Hepatitis C Diagnostic Testing

HCV Diagnostic Tests

Serologic Antibody Assays

Initial testing for the diagnosis of hepatitis C infection uses serologic assays that detect human antibodies generated as a response to hepatitis C virus (HCV) infection.[1,2,3] A positive HCV antibody test indicates one of following three scenarios: (1) active infection, (2) past HCV infection that has resolved, or (3) a false-positive test.[1,4]

- **Enzyme Immunoassay (EIA):** In the United States, three generations of screening EIAs have been developed that can detect anti-HCV antibodies.[5,6] The third-generation EIA detects antibodies that bind to recombinant antigens derived from four viral regions: core, nonstructural 3, nonstructural 4, and nonstructural 5 (Figure 1).[7] The EIA test is reported as positive or negative based on an absorbance signal compared with a cut-off value. The third-generation HCV EIA has a sensitivity of approximately 98%; it is the most sensitive HCV EIA and it detects HCV earlier after acute infection than other HCV EIA tests.[7,8,9,10] Circumstances associated with a false-negative EIA include patients with acute HCV infection, persons with major immunosuppression (advanced HIV infection or organ transplantation recipients), and persons with chronic renal failure on long-term hemodialysis. The third-generation HCV EIA has the highest specificity among the EIAs, with a reported specificity greater than 99%; false-positive tests can occur with increased gammaglobulin production, with autoimmune diseases, and following immunizations.[8] In addition, a false-positive test is more likely when performing widespread testing in populations that have a very low hepatitis C prevalence.

- **Chemiluminescence Immunoassay (CIA):** The CIA test is an antibody test similar to the EIA, but for HCV testing, the CIA is used less frequently than the EIA test. For the diagnosis of HCV, the CIA has similar sensitivity and specificity as the third-generation EIA.[1,11]

- **Point-of-Care Rapid Immunoassays:** The OraQuick HCV Rapid Antibody Test was FDA approved in 2010 as a point-of-care test for use with whole blood samples obtained either by venipuncture or fingerstick. This OraQuick Rapid Antibody Test can be used as an alternative to the third-generation EIA for initial HCV antibody testing.[12,13,14,15] The OraQuick test is read between 20 to 40 minutes after the test device is inserted into the buffer (Figure 2) and the result is either reactive or nonreactive (Figure 3).[12,13] In 2011, the FDA granted a Clinical Laboratory Improvement Amendments (CLIA) waiver for the OraQuick HCV Rapid Antibody Test. Additional point-of-care rapid HCV antibody tests have been developed but are not approved for use in the United States.[16,17]

- **Recombinant Immunoblot Assay (RIBA):** The HCV RIBA is no longer recommended and is no longer available for use in the United States.[2] The RIBA test identifies specific antibodies generated in response to HCV antigens (Figure 4) and the test is interpreted as positive (2 or more antigens), indeterminate (1 antigen), or negative (0 antigens).[5] The RIBA antibody test, when it was available, was used as a follow-up test in persons who had a positive HCV
EIA and a negative HCV RNA test. In this circumstance, the RIBA could differentiate whether the patient had resolved HCV infection or had a biologic false-positive EIA.[1]

Molecular HCV RNA Tests

Molecular diagnostic tests for hepatitis C specifically detect HCV RNA and the process is commonly referred to as a Nucleic Acid Test (NAT) or Nucleic Acid Amplification Test (NAAT).[18] The HCV NAT becomes positive approximately 1 to 2 weeks after initial HCV infection.[19] The NAT test has become the gold standard supplemental test for patients who have a positive HCV EIA screening test.[2,18] The NAT can determine whether a patient with a positive HCV antibody test has current (active) or resolved HCV infection.[2,6] In addition, the NAT can be used in combination with other laboratory studies to diagnose individuals with acute HCV infection.[19] The results for the commercially available quantitative HCV RNA assay are given in International Units (IUs).[6]

- **Qualitative HCV RNA**: The qualitative HCV RNA tests provide a yes or no answer to whether detectable HCV RNA is present in the sample.[18] The qualitative HCV RNA assays are FDA approved for HCV diagnostic purposes. These tests, however, do not provide a quantitative level of HCV and are not used for baseline HCV RNA levels or for monitoring response to therapy.[18]

