WHO PROTOCOL FOR PERFORMANCE LABORATORY EVALUATION OF HCV SEROLOGY ASSAYS
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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 HCV 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 laboratory evaluation of diagnostics for HCV antibody and/or detection

The performance evaluation determines the accuracy of HCV 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 HCV antibody and assays for the detection of both HCV antibody and HCV antigen are covered in this protocol.

All HCV 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 laboratory evaluation determines the accuracy of 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.

The HCV specimen reference panel shall comprise a minimum of 463 anti-HCV positive and anti-HCV 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.
4. Objectives

4.1. Overall objectives
The overall objectives are:
1. To evaluate currently commercially available HCV assays (including EIA and rapid diagnostic tests) for detection of HCV antibodies and/or HCV antigen against established performance criteria.

4.2. Specific objectives
The specific objectives of the evaluation are:
1. To determine the sensitivity, specificity of currently available HCV assays (EIA’s and rapid diagnostic tests and other formats) for the detection of HCV antibodies and/or HCV antigen as compared to a reference result (two EIA’s and one line immunoassay).

2. To evaluate the operational characteristics of HCV 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.


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 HCV positive and negative specimens will also be recorded by taking electronic images. Unexpected test results will also be digitally recorded as well 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 HPE guidelines on laboratory safety should be carefully followed 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. 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 forms. 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 definition1 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.

6. Specimens

6.1. HCV specimen reference panel

6.1.1. Collection of specimens for the HCV 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 WHO HCV specimen reference panel

The panel consists of minimum of 480 serum/plasma specimens of European, African, Latin American and Asian origin. There are 150 anti-HCV positive specimens, and 310 anti-HCV negative specimens.

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1ISO 18113-1:2009 In vitro diagnostic medical devices -Information supplied by the manufacturer (labelling) – Part 1: Terms, definitions and general requirements
Table 1 - HCV specimen reference panel

<table>
<thead>
<tr>
<th>HCV positive specimens</th>
<th>HCV negative specimens</th>
<th>Total number</th>
</tr>
</thead>
<tbody>
<tr>
<td>160</td>
<td>320</td>
<td>480</td>
</tr>
</tbody>
</table>

The HCV specimen reference panel has been characterized using a standardized combination of assays i.e. a testing algorithm. These results are used to determine the HCV status of each specimen for the purpose of this evaluation see Figure 1. **Use of any other combination of assays for characterization of the HCV core Ag specimen evaluation panel shall be communicated, discussed and agreed with WHO beforehand.**

Initially, each specimen is tested on the Murex anti HCV EIA (version 4.0) (DiaSorin S.A. Italy) and Monolisa Anti-HCV Plus version 2.0 (Bio-Rad Laboratories) in parallel. Specimens that are non-reactive on both EIAs are not further tested and are assigned as anti-HCV negative.

Specimens with discrepant EIA results AND with dually reactive EIA results are tested on the RIBA HCV 3.0 Strip Immunoassasy (Chiron) or the HCV Blot 3.0 WB (MP Biomedicals).

Specimens that are positive by line immunoassay/WB are assigned as anti-HCV positive.

Specimens that are indeterminate or negative by line immunoassay / WB are excluded from the HCV specimen reference panel.

Specimens that are indeterminate by line immunoassay are assigned as anti-HCV indeterminate and excluded from the HCV specimen reference panel.

Specimens that are **negative by line immunoassay** are assigned as anti-HCV negative.
6.2. Lot-to-lot variation panel

Lot-to-lot variation is assessed on two separate production lots by testing the same dilution series (comprised of 2-fold dilutions of 10 stock HCV positive specimens in commercially available normal human serum) in the same testing session.

6.3. HCV 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: PHV913, PHV919, PHV 920 and PHV 922 [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 26 specimens collected from four individuals during seroconversion.
6.4. HCV performance panels

One HCV Mixed Titre performance panel containing 16 members, 0810-0175 [sourced from SeraCare, Life Sciences Inc] and one anti-HCV low titre performance panel containing 11 members, 0810-0192 [sourced from SeraCare, Life Sciences Inc] are tested using the assay under evaluation in singular on one lot.

