Annex 5.6

PICO 4 - How to test (HCV)

Diagnostic strategies for hepatitis C antibody detection: a meta-analysis and review of the literature

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1. Executive summary

**Background:** An estimated 130–150 million people have chronic hepatitis C infection worldwide, leading to 350 000–500 000 deaths per year. Although HCV treatment is successful in a majority of people, most HCV-infected individuals remain undiagnosed and untreated. Advances in HCV detection technology create new opportunities for enhancing screening, referral, and treatment.

**Methods:** A comprehensive literature search algorithm, including Internet searches, using the components hepatitis C, screening, and testing strategies were applied. We reviewed observational studies and randomized controlled trials (RCTs) that provided original data from patient specimens. Our goal was to compare the effects of two broad strategies for hepatitis C antibody detection – one-test strategies and two-test strategies on diagnostic accuracy, costs, and resource utilization.

**Results:** Our search resulted in 3060 literature review references and 3 additional Internet references for PICO 4. Screening of titles/abstracts resulted in the selection of 8 articles for possible data extraction. Two of these 8 articles met all of the data extraction inclusion criteria so no articles were identified as final selection for PICO 4; comparing the diagnostic accuracy, cost or effectiveness of two different testing algorithms. These 8 articles are discussed in more detail – 1 of the articles provided a comprehensive overview of antibody/antigen testing; 2 articles delved into core antigen testing; 3 articles exemplified other testing such as recombinant immunoblot (IB) tests, signal-to-cut-off ratios, point-of-care tests (POCT), and antibody-based rapid diagnostic tests (RDT); 1 discussed testing strategies; 1 provided a look at comparison and cost–effectiveness of given testing strategies.

**Conclusions:** Two studies compared the diagnostic accuracy, cost, cost–effectiveness of a 1-test versus 2-test strategy for detection of HCV antibody. One study found that in individuals who are HCV antibody positive, the use of an IB assay with defined signal-to-cut-off ratios can be used to distinguish between those who are viraemic and those who are not. This reduces the number of nucleic acid tests (NATs) required to confirm active infection is a cost–effective strategy. Another study found that screening with a highly sensitive EIA followed by another EIA as confirmation assay in a routine clinical laboratory can be effective in nonimmunocompromised populations. In immunocompromised patients, IB may be more effective as these patients tend to have low antibody levels.

2. Background

**Hepatitis C virus**

Hepatitis C virus (HCV) causes acute infection which can progress to chronic infection and liver disease.\(^1\) An estimated 130–150 million people have chronic hepatitis C infection worldwide, leading to 350 000–500 000 deaths per year.\(^1\) Approximately 15–45% of individuals who have acute HCV infection will spontaneously clear it without any treatment. Most individuals will go on to develop chronic active HCV infection which is defined by the presence of HCV
Although HCV treatment is successful in a majority of people, most HCV-infected individuals remain undiagnosed and untreated. As a result, approximately 15–30% of individuals with chronic HCV infection progress to cirrhosis, leading to end-stage liver disease and hepatocellular carcinoma.

The recent introduction of direct-acting antivirals (DAAs) have led to sustained virological response (SVR) in greater than 90% of all individuals and are recommended by the WHO. DAAs will not only improve SVR rates, but also may simplify HCV management algorithms and allow smaller health facilities to manage HCV-infected individuals.

In April 2014, the World Health Organization published guidelines for the screening, care, and treatment of individuals with HCV infection. These guidelines included recommendations on who to screen for HCV and how to confirm HCV infection, but not which tests are optimal for initial screening. A test for HCV antibody (Ab) is an important first step in the diagnosis of hepatitis C infection as the presence of Ab is a marker of exposure to HCV.

After an initial positive result for HCV Ab, supplementary testing can be undertaken in order to confirm active infection and facilitate entry into a care pathway. The detection of HCV Ab in blood can include rapid diagnostic tests, or enzyme immunoassays (EIA). Confirmation of the specificity of a reactive HCV Ab first-line test result can be carried out by repeating the HCV Ab testing in a different assay of similar sensitivity. Specificity is confirmed when this reagent abolishes reactivity in the assay.

