Technical Specifications Series
for submission to WHO Prequalification – Diagnostic Assessment

TSS-7
Interim
Hepatitis C rapid diagnostic tests for professional use and/or self-testing
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List of contributors

A technical consultation on WHO prequalification requirements for hepatitis C antibody and antigen enzyme immunoassays and rapid diagnostic tests for professional use was held in Geneva, Switzerland from 29 to 30 October 2018.

Meeting participants: J. Alonso, Departamento de Vigilância, Prevenção e Controle das IST, do HIV/AIDS e das Hepatites Virais, Brazil; T. Applegate, Viral Hepatitis Clinical Research Program, Kirby Institute, University of New South Wales, Australia; S. Best, Melbourne, Australia; R. J. S. Duncan, London, United Kingdom; E. Fajardo, HIV/HCV Diagnostic Advisor, Médecins Sans Frontières, Barcelona, Spain; S. Hojvat, Virginia, USA; S. Kamili, Division of Viral Hepatitis, US Centers for Disease Control and Prevention (CDC), Atlanta, USA; S. Lovell, Hepatitis and General Virology, Division of Microbiology Devices, Office of In Vitro Diagnostics and Radiological Health, U.S. Food and Drug Administration (U.S. FDA) Silver Spring, Maryland, USA; R. Njouom, Centre Pasteur du Cameroun, Yaoundé, Cameroon; J. Parry, United Kingdom; A. S. Shah, Blood Safety and Laboratory Technology, WHO Regional office, New Delhi, India; H. Scheiblauer, Paul-Ehrlich-Institut, Langen, Germany; M. Soliman, Ain Shams Faculty of Medicine, Egypt; B. Vetter, Foundation for Innovative New Diagnostics (FIND), Geneva, Switzerland.

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A technical consultation for the second edition to increase the scope to include hepatitis C antibody rapid diagnostic tests for self-testing was held from 26 to 27 October 2020.

Meeting participants: J. Alonso, Departamento de Vigilância, Prevenção e Controle das IST, do HIV/AIDS e das Hepatites Virais, Brazil; R. J. S. Duncan, London, United Kingdom, M. Majam, Wits Reproductive Health and HIV Institute (Wits RHI) South Africa; J. Parry, United Kingdom; E. Pirou, Médecins Sans Frontières, Amsterdam, The Netherlands; S. Scholtmann, Food and Drug Administration FDA, Maryland, United States of America (USA); S. Shilton, Foundation for Innovative New Diagnostics (FIND), Geneva, Switzerland; V. Watson, Liverpool School of Tropical Medicine/STAR Consortium; United Kingdom; K. Whitman, Food and Drug Administration FDA, Maryland, USA.

WHO Secretariat: D. Healy; M. Lanigan; I. Prat; U. Ströher, In Vitro Diagnostics Assessment Team, Regulation and Prequalification Department; P. Easterbrook; N. Luhmann; and M. Jamil, Global HIV, Hepatitis and STIs Programme.

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1 Participated via web conferencing
2 Participated via web conferencing
3 Observer status
The draft technical specifications document was posted on the WHO website for public consultation. Various stakeholders, including manufacturers submitting to WHO Prequalification of IVDs, IVD manufacturing industry associations, various national and international regulatory bodies, and IVD standards organizations were informed of the consultation in order to solicit feedback.

First edition public comments were received for consideration from C. Hayden, R&D Manager, bioLytical Laboratories Inc, Richmond, British Columbia, Canada; the Division of Microbiology Devices, Office of In Vitro Diagnostics and Radiological Health, U.S. Food and Drug Administration, Silver Spring, Maryland, USA; the In Vitro Diagnostic Devices Evaluation Division, Medical Devices Bureau, Therapeutic Products Directorate, Health Canada, Government of Canada, Canada; ISO/TC 212 Secretary on behalf of ISO/TC 212, International Standards Organization, Geneva, Switzerland; MedTech Europe, Brussels, Belgium; R. Meurant, NSF Health Sciences Limited, Paris, France; B. Vetter, Foundation for Innovative New Diagnostics (FIND), Geneva, Switzerland; M. Lanigan, Prequalification Team – Diagnostic Assessment Group, Regulation of Medicines and other Health Products; WHO, Geneva; A Sands, Safety and Vigilance Group, Regulation of Medicines and other Health Products; WHO, Geneva.

Second edition public comments were received for consideration from R. J. S. Duncan, London, United Kingdom; N. Furukawa, Division of Viral Hepatitis, US Centers for Disease Control and Prevention (CDC), Atlanta, USA; M. Hellard, Burnet Institute, Melbourne, Australia.
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 rapid diagnostic tests (RDTs) for the:

- detection of antibodies to Hepatitis C virus (HCV) in whole blood, serum, plasma or oral fluid (professional use);
- detection of antibodies to HCV by self-testing;
- detection of antigens to HCV in whole blood, serum, plasma or oral fluid (professional use).
- Assays which detect both antibodies in combination with antigens to HCV are not within the scope of this specifications.

Minimum performance requirements for WHO prequalification are summarized in this document and apply equally to RDTs intended solely for HCV detection, and to those tests where HCV detection comprises one component of a multidetection assay (e.g. a HIV/HCV RDT). The current version of this document does not address the requirements for accompanying quality control material. However, if quality control material is provided with the assay, it should demonstrate that the IVD is functional and performs as claimed (ISO 15198).

For 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.

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

Where possible, WHO analytical and clinical performance study requirements 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.

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

B. How to apply these specifications

For the purposes of WHO prequalification, the following requirements apply:
• Part 1, Part 2a and Part 3a: antibody detection RDTs for professional use
• Part 1, Part 2a and Part 3b: antibody detection RDTs for self-testing
• Part 1, Part 2b and Part 3a: antigen detection RDTs for professional use

C. Other guidance documents

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

WHO prequalification documents:
• Technical Guidance Series for WHO Prequalification – Diagnostic Assessment;
• Instructions for Compilation of a Product Dossier, WHO document PQDx_018.

