalification Evaluating Laboratory where they are assigned the specimen identification number. Once the specimens have been processed and labelled they are frozen immediately at -20°C. At the WHO Prequalification Evaluating Laboratory, the specimens are aliquotted into working volumes of 250µl and the remainder into 2ml volumes and stored at -20°C until testing commences. During the period of testing the specimens are stored at 2 - 8 °C and this time period does not exceed one week. After the completion of testing, they are again stored at -20°C. Each aliquot does not undergo more than two freeze/thaw cycles.

6.1.2. Characterization of the HIV specimen reference panel

The panel consists of minimum of 1200 serum/plasma specimens of European, African, Latin American and Asian origin. There will include a minimum of 470 anti-HIV positive specimens, of which about 10-20 are anti-HIV 2 positive specimens, and 730 are anti-HIV negative specimens.

Table 1 – HIV specimen reference panel

<table>
<thead>
<tr>
<th>HIV positive specimens</th>
<th>HIV negative specimens</th>
<th>Total number</th>
</tr>
</thead>
<tbody>
<tr>
<td>470 (and 2 x HIV-1 antigen only)</td>
<td>730</td>
<td>1200</td>
</tr>
</tbody>
</table>

The HIV Specimen Evaluation Panel shall be characterized using a standardized combination of assays i.e. a testing algorithm. These results are used to determine the HIV status of each specimen for the purpose of the performance evaluation see Figure 1. **Use of any other combination of assays for characterization of the HIV specimen evaluation panel shall be communicated, discussed and agreed with WHO beforehand.**

Initially, each specimen is tested on the Vironostika® HIV Ag/Ab (bioMérieux) EIA and Enzygnost Anti-HIV 1/2 Plus (Siemens Healthcare Diagnostics) EIA in parallel.

Specimens that are non-reactive on both EIAs are not further tested and are assigned anti-HIV negative.

Specimens with discrepant EIA results AND those with dually reactive results on both EIAs are tested on the INNO-LIA™ HIV I/II Score (Fujirebio) line immunoassay.

Specimens that are negative by line immunoassay are further tested on Innotest® HIV Antigen mAb (Fujirebio) EIA and if found non-reactive then are assigned anti-HIV negative. If found to be neutralisable for HIV-1 antigen, the specimen is considered HIV-1 antigen positive and anti-HIV negative and is retained for the evaluation of 4th generation assay **but not** for 3rd generation assays.

Specimens that are indeterminate by line immunoassay are further tested on Innotest® HIV Antigen mAb (Fujirebio) EIA and if found non-reactive then are excluded from the panel. Specimens that are reactive for antigen (and neutralisable) are assigned as HIV-1 antigen positive and anti-HIV inconclusive. These specimens are retained for the evaluation of 4th generation assay **but not** for 3rd generation assays.
Specimens that are positive by line immunoassay are assigned as anti-HIV-1 positive or anti-HIV-2 positive. Those specimens that cannot be discriminated (i.e. anti-HIV positive) are further tested on the NEW LAV II Blot (BioRad Laboratories). Specimens that are indeterminate or negative by the NEW LAV II Blot are assigned as anti-HIV-1 positive. Specimens that are positive by the NEW LAV II Blot are assigned as anti-HIV positive.
Figure 1 - Testing algorithm for characterization of the WHO HIV specimen reference panel (serum/plasma specimens)
6.2. Lot-to-lot variation panel
Lot-to-lot variation is assessed by testing the same ten dilution series (comprised of 2-fold dilutions of 10 stock HIV positive specimens in commercially available normal human serum) on two separate production lots of the assay under evaluation in the same testing session.

6.3. HIV seroconversion panels
A seroconversion panel is a series of specimens, sequentially collected over a period of time, from an individual developing antibody in response to acute infection. The following commercial seroconversion panels: PRB914, PRB925, PRB926, PRB930, PRB955, PRB965, PRB968, PRB969 [sourced from SeraCare Life Sciences Inc] are tested using the assay under evaluation in singular on one lot. These panels consist of a total of 52 specimens collected from eight individuals during seroconversion.