WHO recommends standardized testing strategies to maximize the accuracy of hepatitis B and C testing while minimizing cost and increasing simplicity. This PICO question addresses the issue of whether a positive result from a single HCV Ab assay has sufficient specificity in order to proceed to supplementary testing and/or entry into a care pathway, or whether confirmatory testing on the same specimen with a different HCV Ab assay performed sequentially after the first assay is required. This is particularly relevant in low-prevalence settings where more than one assay may be required to confirm specificity.

**Description of HCV antibody testing**

Antibodies to HCV infection begin during early infection and persist throughout life in most individuals. Hence, an HCV Ab test is the best marker of exposure to HCV but cannot be used to distinguish between active and treated or resolved past infection.

Screening for exposure to HCV is dependent on assays that detect antibodies to HCV (anti-HCV). Once antibody status is confirmed, the patient can undergo supplementary testing to determine the presence of HCV RNA or core antigen (HCV cAg) as markers of active infection. It is important to note that the latest generation of assays designed to detect anti-HCV are combined with cAg to increase the sensitivity of the assay in detecting active infection.

The question this PICO aims to address is whether one or two serological assays (anti-HCV or HCV Ag/Ab combo assays) performed sequentially are required, in terms of specificity and positive predictive value, in order to proceed to supplementary testing.
<table>
<thead>
<tr>
<th>PICO 4</th>
<th>Among persons identified for hepatitis C testing, what is the best testing strategy (diagnostic accuracy and other outcomes) for detection of HCV antibodies? (One-test versus two-test strategy) (Fig. 1A,1B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>Persons identified for HCV testing</td>
</tr>
<tr>
<td>I</td>
<td>One-test strategy; One HCV Ab test  (Fig. 1A)</td>
</tr>
<tr>
<td>C</td>
<td>Two-test strategy; Two different HCV Ab tests  (Fig. 1B)</td>
</tr>
</tbody>
</table>
| O      | Diagnostic accuracy  
True negatives (TN), who are screen negative, and do not have HCV infection.  
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 prevent progression of liver disease.  
True positives (TP), who are screen positive and have HCV infection.  
False positives (FP), who are screen positive, but do not truly have HBV infection. These will have additional unnecessary tests and evaluation.  
Costs (cost of testing strategy including lab reagents and running costs, cost of further evaluation of a false positive)  
Cost–effectiveness  
Acceptability to health-care worker and patients  
Other outcomes (missed cases of liver disease because of false-negative results, Unnecessary referral, investigations and/or treatment in false positives) |

Two systematic reviews on diagnostic performance of different hepatitis C serological assays focused on evaluating point-of-care tests compared to EIAs and other reference tests.\(^{10,11}\) None of the existing reviews compared one-test and two-test strategies for detection of hepatitis C Ab.
Fig. 1: What is the best testing strategy for detection of HCVAb? (A. One test, B. Two-test strategy)

**Anti-HCV one-assay strategy (HCV-exposure)**

- **Anti-HCV (RTD/EIA) (A1)**
  - **Non-Reactive**
    - **Interpretation:** No serological evidence of exposure to HCV
  - **Reactive**
    - **Interpretation:** Compatible with exposure to HCV
      - Proceed to supplementary testing

Fig. 1a. One-assay testing strategy for exposure to HCV (detection of anti-HCV)

- **Anti-HCV (RTD/EIA) (A1)**
  - **Non-Reactive**
    - **Interpretation:** No exposure to HCV
  - **Reactive**
    - **Anti-HCV one-assay strategy for HCV exposure, with additional step for diagnosis of active HCV infection**
      - **HCV RNA or HCV Ag (A2)**
        - **Detected**
          - **Interpretation:** Active HCV infection
            - Link to care
        - **Not detected**
          - **Interpretation:** No active HCV infection
            - Additional testing as appropriate

Fig. 1b. Two-assay testing strategy for diagnosis of HCV (detection of anti-HCV, followed by HCV RNA/core Ag)

3. **Objectives**
   - To identify quantitative evidence on the sensitivity and specificity of one-test compared to two-test algorithms for detection of hepatitis C antibody
• To evaluate the cost–effectiveness, acceptability, and other outcomes (missed liver disease because of false-negative results, unnecessary referral, investigations) associated with these two types of testing strategies
• To inform models to optimize hepatitis C screening algorithms.