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5 These documents are available at http://www.who.int/diagnostics_laboratory/evaluations/en/
WHO Global Hepatitis programme guidelines:

- Guidelines for the screening, care and treatment of persons with chronic hepatitis C infection\(^6\)
- Guidelines on hepatitis B and C testing\(^7\)
- Guidelines on hepatitis B and C testing - Policy brief\(^8\)

D. Performance principles for WHO prequalification

D.1 Intended use

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

- The assay type and what is detected (e.g. detection of HCV core antigen or antibodies to HCV).
- The clinical indication and function of the IVD (e.g. aid in the diagnosis of HCV infection and to aid in the differential diagnosis of viral hepatitis) and that the result is qualitative. Detection of anti-HCV antibodies cannot determine the status of an infection i.e. acute, resolved or chronic; the presence of HCV core antigen may be an indication that infection is acute or chronic. A multi testing algorithm is required to definitively determine the status of an HCV infection.
- The testing population for which the functions are intended (e.g. see WHO Global Hepatitis programme guidelines).
- The intended operational setting (e.g. for professional use in a laboratory setting, in a community setting, or point of care\(^9\) (POC)).
- The intended user (e.g. trained laboratory professional or by healthcare workers/lay providers).
- The intended specimen types (e.g. oral fluid, capillary whole blood, venous whole blood, serum, plasma).
- Any limitation to the intended use (e.g. differential diagnosis, if test result is negative and clinical symptoms persist follow-up is recommended, etc).

If the assay is being claimed for use as a confirmatory assay, then this should be validated at a regional level. More extensive clinical studies are required which are outside the scope of the current version of this document.

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 that are claimed in the instructions for use (IFU) (e.g. capillary whole blood).

\(\text{\(^6\)http://apps.who.int/iris/bitstream/handle/10665/205035/9789241549615_eng.pdf;jsessionid=215DC1C97558EB1C4233ED2D0CC42729?sequence=1}
\(\text{\(^7\)http://apps.who.int/iris/bitstream/handle/10665/254621/9789241549981-eng.pdf?sequence=1}
\(\text{\(^8\)http://apps.who.int/iris/bitstream/handle/10665/251330/WHO-HIV-2016.23-eng.pdf?sequence=1&isAllowed=y}
\(\text{\(^9\)Point-of-care (POC) in-vitro diagnostic testing 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.}\)
Prequalified IVDs in low- and middle-income countries are likely to be used by laboratory professionals either in clinical/medical laboratories or at POC, or by healthcare workers/lay users trained in the use of the test at POC. Depending on the intended use of the IVD, performance studies shall be designed to consider the diversity of knowledge and skills of potential IVD users, and the likely operational settings in which testing is likely to occur. It is a manufacturer’s responsibility to ensure that the risk assessment for an IVD reflects the intended operational settings, including service delivery complexity and the likely intended user conducting the test.

Laboratory demonstration of equivalence between specimen types without evidence of clinical validation is insufficient (with exception of anticoagulants). For example, studies that comprise the testing of left-over/repository specimens by research and development staff at a manufacturer’s facility shall not, on their own, be considered sufficient to meet many of the performance requirements summarized in this document.

D.3 Applicability of supporting evidence to IVD under review

Performance studies shall be undertaken using the specific, 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, including quality control and quality assurance specifications, is signed off and the finalized method is stated in the IFU. Where this is not possible, a justification shall be provided, and additional supporting evidence may also be required. This may occur in the case of minor variations to design where no 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 a laboratory for studies as outlined in Part 2a and Part 2b 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 prequalification, 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 number of lots required for each study. 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 the IVD, that the minimum numbers of lots chosen for estimating performance characteristics consider 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.

Performance shall be established in comparison to a well-established reference method(s) (e.g. WHO prequalified, FDA-approved, CE-marked or otherwise approved by a stringent conformity assessment body) for which justification shall be provided; comparison with a similar device is insufficient for

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10 Medical technologists, medical laboratory technicians or similar, who have received a formal professional or paraprofessional certification or tertiary education degree.

11 Any person who performs functions related to healthcare delivery and has not received a formal professional or paraprofessional certification or tertiary education degree.

12 [Link](http://apps.who.int/iris/bitstream/handle/10665/251915/WHO-EMP-RHT-PQT-2016.01-eng.pdf;jsessionid=30D58FDB09FFDA3838A1698E65C8B496?sequence=1)
resolution of discrepant specimens (e.g. other method from the same manufacturer or other method using the same antigens or antibodies provided by the same supplier). For WHO purposes, the reference method should be to a level that is currently 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).

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.

For certain analytical studies it may be acceptable to use contrived specimens (e.g. where normal human specimens have been spiked with those containing HCV antibodies or antigen). Clinical studies shall be based on testing in natural specimens in Part 2a and Part 2b.

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

Example: an IVD designed to detect HCV antibodies only, and an IVD by the same developer designed for dual-detection of HCV antibodies and HIV antibodies. It is unlikely that performance evidence presented for the HCV antibody only IVD would be acceptable to support performance claims for the dual-detection IVD.
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## Part 1: Analytical performance and other evidence for HCV antibody and HCV core antigen detection RDTs

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<td><strong>1.1 Stability of specimen(s)</strong></td>
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| 1.1.1 Specimen collection, storage and transport | 1. Real time studies shall account for as applicable:  
   - storage conditions (duration at different temperatures, temperature limits, freeze/thaw cycles);  
   - transport conditions;  
   - intended use (see note 1);  
   - specimen collection and/or transfer devices intended to be used with the RDT;  
   - a minimum of 10 specimens tested.  
2. Using weak reactivity specimens 1 – 2 x RDT’s limit of detection (LOD). (see note 2) | 1. Evidence shall be provided which validates the maximum allowable time between specimen collection, processing of the specimen and its addition to the IVD in the setting where testing takes place.  
2. Specimens may be spiked with HCV antibody or core antigen positive specimens in the appropriate matrix for all claimed specimen types  
   - Multiple specimens from different patients and different disease stages shall be used and documented.  
3. Unless all specimens are expected to be processed as fresh samples within a specified time frame, the RDT performance shall be established under different storage conditions and at the beginning and end of a stated period.  
4. In case the use of archived specimens is considered for Part 2 and 3, evidence of storage stability shall be demonstrated. | |
| **1.2 Validation of specimens** | | | |
| 1.2.1 Demonstration of equivalence between specimen types and between | 1. The equivalence of specimen types shall be determined for all claimed analyte types independently (e.g. anti-hepatitis C antibodies, hepatitis C core antigen)  
   - 50 positive specimens for each claimed specimen type;  
   - 50 negative specimens for each claimed specimen type. | 1. The relationship between IVD performance in claimed specimen types and materials used for analytical studies shall be established. The design of subsequent studies shall then take that relationship into account.  
2. Where a significant difference in performance exists between specimen types, equivalence may need to be investigated as part of a larger clinical study (See Part 2, 3). | TGS-3 (1) European Commission decision on CTS (2) |
### Aspect | Testing requirements | Notes on testing requirements | Source documents
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**contrived specimens and clinical specimens**  
2. If equivalence is claimed between different anticoagulants, testing shall be conducted for all claimed analyte types independently in at least:  
   - 25 spiked positive specimens of each claimed anticoagulant;  
   - 25 negative specimens of each claimed anticoagulant.  
3. The equivalence of specimen types shall be determined for all claimed analytes. If there is no equivalence between claimed specimen types, the level of agreement shall be stated and then the impact that this will have on each subsequent performance claim shall be fully understood and described (see note 2).  
4. Specimens shall be spiked to give a weakly reactive response approx. 1-2 x RDT’s LOD.  
5. If an RDT is intended for testing whole blood and some aspects of performance have been obtained/established using serum or plasma specimens, then  
   - the relationship between analytical sensitivity in serum/plasma to that of the same characteristic in whole blood shall be understood (note 3);  
   - paired specimens shall be used for RDTs intended to test capillary blood and oral fluid (see note 4).  
6. Similarly, the relationship between analytical sensitivity in spiked HCV antibody or core antigen positive paired spiked oral fluid specimens and demonstration of the comparability of specimen types may be achieved by comparing RDT results between end-point dilution series of several HCV core antigen or antibody (as applicable) positive whole blood specimens titrated into whole blood and compared with the serum from those same specimens titrated into serum.  
4. Positive HCV specimens (undiluted), as determined by testing with reference method that includes state of the art (HCV antibody or HCV core antigen assay as applicable), should be chosen so that the majority are near the RDT LOD.
### 1.3 Metrological traceability of calibrator and control material values

