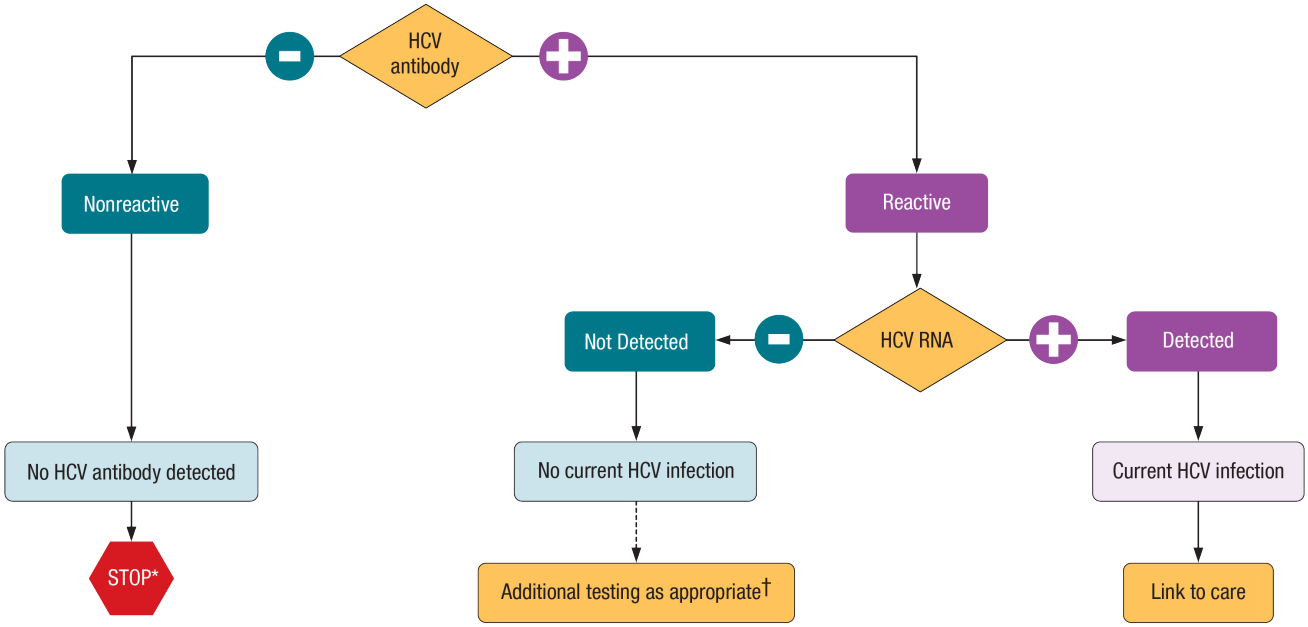
Testing For Hepatitis C Viral Infections:

FREQUENTLY ASKED QUESTIONS

In May 2013, the Centers for Disease Control and Prevention released Testing for HCV Infections: An Update of Guidance for Clinicians and Laboratorians. The updated guidelines emphasize identifying persons with current hepatitis C virus (HCV) infections and incorporate recent changes in the availability of certain commercial HCV antibody tests. The new recommended testing sequence includes an initial test with an FDA-approved test for HCV antibodies, followed by an FDA-approved diagnostic nucleic acid test (NAT) intended for the detection of HCV RNA in serum or plasma if the initial HCV antibody test is reactive (Figure 1). This document intends to provide answers to some frequently asked questions regarding the recommended testing sequence and outlines FDA-approved diagnostic HCV RNA tests (Table 1).

Figure 1: Recommended testing sequence for identifying current HCV infection[1]



* 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.

1 http://www.cdc.gov/hepatitis/hcv/labtesting.htm

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1. **Our laboratory's requisition specifies HCV antibody testing and HCV RNA testing as separate tests. Is the clinician required to order both types of tests in order for the laboratory to perform the full algorithm?** If the requisition specifies antibody testing only, it may be necessary for the clinician to order an HCV RNA test, as needed. A reflex option for an RNA test may be offered for positive antibody tests. Laboratories are encouraged to review their requisition process and consider revising the test menu to indicate HCV diagnostic testing without specifying the type of test.

2. **The new algorithm begins with an HCV antibody test. Can a rapid test that detects HCV antibodies be used for this step?** Yes, a test that has been approved by the FDA to screen for HCV antibodies for diagnostic purposes may be used for the HCV antibody test step at the beginning of the algorithm. This may be an FDA-approved HCV rapid test or a conventional HCV antibody immunoassay. If the laboratory receives an appropriate specimen from a patient who has already tested reactive on a rapid test, no additional testing to confirm antibodies is needed. A suitable specimen may be submitted directly for RNA testing to determine current infection status.

