Annex 5.8

PICO 5a and PICO 9 - How to test (confirmation of HCV viraemia)

HCV core antigen testing for presence of active HCV infection and monitoring for treatment response and cure: a systematic review

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Abstract

Background: Chronic hepatitis C virus (HCV) infection with viraemia is prevalent in approximately 1.1% of the world population. Current diagnosis of active infection requires a positive HCV antibody (Ab) as well as nucleic acid testing (NAT) to detect HCV ribonucleic acid (RNA) indicative of active replication. HCV core antigen (HCVcAg) testing was developed as an alternative to NAT. This systematic review aims to summarize (1) the diagnostic accuracy of HCVcAg testing in those with and without positive HCV Ab (PICO 5a), (2) inform the best testing strategy for identification of active HCV infection (PICO 5b), and (3) examine the utility of HCVcAg monitoring for those on HCV treatment (PICO 9).

Methods: We performed a literature search in multiple databases for all published and peer reviewed literature without language restriction through March 2015. Studies were included if a commercially available HCV Core Ag test result was compared with NAT in at least 10 independent clinically collected samples. We contacted authors for missing data to complete extraction. We assessed the quality of studies using an adapted QUADAS-2 tool. Data were classified by HCV Core Ag test manufacturer. For PICO 5a, bivariate meta-analyses were performed for the Abbott ARCHITECT, Hunan Jynda, and Ortho ELISA to obtain pooled sensitivity (Se) and specificities (Sp) with 95% confidence intervals (CI). Due to limited number of studies and specificity data descriptive statistics were derived for the Murex EIA, Bio-RAD Monolisa, EIKEN Lumispot and Fujirebio Lumipulse. We assessed non-parametric regression of quantitative data and identified outliers. Due to the absence of published studies to inform PICO 5b, a decision analysis was performed and is reported separately. Only a descriptive analysis was possible on the use of HCV core Ag in treatment monitoring and assessment of SVR (PICO 9).

Results: We identified 50 published studies for inclusion in the analysis of PICO 5a, 1 study relevant to PICO 5b, and 5 studies relevant to PICO 9. For PICO 5a, 7 index tests were included with 30 studies utilizing Abbott ARCHITECT, 5 studies for Bio-RAD Monolisa, 4 for Murex Ag/Ab EIA, 6 for Ortho ELISA-Ag, 2 for EIKEN Lumispot HCV Ag, 1 for Ortho Lumipulse-Ag, and 4 for Hunan Jynda Bioengineering Group HCV Core Ag ELISA. Among these, 1 directly compared the ARCHITECT with the Lumipulse and Lumispot, and 1 compared the Monolisa with the Murex. From bivariate analyses, the pooled sensitivity and specificity with 95% CI were: ARCHITECT 93.4% (88.7, 96.2) and 98.7% (96.9, 99.4), Ortho ELISA 93.2% (81.6, 97.7) and 99.2% (87.9, 100), and Hunan Jynda 59.5% (46% 71.7) and 82.9% (58.6, 94.3). The sensitivity for the Lumipulse was 95% (90.2, 99.8) in one study; specificities could not be calculated. Three studies using the ARCHITECT provided quantitative data. The few points with negative HCVcAg were shown to occur at RNA levels below 3000 IU/mL where loss of linearity was also noted in pooled non-parametric regression. Accuracy of HCVcAg for treatment monitoring and as a test of cure was assessed by descriptive analysis in 5 studies (PICO 9). The sensitivity of ARCHITECT in EVR ranged from 74–100% with specificity from 70% to 100%. SVR was only assessed in 2 studies with 100% sensitivity and specificity ranging from 94% to 100%. Data on accuracy in prediction of SVR were limited and assessed in only 3 small studies.
Conclusions: HCV core antigen assays can have high sensitivity (up to 93.4% for Abbott ARCHITECT HCVcAg test), high specificity, and good correlation with HCV RNA to a detection limit of roughly 3000 IU/mL. The data on core antigen for treatment monitoring and as a test of cure is too limited to reach reliable conclusions.

GRADE summary tables

I. PICO 5a: What is the best strategy (diagnostic accuracy and other outcomes); comparing HCV core Ag test versus NAT for HCV RNA for detection (and/or) quantification to confirm active HCV infection?

SR Outcome: Diagnostic accuracy, sensitivity and specificity

Patients/population: Persons with detectable HCV RNA with or without positive HCV antibody

Setting: Any

Index tests: HCV core antigen assay

Importance: Inform best strategy for HCV diagnosis in a variety of clinical settings and economies

Reference standard: HCV RNA testing

Studies: Cohort, cross-sectional, or randomized controlled trials that use HCV NAT as gold standard reference test compared with a commercially available HCV core Ag index test

A) Strength of evidence

<table>
<thead>
<tr>
<th>Index test</th>
<th>Outcome Measure</th>
<th># Studies (# samples)</th>
<th>Design</th>
<th>Risk of Bias</th>
<th>Inconsistency</th>
<th>Indirectness</th>
<th>Imprecision</th>
<th>Strength of Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott ARCHITECT HCV Ag Assay</td>
<td>Sensitivity</td>
<td>30 (12,788)</td>
<td>Cohort and cross-sectional</td>
<td>Low</td>
<td>Low</td>
<td>Moderate</td>
<td>Low</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>Specificity</td>
<td>20 (11,820)</td>
<td>Cohort and cross-sectional</td>
<td>Low</td>
<td>Low</td>
<td>Moderate</td>
<td>Low</td>
<td>Moderate</td>
</tr>
<tr>
<td>Ortho ELISA-Ag</td>
<td>Sensitivity</td>
<td>6 (1,423)</td>
<td>Cohort and cross-sectional</td>
<td>High</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Low</td>
<td>Very low</td>
</tr>
<tr>
<td></td>
<td>Specificity</td>
<td>5 (1,177)</td>
<td>Cohort and cross-sectional</td>
<td>High</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Low</td>
<td>Very low</td>
</tr>
<tr>
<td>Bio-RAD Monolisa HCV Ag-Ab ULTRA</td>
<td>Sensitivity</td>
<td>5 (525)</td>
<td>Cohort and cross-sectional</td>
<td>Low</td>
<td>High</td>
<td>Moderate</td>
<td>Low</td>
<td>Very low</td>
</tr>
<tr>
<td></td>
<td>Specificity</td>
<td>1 (337)</td>
<td>Cross-sectional</td>
<td>Moderate</td>
<td>NA</td>
<td>Moderate</td>
<td>NA</td>
<td>Very low</td>
</tr>
<tr>
<td>EIKEN Lumispot HCV Ag</td>
<td>Sensitivity</td>
<td>2 (235)</td>
<td>Cross-sectional</td>
<td>Moderate</td>
<td>Low</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Very low</td>
</tr>
<tr>
<td></td>
<td>Specificity</td>
<td>Sensitivity</td>
<td>Cohort and cross-sectional</td>
<td>Moderate</td>
<td>High</td>
<td>Moderate</td>
<td>Low</td>
<td>Very low</td>
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</tr>
<tr>
<td>Fujirebio Lumipulse</td>
<td>0</td>
<td>1 (80)</td>
<td>Cross-sectional</td>
<td>Moderate</td>
<td>High</td>
<td>Moderate</td>
<td>Low</td>
<td>Very low</td>
</tr>
<tr>
<td>Ortho HCV Ag</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Specificity</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Hunan Jynda HCV Core</td>
<td>0</td>
<td>4 (524)</td>
<td>Cohort and cross-sectional</td>
<td>Moderate</td>
<td>High</td>
<td>Moderate</td>
<td>Low</td>
<td>Very low</td>
</tr>
<tr>
<td>Ag ELISA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Specificity</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>DiaSorin S.A. Murex</td>
<td>3 (658)</td>
<td>4 (770)</td>
<td>Cohort and cross-sectional</td>
<td>Low</td>
<td>High</td>
<td>Moderate</td>
<td>Low</td>
<td>Very low</td>
</tr>
<tr>
<td>Ag/Ab EIA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Specificity</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA = not applicable

Footnotes:

For each index test, quality of evidence started high when there were several high-quality observational studies (prospective cohort studies, cross-sectional studies with direct comparison of index test results with a reference standard). We then downgraded one point when there was moderate concern identified and two points when there was a high concern identified in any of the four factors that may decrease the quality of evidence: risk of bias, inconsistency, indirectness, and imprecision.

1 We used QUADAS-2 to assess risk of bias.

- For ARCHITECT, in half of the studies it was unclear how participants were selected and one study used only healthy blood donors; however, the data from all studies is consistent and unclear selection does not appear to cause bias thus we did not downgrade.

- For the Ortho ELISA, two studies of five used convenience enrolment for participant selection, and one enrolled only healthy blood donors thus we downgraded 2 points.

- For the Monolisa, four of five studies had unclear patient selection. For one it was unclear if the index and reference test were performed within 30 days. Given that there were no high-risk concerns for bias we did not downgrade.

- For specificity, there was only one study with data that had unclear participant selection, thus we downgraded one point, as there were no data from studies with random or consecutive selection to compare to and identify possible selection bias (as was possible with the ARCHITECT).

- For the Lumispot, both studies had unclear patient selection. As there were no data from studies with random or consecutive selection to compare, we downgraded one point.

- The Lumipulse only included one study with unclear participant selection and was downgraded 1 point.

- The Hunan Jynda had one of four studies with unclear participant selection, one in only healthy blood donors, and one for which it was unclear whether the index and reference were performed within 30 days. As the use of only healthy blood donors was considered a high-risk category, in combination with the other unclear factors, we downgraded one point.

- For the Murex test, three of four studies had unclear participant selection but no other high-risk concerns for bias and thus we did not downgrade.

2 Unexplained heterogeneity in remaining studies may be related to covariates that could not be adjusted for in meta-regression due to limited data (HIV and HBV coinfections, HCV genotype). Additionally, not all studies identified HCV antibody status or stratified by acute and chronic infection thus variability of HCV replication could contribute to higher false negative HCVcAg.
- There was little heterogeneity noted in the ARCHITECT studies; thus we did not downgrade.
- For the Ortho ELISA, there was moderate heterogeneity with largely one outlier study, thus we downgraded 1 point.
- For the Monolisa sensitivity outcome, heterogeneity between studies precluded meta-analysis and thus we downgraded 2 points. For specificity, there is only 1 study and we cannot assess heterogeneity and downgrade 1 point.
- For the Murex sensitivity outcome there was too much heterogeneity to pool the data, and thus we downgraded 2 points. For specificity, there were not enough studies to perform meta-analysis and heterogeneity could not be formally assessed, however there is a broad range among results and thus we downgraded one point.
- The EIKEN Lumispot was only used in 2 studies. Sensitivity was similar in both studies suggesting little heterogeneity, thus we did not downgrade.
- For the Fujirebio Lumipulse, there is only 1 study and we cannot assess heterogeneity and downgrade 1 point.

3 All studies were performed in reference laboratories, and the majorities were in high and middle-income countries. Thus the patient population, the viral population tested (e.g. genotype distribution), and the test users are not representative of the limited-resource settings for which these guidelines are envisioned. All were downgraded 1 point.

4 We considered imprecision as present when the pooled confidence intervals were >10% and when there were fewer than 250 samples in the analysis. As such, we downgrade the Ortho ELISA, and Hunan Jynda one point for wide confidence intervals, and downgraded the Lumispot one point for small sample size. Additionally, imprecision could not be graded for the Monolisa specificity outcome, and the Lumipulse test as these only included one study.

B) Summary of findings, PICO 5a

<table>
<thead>
<tr>
<th>SR outcome: diagnostic accuracy</th>
<th>Index test</th>
<th># Studies (# samples)</th>
<th>Unit of analysis</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive LR</th>
<th>Negative LR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Abbott ARCHITECT HCV Ag Assay</td>
<td>20 (11,820)</td>
<td>Sample</td>
<td>93.4% (88.7, 96.2)</td>
<td>98.7% (96.9, 99.4)</td>
<td>71.8 (28.6, 160.3)</td>
<td>0.07 (0.04, 0.12)</td>
</tr>
<tr>
<td></td>
<td>Ortho ELISA-Ag</td>
<td>5 (1,177)</td>
<td>Sample</td>
<td>93.2% (81.6, 97.7)</td>
<td>99.2% (87.9, 99.9)</td>
<td>116.5 (6.7, 977)</td>
<td>0.06 (0.02, 0.07)</td>
</tr>
<tr>
<td></td>
<td>Bio-RAD Monolisa HCV Ag-Ab ULTRA</td>
<td>5 (525)</td>
<td>Sample</td>
<td>28.6–95%*</td>
<td>94.9% (89.9, 99.8)**</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>EIKEN Lumispot HCV Ag</td>
<td>2 (235)</td>
<td>Sample</td>
<td>97.5–98.1%*</td>
<td>ND</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Fujirebio Lumipulse Ortho HCV Ag</td>
<td>1 (80)</td>
<td>Sample</td>
<td>95% (90.2, 99.8)**</td>
<td>ND</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Hunan Jynda HCV Core Ag ELISA</td>
<td>4 (524)</td>
<td>Sample</td>
<td>59.5% (46, 71.7)</td>
<td>82.9% (58.6, 94.3)</td>
<td>3.5 (1.1, 12.6)</td>
<td>0.28 (0.2, 0.3)</td>
</tr>
<tr>
<td></td>
<td>DiaSorin S.A. Murex Ag/Ab ELIA</td>
<td>4 (730)</td>
<td>Sample</td>
<td>50–100%*</td>
<td>83.8–100%*</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

ND: no data, NA = not applicable – if sensitivity and specificity results were not available from meta-analysis, likelihood ratios were not calculated.

