Hepatitis C Virus Diagnostics
Case Studies and Updates

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2023 HIV, HCV, and Syphilis Diagnostic Testing Workshop
12 March 2023
Natural History of HCV Infection

Acute
Symptoms +/-
HCV core Ag
HCV RNA

Anti-HCV

Chronic
Symptoms +/-

ALT levels

Months after exposure

Months
Years
Markers of HCV Infection

Days after exposure

- Acute phase
- Chronic phase
- Window period

HCV RNA

Anti-HCV
## FDA-approved HCV RNA Tests

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Assay</th>
<th>LoD&lt;sup&gt;a&lt;/sup&gt; serum IU/mL</th>
<th>% Sensitivity [95% CI]</th>
<th>% Specificity [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hologic</td>
<td>Aptima HCV Quant Dx Assay</td>
<td>3.4</td>
<td>98.8 [96.7-99.6]</td>
<td>100 [95.4-100]</td>
</tr>
<tr>
<td>Roche Molecular</td>
<td>COBA-HCV</td>
<td>13.7</td>
<td>100.0 [97.5-100]</td>
<td>98.8 [93.3-99.8]</td>
</tr>
<tr>
<td>Abbott Molecular</td>
<td>Abbott RealTime HCV&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12</td>
<td>N/A&lt;sup&gt;b&lt;/sup&gt;</td>
<td>N/A&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Roche Molecular</td>
<td>COBAS AmpliPrep/ COBAS TaqMan HCV Test</td>
<td>18</td>
<td>100 [97.3-100]</td>
<td>100 [95.5-100]</td>
</tr>
<tr>
<td>Hologic (Gen-Probe)</td>
<td>VERSANT TM HCV RNA Qualitative Assay</td>
<td>7.5</td>
<td>99.7&lt;sup&gt;d&lt;/sup&gt;</td>
<td>97.9&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> LoD as assessed with the WHO IS for HCV RNA (Genotype 1a); LoDs can vary by genotype

<sup>b</sup> Performance metrics were related to the ability of the test result to predict SRV (PPV/NPV)

<sup>c</sup> Diagnostic claim added to P060030 per PMA supplement

<sup>d</sup> Performance against another PCR assay

*Courtesy: Silke Schlottmann (FDA)*
Case Study I

**HCV NAT Results**

- **HCV RNA <3.4 IU/mL** (Hologic Aptima HCV Quant Dx Assay)
- **HCV RNA <13.7 IU/mL** (Roche Molecular)
- **HCV RNA <12 IU/mL** (Abbott RealTime HCV)
- **HCV RNA <18 IU/mL** (COBAS AmpliPrep/ COBAS TaqMan HCV Test)
- **HCV RNA <7.5 IU/mL** (VERSANT TM HCV RNA Qualitative Assay)

**Problem:** When HCV RNA levels are at the lowest limit of quantitation and are not quantifiable, results can be **misinterpreted** as if the patient is HCV RNA negative.
Antibodies to HCV

• Indicates exposure to HCV
• Present throughout acute, resolved and chronic phases of infection
• Laboratory tests
  • Enzyme immunoassays (EIAs) and Chemiluminescence assays (CIAs)
    • Serum, plasma, dried blood spot
  • Rapid diagnostic assays (RDTs)
    • Whole blood, serum, plasma
  • Confirmatory Immunoblot assays
    • Serum, plasma
Case Study II

Confirmation of anti-HCV screening test results performed by using incorrect signal-to-cutoff ratio thresholds
CDC’s HCV Testing Guidelines - 2003

Guidelines for Laboratory Testing and Result Reporting of Antibody to Hepatitis C Virus. MMWR. 2003 /52 / RR-33
CDC’s Updated HCV Testing Guidelines - 2013

- HCV antibody
  - Nonreactive
    - Never infected
    - Susceptible
  - Reactive
    - HCV RNA
      - Not detected
        - Past / Resolved infection
        - Or False positive anti-HCV
      - Detected
        - Current HCV infection
        - Additional follow-up needed