- **Quantitative HCV RNA**: The quantitative HCV RNA tests are not FDA approved for HCV diagnostic purposes. More recently, however, with the introduction of ultrasensitive HCV quantitative RNA assays (that detect as few as 5 copies/mL), the quantitative HCV RNA has achieved a similar level of diagnostic sensitivity as the qualitative assay.[6] In addition, quantitative HCV RNA assays generate an actual HCV RNA level that may provide useful information as a baseline HCV RNA. Because the sensitivity of the quantitative HCV RNA assays has dramatically improved, many clinicians utilize the quantitative HCV RNA for diagnostic purposes instead.[6] Most often, the quantitative HCV RNA test is used to monitor response to HCV therapy.[6,18]

**Immunoaassays for HCV Core Antigen**

As an HCV diagnostic marker, HCV core antigen has been studied, either alone or as an HCV antibody-HCV antigen combination assay.[20,21] Some experts have proposed use of an HCV core antigen test as a less expensive option than HCV RNA testing, but there are no HCV antigen assays (or HCV antigen-antibody combination assays) that are FDA approved for use in the United States.[22,23]
HCV Testing Sequence

Recommended Testing Sequence

In May 2013, the Centers for Disease Control and Prevention published a new recommended testing sequence for diagnosing current (active) hepatitis C infection (Figure 5). The new recommended sequence consists of initial testing for HCV antibody (using either a rapid or laboratory-conducted assay), followed by HCV RNA testing for all positive HCV antibody tests. Persons who have a negative screening HCV antibody test are considered not infected with HCV and do not need further diagnostic evaluation, unless they have a known risk factor for a false-negative test, such as suspected acute HCV infection, chronic hemodialysis, or an immunocompromising condition. Individuals who have a positive HCV antibody test and a positive HCV RNA are considered to have current (active) HCV infection. If an individual has a positive HCV antibody test and a negative HCV RNA assay, they are considered to have no evidence of current HCV infection; in this situation, further testing with a different HCV antibody assay can usually help to differentiate past (resolved) infection from a biologic false positive result. The 2013 HCV diagnostic testing sequence recommended by the CDC is not intended for diagnosing acute HCV infection.

Reflexive HCV RNA Testing with Positive Initial HCV EIA

Ideally, samples that test positive with a laboratory-conducted HCV antibody assay would then undergo reflexive HCV RNA testing using the same patient blood sample. Some laboratories now offer reflexive HCV RNA testing on HCV antibody-positive samples. Other less efficient approaches for follow-up HCV RNA testing include (1) collecting two separate venipuncture samples at the initial blood draw (with the option of ordering the HCV RNA test if the antibody test is positive), or (2) having the patient return for another venipuncture after receiving a positive antibody test result. From a practical standpoint, it is clearly preferable to have the laboratory reflexively perform the HCV RNA testing for positive HCV EIA tests utilizing the same blood sample.

Use of Signal-to-Cut-Off Ratio

A prior and now obsolete CDC HCV testing algorithm used HCV EIA signal-to-cut-off ratios (optical density value of the patient sample divided by the optical density of the assay cutoff for that specific run) as a major factor in determining the sequence of subsequent HCV tests. With this approach, a high signal-to-cut-off ratio (greater than or equal to 3.8) was generally considered to indicate a true-positive EIA. The use of signal-to-cut-off ratio was eliminated from the modern HCV testing algorithm because most clinicians are not familiar with HCV EIA signal-to-cut-off ratios and newer antibody tests have much greater sensitivity than the older antibody tests where signal-to-cut-off ratios may have been useful.
Interpreting and Communicating Test Results

Interpretation of HCV Test Results and Recommended Action

Prior to discussing the HCV test results with the individual who has undergone testing, it is important to interpret the test results and have a plan for communicating the test results and your recommended further action (Figure 6).[2]

**Negative HCV Antibody**

Individuals with a negative HCV antibody test result should be informed they are not infected with HCV, as long as they have not had a potential exposure to HCV in the prior 6 months.[2] If they have not had a potential exposure to HCV in the prior 6 months, they do not need further follow-up HCV testing, unless they have an exposure to HCV in the future.[2]

**Positive HCV Antibody and Negative HCV RNA**

Individuals with a positive HCV antibody test and a negative HCV RNA should be informed they do not have evidence of current hepatitis C infection.[2] It should be explained to the patient that most likely they were infected at some time with HCV, but cleared the infection on their own and they do not have current infection. They do not need further follow-up HCV testing, unless they had recent (within 6 months) exposure to HCV; they should be reevaluated if they have exposure to HCV in the future. In addition, they should receive counseling that prior infection does not make them immune to reinfection with HCV.[24,25,26]