6.5. External quality control specimen

See section 6 for further details.

Table 2 - Overview of specimen panels used in laboratory evaluation

<table>
<thead>
<tr>
<th>Panel name</th>
<th>Number of specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO HCV specimen reference panel</td>
<td>163 HCV positive, 320 HCV negative</td>
</tr>
<tr>
<td>Lot-to-lot variation panel</td>
<td>16 member dilution series of 10 specimens, (160 in total)</td>
</tr>
<tr>
<td>Commercial HCV seroconversion panels</td>
<td>4 panels comprising 26 specimens in total</td>
</tr>
<tr>
<td>Commercial HCV performance panels</td>
<td>2 panels comprising 27 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 HCV specimen reference panel should initially be tested in singular and in a blinded manner.

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

The seroconversion panels, and performance panels 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 diagnostic 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

<table>
<thead>
<tr>
<th>Scoring index</th>
<th>Intensity reading scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Negative</td>
</tr>
<tr>
<td>1</td>
<td>Uncertain Reactivity, Indeterminate</td>
</tr>
<tr>
<td>2</td>
<td>Very Weak, but Definitely Reactive</td>
</tr>
</tbody>
</table>
All test results are recorded electronically directly from the plate reader and then entered in a Microsoft Excel spreadsheet for further data analysis. For subjectively read assays such as rapid diagnostic tests or line immunoassay, the intensity of band/line/spot is additionally entered into the data collection sheet. The intensity rating system reads as described in Table 3

Table 3 - Results legend for data collection sheets for subjectively read assays

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Medium to Strong Reactivity</td>
</tr>
<tr>
<td>4</td>
<td>Invalid (including no control line/band/dot/spot visible, or obviously defective test device, no flow, debris present)</td>
</tr>
</tbody>
</table>

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 room to record 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 at the control specimen, see section 9.3.

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

Generally, rapid diagnostic tests contain a control band, line or spot to determine that the test device is operating correctly. 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 recorded 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 lowly reactive HCV positive specimen, and thus may be different for different assays and different assay formats. The QC specimen is supplied by the WHO prequalification evaluating laboratory or acquired commercially, depending on the assay under evaluation.
8.4. Proficiency panels
A proficiency panel must be run successfully for each assay by each operator before the evaluation commences. This may be the same panel as that used 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, and WHO reference preparation).

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 entire evaluation.

Invalid results may mean invalid test results as defined by the IFU 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 assay readings must be performed 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.

9.3. Performance characteristics from WHO HCV 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 4 - 2 x 2 table for calculation of performance characteristics

<table>
<thead>
<tr>
<th>Results of assay under evaluation</th>
<th>Reference testing results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive</td>
<td>Reactive (true positives)</td>
</tr>
<tr>
<td>Reactive</td>
<td>a</td>
</tr>
<tr>
<td>Non-reactive</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 HCV antibodies and/or HCV antigen (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.

\[
\text{Sensitivity} = \frac{a}{a+c}
\]

9.3.2. Specificity

Specificity is the ability of the assay under evaluation to detect correctly specimens that do not contain HCV antibodies and/or HCV antigen (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.

\[
\text{Specificity} = \frac{d}{b+d}
\]

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 HCV antibodies and/or HCV antigen. PPVs were calculated using the formula.

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

9.3.5. Negative predictive value (NPV)

The probability that when the test is negative that a specimen does not have contain HCV antibodies and/or HCV antigen. NPVs were calculated using the formula.

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

The probability that a test result will accurately determine the true infection status of a person being tested varies with the prevalence of HCV infection in the population from which the person comes. In general, the higher the prevalence of HCV 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. Therefore, as prevalence increases, so does the proportion of individuals testing false-negative.

The PPV and NPV are calculated at prevalences of 0.1%, 1% and 5%.
9.4. Indeterminate results
For the WHO 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. 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 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 final 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.