- Exposure within prior 6 months
- Other issue

MMWR. 2013;62(18)
# Case Study II

<table>
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<tr>
<th>Manufacturer</th>
<th>Assay</th>
<th>% Sensitivity [95% CI]</th>
<th>% Specificity [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>OraSure Technologies</td>
<td>OraQuick HCV Rapid Antibody Test</td>
<td>99.5 [98.4-99.9]</td>
<td>99.0 [98.0-99.6]</td>
</tr>
<tr>
<td>Roche</td>
<td>Elecsys Anti-HCV II</td>
<td>99.6 [98.7-99.96]</td>
<td>98.8 [98.2-99.3]</td>
</tr>
<tr>
<td></td>
<td>Elecsys Anti-HCV</td>
<td>99.6 [98.5-99.5]</td>
<td>96.9 [95.9-97.7]</td>
</tr>
<tr>
<td></td>
<td>Elecsys Anti-HCV</td>
<td>99.6 [98.5-99.95]</td>
<td>97.1 [96.2-97.9]</td>
</tr>
<tr>
<td></td>
<td>Elecsys Anti-HCV</td>
<td>99.4 [98.1-99.9]</td>
<td>97.2 [96.3-98.0]</td>
</tr>
<tr>
<td>Abbott Laboratories</td>
<td>ARCHITECT anti-HCV</td>
<td>99.53 [97.4-99.99]</td>
<td>97.6 [96.5-99.8]</td>
</tr>
<tr>
<td>Siemens Healthcare</td>
<td>ADVIA Centaur HCV</td>
<td>99.9 [99.5-100]</td>
<td>97.5 [96.4-98.3]</td>
</tr>
<tr>
<td>Ortho Clinical Diagnostics</td>
<td>Vitros Anti-HCV</td>
<td>99.5 [98.7-99.9]</td>
<td>98.2 [97.5-98.8]</td>
</tr>
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</table>

### Screening Test Kit

<table>
<thead>
<tr>
<th>Test Kit</th>
<th>S/CO</th>
</tr>
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<tbody>
<tr>
<td>Ortho HCV V3.0 ELISA</td>
<td>≥3.8</td>
</tr>
<tr>
<td>Abbott HCV EIA 2.0</td>
<td>≥3.8</td>
</tr>
<tr>
<td>Ortho VITROS Anti-HCV CIA</td>
<td>&gt;8.0</td>
</tr>
<tr>
<td>Abbott AxSYM Anti-HCV MEIA</td>
<td>&gt;10.0</td>
</tr>
<tr>
<td>Abbott Architect Anti-HCV CIA</td>
<td>&gt;5.0</td>
</tr>
<tr>
<td>Bayer Advia Centaur HCV CIA</td>
<td>≥11.0</td>
</tr>
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MMWR. 2003 /52 / RR-33
CDC’s Updated HCV Testing Guidelines - 2013

HCV antibody
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- Reactive
  - HCV RNA
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    - Detected
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  Additional follow-up needed

MMWR. 2013;62(18)
Hepatitis Testing Guidelines - Updates
Four possible operational strategies to accomplish the two-step testing sequence to diagnose current HCV infection:

1. Blood from a subsequent venipuncture is submitted for HCV RNA testing if the blood sample collected is reactive for HCV antibody during initial testing;

2. From a single venipuncture, two specimens are collected in separate tubes, one tube for initial HCV antibody testing, and a second tube for HCV RNA testing if the HCV antibody test is reactive;

3. The same sample of venipuncture blood used for initial HCV antibody testing, if reactive, is reflexed for HCV RNA testing without another blood draw;

4. A separate blood sample is submitted for HCV RNA testing if the initial testing of HCV antibody has used fingerstick blood.

Operational strategy 1 should no longer be used as it can lead to incomplete HCV testing and gaps in the HCV care cascade.

Emily Cartwright et al. unpublished
Draft hepatitis C virus (HCV) testing recommendations for perinatally exposed infants and children

- Perinatally exposed infants should receive a nucleic acid test (NAT) for HCV ribonucleic acid (RNA) at age 2-6 months to identify children who might go on to develop chronic HCV infection
  - Infants and children aged 7-17 months who are perinatally exposed to HCV and have not previously been tested should receive a NAT for HCV RNA
  - Children aged ≥ 18 months who are perinatally exposed to HCV and have not previously been tested should receive an anti-HCV test with reflex* to NAT for HCV RNA

* A NAT for HCV RNA performed on specimens that are anti-HCV reactive

Courtesy: Carolyn Wester, DVH
Status: perinatal HCV testing recommendations

- **External peer review**
  - Completed November 16, 2022

- **Public webinar**
  - Held on December 6, 2022
  - [https://www.cdc.gov/hepatitis/policy/pdfs/CDC_perinatal_hep_c_testing_508.pdf](https://www.cdc.gov/hepatitis/policy/pdfs/CDC_perinatal_hep_c_testing_508.pdf)

- **Federal Register Notice:**
  - Public comment period closed January 27, 2023

*Courtesy: Carolyn Wester, DVH*
Next steps: perinatal HCV testing recommendations

- Winter 2022/23: Review and respond to external peer review and FRN comments

- Winter/Spring 2023: Supplemental literature review

- Summer 2023: Submit revised guidelines to CDC clearance

- Fall 2023: MMWR publication (tentative)

Courtesy: Carolyn Wester, DVH
HCV Core Antigen

• Detectable within 1-2 weeks after exposure to HCV
• Test samples
  • Serum, plasma, dried blood spots
  • Lower sample volume than NAT
  • No pristine sample needed
• Undetectable when HCV RNA <2000 IU/ml
• Abbott and Roche
POCTs for HCV RNA and HCV core Antigen

FIND Presentation at EASL 2019, Vienna
For more information, contact CDC
1-800-CDC-INFO (232-4636)

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.