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<td>1.3.1</td>
<td>Only applicable for HCV core antigen detection RDTs</td>
<td>1. If a control material has an assigned concentration value, the metrological- (not commercial- nor documentary-) traceability to an accepted international standard shall be demonstrated&lt;br&gt;• In some jurisdictions there is a requirement for use of a ‘National Testing Panel’ for lot release and IVD validation. Such a national requirement does not obviate (or remove) the need for evidence of traceability to a validated reference material such as, the WHO Hepatitis C Virus Core Antigen (HCV core Ag) for HCV core antigen assays 1st International Standard, 2014 PEI code 129096/12.</td>
<td>PQDx_018 (3) ISO 15198 (4) ISO 17511 (5)</td>
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### 1.4 Precision

| 1.4.1 | Both repeatability and reproducibility shall be determined for each analyte (HCV antibody or HCV core antigen) for which detection is claimed (see note 1). The panel of spiked specimens shall include at least:<br>• 1 non-reactive specimen;<br>• 1 weak anti-HCV or HCV core antigen reactivity positive specimen (approx. 1-2 x RDT’s LOD);<br>• 1 medium anti-HCV or HCV core antigen reactivity positive specimen (approx. 2-3 x RDT’s LOD);<br>• the panel shall include whole blood specimens if claimed. | 1. E.g. within- or between-run, -lot, -day, -site, etc.<br>2. Where possible, the testing panel should be the same for all operators, lots and sites.<br>3. Test line intensity shall be measured in a graduated form to be able to detect reaction differences.<br>4. Each lot shall comprise different production (or manufacturing, purification, etc.) runs of critical reagents.<br>5. Results shall be statistically analysed by ANOVA or similar methods to identify and isolate the sources and extent of any variance.<br>6. The percentage of correctly identified, incorrectly-identified and invalid results shall be tabulated for each | EN 13612 (6) CLSI EP12-A2 (7) VIM (8) |

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## Part 1: Analytical Performance

### Technical Specifications for submission to WHO Prequalification – Diagnostic Assessment: Hepatitis C rapid diagnostic tests for professional use and/or self-testing

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| 2.     | Each panel member shall be tested:  
• in 5 replicates per test  
• over 5 days (not necessarily consecutive) with one run per day (alternating morning/afternoon)  
• repeated in total with 3 different lots (at least 2 lots should be tested at each of the sites)  
• tested at each of 3 different sites  
3.     | The effect of operator-to-operator variation on IVD performance should be included as part of the precision studies (see also note 8). Testing should be done:  
• by personnel representative of intended users; and  
• unassisted.  
4.     | Testing shall be conducted using only those materials provided with the RDT (e.g. IFU, labels and other instructional materials).  
specimen and be separately stratified according to each site, lot, etc. This type of analysis is especially important for RDTs that may not have results with any numerical values.  
7.     | To understand manufacturing irregularities in results obtained, at least 2 lots should be tested at each of the 3 testing sites. (3 different lots are required to be tested overall across the 3 testing sites)  
8.     | The effect of operator-to-operator variation on RDT performance may also be considered as a human factor when designing flex studies (see 1.9.1 flex studies) and may be addressed as part of clinical studies in representative populations (see Part 2, 3).  
9.     | Users shall be selected based on a pre-determined and contextually appropriate level of education, with literacy and auxiliary skills that will challenge the usability of the RDT and reflect the diversity of intended users and operational settings. These characteristics shall be detailed in the study report.  
| 1.5 Analytical sensitivity |  
1.5.1 Limit of detection for HCV core antigen or antibody |  
1. | Analytical sensitivity shall be determined relative to the international standards or to secondary standards metrologically traceable to it:  
• WHO 1st International Standard, 2014 for HCV Core antigen PEI code 129096/12;  
• The determination should comprise a minimum of 20 replicate tests of an 8-member dilution panel  
2. | For analytes for which no international standards exist, analytical sensitivity shall be determined | WHO TRS 1004 (9)  
1. | For the international standard, the result shall be expressed in international units as an analytical end-point sensitivity with its associated metrological uncertainty.  
2. | If the listed international standards are not available any more, then the version of the international standard used shall be stated.  
3. | End-point titres and interlot variation must be evaluated by appropriate statistical means. |
### Part 1: Analytical Performance