6.4. HIV performance panels
One anti-HIV mixed titer performance panel containing 25 members, PRB205 [sourced from SeraCare, Life Sciences Inc] and, if evaluating a 4th generation antigen/antibody detection assay, one HIV-1 p24 antigen performance panel containing 25 members, PRA204 [sourced from SeraCare Life Sciences Inc] are tested using the assay under evaluation in singular on one lot.

6.5. WHO international reference preparations
The WHO international biological reference preparation panel with the catalogue number 02/210 (Anti-HIV antibodies [HIV-1 subtypes A, B, C, CRF01_AE, group O and HIV-2]) is tested using the assay under evaluation in singular on one lot. If evaluating a 4th generation antigen/antibody detection assay, the WHO international biological standard with the catalogue number 90/636 (HIV-1 p24 Antigen) is tested in 2-fold dilutions in singular on one production lot.

6.6. HIV culture supernatant panel
If evaluating a 4th generation antigen/antibody assay, ten commonly occurring subtypes derived from culture supernatant studies are tested using the assay under evaluation. A 3-fold dilution series for each of 10 subtypes is made and six members of the dilution series are tested, in singular on one lot.

6.7. External quality control specimen
See section 9 for further details

Table 2 - Overview of specimen panels used in laboratory evaluation

<table>
<thead>
<tr>
<th>Panel name</th>
<th>Sub groups</th>
<th>Number of specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO HIV specimen reference panel</td>
<td></td>
<td>470 HIV positive (and 2 x HIV-1 antigen only*) and 730 HIV negative</td>
</tr>
<tr>
<td>Lot-to-lot variation panel</td>
<td></td>
<td>16 member dilution series of 10 specimens (160 in total)</td>
</tr>
<tr>
<td>Commercial HIV seroconversion panel</td>
<td></td>
<td>8 panels comprising 52 specimens in total</td>
</tr>
<tr>
<td>Commercial HIV performance</td>
<td>Anti-HIV mixed titer panel</td>
<td>1 panel comprising 25 specimens in total</td>
</tr>
</tbody>
</table>
### 7. Laboratory testing

#### 7.1. Sequence of testing

Each product under evaluation is used strictly in accordance with the instructions for use (IFU) issued by the manufacturer. The WHO Prequalification Evaluating Laboratory will send a hard or electronic copy of the IFU to WHO/PQDx upon delivery of the test kits and prior to the commencement of the laboratory evaluation. The IFU must be reviewed against the IFU submitted to WHO/PQT as part of the dossier assessment for the prequalification assessment. If the IFU has been updated since dossier submission, a letter from the manufacturer detailing changes made must be sent to WHO/PQDx prior to the laboratory evaluation commencing.

The specimen reference panel is run in order that approximately one half of the specimen panel will be run with the one lot and the other half of the panel with the other lot. The specimens of the WHO HIV specimen reference panel should initially be tested in singular and in a **blinded manner**.

Lot-to-lot variation is assessed by testing the same set of dilution series (comprised 2-fold dilutions of 10 stock HIV positive specimens) on two separate production lots of the assay under evaluation.

The seroconversion panels, performance panels [PRB205 for both 3rd and 4th generation assays, PRA204 for 4th generation assays only], WHO international biological reference preparation panel [WHO p24 antigen standard for 4th generation assays only] and culture supernatant panel [for 4th generation assays only] are then tested in singular on a single lot.

For the purpose of evaluating the rapid diagnostic tests, a ‘test run’ is defined as a consecutive run of simple/rapid tests of the same production lot performed during the same ‘session’. A ‘testing session’ might be considered to be a morning or afternoon.