* Meta-analysis not possible. Range of results seen across studies reported. **Result from one study only.
C) Impact of findings in different prevalence settings

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Effect per 1000 patients with presumed HCV for varying prevalence settings comparing HCV core Ag against HCV RNA</th>
<th>Prevalence 2%*</th>
<th>Prevalence 10%*</th>
<th>Prevalence 30%*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Abbott ARCHITECT HCV Ag Assay</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>True positives (patients with HCV)</td>
<td>19 (18, 19)</td>
<td>93 (89, 96)</td>
<td>279 (267,288)</td>
<td></td>
</tr>
<tr>
<td>True negatives (patients without HCV)</td>
<td>967 (951,974)</td>
<td>888 (873,895)</td>
<td>691 (697,696)</td>
<td></td>
</tr>
<tr>
<td>False positives (patients incorrectly classified as having HCV)</td>
<td>13 (6, 29)</td>
<td>12 (5, 27)</td>
<td>9 (4, 21)</td>
<td></td>
</tr>
<tr>
<td>False negatives (patients incorrectly classified as not having HCV)</td>
<td>1 (1, 2)</td>
<td>7 (4, 11)</td>
<td>21 (12, 33)</td>
<td></td>
</tr>
<tr>
<td><strong>Ortho ELISA-Ag</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>True positives (patients with HCV)</td>
<td>19 (16, 20)</td>
<td>93 (82,98)</td>
<td>279 (246,294)</td>
<td></td>
</tr>
<tr>
<td>True negatives (patients without HCV)</td>
<td>970 (862,980)</td>
<td>891 (792,900)</td>
<td>693 (616,700)</td>
<td></td>
</tr>
<tr>
<td>False positives (patients incorrectly classified as having HCV)</td>
<td>10 (0, 118)</td>
<td>9 (0, 108)</td>
<td>7 (0, 84)</td>
<td></td>
</tr>
<tr>
<td>False negatives (patients incorrectly classified as not having HCV)</td>
<td>1 (0,4)</td>
<td>7 (2, 18)</td>
<td>21 (6,54)</td>
<td></td>
</tr>
<tr>
<td><strong>Hunan Lynda HCV Core Ag ELISA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>True positives (patients with HCV)</td>
<td>12 (9, 14)</td>
<td>60 (46,72)</td>
<td>179 (138,216)</td>
<td></td>
</tr>
<tr>
<td>True negatives (patients without HCV)</td>
<td>813 (578,921)</td>
<td>747 (531,846)</td>
<td>581 (413,658)</td>
<td></td>
</tr>
<tr>
<td>False positives (patients incorrectly classified as having HCV)</td>
<td>167 (59,402)</td>
<td>153 (54,369)</td>
<td>119 (42,287)</td>
<td></td>
</tr>
<tr>
<td>False negatives (patients incorrectly classified as not having HCV)</td>
<td>8 (6,11)</td>
<td>41 (28,54)</td>
<td>122 (84,162)</td>
<td></td>
</tr>
</tbody>
</table>

*Numbers in parentheses consider 95% confidence intervals of accuracy estimate

II. PICO 9: Among patients receiving treatment for HCV, what is the diagnostic accuracy of HCV core Ag test versus NAT for HCV RNA detection (and/or) quantification to confirm successful treatment response with viral clearance?

**SR Outcome 1:** Diagnostic accuracy, sensitivity and specificity of HCVcAg at SVR

**SR Outcome 2:** Timing and predictive accuracy of HCVcAg for SVR

**Patients/population:** Persons with detectable HCV RNA with or without positive HCV antibody

**Setting:** Any

**Index tests:** HCV core antigen assay
**Importance:** Inform best strategy for treatment monitoring and test of cure in a variety of clinical settings and economies

**Reference standard:** HCV RNA Testing

**Studies:** Longitudinal cohort or randomized controlled trials that use HCV NAT as gold standard reference test compared with a commercially available HCV core Ag index test

### SR outcome 1: Diagnostic Accuracy at SVR

<table>
<thead>
<tr>
<th>Index test</th>
<th>Outcome measure</th>
<th># Studies (# samples)</th>
<th>Design</th>
<th>Risk of bias</th>
<th>Inconsistency</th>
<th>Indirectness</th>
<th>Imprecision</th>
<th>Effect*</th>
<th>Strength of evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott ARCHITECT HCV Ag Assay</td>
<td>Sensitivity</td>
<td>2 (67)</td>
<td>RCT, cohort</td>
<td>Low¹</td>
<td>Low²</td>
<td>Moderate³</td>
<td>Low⁴</td>
<td>100%*</td>
<td>Moderate ○○○</td>
</tr>
<tr>
<td></td>
<td>Specificity</td>
<td>2 (67)</td>
<td>RCT, cohort</td>
<td>Low¹</td>
<td>Moderate² (-1)</td>
<td>Moderate³ (-1)</td>
<td>Low⁴</td>
<td>94–100%*</td>
<td>Low ○○○</td>
</tr>
</tbody>
</table>

### SR outcome 2: Predictive accuracy of SVR

<table>
<thead>
<tr>
<th>Index test</th>
<th>Outcome measure</th>
<th># Studies (# individuals)</th>
<th>Design</th>
<th>Risk of bias</th>
<th>Inconsistency</th>
<th>Indirectness</th>
<th>Imprecision</th>
<th>Effect*</th>
<th>Strength of evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott ARCHITECT HCV Ag Assay</td>
<td>Sensitivity</td>
<td>1 (23)</td>
<td>Cohort</td>
<td>Low¹</td>
<td>NA² (-1)</td>
<td>Moderate³ (-1)</td>
<td>NA⁴</td>
<td>95.2%**</td>
<td>Low ○○○</td>
</tr>
<tr>
<td></td>
<td>Specificity</td>
<td>1 (23)</td>
<td>Cohort</td>
<td>Low¹</td>
<td>NA² (-1)</td>
<td>Moderate³ (-1)</td>
<td>NA⁴</td>
<td>70%**</td>
<td>Low ○○○</td>
</tr>
<tr>
<td>Fujirebio Lumipulse Ortho HCV Ag</td>
<td>Sensitivity</td>
<td>2 (134)</td>
<td>Cohort</td>
<td>Moderate¹ (-1)</td>
<td>Moderate² (-1)</td>
<td>Moderate³ (-1)</td>
<td>Moderate⁴ (-1)</td>
<td>57.1–79.4%*</td>
<td>Very low ○○○</td>
</tr>
<tr>
<td></td>
<td>Specificity</td>
<td>2 (134)</td>
<td>Cohort</td>
<td>Moderate¹ (-1)</td>
<td>Moderate² (-1)</td>
<td>Moderate³ (-1)</td>
<td>Moderate⁴ (-1)</td>
<td>88.5–99.3%*</td>
<td>Very low ○○○</td>
</tr>
</tbody>
</table>

* Results reported are range across studies or **individual result, NA= not applicable

**Footnotes:**
For each index test, quality of evidence started high when there were several high quality observational studies (prospective cohort studies, cross-sectional studies with direct comparison of index test results with a reference standard). We then downgraded one point when a serious issue was identified and two points when a very serious issue was identified in any of the four factors that may decrease the quality of evidence: risk of bias, inconsistency, indirectness, and imprecision.

**SR outcome 1: diagnostic accuracy at SVR**

1. We used QUADAS-2 to assess risk of bias. There were no concerns raised for the two studies that utilized the ARCHITECT assay.
2. The limited number of studies precluded a meta-analysis and formal assessment of heterogeneity. However, the data between studies for sensitivity is consistent thus we did not downgrade. There is some variability seen in the data for specificity, thus we downgrade 1 point.

3. Both studies were performed in reference laboratories in high and middle-income countries, which is not representative of broad use throughout the world thus we downgraded 1 point.

4. The range in specificity results was attributed to possible unexplained heterogeneity and is less likely from verification bias given the excellent reference standard. Given that we already downgraded for heterogeneity, we did not downgrade for imprecision.

SR outcome 2: predictive accuracy of early HCV Ag on SVR

1. We used QUADAS-2 to assess risk of bias. For the ARCHITECT, there were no concerns raised so we did not downgrade. In the 2 Fujirebio Lumipulse studies, participant selection was unclear in one, and one did not include all patients initially enrolled in the analysis so we downgraded 1 point.

2. For the ARCHITECT, there was only one study thus we could not assess heterogeneity and downgrade one point. For the Lumipulse assay, there were only 2 small studies and no formal heterogeneity could be assessed. However, neither study included covariate information aside from genotype and the results between studies are broad, thus we downgraded one point.

3. All studies were performed in reference laboratories in high- and middle-income countries, which is not representative of broad use throughout the world. All were downgraded 1 point.

4. For the ARCHITECT, imprecision could not be graded as there was only one study. There is a broad range of effect between the Fujino studies, which may in part be from unexplained heterogeneity already discussed, but may also be from imprecision as only absolute values of decline in HCVcAg were examined instead of log decline thus we downgraded 1 point.

2. Background

Chronic hepatitis C virus (HCV) infection with viraemia is prevalent in approximately 1.1% of the world population, or 64–103 million people, with an estimated 75% of all cases occurring in low- to middle-income countries (LMICs). HCV is a small, enveloped, single stranded ribonucleic acid (RNA) virus belonging to the Flaviviridae family with seven genotypes and more than sixty-seven subtypes. The genome is contained in an internal capsid formed by three domains of the HCV core protein, which is highly conserved and antigenic. During viral assembly, nucleocapsid peptides 22 (p22) are released into plasma and can be detected early in the course of infection.

Screening assays to assess for anti-HCV antibodies (HCV Ab) were among the first diagnostic tools developed to identify HCV infection, but can only inform about exposure to the virus and not active replication or ongoing infection. The serological window for conversion to a positive antibody is highly variable with an average of 60 days and antibodies may remain persistently negative among patients on haemodialysis and those with poorly controlled HIV infection or other immunocompromised states. Thus, diagnosis of active HCV infection requires antibody testing followed by an assessment for viraemia both for confirmation of true infection in antibody-positive patients and for high-risk antibody negative patients. Confirmatory testing can be based on nucleic acid testing (NAT) to detect HCV-RNA or an antigen testing to detect core antigen.

HCV core antigen (HCVcAg) tests largely targeting p22 have been in development as an alternative to NAT since Tanaka et al. first demonstrated detection of circulating antigen in those
with chronic HCV infection in 1995\textsuperscript{7} and the first commercial assay was released in 2000.\textsuperscript{8} HCVcAg tests have the potential to be less costly and less centralized than NAT.

Detection of HCV viraemia is also important during treatment of chronic HCV infection. Current guidelines recommend virological confirmation pretreatment with the measurement of a baseline viral load with NAT. For interferon-based treatments, viral load is assessed at week 4 of therapy for the "rapid viral response" (RVR) to help predict efficacy of therapy, and repeated at week 6 if elevated at week 4 to see further viral response and guide whether treatment should be continued. NAT is performed again at week 12 (early viral response, EVR), at the end of treatment, and 12 and 24 weeks after therapy is completed to test for cure, "sustained viral response" (SVR). With the development of direct-acting antivirals (DAAs), NAT during therapy may no longer be necessary.\textsuperscript{9} Additionally, DAA has made treatment for HCV possible in LMICs\textsuperscript{10} making access to an affordable diagnostic and monitoring test even more important.

This systematic review of the published literature aims to assess the diagnostic accuracy of HCVcAg testing for HCV detection and inform the best testing strategy for identification of chronic HCV infection. Furthermore, the review looks at the utility of HCVcAg for monitoring on HCV treatment and to test for cure.

**Tests included in this systematic review**

Only commercially available tests were included in the systematic review. The most widely studied is the Abbott ARCHITECT HCV Ag assay, a two-step automated chemiluminescent microparticle immunoassay (CMIA) that allows quantitative determination of HCVcAg in serum or plasma. The assay uses the Abbott ARCHITECT i System (i2000/i2000SR/i1000SR modules), a reference laboratory instrument with ARCHITECT System Software version 5.0 or higher. The Fujirebio Lumipulse Ortho HCV Ag test and EIKEN Lumispot HCV Ag are similar automated chemiluminescent enzyme immunoassays (CLEIA) available in Japan and China.

There are two available Ab–Ag combination enzyme immunoassays (EIA), the DiaSorin S.A. Murex HCV Ag–Ab combination and Bio-RAD Monolisa\textsuperscript{TM} HCV Ag-Ab ULTRA. The Monolisa uses a spectrophotometer to read absorbance values that detects presence or absence of Ab and/or HCVcAg with the colour intensity being proportional to quantity of Ab or Ag to HCV bound on the solid phase. Lastly, there are two enzyme-linked immunosorbent assay (ELISA)-based HCVcAg tests, Hunan Jynda Bioengineering Group HCV Core Ag ELISA and Ortho ELISA-Ag.
3. Objectives

This systematic review addresses predefined PICO questions 5a and 9. Question 5b will be addressed in a separate report.

<table>
<thead>
<tr>
<th>PICO 5a</th>
<th>What is the best strategy (diagnostic accuracy and other outcomes); comparing HCV core Ag test versus NAT for HCV RNA for detection (and/or) quantification to confirm active HCV infection?</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P</strong></td>
<td>Persons with detectable HCV RNA with or without positive HCV antibody</td>
</tr>
<tr>
<td><strong>I</strong></td>
<td>HCV core antigen assay</td>
</tr>
<tr>
<td><strong>C</strong></td>
<td>HCV RNA testing</td>
</tr>
<tr>
<td><strong>O</strong></td>
<td>• <strong>Diagnostic accuracy</strong></td>
</tr>
<tr>
<td></td>
<td>1. True negatives (TN), who are screen negative, and do not have HCV infection.</td>
</tr>
<tr>
<td></td>
<td>2. False negatives (FN), who are screen negative but have HCV infection. These are incorrectly misclassified, and this may result in missed opportunity to recognize and present progression of liver disease.</td>
</tr>
<tr>
<td></td>
<td>3. True positives (TP), who are screen positive and have HCV infection.</td>
</tr>
<tr>
<td></td>
<td>4. False positives (FP), who are screen positive, but do not truly have HCV infection. These will have additional unnecessary tests and evaluation.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PICO 5b</th>
<th>What is best testing strategy (diagnostic accuracy and other outcomes); between using sequential testing strategy (HCV core Ag followed by NAT if negative) versus NAT alone for diagnosis of active HCV infection?</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P</strong></td>
<td>Persons with detectable HCV RNA with or without positive HCV antibody</td>
</tr>
<tr>
<td><strong>I</strong></td>
<td>Sequential testing strategy (HCV core Ag followed by NAT if negative) (Fig. 4A)</td>
</tr>
<tr>
<td><strong>C</strong></td>
<td>Standalone NAT test (Fig. 4B)</td>
</tr>
<tr>
<td><strong>O</strong></td>
<td>• <strong>Diagnostic accuracy</strong></td>
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</tbody>
</table>
Among patients receiving treatment for HCV, what is the diagnostic accuracy of HCV core Ag test versus NAT for HCV RNA detection (and/or) quantification to confirm successful treatment response with viral clearance? (Fig. 5A, 5B)

| P | Patients receiving treatment for HCV |
| I | HCV core Ag test (Fig. 5A) |
| C | NAT for HCV RNA detection (and/or) quantification (Fig. 5B) |
| O | • Diagnostic accuracy |
|  | 1. True negatives (TN), who are screen negative, and cleared the HCV infection. |
|  | 2. False negatives (FN), who are screen negative but have HCV infection. (These will be misclassified, and treatment will be stopped resulting in disease progression leading to Liver related morbidity (fibrosis, cirrhosis, end-stage liver disease, hepatocellular carcinoma), progression of liver disease, and mortality. |
|  | 3. True positives (TP), who are screen positive and truly have HCV infection, this will increase the number of treated cases and cured rate. |
|  | 4. False positives (FP), who are screen positive, but do not have HCV infection. (These will continue treatment inappropriately, and will have unnecessary referral). |

4. Methods
We followed standard guidelines and methods for systematic review and meta-analyses of diagnostic tests.11–13 We prepared a protocol for the literature search, article selection, data extraction, and assessment of methodological quality.