**Technical Specifications for submission to WHO Prequalification – Diagnostic Assessment**

**Hepatitis C rapid diagnostic tests for professional use and/or self-testing**

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<tr>
<td><strong>1.6 Analytical specificity</strong></td>
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</table>
| **1.6.1 Potentially interfering substances** | | | CLSI EP07-A3 (10)  
CLSI EP37 (11)  
ISO 14971 (12)  
Mane A, et al (13)  
Gifford JL et al (14)  
Trambas CM,et al (15) |
| 1. The potential for false results (false-negatives and false-positives) arising from interference from, at least, the substances/conditions listed below shall be determined (see note 1):  
• using a minimum of 100 specimens;  
• with each substance represented by at least 5 to 10 specimens from different individuals. | 1. The risk assessment conducted for the RDT should identify substances/conditions where the potential for interference can reasonably be expected for the analyte being detected in the areas of intended use and not simply rely on published lists of such compounds and conditions which might be of limited relevance in resource limited settings (and overlook those which might be of relevance).  
• By conducting appropriate risk assessment, testing can be performed on the substances or conditions identified as likely to be significant and testing of potentially irrelevant substances/conditions can be avoided. | |
| 2. Testing shall be undertaken in both HCV-negative and HCV antibody or core antigen weak reactivity positive (see note 2), un-spiked or spiked with each potentially interfering substance at physiologically relevant levels or medically relevant dosages. | 2. Interference studies should be performed with specimens with an analyte response near the RDT LOD (approx. 1-2 x RDT’s LOD).  
3. The methods and concentrations used shall be validated so that any effect of clinical importance would be detected.  
4. Any observed interference shall be investigated and performance limitations of the RDT reported in the IFU. | |
| **1.6.1.1 Endogenous** | | | |
| 1. Human antibodies to the expression system (for recombinants), e.g. anti-<i>Escherichia coli</i> (anti-<i>E. coli</i> positive), human anti-mouse antibody (HAMA).  
2. Recipients of multiple blood transfusions.  
3. Pregnant (including multiparous) women.  
5. Elevated immunoglobulin concentrations.  
6. Rheumatoid factor.  
7. Sickle-cell disease. | | |
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<td>1.6.2</td>
<td><strong>Cross-reactivity</strong></td>
<td>The potential for false-positive results arising from cross-reactivity (see note 1) should be determined for a minimum of 100 specimens, including, at least 5 to 10 of each:</td>
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<tr>
<td>1.6.1.2</td>
<td>Exogenous</td>
<td>1. Medicines, relevant to the populations intended to be tested including: interferon, direct acting antivirals, antiparasitic, antimalarial and antituberculosis medicines.</td>
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<td></td>
<td></td>
<td>2. Common over-the-counter analgesic medications (aspirin, paracetamol).</td>
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<td>3. Ethanol, caffeine, biotin (see note 7).</td>
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<td>8.</td>
<td>Other autoimmune conditions including systemic lupus erythematosus anti-nuclear antibodies (ANA), and autoimmune hepatitis type 2.</td>
<td>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. Any effect must be evaluated against the probability of that effect occurring and causing clinically significant issues in the population tested in resource limited settings. Evaluation of endogenous interfering substances may be addressed as part of the clinical studies but the number of specimens of each type evaluated shall be in accord with the requirement in this section. If biotin is commonly used as a supplement and the technology of the test employs streptavidin, then biotin levels of up to 1200 ng/ml should be tested as part of this study. In addition to the substances listed here, RDTs that are used to test oral fluid shall consider the effect of oral infections, such as Candida, as well as tobacco, mouthwash, concomitant medications, dental fixtures, toothpaste, food or drink (consumed immediately prior to testing), consumption of alcohol and teeth brushing.</td>
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<tr>
<td>5.</td>
<td>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.</td>
<td>• Any effect must be evaluated against the probability of that effect occurring and causing clinically significant issues in the population tested in resource limited settings.</td>
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<td></td>
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<td>6. Evaluation of endogenous interfering substances may be addressed as part of the clinical studies but the number of specimens of each type evaluated shall be in accord with the requirement in this section.</td>
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<td></td>
<td></td>
<td>7. If biotin is commonly used as a supplement and the technology of the test employs streptavidin, then biotin levels of up to 1200 ng/ml should be tested as part of this study.</td>
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<tr>
<td></td>
<td></td>
<td>8. In addition to the substances listed here, RDTs that are used to test oral fluid shall consider the effect of oral infections, such as Candida, as well as tobacco, mouthwash, concomitant medications, dental fixtures, toothpaste, food or drink (consumed immediately prior to testing), consumption of alcohol and teeth brushing.</td>
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## Part 1: Analytical Performance

### Technical Specifications for submission to WHO Prequalification – Diagnostic Assessment: Hepatitis C rapid diagnostic tests for professional use and/or self-testing

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<td>3. Specimens from patients with non-viral hepatitis.</td>
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<td>2. Any observed cross-reactivity shall be investigated and performance limitations of the RDT reported in the IFU.</td>
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<td>4. Acute cytomegalovirus, acute Epstein–Barr virus, varicella zoster virus, herpes simplex virus, human papillomavirus.</td>
<td></td>
<td>3. For RDTs that are not specific for immunoglobulin G (IgG), but also detect immunoglobulin M (IgM), it is important to include IgM positive specimens as this isotype is known to be less specific.</td>
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<td>5. HIV 1 and 2, and Individuals with HIV co-infection, both those who are virally suppressed and not virally suppressed.</td>
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<td>7. Other unrelated conditions known to cause cross-reactivity in HCV RDTs.</td>
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<td>8. Recent vaccinations for example against: influenza, hepatitis B virus, yellow fever.</td>
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### 1.7 High dose hook effect

#### 1.7.1 High dose hook effect for antibody detection RDTs

- The potential for a prozone/high dose hook effect shall be determined:
  1. Using multiple, highly-reactive natural specimens (minimum of 20).
  2. Using at least 2 different concentrations (diluted by at least a factor of 10).
  3. Using at least 1 lot of the IVD.
  4. Specimens shall be chosen that have a high analyte concentration, as determined using an IVD method other than the RDT intended to be prequalified e.g. by concordance with a stringently approved enzyme immunoassay or molecular assay. These second methods shall be of a design not subject to competitive inhibition.
  5. If there is evidence of competitive inhibition, this information shall be added to the IFU and mitigation actions identified.

#### 1.7.2 High dose hook effect for core antigen

- The potential for a prozone/high dose hook effect shall be determined:
  1. Using multiple, highly-reactive specimens (minimum of 20).
  2. Specimen selection may be guided by a molecular HCV assay.

Butch, AW (16)  
TGS-6 (17)
### Part 1: Analytical Performance

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**TSS-7**

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<td>detection RDTs</td>
<td>2. Using at least 2 different concentrations (diluted by at least a factor of 10). 3. Using at least 1 lot of the IVD.</td>
<td>2. If there is evidence of competitive inhibition, this information shall be added to the IFU and mitigation actions identified.</td>
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#### 1.8 Validation of the assay procedure

**1.8.1 Validation of reading times**

1. For RDTs where a reading interval is specified, validation of critical time points shall be provided:
   - the result at the minimal allowable time, and
   - the result at the maximal allowable time shall be recorded
2. Performance studies shall be conducted at the extremes of the intended operational temperature range; the effect of humidity on reading times shall also be investigated.
3. Specimen panel to be tested shall be as follows:
   - non-reactive specimen;
   - 1 HCV antibody or core antigen weak reactivity positive specimen (approx. 1-2 x RDT’s LOD);
   - 1 medium reactivity positive specimen (approx. 2-3 x RDT’s LOD);
   - in 3 replicates;
   - the panel shall include whole blood and anticoagulated plasma (e.g. EDTA) if claimed.