#### 7.2. Recording test results

All test results are recorded on standardized test result worksheets and then entered in a Microsoft Excel spreadsheet for further data analysis. For subjectively read assays such as rapid diagnostic tests or line immunonassay, the intensity of band/line/spot is additionally entered into the data collection sheet. The intensity rating system reads as described in Table 2
### Scoring index

<table>
<thead>
<tr>
<th>Scoring index</th>
<th>Intensity reading scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Non-reactive</td>
</tr>
<tr>
<td>1</td>
<td>Uncertain Reactivity, Indeterminate</td>
</tr>
<tr>
<td>2</td>
<td>Very Weak, but Definitely Reactive</td>
</tr>
<tr>
<td>3</td>
<td>Medium to Strong Reactivity</td>
</tr>
<tr>
<td>7</td>
<td>Debris</td>
</tr>
</tbody>
</table>

Table 2 - Results legend for data collection sheets for subjectively read assays

Visual interpretation of results of subjectively-read assays is made independently by three readers (without the knowledge of the other two sets of results and blinded to the reference result for the specimen) and entered onto the data collection sheets. These results are compared by the operator carrying out the assay so that any mistakes may be identified and rectified immediately. Should recording errors be identified, both the original and corrected result are recorded and initialed by the reader. When the three readers interpret the results differently from each other, the consensus is recorded as that interpretation which occurs two out of three times. In cases where all three interpretations are different, the result is recorded as indeterminate.

A technician’s appraisal is made of each assay under evaluation and is completed by the operator performing the testing. It includes questions about the ease of the procedure, reading of results, clarity of IFU, as well as a record of any specific difficulties encountered during the evaluation.

#### 8. Quality control and interpretation of test results

##### 8.1. Test kit controls

Manufacturer-supplied positive and negative test kit controls will be run as indicated in the IFU for all test formats included in each test run for EIAs and at the commencement of each testing session for rapid diagnostic tests and other formats. Where positive and negative test kit controls are not supplied by the manufacturer, as will be the case for many rapid diagnostic tests, the external quality control specimen will act as the control specimen, see section 9.3 for more details.

##### 8.2. Internal control lines for rapid diagnostic tests

Generally, rapid diagnostic tests contain a control band, line or spot to determine migration of the reagents or the sample has occurred. Most control bands/lines/spots will become visible with the addition of reagent (i.e. buffer). However, some rapid diagnostic tests will contain a control band/line/spot that becomes visible with addition of specimen (i.e. presence of IgG). It is imperative that the exact nature of the control band/line/spot is ascertained and included in the report. An experiment is performed to verify this point, if not explicitly mentioned in the IFU.

##### 8.3. External quality control specimen

The WHO prequalification evaluating laboratory supplies an external quality control (QC) specimen which is tested in singular at the beginning of each test session for rapid diagnostic tests and other formats and in
triplicate for each test run for EIAs. See definition of testing session in section 5.1. The QC specimen represents a weakly reactive HIV positive specimen, and thus may be different for different assays and different assay formats. The QC specimen are made by the WHO Prequalification Evaluating Laboratory or acquired commercially, depending on the assay under evaluation.

8.4. Proficiency panels
User proficiency must be established for each assay by each operator before the evaluation commences. This may be established at the time of assay demonstration by the manufacturer or for training purposes.

8.5. Limits of acceptability
All results on test kits controls and QC specimens are entered on the data collection sheets. Should the test kit controls or the QC specimen not give results within the expected ranges, evaluation testing on that assay is suspended until the cause has been identified and a satisfactory solution identified. Such problems must be communicated immediately to WHO and recorded on the data sheets. The PI is responsible for carefully checking all data entry forms for legibility, accuracy and completeness.

8.6. Interpretation of results
The interpretation of results for each assay under evaluation is made strictly according to the manufacturers’ instructions within the IFU. Invalid test results are recorded on the data collection sheets including where the control line does not appear or in any other way the test result is invalid as defined by the IFU. For test results that are indeterminate according to the IFU, the results are recorded on data collection sheets.

9. Analysis of data

9.1. Invalid runs/devices
The number of invalid test runs (if EIA) is recorded as the number of invalid runs as a percentage of the total number of runs performed for clinical specimens only (excluding the commercially acquired panels, lot variation panels, culture supernatant panels and WHO reference preparations).

The number of invalid devices (if rapid diagnostic test or other format) is recorded as the number of invalid test devices as a percentage of the total number of devices used for the evaluation testing with clinical specimens (excluding commercially acquired panels, WHO reference preparations, culture supernatant panels, lot to lot variation panels).

Invalid results may mean invalid test results as defined by the instructions for use such as where the control line/band/spot does not appear or invalid due to obviously defective test device or defective transfer pipette.