3. **In the past, laboratories were encouraged to report the signal-to-cutoff ratios from laboratory-based HCV enzyme immunoassays (IA). Should laboratories continue to report signal-to-cutoff ratios?** The signal-to-cutoff ratio is not needed to interpret results in the newly recommended testing sequence. However, package inserts for some HCV IAs may recommend reporting signal-to- cutoff ratios. For information on reporting the signal-to-cutoff ratio, refer to the package inserts of assays and consider your jurisdiction's individual surveillance needs. Note: An HCV RNA test is indicated when an HCV IA test is reactive, regardless of signal-to-cutoff ratio.

4. **The algorithm indicates that an HCV RNA test should be performed for all patients who have a reactive HCV antibody test result. Are laboratories allowed to reflex directly to the RNA test?** If the specimen submitted to the laboratory is acceptable for the HCV RNA test, the laboratory may reflex directly to the HCV RNA test. In some cases, a separate sample tube or a pristine aliquot may be submitted and processed for RNA testing if needed. If the original specimen is not suitable for RNA testing, or if insufficient volume remains, the laboratory should request another blood specimen and provide appropriate collection instructions. Laboratories may need to alter their requisition forms to include an option to specifically request an HCV RNA test or to reflex to HCV NAT following a positive antibody test according to the algorithm.

5. **Can a quantitative HCV RNA test (i.e. a viral load test) be used in the HCV RNA test step of the algorithm?** Currently, available HCV quantitative RNA tests are approved by FDA for the management of patients undergoing antiviral therapy and should only be performed after a confirmed diagnosis of active HCV. Quantitative HCV RNA tests are not intended for diagnostic use, and any use in the diagnostic algorithm would be off-label. To use a quantitative HCV RNA test in the diagnostic algorithm, the laboratory must have performed an appropriate validation study. Quantitative HCV RNA tests should only be used after the

validation is completed or if a physician has specifically ordered the test.

6. **If the laboratory performs HCV antibody testing on serum specimens, can the same serum specimen be used for the HCV RNA test step?** Laboratories must adhere to the specimen collection, processing and storage criteria for the RNA test as approved by the FDA. Specimens must be handled with care to minimize the chance of cross contamination. If serum is an acceptable specimen type listed in the package insert, then it may be used.

7. **What testing should be recommended if an individual has a reactive HCV antibody test, but the HCV RNA test is negative?** If the HCV antibody test is reactive, but HCV RNA is not detected, the laboratory should report the results with an interpretation of "HCV RNA not detected." The laboratory may recommend further actions that include re-testing with a different HCV antibody test, repeat testing if the person may have had a recent (within 6 months) exposure or has clinical evidence of HCV disease.

Table 1: FDA Approved Diagnostic HCV RNA Tests

	Test Name	Manufacturer	Intended Use	LOD/LLOQ	Specimen Type
Qualitative HCV RNA Tests	**VERSANT HCV RNA Qualitative Assay/APTIMA HCV RNA Qualitative Assay**	Gen-Probe	Diagnostic	7.5 IU/mL (genotype 1) 9.6 IU/mL overall	Serum or plasma (EDTA, sodium heparin, sodium citrate, and ACD)
	COBAS Amplicor HCV Test, v2.0 *and* **COBAS AmpliPrep/COBAS Amplicor HCV Test, v2.0**	Roche	Diagnostic	100 IU/mL	Serum or plasma (EDTA)
	AMPLICOR HCV Test, v2.0	Roche	Diagnostic	50 IU/ml	Serum or plasma (EDTA)
Quantitative HCV RNA Tests	**Abbott RealTime HCV**	Abbott	Aids in the management of HCV-infected patients undergoing antiviral therapy	12/12 IU/mL	Serum and plasma (EDTA)
	COBAS AmpliPrep/COBAS TaqMan HCV Test *and* **COBAS TaqMan HCV Test For Use With The High Pure System**	Roche	Aids in the management of HCV-infected patients undergoing antiviral therapy	15/15 IU/mL 20/25 IU/mL	Serum and plasma (EDTA)
	VERSANT HCV RNA 3.0 bDNA	Siemens	Aids in the management of HCV-infected patients undergoing antiviral therapy	LOD 988 (340 system) 1,100 IU/mL (440 system) Detection Cutoff 615 IU/mL	Serum and plasma (EDTA, ACD)

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