4.4. How to test HCV

Decision-making tables – PICO 2

To ascertain exposure to HCV through anti-HCV testing: Among individuals identified for hepatitis C testing, what is the diagnostic accuracy of available assays for detecting anti-HCV (RDT, EIA)?

1. **Topic for analysis:** How to test

**Population:** Individuals identified for HCV testing to ascertain exposure to HCV

**Intervention:** Rapid diagnostic tests and enzyme immunoassays for detection of antibodies to HCV

**Comparison:**

1. Nucleic acid testing (NAT)
2. EIA and immunoblot
3. EIA only

**Outcomes:** Diagnostic accuracy (Sensitivity, Specificity, Positive predictive value, Negative predictive value, TN, TP, FN and FP).

2. **Background:**

- Screening for exposure to hepatitis C virus (HCV) is dependent on assays that detect antibodies to HCV (anti-HCV) in the first instance.
- Once antibody status is confirmed, the individual should undergo supplementary testing for active HCV infection using an assay designed to detect viral replication, such as HCV RNA or core antigen (HCV cAg).
- Assays designed solely to detect antibodies to viral antigens will inevitably have a “window period” of infectivity in early infection in an individual who has been recently infected whose infection will not be detected by a given serological assay. This diagnostic window period can be shortened by direct detection of viral antigen or nucleic acid.
- The improvements in assay performance over time, in particular the EIAs, have been termed as “generations” of the assays. *(see footnote)*
- It is important to note that the latest generation of assays designed to detect anti-HCV also are combined with cAg in order in increase seroconversion sensitivity of the assay, but these “4th generation” or “combination” assays should not be used to differentiate exposure from chronic infection.
• Note that sensitivity of antibody-only assays may be reduced if the patient is immunocompromised, e.g. HIV, immunosuppressive therapy, renal dialysis.

**Imunoassays (laboratory-based)**

• The most widely used anti-HCV assays are laboratory-based immunoassays.
• They detect antibodies to core and non-structural antigens. In the case of 4th generation assays, the assay combines detection of antibodies to HCV along with detection of hepatitis C core (P22 Ag) antigen directly.
• These can be in the form of an enzyme immunoassay (EIA), chemiluminescence immunoassay (CLIA), electrochemiluminescence immunoassay (ECL) or recombinant immunoblot assay.
• These are best suited to settings with high throughput of specimens and where infrastructure (electricity, cold storage, climate-controlled rooms) and skilled staff are consistently available.

**Rapid diagnostic tests (RDTs)**

• Many laboratories in resource-limited settings may not have access to this specialized equipment and process fewer specimens, per day. Hence, individual tests, including rapid diagnostic tests (RDTs), may be more appropriate.
• RDTs for detection of anti-HCV are simple to perform and do not require instrumentation, they come in immunofiltration (flow through) and immunochromatographic (lateral flow) formats and may be read visually.
• In general, RDTs do not require cold storage and may be tested using capillary (fingerstick) whole blood or oral fluid. However, the manufacturer’s instructions for use should always be followed.
• RDTs may be deliverable at the point of care (POC).
• The expansion of their use depends on their performance and operational characteristics in the setting of intended use, ultimately with the aim being to reach resource-limited settings and offer cost-efficient testing services as an alternative to assays that require specific laboratory infrastructure and staff skills to perform.

*Summary of assay “generations”*

**1st generation assays:**

• Detection of antibodies to NS4 antigen only
• Becomes detectable 12–26 weeks after exposure
• High false-positive rate, i.e. poor positive-predictive value in low-prevalence populations.

**2nd generation assays:**

• Detects antibodies to NS3, NS4 and core antigen
• Decreased window period of infectivity to 10–24 weeks.
3rd generation assays:

- Detects antibodies to NS3, NS4, NS5 and core antigen
- Further decreased window period of infectivity.

4th generation assays A.K.A. combination assays:

- Combination of detection of circulating antibodies to viral antigens as above, but also addition of monoclonal antibodies to detect hepatitis C antigens (P22 Ag) directly.
- Immunoassays solely for the detection of HCV core antigen were developed initially to close the diagnostic window in seronegative infection and subsequently for the detection of antigenemia in the presence of antibody.

3. Draft recommendation(s):

4. Summary and quality of evidence

Systematic review report

A systematic review was commissioned in order to assess this PICO question (see SR PICO 2). The purpose of this review was to determine the sensitivity and specificity of assays used to detect hepatitis C antibody using multiple specimen types, including serum, whole blood and oral fluid.