4. Methods

We reviewed observational studies and RCTs that provided original data from patient specimens. Our goal was to compare two broad strategies for hepatitis C antibody detection – one-test strategies and two-test strategies.

Search algorithm

Literature search strategies were developed by a medical librarian with expertise in systematic review searching. Our search algorithm consisted of the following components: hepatitis C, screening, and testing strategies (Annex 1).

We searched MEDLINE (OVID interface, 1946 onwards), EMBASE (OVID interface, 1947 onwards), the Cochrane Central Register of Controlled Trials (Wiley interface, current issue), Science Citation Index Expanded (Web of Science interface, 1970 onwards), Conference Proceedings Citation Index-Science (Web of Science interface, 1990 onwards), SCOPUS (1960 onwards), Literatura Latino-Americana e do Caribe em Ciências da Saúde (LILACS) (BIREME interface) and WHO Global Index Medicus. The search was supplemented by searching for ongoing studies in WHO’s International Clinical Trials Registry. The literature search was limited to the English language and human subjects.

We formulated a comprehensive and exhaustive search strategy in an attempt to identify all relevant studies. After the MEDLINE strategy was finalized, it was adapted to the syntax and subject headings of the other databases.

In addition to searching databases, we also searched the Internet for any peer-reviewed articles and conference abstracts that might have been missed through our librarian search and also expanded our search to national guidance documents.

5. Results

Study selection

The librarian search resulted in 3060 references for PICO 4. Because of overlap with objectives and search strategies between PICOs 3 and 4, and to expedite the initial screening, PICO 4 references were combined with the 3655 references identified through the librarian search for PICO 3 (HBV) for a total of 6715 references. 2388 searches were immediately excluded: the librarian excluded 835 as not relevant and there were 1553 duplicates; 4327 remained for screening. Titles/abstracts were screened according to protocol inclusion and exclusion criteria, for both PICOs 3 and 4; 4307 reports were excluded. Reasons for excluding them were noted (Fig. 2).
From the librarian search, 5 reports were identified for possible data extraction. The Internet searches resulted in 3 additional reports for possible data extraction. Full documents (manuscripts, abstracts, guidelines, etc.) were obtained and assessed against inclusion criteria. Papers were either accepted or rejected and reasons for rejection were explained.

**Fig. 2. PRISMA for PICO 4 HCV** (diagnostic strategies for hepatitis C antibody detection)
The following inclusion criteria were used to evaluate the final selection: evaluations of HCV testing strategies; evaluations based on human clinical materials. The following exclusion criteria were used: studies only focused on evaluation of single-test assays without a two-test comparator group; studies focused on two-test strategies that include other types of test (e.g. HCV RNA) studies with primary aims other than evaluation of testing strategies; studies related to disease prevalence, drug resistance, genotyping, sequencing, or non-diagnostic purposes; articles in languages other than English, conference abstracts.

Study characteristics

Of the 8 selected for possible data extraction, the following variables were collected, when available: first author, title, year, objective, and exclusion criteria (Table 2).

