

Optimizing Hepatitis C Virus Testing: Key Considerations for Reflexing HCV Antibody Reactive Specimens to Confirmatory HCV RNA Testing

In July of 2023 the US Centers for Disease Control and Prevention (CDC) released [Updated Operational Guidance for Implementing CDC's Recommendations on Testing for Hepatitis C Virus \(HCV\) Infection](#), which clarified that laboratories should implement operational strategies to enable completion of the entire HCV testing algorithm with a specimen(s) collected during a single patient visit and recommends against stand-alone HCV antibody testing.¹

This document is intended for laboratorians, health departments and healthcare providers, and discusses considerations and factors that affect reflex of HCV antibody reactive specimens for HCV ribonucleic acid (RNA) testing. Reflex testing includes testing performed on-site or by direct referral to another laboratory.

Introduction

Accurate diagnosis of current infection with HCV is dependent upon completion of a [two-step algorithm](#), consisting of HCV antibody (also known as anti-HCV) testing followed by HCV RNA testing of specimens with reactive HCV antibody. An RNA test must be performed to determine whether a patient has an active HCV infection; a reactive HCV antibody test indicates that a patient either currently has or previously had an HCV infection—not whether the virus is still present in the blood. The HCV RNA test is the only available test that detects if a patient has current HCV infection (HCV RNA detected). Efficient diagnosis of current HCV infection facilitates timely curative treatment and is essential to reducing future transmission. As such, completion of the entire HCV testing algorithm is necessary for proper patient management, to accurately characterize incidence within a given population and to support HCV elimination efforts.

Data and Recommendations

Data from the APHL 2019-2020 [HIV and HCV Diagnostics Survey Report](#) shows that 54% of responding public health laboratories automatically reflex HCV antibody reactive specimens for HCV RNA testing, using specimen(s) already submitted. Similarly, a meta-analysis that focused on US-based studies conducted in healthcare organizations, the Veterans Administration or via the National Health and Nutrition Examination Survey identified gaps between the number of patients with a reactive HCV antibody and those that receive an HCV RNA test.² According to these data, specimens that test reactive for HCV antibody are not always reflexed to an HCV RNA test. To help address this issue and avoid disruptions in the HCV Care Cascade, the CDC recommends that laboratories discontinue use of operational strategies that require patients to make multiple visits to a health care facility in order to complete two-step HCV testing algorithm and recommend against standalone HCV antibody testing.¹

Test Orders

Attention to ordering options is warranted in laboratories that provide HCV antibody testing. Laboratory information management systems (LIMS) should be set up to handle the reflexive order, by incorporating automatic ordering of the HCV RNA test when the HCV antibody test is reactive. Whether HCV RNA testing is offered in-house or via referral, laboratories should ensure that HCV antibody assay orders lead to reflexive HCV RNA testing when an HCV antibody test result is reactive. This applies to HCV antibody testing that is ordered as either a single test or as part of a multi-test panel.

Language in the test directory should specify the specimen submission requirements, the HCV RNA test that will be automatically performed if a specimen is reactive for HCV antibody (i.e., qualitative or quantitative) and which CPT codes apply. It is important to be specific about the tests that will be performed; the examples provided here refer to a quantitative transcription-mediated amplification test, but the formatting can be adapted for qualitative tests and other methods of HCV RNA detection.* If a specimen will be referred to another laboratory for HCV RNA testing, that should be stated as well. In situations where the HCV antibody result is equivocal, another specimen should be obtained from the patient for repeat HCV antibody testing or follow CDC recommendations for supplemental testing.³

Example Test Directory Language

To ensure that reflexive HCV RNA testing is included in a test order, the test directory should specify all potential courses of action.

Below is an example of how a quantitative reflexive order might look in your test directory:*

Test Order Name:

Hepatitis C Antibody with Reflex to HCV RNA, Quantitative, Transcription-mediated Amplification (CPT code: 86803)

Note:

If anti-HCV is reactive, then HCV RNA, Quantitative, Transcription-mediated Amplification will be performed at an additional charge (CPT code: 87522).

Reporting

The preferred practice is to report reactive HCV antibody results when the HCV RNA testing is completed. This will help avoid confusion by ensuring that the result of the testing algorithm, driven by the HCV RNA result, is given to providers so that they have all the information required to determine the next steps. Reporting language must be appropriately worded so both the provider and the health department are aware of the patient's overall HCV infection status. When deciding how to release results, laboratories should consider the way their reports look in the medical record and work to mitigate misinterpretation that can occur (e.g., when the specimen is HCV antibody reactive, but HCV RNA negative). See **Figure 1** for examples of abbreviated results reports.

Figure 1. Examples of abbreviated result reports for HCV quantitative test*

Hepatitis C Antibody With Reflex to HCV RNA, Quantitative, TMA	
Clinical Interpretation	No HCV antibody detected
HCV Antibody Interpretation	Nonreactive
HCV RNA Interpretation	N/A. Based on the result of the HCV antibody screening test, the HCV RNA test was not performed.