Summary of the evidence

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

Results:

A total of 52 studies were included, evaluating 30 RDT devices of varying generation of assay.
- **RDTs vs EIA only:**
  Pooled clinical sensitivity and specificity were 0.98 and 1.00, respectively.

- **RDTs vs EIA, immunoblot and NAT:**
  Pooled clinical sensitivity and specificity were 0.96 and 1.00, respectively.

- **RDTs vs NAT or immunoblot:**
  Pooled clinical sensitivity and specificity were 0.93 and 0.98, respectively.

- **RDTs vs Ag/Ab combination assay:**
  Pooled clinical sensitivity and specificity were 0.86 and 0.99, respectively.

- **RDTs on oral fluid compared to a serological reference standard using serum/plasma:**
  Pooled clinical sensitivity and clinical specificity were 0.94 and 1.00, respectively.

- **Results were comparable across general populations, hospital patients and key populations.**

### Pooled diagnostic accuracy for HCV antibody tests

**Pooled test accuracy for different tests (52 research studies).**

<table>
<thead>
<tr>
<th>Comparison (number of studies)</th>
<th>Pooled SE</th>
<th>95% CI</th>
<th>Tau-square P-value for heterogeneity</th>
<th>Pooled SP</th>
<th>95% CI</th>
<th>Tau-square P-value for heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>RDT versus EIA only (n = 5)</td>
<td>0.99</td>
<td>0.98</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>RDT versus NAT or immunoblot (n = 13)</td>
<td>0.93</td>
<td>0.91</td>
<td>0.95</td>
<td>0.98</td>
<td>0.97</td>
<td>0.99</td>
</tr>
<tr>
<td>RDT versus EIA, NAT or immunoblot (n = 14)</td>
<td>0.97</td>
<td>0.96</td>
<td>0.98</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Oral RDT versus blood reference (n = 12)</td>
<td>0.94</td>
<td>0.93</td>
<td>0.96</td>
<td>&lt;0.001</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Sample type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood samples (n = 45)</td>
<td>0.98</td>
<td>0.97</td>
<td>0.98</td>
<td>&lt;0.001</td>
<td>0.98</td>
<td>0.99</td>
</tr>
<tr>
<td>Oral samples (n = 12)</td>
<td>0.94</td>
<td>0.93</td>
<td>0.96</td>
<td>&lt;0.001</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Source population</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>General screening (n = 17)</td>
<td>0.95</td>
<td>0.94</td>
<td>0.96</td>
<td>&lt;0.001</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>Key population (n = 19)</td>
<td>0.97</td>
<td>0.96</td>
<td>0.98</td>
<td>&lt;0.001</td>
<td>0.94</td>
<td>0.95</td>
</tr>
<tr>
<td>Hospital patients (n = 16)</td>
<td>0.97</td>
<td>0.96</td>
<td>0.98</td>
<td>&lt;0.001</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Antibody and Antigen Combo testing (n=6)</td>
<td>0.86</td>
<td>0.79</td>
<td>0.94</td>
<td>&lt;0.001</td>
<td>0.99</td>
<td>0.98</td>
</tr>
<tr>
<td>Oral kits brand</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OraQuick (n = 8)</td>
<td>0.98</td>
<td>0.97</td>
<td>0.99</td>
<td>&lt;0.001</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Page | 69
Other brands (n = 6) 0.88 0.84 0.92 <0.001 0.99 0.99 1.00 <0.001

SE: sensitivity; SP: specificity; CI: confidential interval; RDT: rapid diagnostic test; EIA: enzyme immunoassay; NAT: nucleic acid testing

Note:* 
*Studies conducted across these regions were not included here.

Conclusions:

• Rapid diagnostic tests, including RDTs for oral fluid, have excellent sensitivity and specificity compared to laboratory-based methods, across different populations for detection of antibodies to HCV. This suggests that RDTs can be used to test for HCV antibody.
• Sensitivity/specificity was less for RDTs for anti-HCV compared to newer combination antibody/antigen assays.

Issues raised from the review:

• The comparison of RDT versus immunoblot would include HCV-cleared person: HCV Ab⁺ but HCV RNA⁻.
• Publication bias, as studies with poor test performance were less likely to be published, lead to exaggerated estimates of the accuracy.

