Annex 5.7

PICO 6 - How to test (confirmation of HCV viraemia)

Diagnostic accuracy of HCV RNA tests to detect active HCV infection: a meta-analysis and review of the literature

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*Co-leaders of this review

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1. Executive summary

**Background:** Advances in hepatitis C virus detection technology create new opportunities for enhancing screening, referral and treatment. The purpose of this review was to determine the accuracy of qualitative NAT methods versus quantitative NAT methods for HCV RNA for detection and/or quantification to confirm active HCV infection.

**Method:** A literature search was conducted focused on hepatitis C, diagnostic tests and diagnostic accuracy. Studies were included if they evaluated an assay to determine the sensitivity and specificity of a single qualitative hepatitis C RNA test compared to a quantitative HCV RNA reference among humans. Two reviewers performed a quality assessment of the studies and extracted data for estimating test accuracy.

**Results:** Traditionally, qualitative nucleic acid amplification (NAT) assays are at least 10 times more sensitive than quantitative assays. This systematic review shows that for HCV, the lower limit of detection of most commercial qualitative assays was in the 10–15 IU/mL range measured against a WHO standard, whereas the lower limit of detection for quantitative assays is at 600–1100 IU/mL. This systematic review shows that the sensitivity of HCV viral quantitative assays range from 87% to 100% compared to qualitative assays.

**Conclusions:** Although HCV qualitative assays have a lower limit of detection than quantitative assays, the range of sensitivity found in this systematic review demonstrate that HCV viral loads are rarely in the lower range of the limit of detection of these quantitative assays. New technology platforms are now available which have linear range of quantitation between 12 and $10^8$ IU/mL, with the result that there is no longer any difference between the lower limit of detection of a qualitative assay compared to a quantitative assay. New point-of-care (POC) devices for quantitation of HCV viral load will soon be available. These devices are more affordable than the laboratory-based assays and can potentially be used to improve access to HCV detection and treatment monitoring.

2. Background

Hepatitis C is a liver disease caused by the hepatitis C virus (HCV) that causes acute and chronic infection. An estimated 130–150 million people have chronic hepatitis C infection worldwide, leading to 350 000–500 000 deaths per year. Although HCV treatment is successful in a majority of people, most HCV-infected individuals remain undiagnosed and untreated. As a result, approximately 15–30% of individuals with chronic HCV infection progress to cirrhosis, leading to end-stage liver disease and hepatocellular carcinoma.

Rapid detection of HCV is essential for prevention of the progression of the disease into the chronic phase. Qualitative nucleic acid testing (NAT) allows for a rapid and sensitive detection of the virus as well as evidence of viral RNA load falling below a clinical threshold. Quantitative testing is useful for measuring of viral burden and treatment response. Both methods are essential in the detection of active HCV infection, though there is scare research comparing the two NAT methods for this purpose.

In April 2014, the World Health Organization (WHO) published guidelines for the
screening, care and treatment of individuals with HCV infection. These guidelines included recommendations on who to screen for HCV and how to confirm HCV infection, but not which tests are optimal for initial screening. The World Health Assembly has passed several resolutions highlighting the importance of viral hepatitis for global health.

Advances in HCV detection technology create new opportunities for enhancing screening, referral and treatment. Previous systematic reviews on hepatitis C infection have focused on treatment response, clinical complications, and epidemiology. Two systematic reviews on hepatitis C testing focused on evaluating point-of-care tests compared to EIAs and other reference tests. This review instead focuses on individuals with detectable HCV antibodies to evaluate qualitative versus quantitative detection methods to confirm active HCV infection.

The purpose of this review was to identify evidence on the sensitivity and specificity of qualitative HCV RNA tests compared to quantitative HCV RNA tests for the detection of active HCV infection, to summarize the key test characteristics associated with detection of active HCV infection.

<table>
<thead>
<tr>
<th>PICO 6</th>
<th>Among HCV Ab positive patients, what is diagnostic test accuracy of qualitative NAT methods versus quantitative NAT methods for HCV RNA for detection and/or quantification to confirm active HCV infection?</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>Persons with detectable HCV antibodies</td>
</tr>
<tr>
<td>C</td>
<td>Quantitative NAT methods</td>
</tr>
</tbody>
</table>

3. Objectives
The purpose of this review was to identify evidence on the sensitivity and specificity of qualitative HCV RNA tests compared to quantitative HCV RNA tests for the detection of active HCV infection and to summarize the key test characteristics associated with detection of active HCV infection.
4. **Methodology**

We followed standard guidelines and methods for systematic review and meta-analyses of diagnostic tests.\textsuperscript{14,15} We prepared a protocol for the literature search, article selection, data extraction and assessment of methodological quality.