Table 2. Eight reports assessed for eligibility

<table>
<thead>
<tr>
<th>Author or source, year</th>
<th>Title</th>
<th>Objective</th>
<th>Exclusion criteria</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Cresswell. et al. 2014</td>
<td>Hepatitis C core antigen testing: a reliable, quick and potentially cost-effective alternative to hepatitis C polymerase chain reaction in diagnosing acute hepatitis C virus infection</td>
<td>To compare the utility of HCV core-antigen compared to qRT-PCR in the diagnosis on acute HCV in an HIV-positive cohort</td>
<td>No comparison of testing strategies</td>
<td>HCV core-antigen detection compared to HCV PCR is a quick, simple, cost-effective test in screening for acute HCV</td>
</tr>
<tr>
<td>2. Krajden 2000</td>
<td>Hepatitis C virus diagnosis and testing</td>
<td>To identify how anti-HCV serology and NAT can be combined to provide a definitive answer as to whether or not an individual has been or is actively infected</td>
<td>No data/not a study</td>
<td>Report describes how anti-HCV serology and NAT can be combined to provide a definitive answer as to whether or not an individual has been or is actively infected</td>
</tr>
<tr>
<td>3. Njouom 2006</td>
<td>A cost-effective algorithm for the diagnosis of hepatitis C virus infection and prediction of HCV viraemia in Cameroon</td>
<td>To describe the accuracy of an algorithm that combines two HCV rapid tests to diagnose and predict viraemia of HCV in Cameroon</td>
<td>No comparison of testing strategies</td>
<td>A comparison of 2 HCV rapid tests suggests an algorithm using the more sensitive test first to screen followed by the 2nd test to discriminate between viraemic and non-viraemic HCV seropositive subjects. Not relevant for this review as the second test is for HCV RNA</td>
</tr>
<tr>
<td>No.</td>
<td>Author(s)</td>
<td>Title</td>
<td>Study Design</td>
<td>Findings</td>
</tr>
<tr>
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<tr>
<td>4.</td>
<td>Shivkumar (2012)</td>
<td>Accuracy of rapid and point-of-care screening tests for hepatitis C: a systematic review and meta-analysis</td>
<td>To review evidence on the diagnostic performance of globally available RDTs and POCTs to screen for hepatitis C</td>
<td>POCTs (blood) have highest accuracy, followed by RDTs (serum, plasma) and POCTs (oral fluids). RDTs and POCTs may be useful in expanding first-line screening for hepatitis C</td>
</tr>
<tr>
<td>5.</td>
<td>Tillmann (2014)</td>
<td>Hepatitis C virus core antigen testing: role in diagnosis, disease monitoring and treatment</td>
<td>To review the current knowledge on 4 newer assays with decreased sensitivity, in different scenarios and reflect on their utility</td>
<td>HCV core antigen has relative strong role in a diagnostic algorithm for HCV infection, while it is too insensitive in its present form to substitute for HCV RNA testing in the blood bank setting</td>
</tr>
<tr>
<td>6.</td>
<td>Barreto (2008)</td>
<td>Cost–effective analysis of different algorithms for the diagnosis of hepatitis C virus infection</td>
<td>To compare diagnostic performance and cost–benefit of two new algorithms with the conventional one in Brazilian blood donors who showed positive or inconclusive anti-HCV results in screening tests</td>
<td>Study evaluated and costed 3 algorithms (2 CDC algorithms and Brazilian). The more practical and economical algorithm requires the establishment of a specific level of signal-to-noise ratio to determine the need for reflex supplemental testing (i.e. immunoblot anti-HCV)</td>
</tr>
<tr>
<td>7.</td>
<td>Vermeersch (2008)</td>
<td>Validation of a strategy for HCV antibody testing with two enzyme immunoassays in a routine clinical laboratory</td>
<td>To compare the performance of a strategy using AxSYM HCV 3.0 as screening test and Monolisa Plus anti-HCV version 2 as confirmation to AxSYM- pos sera with PCR and immunoblot</td>
<td>Monolisa Plus can be used as an alternative to immunoblot for the confirmation of AxSYM-positive sera</td>
</tr>
<tr>
<td>8.</td>
<td>CDC MMWR (2013)</td>
<td>Testing for HCV infection: an update of guidance for clinicians and laboratorians</td>
<td>To provide guidance to for clinicians and laboratorians on testing for HCV infection</td>
<td>Update to CDC guidance for diagnosis of acute hepatitis C: rapid or a laboratory-conducted assay for HCV antibody, reactive followed by NAT for HCV RNA</td>
</tr>
</tbody>
</table>

References listed in Annex 2.