**1.8.2 Validation of the control line or dot**

1. The RDT shall have a procedural control.
   - The nature of the procedural control (specimen addition or only reagent addition) shall be explained.

1. The ranges of humidity tested for shall be risk-based, taking into consideration likely operational settings.
2. The intended operating temperature, upon which reading time has been validated, shall be clearly stated in the IFU.
3. The studies should consider possible differences between use of freshly made devices and those stored until near the end of their assigned shelf-lives under the conditions expected in resource limited settings and being used under those conditions.
4. Some of these aspects may be evaluated within section 1.9 Usability/human factors studies.

Source documents:

- PQDx_018 (3)
- IMDRF IVD MA ToC (18)
### Part 1: Analytical Performance

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- **Hepatitis C rapid diagnostic tests for professional use and/or self-testing**

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<td>1.8.3  Validation of controls</td>
<td>If control materials (positive controls) are provided, the controls materials should be validated as showing that if the RDT would not meet the claims, that the positive control will indicate the failure.</td>
<td>1. If control materials (positive controls) are provided, the controls materials should be validated as showing that if the RDT would not meet the claims, that the positive control will indicate the failure.</td>
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</table>
| 1.8.4  Establishment of reader cut-off | In RDTs provided with a reader, the way in which the reader has been designed to differentiate positive specimens from negative specimens shall be described in detail and demonstrated:  
- if both manual and automated digital read-out versions of the reader are available, equivalence of the 2 modes should be demonstrated. | 1. If the manufacturer supplies a reader for use with the IVD, safety and performance data shall be provided in the dossier with and without the use of the reader. |  |
| 1.9 Usability/human factors | 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. | 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 specimen shall reflect the most challenging matrix claimed in the IFU (e.g. oral fluid, whole blood).  
3. The factors listed opposite should be investigated in ways that not only reflect, but also exceed, likely operating conditions in low- 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, temperature ranges should be investigated that exceed those of claimed operating conditions and which cause test failure (incorrect/invalid results). | PQDx_018 (3)  
U.S FDA (19-21)  
Page | 19
### Aspect: Testing requirements

| Testing requirements                                                                                                                                                                                                 | Notes on testing requirements                                                                                                                                     | Source documents |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|
| • permanence of component labels: print legibility, adhesiveness;                                                                                                                                                     | 4. The impact of lighting can be twofold – i.e. the impact of lighting on packaging e.g. fading, and the sufficiency of lighting to read the test lines.              | ISO 23640 (22)                                                                 |
| • effects of lighting and humidity (See note 3);                                                                                                                                                                      | 5. The factors should be investigated using “designed experimentation” so that potential critical interactions between them can be understood e.g. the effect of low or high operating temperature with low or high volume of specimen at an incorrect reading time. | CLSI EP25-A (23)                                                                 |
| • residual volumes and characteristics of liquids (potential evaporation, pH changes, microbial growth, antimicrobial efficacy).                                                                                 |                                                                                                                                                                  | TGS-2 (24)                                                                                          |
| 3. Review of instrumentation (if applicable and based on a risk assessment) including:                                                                                                                                  |                                                                                                                                                                  | ASTM D4169-16 (25)                                                                                 |
| • ruggedness (see note 4);                                                                                                                                                                                                 |                                                                                                                                                                  |                                                                                                   |
| • impact of dust and mould on componentry (e.g. optics if applicable);                                                                                                                                                  |                                                                                                                                                                  |                                                                                                   |
| • software validation, if instrument read mode.                                                                                                                                                                         |                                                                                                                                                                  |                                                                                                   |

### 1.10 Stability of the IVD

#### 1.10.1 Shelf-life (including transport stability)

1. Stability studies shall be conducted using the conditions expected in the environment of intended use.
2. Replicate testing (n=3) shall be undertaken using a panel of spiked specimens of at least:
   - 1 non-reactive specimen;
   - 2 weak reactivity specimens, (approx. 1 - 2x RDT’s LOD);
   - 1 medium reactivity specimen (approx. 2 – 3 x RDT’s LOD).
3. Wherever possible, specimens chosen for the testing panel shall include panel members that reflect the main specimen types intended for use with the RDT (e.g. capillary whole blood/oral fluid, as appropriate).
4. A minimum of 3 lots in final packaging.
5. The testing panel shall include all claimed critical epitopes, for example HCV core antigen, NS3, NS4 and NS5 as verified by 3rd or 4th generation HCV antibody assays and currently available HCV core antigen assays and include whole blood and other specimen types, in accordance with intended use (for example to verify proper flow, no background interference and account for other variables).
6. Each lot should comprise different production (or manufacturing, purification, etc.) runs of critical reagents.
7. The numbers of invalid tests with each kit lot shall be reported.
8. 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
### Part 1: Analytical Performance

#### Technical Specifications for submission to WHO Prequalification – Diagnostic Assessment: Hepatitis C rapid diagnostic tests for professional use and/or self-testing

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<td>5. Lots shall be subjected to simulated “transport stress” before real time studies are undertaken on these lots. This mimics the real situation.</td>
<td>bulk of lots will be expected to meet the claimed life. For example: for testing conducted at 3, 6, 9, 12 and 15 months, if stability was observed at 15 months, then the maximum stability claim can be 12 months. 5. Accelerated studies do not replace the need for real time studies.</td>
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<td>1.10.2 In-use stability (open pack/open vial)</td>
<td>1. There shall be evidence that once the unit is removed from its primary packaging, it is stable at the expected temperature and humidity ranges for a defined period of time at the beginning and end of its assigned shelf-life. 2. Testing shall be performed: • of all labile components (e.g. buffers vials, etc.) (see note 1); • liquid components, once opened, shall have a validated life and number of stated uses under environmental (including microbial) conditions expected. • using a minimum of 1 lot 3. The test panel shall include at least: • 1 non-reactive specimen; • 2 weak reactivity specimens (approx. 1 - 2x RDT’s LOD); • 1 medium reactivity specimen (approx. 2 - 3 x RDT’s LOD).</td>
<td>1. In-use stability of labile components shall be conducted using components in their final configuration. 2. In-use stability should be conducted with lots at the beginning and end of their shelf-lives.</td>
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<td>1.11 Performance panels</td>
<td>1. A minimum of 25 well characterized (note 4) or commercial HCV seroconversion panels shall be tested.</td>
<td>1. Panels should start with negative bleeds, have a narrow bleeding interval to cover the seroconversion</td>
<td>European Commission</td>
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### Part 1: Analytical Performance