**Selection criteria**

**Types of studies**

We included observational and RCT studies that provide original data from patient specimens, including cross-sectional and case–control studies and studied qualitative NAT tests used to detect HCV RNA compared to a reference standard of quantitative HCV RNA tests.

**Participants**

Little information on participants was provided in the selection of papers included in the systematic review; therefore, we set a wide inclusion criterion. We included patients of all age groups from all settings and countries as well as all types of specimens.

**Index tests**

Studies that utilized a commercially available HCV NAT test were eligible for inclusion. The following seven are the index tests included:

- AMPLICOR HCV test, version 2.0, Roche
- CAP/CTM, Roche
- COBAS AMPLICORTM HCV Test v1.0 assay, Roche
- COBAS AMPLICORTM HCV Test v2.0 assay, Roche
- COBAS HCM-2, Roche
- Real-Time Assay, Abbott
- Versant HCV genotype assay, Bayer.

**Reference standard**

The reference standards accepted for a definitive diagnosis included tests for detection of HCV RNA by the following quantitative NAT techniques: polymerase chain reaction (PCR), branched-chain DNA (bDNA), or transcription mediated amplification (TMA). The performance characteristics of NATs are very similar above 50 IU/mL; thus all NATs were considered as one reference standard.

**Outcome measures**

Sensitivity refers to the proportion of samples with true HCV infection diagnosed with positive qualitative NAT tests confirmed with a positive quantitative NAT tests.
Specificity refers to the proportion of samples with negative qualitative NAT tests confirmed with a negative quantitative NAT tests.

**Search methods**

A database search of LILACS, MEDLINE, EMBASE, PubMed, Scopus, Web of Science, Cochrane and WHO Global Index Medicus was performed through April 2015. No language restriction was applied. The references of published articles found in the above databases were searched for additional pertinent materials.

Study selection proceeded in three stages. First, titles/abstracts were screened by a single reviewer according to standard inclusion and exclusion criteria. Second, full manuscripts were obtained and assessed against inclusion criteria. Papers were accepted or rejected and reasons for rejection were specified. Third, two independent reviewers assessed each manuscript and differences were resolved by a third independent reviewer.

**Data extraction**

Information on the following variables were extracted by a reviewer if the study met the exclusion and inclusion criteria: first author, total sample size, country (and city) of sampling, sample type (oral fluid, finger-prick, venous blood, etc.), point-of-care (Y/N), eligibility criteria, reference standard, manufacturer, raw cell numbers (true positives, false negatives, false positives, true negatives), sources of funding and reported conflict of interest. We define point-of-care as being able to give a result within 60 min and having the results guide clinical management at the same encounter.

**Assessment of methodological quality**

Study quality was evaluated using the QUADAS-2 tool, the STARD checklist and the GRADE method. QUADAS includes domains to evaluate bias in the following categories: risk of bias (patient selection, index test, reference standard, flow and timing); applicability concerns (patient selection, index test, reference standard). The GRADE method evaluates the strength of evidence by assessing the risk and probability of bias, imprecision and inconsistency as well as dose-respondent gradient and residual confounding.

5. **Results**

PRISMA flowchart

A total of 17 109 citations were identified and 9623 duplicates were removed. Each of the 7486 titles was examined according to pre-specified inclusion and exclusion criteria. A total of 4 research studies were included in the final analysis (Fig. 1 below).
Characteristics of included studies

A total of four studies met the PICO criteria and data was extracted from each of these studies. Two of the four studies took place in the United States of America, with the remaining two in Taiwan and Germany. Of these studies only one included a population of patients at risk of HCV infection, while the others were either patients who have an acute or chronic HCV infection. The assays evaluated in these analyses were Abbott Real-Time Assay, AMPLICOR
HCV test, v2.0 assay, COBAS AMPLICOR™ HCV Test v2.0 assay and Versant HCV genotype assay.

This systematic review shows that for HCV, the lower limit of detection of most commercial qualitative assays was in the 10–15 IU/mL range measured against a WHO standard, whereas the lower limit of detection for quantitative assays is at 600–1100 IU/mL. The sensitivities of qualitative NAT methods reported in the selected articles showed a relatively wide range (87–100%), contrary to the narrow range reported for specificity (97–100%).

A large limitation in the quality of the studies was a lack of information on the populations studied, randomization and sample collection as well as poor standardization in the evaluation of diagnostic test accuracy.