Hepatitis C Antibody With Reflex to HCV RNA, Quantitative, TMA	
Clinical Interpretation	No Current HCV Infection
HCV Antibody Interpretation	Reactive
HCV RNA Interpretation	HCV RNA Not Detected

Hepatitis C Antibody With Reflex to HCV RNA, Quantitative, TMA	
Clinical Interpretation	Current HCV Infection
HCV Antibody Interpretation	Reactive
HCV RNA Interpretation	HCV RNA Detected and Quantified: <ul style="list-style-type: none"> • 616,595 IU/mL • 5.79 log₁₀ IU/mL

* Adjust language to reflect type of RNA test used (e.g., quantitative, qualitative, real-time PCR or other methods of HCV RNA detection)

Specimen Collection and Submission

Regardless of whether reflex HCV RNA testing will be conducted on-site or by referral to another laboratory, it is essential that laboratories thoroughly consider the specimen collection, handling and transport requirements for both HCV antibody and HCV RNA tests when establishing procedures and workflows for complete HCV testing.

When collecting a specimen for HCV testing, it is important to consider that the specimen may require both HCV antibody and HCV RNA testing. Serum and plasma are both acceptable in all FDA-approved anti-HCV and RNA assays, but due to the highly sensitive nature of nucleic acid tests for HCV RNA detection, utmost precautions should be taken at all stages of pre-analytic specimen processing—including specimen collection, separation of serum/plasma, aliquoting and shipping—to avoid any chances of specimen contamination. Moreover, laboratories must ensure that the volume of specimen collected is sufficient for both HCV antibody testing and reflex HCV RNA testing, as well as any other testing that may be performed as part of a panel. **Tables 1** and **2** provide details related to specimen volumes required in various FDA-approved assays.

Testing platforms are available that allow for multiple assays to be completed using a single sample; specifics on these have been compiled in APHL's [FDA-Approved HIV, HAV, HBV, HCV and STD Diagnostic or Monitoring Assays by Manufacturer and Platform](#).

When collecting specimens from special populations (babies, infants, children, pregnant women, immunosuppressed individuals, cadavers, etc.) it is important to understand the individual assay's limitations and intended use, as tests may have different applications defined in their regulatory claims.

Once determined, specimen collection and transport instructions should be made available to the laboratory's clients in a clear and accessible manner, via inclusion in the test directory and other appropriate channels.

Laboratory Workflows

Common approaches to HCV laboratory workflow include either requiring one or two blood tubes for HCV antibody testing:

One Tube

If one tube is required, the laboratory may choose to aliquot the specimen for HCV antibody and HCV RNA testing following clot or cell separation or to use a single tube of separated serum or plasma for both HCV antibody and HCV RNA testing. If the same tube is used for both tests, laboratories must ensure that carryover on the serology instrument does not compromise the integrity of nucleic acid testing. Data from manufacturer's studies and laboratory verification/validations may be used to evaluate carryover. Laboratories that use serology instruments that employ disposable tips when aspirating specimens for HCV antibody testing may cite published studies showing that disposable tips reduce carryover.^{4,5}

Two Tubes

If two tubes are collected for the HCV antibody test, with one being used for the HCV RNA test when HCV antibody is reactive, laboratories must ensure that they have freezer space to store the extra specimen at -20°C or lower, plan for the staff time required to find specimens that require HCV RNA testing, and plan for supply chain disruptions that may make using two blood collection tubes for each HCV antibody order difficult.