Should a negative specimen from the HCV specimen reference panel give a repeatedly reactive result in the assay under evaluation it will be further characterized with additional serology (antigen only; antibody only) and molecular techniques. This additional testing will be conducted to rule out the remote possibility that the reactivity in the assay under evaluation is true, and is more sensitive than the assays with which the panel specimens were characterized.

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 DiaSorin Murex Anti-HCV EIA (version 4.0) 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 DiaSorin Murex Anti-HCV EIA (version 4.0) EIA is assigned the value “0”. Results from the assay under evaluation are compared with DiaSorin Murex Anti-HCV EIA (version 4.0) EIA 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 DiaSorin Murex Anti-HCV EIA (version 4.0) EIA, the value assigned for that series in that assay would be -2. Similarly, if an assay becomes reactive one specimen later than DiaSorin Murex Anti-HCV EIA (version 4.0) EIA, the value assigned would be +1. The assigned values over the four seroconversion panels (series) are averaged to determine a mean relative seroconversion sensitivity index for each assay and the 95% confidence interval is determined.

9.8. Interpretation of results from performance panels
The number of specimens correctly identified by the assay under evaluation in the HCV performance panels is determined by comparison with DiaSorin Murex Anti-HCV EIA (version 4.0) EIA.
9.9. Interpretation of results from WHO international reference preparations

The number of specimens identified by the assay under evaluation for the WHO international reference preparation are determined by comparison with the combined reference results generated by NRLHPA: DiaSorin Murex Anti-HCV EIA (version 4.0) EIA HCV ELISA Test System 3.0 with Enhanced SAVe [short incubation] (Ortho-Clinical Diagnostics), Monolisa Anti-HCV Plus version 2.0 (Bio-Rad Laboratories), and CHIRON RIBA HCV 3.0 (Ortho-Clinical Diagnostics).

9.10. Technician's appraisal

The technical aspects of the assays 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.

9.11. Report preparation

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/PQ Diagnostics assessment on the following forms:

A. Data collection Microsoft Excel spreadsheet for the rapid diagnostic tests
B. Data collection Microsoft Excel spreadsheet for EIAs (print-outs)
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

1. Act as repository for the HCV specimen reference panel, lot-to-lot variation, seroconversion and performance panels;
2. Conducting the laboratory evaluation according to the agreed protocol;
3. Preparation of QC specimens and proficiency panels;
4. Preparation of draft report on laboratory evaluation;
5. 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/PQ Diagnostics assessment

1. Technical advice to the PI;
2. Technical and administrative management of the laboratory evaluation;
3. Verification of the draft report, seeking of comments from manufacturer;
4. Preparation and dissemination of the final report;
5. 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:

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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_136 PQDx report template for 3rd generation HCV EIAs including excel spreadsheets of results
SOP_PQDx_137 PQDx report template for 3rd generation HCV simple/rapid assays for non-discriminatory detection including excel spreadsheets of results
### 14. DOCUMENT REVISION HISTORY

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<tr>
<th>SOP version</th>
<th>Effective date</th>
<th>Reason for revision</th>
<th>Prepared by</th>
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<tr>
<td>1.0</td>
<td>21 Sept 2012</td>
<td>First issue</td>
<td>Willy Urassa, Anita Sands</td>
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<tr>
<td>2.0</td>
<td>11 Jan 2013</td>
<td>Second version</td>
<td>Anita Sands</td>
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<tr>
<td>3.0</td>
<td>04 June 2014</td>
<td>Changes to reflect dept changes, added first page Adopt PQT-SOP format</td>
<td>Willy Urassa</td>
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<td>4.0</td>
<td>21 November 2015</td>
<td>Name the protocol PQDX_040_PHE to differentiate it from PQDX_040_NRL</td>
<td>Willy Urassa</td>
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<td>5.0</td>
<td>15 December 2016</td>
<td>Made several changes in the methodology to align with changes implemented in NRL</td>
<td>Willy Urassa</td>
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<tr>
<td>6.0</td>
<td>1 February 2017</td>
<td>Update the protocol to remove the name of the WCC and update it to suit posting to the website</td>
<td>Willy Urassa</td>
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