9.2. Inter-reader variability
The inter-reader variability is calculated when test results must be read without any objective reading instruments i.e. rapid diagnostic tests. Three persons independently interpret each test result. The inter-reader variability is expressed as the percentage of specimens for which initial test results are differently interpreted (i.e. reactive or non-reactive or indeterminate) by the independent readers for the clinical specimens (excluding commercially acquired panels, WHO reference preparations, culture supernatant panels, lot to lot variation panels).
Inter-reader variability is assessed for each test band i.e. HIV-1 and HIV-2 test bands in discriminatory HIV-1/2 antibody detection assays, and HIV-1/2 antibody and HIV-1 p24 antigen test bands for antibody/antigen detection assays.

9.3. Performance characteristics from the HIV specimen reference panel

The following strategies are used to calculate the performance characteristics for each assay under evaluation and is closely linked to the reference testing results gained during specimen panel characterization.

Table 3 - 2 x 2 table for calculation of performance characteristics

<table>
<thead>
<tr>
<th>Results of assay under evaluation</th>
<th>Reference testing results</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive</td>
<td>Reactive</td>
<td>Non-reactive</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Reactive</td>
<td>a</td>
<td>b</td>
<td>a + b</td>
<td></td>
</tr>
<tr>
<td>(true positives)</td>
<td>(false positives)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-reactive</td>
<td>c</td>
<td>d</td>
<td>c + d</td>
<td></td>
</tr>
<tr>
<td>(false negatives)</td>
<td>(true negatives)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>a + c</td>
<td>b + d</td>
<td>a+b+c+d</td>
<td></td>
</tr>
</tbody>
</table>

9.3.1. Sensitivity

Sensitivity is the ability of the assay under evaluation to detect correctly specimens that contain the analyte (reference results positive). Thus sensitivity is the number of true positive specimens identified by the assay under evaluation as positive (a), divided by the number of specimens identified by the reference assays as positive (a+c), expressed as a percentage.

Sensitivity = $\frac{a}{a + c}$

9.3.2. Specificity

Specificity is the ability of the assay under evaluation to detect correctly specimens that do not contain the analyte (reference results negative). Thus specificity is the number of true negative specimens identified by the assay under evaluation as negative (d), divided by the number of specimens identified by the reference assays as negative (b+d), expressed as a percentage.

Specificity = $\frac{d}{b + d}$

9.3.3. Confidence intervals

The 95% confidence intervals are calculated for both sensitivity and specificity in order to assess the level of uncertainty introduced by sample size, etc. Exact 95% confidence intervals for binomial proportions were calculated from the F-distribution. [Armitage, 2002; Kirkwood, 2003]

9.3.4. Positive predictive value (PPV)

The probability that when the test is reactive that the specimen does contain the analyte. PPVs were calculated using the formula.
9.3.5. Negative predictive value (NPV)

The probability that when the test is negative that a specimen does not contain the analyte. NPVs were calculated using the formula.

\[ \text{NPV} = \frac{(1 - \text{prevalence}) \times \text{specificity}}{(1 - \text{prevalence}) \times \text{specificity} + (\text{prevalence}) \times (1 - \text{sensitivity})} \]

The probability that a test result will accurately determine the true infection status of a person being tested varies with the prevalence of HIV infection in the population from which the person comes. In general, the higher the prevalence of HIV infection in the population, the greater the probability that a person testing positive is truly infected (i.e., the greater the positive predictive value [PPV]). Thus, with increasing prevalence, the proportion of individuals testing false-positive decreases; conversely, the likelihood that a person whose test result is negative is truly uninfected (i.e., the negative predictive value [NPV]), decreases as prevalence increases.

The PPV and NPV are calculated at a prevalence of 0.1%, 1% and 5%.

9.4. Indeterminate results

For the HIV specimen reference panel only: specimens which are found to be indeterminate (grey zone) by the criteria stated in the instructions for use should be retested in duplicate on the same lot number of assay and singular on the other lot. In the case that the testing result cannot be resolved after all testing, the specimen is to be called indeterminate and included in sensitivity/specificity calculations. A value for initial sensitivity and specificity are calculated based on the results obtained for the assay under evaluation on the first lot. The final sensitivity and specificity values are calculated taking into consideration the repeat testing performed on a same lot and further testing second lot of the assay under evaluation.