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| 2.     | At least 30 early seroconversion specimens (see note 2 and 5) | period and should also cover the whole window period.  
2. Testing shall be undertaken in at least NS3 first seroconversion specimens and core antigen first seroconversion specimens.  
3. Seroconversion sensitivity shall be reported to the user in the IFU.  
4. Previous characterization of seroconversion samples shall demonstrate that the specimens are  
   • recognized by all HCV enzyme immunoassay antibody tests (published in the supplier’s data sheets);  
   • positive or indeterminate in confirmatory tests (HCV immunoblot).  
5. Early seroconversion shall be core antigen or HCV RNA-positive and/or anti-NS3.  
6. Where a claim is made for detection of specific critical HCV epitopes, performance characteristics should be determined in each epitope claimed. This may be avoided if the manufacturer demonstrates that each epitope system is equivalent. The estimate of analytical sensitivity should be confirmed by separately testing an additional 20 replicates.  
Testing should be conducted using more than 1 lot of the RDT. | decision on CTS (2)  
CLSI EP12-A2 (7)  
TGS-6 (17) |

1.11.2 Genotype panels  
Testing of WHO International Reference Preparations and/or commercial HCV worldwide genotype panels shall include (if available):  
• genotypes 1-4 and 6: at least 10 samples per genotype (including non-a subtype of genotype 4);  
1. Testing should be performed using a minimum of 2 different lots. |
### Aspect | Testing requirements | Notes on testing requirements | Source documents
--- | --- | --- | ---
|  | • genotype 5: at least 3 samples; | 2. All genotype specimens, confirmed as positive by HCV genotyping tests (stringently regulated by a GHTF founding member 13) shall be detected by the RDT.  
• Genotypes listed in the certificate of analysis do not require confirmation by reference methods.  
3. All reasonable attempts shall be made to test rare subgenotypes. |  

---

### Part 2a: Clinical evidence for HCV antibody detection RDTs (clinical performance characteristics)

| Aspect                                      | Testing requirements                                                                                                                                                                                                 | Notes on testing requirements                                                                                                                                                                                                 | Source documents |
|----------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|
| **2a.1 Diagnostic sensitivity and specificity** | **1. Diagnostic sensitivity and specificity shall be determined for each claimed specimen type.**  
**2. Testing shall be conducted:**  
- at different geographical settings (minimum of 2 regions) representing different genotypes;  
- by a variety of intended users (see note 1) in the intended testing settings;  
- using at least 2 different lots (see note 2);  
**3. The specimens shall be collected from the intended use population and include specimens from different stages of infection** | **1. Prequalified RDTs are generally used by lay providers and health care workers. For WHO prequalification purposes, these should be considered as the intended user rather than a trained laboratory professional.**  
**2. Each lot shall comprise different production (or manufacturing, purification, etc.) runs of critical reagents, representative of routine manufacture.**  
**3. A separate specimen shall be collected to have sufficient volume to establish the reference result. The testing algorithm used to determine the reference results shall include a state of the art method such as 4th generation immunoassay (detecting antibodies and core antigen of HCV) as applicable, with all initially reactive specimens reflexed for full characterization of the HCV status.**  
**4. Characterization of antibody positive specimens shall be undertaken using HCV immunoblot (if available).**  
**5. Criteria for the selection of archived specimens shall be explained. Archived samples shall be randomized and blinded for testing.**  
**6. Consideration shall be given to the influence of antiviral medications in a specimen on the serostatus of such specimens, and how this might affect specimen selection.**  
**7. Problematic specimens, those with unexpected results but which otherwise meet selection criteria** | **Source documents** |
### Part 2a: Clinical evidence (HCV antibody RDT)

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**Hepatitis C rapid diagnostic tests for professional use and/or self-testing**

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<td>for a study, shall not be systematically excluded from analysis.</td>
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<td>8.</td>
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<td>If sample is available in sufficient volume, all discrepant results (between assay under evaluation and the reference results) shall be repeated using the same lot, and then on all available lots and the variability noted.</td>
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<td>9.</td>
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<td>The protocol should specify the criteria for unbiased patient selection with associated risk analysis but in general there should be no exclusions except for ethical reasons.</td>
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<td>- The patients should be classified, and results analysed accordingly (e.g. first time or repeat blood donors, concomitant infections, age, gender, medications taken, including direct acting antivirals agents, recent vaccinations).</td>
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<td>10.</td>
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<td>Performance characteristics shall be reported using initial results, only. The results of further testing of specimens with discrepant results shall be reported separately as additional information about RDT performance.</td>
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<td>11.</td>
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<td>Inconclusive results (where the results on a test cannot be definitively determined as either HCV antibody/HCV core antigen positive or negative) shall not be systematically excluded from the denominator data for analysis.</td>
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<td>12.</td>
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<td>All invalid test results shall be recorded.</td>
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<td>13.</td>
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<td>Estimates of diagnostic/clinical sensitivity and specificity shall be reported with 95% confidence intervals.</td>
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| 2a.1.2 Diagnostic sensitivity | Testing of:  
1. At least 400 subject specimens confirmed HCV antibody positive;  
2. Consolidation of results from archived specimen collections and clinical evaluation studies is permissible. | 1. At least 50% of the results from which the diagnostic sensitivity is calculated shall be from fresh specimens for each of the specimen types claimed in the assay intended use, with the exception of venous whole blood and capillary blood specimens.  
   • Specimen/specimen types should reflect intended use/POC setting.  
2. Whole blood specimens shall always be required to be freshly collected. | European Commission decision on CTS (2) |
| 2a.1.3 Diagnostic specificity | 1. Testing of at least 1000 HCV antibody and core antigen negative specimens. | 1. At least 80% of the results from which the diagnostic specificity is calculated shall be from fresh specimens for each of the specimen types claimed in the assay intended use, with the exception of venous whole blood and capillary blood specimens.  
2. Specimen/specimen types should reflect intended use/POC setting. If the RDT claim is for aid for diagnostic use, blood bank specimens will be insufficient. | European Commission decision on CTS (2) |
### Part 2b: Clinical evidence for HCV antigen detection RDTs (clinical performance characteristics)