Selection criteria

Types of studies
We included case–control, cross-sectional, cohort studies and randomized trials that used HCV NAT as gold standard reference test compared with a commercially available HCV core Ag index test for the diagnosis of active HCV infection or in the monitoring of HCV infection while on treatment.

Participants
We included patients of all age groups from all settings and countries. Specimen types were limited to whole blood, plasma or serum, and we only included studies that examined at least 10 independent HCV NAT positive samples. Saliva specimens were also considered, but only one study was identified during the search and it did not use NAT as reference test and was thus excluded from further analysis. Commercially prepared reference panel specimens were excluded.

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

- Abbott ARCHITECT HCV Ag
- Bio-RAD Monolisa™ HCV Ag-Ab ULTRA
- EIKEN Lumispot HCV Ag
- Fujirebio Lumipulse Ortho HCV Ag
- Hunan Jynda Bioengineering Group HCV Core Ag ELISA
- DiaSorin S.A. Murex Ag/Ab EIA
- Ortho ELISA-Ag

**Target conditions**

**PICO 5a/b**

- Acute HCV infection: the 6-month time period following acquisition of hepatitis C virus. HCV Ab may be positive or negative; the time period between initial infection to seroconversion of antibody is the “window period”. HCV RNA is detectable.
- Chronic HCV infection: duration of HCV infection more than 6 months from time of acquisition. HCV Ab and RNA are detectable.

**PICO 9:**

- Monitoring of viral clearance while on treatment with rapid viral response (RVR) at 4 weeks and early viral response (EVR) at 12 weeks and sustained viral response (SVR) at 24 weeks after completion of treatment

**Reference standard**

The reference standards accepted for a definitive diagnosis included tests for detection of HCV RNA by any of the following NAT techniques: polymerase chain reaction (PCR), branched-chain DNA (bDNA), or transcription mediated amplification (TMA). Tests were noted to be either qualitative or quantitative. 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 HCV Core Ag test confirmed with a positive NAT result.

**Specificity** refers to the proportion of samples with negative HCV Core Ag test and no evidence of active HCV infection confirmed with a negative NAT result.

**Search methods**
A database search of EMBASE, PubMed, Scopus, Web of Science, and Cochrane was performed through March 2015. No language restriction was applied. The search terms used for each database are outlined in Appendix A.

Two review authors (JMF and TT) independently assessed titles and abstracts identified by the literature search to select potentially eligible studies (screen 1). Any citation identified by either review author during screen 1 was selected for full text-review. Full papers of each potentially eligible article were retrieved. Two review authors (JMF and TT) independently assessed the full text articles for inclusion using the predefined inclusion and exclusion criteria (screen 2). Three articles were excluded because of inability to find appropriate language interpretation. Discrepancies were resolved by discussion between the review authors, and for several studies by the decision of a third review author (CMD). The included studies were divided into those applicable for each PICO question. A list of excluded studies and reasons for exclusion can be found in Appendix D.

Data extraction
We created a data extraction form, pilot-tested the form with a subset of eligible studies, and then finalized the form (Appendix B). Two review authors (JMF and TT) independently extracted data from the included studies with the standardized form and crosschecked to ensure accuracy. Disagreement between review authors on data extraction was resolved by discussion or by a third reviewer (CMD). For studies without complete extraction information available, authors were contacted to request further data. Studies without extractable sensitivity and specificity data were excluded if no further information was acquired after three attempts to contact the study authors.

Assessment of methodological quality
We adapted the QUADAS-2 instrument, a validated tool for diagnostic studies, to assess study quality. The information needed to answer QUADAS-2 questions was incorporated in the data extraction sheet. A description of the QUADAS-2 items and the interpretation in the study context can be found in Appendix C.

Statistical analysis and data synthesis
Statistical analyses were performed using STATA (version 14; STATA corporation, College Station, TX). The studies were grouped by type of index test used. QUADAS analysis was performed using Excel (version 14.5.3; Microsoft, Seattle, WA).

Approach to indeterminate index test results
We excluded indeterminate test results from the analyses for determination of sensitivity and specificity, as it was less than 1% for all index tests.

Assessment of publication bias
We did not perform formal assessment of publication bias (tests for funnel plot asymmetry), as these techniques are not recommended for diagnostic test accuracy studies. We reviewed the EASL and AASLD conference abstract books for abstracts of studies that have not been published subsequently and did not find anything between 2010 and 2013. We did not include unpublished data in this review.

**Meta-analysis**

Meta-analysis for each index test type was performed if at least four studies were available with the same index test with at least ten independent samples in each study. Bivariate random effects meta-analyses were performed for index tests with enough studies that included data to calculate sensitivity, specificity, and 95% confidence intervals for each. Several studies did not contribute to both sensitivity (no true positives and false negatives) and specificity (no false positives and true negatives) but only to one of the two. In such cases, we examined the correlation between sensitivity and specificity visually from a scatter plot of the sensitivity versus 1-specificity across studies. If the correlation was limited, we performed a univariate random effects meta-analysis of the sensitivity and/or specificity estimates separately, so as to make complete use of the available data. We then compared the results from the univariate analysis (including all studies) with the results from the bivariate analysis of the subset of studies that contributed to both sensitivity and specificity estimates. For index tests with data that contributed only to sensitivity but had at least 4 studies, we performed univariate random effects meta-analysis only for sensitivity. A descriptive analysis was performed for index tests with less than four studies available or when substantial heterogeneity was evident on forest plots that precluded a meta-analysis.

We visually assessed forest plots for heterogeneity among the studies within each index test and in the summary plots we examined the variability in estimates and the width of the confidence region, with a wider prediction region suggesting more heterogeneity. We also report an estimate of $\tau^2$ (along with its standard error) corresponding to the variance of the logit-transformed specificity and sensitivity, which can be interpreted as a measure of between-study variability.

The initial protocol planned for sensitivity analysis excluding case-control studies, but none were identified amongst the studies included. We anticipated that studies included in the meta-analysis would be heterogeneous in many respects. Therefore, we pre-specified subgroups by antibody status. Furthermore, we planned to examine the effect of specimen condition (fresh vs. frozen), HBV and HIV status and genotype in a meta-regression. Where meta-regression to assess impact of covariates was not possible due to limited data, we showed descriptive statistics for HIV and HBV coinfection and genotype distribution. The impact of specimen condition could not be assessed as all studies either used frozen samples or did not specify condition.
Analysis of quantitative data

Where quantitative data were available from the studies, a locally weighted regression smoother was used to visually assess the correlation between quantitative HCV Ag measured in fmol/L to HCV RNA measured in IU/mL.\textsuperscript{15} We identified outliers and performed descriptive statistics of these points. There was only enough quantitative data to assess the Abbott ARCHITECT assay.

5. Results

Results of the search

From the literature search, 8,146 citations were identified and a total of 313 full-text articles were reviewed: 283 applied to PICO 5a, 11 applied to PICO 5b (reported separately), and 44 applied to PICO 9. For PICO 5a, 50 studies were included. For PICO 9, 4 studies were included. Figure 1a-d shows the PRISMA diagram with the flow of studies for each PICO and reasons for exclusion.

Description of studies

Core antigen for HCV detection (PICO 5a) – included studies

Fifty included studies utilized the 7 different HCV cAg assays described above, with two performing direct comparisons between two or more antigen tests.\textsuperscript{16,17} Four studies were translated from Mandarin,\textsuperscript{18} 1 from German,\textsuperscript{19} 1 from French,\textsuperscript{20} and 2 from Japanese.\textsuperscript{21, 22} Characteristics for each study are presented in Table 1a.

The Abbott ARCHITECT HCV Ag assay was assessed in 30 studies.\textsuperscript{5, 16, 18, 19, 23–49} All study designs were either cross-sectional or cohort, with a broad study population (included patients with HCV disease, and those susceptible to HCV disease) with the exception of one study that evaluated only healthy blood donors.\textsuperscript{35} Only 20 had enough data to be included in the bivariate analysis.\textsuperscript{34} Ten did not have enough data to calculate specificity\textsuperscript{16, 17, 18, 28, 31, 36, 37, 41, 42, 45} and were only included in the univariate pooled sensitivity estimate. All but 3 studies specified positive HCV Ab status of specimens\textsuperscript{19, 25, 26} and 4 included data for HCV Ab negative samples.\textsuperscript{18,24,42,45, 48} Demographic data was available in 18 studies, the remainder utilized anonymous specimens and authors were unable to provide further information. HIV status was known in 15 of the studies with 2 including only HIV-coinfected subjects.\textsuperscript{46, 48} HBV status was known in 13 studies and all but 4 excluded patients with HBV coinfection. The study with highest prevalence included 50.5\% with HBV coinfection.\textsuperscript{40} Only 1 study included children.\textsuperscript{28}

The Bio-RAD Monolisa\textsuperscript{TM} HCV Ag-Ab ULTRA was used in 5 studies;\textsuperscript{17, 20, 50–52} all were cohort or cross-sectional in design with a broad study population. One study had an unknown amount of participants with at least 25 known subjects and an additional 94 samples from an unknown amount of donors.\textsuperscript{48} Two included only HIV-coinfected adult subjects,\textsuperscript{20, 51} the remaining 3 had unknown subject demographic information.

The EIKEN Lumispot HCV Ag was performed in one cross-sectional study with a broad study population.\textsuperscript{53} Further demographic information was unavailable.
The EIKEN Lumispot HCV Ag, Fujirebio Lumipulse Ortho HCV Ag, and Abbott ARCHITECT HCV Ag were compared in 1 cross-sectional study,\textsuperscript{16} with unknown demographic information.

Four studies assessed the Hunan Jynda Bioengineering Group HCV Core Ag ELISA.\textsuperscript{54–57} Two studies had a cohort design, 2 cross-sectional, and 1 assessed a healthy blood donor population\textsuperscript{56} while the others included broad study populations. HIV and HBV coinfection status was unknown in all studies. One included children,\textsuperscript{57} and the remaining had unknown age groups included.

The DiaSorin S.A. Murex Ag/Ab EIA was used in 4 articles, 3 adult cohort studies\textsuperscript{58–60} and 1 cross-sectional study that compared performance with the Bio-RAD Monolisa\textsuperscript{TM} HCV Ag-Ab ULTRA;\textsuperscript{17} this is the same study as above with an unknown total number of participants. One study included 25% HIV-coinfected patients,\textsuperscript{59} and one included 6.1% HBV co-infected patients.\textsuperscript{60}

Finally, 6 articles utilized the Ortho ELISA-Ag test.\textsuperscript{61–64} All were either cross-sectional or cohort designs in broad study populations except for 1 study performed in healthy blood donors.\textsuperscript{63} All had unknown demographic information.

**Core antigen in best testing strategy for identification of active HCV infection (Pico 5b) – included studies**

Only 1 study was found to meet inclusion criteria.\textsuperscript{43} Given limited data, a decision analysis was performed to address this PICO question and was reported separately.

**Core antigen for treatment monitoring and test of cure (PICO 9) – included studies**

Two studies evaluated the Abbott ARCHITECT compared to NAT at baseline, EVR and SVR, one used patients enrolled in a randomized controlled trial\textsuperscript{65} and one used a cohort design.\textsuperscript{66} One study used the ARCHITECT to assess correlation of HCVcAg and NAT at EVR only. Three cohort studies evaluated HCVcAg kinetics during EVR to assess predictive accuracy of SVR, but did not compare HCVcAg to NAT at SVR. One used the ARCHITECT,\textsuperscript{67} and 2 employed the Fujirebio Lumipulse.\textsuperscript{68,69} None required translation. Characteristics are presented in Table 1b. All studies included patients with active HCV infection who were initiated on interferon based treatment regimens.

**Excluded studies**

A list of excluded studies for each PICO and the reasons for exclusion is presented in appendix D.

**Methodological quality (QUADAS-2)**

The overall methodological quality of all included studies for each PICO question organized by QUADAS-2 domain is summarized in Fig. 2 and presented for each individual study in Fig. 3.
Patient selection
PICO 5a
In the “patient selection” domain, we judged 2 studies to have “high risk of bias” because they used convenience sampling of participants for enrolment. Twenty-three studies were judged to be “low risk of bias”. In 25 studies risk of bias was “unclear” with 12 having both unspecified enrolment and prior exclusion strategies and the remaining 13 with a mix of the two. Applicability in this domain was judged to be “high risk” in 3 studies that included only healthy blood donors, and the remaining 47 were determined to be “low risk”. Setting of testing was not considered for this review as currently available tests can only be operated in specialized laboratories.

PICO 9
In the “patient selection” domain, 4 studies were judged to be “low risk of bias” as sampling was consecutive or random. One was judged to be “unclear risk of bias” as patient selection was not specified. Applicability was judged to be “low risk” in all studies as all included patients with active HCV infection.