### Table 1. Description of study design, study population and setting of all studies (n=4)

<table>
<thead>
<tr>
<th>No</th>
<th>First author, country</th>
<th>Sample type and number</th>
<th>Study population</th>
<th>Diagnostic test (quantitative)</th>
<th>Reference test (qualitative)</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lee, 2000, United States of America</td>
<td>Serum N = Not stated</td>
<td>Patients at risk of HCV infection</td>
<td>AMPLICOR HCV test, version 2.0 Roche</td>
<td>COBAS AMPLICOR™ HCV Test v2.0 assay. Roche</td>
<td>94%</td>
<td>97%</td>
</tr>
<tr>
<td>2</td>
<td>Yu 2000, Taiwan</td>
<td>Serum N = 215</td>
<td>Patients with chronic hepatitis C</td>
<td>COBAS HCM-2 Roche</td>
<td>COBAS AMPLICOR™ HCV Test v2.0 assay. Roche</td>
<td>95%</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>Ferreira-Gonzalez, 2007, United States of America</td>
<td>Plasma N = 76</td>
<td>Patients with HCV infection</td>
<td>Versant HCV genotype assay, Bayer</td>
<td>COBAS AMPLICOR™ HCV Test v1.0 assay Roche</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

*CAP/CTM = Roche Cobas Ampliprep/Cobas Taqman HCV assay

Lee et al. (2000) investigated the performance characteristics of AMPLICOR HCV test, version 2.0 Roche (quantitative assay) and COBAS AMPLICOR™ HCV Test v2.0 assay Roche (qualitative assay). This was done by measuring the limit of detection, sensitivity, specificity, linear range, agreement between test formats and genotypic reactivity for both tests. The genotypic reactivity for both tests showed that samples with 10 copies per reaction yielded positive results at least 95% of the time for all genotypes with the exception of genotype 5. When testing for the agreement between test formats, it was shown that the COBAS AMPLICOR v.2 assay format produced values with a range of 0.02–0.13 log₁₀ higher than those obtained for the AMPLICOR assay with samples containing titres within the linear range of the assays.
Yu et al. (2000) investigated the performance characteristics of COBAS HCM-2 (quantitative assay) compared to the COBAS AMPLICORTM HCV Test v2.0 assay (qualitative) and evaluated the clinical utility of COBAS HCM-2. This study looked at quantitative range, reproducibility of COBAS HCM-2 and linearity of HCV RNA quantifications. The quantitative range for the COBAS HCM-2 assay from $1.0 \times 10^3$ to $3.88 \times 10^6$ copies/mL and the within-run reproducibility showed serum HCV RNA levels with standard deviations of 0.03, 0.09 and 0.12. The linearity of HCV RNA quantifications ranged from 6.11, 6.44, 6.46 to 6.49 logs for genotypes 1b, 2a, 2b and 1b.

Ferreira-Gonzalez et al. (2007) evaluated the qualitative (COBAS Taqman HCV) against the quantitative (Versant HCV genotype assay, Bayer) using the same clinical specimens. Analytical sensitivity was measured by the ability of a system to detect replicates, both tests were capable of detecting all six replicates with 10 HCV RNA IU/mL and 100% of all replicates with 1.0 \log_{10} HCV RNA IU/mL.

Sarrazin et al. (2008) focuses on the evaluation and comparison of performance characteristics of HCV qualitative (CAP/CTM Roche Cobas Ampliprep/Cobas Taqman HCV assay, Roche) and quantification (Versant HCV genotype assay, Bayer) methods. The study compared intra-assay variability, analytic sensitivity, limit of detection, HCV WHO standard RNA unitage, genotypic specific assay linearity. The intra-assay variability of the two assays varied from 0.72% to 1.3% for CAP/CTM assay and 1.4–3.02% for Versant HCV genotype assay. For sensitivity, the positive hit rates for WHO HCV RNA standards for the Abbott RealTime HCV assay were 87% at 15 IU/mL, where as for the CAP/CTM they were 100% at 15 IU/mL. The limit of detection was higher for RealTime HCV assay at 16.8 (95% CI; 13.1 to 27.9) than for CAP/CTM 10.3 (95% CI; 8.4 to 15.1). HCV WHO standard RNA unitage deviated between $-0.2 \log_{10}$ IU/mL at 3.2 $\log_{10}$ for RealTime HCV assay and $-0.3 \log_{10}$ IU/mL at 3.2 $\log_{10}$. For RealTime HCV assay and CAP/CTM assay, the quantification of HCV RNA of five different HCV genotypes was mostly linear between concentrations of $4.0 \times 10^3$ and $1.0 \times 10^6$ IU/mL though the sample harbouring genotype 4 showed lower results than expected at concentrations above $1.0 \times 10^4$ IU/mL.

