An Update on Hepatitis C Virus Diagnostic Testing

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Faculty Disclosure

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Monica Parker, Ph.D.
“Nothing to disclose”.
Program Objectives

• Describe the test methods available for HCV diagnostic testing
• Provide information on new testing strategies for identifying HCV infections.
Hepatitis C Virus (HCV)

- Family: Flaviviridae, genus: *Hepacivirus*
- 9.6 kb, positive-strand RNA genome
- Humans are only known natural host
  - Primary target is liver cells (hepatocytes)
- Transmitted by percutaneous (through the skin) exposure to infected blood
- No vaccine is available
Acute Phase of HCV Infection

- Liver enzymes typically increase
  - May reach levels >10X above normal
- HCV RNA becomes detectable in 1 to 2 wks
- ~80% of people who become infected will have no symptoms or symptoms will be non-specific
  - Hepatitis C infection is usually not diagnosed in the acute stage
- In some cases, virus will be cleared
  - Within 6 months of exposure
HCV Antibody Production

- Antibodies to HCV are generally detectable by 6 to 8 weeks after infection
  - In some cases, it can take several months
  - >97% of persons have detectable anti-HCV antibodies at 6 months after exposure
- When infection resolves on its own
  - RNA will become undetectable
  - Liver enzymes return to normal levels
  - Antibodies will persist
Chronic HCV Infection

• Majority of infected persons advance to chronic infection
  o Signified by detectable HCV RNA > 6 months after onset of infection
• Liver enzymes typically remain above normal and may fluctuate
• There may be no noticeable symptoms
• All chronically infected persons carry virus in their blood and have the potential to transmit
Natural History of HCV Infection

Exposure (Acute Phase)

- Resolved: 15-25%
- Chronic: 75-85%

Chronic liver disease - stable: 80% (60-70)

Cirrhosis: 20% (15-20)

- Slowly Progressive Disease: 75% (15)
- HCC: 25% (1-5)

Transplant

Death
1998 HCV Testing Recommendations

- High-risk individuals, including persons who:
  - Ever injected drugs
  - Had chronic hemodialysis
  - Rec’d donated blood or organs prior to 1992
  - Rec’d clotting factor conc. made before 1987
  - People with known exposure
  - HIV-infected individuals
  - Children born to HCV + mothers
HCV Laboratory Testing Guidelines

• 2003 lab guidelines emphasized testing and result reporting of HCV antibody tests
  o RNA testing was provided as optional supplemental test
• Testing sequence frequently stopped once antibody status was determined
• Better treatment options have increased the incentive to identify currently infected individuals
  o Improve health outcomes for infected individuals
  o Reduce new infections by effectively treating chronic carriers
Rationale for Revising the Strategy

• Most HCV-infected people are unaware of their infection
  o 45-85% of infected people have not been identified

• Disease burden from hepatitis C is on the rise
  o Prevalence of HCV is declining, but prevalence of liver disease will continue to rise (Razavi et al. 2012 Hepatology 57: 2164-70)

• New drugs have improved treatment outcomes
  o Several more highly effective drugs are progressing towards FDA approval
New HCV Recommendations

August 2012
1-time testing of birth cohort

MMWR Vol. 61/No 4, 8/17/12

May 2013
Update to Testing Sequence

MMWR Vol. 62, 5/7/13
Birth Cohort Testing

• New recommendation: One-time testing of all persons born between 1945 and 1965
  ○ Studies indicate higher prevalence in this population than other birth years
  ○ No prior assessment of risk needed
• Previous recommendations for testing other groups at increased risk continue to hold
New Diagnostic Testing Guidelines

• New guidelines are designed to identify people with current (active) HCV infection
  o Previous strategy emphasized antibody testing – not sufficient to identify current infection
• Guidelines incorporate changes in the availability of FDA-approved HCV tests
  o 1st HCV rapid test rec’d CLIA waiver in 2011
  o RIBA test for HCV antibody confirmation was discontinued in 2013
Recommended Testing Sequence for Identifying Current Hepatitis C Virus (HCV) Infection

1. **Nonreactive**
   - No HCV antibody detected
   - STOP*

2. **Reactive**
   - HCV RNA
     - Not Detected
     - No current HCV infection
     - Additional testing as appropriate†
   - Detected
     - Current HCV infection
     - Link to care

* For persons who might have been exposed to HCV within the past 6 months, testing for HCV RNA or follow-up testing for HCV antibody is recommended. For persons who are immunocompromised, testing for HCV RNA can be considered.

† To differentiate past, resolved HCV infection from biologic false positivity for HCV antibody, testing with another HCV antibody assay can be considered. Repeat HCV RNA testing if the person tested is suspected to have had HCV exposure within the past 6 months or has clinical evidence of HCV disease, or if there is concern regarding the handling or storage of the test specimen.