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| 2b.1 Diagnostic sensitivity and specificity | 1. Diagnostic sensitivity and specificity shall be determined.  
2. Testing shall be conducted:  
   • in specimens from different geographical settings (minimum of 2 regions, see note 1)  
   • by a variety of intended users (see note 1) in the intended testing settings;  
   • in a minimum of 2 lots.  
3. Reactive, discrepant and unexpected results shall be fully evaluated. | 1. Prequalified RDTs are generally used by lay providers and health care workers. For WHO prequalification purposes, these should be considered as the intended user rather than a trained laboratory professional.  
2. Specimens for testing include:  
   • freshly taken, unfrozen routine specimens stored as described in the IFU should be used for determination of specificity and sensitivity (see note 2b.1.2 and 2b.1.3);  
   • appropriately stored, well characterized sera that have not undergone more than one freeze-thaw cycle may also be used for clinical evaluation testing if necessary assuming that such specimens have been validated during analytical studies (see section 1.1);  
   • if necessary, the sensitivity for antigen detection may be verified using specimens archived at the chosen clinical sites assuming that such stored/archived specimens have been validated during analytical studies (see section 1.1)  
   • at collection, an aliquot of an appropriate specimen type should be frozen at -70°C for use should HCV RNA testing be required.  
3. Criteria for the selection of archived specimens shall be explained. Archived samples shall be randomized and blinded for testing.  
4. The protocol should specify the criteria for unbiased patient selection with associated risk analysis but in |
### Part 2b: Clinical evidence (HCV antigen RDT)

#### Technical Specifications for submission to WHO Prequalification – Diagnostic Assessment:
Hepatitis C rapid diagnostic tests for professional use and/or self-testing

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<td>General there should be no exclusions except for ethical reasons:</td>
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<td>• the patients should be classified, and results analysed accordingly (e.g. concomitant infections, age, gender, medications taken, recent vaccinations).</td>
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<td>5. All specimens shall be characterized using a reference HCV RNA test.</td>
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<td>• all specimens shall be subjected to full characterization of their HCV status.</td>
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<td>• all initially reactive specimens on RDT under evaluation shall be subjected to full characterization of their HCV status;</td>
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<td>• the algorithm shall include a reference HCV RNA assay;</td>
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<td>• frozen specimens, if used for verification of diagnostic sensitivity of antigen detection, shall be (or have been) similarly characterized.</td>
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<td>6. Performance characteristics shall be reported using initial results only. The results of further testing of specimens with discrepant results shall be reported separately as additional information about RDT performance.</td>
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<td>7. Inconclusive results (where the results on a test cannot be definitively determined as HCV core antigen positive or negative) shall not be systematically excluded from the denominator data for analysis.</td>
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<td>8. All invalid test results shall be recorded.</td>
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<td>9. Estimates of diagnostic/clinical sensitivity and specificity shall be reported with 95% confidence intervals.</td>
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## Part 2b: Clinical evidence (HCV antigen RDT)

### Technical Specifications for submission to WHO Prequalification – Diagnostic Assessment:
**Hepatitis C rapid diagnostic tests for professional use and/or self-testing**

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| 2b.1.2 Diagnostic sensitivity        | 1. Testing of at least 400 subject specimens confirmed HCV antigen positive           | 1. At least 50% of the results from which the diagnostic sensitivity is calculated shall be from fresh specimens for each of the specimen types claimed in the assay intended use, with the exception of venous whole blood and capillary blood specimens.  
- Specimen/specimen types should reflect intended use/POC setting  
- Whole blood specimens shall always be required to be freshly collected. |                  |
| 2b.1.3 Diagnostic specificity        | 1. Testing of at least 1000 HCV antibody and core antigen negative specimens.         | 1. At least 80% of the results from which the diagnostic specificity is calculated shall be from fresh specimens for each of the specimen types claimed in the assay intended use, with the exception of venous whole blood and capillary blood specimens.  
- Specimen/specimen types should reflect intended use/POC setting  
- Whole blood specimens shall always be required to be freshly collected. |                  |
## Part 3a: Qualification of usability for professional use

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<td>3a.1.1 Label comprehension study</td>
<td>1. Testing shall be undertaken to assess the ability of intended users to correctly comprehend key messages from packaging and labelling: • understanding key warnings, limitations and/or restrictions; • proper test procedure; • test result interpretation; • using only the information available to all users (IFU and any job aid). 2. Studies shall include: • at least 15 intended users including those whose native language may not be the language of the IFU if necessary; • in their usual working environment, not employees of the manufacturer; • from 2 geographically diverse populations to demonstrate comprehension of key messages in each user group.</td>
<td>1. If the labelling is available in different languages, the labelling comprehension study should be performed for each language. 2. Requirements listed may be investigated as separate studies or included as part of clinical studies. 3. Testing may be conducted using questionnaire-based surveys.</td>
<td>European Parliament IVD regulations (26) Center for Devices and Radiological Health CLIA waiver, USFDA (20, 21)</td>
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| 3a.1.2 Results interpretation study | 1. Intended users shall interpret the results of contrived RDTs (e.g. static/pre-made tests) to assess their ability to correctly interpret predetermined test results. 2. Contrived RDTs shall be made to demonstrate the following potential test results: • non-reactive; | 1. The contrived tests shall be prepared by persons different from those reading the results. The tests shall be randomized prior to the users reading the results. | |
### Part 3a: Qualification of usability for professional use

#### Technical Specifications for submission to WHO Prequalification – Diagnostic Assessment:
Hepatitis C rapid diagnostic tests for professional use and/or self-testing

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<td>• range of invalid results;</td>
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<td>• reactive;</td>
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<td>• weak reactive.</td>
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<td>3.</td>
<td>Testing subjects shall consist of:</td>
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<td>• at least 15 intended users, including those whose native language may not be the IFU language;</td>
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<td>• in their usual working environment, not employees of the manufacturer;</td>
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<td>from 2 geographically diverse populations to demonstrate correct interpretation of simulated test results.</td>
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Part 3b: Qualification of usability for self-testing

3b.1 Purpose of qualification of usability for self-testing:
Assessment of product design, labelling and usability of rapid diagnostic tests (RDTs) for self-testing by analysis of the following:

- Results of a questionnaire to assess whether the key messages and instructions from packaging and labelling are understood and easily followed by untrained intended users (i.e. self-testers) including whether the test is appropriate for use by that individual (understanding the limitations of the assay, for example it is not for use in monitoring or evaluation of treatment with direct acting antiviral agents).

- Results of the interpretation of test-results study in untrained intended users (i.e. self-testers) of simulated RDTs (e.g. pre-made and with contrived results).

- Test results and interpretations when the assay is performed by untrained intended users (i.e. self-testers), in an observed setting.

- Evaluation of the actions taken by lay users on completion of the test (e.g. an understanding of the meaning of a negative test and that a positive test requires professional verification).

3b.1.1 Additional points:

- Manufacturers are encouraged to conduct a small-scale human factors study prior to starting the full validation.