**Narrative summary of each systematic review’s findings**

Traditionally, qualitative nucleic acid amplification (NAT) assays are at least 10 times more sensitive than quantitative assays. This systematic review shows that for HCV, the lower limit of detection of most commercial qualitative assays was in the 10–15 IU/mL range measured against a WHO standard, whereas the lower limit of detection for quantitative assays is at 600–1100 IU/mL.

Though very sensitive for determining the presence of virus, qualitative assays do not allow for determination of viral load. The sensitivity of qualitative assays makes them essential during screening blood donors and monitoring treatment progression as they are used to show the presence of virus as a marker of an on-going HCV infection. This systematic review showed sensitivities as low as 5 IU/mL for HCV NAATs. Verification of the presence of RNA is complicated due to the lack of a standardized commercial HCV assay with sufficient sensitivity that is capable of testing discrepant specimens at such low concentrations. This is
particularly important when qualitative assays are used prove the absence of HCV-RNA at the end of treatment (ETR) or at the end of follow up (EFU).\textsuperscript{22} Another use of qualitative assays is to discriminate sustained responders (SRs) from relapsers (RELs).\textsuperscript{23–25}

Despite the lower sensitivity, quantitative assays have been found to be a reproducible method to detect and quantify HCV RNA in plasma or serum.\textsuperscript{25} This systematic review also highlighted potential issues with accuracy as the level of imprecision in a number of quantitative tests (COBAS AMPLICOR HCV Monitor assay) was seen to be between a factor of 3–5 (0.5–0.7 log\textsubscript{10}) difference from the actual titre.\textsuperscript{26} These assays have been proven to be crucial in the measurement of the viral load at the start of therapy and after 12 weeks of treatment to decide about the usefulness of further treatment (stopping rule). These assays have also been proven to have a broad dynamic range of 615–7 700 000 IU/mL ([COBAS] AMPLICOR HCV Monitor assay, Roche). The ability of a quantitative tests to detect viral loads as low as 650 IU/mL allow them to measure early response to treatment as often viral loads drop rapidly at the start of interferon treatment. Viral loads seldom exceed the upper limit of this assay so that retesting of diluted samples is often unnecessary.\textsuperscript{27–28} This explains the reasoning behind laboratories in high-income countries employ HCV RNA quantitative assays in serum or plasma, notwithstanding its higher cost, as it can be used to monitor treatment efficacy and chronic HCV disease progression.\textsuperscript{29–32}

Since these studies were published, companies have been steadily improving the sensitivity of quantitative assays. Table 1 shows that currently 5 HCV quantitative assays are commercially available with another two in the pipeline (UNITAID Hepatitis C diagnostic technology landscape report, 2015).\textsuperscript{39} These assays have linear range of quantitation of 12–10\textsuperscript{8} IU/mL using plasma or serum and the time to result ranges from 70 min to 5–6 h. With these new technology platforms, there is no longer any difference between the lower limit of detection of a qualitative assay compared to a quantitative assay. From this systematic review, the finding of sensitivities of 87–100\% for a quantitative assay compared to qualitative assays demonstrates that viral loads are rarely in the lower range of the limit of detection of these older quantitative assays.

These new quantitative assays and the equipment are costly and can be prohibitive to control programmes. However, since other assays such as HIV viral load can be performed on these platforms, HCV control programmes can leverage investments made by HIV programmes for the procurement of these technologies. In resource-limited settings where these assays are not affordable, HCV antigen detection can be considered as a surrogate marker of ongoing virus replication.

Investments in the development of point-of-care (POC) devices that can be used to measure HIV viral load have yielded several technological platforms that can be used to quantitate HCV viral load. Table 2 shows that four companies have developed HCV quantitative assays that would soon be available with another three in the pipeline. These devices cost much less than the laboratory based instruments and can be used outside of laboratory settings as some of them can run on batteries. They are sample in-answer out type of technologies that will require minimal training and all have connectivity capacity so that surveillance for hepatitis C can be automated. The evaluation of these POC platforms will be
important to inform countries of whether these devices can be used to improve access to HCV detection and treatment monitoring.
### Table 2. Qualitative/quantitative HCV RNA platforms currently available (1–5) and soon to be available (6–7)