Quality of evidence

*Refer GRADE table in footnote

5. Risks/benefits

Benefits

Advantages of testing by RDT compared to laboratory-exclusive EIAs

• Does not require capital investment in laboratory infrastructure, e.g. EIA washers, readers, incubators, analysers, cartridge or random-access analysers
• Concurrent reduction in maintenance costs and reagents
• May be deliverable at the point of care (POC). This may allow greater access to testing and eliminate need for mechanisms for transportation of specimens to the laboratory
• If testing at POC, may reduce number of individuals “lost to follow-up”, i.e. never receive their test results
• May be carried out by trained lay-providers and health-care workers, in addition to trained laboratory scientists
• Dedicated venepuncture may not be required as some assays are validated

□ Benefits clearly outweigh harms
□ Benefits and harms are balanced
□ Potential harms clearly outweigh potential benefits

Are the desirable anticipated effects large?
for capillary whole blood or oral fluid.

- Rapid diagnostic tests (RDTs) have potential for scaling up access to hepatitis B testing, particularly for key populations.

Risks

Disadvantages of testing by RDTs compared to laboratory-exclusive EIAs

- Increased cost per test after expense of laboratory infrastructure has been met
- User variability and subjectivity in reading the visual assay, suggest second reader
- Performance characteristics may vary with environmental factors, e.g. heat, humidity, storage conditions.
- Internal quality control measures may be inferior to standardized laboratory assays, e.g. lack of test kit controls, no specimen addition controls.
- Performance characteristics may vary in certain individuals, e.g. HIV infection, immunosuppressed – lower sensitivity of anti-HCV compared to NAT testing.
- Recording of results in a database which can be subsequently interrogated and audited as is the case with centralized laboratory testing may be compromised with testing at POC. This may impact on reporting and epidemiological surveillance of the burden of disease.

6. Acceptability, values and preferences

A values and preferences survey of implementers and users of hepatitis B and C testing services was carried out by FIND in September 2015. A total of 104 respondents from 43 (20 high-income, 23 low- and middle-income) countries participated. Relating to this PICO,

- 47% of respondents from low- and middle-income countries would prefer an RDT method of testing, even at the cost of reduced sensitivity).

Community:

- Support for the most effective testing approach in order to impact on availability of testing, especially in resource-limited and remote areas and optimize access to at-risk groups.

Patients/caretakers:

- In the setting of HIV, use of RDTs has facilitated scaling up of testing services in terms of widening access to testing services.

Health-care workers:

- If RDTs are utilized at POC, this will allow HCWs to carry out testing and...
organize follow up potentially in one consultation. There is a need for appropriate training of testing providers and laboratory staff.

- Appropriate pre- and post-test counselling was mentioned in the values and preferences survey, which suggested that in low- and middle-income countries, not all individuals were being offered this service.
- 47% of respondents \( n = 23 \) preferred POC testing using capillary whole blood even at the expense of clinical sensitivity.
- The intervention was considered likely to be acceptable to key stakeholders as clinical sensitivity and clinical specificity of RDTs for ascertaining HCV exposure are comparable with EIAs.

<table>
<thead>
<tr>
<th>7. Equity, ethics and human right implications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Will the recommendation raise questions around equity?</td>
</tr>
<tr>
<td>- No. The recommendation of the possibility of testing using RDTs offers new opportunities for enhancing screening, referral, and treatment for the individuals with chronic HCV infection especially in the resource-limited settings, thus will reduce transmission, morbidity and mortality associated with undetected and untreated HCV infection.</td>
</tr>
<tr>
<td>Are there ethical implications to this recommendation?</td>
</tr>
<tr>
<td>- No major concerns.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Resource use and financial implications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Materials:</td>
</tr>
<tr>
<td>- Cost of test kits</td>
</tr>
<tr>
<td>- Cost of sterile lancets, alcohol swabs, gloves, sharps-bins or other method of disposal of used-kits</td>
</tr>
<tr>
<td>- Cost of automated RDT readers, if applicable</td>
</tr>
<tr>
<td>- Quality control reagents, if applicable (some kits are supplied with positive and negative test kit controls)</td>
</tr>
<tr>
<td>Training and supervision:</td>
</tr>
<tr>
<td>- Cost of training testing providers and appropriate competency assessment, certification and re-certification of their skills</td>
</tr>
<tr>
<td>- From included studies, excellent specificity of all assays is reassuring in</td>
</tr>
</tbody>
</table>

☐ Less equitable
☐ More equitable

☐ No
☐ Probably
☐ Uncertain
☐ Yes
☐Varies
terms of ensuring cost–effective initiation of algorithms for further investigation and treatment.