Recommended Testing Sequence

• Begin with a FDA-approved immunoassay to test for HCV antibodies in blood
• This may be an instrument-based immunoassay or a rapid test
• Several kits and platforms available for instrument-based screening assays
• Currently, only one FDA-approved rapid test
  o CLIA-waived for whole blood collected by fingerstick or venipuncture
# Interpretation of Results of Tests for Hepatitis C Virus (HCV) Infection and Further Actions

<table>
<thead>
<tr>
<th>TEST OUTCOME</th>
<th>INTERPRETATION</th>
<th>FURTHER ACTIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV antibody nonreactive</td>
<td>No HCV antibody detected</td>
<td>Sample can be reported as nonreactive for HCV antibody. No further action required.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>If recent exposure in person tested is suspected, test for HCV RNA.*</td>
</tr>
<tr>
<td>HCV antibody reactive</td>
<td>Presumptive HCV infection</td>
<td>A repeatedly reactive result is consistent with current HCV infection, or past HCV infection that has resolved, or biologic false positivity for HCV antibody. Test for HCV RNA to identify current infection.</td>
</tr>
<tr>
<td>HCV antibody reactive, HCV RNA detected</td>
<td>Current HCV infection</td>
<td>Provide person tested with appropriate counseling and link person tested to care and treatment.</td>
</tr>
<tr>
<td>HCV antibody reactive, HCV RNA not detected</td>
<td>No current HCV infection</td>
<td>No further action required in most cases.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>If distinction between true positivity and biologic false positivity for HCV antibody is desired, and if sample is repeatedly reactive in the initial test, test with another HCV antibody assay. In certain situations, follow up with HCV RNA testing and appropriate counseling.</td>
</tr>
</tbody>
</table>

* If HCV RNA testing is not feasible and person tested is not immunocompromised, do follow-up testing for HCV antibody to demonstrate seroconversion. If the person tested is immunocompromised, consider testing for HCV RNA.

* It is recommended before initiating antiviral therapy to retest for HCV RNA in a subsequent blood sample to confirm HCV RNA positivity.

* If the person tested is suspected of having HCV exposure within the past 6 months, or has clinical evidence of HCV disease, or if there is concern regarding the handling or storage of the test specimen.

Antibody Test Results

• Antibody test result is reported as “Reactive” or “Nonreactive”

• A reactive result can indicate:
  o Current HCV infection
  o Past (resolved) HCV infection
  o False positive result

• If result is reactive for HCV antibodies, an HCV RNA test should be performed next
  o No additional test to confirm antibodies
HCV RNA Test

• Necessary to determine current infection status
• If RNA is detected, current HCV infection is present
• If RNA is not detected, the interpretation is “No current HCV infection”
• No further testing is required, unless
  o Exposure within past 6 months is suspected
  o Clinical signs of Hepatitis C are present
  o Improper specimen handling occurred
• If these exceptions exist, HCV RNA testing should be repeated
Specimen Considerations

- Reflexing directly to the RNA test is optimal
- RNA tests have more stringent specimen handling requirements than antibody tests
- Laboratories should:
  - Review package inserts for each test in the algorithm
  - Develop specimen collection and storage guidance for submitters
- Several options exist for specimen collection that will ensure RNA testing can take place, if needed
Specimen Collection Options

- A single specimen may be used for Ab and RNA testing if requirements for both tests are met.
- Two separate specimens, one for Ab and one for RNA, may be collected at the time of initial testing.
- If the OraQuick HCV Rapid Test is performed from fingerstick blood and is reactive, a venipuncture specimen must be collected for RNA testing.
- If RNA testing is indicated and the initial specimen is not suitable, one should be requested.
HCV RNA Test Considerations

• Laboratories are required to adhere to the package insert of FDA-approved tests
• FDA-approved qualitative HCV RNA tests for diagnostic use are available
• Currently, quantitative RNA (viral load) tests have not been approved for diagnostic use
  o Laboratories may conduct validation of a viral load test to allow its use in a diagnostic algorithm
  o Validation must include comparison to an approved diagnostic test method
Proficiency Testing

- Viral Markers-Series 1 (VM1) for anti-HCV, not for rapid (waived) tests
- Anti-HCV Rapid Methods (RHCVW) for anti-HCV waived methods only
- Nucleic acid testing (NAT)
  - Includes HCV along with HIV, HBV and WNV
  - Designed for blood donor testing, but may be used for diagnostic tests
Summary

• New HCV testing recommendations were developed to identify currently infected persons
• RNA testing should be performed on all HCV-antibody reactive specimens
• Laboratories should develop protocols to facilitate completion of the full testing sequence as efficiently as possible
Questions?

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