- For each of the studies summarized below the study group shall comprise untrained subjects whose age, gender, level of education, literacy and additional, supplementary skills can challenge the usability of the IVD in intended users.

- These assessment activities will determine the changes needed to optimize the IVD for use by self-testers. Changes may range from minor (simplification of instructions for use without change to the method) to major (e.g. change to the method of specimen collection). The impact of any change on safety and performance shall be determined for both professional and lay use.

- Results from any one of the stages summarized below may indicate that assay redesign is necessary. This may in turn result in a need to revalidate the IVD or to perform additional specific performance studies and to update the risk analysis.

- Use by lay users may necessitate changes in the packaging, including changes to volumes of liquids, the printing on the outer and inner packaging and the number of devices and hence to the size of the individual primary and secondary packaging. In these
cases, the effect on stability and transport ruggedness shall be assessed (refer to “TSS-7 Rapid diagnostic tests to detect hepatitis C antibody or antigen” Part 1: Analytical performance and other evidence).

- In the observed untrained user study, the test result of the venous whole blood specimen shall be used to link participants to clinical management.

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<td><strong>3b.2 Qualification of usability (self-testing) testing requirements</strong></td>
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| **3b.2.1. Labelling comprehension study** | 1. Questionnaire-based testing of subjects representative of intended users, to assess the ability of such users to correctly comprehend key messages from packaging and labelling with regard to:  
   - proper self-selection (whether users understand if it is appropriate for them to undertake testing);  
   - understanding key warnings, limitations and/or restrictions;  
   - proper test procedure;  
   - test result interpretation.  
2. Questionnaire shall be administered to at least 200 subjects, representative of intended users, in order to demonstrate comprehension of key messages, see note 2. | 1. Labelling shall be clear and easy to understand; instruction material shall include pictures, quick guides or job aids.  
   - Additional resources such as a QR code linking to a demonstration video in local language(s) is encouraged.  
2. Some of the subjects ideally should be naïve to testing with RDTs, whereas others have been tested professionally/self-testing for e.g. malaria, hepatitis C, or HIV.  
   - Subjects who are trained in laboratory procedures/have experience with laboratory techniques shall be excluded.  
   - Subjects shall be from low and high prevalence settings and from both low and high-risk groups (e.g. MSM, PWID), for HCV from those settings.  
   - The range of subjects shall include statistically meaningful numbers of all intended users: by e.g. educational level, economic status, gender, age.  
3. Subjects identify that a positive test can mean either current or past (naturally cleared or treated) HCV | European Commission decision on CTS (2)  
ISO 18113-1:2009 (27)  
IEC 62366-1:2015 (29)  
MHRA (30)  
European Parliament and European Council IVDD (26)  
Center for Devices and Radiological Health CLIA waiver, USFDA (20, 21) |
### 3b.2.2 Results interpretation study

1. A minimum of 400 subjects to interpret the results of contrived IVDs (for example, static/pre-made tests) to assess their ability to correctly interpret pre-determined test result (see note 2). Contrived tests shall be made to demonstrate the following potential test results:
   - non-reactive;
   - range of invalid results;
   - reactive;
   - weak reactive (the colour intensity of the line shall be faint and resemble a real clinical test line with reactivity close to that of the assay cut-off).
   - failed control lines

2. Study group to consist of at least 200 subjects in approximately equal numbers from 2 high-prevalence (>2%) and geographically diverse populations, and at least 200 subjects from a low-prevalence (<2%) population to demonstrate correct interpretation of simulated test results.

### 3b.2.3 Observed untrained user study

1. Testing by at least 900 self-testing subjects comprising at least 200 self-testers in each of two high-prevalence (>2%) geographically diverse populations, and at least 500 self-testers from a low-prevalence (<2%) population. (see note 1)
   - Each subject to self-collect test specimen and perform test according to only those

### Notes on testing requirements

1. The study group may include subjects recruited as part of the labelling comprehension study (see also note 2 of requirement 3b.2.1).
2. The test and control lines shall be static and stable. The colour intensity grading of the lines shall not change over time.

### Source documents

Center for Devices and Radiological Health, USFDA (30)
### Part 3b: Qualification of usability for self-testing

**Technical Specifications for submission to WHO Prequalification – Diagnostic Assessment:**

**Hepatitis C rapid diagnostic tests for professional use and/or self-testing**

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<td>materials to be provided with the IVD (e.g., instructions for use, labels and other instructional materials).</td>
<td>• Concordance between professional result and self-testing result on the RDT shall be reported.</td>
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<td>2. Each such test to be observed by a trained laboratory or health-care professional, see note 4. The observing professional shall not tutor nor interact with the subject conducting the test but shall note errors and other observations about the self-tester.</td>
<td>3. A separate venous whole blood specimen shall be collected prior to testing to establish the reference results for HCV status, both reactive and non-reactive. The testing algorithm used to determine the reference results shall include use of a state-of-the-art immunoassay, with all initially reactive specimens subject to the confirmation algorithm used for professional validation of the IVD.</td>
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<td>• Observation may also be conducted by viewing a video recording of self-testing.</td>
<td>• Concordance between the self-test RDT result and the reference method result shall be reported.</td>
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<td>• Particular attention shall be paid to documenting the subjects’ compliance with each of the factors raised during risk assessment (ISO 14971) of the process, see note 6.</td>
<td>4. For WHO purposes, the term “professional use” encompasses a diversity of skills, training and experience, and does not necessarily imply “highest standard of skills, training and experience”.</td>
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<td>• User techniques and difficulties in operating the system and applying the sample shall be documented and reported (“human factors”)</td>
<td>5. There may be a likelihood of bias at the community level when simple sampling methodologies are applied. Efforts shall be made to avoid convenience sampling of people (subject) who already know they are HCV positive.</td>
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<td>• The observing professional shall also interpret the test result, in a blinded fashion and within the validated reading time stated in the instructions for use.</td>
<td>6. Factors likely to arise during risk evaluation could be for example</td>
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<td>• Paying attention to the instructions before starting;</td>
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<td>• Correct use and disposal of the specimen collection accessories e.g. lancet, swab;</td>
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<td>• Application of correct volumes to the IVD;</td>
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<td>• Use a timing device to read within the required times; • Correct assignment of invalid tests</td>
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Source documents:

- ...
F. Source documents


The Technical Specifications Series for submission to WHO Prequalification – Diagnostic Assessment set out appropriate performance evaluation criteria to meet prequalification requirements. Each Technical Specification provides information on the minimum performance requirements for WHO Prequalification that should be met by a manufacturer to ensure that their in vitro diagnostic medical device is safe and performs optimally.