HCV: Diagnostic Testing and Staging

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HCV: Diagnostic Tests

Necessary
• HCV Antibody
• HCV-RNA
• Genotype

Not Necessary
• ALT
• IL28 B
ALT as a Sole Trigger for Screening Misses Some Infected Patients

- 42% Persistently Normal ALT
- 15% Persistently Elevated ALT
- 43% Intermittently Elevated ALT

*4 serum ALT level measurements during 25 months of follow-up (n = 1042). These results are from a prospective community-based study that evaluated liver enzymes in patients with Hepatitis C who had a history of prior drug use.

ALT = alanine aminotransferase.


Hepatitis C Antibody Testing

- 2 types of tests
  - Standard—goes to lab
  - Point of Care Testing
Detection of Anti-HCV IgG: Rapid Tests

- Do not require complicated instrumentation/testing by skilled staff
- Potentially generate results within an hour (potential point-of-care use)
- CDC evaluation of 3 rapid tests (Orasure, Chembio, and Medmira)
  - Assays based on recombinant antigens derived from core, NS3, NS4, and NS5 proteins in an immunochromatographic format
  - Specificity: >99%; sensitivity: 86% to 99%
- OraQuick HCV Rapid Antibody Test (CLIA-waived)
  - FDA approved with fingerstick, whole-blood, and venous blood specimens from individuals aged ≥15 years and at risk for HCV infection or persons with signs and symptoms of hepatitis
  - Not approved for general screening
- Typically more expensive versus conventional immunoassays
- Not designed for testing large batches of specimens
- Results available in ~20 minutes


Steps for Fingerstick or Venipuncture Specimen Collection
Detection of Anti-HCV IgG: Immunoassays

- Diagnostic specificity
  - >99% for 3rd generation assays
- False-negative results
  - Undergoing hemodialysis
  - Immunocompromised patients
- Signal-to-cutoff ratios
  - Predict a true antibody positive results >95% of the time, regardless of the anti-HCV prevalence or characteristics of the population tested

<table>
<thead>
<tr>
<th>Signal-to-Cutoff Ratios (FDA-Approved, Screening Assays)</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme immunoassay (manual)</td>
<td>≥3.8</td>
</tr>
<tr>
<td>Ortho HCV Version 3.0</td>
<td>≥3.8</td>
</tr>
<tr>
<td>Abbott HCV EIA 3.0</td>
<td></td>
</tr>
<tr>
<td>Chemiluminescence immunoassay (automated)</td>
<td></td>
</tr>
<tr>
<td>Vitros anti-HCV</td>
<td>≥8.0</td>
</tr>
<tr>
<td>Advia Centaur HCV</td>
<td>≥11.0</td>
</tr>
<tr>
<td>Microparticle immunoassay (automated)</td>
<td></td>
</tr>
<tr>
<td>Architect anti-HCV</td>
<td>≥10.0</td>
</tr>
<tr>
<td>Chemiluminescence microparticle immunoassay (automated)</td>
<td></td>
</tr>
<tr>
<td>Architect anti-HCV</td>
<td>≥5.0</td>
</tr>
</tbody>
</table>


HCV Antibody

- Does not diagnose disease
- Positive antibody may mean:
  - Active disease
  - Previous infection now cleared
  - False positive
- All positive antibodies requires confirmatory HCV-RNA testing
Detection of Virus-Specific Molecules: HCV RNA and Genotype

- Nucleic acid testing for HCV RNA remains the gold standard
  - PCR, branched DNA signal amplification, and transcription-mediated amplification
  - Specificity: up to 99% across all genotypes
- Essential for monitoring response to therapy

<table>
<thead>
<tr>
<th>Qualitative</th>
<th>Method</th>
<th>LLD (IU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplisor HCV v2.0 (Roche)</td>
<td>RT-PCR (manual)</td>
<td>50</td>
</tr>
<tr>
<td>COBAS Amplisor HCV 2.0 (Roche)</td>
<td>RT-PCR (semi-auto)</td>
<td>50</td>
</tr>
<tr>
<td>Ampliscreen (Roche)</td>
<td>RT-PCR (semi-auto)</td>
<td>10</td>
</tr>
<tr>
<td>Versant HCV RNA (Gen-Probe)</td>
<td>TMA (manual)</td>
<td>10</td>
</tr>
<tr>
<td>UltraQual HCV (National Genetics)</td>
<td>RT-PCR</td>
<td>10</td>
</tr>
<tr>
<td>Procleix HIV-1/HCV (Gen-Probe)</td>
<td>TMA (manual)</td>
<td>&lt;50</td>
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</tbody>
</table>