9.5. Discrepant results

Those specimens with results that are consistent with the reference assays results i.e. the characterized specimen results undergo no further testing. Those specimens with results discrepant from the reference result are retested in duplicate using the same lot number by the same operator. The results that occur two out of three times are recorded as the test result. If the result is again discrepant, the specimen is retested on a second lot number, if available. If the result on the second lot is concordant with the reference result, no further testing is required. If the result is still discrepant from the reference results, the result is recorded as is.

An initial sensitivity and specificity are calculated based on the initial results obtained for the assay under evaluation on the first lot. The final sensitivity and specificity are calculated taking into consideration the results of repeat testing performed on a second lot of the assay under evaluation i.e. if found to be concordant on the second lot will be recorded as such, and if found to be discrepant on the second lot will be recorded as such.
9.6. Interpretation of results from lot-to-lot variation panel

The results of the lot-to-lot panel for the two production lots are compared and a variation of +/- 1 dilution series is considered acceptable.

9.7. Interpretation of seroconversion sensitivity

The results obtained from seroconversion panels using the assay under evaluation are compared with those obtained using Enzygnost Anti-HIV 1/2 Plus (Siemens Healthcare Diagnostics) EIA; the assay arbitrarily designated the reference for determination of relative sensitivity in these panels. For each seroconversion series (panel) the first specimen in the sequence to become reactive with Enzygnost Anti-HIV 1/2 Plus is assigned the value “0”. Results from the assay under evaluation are compared with Enzygnost Anti-HIV 1/2 Plus by determining the difference between the specimen assigned value “0” and the relative position in the sequence of the first specimen showing a reactive result for the assay under evaluation. For example, if an assay becomes reactive two specimens earlier in a series than Enzygnost Anti-HIV 1/2 Plus, the value assigned for that series in that assay would be -2. Similarly, if an assay becomes reactive one specimen later than Enzygnost Anti-HIV 1/2 Plus, the value assigned would be +1. The assigned values over the eight seroconversion panels (series) are averaged to determine a mean relative seroconversion sensitivity index for each assay and the 95% confidence interval is determined.

In addition, each specimen is tested on the following assays: Vironostika® HIV Ag/Ab (bioMérieux) EIA, INNOTEST® HIV Antigen mAb (Fujirebio) EIA, and INNO-LIA™ HIV I/II Score (Fujirebio) line immunoassay.

9.8. Interpretation of results from performance panels

The number of specimens detected by the assay under evaluation in the HIV-1 mixed titer and HIV-1 p24 antigen mixed titer performance panels is determined by comparison with the combined reference results generated by the WHO Prequalification Evaluating Laboratory: Enzygnost HIV Anti-1/2 Plus (Siemens Healthcare Diagnostics) EIA, Vironostika HIV Ag/Ab (bioMérieux) EIA, INNOTEST HIV Antigen mAb (Fujirebio) EIA and INNO-LIA HIV I/II Score (Fujirebio) line immunoassay.

9.9. Interpretation of results from WHO international reference preparations

The number of specimens detected by the assay under evaluation for the WHO international reference preparation are determined by comparison with the combined reference results generated by the WHO prequalification evaluating laboratory: Enzygnost HIV Anti-1/2 Plus (Siemens Healthcare Diagnostics) EIA, Vironostika HIV Ag/Ab (bioMérieux) EIA, INNOTEST HIV Antigen mAb (Fujirebio) EIA and INNO-LIA HIV I/II Score (Fujirebio) line immunoassay.

9.10. Interpretation of results from culture supernatant panel

The number of specimens detected by the assay under evaluation on the culture supernatant panel are determined in comparison with the reference testing results using the Vironostika HIV Ag/Ab (bioMérieux) EIA and INNOTEST® HIV Antigen mAb (Fujirebio) EIA.

9.11. Technician’s appraisal

The technical aspects of the assay under evaluation is assessed by the technician who performed the testing. These assessments, along with other selected assay characteristics, contribute to an overall appraisal of each assay’s suitability for use in small laboratories. To enable comparison between assays, a scoring system is used to rate specified operational characteristics.