Index test
PICO 5a
All studies were determined to have “low risk of bias” as all index tests had predefined thresholds of positivity and interpretation does not require judgement thus all were considered blinded with respect to the results of the reference test. Applicability in this domain was assessed by whether or not the index test was performed per recommendations of the manufacturer. In 3 studies, this was unclear and information could not be obtained from the study authors, thus 3 were determined to be “unclear risk” while the remaining 47 were “low risk”.

PICO 9
All studies were determined to have “low risk of bias” as all index tests had predefined thresholds of positivity and interpretation does not require judgement. For applicability, it was unclear in 1 study whether the index test was performed per recommendation of the manufacturer and was thus judged “unclear”. The remaining 4 studies were "low risk”.

Reference standard
PICO 5a and PICO 9
All studies were judged to be “low risk of bias” per our QUADAS-2 rules. Though studies used a variety of NAT techniques, all are considered highly sensitive and results are objective and do not require interpretation. As far as “applicability”, this was also determined to be “low risk” for all studies as circulating virus detected by NAT is by definition associated with active infection and the specificity of the reference standard is high.
Flow and timing

PICO 5a

In the “flow and timing” domain, 42 studies were judged “low risk of bias”. Eight studies were judged to be “unclear risk of bias”. In 7 it could not be determined whether the index and reference tests were performed on the same specimen or within <1 month, and in 1 there were an unknown number of participants so we could not judge if all were included in the final analysis.

PICO 9

Four studies were judged to be “low risk” as index test and reference testing were performed on the same specimens at various time points throughout, and all patients were included in the final analyses. One study was judged to be “high risk” as not all patients enrolled were included in the analysis, only those who completed protocol.

HCV core Ag for diagnosis of active HCV infection

Abbott ARCHITECT

There were 20 studies included in the bivariate analysis with 11,820 total samples. Based on studies reporting paired (sensitivity and specificity) data, the pooled sensitivity regardless of HCV Ab status was 93.4% (95% CI 88.7, 96.2), sensitivity was 98.7% (95% CI 96.9, 99.4), positive likelihood ratio (LR) was 71.8 (95% CI 28.6, 160.3), and negative LR 0.07 (0.04, 0.12) (Table 2, Fig. 4a). The pooled sensitivity estimate from a univariate analysis was 94.1% (95% CI 92.4, 95.7) and included 10 additional studies that only contributed data for sensitivity with a total of 12,788 samples (Table 2, Figure 4b). Among 16 studies with known HCV Ab positive samples, the sensitivity was 92.5% (95% CI 86.9, 95.8) and specificity 97.8% (95% CI 94.7, 99.1) (Table 2, Figure 4c). From 4 studies that analysed HCV Ab negative samples, the pooled sensitivity was 74.4% (95% CI 6.2, 99.2) and specificity was 98.8% (97.2, 99.5) (Table 2, Fig. 4d). Figure 5 presents the pooled sensitivity and specificity estimates (a) regardless of HCV Ab status, (b) for HCV Ab-positive samples only, and (c) for HCV Ab-negative samples. In plots (a) and (b) the summary point approached the upper left corner suggesting good accuracy of the ARCHITECT test for diagnosis of HCV infection. Plot (c) demonstrated the broad 95% confidence interval among Ab-negative specimens.

Heterogeneity was visually assessed in Figs 4 and 5 and with $\tau^2$ (Fig. 2). The studies were relatively homogeneous (Fig. 4a). A meta-regression was not possible given the limited amount of data on predefined covariates. There were three outlier studies in respect to sensitivity: Ergünay, Florea and Gu (72%, 74% and 44% sensitivity, respectively). Antibody status was known for Gu and performance was similar across antibody-positive and antibody-negative samples (44.0% and 41.7%, respectively). Other covariates were examined to assess reasons for low sensitivity. In the Ergünay study, HIV and HBV coinfection status were unknown, 60.2% of participants had HCV genotype 1b infection, 2.2% genotype 1a, 0.8% genotypes 3 and 4, and 35.8% were unknown (Table 3). In the Florea study, there were...
no HIV or HBV infected patients, but genotype status was unknown. For specificity, the results are even more homogeneous with only 1 outlier, the Medici study. There are no demographic data for this study as it was performed on anonymous samples. Overall, genotype distribution was reported for 15 studies (Table 3a) with genotype 1b being the most prevalent and genotypes 5 and 6 minimally studied.

Ortho ELISA-Ag
Five studies were included in the bivariate analysis with 1177 total samples. The pooled sensitivity was 93.2% (95% CI 81.6, 97.7), specificity 99.2% (95% CI 87.9, 100), positive LR 116.5 (95% CI 6.7, 977), and negative LR 0.06 (95% CI 0.02, 0.07) (Fig. 6, Table 2). Univariate analysis with one additional study by Agha resulted in a pooled sensitivity of 90.8% (95% CI 83.5, 98.2) (Table 2). Figure 7 demonstrates the bivariate pooled sensitivity and specificity estimates, with the summary point approaching the upper left corner suggesting good accuracy of the Ortho ELISA-Ag test for diagnosis of HCV infection though the data exhibit some heterogeneity demonstrated by the wide 95% CI. Heterogeneity was also visually assessed in the forest plot (Fig. 6) and with $\tau^2$ (Fig. 2) with two outlier studies, Nübling and El-Sayed. Both studies reported unknown HIV or HBV coinfection information, and genotype distribution was unknown for El-Sayed. The genotype distribution in the Nübling study was 11.5% genotype 1 not specified, 42.3% genotype 1a, 19.2% genotype 1b, 11.5% genotype 2, and 15.4% genotype 3. This study was performed in 494 total plasma samples from 52 subjects at various time points during HCV infection with varying levels of HCV RNA. The data were not stratified by antibody status, and the raw quantitative information was no longer available. The authors noted that panels later in the course of infection with higher and more consistent HCV RNA levels had improved correlation with HCVcAg detection but no sensitivity or specificity data were calculated.

Bio-RAD Monolisa HCV Ag-Ab ULTRA
Five studies with 525 total samples were included. Given heterogeneity observed in the forest plot, a pooled analysis was not performed and only descriptive statistics were examined (Fig. 8). The Nastouli and Schnuriger studies have substantially different results – sensitivities of 61.9 (95% CI 38.6, 81.9) and 95% (95% CI 75.1, 99.9), respectively. Each study was performed in participants with 100% HIV coinfection, though the genotype distribution differed with more genotype 1 patients in the Nastouli study, and more genotype 3 and 4 in the Schnuriger study (Table 3). The Tuke study demonstrated the lowest sensitivity of 28.6% (95% CI 20.4, 37.7). This study was performed in pre-seroconversion HCV Ab negative specimens only. Among the HCV genotypes, sensitivity was 33% for genotype 1a, 41% for genotype 1b, 29% for genotype 2, 0% for genotype 3, and 0% for unknown genotype (data not shown, obtained from original article). The authors also noted the sensitivity improved to 71% when limited to specimens with HCV RNA >10^6 IU/mL, though remained negative in 7 genotype 3 samples with viral load >2 million IU/mL. The Laperche study was also performed in HCV Ab negative specimens with a broad distribution
of genotypes: 11.4% genotype 1a, 34.3% genotype 1b, 25.7% genotype 2, 14.3% genotype 3, 5.7% genotype 4, and 2.9% unknown. The sensitivity was 40.9% (95% CI 29.3, 53.2). The Vermeersch study was the largest with 337 samples and was the only with data to calculate specificity. The reported sensitivity was 93.83% (95% CI 90.2, 96.4) and specificity 94.9% (95% CI 89.9, 99.8). The study was done on anonymous samples without known HIV or HBV status or genotype. The reference standard was incomplete as RNA testing was done only on 61 random samples and all samples with discordant Ab and Ag result. Seventy-eight samples were antibody negative. Genotype distribution was unknown.

**EIKEN Lumispot HCV Ag**

Two studies only utilized the Lumispot assay. The first included 155 samples and the sensitivity reported was 98.1% (95% CI 95.9, 100) (Table 2).53 The majority of samples were genotype 1 (65.2%) with the remaining genotype 2. The second study (Murayama et al.) compared the Lumispot to Fujirebio Lumipulse and Abbott ARCHITECT.16 There were 80 participants, and the reported sensitivity was 97.5% (95% CI 94.1, 100). The Abbott ARCHITECT sensitivity in that study was 100%, suggesting a bias towards better performance. Not enough data were reported to determine specificity in either study.

**Fujirebio Lumipulse Ortho HCV Ag**

Only one study was performed using the Lumipulse test with 80 participants comparing against Lumispot and Abbott ARCHITECT.16 Sensitivity for the Lumipulse was reported as 95% (95% CI 90.2, 99.8) (Table 2). The Abbott ARCHITECT sensitivity in that study was 100%, suggesting a bias towards better performance. Not enough data was reported to determine specificity.

**Hunan Jynda Bioengineering Group HCV Core Ag ELISA**

There were 4 studies included in the bivariate analysis with 524 total samples. The pooled sensitivity was 59.5% (95% CI 46, 71.7), specificity 82.9% (95% CI 58.6, 94.3), positive LR 3.5 (95% CI 1.1, 12.6), and negative LR 0.28 (95% CI 0.2, 0.3; Table 2). Both the forest plot (Fig. 9) and bivariate analysis (Fig. 10; Table 2) demonstrated heterogeneity among the four studies, which limited confidence in the pooled estimate. No covariate assessment was performed as HIV status, HBV status and genotype distribution were unknown for all studies.

**DiaSorin S.A. Murex Ag/Ab EIA**

Four studies with a total sample size of 770 were available; however, given substantial heterogeneity in the forest plot (Fig. 11) a pooled estimate was not calculated. The sensitivity estimates varied between 50% and 100%. Heterogeneity was largely secondary to one outlier study by Tuke where a sensitivity of 50% (95% CI 40.4%, 59.6%) was reported. As reported above, this study was performed in HCV Ab-negative specimens in the “window period” of acute HCV infection. The authors note that when analysis was limited to specimens with viral load HCV RNA >10^6 IU/mL, there was an increase in sensitivity from 50% to 98%
Specificity could not be calculated. The El-Emshaty study is the smallest with 39 participants and reported a sensitivity of 91.3% (95% CI 71.9, 98.9), and specificity of 100% (95% CI 75.9, 100). Genotype distribution was unknown, though the study was performed in Egypt where genotype 4 is most prevalent. The Alzahrani study included 418 samples from 118 female adult participants and reported a sensitivity of 97.4% (95% CI 92.6%, 99.5%) and specificity of 100% (95% CI 98.4, 100). Finally, the Yang study conducted in Taiwan included 201 participants, 25% with HIV coinfection, and unknown genotype distribution. The reported sensitivity was 100% (95% CI 96.5, 100) and specificity 83.8% (95%CI 74.8, 90.2).

Quantitative data
Three studies provided quantitative data for analysis. All used the Abbott ARCHITECT HCV Core Ag Assay in comparison with NAT. Non-parametric regression of these pooled quantitative data was used to visually assess the correlation between HCVcAg and RNA (Fig. 12). The few points with negative HCVcAg were shown to occur at RNA levels < 3000 IU/mL where loss of linearity was also noted. There were two outlier points between 10 000 and 100 000 IU/mL and an additional point on the threshold cut-off for positivity. No further data on genotype or coinfection information was provided to further characterize these points.

HCV core Ag for treatment monitoring
Two studies evaluated HCVcAg compared to NAT at SVR, both using the Abbott ARCHITECT index test. One additional study assessed the accuracy of HCVcAg compared to NAT at EVR only. There were not enough studies to perform a meta-analysis. Results for sensitivity and specificity of the index test compared to NAT at baseline, EVR, and SVR were calculated and summarized in Table 4. These data do not evaluate the accuracy of HCVcAg at EVR to predict SVR but rather assess how the tests correlate at each specific time point and thus shed light on differences in the kinetics of core antigen and RNA. Two additional studies assessed timing of HCVcAg in EVR as a predictor of SVR using the Fujiribio Lumipulse test. Descriptive statistics of each including demographic data, HIV and HBV coinfection, and HCV genotype distribution are presented in Tables 1b and 3b.

HCV core Ag performance at different time-points during treatment
The Feng study included 32 adults without HIV or HBV coinfection. All participants had genotype 1b chronic HCV infection with viral loads >2000 IU/mL. The sensitivity of HCVcAg at baseline, EVR, and SVR was reported to be 100%, though specificity of EVR was 88.9% (95% CI 68.4, 100). There were 21 patients who achieved SVR, and 11 whose HCVcAg and HCV RNA remained positive 24 weeks after completion of therapy. In all 11 patients, the HCV viral load was >10^4 IU/mL.

The Loggi-study enrolled 35 adult patients without HIV or HBV coinfection; 20% had genotype 1a, 80% had genotype 1b HCV infection. Seventeen patients achieved sustained
virological response. The baseline sensitivity of the HCVcAg was 100% without enough data to calculate specificity. Sensitivity at EVR was 73.5% (95% CI 58.7, 88.4) with 100% specificity. The false negatives occurred in samples with viral loads between 15 and 10 000 IU/mL. For SVR, the sensitivity was 100% with 94.1% specificity (95% CI 82.9, 100).

The Moscato study analysed samples from 23 patients with unknown demographic information and included 4% genotype 1a, 39.1% genotype 1b, 26.1% genotype 2, 21.7% genotype 3, and 8.7% genotype 4. Baseline and SVR data comparing HCVcAg to NAT were not reported. Direct comparison of HCVcAg compared to NAT at 4 weeks for 10 patients had 100% sensitivity with 70% specificity (95% CI 41.6, 98.4). Three false-positive HCVcAg results were obtained: for 1 of which HCVcAg turned negative 1 month later and 2 turned negative 2 months later (12 weeks into therapy). For three patients tested at 12 weeks, correlation between qualitative results of HCVcAg and HCV NAT was complete. In 9 additional patients with unknown demographic data, correlation between quantitative HCV RNA and HCVcAg was assessed in 54 serum specimens collected at various time points during treatment. Among these samples, authors reported 100% sensitivity of cAg, including 9 specimens with low-level viraemia between 100 and 1000 IU/mL.