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<tr>
<th>Quantitative</th>
<th>Method</th>
<th>LLD (IU/mL)</th>
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<tr>
<td>Amplisor HCV Monitor (Roche)</td>
<td>RT-PCR (manual)</td>
<td>50</td>
</tr>
<tr>
<td>COBAS Amplisor HCV Monitor (Roche)</td>
<td>RT-PCR (semi-auto)</td>
<td>50</td>
</tr>
<tr>
<td>Versant HCV RNA 3.0 (Gen-Probe)</td>
<td>qDNA (semi-auto)</td>
<td>615</td>
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<tr>
<td>COBAS Ampliprep/TaqMan (Roche)</td>
<td>qPCR (semi-auto)</td>
<td>18</td>
</tr>
<tr>
<td>Real Time HCV/m2000sp/m2000rt (Abbott)</td>
<td>qPCR (semi-auto)</td>
<td>12</td>
</tr>
<tr>
<td>HCV SuperQuant (National Genetics)</td>
<td>RT-PCR (semi-auto)</td>
<td>20</td>
</tr>
<tr>
<td>LCx HCV RNA Quantitative (Abbott)</td>
<td>RT-PCR (manual)</td>
<td>25</td>
</tr>
</tbody>
</table>


General Statements on HCV Viral Load

- Amount of virus not clinically significant
- 10,000,000 IU/ml may be mild disease or cirrhosis
- 50,000 IU/l may be mild disease or cirrhosis
- Viral load not predictive of disease progression
- Viral load predictive of maternal-fetal transmission
- Viral load critical for assessing response to therapy
- Viral load critical for assessing adherence to therapy
Hepatitis C Genotype

- Important in determining which therapy to use
- Important in determining duration of therapy
- Prognosis
- Sub-typing important
- Common US genotypes
  - 1a, 1b, 2, 3, 4a -?
  - Less common 5 and 6

Recommended Laboratory Tests for Chronic HCV Infection

<table>
<thead>
<tr>
<th>Test</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis C antibody by enzyme immunoassay (EIA)</td>
<td>Screening for past or present HCV infection</td>
</tr>
<tr>
<td>PCR for HCV RNA</td>
<td>Confirmation of positive EIA</td>
</tr>
<tr>
<td>Genotype</td>
<td>Determines therapy and duration</td>
</tr>
</tbody>
</table>

HCV Diagnostic Assays: What the Results Mean

<table>
<thead>
<tr>
<th>Anti-HCV</th>
<th>HCV RNA</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>Acute or chronic HCV depending on the clinical context</td>
</tr>
</tbody>
</table>
| +        | −       | False positive HCV antibody  
Resolved infection  
Low-level intermittent viremia |
| −        | +       | Early acute HCV infection  
Chronic HCV in setting of immunosuppressed state  
False positive HCV RNA test |
| −        | −       | Absence of HCV infection |

AASLD and IDSA.  

Pre-Treatment Assessment

Medically necessary  
• HgB  
• Cr  
• Liver enzymes  
• HCV-RNA  
• Genotype  
• Assessment of disease severity

May be required by payers  
• Urine drug testing  
• Serum ETOH  
• INR
Options for Liver Fibrosis Assessment:
Degree of Fibrosis = Disease severity

- Liver Biopsy
- Serum Biomarkers
- FibroScan

Serum Biomarkers assessing Fibrosis in Chronic HCV

- Fibrotest
- Fibrosis Score 4
- AST to platelet ratio (APRI)
- FibroSpect II
- Enhanced liver fibrosis score (ELF)
- Fibrosis probability index
- Hepascore
- Lok Index
- Virahep
- Fibroindex
- AST to ALT ratio
- Platelet count

Serum FibroTest: A Continuous Variable (n=1,270)

<table>
<thead>
<tr>
<th>FibroTest</th>
<th>Expected Fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.75-1.00</td>
<td>F4</td>
</tr>
<tr>
<td>0.73-0.74</td>
<td>F3-F4</td>
</tr>
<tr>
<td>0.59-0.72</td>
<td>F3</td>
</tr>
<tr>
<td>0.49-0.58</td>
<td>F2</td>
</tr>
<tr>
<td>0.32-0.48</td>
<td>F1-F2</td>
</tr>
<tr>
<td>0.28-0.31</td>
<td>F1</td>
</tr>
<tr>
<td>0.22-0.27</td>
<td>F0-F1</td>
</tr>
<tr>
<td>0.00-0.21</td>
<td>F0</td>
</tr>
</tbody>
</table>


Serum biomarkers

- Excellent at predicting minimal fibrosis
- Excellent at predicting cirrhosis
- May need 2-3 tests
- Unclear if cost effective
Liver Biopsy for assessing fibrosis

- “Gold standard”
- Sampling error (< 1/50,000th of the liver)
  - Cirrhosis missed in up to 20%
  - Grade of inflammation and stage of fibrosis under-scored in shorter and narrower specimens