Of the 8 included reports, 6 described algorithms with the types of tests used (Table 3). Reports 1–4 were excluded from the systematic review as they did not compare testing
strategies. Two studies, Boretto et al. (2008) and Vermeersch et al. (2008), determined costs and effectiveness.

Table 3. Six reports of HCV testing algorithms

<table>
<thead>
<tr>
<th>Report</th>
<th>Test 1</th>
<th>Test 2</th>
<th>Test 3</th>
<th>Test 4</th>
<th>Exclusion Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Cresswell 2014</td>
<td>HCV core-antigen</td>
<td>HCV RNA</td>
<td>HCV Ab</td>
<td>No comparison of testing strategies</td>
<td></td>
</tr>
<tr>
<td>2* Njouom 2006</td>
<td>Anti-HCV EIA</td>
<td>HCV RNA PCR</td>
<td></td>
<td>No comparison of testing strategies</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anti-HCV EIA</td>
<td>HCV RNA PCR</td>
<td>RT</td>
<td>RT</td>
<td></td>
</tr>
<tr>
<td>3* Tillmann 2014</td>
<td>Anti-HCV testing</td>
<td>RIBA</td>
<td>RNA PCR</td>
<td>No comparison of testing strategies</td>
<td></td>
</tr>
<tr>
<td>4* CDC 2013</td>
<td>HCV antibody</td>
<td>HCV RNA</td>
<td></td>
<td>No data/not a study</td>
<td></td>
</tr>
<tr>
<td>5* Barreto 2008</td>
<td>See schematic below (algorithm depends on a specific level of signal-to-cut-off ratio)</td>
<td></td>
<td>Study was performed using blood donors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Vermeersch</td>
<td>MEIA</td>
<td>Confirm by EIA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MEIA</td>
<td>Confirm by PCR</td>
<td>Confirm by immunoblot</td>
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</tbody>
</table>

* Algorithm schematics shown in Annex 3.

Although the study of Barreto et al. was conducted in a blood donor setting, the study did compare 3 testing strategies and determined cost–effectiveness. In this study the authors recognized that new anti-HCV tests have increased sensitivity but it means that there may be more false-positive results. These tests would be falsely negative in individuals who are newly infected as antibodies are absent or at low levels during this immunological window period. The use of a confirmatory diagnostic assay that targets different antigens can lower the risk of detecting false reactive results. Supplemental testing can be used to ensure a reliable diagnosis but this also means increased costs.

The authors compared 2 CDC algorithms to the national Brazilian algorithm to determine effectiveness and cost–benefit. The figure below depicts the testing of 517 individuals identified as ELISA-positive or inconclusive by anti-HCV test using 3 different algorithms. Algorithms A and B are the CDC recommended algorithms while Algorithm C is the national Brazilian algorithm.
The authors found that all three algorithms had similar diagnostic performance, revealing a remarkable agreement in the results obtained by the algorithms. As shown above, PCR was performed to resolve indeterminate results from immunoblots (139 samples from algorithm A and 141 samples from conventional algorithm C).

Algorithm A (CDC) was recommended for populations with a high prevalence of HCV infection. The algorithm showed high concordance with true-positive results. IB testing was required only for weakly reactive samples.

Algorithm B (CDC) used PCR to speed up clinical decision and was found more suitable for the immunosuppressed patient population for whom the IB test could represent a problem because of its low antibody level, leading to occasional false-negative results.

Algorithm C (Brazil) was found to be useful for determining the immune status of the patients against HCV infection and also for confirming the specificity of positive enzyme-linked immunoassay (ELISA) results. It is recommended for low prevalence populations for which false-positive antibody results are usually high. However, in the present study, this algorithm yielded a high frequency of IB-indeterminate results, producing no conclusive diagnosis. This algorithm also did not differentiate between active and past infections.

While algorithms A and B were found to be highly sensitive, the choice of an algorithm must take into account its purpose, the population and the prevalence of HCV infection, as well as the financial and infrastructure conditions of the laboratory. In the end they concluded that algorithm A is the best in terms of cost and feasibility, and particularly suitable for laboratories in resource-limited settings as it minimizes the number of samples requiring supplemental testing. Supplemental PCR tests were still required to detect active infection.