Other:

- Creation of a database into which results obtained by POC can be recorded
- Linkage to care, e.g. antenatal clinics

Possible test-procurement costs:

<table>
<thead>
<tr>
<th>Tests</th>
<th>Cost (US$) per test</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>RDTs</td>
<td>0.50–1.70 (&gt;10 for oral fluid)</td>
<td>MSF, WHO database</td>
</tr>
<tr>
<td>Laboratory-based assays</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV EIA</td>
<td>2.60–4.30 (procurement costs)</td>
<td>WHO database</td>
</tr>
<tr>
<td>HCV RNA</td>
<td>~20</td>
<td></td>
</tr>
</tbody>
</table>

8. Feasibility and constraints to implementation

Are any major barriers expected for the implementation of this recommendation?

- High-throughput EIAs require extensive laboratory infrastructure and equipment with staff expertise in its operation. Batching can lead to long delays before results are available.
- Delivery of RDTs requires appropriate training of test providers in performing and reading of the test result, storage of materials and recording and reporting of status.
- Decentralization of testing puts tremendous stress on already fragile health systems in terms of training needs, supply chain management, quality assurance, and monitoring and evaluation of effectiveness and impact. External quality assessment of quality of tests and testing possible but challenging when the need for proficiency panels is increased from a few laboratories to hundreds and possibly thousands of POC sites.

Feasibility survey report to be presented at meeting.

9. Relevance to different settings/populations

Will this recommendation be most relevant for particular settings (e.g. endemicity)?

- This recommendation of the introduction of RDTs will be most relevant in settings where there is poor provision of laboratory testing-services, either access to centralized laboratory testing or lack of testing infrastructure in existing laboratories.
- Delivery of RDTs at the POC will have relevance more in remote or resource-limited settings,
compared to settings where there is good access to health care and established screening programmes.

- It will be most relevant to groups of patients at risk of infection but who may be reluctant to or have poor access to health-care services, such as individuals who attend drug-rehabilitation clinics or closed settings such as prisoners. These individuals require screening and may require assessment and treatment if found to be infected.
- It will be less relevant in individuals who have good access to health care and in settings where laboratory testing for hepatitis C is already well established.

10. Rationale for recommendation:

11. Strength of recommendation

12. Implementation considerations

13. Research Gaps
- Impact of testing at POC using RDTs to rule out/rule in HCV exposure on HCV-associated morbidity and mortality and onward transmission testing and treatment programmes.
**GRADE Summary of findings**

**Table: Strength of evidence for diagnostic accuracy**

<table>
<thead>
<tr>
<th></th>
<th>Unit of analysis</th>
<th>Type of samples</th>
<th>Studies, n</th>
<th>Risk of bias</th>
<th>Consistency</th>
<th>Directness</th>
<th>Precision</th>
<th># of samples</th>
<th>Strength of evidence</th>
<th>Sen (95% CI)</th>
<th>Sp (95% CI)</th>
<th>Pretest probability (%)</th>
<th>Positive LR (95% CI)</th>
<th>PPV</th>
<th>Negative LR (95% CI)</th>
<th>NPV</th>
<th>Strength evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RDT versus EIA,</strong></td>
<td>General population, hospital patients, blood donors, injection drug users and other high-risk populations</td>
<td>Oral fluid, serum or plasma</td>
<td>14</td>
<td>Mod</td>
<td>Se: Inconsistent</td>
<td>Indirect</td>
<td>Precise</td>
<td>42,239</td>
<td>Se: Mod</td>
<td>0.97 (0.96–0.98)</td>
<td>1.00 (1.00–1.00)</td>
<td>0.05</td>
<td>372.92 (267.56–574.12)</td>
<td>0.95</td>
<td>0.03 (0.02–0.04)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td><strong>or immunoblot</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<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></td>
<td>Oral fluid, serum or plasma</td>
<td></td>
<td>12</td>
<td>Mod</td>
<td>Se: Inconsistent</td>
<td>Indirect</td>
<td>Precise</td>
<td>14,547</td>
<td>Se: Mod</td>
<td>0.94 (0.93–0.96)</td>
<td>1.00 (1.00–1.00)</td>
<td>0.05</td>
<td>314.5 (202.02–684.07)</td>
<td>0.94</td>
<td>0.06 (0.04–0.07)</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>

Mod: Moderate; Sen: sensitivity; Sp: specific