HCV: Diagnostic Testing and Staging

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

Patients* With Hepatitis C Infection

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

*Four serum ALT level measurements during 25 months of follow-up (n = 1042). These results are from a prospective community-based study that evaluated liver enzyme levels 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

Collect

Mix

Read
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 99% across all genotypes 1-6
  - Essential for monitoring response to therapy

<table>
<thead>
<tr>
<th>HCV RNA Assays</th>
<th>Method</th>
<th>LLD (IU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qualitative</td>
<td></td>
<td></td>
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<tr>
<td>Amplicor HCV v2.0 (Roche)</td>
<td>RT-PCR (manual)</td>
<td>50</td>
</tr>
<tr>
<td>COBAS Ampliprep HCV 2.0 (Roche)</td>
<td>RT-PCR (semi-auto)</td>
<td>50</td>
</tr>
<tr>
<td>Amplicor (Roche)</td>
<td>RT-PCR (semi-auto)</td>
<td>&lt;50</td>
</tr>
<tr>
<td>Versant HCV RNA (Gen-Probe)</td>
<td>TMA (manual)</td>
<td>10</td>
</tr>
<tr>
<td>Ultra2000 HCV (National Genetics)</td>
<td>RT-PCR</td>
<td>10</td>
</tr>
<tr>
<td>Precise HIV-1/HCV (Gen-Probe)</td>
<td>TMA (manual)</td>
<td>&lt;50</td>
</tr>
<tr>
<td>Quantitative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplicor HCV Monitor (Roche)</td>
<td>RT-PCR (manual)</td>
<td>50</td>
</tr>
<tr>
<td>COBAS Ampliprep HCV Monitor</td>
<td>RT-PCR (semi-auto)</td>
<td>50</td>
</tr>
<tr>
<td>(Roche)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Versant HCV RNA 1.2 (Gen-Probe)</td>
<td>qPCR (semi-auto)</td>
<td>12</td>
</tr>
<tr>
<td>Versant HCV RNA 2.0 (Gen-Probe)</td>
<td>qPCR (semi-auto)</td>
<td>20</td>
</tr>
<tr>
<td>Real-Time RT-PCR/pcR0000/h2000</td>
<td>qPCR (semi-auto)</td>
<td>25</td>
</tr>
</tbody>
</table>


General Clinical 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/I 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
- Sub-typing important
- Common US genotypes
  - 1a, 1b, 2, 3, 4
  - 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>

AASLD and IDSA.
**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.  

**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

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<table>
<thead>
<tr>
<th>Tissue</th>
<th>Stiffness (Pa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal liver</td>
<td>10^2</td>
</tr>
<tr>
<td>Cirrhotic liver</td>
<td>10^3</td>
</tr>
<tr>
<td>Palpable nodules</td>
<td>10^4</td>
</tr>
<tr>
<td>Contracted muscle</td>
<td>10^5</td>
</tr>
<tr>
<td>Cartilage</td>
<td>10^6</td>
</tr>
<tr>
<td>Bone</td>
<td>10^7</td>
</tr>
</tbody>
</table>
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“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

Shear Wave Propagation Map

Explored Tissue

Skin

2.5 cm

6.5 cm

Speed = Distance / Time
Summary Data Display

Median shear wave speed, stiffness & IQR data

FIBROSCAN

Correlation between liver stiffness (kPa) & fibrosis stage

Hepatitis B
HCV/HIV co-infection
Hepatitis C recurrent after liver transplantation
Hepatitis C
Autoimmune chronic liver disease
Alcohol
NASH

Fibroscan, a reliable tool in hepatology
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