The Vermeersch study also investigated the CDC guidelines, specifically the required confirmation of HCV screening-test-positive sera with a low signal/cut-off (S/CO) ratio by
recombinant immunoblot or PCR. The UK Health Protection Agency suggested that a second EIA could be used as an alternative for confirmation in non-immunocompromised patients. A total of 17 936 consecutive in-house sera were evaluated in this study; AxSYM-positive sera were tested by Monolisa Plus and confirmed with IB (per CDC guidelines) or PCR.

This study specifically determined the performance of a strategy using AxSYM as screening test and Monolisa Plus as confirmation assay in a routine clinical laboratory and found that Monolisa Plus can be used as an alternative to immunoblot for the confirmation of AxSYM-positive sera in nonimmunocompromised. Although the study of Barreto et al. was conducted in a blood donor setting, the study did compare 3 testing strategies and determined cost–effectiveness.

Cost

Barreto et al. performed a cost–effective analysis of the two CDC-recommended algorithms compared to the current Brazilian national algorithm for the diagnosis of HCV infection. The cost of each algorithm depended on the number of supplemental tests required.

<table>
<thead>
<tr>
<th>Algorithm A (CDC)</th>
<th>Algorithm B (CDC)</th>
<th>Algorithm C (Brazil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Based on signal-to-cut-off (s/co) ratio of ELISA anti-HCV samples that show s/co ratio ≥95% concordance with immunoblot (IB) positivity.</td>
<td>Reflex nucleic acid amplification testing by PCR was required for ELISA-positive or -inconclusive samples and IB for PCR-negative samples</td>
<td>All positive or inconclusive ELISA samples were submitted to immunoblot</td>
</tr>
<tr>
<td><strong>US$ 21 299.39</strong></td>
<td><strong>US$ 32 397.40</strong></td>
<td><strong>US$ 37 673.79</strong></td>
</tr>
<tr>
<td>This was determined to be the more practical and economical one since it requires supplemental tests for only 54% of the samples</td>
<td>This one provided early information about the presence of viraemia</td>
<td></td>
</tr>
</tbody>
</table>

Quality assessment

Study quality was not evaluated using the QUADAS-2 tool and the STARD checklist, as these do not apply to the two studies.

6. Discussion

Although none of the studies met inclusion criteria, eight references were identified that might be useful for modelling exercises to address this PICO question. This short narrative will provide an overview of these 8 articles, also drawing on other informative reviews and personal communications.
Antibody and antigen tests

In 2000, Krajden described strengths and weaknesses of serological and molecular tests for diagnosing hepatitis C. In general, serological tests detect antibodies to hepatitis C while molecular tests detect or quantify HCV RNA. This combination of an antibody test followed by a confirmatory NAT (RNA) has generally been accepted.

HCV antibody detection by enzyme immunotests (EIA) are simple, inexpensive, and often less time consuming, although they cannot distinguish between acute, active or chronic, non-viraemic HCV infection. In chronically infected persons, EIA sensitivity approaches 97–99% while in acutely infected individuals, EIA sensitivity is as low as 50–70%. The rapid antibody tests are typically more expensive and not designed for testing large batches of specimens. However, in non-clinical (field) settings and laboratories that conduct low-volume testing, adoption of rapid testing can be cost-effective.

Nucleic acid testing (NAT) remains the gold standard for identifying active infection (HCV RNA is detectable in serum or plasma as early as 1 week after exposure) but is costly, requires skilled technicians, extensive equipment and reagents, and a robust transport system to ensure sample integrity. The various forms of NAT testing include polymerase chain reaction (PCR), branched DNA signal amplification, and transcription-mediated amplification. NATs exhibit high specificities of up to 99% across all 6 genotypes of HCV.