<table>
<thead>
<tr>
<th></th>
<th>Roche Molecular Systems (1)</th>
<th>Abbott Diagnostics (2)</th>
<th>Siemens Healthcare Diagnostics (3)</th>
<th>Sacace Biotechnologies (4)</th>
<th>QIAGEN (5)</th>
<th>Beckman Coulter (6)</th>
<th>Hologic Inc (7)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Qualitative assays</strong></td>
<td>COBAS Amplicor /COBAS TaqMan HCV Qualitative Test v.2 (LLOD: 15 IU/mL)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Quantitative assays</strong></td>
<td>COBAS Amplicor /COBAS TaqMan HCV Quantitative Test v.2</td>
<td>Abbott RealTime HCV Assay</td>
<td>VERSANT kPCR HCV RNA Assay</td>
<td>HCV Real-TM Quant Dx Assay</td>
<td>artus HCV QS-RCQ Kit</td>
<td>VERIS MDx</td>
<td>RT-TMA Technology for the Panther® System</td>
</tr>
<tr>
<td><strong>Linear range of quantitation, IU/mL</strong></td>
<td>15–10^6</td>
<td>12–10^6</td>
<td>15–10^6</td>
<td>13–10^6</td>
<td>35–1.77x10^4</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Sample type (mL)</strong></td>
<td>0.650 mL plasma/serum</td>
<td>0.5 mL plasma/serum</td>
<td>0.5 mL plasma or serum</td>
<td>1 mL plasma</td>
<td>1 mL plasma</td>
<td>plasma, serum</td>
<td>0.24 mL plasma</td>
</tr>
<tr>
<td><strong>Price of instrument (US$)</strong></td>
<td>COBAS Amplicor: 80 000–100 000 COBAS Taqman 48: 248 000 (45 000 + 162 000 + 80 000)</td>
<td>Pricing for the assay and instrument is available from Siemens</td>
<td>Pricing for the assay and instrument is available from Siemens</td>
<td>Pricing for the assay and instrument is available from Siemens</td>
<td>Pricing for the assay and instrument is available from Siemens</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td># Specimen/run and Time to result</td>
<td>24 specimens in 2 h, can process up to 72 samples at one time</td>
<td>96 samples at a time in about 3 h</td>
<td>89 samples per run with a total time to result of &lt;6 h</td>
<td>24 samples/run in 5–6 h</td>
<td>Continuous loading in batches of up to 24 samples plus internal controls</td>
<td>48 samples can be lined up on 12 racks; DNA tests takes ~70 min and RNA tests ~110 min</td>
<td>First results available 3 h after loading samples and five results after every 5 min thereafter. Samples can be continuously loaded, with up to 120 samples on the Panther® System</td>
</tr>
</tbody>
</table>

| Table 3. Point-of-care HCV RNA platforms in the pipeline |

<table>
<thead>
<tr>
<th></th>
<th>Alere</th>
<th>Molbio Diagnostics Pvt Ltd</th>
<th>Cepheid</th>
<th>Ustar Biotechnologies</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Quantitative Assays</strong></td>
<td>Alere q HCV VL</td>
<td>TrueLab Real Time micro PCR System</td>
<td>GeneXpert HCV quantitative assay</td>
<td>RT-CPA HCV Viral Load Test</td>
</tr>
<tr>
<td>Linear range of quantitation, IU/mL</td>
<td>–</td>
<td>–</td>
<td>$10^3$–$10^8$</td>
<td>$10^4$–$10^6$</td>
</tr>
<tr>
<td>Sample type (mL)</td>
<td>0.5–1 plasma</td>
<td>0.1 plasma</td>
<td>1 plasma/serum</td>
<td>0.1 blood</td>
</tr>
<tr>
<td>Cost/test (US$)</td>
<td>15–25</td>
<td>14 per chip; 2 per extraction</td>
<td>&lt;US$ 20</td>
<td>–</td>
</tr>
<tr>
<td>Price of instrument (US$)</td>
<td>–</td>
<td>8000</td>
<td>17 000</td>
<td>–</td>
</tr>
<tr>
<td>Time to result (min)</td>
<td>&lt;60</td>
<td>60</td>
<td>105</td>
<td>20–45 (&lt;500 IU/mL)</td>
</tr>
<tr>
<td>Other tests on platform</td>
<td>HIV, Ebola</td>
<td>MTB, HBV, dengue, chikungunya, HINI, malaria, HIV VL, HCV VL</td>
<td>HIV</td>
<td>–</td>
</tr>
</tbody>
</table>
Hepatitis C virus point-of-care diagnosis and treatment monitoring platforms: pipeline*

*Estimated as of September 2014 - timeline and sequence may change. ---- No market launch date set by company.
References

A. Reference list of studies that met criteria for inclusion in the analysis


B. Reference list from background

1. WHO. Hepatitis C (No 164) [Fact sheet]. 2014.