**Positive HCV Antibody and Positive HCV RNA**

Individuals with a positive HCV EIA and positive HCV RNA should be told they have evidence of active hepatitis C infection and they should clearly understand they need medical follow-up evaluation and potential treatment of liver disease.[2] For persons with a positive HCV EIA and positive HCV RNA, the CDC has generated counseling messages that focus on four areas: (1) contacting a health-care provider for further evaluation and management of their HCV infection, (2) means of protecting their liver from further harm, (3) considerations for overweight and obese persons, and (4) minimizing transmission of their HCV to others (Figure 7).[27] In addition, the CDC recommends performing alcohol screening and brief intervention, which consists of screening for excessive alcohol consumption, brief counseling for individuals who screen positive, and referral to a specialized alcohol treatment program for individuals with possible alcohol dependence.[27]
Linkage to Care

Recommendations Regarding Linkage to Care

All persons identified with active hepatitis C infection (positive HCV RNA) should be linked to a medical provider who can provide competent and comprehensive management of HCV.[27,28] Available data suggest that in the current era, nonspecialists can effectively manage HCV, especially with back-up and consultation for more complicated issues.[29,30,31] The management of patients with decompensated cirrhosis should always involve a hepatologist. In addition, persons with HCV who have renal insufficiency or extra-hepatic complications of HCV infection will likely require referral to a specialist. Individuals with positive HCV antibody test but negative HCV RNA levels should not be referred to a hepatitis C expert for further evaluation and management of HCV infection.

Recommended Laboratory Evaluation Prior to Referral

All patients referred for further evaluation and management of HCV infection should have a confirmed positive HCV RNA level, preferably a quantitative HCV RNA level (viral load) and not a qualitative HCV RNA level.[28] It is ideal, but not imperative, that the clinician who makes the diagnosis of HCV infection can perform some preliminary tests to maximize the initial referral visit. These initial preliminary tests that can be obtained with a blood draw include an HCV genotype, tests of synthetic liver function (platelet count, albumin, prothrombin time [PT]), hepatic inflammation (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]), and assays to detect relevant coinfection (hepatitis A antibody, hepatitis B antibody, and HIV antibody). For primary care providers taking on a more comprehensive role for the initial evaluation and management, see Module 2, Lesson 1 for a detailed discussion in the Core Concept Initial Evaluation of Persons with Chronic Hepatitis C.

Gap in Linkage to Care

Unfortunately, many individuals diagnosed with HCV infection (positive HCV RNA) do not get linked to appropriate care for their HCV infection.[32] Multiple reasons for the gap in linkage to care have been cited, including failure of the medical provider to make the referral, patient failure to follow-up on the referral, lack of medical insurance, and substance abuse problems that interfere with making or keeping the referral appointment. Linkage to care has been particularly problematic among racial and ethnic minorities. Failure to link to care negatively impacts health outcomes in persons living with HCV infection.[33] With highly effective HCV treatment now available for all HCV genotypes, referral for evaluation and management of HCV has taken on even great importance.

Strategies for Improving Linkage to Care

Attempts at the public health level to implement an HCV testing and linkage-to-care program have shown that additional funds can be used to leverage existing program and provider networks.[34,35] The CDC and other organizations are actively working to explore strategies, such as the Hepatitis Testing and Linkage to Care (HepTLC) initiative, to enhance linkage to care for persons infected with HCV.[36] It should also be noted that patients who have been previously diagnosed many years ago in the interferon era may have been counseled to not seek treatment given the relatively poor efficacy, long duration, and high rate of adverse effects associated with interferon-based therapy. These patients may require more intensive efforts to educate and update on new greatly improved medications that are now available.
Summary Points

- Diagnostic tests for hepatitis C include serologic assays that measure human antibodies generated in response to HCV infection and molecular virologic assays that directly detect HCV RNA.
- The third-generation HCV EIA test is the most frequently used antibody test to initially screen for HCV infection. The test has high sensitivity and specificity, but does not distinguish between current and resolved hepatitis C infection.
- The OraQuick HCV Rapid Antibody Test is available as a point-of-care rapid test and this test can be used for initial HCV antibody screening.
- Quantitative HCV RNA assays are the preferred supplemental test for persons who have a positive screening HCV antibody test. Testing positive for HCV RNA indicates current (active) HCV infection. Qualitative HCV RNA assays can be used for supplemental testing but do not offer any advantage over the quantitative assay.
- The 2013 CDC recommended testing sequence for identifying current HCV infection consists of initial HCV antibody testing (either rapid or laboratory-conducted assay) followed by an HCV RNA assay for all positive antibody tests.
- All HCV testing results should be communicated to the patient; individuals who have a positive HCV RNA assay should understand they have current (active) hepatitis C infection.
- Patients who have a positive HCV RNA assay and newly diagnosed hepatitis C should receive preliminary counseling about protecting their liver from further harm, strategies for weight loss if they are overweight, and how to minimize their risk of transmitting HCV to others.
- Individuals with newly diagnosed hepatitis C infection should be linked to clinical care for further liver disease evaluation and for potential HCV treatment.
Citations