Recently, HCV core antigen testing has become widely commercially available. Two of the eight papers selected for this narrative discussed HCV core antigen testing (Cresswell 2014; Tillmann 2014). Tillmann describes the use of core testing as a serological test capable of identifying active infection, and as a possible replacement for NAT as a confirmatory test. Overall, the core test is less sensitive than HCV RNA tests, but as Tillman reports, more than 50% of anti-HCV positive persons will be HCV core antigen positive making core antigen testing a cost–effective reflex test to confirm infection, and can easily be applied on the same platform.

(Current HCV RNA assays have a lower level of detection between about 5–15 IU/mL. The sensitivity for the currently available HCV core antigen assay by Abbott was improved to about 3.00 fmol/L [0.0 6 pg/mL].)

Cresswell examined the efficacy and cost of HCV core antigen in diagnosing acute HCV in a high-risk, high-prevalence population (HIV-positive cohort of MSM). Compared to HCV NAT PCR, core antigen proved sensitive (100%), specific (97.9%), and cost–effective. In their cohort, they calculated cost per individual tests to be $108 for PCR versus $23.4 for HCV cAg. Their conclusion was that in high-risk, high-prevalence populations, the core test can be used as a quick, simple and cost–effective test in screening for acute HCV.

Other possible tests

Three other possibilities for testing were discussed in the literature and briefly mentioned here; recombinant immunoblot tests, signal-to-cut-off ratios and point-of-care tests (POCT) and antibody-based rapid diagnostic tests (RDT).
IBs are highly specific serological tests. They can be performed on the same sample used in the screening test; however, they are not amenable to routine use, as they do not have high sensitivity, are costly, with a testing procedure that is technically complex, and lengthy. Confirmation of active infection still requires testing for HCV RNA.

The CDC guidelines now include an option to use signal-to-cut-off ratios to limit the number of samples needing supplemental testing. Signal-to-cut-off ratios are test specific and slightly complicated to put in use and interpret. This approach might be better suited in a clinical laboratory setting (reference laboratory) that would use only one test, employ skilled technicians, and have a high volume throughput.

Shivkumar (2012) published a meta-analysis specifically on diagnostic accuracy of POCTs and RDTs to screen for hepatitis C. This analysis showed POCTs of blood (serum, plasma, or whole blood) have the highest accuracy, followed by RDTs of serum or plasma and then by POCTs of oral fluids. More evidence is needed to consider using these newer tests in a diagnostic algorithm.

Testing recommended for select populations

Many of the articles identified through the librarian search did not meet the inclusion criteria because they recommended HCV testing in select populations based on demography, prior exposures, high-risk behaviours, and medical conditions.

For example, one-time HCV testing is recommended for persons born between 1945 and 1965, without prior ascertainment of risk. Smith et al. (2012) note that the cost-effectiveness of one-time birth cohort testing is comparable to that of current risk-based screening strategies. Other major groups discussed under “risk behaviours” or “risk exposures” include injection drug use, children born to HCV-infected women, HIV infection. Because these studies were so specific to populations, they were not included as applicable to PICO 4.

Testing strategies

CDC MMWR (2013) describes CDC guidelines for HCV diagnostic testing: an anti-HCV test, and if the result is positive, active infection should be confirmed by a sensitive HCV RNA test. CDC recommends using US Food and Drug Administration (FDA)-approved tests (laboratory-based tests and POCT) such as OraQuick HCV rapid antibody test which has sensitivity and specificity similar to those of FDA-approved laboratory-based HCV antibody tests). An FDA-approved quantitative or qualitative NAT with a detection level of 25 IU/mL or lower should be used to detect HCV RNA.

Persons positive for an anti-HCV test and negative for HCV PCR RNA are informed that they do not have current (active) HCV infection, with no further testing necessary, unless there are ongoing risk factors for and suspicion of recent infection. In this case, repeat HCV RNA test is recommended.

To determine if the HCV antibody test represents a remote HCV infection that has resolved (true positivity) or a false-positive result (biological false positivity), CDC recommends
a second FDA-approved HCV antibody test that is different from the test used for initial antibody testing. A biological false result is not likely to occur with 2 different tests (Vermeersch 2008).