**HCV Core Ag as a predictor of SVR**

*Abbott ARCHITECT*

Included in the Feng study presented above, was an assessment of measurement of HCVcAg for EVR as a predictor of SVR. The study found a sensitivity of 100% with a specificity of 28% of EVR to predict SVR. This translated into a positive predictive value (PPV) 72% and a negative predictive value (NPV) of 100%. At 4 weeks after therapy initiation (RVR), the performance was inversed with a sensitivity of 29% and a specificity of 100%. The best measure was identified to be a log10 reduction in HCVcAg (ΔHCV Ag) at 144 hours with 95% sensitivity and 73% specificity.

*Fujirebio Lumipulse*

The Takahashi study included 60 genotype 1b patients, and 30 genotype 2 patients with unknown HIV and HBV coinfection status. Serum HCV core Ag was measured at baseline and at 3 days, 1 week, 2 weeks, 4 weeks, and 12 weeks of treatment while qualitative NAT was performed at 12 weeks to assess EVR, and 24 weeks after completion of therapy for SVR. SVR was achieved in 50% of genotype 1b patients, and 90% of genotype 2 patients. In genotype 1b patients, HCVcAg was higher at each time point among the non-SVR group compared to the SVR group, while in genotype 2 patients there was no difference seen in HCVcAg quantity over time between the 3 non-SVR patients and those who achieved SVR, and HCVcAg was below detection limit in all genotype 2 patients by day 14. For genotype 1b, HCVcAg level on day 7 was found to be the best predictor for SVR with sensitivity 79.4%, specificity 88.5%, PPV 90%, NPV 76.7%, and accuracy of 83.3% (Table 5).
In the Fujino study, 49 adult genotype 1b patients were initially enrolled, though 44 completed protocol and were included in the analysis. Patients with HBV were excluded and HIV status was not described. SVR was achieved in 10 patients. HCVcAg and RNA were measured on days 1, 7, and 14 of therapy. Four of the SVR group had negative HCVcAg on day 1 of therapy while all had positive NAT. Negative HCVcAg on day 7 of therapy gave sensitivity 57.1%, specificity 93.3%, PPV 80%, NPV 82.4%, and accuracy of 81.8% in prediction of SVR (Table 5), while undetectable HCV RNA on day 7 yielded sensitivity 100%, specificity 87.2%, PPV 50%, NPV 100%, and accuracy of 88.6%.

6. Discussion

This systematic review addressed diagnostic accuracy of HCV core antigen tests for identification of active HCV infection among those with and without positive HCV antibody through an analysis of 50 published studies (PICO5a) that utilized 7 different index tests. Additionally, accuracy of HCV core antigen tests for treatment monitoring and as a test of cure was assessed in 5 published studies (PICO 9).

The Abbott ARCHITECT HCV Core Antigen test had the highest sensitivity (93.4%), while specificity was similar to that of the Ortho ELISA-Ag (98.7% vs 99.2%). The estimates for both sensitivity and specificity were more precise for the ARCHITECT assay. This was partly because the ARCHITECT was the most extensively studied, with 30 publications included in this review compared to 5 studies included for Ortho, but also partly because of the greater homogeneity among the ARCHITECT studies. The likelihood ratios for both tests were also very favourable with the positive LR >10 indicative of a large increase in probability of disease with a positive result and negative LR <0.1 indicative of a moderate decrease in the probability of disease with a negative result. The EIKEN Lumispot and Fujirebio Lumipulse were designed with the same principle of technology as the ARCHITECT and have similar sensitivity and specificity, though assessment was limited to 1 and 2 studies. Tests such as the Hunan Jynda assay have the lowest sensitivity (59.5%), which supports the notion that signal amplification (as with chemiluminescence) is necessary to achieve adequate detection limits.

Quantitative analysis of data available from 3 studies using the ARCHITECT demonstrated close correlation between HCVcAg and RNA, though the linearity declined around an HCV RNA level of 3000 IU/mL, which is consistent with the analytical limit of detection reported by Abbott.

All studies included with treatment monitoring and SVR data were in patients on interferon (IFN)-based therapies. Data was limited and a meta-analysis was not possible. Descriptive analysis found the sensitivity of ARCHITECT at EVR in comparison to RNA ranging from 74% to 100% with specificity from 70% to 100% and at SVR (only assessed in 2 studies) sensitivity was 100% and specificity ranged from 94% to 100%. HCVcAg predictive accuracy for SVR was described in only 3 studies, 1 using the ARCHITECT and 2 using the Lumipulse. All three included mostly genotype 1b patients, and results indicated best
predictive accuracy of core antigen for SVR from the decline or reversion to negative early on in therapy at 6–7 days.

There were limitations in the data summarized in this review. For several index tests, there were not enough studies to derive pooled estimates and descriptive analyses only could be completed. There was substantial heterogeneity among all index tests aside from the ARCHITECT, and there were not enough data to perform planned sensitivity analyses of covariates and meta-regression of subgroups; thus descriptive statistics were substituted. From the limited data available, it is clear that data on core antigen test performance in genotypes 4, 5 and 6 is largely lacking, which limits the conclusions. Most of the studies were performed in high-resource settings and might not be reflective of the population that will be tested if HCVCAg diagnostics are implemented in LMICs. Furthermore, most studies were performed in reference laboratories and test performance might be decreased if tests are applied in routine laboratories.

To assess treatment monitoring, only 2 studies measured HCVCAg in comparison with NAT at SVR and only descriptive analyses could be conducted. Several of the studies found in the search were designed to answer a different question from that of the PICO structure – whether an early decline in HCVCAg could predict SVR and at what time period this was most accurate. These data were also described, though again meta-analysis could not be performed. Additionally, there were no studies using DAA IFN-free treatment regimens, thus the results from this descriptive analysis might not be reflective of the results that are to be expected with DAAs. The timing of this review has also occurred during a rapidly changing landscape; the utility of viral load monitoring while on treatment with these highly effective therapies, and thus utility of HCVCAg as a surrogate of NAT, has been called into question.9

Strengths and limitations of the systematic review
Strengths of this review include the development of an a priori protocol for the literature search, article selection, data extraction, and assessment of methodological quality. The search was performed without language restriction, though ultimately 3 articles were excluded for inability to find appropriate translation for Russian, Korean and Polish. Nevertheless, studies may have been missed in the comprehensive search, and subsequent studies published after the search date could not be included. Article selection and standardized data extraction in accordance with the predefined protocol was ensured by independent reviewers. Authors were contacted for missing data and clarifications, though some studies were excluded due to lack of author response or inability to provide original data. In the analysis, bivariate random effects modeling was used when appropriate to derive pooled estimates and univariate analyses were performed in effort to utilize all available data.

Further research suggested
The data limitations in this review highlight a need for better surveillance data that will inform an understanding of how many patients are missed by assays that have higher limits
of detection (e.g. 3000 IU/mL for ARCHITECT). Furthermore, a better understanding is necessary on the outcomes of patients with low viral loads: are these patients more likely to resolve their infection or at least less prone to develop HCV disease, or do they still have notable disease progression that would make them eligible for treatment? Similarly, more information is necessary on patients with high viral loads and negative HCVcAg to inform the optimization of antigen detection. The fluctuation in RNA during the pre-seroconversion phase and correlation of core antigen is also poorly understood.

Additionally, research is required to determine how covariates such as HIV or HBV coinfection or genotype may impact the accuracy of HCVcAg for diagnosing active infection as well as for monitoring treatment outcomes. The kinetics of HCVcAg with treatment also need to be evaluated further, particularly in the context of new DAA regimens.

7. Summary

In summary, this systematic review showed that there are several HCVcAg assays associated with high sensitivity (>90%) and specificity (>98%) compared with NAT. While even those with the highest performance do not reach the sensitivity of NAT, well-performing HCVcAg tests with an analytical sensitivity reaching into the femtomolar range (~3000 IU/mL), which translates into diagnostic sensitivity of about 95%, could serve as a replacement for NAT for HCV detection. This is the case particularly if HCVcAg are more affordable than NAT, which is conceivable from the cost of goods for the test. Furthermore, HCVcAg tests could be applied for a one-step screening test as they turn positive earlier than antibody tests (1–2 days after HCV RNA appears) and have a high specificity, thus not requiring any further confirmatory testing.

For both core antigen tests and NATs to reach a larger population at risk in LMICs, tests with better point-of-care (POC) suitability need to be developed and sample processing and transport mechanisms need to be improved to optimize the use of platforms requiring reference laboratories. HCVcAg tests are possible on a POC platform; however, given the need for signal amplification (as suggested by this review), an instrument-free assay is not conceivable in the near future. Furthermore, sample processing is necessary.

The role for HCVcAg as a substitute for NAT in assessment for SVR remains less clear. While the two studies presented show excellent results, and the quantitative data from PICO5a supports close correlation of HCVcAg with RNA above 3000 IU/mL, the kinetics of HCVcAg with treatment are not fully understood. Particularly, data on the early kinetics of HCVcAg, and the appropriate timing of assessment for predicting SVR are limited.

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References


## Tables

### Table 1a. Characteristics of included studies for PICO 5a grouped alphabetically by index test type

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Country and income category</th>
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<th>Sample condition</th>
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<tr>
<td>---------------</td>
<td>-----------------------------</td>
<td>--------------</td>
<td>------------------</td>
<td>-----------</td>
<td>--------------------</td>
<td>------------------------------</td>
<td>------------------------------</td>
<td>------------------</td>
<td>-------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Laperche, 2005</td>
<td>France (A)</td>
<td>Cohort</td>
<td>Broad</td>
<td>Unknown</td>
<td>35</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Plasma</td>
<td>Frozen</td>
</tr>
<tr>
<td>Nastouli, 2008</td>
<td>UK (A)</td>
<td>Cohort</td>
<td>Broad</td>
<td>Adults</td>
<td>25</td>
<td>100%</td>
<td>Unknown</td>
<td>0%</td>
<td>Serum</td>
<td>Frozen</td>
</tr>
<tr>
<td>Schnuriger, 2006</td>
<td>France (A)</td>
<td>Cohort</td>
<td>Broad</td>
<td>Adults</td>
<td>20</td>
<td>100%</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Serum</td>
<td>Frozen</td>
</tr>
<tr>
<td>Tuke, 2008</td>
<td>UK (A)</td>
<td>Cross-sectional</td>
<td>Broad</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Plasma</td>
<td>Frozen</td>
</tr>
<tr>
<td>Vermeersch, 2010</td>
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<td>Unknown</td>
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</tr>
<tr>
<td>Murayama, 2012</td>
<td>Japan (A)</td>
<td>Cross-sectional</td>
<td>Broad</td>
<td>Unknown</td>
<td>80</td>
<td>Unknown</td>
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<tr>
<td>Murayama, 2012</td>
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<td>Broad</td>
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</tr>
<tr>
<td>Lu, 2007</td>
<td>China (B)</td>
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<td>Broad</td>
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<td>Broad</td>
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<td>Cohort</td>
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<td>Broad</td>
<td>Mixed</td>
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<td>Unknown</td>
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<td>6.1%</td>
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<td>Serum</td>
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<tr>
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<td>Egypt (B)</td>
<td>Cohort</td>
<td>Broad</td>
<td>Adults</td>
<td>39</td>
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<td>Unknown</td>
<td>Serum</td>
<td>Frozen</td>
</tr>
<tr>
<td>Tuke, 2008</td>
<td>UK (A)</td>
<td>Cross-sectional</td>
<td>Broad</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Plasma</td>
<td>Frozen</td>
</tr>
<tr>
<td>Yang, 2011</td>
<td>Taiwan (A)</td>
<td>Cohort</td>
<td>Broad</td>
<td>Adults</td>
<td>201</td>
<td>25%</td>
<td>0%</td>
<td>39%</td>
<td>Serum</td>
<td>Frozen</td>
</tr>
<tr>
<td>Author, year</td>
<td>Country and income category</td>
<td>Study design</td>
<td>Study population</td>
<td>Age group</td>
<td>Number of subjects</td>
<td>Proportion with HIV infection</td>
<td>Proportion with HBV infection</td>
<td>Proportion female</td>
<td>Sample type</td>
<td>Sample condition</td>
</tr>
<tr>
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<td>------------------</td>
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<td>-----------------------------</td>
<td>-------------------------------</td>
<td>-----------------</td>
<td>-------------</td>
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</tr>
<tr>
<td><strong>Abbott ARCHITECT HCV Ag</strong></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Feng, 2014</td>
<td>China (B)</td>
<td>RCT</td>
<td>Broad</td>
<td>Adults</td>
<td>32</td>
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<td>0%</td>
<td>50%</td>
<td>Serum</td>
<td>Unknown</td>
</tr>
<tr>
<td>Loggi, 2013</td>
<td>Italy (A)</td>
<td>Cohort</td>
<td>Broad</td>
<td>Adults</td>
<td>35</td>
<td>0%</td>
<td>0%</td>
<td>34.4%</td>
<td>Serum</td>
<td>Frozen</td>
</tr>
<tr>
<td>Moscato, 2010</td>
<td>Italy (A)</td>
<td>Cohort</td>
<td>Broad</td>
<td>Unknown</td>
<td>23</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Serum</td>
<td>Frozen</td>
</tr>
<tr>
<td><strong>Fujirebio Lumipulse Ortho HCV Ag</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fujino, 2009</td>
<td>Japan (A)</td>
<td>Cohort</td>
<td>Broad</td>
<td>Adults</td>
<td>90</td>
<td>Unknown</td>
<td>Unknown</td>
<td>24%</td>
<td>Serum</td>
<td>Unknown</td>
</tr>
<tr>
<td>Takahashi, 2005</td>
<td>Japan (A)</td>
<td>Cohort</td>
<td>Broad</td>
<td>Adults</td>
<td>44</td>
<td>Unknown</td>
<td>0%</td>
<td>31.8%</td>
<td>Serum</td>
<td>Unknown</td>
</tr>
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</table>

Table 2. Diagnostic accuracy by HCVcAg index test type for diagnosis of active HCV infection compared to nucleic acid testing as the reference standard. Results from bivariate, univariate, range of studies, and single studies are all reported.