Table 1. Summary of Serologic (Antibody) Assays

Manufacturer	Platform	Assay	Type Of Claim	Specimen Types Accepted	Specimen Volume	Specimen Stability	Special Notes	CPT Code
Abbott	ARCHITECT	ARCHITECT anti-HCV	Diagnostic	Serum/Plasma	150 µL	<ul style="list-style-type: none"> Up to three days at 20–23 °C Up to seven days at 2–8 °C Once removed from clot or separated from cells or gel: store at -20 °C indefinitely (no more than three freeze/thaws) 	<ul style="list-style-type: none"> Utilizes reusable probe for sampling Random access testing Kit size(s): 100/500 tests 	86803 (G0472)
	Alinity i	Alinity I Anti-HCV	Diagnostic	Serum/Plasma	150 µL	<ul style="list-style-type: none"> Up to three days at 20–23 °C Up to seven days at 2–8 °C Once removed from clot or separated from cells or gel: store at -20 °C up to three months (no more than three freeze/thaws) 	<ul style="list-style-type: none"> Utilizes reusable probe for sampling Random access testing Kit size(s): 200/1000 tests 	86803
Diasorin	LIAISON® XL	Murex HCV Ab	Diagnostic	Serum/Plasma	175 µL	<ul style="list-style-type: none"> Up to four days at 18–30 °C Up to seven days at 2–8 °C Once removed from clot or separated from cells or gel: store at -20 °C for up to three months (no more than seven freeze/thaws) 	<ul style="list-style-type: none"> Utilizes disposable pipette tips Random access testing Kit size(s): 100 tests 	86803
Quidel Ortho-clinical diagnostics	Vitros ECI	Vitros Immunodiagnosics Anti-HCV	Diagnostic	Serum/Plasma	20 µL + system required dead volume	<ul style="list-style-type: none"> Up to eight hours at 22 °C Up to two days at 2–8 °C Once removed from clot or separated from cells or gel: store at -20 °C up to three months (no more than one freeze/thaw) 	<ul style="list-style-type: none"> Utilizes disposable pipette tips Random access testing Kit size(s): 100 tests 	86803
Roche	Cobas e 601	Elecsys Anti-HCV II	Diagnostic	Serum/Plasma	30 µL + system required dead volume	<ul style="list-style-type: none"> Up to three days at 20–23 °C Up to seven days at 2–8 °C Up to three months at -20 °C (no more than six freeze/thaws) 	<ul style="list-style-type: none"> Utilizes disposable pipette tips Random access testing Kit size(s): 300 tests Samples should not be taken from patients receiving therapy with high biotin doses (i.e., > 5 mg/day) until at least eight hours following the last biotin administration 	86803
Siemens	Advia Centaur	HCV (aHCV)	Diagnostic	Serum/Plasma	30 µL + system required dead volume	<ul style="list-style-type: none"> Up to two days at 25 °C Up to seven days at 2–8 °C Once removed from clot or separated from cells or gel: store at -20 °C (no more than four freeze/thaws) 	<ul style="list-style-type: none"> Utilizes disposable pipette tips Random access testing Kit size(s): 200 tests 	86803

Table 2. Summary of Molecular (RNA) Assays

Manufacturer	Platform	Assay	Type Of Claim	Specimen Types Accepted	Specimen Volume	Specimen Stability	Special Notes	CPT Code
Abbott	m2000sp and m2000rt	RealTime HCV Viral Load	Viral Load	Serum/Plasma	900 µL	<p>Whole blood: Up to six hours at 2–30 °C</p> <p>Serum/plasma:</p> <ul style="list-style-type: none"> Up to 24 hours at 15–30 °C Up to three days at 2–8 °C Up to 60 days at -10 – -30 °C Up to 60 days at -70 °C (no more than three freeze/thaws) 	<ul style="list-style-type: none"> LoD serum/plasma: 12.0 IU/mL LLoQ serum/plasma: 12.0 IU/mL Kit size(s): 96 tests Though rare, mutations within the highly conserved regions of the viral genome covered by the Abbott RealTime HCV assay primers and/or probe may result in the under-quantitation of or a complete lack of virus detection. Precision was established with HCV Genotypes 1 and 3 only. 	87522
	Alinity m	Alinity m HCV (Viral Load)	Diagnostic, Viral Load	Serum/Plasma	750 µL	<p>Whole blood:</p> <ul style="list-style-type: none"> Up to four hours at 15–30 °C Up to three days at 2–8 °C <p>Serum/plasma:</p> <ul style="list-style-type: none"> Up to 20 hours at 15–30 °C Up to three days at 2–8 °C Up to 60 days at -20 °C Long term storage at -70 °C 	<ul style="list-style-type: none"> Random access testing Kit size(s): 192 tests LoD serum: 7.96 IU/mL LoD plasma: 8.5 IU/mL LLoQ: 12 IU/mL 	87522
Hologic	Panther	HCV Quant Dx	Diagnostic, Viral Load	Serum/Plasma	700 µL	<ul style="list-style-type: none"> Prior to plasma/serum preparation: Up to six hours at 2–30 °C Centrifuged in primary collection tube: Store up to five days at 2–8 °C In secondary tube: Store up to 60 days at -20 °C 	<ul style="list-style-type: none"> Random access testing Kit size(s): 100 tests LoD serum: 3.4 IU/mL LoD plasma: 3.9 IU/mL LLoQ serum/plasma: 10.0 IU/mL 	87522
Roche	Cobas 6800/8800	cobas HCV	Diagnostic, Viral Load	Serum/Plasma	650 µL	<p>Prior to plasma/serum preparation: Up to 24 hours at 2–25 °C</p> <p>After separation from cells:</p> <ul style="list-style-type: none"> Up to six days at 2–8 °C Up to 12 weeks at ≤ -18 °C Long-term storage (up to six months): Temperatures at ≤ -60 °C are recommended; stable for up to four freeze/thaws. 	<ul style="list-style-type: none"> Random access testing Kit size(s): 96 tests LoD serum: 13.7 IU/mL LoD plasma: 12.0 IU/mL LLoQ serum/plasma: 15.0 IU/mL 	87522

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