The data analysis and report drafting is carried out by the WHO prequalification evaluating laboratory and sent to WHO for List 1 laboratories. In case of list two laboratories the data and the report will be sent simultaneously to WHO and the Manufacturer in a timely manner. WHO verifies the draft report from List one laboratories and sends to the authorized contact designated by the manufacturer for comment. The company has one month right of reply. The final report is prepared after one month has elapsed. The WHO scientist ensures that the comments of the authorized contact are reviewed and any outstanding issues are resolved before final publication. The final report is prepared and disseminated by WHO. A copy of the final report is sent to the authorized contact designated by the manufacturer and to WHO prequalification evaluating laboratory.

10. Materials and supplies

10.1. Data collection sheets

All data will be reported to WHO/PQDx on the following forms:

   A. Data collection Microsoft Excel spreadsheet for the rapid diagnostic tests
      - Anti-HIV-1/2 non-discriminatory detection
      - Anti-HIV-1/2 discriminatory detection
      - Anti-HIV-1/2 and HIV-1 antigen discriminatory detection
   B. Data collection Microsoft Excel spreadsheet for EIAs
      - Anti-HIV-1/2 non-discriminatory detection
      - Anti-HIV-1/2 and HIV-1 antigen non-discriminatory detection
   C. Technician's appraisal worksheet
   D. Findings of supplementary testing on discrepant specimens

10.2. Supplies

The manufacturers of products will provide the products and any equipment necessary for the evaluation free of charge.

11. Roles and responsibilities

11.1. Responsibilities of the WHO Prequalification Evaluating Laboratory

   i. Ensure availability of HIV specimen reference panel, lot-to-lot variation, culture supernatant, seroconversion and performance panels;
   ii. Conducting the performance evaluation in accordance with internationally recognized best practice;
   iii. Preparation of QC specimens and proficiency panels;
   iv. Preparation of draft report on laboratory evaluation;
   v. Advising WHO on operational characteristics of assays evaluated.

All source data, data analysis records and all correspondence are retained and archived for a period of at least ten years.

11.2. Responsibilities of WHO/PQDx

   i. Technical advice to the PI;
   ii. Technical and administrative management of the laboratory evaluation;
   iii. Verification of the draft report, seeking of comments from manufacturer in case of List one laboratories;
iv. Preparation and dissemination of the final report;
v. Formal contacts with authorized contacts of the manufacturers.

Any publication by WHO of the results of these evaluations and the WHO recommendations derived therefrom will, however, be accompanied by the following disclaimer:

*The mention of specific companies or of certain manufacturers’ products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.*

*WHO and the WHO Prequalification Evaluating Laboratory, do not warrant or represent that the evaluations conducted with the HIV test kits referred to in this document are accurate, complete and/or error-free. WHO and the WHO Prequalification Evaluating Laboratory disclaim all responsibility for any use made of the data contained herein, and shall not be liable for any damages incurred as a result of its use. This document must not be used in conjunction with commercial or promotional purposes.*

12. References


World Health Organization Guidelines on HIV Safety Precautions, and Guidelines for the Safe Transport of Specimens (WHO/EMC/97.3)

International Standards
EN 13612:2002 Performance evaluation of in vitro diagnostic medical devices
ISO 17025 (General requirements for the competence of testing and calibration laboratories)
ISO15189 (Medical laboratories — Particular requirements for quality and competence)

13. Other documents required

**Work Instructions**

SOP_PQDx_072 PQT work instruction for laboratory testing at the WHO Prequalification Evaluating Laboratory
SOP_PQDx_074 PQT work instruction for data entry and analysis
SOP_PQDx_075 PQT work instruction for report preparation and dissemination

**Master Templates**

SOP_PQDx_079 PQT report template for 3rd generation EIAs
SOP_PQDx_080 PQT report template for 3rd generation simple/rapid assays
SOP_PQDx_130 PQT report template for 4th generation HIV EIAs
SOP_PQDx_131 PQT report template for 4th generation HIV simple/rapid assays
SOP_PQDx_132 PQT report template for 3rd generation HIV discriminatory simple/rapid assays