28. AASLD-IDSA. HCV Guidance: Recommendations for testing, management, and treating hepatitis C. [AASLD-IDSA Hepatitis C Guidance] -


**Figures**

**Figure 1 HCV Third-Generation EIA Test**

The third-generation HCV EIA is a qualitative test that detects human antibodies. This image shows several combinations of proteins used in different third-generation HCV EIA tests.
Figure 2 OraQuick HCV Rapid Antibody Test

This image shows the test device after it has been placed into the buffer in the developer solution vial. The test should be read 20 to 40 minutes after it is placed in the buffer.

Source: Image courtesy of OraSure Technologies, Inc.
Figure 3 OraQuick HCV Rapid Antibody Test Device

The OraQuick HCV Rapid Antibody Test can detect HCV antibodies in whole blood (obtained by fingerstick or venipuncture). The test device has control (C) and test (T) zones. If a line is visible only in the C zone (device on left), the test is considered Non-Reactive. If lines are visible in both the T and C zones (device on right), the test is considered Reactive.

Source: Image courtesy of OraSure Technologies, Inc.
The HCV RIBA is a highly sensitive and specific antibody test, but it is a more complex and expensive test to perform than the HCV EIA. The HCV RIBA is no longer available in the United States.
Figure 5 HCV Testing Sequence for Identifying Current HCV Infection

This diagram shows the HCV testing sequence recommended by the Centers for Disease Control and Prevention in May 2013.


Recommended Testing Sequence for Identifying Current HCV Infection

```
Nonreactive

HCV Antibody

Reactive

Not Detected

HCV RNA

Detected

No HCV antibody detected

No current HCV infection

Current HCV infection

STOP*

Additional testing as appropriate†

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.
**Figure 6 Interpretation of HCV Test Results and Recommended Action**


<table>
<thead>
<tr>
<th>Test Outcome</th>
<th>Interpretation</th>
<th>Further Action</th>
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| HCV antibody nonreactive | No HCV antibody detected | • Sample can be reported as nonreactive for HCV antibody. No further action required.  
• If recent HCV exposure in person tested is suspected, test for HCV RNA.* |
| HCV antibody reactive | Presumptive HCV infection | • 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. |
| HCV antibody reactive, HCV RNA detected | Current HCV infection | • Provide person tested with appropriate counseling and link person tested to medical care and treatment.† |
| HCV antibody reactive, HCV RNA not detected | No current HCV infection | • No further action required in most cases.  
• 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. |

* 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.
Figure 7 CDC Recommended Counseling for Persons with a Positive HCV RNA Test

The CDC recommends that persons newly diagnosed with HCV infection should receive some basic post-test counseling messages.


New Diagnosis of Hepatitis C Infection: Post-Test Counseling Messages

<table>
<thead>
<tr>
<th>Persons infected with HCV can benefit from the following messages</th>
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<tbody>
<tr>
<td>• Contact a health-care provider (either a primary-care clinician or specialist [e.g., in hepatology, gastroenterology, or infectious disease]), for</td>
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<tr>
<td>- medical evaluation of the presence or development of chronic liver disease;</td>
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<tr>
<td>- advice on possible treatment options and strategies; and</td>
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<td>- advice on how to monitor liver health, even if treatment is not recommended.</td>
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<td>• Protect the liver from further harm by,</td>
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<td>- considering hepatitis A and B vaccination if susceptible and if liver disease is present;</td>
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<td>- reducing or discontinuing alcohol consumption;</td>
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<td>- avoiding new medicines, including over-the-counter and herbal agents, without first checking with their health-care provider; and</td>
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<td>- obtaining HIV risk assessment and testing.</td>
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<td>• For persons who are overweight (BMI ≥25kg/m²) or obese (BMI ≥30kg/m²),</td>
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<td>- consider weight management or losing weight and</td>
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<td>- follow a healthy diet and stay physically active.</td>
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<td>• To minimize the risk for transmission to others,</td>
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<td>- do not donate blood, tissue, or semen and</td>
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<td>- do not share appliances that might come into contact with blood, such as toothbrushes, dental appliances, razors, and nail clippers.</td>
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