Adequate specimen:

≥ 1.5 cm long (≥ 2 cm preferable; ≥ 2.5 cm best)
≥ 1.0 mm wide (≥ 1.4 mm preferable)
≥ 6 portal triads (≥ 11 is better)


Sampling Error of Liver Biopsy

Fibrosis Area: 65%

Fibrosis Area: 15%

Courtesy of M. Pinzani, Florence
Liver Biopsy: Potential Complications

- Pain
- Bleeding
- Bile Peritonitis
- Puncture of gallbladder, colon, kidney
- Pneumothorax, hemothorax, pleural effusion
- Arteriovenous fistula
- Sepsis
- Needle track seeding with tumor


Bleed following Liver Biopsy
Summary: Liver Biopsy

- Gold standard
- Poor patient acceptance
- Safe with potential complications
- Limited availability

Fibrosis = Stiffness

- A key parameter of soft biological tissues
  - Often related to a pathological state
  - Palpation widely used in routine clinical practice

"When the liver is stiff, prognosis is bad"
Aphorisms, Hippocrates (460-370 BC)
Transient Elastography measures stiffness

- Fibroscan®
- MRI elastography

Fibroscan

- Examination time < 5 minutes
- Median value of 10 successful acquisitions
- Fasting 2-4 hours
- Performed on expiration
The FibroScan Probe

Ultrasound (Shear wave speed measurement)

Actuator (Shear wave generator)

Measurement Position

Explored Tissue

Skin

2.5 cm

6.5 cm

1 cm

4 cm

4 cm³
Shear Wave Propagation Map

\[ \text{Speed} = \frac{\text{Distance}}{\text{Time}} \]

Summary Data Display

Median shear wave speed, stiffness & IQR data
Transient elastography: What the Clinician needs to know

- What is the underlying disease
- Other evidence of advanced liver disease
- Factors that can affect the test
  - Is the patient fasting?
  - BMI
  - Burden of inflammation (ALT level)
  - ETOH use
  - Cholestasis

### kPa Cutoffs

<table>
<thead>
<tr>
<th>Disease</th>
<th>Cirrhosis</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis C</td>
<td>12.5 kPa</td>
<td>7.3 kPa suggests significant fibrosis</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>11.7 kPa</td>
<td>If normal ALT, consider treating at 9.0 kPa</td>
</tr>
<tr>
<td>NAFLD</td>
<td>10.3 kPa</td>
<td>Consider performing CAP* (controlled attenuation parameter) assessment</td>
</tr>
<tr>
<td>ETOH</td>
<td>22.7 kPa if drinking</td>
<td>12.5 kPa if abstinent</td>
</tr>
<tr>
<td>Biliary liver disease</td>
<td>17.9 kPa</td>
<td>Must know alkaline phosphatase</td>
</tr>
<tr>
<td>Portal HTN</td>
<td></td>
<td>20 kPa suggests HVPG &gt; 10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50.7 kPa suggests high risk of variceal bleeding</td>
</tr>
</tbody>
</table>

* Assessed in decibels per meter, currently proprietary algorithm

### Confounders of VCTE

- **Know the ALT**
- **Fast 3-4 hours**
- **Inflammation**
- **Non-fasting**
- **Cholestasis**
- **Obesity**
- **Use XL probe for BMI > 30**
- **Alcohol**
- **Examine for Right heart failure**
- **Determine drinking status**
- **Inexperience**
- **> 100**
Magnetic resonance elastography for detecting hepatic fibrosis. Magnetic resonance elastography uses a vibrating device to induce shear waves in internal organs, which are detected by a modified magnetic resonance imaging machine.

**Importance of Determining Degree of Fibrosis**

- Determines prognosis
- With new therapies, timing may be before, during or after therapy
  - If possible, do not delay therapy
- May determine eligibility for treatment with anti-viral therapy
**Recommendations for when and in whom to initiate treatment**

- Treatment is recommended for patients with chronic HCV infection.
- Rating: Class I, Level A
- Treatment is assigned the highest priority for those patients with advanced fibrosis (Metavir F3), those with compensated cirrhosis (Metavir F4), liver transplant recipients, and patients with severe extrahepatic hepatitis C
- Based on available resources, treatment should be prioritized as necessary so that patients at high risk for liver-related complications and severe extrahepatic hepatitis C complications are given high priority


**Diagnosis and Staging Combined for Efficiency**

- Initial Visit (PCP)
  - HCV Antibody
    - POCT
  - Send off
    - HCV-RNA
    - Genotype
- Second visit (Specialist)
  - Review HCV-RNA
  - Review genotype
  - Obtain Fibroscan®
  - Initiate anti-viral therapy