<table>
<thead>
<tr>
<th>Index Test</th>
<th>HCV Ab status</th>
<th># Studies (# samples)</th>
<th>Sensitivity 95% CI</th>
<th>Specificity 95% CI</th>
<th>Positive LR 95% CI</th>
<th>Negative LR 95% CI</th>
<th>$\tau^2$ [Covariance]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott ARCHITECT(^1)</td>
<td>All</td>
<td>20 (11,820)</td>
<td>93.4%(^i) (88.7, 96.2)</td>
<td>98.7%(^i) (96.9, 99.4)</td>
<td>71.8 (28.6, 160.3)</td>
<td>0.07 (0.04, 0.12)</td>
<td>Sens: 1.5 (SE 0.6) Spec: 2.3 (SE 1.0); [0.03]</td>
</tr>
<tr>
<td>Abbott ARCHITECT(^2)</td>
<td>All</td>
<td>30 (12,788)</td>
<td>94.1%(^i) (92.4, 95.7)</td>
<td>ND</td>
<td>NA</td>
<td>NA</td>
<td>Sens: 14.1</td>
</tr>
<tr>
<td>Abbott ARCHITECT(^1)</td>
<td>Known Ab positive</td>
<td>16 (5,246)</td>
<td>92.5%(^i) (86.9, 95.8)</td>
<td>97.8%(^i) (94.7, 99.1)</td>
<td>42 (16.4, 106.4)</td>
<td>0.05 (0.03, 0.08)</td>
<td>Sens: 1.4 (SE 0.5) Spec: 1.7 (SE 1.0); [0.02]</td>
</tr>
<tr>
<td>Abbott ARCHITECT(^2)</td>
<td>Known Ab positive</td>
<td>26 (6,214)</td>
<td>93.3%(^i) (91.2, 95.3)</td>
<td>ND</td>
<td>NA*</td>
<td>NA*</td>
<td>Sens: 19.4</td>
</tr>
<tr>
<td>Abbott ARCHITECT(^1)</td>
<td>Known Ab negative</td>
<td>4 (3,450)</td>
<td>74.4%(^i) (6.2, 99.2)</td>
<td>98.8%(^i) (97.2, 99.5)</td>
<td>62 (2, 198.5)</td>
<td>0.25 (0.003, 0.94)</td>
<td>Sens: 8.4 (SE 16.6) Spec: 0.2 (SE 0.6)</td>
</tr>
<tr>
<td>Ortho ELISA-Ag(^1)</td>
<td>All</td>
<td>5 (1,177)</td>
<td>93.2%(^i) (81.6, 97.7)</td>
<td>99.2%(^i) (87.9, 100)</td>
<td>116.5 (67, 977)</td>
<td>0.06 (0.02, 0.07)</td>
<td>Sens: 1.4 (SE 1.0) Spec: 3.8 (SE 5.1); [-0.4]</td>
</tr>
<tr>
<td>Ortho ELISA-Ag(^2)</td>
<td>All</td>
<td>6 (1,423)</td>
<td>90.8%(^i) (83.5, 98.2)</td>
<td>ND</td>
<td>NA</td>
<td>NA</td>
<td>122.0</td>
</tr>
<tr>
<td>Bio-RAD Monolisa™ HCV Ag-Ab ULTRA*</td>
<td>All</td>
<td>5 (525)</td>
<td>28.6–95%* (89.9, 99.8)</td>
<td>ND</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>EIKEN Lumispot HCV Ag</td>
<td>All</td>
<td>2 (235)</td>
<td>97.5–98.1%*</td>
<td>ND</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Fujirebio Lumipulse Ortho HCV Ag(^1)</td>
<td>All</td>
<td>1 (80)</td>
<td>95%** (90.2, 99.8)</td>
<td>ND</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Hunan Iynda Bioengineering Group HCV Core Ag ELISA(^1)</td>
<td>All</td>
<td>4 (524)</td>
<td>59.5%(^i) (46.7, 71.7)</td>
<td>82.9%(^i) (58.6, 94.3)</td>
<td>3.5 (1.1, 12.6)</td>
<td>0.28 (0.2, 0.3)</td>
<td>NA*</td>
</tr>
<tr>
<td>Murex Ag/Ab EIA</td>
<td>All</td>
<td>4 (770)</td>
<td>50–100%* (46.7, 71.7)</td>
<td>83.8–100%*</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

HCV: hepatitis C virus, cAg: core antigen, Ab: antibody, CI: confidence interval, LR: likelihood ratio, ELISA: enzyme linked immunosorbent assay, EIA: enzyme immunoassay, $\tau^2$: Tau squared, SE: standard error. 1. Determined by bivariate meta-analysis = “metandi” command in STATA, 2. Determined by univariate meta-analysis = “metan” command in STATA, *: Meta-analysis not possible, range of results seen across studies reported, **: results from one study only, ND: no data, NA: not applicable – if sensitivity and specificity results were not available from meta-analysis, likelihood ratios were not calculated; +: output of $\tau^2$ not interpretable given small number of studies.
Table 3a. Available genotype information for studies included in PICO 5a grouped alphabetically by index test type

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Number of subjects</th>
<th>% G1</th>
<th>% G1a</th>
<th>% G1b</th>
<th>%G2</th>
<th>%G3</th>
<th>%G4</th>
<th>%G5</th>
<th>%G6</th>
<th>% Other or unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chevaliez, 2014</td>
<td>514</td>
<td>59.3%</td>
<td></td>
<td></td>
<td>5%</td>
<td>12.3%</td>
<td>19.2%</td>
<td>1%</td>
<td>1.9%</td>
<td>1.9%</td>
</tr>
<tr>
<td>Descamps, 2012</td>
<td>22</td>
<td>68.2%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>31.8%</td>
</tr>
<tr>
<td>Durante-Mangoni, 2013</td>
<td>114</td>
<td>49%</td>
<td></td>
<td></td>
<td>31%</td>
<td>20%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duy Thong, 2015</td>
<td>189</td>
<td>35.4%</td>
<td>0%</td>
<td></td>
<td>44.9%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>19.6%</td>
</tr>
<tr>
<td>Ergünay, 2011</td>
<td>272</td>
<td>0.8%</td>
<td>2.2%</td>
<td>60.2%</td>
<td>0.4%</td>
<td>0.4%</td>
<td></td>
<td></td>
<td></td>
<td>35.8%</td>
</tr>
<tr>
<td>Garbuglia, 2014</td>
<td>292</td>
<td>17.1%</td>
<td>14.5%</td>
<td>9.4%</td>
<td>1%</td>
<td>27.6%</td>
<td>15.4%</td>
<td></td>
<td></td>
<td>15%</td>
</tr>
<tr>
<td>Hadziyannis, 2013</td>
<td>105</td>
<td>36%</td>
<td></td>
<td></td>
<td>6%</td>
<td>37%</td>
<td>21%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kesli, 2011</td>
<td>212</td>
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<td></td>
<td>100%</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Li Cavoli, 2012</td>
<td>92</td>
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<td></td>
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<tr>
<td>Mederacke, 2009</td>
<td>118</td>
<td>45.8%</td>
<td>10%</td>
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<td>19%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24.6%</td>
</tr>
<tr>
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<td>2850</td>
<td>2%</td>
<td>8.2%</td>
<td>17.3%</td>
<td>15.3%</td>
<td>17.3%</td>
<td>11.2%</td>
<td>8.2%</td>
<td>3.1%</td>
<td>17.3%</td>
</tr>
<tr>
<td>Ottiger, 2013</td>
<td>97</td>
<td>30.9%</td>
<td>19.5%</td>
<td>10.3%</td>
<td>23.7%</td>
<td>15.5%</td>
<td></td>
<td></td>
<td></td>
<td>30.9%</td>
</tr>
<tr>
<td>Russi, 2014</td>
<td>102</td>
<td>50%</td>
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<td></td>
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<td>48.1%</td>
<td>1.9%</td>
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</tr>
<tr>
<td>Tedder, 2013</td>
<td>54</td>
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<td>40.7%</td>
<td>22.2%</td>
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<td>16.7%</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vermehren, 2012</td>
<td>160</td>
<td>19%</td>
<td>29%</td>
<td>51%</td>
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<td></td>
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</table>
### Bio-RAD Monolisa™ HCV Ag-Ab ULTRA

<table>
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<tr>
<th>Study</th>
<th>N</th>
<th>5.7%</th>
<th>11.4%</th>
<th>34.3%</th>
<th>25.7%</th>
<th>14.3%</th>
<th>5.7%</th>
<th>2.9%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laperche, 2005</td>
<td>35</td>
<td>5.7%</td>
<td>11.4%</td>
<td>34.3%</td>
<td>25.7%</td>
<td>14.3%</td>
<td>5.7%</td>
<td>2.9%</td>
</tr>
<tr>
<td>Nastouli, 2008</td>
<td>25</td>
<td>68%</td>
<td>4%</td>
<td>4%</td>
<td>16%</td>
<td>8%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schnuriger, 2006</td>
<td>20</td>
<td>20%</td>
<td>15%</td>
<td>5%</td>
<td>30%</td>
<td>45%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tuke, 2008*</td>
<td></td>
<td></td>
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</table>

### EIKEN Lumispot HCV Ag

<table>
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<th>Study</th>
<th>N</th>
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<th></th>
<th>35.80%</th>
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<tbody>
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<td>Saito, 2003</td>
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### Ortho ELISA-Ag

<table>
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<th>5.8%</th>
<th>47.3%</th>
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<tbody>
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<td>Agha, 2004</td>
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<td></td>
<td></td>
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<td>11.5%</td>
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<td>Nübling, 2002</td>
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<td>42.3%</td>
<td>19.2%</td>
<td>11.5%</td>
<td>15.4%</td>
</tr>
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</table>


* Data are the same for DiaSorin S.A. Murex Ag/Ab EIA
### Table 3b. Genotype information for studies included in PICO 9 grouped alphabetically

<table>
<thead>
<tr>
<th>Author, Year</th>
<th>Number of subjects</th>
<th>% G1</th>
<th>% G1a</th>
<th>% G1b</th>
<th>% G2</th>
<th>% G3</th>
<th>% G4</th>
<th>% G5</th>
<th>% G6</th>
<th>% Other or unknown</th>
</tr>
</thead>
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<tr>
<td>Feng, 2014</td>
<td>32</td>
<td></td>
<td>100%</td>
<td>100%</td>
<td></td>
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</tr>
<tr>
<td>Loggi, 2013</td>
<td>35</td>
<td>100%</td>
<td>20%</td>
<td>80%</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Moscato, 2010</td>
<td>23</td>
<td>4%</td>
<td>39.1%</td>
<td>26.1%</td>
<td>21.7%</td>
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<tr>
<td>Fujirebio Lumipulse Ortho HCV Ag</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td>Fujino, 2009</td>
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<td>66.7%</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Takahashi, 2005</td>
<td>44</td>
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<td></td>
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</tr>
</tbody>
</table>

HCV: hepatitis C virus, Ag: antigen, Ab: antibody, G1 = genotype 1, G1a = genotype 1a, G1b = genotype 1b, G2 = genotype 2, G3 = genotype 3, G4 = genotype 4, G5 = genotype 5, G6 = genotype 6

### Table 4. Sensitivity and specificity of Abbott ARCHITECT HCV Ag assay compared to nucleic acid testing (NAT) assessed at baseline, at week 4 of interferon based therapy (early viral response), and at week 24 after completion of treatment (sustained viral response)

<table>
<thead>
<tr>
<th>Author, Year</th>
<th>Number of subjects</th>
<th>Baseline</th>
<th>Early viral response (EVR)</th>
<th>Sustained viral response (SVR)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Se (95% CI)</td>
<td>Sp (95% CI)</td>
<td>Se (95% CI)</td>
</tr>
<tr>
<td>Feng, 2014</td>
<td>32</td>
<td>100%</td>
<td>NA</td>
<td>100%</td>
</tr>
<tr>
<td>Loggi, 2013</td>
<td>35</td>
<td>100%</td>
<td>NA</td>
<td>73.5% (58.7%, 88.4%)</td>
</tr>
<tr>
<td>Moscato, 2010</td>
<td>23</td>
<td>NA</td>
<td>NA</td>
<td>100%</td>
</tr>
</tbody>
</table>

HCV: hepatitis C virus, Ag: antigen, Se: sensitivity, Sp: specificity, CI: confidence interval, NA: not applicable as cannot be calculated from study data
Table 5. Sensitivity and specificity of HCV core antigen assay in prediction of sustained viral response (SVR) after initiation of interferon-based treatment

<table>
<thead>
<tr>
<th>Author, Year</th>
<th>No. of subjects (no.to achieve SVR)</th>
<th>Index test</th>
<th>Timing of test after treatment start</th>
<th>Change in HCVcAg</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feng, 2014</td>
<td>32 (21)</td>
<td>Abbott ARCHITECT</td>
<td>6 days</td>
<td>Log 10</td>
<td>95.2%</td>
<td>70%</td>
</tr>
<tr>
<td>Fujino, 2009</td>
<td>90 (57)</td>
<td>Fujirebio Lumipulse</td>
<td>7 days</td>
<td>Absolute</td>
<td>79.4%</td>
<td>88.5%</td>
</tr>
<tr>
<td>Takahashi, 2005</td>
<td>44 (10)</td>
<td>Fujirebio Lumipulse</td>
<td>7 days</td>
<td>Absolute</td>
<td>57.1%</td>
<td>93.3%</td>
</tr>
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</table>

HCV: hepatitis C virus, No.: number
Figures

**Fig. 1a.** PRISMA diagram of studies excluded from screen one, and those full papers retrieved for more detailed evaluation

Potentially relevant citations identified from electronic databases: 8146

Excluded screen one: 7833

*Reason: Not relevant based on assessment of title and abstract*

Full papers retrieved for more detailed evaluation: 313

Full studies relevant to PICO 5a: 283

Full studies relevant to PICO 5b: 11

Full studies relevant to PICO 9: 44
Fig. 1b. PRISMA diagram of studies included in the review for PICO 5a