For patients with no apparent risk for HCV infection, the likelihood of a false-positive HCV antibody test is directly related to the HCV prevalence in the tested population; false-positive test results for anti-HCV are most common for populations with a low prevalence of HCV infection.

Njoum et al. (2006) performed a study in Cameroon comparing HCV rapid tests. In this study, using the more sensitive test first to screen followed by the second test to discriminate between viraemic and non-viraemic HCV seropositive proved to be a cost–effective algorithm for the diagnosis of HCV infection and prediction of HCV viraemia in Cameroon.

The two rapid tests evaluated were the ImmunoComb® II HCV assay and Hexagon® HCV assay. The ImmunoComb® II HCV test had a higher sensitivity than the Hexagon® HCV assay for detecting anti-HCV.

<table>
<thead>
<tr>
<th></th>
<th>ImmunoComb II</th>
<th>Hexagon</th>
<th>Reference assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>99.4</td>
<td>64.0</td>
<td>HCV antibody detection</td>
</tr>
<tr>
<td>Specificity</td>
<td>89.9</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>100.0</td>
<td>87.7</td>
<td>HCV RNA detection</td>
</tr>
<tr>
<td>Specificity</td>
<td>2.1</td>
<td>93.6</td>
<td></td>
</tr>
</tbody>
</table>

Their study did not actually report on cost but mentioned that EIAs are less expensive than PCR technology and in this case a second EIA can be substituted in the algorithm for the confirmatory PCR test.

7. Conclusions and recommendations for research

- Two studies compared the diagnostic accuracy, cost, cost–effectiveness of a one-test versus two-test strategies for detection of HCV antibody. One study found that in individuals who are HCV antibody positive, the use of an immunoblot assay with a defined signal-to-cut-off ratio can be used to distinguish between those who are viraemic and those who are not. This reduces the number of NATs required to confirm active infection is a cost–effective strategy.
- The challenge of using immunoblot assays is that they are lengthy and technically complex laboratory procedures, often leading to indeterminate results.
- Another study found that screening with a highly sensitive EIA followed by another EIA as confirmation assay in a routine clinical laboratory can be effective in non-immunocompromised populations. In immunocompromised patients, immunoblot is more effective as these patients tend to have low antibody levels.
References

Appendices

Appendix 1. Librarian search

31. Hepatitis, Viral, Human/
32. Hepatitis Viruses/
33. Hepatitis Antibodies/
34. exp Hepadnaviridae Infections/
35. Hepatitis C Antibodies/
36. Hepatitis B virus/
37. Hepadnaviridae/
38. Hepatitis B Surface Antigens/
39. (hepatitis-b or hep-b or (hepatitis adj5 b) or (hep adj5 b) or hbv).ti,ab.
40. hbsag.ti,ab.
41. exp Hepatitis C/
42. Hepacivirus/
43. Hepatitis C Antibodies/
44. (hepatitis-c or hep-c or (hepatitis adj5 c) or (hep adj5 c) or hcv or aghcv or
     hepacivirus*).ti,ab.
45. hcvab.ti,ab.
46. or/1-15 [HEP B or HEP C]
47. exp Mass Screening/
48. screen*.ti,ab.
49. 17 or 18 [MASS SCREENING]
50. (one-test* or two-test*).ti,ab.
51. ("1-test**" or "2-test**").ti,ab.
52. ((one or two or "1" or "2" or strateg* or algorithm* or approach or procedure* or
     system*) adj5 (test or tests or testing or detect* or diagnos* or kit or kits or assay* or
     device*)).ti,ab.
53. or/20-22 [TESTING STRATEGIES]
54. 16 and 19 and 23
55. Humans/
56. Animals/
57. 25 and 26
58. 26 not 27
59. 24 not 28
60. limit 29 to english language
Appendix 2. Eight full-text articles assessed for eligibility (comparing algorithms, including costing).

Appendix 3. Testing schematics

Vermeersch et al. 2008

Tillman et al. 2014
Njoum et al. 2006

Baretto et al. 2008