Full papers retrieved for more detailed evaluation: 283

Excluded screen two: 229

Reasons:
- Abstract or poster: 53
- Duplicate data/study: 6
- Editorial/Comment: 9
- Inappropriate ref standard: 6
- Less than 10 samples: 16
- Non-commercial or off-market assay: 65
- No core antigen, does not apply to study question: 25
- Non-blood specimen: 20
- Non-human specimens or commercial sera panels: 5
- Review article: 21
- Unable to translate: 3
- Unable to retrieve full article: 1

Excluded for non-extractable data with no response from authors: 4

Papers (studies) included in the systematic review: 50
**Fig. 1c.** PRISMA diagram of studies included in the review for PICO 5b

- Full papers retrieved for more detailed evaluation: **11**

- Excluded screen two: **10**
  - Reasons:
    - Abstract or poster: 2
    - Inappropriate ref standard: 1
    - Does not apply to study question: 4
    - Review article: 3

- Papers (studies) included in the systematic review: **1**
Fig. 1d. PRISMA diagram of studies included in the review for PICO 9

Full papers retrieved for more detailed evaluation:

44

Excluded screen two: 35

Reasons:
- Abstract or poster: 13
- Duplicate data/study: 1
- Less than 10 samples: 2
- Non-commercial or off-market assay: 14
- Does not apply to study question: 4
- Review article: 1

Excluded for non-extractable data with no response from authors or authors unable to provide needed information: 4

Papers (studies) included in the systematic review:

5
**QUADAS figures**

Fig. 2a. Risk of bias and applicability summary as judged by review authors about each QUADAS-2 domain presented as percentages across the 50 included studies for PICO 5a

**Risk of bias**

- **Flow and Timing**
- **Reference Standard**
- **Index Test**
- **Patient Selection**

Legend:
- Low risk of bias
- High risk of bias
- Unclear risk of bias
**Fig. 2b.** Risk of bias and applicability summary as judged by review authors about each QUADAS-2 domain presented as percentages across the 5 included studies for PICO 9

**Risk of bias**

- **Flow and Timing**
- **Reference Standard**
- **Index Test**
- **Patient Selection**

**Applicability**

**Fig. 3a.** Risk of bias and applicability summary as judged by review authors about each QUADAS-2 domain presented by individual study included in PICO 5a.
**Fig. 3a (cont).** Risk of bias and applicability summary as judged by review authors about each QUADAS-2 domain presented by individual study for PICO 5a
<table>
<thead>
<tr>
<th>Risk of Bias, ARCHITECT Studies (Cont.)</th>
<th>ARCHITECT, Applicability Concerns (Cont.)</th>
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</thead>
<tbody>
<tr>
<td><strong>Patient Selection</strong></td>
<td><strong>Patient Selection</strong></td>
</tr>
<tr>
<td><strong>Index Test</strong></td>
<td><strong>Index Test</strong></td>
</tr>
<tr>
<td><strong>Reference Standard</strong></td>
<td><strong>Reference Standard</strong></td>
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<tr>
<td><strong>Flow and Timing</strong></td>
<td><strong>Flow and Timing</strong></td>
</tr>
<tr>
<td>Mederacke, 2009</td>
<td>Mederacke, 2009</td>
</tr>
<tr>
<td>Mederacke, 2012</td>
<td>Mederacke, 2012</td>
</tr>
<tr>
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<td>Medici, 2011</td>
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<td>Miedouge, 2010</td>
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<td>Murayama*, 2012</td>
<td>Murayama*, 2012</td>
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<td>Ottiger, 2013</td>
<td>Ottiger, 2013</td>
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<td>Park, 2010</td>
<td>Park, 2010</td>
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<td>Reyes-Méndez, 2014</td>
<td>Reyes-Méndez, 2014</td>
</tr>
<tr>
<td>Rouet, 2015</td>
<td>Rouet, 2015</td>
</tr>
<tr>
<td>Russi, 2014</td>
<td>Russi, 2014</td>
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<tr>
<td>Tedder, 2013</td>
<td>Tedder, 2013</td>
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<tr>
<td>van Helden, 2014</td>
<td>van Helden, 2014</td>
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<tr>
<td>Vanhommerig, 2015</td>
<td>Vanhommerig, 2015</td>
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<td>Vermehren, 2012</td>
<td>Vermehren, 2012</td>
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*Same data for Fujirebio Lumipulse Ortho HCV Ag and EIKEN Lumispot HCV Ag*
Fig. 3a (cont). Risk of bias and applicability summary as judged by review authors about each QUADAS-2 domain presented by individual study for PICO 5a.
Fig. 3 (cont). Risk of bias and applicability summary as judged by review authors about each QUADAS-2 domain presented by individual study for PICO 5a.
**Fig. 3b.** Risk of bias and applicability summary as judged by review authors about each QUADAS-2 domain presented by individual study for PICO.
**Fig. 4a.** Forest plot of Abbott ARCHITECT HCV Ag assay sensitivity and specificity for the diagnosis of active HCV infection compared to NAT reference test for all samples regardless of HCV Ab status.
**Fig. 4b.** Univariate analysis of Abbott ARCHITECT HCV Ag Assay sensitivity for the diagnosis of active HCV infection compared to NAT reference test for all studies with sensitivity data regardless of HCV Ab status

<table>
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<td>43</td>
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</tr>
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<td>0</td>
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<tr>
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<td>34</td>
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</table>

**Sensitivity (95% CI)**

- Gu: 43.68 (33.66, 54.74)
- Ergünay: 72.40 (66.00, 78.18)
- Florea: 74.14 (60.96, 84.74)
- Buket: 86.46 (77.96, 92.59)
- Kadkhoda: 86.67 (77.87, 92.92)
- Roulet: 87.18 (72.57, 95.70)
- Li Cavoli: 90.00 (68.30, 98.77)
- Park: 90.23 (84.82, 94.20)
- Garbuglia: 90.48 (86.68, 93.48)
- Reyes-Méndez: 91.67 (77.53, 98.25)
- Miedouge: 92.00 (84.84, 96.48)
- Heidrich: 92.57 (90.42, 94.37)
- Medici: 96.12 (94.85, 97.16)
- Kesli: 96.25 (92.02, 98.61)
- Hadziyannis: 97.65 (91.76, 99.71)
- Kuo: 97.85 (92.45, 99.74)
- Chevaliez: 98.11 (95.93, 99.30)
- Mederacke: 98.31 (95.15, 99.65)
- van Helden: 99.47 (98.45, 99.89)
- Vanhommerig: 100.00 (88.78, 100.00)

HCV: hepatitis C virus, Ag: antigen, NAT: nucleic acid testing, Ab: Antibody, TP: true positive, FP: false positive, FN: false negative, TN: true negative, CI: confidence interval
**Fig. 4c.** Forest plot of Abbott ARCHITECT HCV Ag assay sensitivity and specificity for the diagnosis of active HCV infection compared to NAT reference test for known HCV antibody-positive samples.

HCV: hepatitis C virus, Ag: antigen, NAT: nucleic acid testing, Ab: antibody, TP: true positive, FP: false positive, FN: false negative, TN: true negative, CI: confidence interval
Fig. 4d. Forest plot of Abbott ARCHITECT HCV Ag assay sensitivity and specificity for the diagnosis of active HCV infection compared to NAT reference test for known HCV antibody-negative samples.

HCV: hepatitis C virus, Ag: antigen, NAT: nucleic acid testing, Ab: antibody, TP: true positive, FP: false positive, FN: false negative, TN: true negative, CI: confidence Interval.
**Fig. 5.** Bivariate analysis of Abbot ARCHITECT HCV antigen assay sensitivity and specificity for diagnosis of active HCV infection compared to gold standard nucleic acid testing in (a) all samples regardless of HCV antibody (Ab) status, (b) HCV Ab-positive samples (c) HCV Ab-negative samples. These plots show pooled summary estimates (red squares), the dashed red line represents the 95% confidence region and the dashed green line represents the 95% prediction region. The individual circles represent each study and the size of the circle is proportional to the total sample size.
Fig. 6. Forest plot Ortho ELISA-Ag sensitivity and specificity for diagnosis of active HCV infection compared to NAT reference test for all samples regardless of HCV Ab status.

<table>
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<tr>
<th>Author</th>
<th>Year</th>
<th>TP</th>
<th>FP</th>
<th>FN</th>
<th>TN</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
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<tr>
<td>Nübling</td>
<td>2002</td>
<td>218</td>
<td>0</td>
<td>91</td>
<td>185</td>
<td>70.55 (65.13, 75.58)</td>
<td>100.00 (98.03, 100.00)</td>
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<td>El-Sayed</td>
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<td>28</td>
<td>0</td>
<td>6</td>
<td>16</td>
<td>82.35 (65.47, 93.24)</td>
<td>100.00 (79.41, 100.00)</td>
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<td>Okazaki</td>
<td>2008</td>
<td>250</td>
<td>0</td>
<td>9</td>
<td>41</td>
<td>96.53 (93.51, 98.40)</td>
<td>100.00 (91.40, 100.00)</td>
</tr>
<tr>
<td>Ohta</td>
<td>2004</td>
<td>99</td>
<td>9</td>
<td>3</td>
<td>98</td>
<td>97.06 (91.64, 99.39)</td>
<td>91.59 (84.63, 96.08)</td>
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<tr>
<td>Letowska</td>
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<td>96</td>
<td>100.00 (86.28, 100.00)</td>
<td>96.97 (91.40, 99.37)</td>
</tr>
</tbody>
</table>

ELISA: enzyme linked immunosorbent assay, Ag: antigen, HCV: hepatitis C virus, NAT: nucleic acid testing, Ab: antibody, TP: true positive, FP: false positive, FN: false negative, TN: true negative, CI: confidence interval.
**Fig. 7.** Bivariate analysis of Ortho ELISA-Ag sensitivity and specificity for diagnosis of active HCV infection compared to gold standard nucleic acid testing in all samples regardless of HCV antibody status. This plot shows pooled summary estimates (red squares), the dashed red line represents the 95% confidence region and the dashed green line represents the 95% prediction region. The individual circles represent each study and the size of the circle is proportional to the total sample size.
**Fig. 8.** Forest plot of Bio-RAD Monolisa™ HCV Ag-Ab ULTRA sensitivity for diagnosis of active HCV infection compared to NAT reference test for all samples regardless of HCV Ab status

HCV: hepatitis C virus, Ag: antigen, Ab: antibody, NAT: nucleic acid testing, TP: true positive, FP: false positive, FN: false negative, TN: true negative
**Fig. 9.** Forest plots of Hunan Jynda HCV Core Ag ELISA sensitivity and specificity for diagnosis of active HCV infection compared to NAT reference test for all samples regardless of HCV Ab status

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>TP</th>
<th>FP</th>
<th>FN</th>
<th>TN</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
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<td>69.63 (61.13, 77.24)</td>
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<td>Ouyang</td>
<td>2006</td>
<td>44</td>
<td>30</td>
<td>38</td>
<td>37</td>
<td>53.66 (42.30, 64.75)</td>
<td>55.22 (42.58, 67.40)</td>
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<td>26</td>
<td>84</td>
<td>69.77 (58.92, 79.21)</td>
<td>96.55 (90.25, 99.28)</td>
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<td>27</td>
<td>2</td>
<td>9</td>
<td>11</td>
<td>75.00 (57.80, 87.88)</td>
<td>84.62 (54.55, 98.08)</td>
</tr>
</tbody>
</table>

HCV: hepatitis C virus, ELISA: enzyme linked immunosorbent assay, Ag: antigen, NAT: nucleic acid testing, Ab: Antibody, TP: true positive, FP: false positive, FN: false negative, TN: true negative, CI: confidence interval
**Fig. 10.** Bivariate analysis of Hunan Jynda Bioengineering Group HCV Core Ag ELISA sensitivity and specificity for diagnosis of active HCV infection compared to gold standard nucleic acid testing for all samples regardless of HCV antibody status. This plot shows pooled summary estimates (red squares), the dashed red line represents the 95% confidence region and the dashed green line represents the 95% prediction region. The individual circles represent each study and the size of the circle is proportional to the total sample size.
**Fig. 11.** Forest plot of DiaSorin S.A. Murex Ag/Ab EIA sensitivity for diagnosis of active HCV infection compared to NAT reference test for all samples regardless of HCV Ab status

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
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<th>FP</th>
<th>FN</th>
<th>TN</th>
<th>Sensitivity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuke</td>
<td>2008</td>
<td>56</td>
<td>0</td>
<td>56</td>
<td>0</td>
<td>50.00 (40.40, 59.60)</td>
</tr>
<tr>
<td>El-Emshaty</td>
<td>2011</td>
<td>21</td>
<td>0</td>
<td>2</td>
<td>16</td>
<td>91.30 (71.96, 98.93)</td>
</tr>
<tr>
<td>Alzahrani</td>
<td>2008</td>
<td>112</td>
<td>0</td>
<td>3</td>
<td>303</td>
<td>97.39 (92.57, 99.46)</td>
</tr>
<tr>
<td>Yang</td>
<td>2011</td>
<td>102</td>
<td>16</td>
<td>0</td>
<td>83</td>
<td>100.00 (96.45, 100.00)</td>
</tr>
</tbody>
</table>

Ag: antigen, Ab: antibody, EIA: enzyme immunoassay, HCV: hepatitis C infection, NAT: nucleic acid testing, TP: true positive, FP: false positive, FN: false negative, TN: true negative
Fig. 12. Non-parametric regression smoother of pooled quantitative data assessing correlation between Abbott ARCHITECT HCV core Ag measured in log fmol/L and HCV RNA measured in log IU/mL. The red line indicates the positivity threshold of the core antigen index test corresponding to 3 fmol/L.

HCV = hepatitis C virus, Ag = antigen, RNA = ribonucleic acid
Appendices

Appendix A. Search report for systematic review on HCV Antigen use for diagnostics as well as treatment monitoring

Date of search = March 2015. The following tables shows the sources that have been searched and the hits retrieved from those searches

<table>
<thead>
<tr>
<th>Source</th>
<th>Date range searched</th>
<th>Hits retrieved (before duplicate removal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electronic databases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medline (PubMed)</td>
<td>All available</td>
<td>2820</td>
</tr>
<tr>
<td>Cochrane</td>
<td>All available</td>
<td>127</td>
</tr>
<tr>
<td>Embase</td>
<td>All available</td>
<td>5501</td>
</tr>
<tr>
<td>Web of Science</td>
<td>All available</td>
<td>2635</td>
</tr>
<tr>
<td>Scopus</td>
<td>All available</td>
<td>3549</td>
</tr>
<tr>
<td><strong>Final number of records in EndNote database after deleting duplicates</strong></td>
<td></td>
<td><strong>8146</strong></td>
</tr>
</tbody>
</table>

**Search strategy Embase**

1. ‘hepatitis c antigen’/exp OR ‘hepatitis c antigen’ OR ‘hepatitis c'/exp OR ‘hepatitis c virus’/exp OR ‘hepatitis c virus’
2. ‘hepatitis c antigen’ OR ‘hepatitis C’ OR hepatitis c virus’ or ‘hcv’
3. #1 OR #2
4. ‘antigen’/exp OR ‘antigen' OR ‘virus antigen’/exp OR ‘virus antigen’
5. ‘antigen’ OR ‘virus antigen’
6. #4 OR #5
7. ‘nucleic acid amplification’/exp OR ‘nucleic acid amplification’ OR ‘virus rna’/exp OR ‘virus rna’ OR ‘rna’/exp OR ‘rna’
8. ‘nucleic acid amplification’ OR ‘virus rna’ OR ‘rna’ OR ‘nucleic acid test’
9. #7 OR #8
10. #3 AND #6 AND #9

‘hepatitis c antigen’/exp OR ‘hepatitis c’/exp OR ‘hepatitis c virus’/exp OR ‘hepatitis c antigen' OR ‘hepatitis c' OR ‘hepatitis c virus’ OR 'hcv' AND (‘antigen’/exp OR ‘virus antigen’/exp OR ‘antigen' OR ‘virus antigen’) AND (‘nucleic acid amplification’/exp OR ‘virus rna’/exp OR ‘rna’/exp OR ‘nucleic acid amplification’ OR ‘virus rna’ OR ‘rna’ OR ‘nucleic acid test’
Search strategy Web of Knowledge (SCI-expanded, SSCI, Conference Proceedings science, BIOSIS previews)

1. Hepatitis C OR HCV (topic)
2. Antigen* OR core antigen* (topic)
3. RNA OR NAT or nucleic acid test* (topic)
4. #1 AND #2 AND #3

Search strategy PubMed


Search strategy SCOPUS

( TITLE-ABS-KEY ( "Hepatitis C"OR hcv )AND TITLE-ABS-KEY ( antigen*OR "core antigen*" )AND TITLE-ABS-KEY ( rna OR"nucleic acid test" ) )AND( LIMIT-TO ( DOCTYPE ,"ar" )OR LIMIT-TO ( DOCTYPE ,"ip" ) )

Search strategy Cochrane

1. MeSH descriptor: Hepatitis C
2. MeSH Descriptor: Hepacivirus
3. MeSH Descriptor: Hepatitis C Antigens
4. Hepatitis C
5. HCV
6. #1 or #2 or #3 or #4 or #5
7. MeSH Descriptor: Nucleic Acid Amplification Techniques
8. RNA
9. Nucleic acid test
10. #7 or #8 or #9
11. Antigen
12. “Core antigen”
13. #11 or #12
14. #6 AND #10 AND #13
# Appendix B. Data extraction form

<table>
<thead>
<tr>
<th>ID</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>First Author</td>
<td></td>
</tr>
<tr>
<td>Corresponding author and email</td>
<td></td>
</tr>
</tbody>
</table>
| Was author contacted? | 1 – Yes  
2 – No  
If yes, dates(s) |
| Title |  |
| Year (of publication) |  |
| Year (study start date) |  |
| Language | 1 – English  
2 – Other  
If other, specify: |
| HCV Genotypes specified | 1 – n, % Genotype 1  
Genotype 1a  
Genotype 1b  
2 – n, % Genotype 2  
3 – n, % Genotype 3  
4 – n, % Genotype 4  
5 – n, % Genotype 5  
6 – n, % Genotype 6 |
| % HIV positive |  |
| % HBsAg + (chronic HBV infection) |  |
| % Adults/children |  |
| Age (mean SD, median IQR, range) |  |
| Gender, % Female |  |
| Country where study was conducted |  |
| Country World Bank Classification (at time of study start date) | 1 – Middle/Low  
2 – High  
3 – Both middle/low and high |
| Study design | 1 – Randomized trial  
2 – Cross-sectional  
3 – Cohort  
4 – Case Control  
5 – Other, specify  
9 – Unk/NR  
If other, specify: |
| Participant selection | 1 – Consecutive  
|                      | 2 – Random  
|                      | 3 – Convenience  
|                      | 4 – Other  
|                      | 9 – Unk/NR  
| Study population | 1 – Broad  
|                  | 2 – Healthy persons only  
|                  | 3 – Unk/NR  
|                  | Comments: ___________________________________  
| Direction of study data collection | 1 – Prospective  
|                                    | 2 – Retrospective  
|                                    | 9 – Unk/NR  
| Comments about study design |  
| Were samples excluded based on prior testing of the sample? | 1 – yes (specify below)  
|                                                            | 2 – no  
|                                                            | 9 – Unk/NR  
|                                                            | Comments: ___________________________________  
| Number after screening by exclusion and inclusion criteria | _____  
|                                                        | 9 – Unk/NR  
| Sample size (total number included in 2/2 table) | _____  
|                                                  | 9 – Unk/NR  
| Unit of analysis | 1 – One specimen per patient  
|                      | 2 – Multiple specimens per patient  
|                      | 3 – Unknown number of specimens per patient  
|                      | 9 – NR/Unclear  
|                      | Describe as in paper, if unclear:  
| Types of specimen and number | 1 – Serum ___  
|                                  | 2 – Plasma ___  
|                                  | 3 – Whole blood___  
| HCV NAT method used | 1 – PCR  
|                      | 2 – bDNA  
|                      | 3 – TMA  
|                      | 9 – Unk/NR  
| HCV NAT method quantitative? | 1 – Yes  
|                                 | 2 – No  
|                                 | 9 – Unk/NR  
| HCV Ag test manufacturer | 1 – Abbott ARCHITECT  

<table>
<thead>
<tr>
<th>Question</th>
<th>Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV Ag method quantitative?</td>
<td>1 – Yes</td>
</tr>
<tr>
<td></td>
<td>2 – No</td>
</tr>
<tr>
<td></td>
<td>9 – Unk/NR</td>
</tr>
<tr>
<td>Were reference NAT test and HCV Ag test performed on specimen within 30 days</td>
<td>1 – Yes</td>
</tr>
<tr>
<td></td>
<td>2 – No</td>
</tr>
<tr>
<td></td>
<td>3 – Unk/NR</td>
</tr>
<tr>
<td>Was Ag test obtained/repeated while subject was on treatment for HCV infection or after treatment completed?</td>
<td>1 – yes (Specify below)</td>
</tr>
<tr>
<td></td>
<td>2 – No</td>
</tr>
<tr>
<td>Specify timing of Ag Collection</td>
<td>1 – Baseline, prior to treatment</td>
</tr>
<tr>
<td></td>
<td>2 – EVR</td>
</tr>
<tr>
<td></td>
<td>3 – SVR 12 weeks</td>
</tr>
<tr>
<td></td>
<td>4 – SVR 24 weeks</td>
</tr>
<tr>
<td>What treatment regimen was used?</td>
<td>1 – Interferon based therapy</td>
</tr>
<tr>
<td></td>
<td>2 – Interferon free direct acting antivirals</td>
</tr>
<tr>
<td></td>
<td>3 – Unk/NR</td>
</tr>
<tr>
<td>Did all patients NAT within the study?</td>
<td>1 – yes</td>
</tr>
<tr>
<td></td>
<td>2 – no</td>
</tr>
<tr>
<td></td>
<td>9 – Unk/NR</td>
</tr>
<tr>
<td>Comments:</td>
<td>______________________________</td>
</tr>
<tr>
<td>Was index test performed per recommendation of the manufacturer?</td>
<td>1 – Yes</td>
</tr>
<tr>
<td></td>
<td>2 – No</td>
</tr>
<tr>
<td></td>
<td>9 – Unk/NR</td>
</tr>
<tr>
<td>Comments:</td>
<td>______________________________</td>
</tr>
</tbody>
</table>
Appendix C. QUADAS-2 protocol

Domain 1. Patient selection

Risk of bias: could the selection of patients have introduced bias?

- Signalling question 1: Was a consecutive or random sample of patients or specimens enrolled? Score “yes” if the study enrolled a consecutive or random sample of eligible patients; “no” if the study selected patients by convenience, and “unclear” if the study did not report the manner of patient selection or unable to tell.

- Signalling question 2: Was a case-control design avoided? Rate “no” if case-control study, “yes” if prospective or cross-sectional study.

- Signalling question 3: Did the study avoid inappropriate exclusions? Score “no” if the study excluded samples based on prior testing of the sample and “unclear” if unable to tell.

Risk of Bias is scored as “low risk” if selection was done in a random or consecutive manner and the study was prospective and did not exclude samples based on prior testing; “high risk” if selection was by convenience, from case-control study or excluded samples; and “unclear risk” if the manner of participant selection is unclear and no clinical information is provided.

Applicability: Are there concerns that the included patients and setting do not match the review question?

We are interested in how HCV AG test performs across HCV genotypes and among HIV-infected (immunocompromised) persons. If a study includes only very selected persons, only healthy or blood donors, it would not be relevant to the study question. Setting of testing is not relevant to the review question. We will score “low risk” if broad study population, “high risk” if population is blood donors or healthy persons only, and “unclear risk” if the population is not well characterized.

Domain 2. Index test

Risk of bias: could the conduct or interpretation of the index test have introduced bias?

- Signalling question 1: Were the index test results interpreted without knowledge of the results of the reference standard? Rate “yes” if results of reference standard were blinded. Rate “no” if reference standard results were unblinded.

- Signalling question 2: If a threshold was used, was it pre-specified? Answer “yes” for all studies as limit of detection for all commercially available HCV Ag tests are pre-specified. Score “low risk” for all tests interpreted with blinded results of reference standard. Score “high risk” for antigen tests interpreted with results from reference standard available. Score “unclear risk” if availability of reference test is not specified.

Applicability: Are there concerns that the index test, its conduct, or its interpretation differ from the review question? Variations in test technology, execution, or interpretation may affect estimates of the diagnostic accuracy of a test.
• Score “low concern” if the test was done as per recommendation of the manufacturer. Score “high concern” if additional processing steps were added. Score “unclear” if not discussed in the study.

**Domain 3. Reference standard**

*Risk of bias: could the reference standard, its conduct, or its interpretation have introduced bias?*

• Signalling question 1: Is the reference standard likely to correctly classify the target condition?
  
  There are multiple methods of NAT, each with slightly varying sensitivity, however overall the tests are highly sensitive and the verification should be minimal. We will score “yes” for all studies.

• Signalling question 2: Were the reference standard results interpreted without knowledge of the results of the index test?
  
  The reference standard in this case also does not allow for interpretation. Therefore it is unlikely to introduce bias even if reference standard was resulted with knowledge of the index test result.

For risk of bias, score “low risk” for all studies.

*Applicability: Are there concerns that the target condition as defined by the reference standard does not match the question?*

Judge applicability to be of “low risk” for all studies as circulating virus is by definition associated with active infection and the specificity of the reference standard is high. While the reference standard is not able to differentiate between acute or chronic infection, the core antigen is also not expected to do so. The differentiation will be done based on the constellation of NAT results with serology results. This will be assessed in a stratified analysis.

**Domain 4. Flow and timing**

*Risk of bias: Could the patient flow have introduced bias?*

• Signalling question 1: Was there an appropriate interval between the index test and reference standard? We will limit time between reference and index testing to <1 month. Score “yes” if time between tests is <1 month, score “no” if time between tests is more than 1 month.

• Signalling question 2: Did all patients in the study receive the same reference standard? Answer “yes” if all patients had NAT, answer “no” if reference standard NAT was not used for all patients, answer “unclear” if it is not specified.

• Signalling question 3: Were all patients included in the analysis? Determined the answer to this question by comparing the number of patients enrolled with the number of patients included in the two-by-two tables.
For risk of bias, score “low risk” if the number of participants enrolled was clearly stated and corresponded to the number presented in the analysis or if exclusions were adequately described. Score “high risk” if there were participants missing or excluded from the analysis and there was no explanation given; and “unclear risk” if not enough information was given to assess whether participants were excluded from the analysis; usually this means that the number of participants originally enrolled in the study was not explicitly stated.

Appendix D. List of excluded studies and reasons for exclusion organized by PICO.

**PICO 5a:**


30. Busch MP, Wright DJ, Hirschhorn DF, Baggett D, Maret S, Lee SR, et al. Sensitivity of 1(st) and 2(nd) generation HCV antigen assays versus nucleic acid testing (NAT) for detection of ramp-up phase of HCV infection. Transfusion. 2001;41(9):3S. Abstract or poster


66. Frank K, Karl A. Comparison of different confirmation test methods to anti-HCV used for repeatedly HCV-reactive samples. Infusionsther Transfusionsmed. 2001;28(Suppl. 1):47. No HCV core antigen performed


71. Grant PR, Sims CM, Tedder RS. Quantification of HCV RNA levels and detection of core antigen in donations before seroconversion. Transfusion. 2002;42(8):1032–6. Non-commercial or off-market assay


Kuo YH, Lu SN. Is HCV core antigen (HCV Ag) an adequate marker for community screening? Hepatol Int. 2012;6(1):201–2. Abstract or poster


Editorial or comment


Tanaka E, Ohue C, Aoyagi K, Yamaguchi K, Yagi S, Kiyosawa K, et al. Evaluation of a new enzyme immunoassay for hepatitis C virus (HCV) core antigen with clinical sensitivity approximating...
that of genomic amplification of HCV RNA. Hepatology. 2000;32(2):388–93. *Non-commercial or off market assay*


Page | 443


**PICO 5b:**


**PICO 9:**


37. Yang R, Rao H, Wei L. Hepatitis C virus core antigen was correlated with RNA load and had similar dynamics during treatment for chronic hepatitis C. Hepatol Int. 2011;5(1):220. Abstract or poster
