



## Hepatitis C Viral Load Testing: Expanded Reportable Range and Improved Sensitivity with Real-Time PCR

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Nucleic acid tests that detect hepatitis C virus (HCV) RNA are commonly used to diagnose acute and chronic HCV infections and to evaluate and manage patients with chronic hepatitis C.<sup>1</sup> Quantitative HCV RNA testing is particularly useful for determining baseline viral loads before therapy, for monitoring viral RNA levels during therapy, and for evaluating patients for end-of-treatment response and sustained virological response. The new viral load tests using real-time PCR have an expanded dynamic range and an improved sensitivity compared with traditional viral load tests.

PAML is pleased to announce the addition of *HCV RNA Real-Time PCR Quantification* using COBAS HCV TaqMan™ (Roche Diagnostics) to our HCV RNA test menu. **This test replaces Hepatitis C Virus RNA Quantitative by PCR (discontinued order code: HCVTPC) and Hepatitis C Virus RNA Qualitative by PCR (discontinued order code: HCVLPC).**

### Expanded Reportable Range

Clinical trials have shown that therapeutic response can often be predicted early in the course of therapy by monitoring HCV RNA levels,<sup>2</sup> and a recent NIH consensus statement underscores the importance of accurate quantitative results.<sup>3</sup> The narrow dynamic-range of the conventional PCR test (600–700,000 IU/mL) often initiates a practice of sample dilution and re-testing before accurate quantitative results are achieved—a labor-intensive process that delays reports. The expanded reportable range of the new real-time test is 25–25,000,000 IU/mL, which virtually eliminates repeat testing—and its associated reporting delays.

### Confirmation of Positive Antibody Screens

The CDC advocates confirmation of positive antibody screens with an alternative method, either serologic or nucleic acid. Because HCV RNA testing can also detect active HCV infection, it is a practical choice for antibody confirmation. Supplemental serologic testing (RIBA) for confirmation of a positive screen can then be reserved only for instances in which the HCV RNA result is negative. PAML is adopting this model and is eliminating from our test menu all HCV antibody panels that include a reflex to RIBA confirmation, which may still be ordered as a single test as needed (see accompanying table for order code). Note that HCV RNA confirmation requires a separate sample collected and handled appropriately for nucleic acid testing (refer to the accompanying table for details on sample requirements).

### Improved Sensitivity

The principal goal of HCV therapies is persistently undetectable levels of HCV RNA. Therefore, highly sensitive molecular testing is warranted to ascertain very low viral levels that may exist at the end of treatment. Traditional molecular methods for HCV RNA quantification do not reliably detect RNA levels below 600 IU/mL. HCV RNA Real-Time PCR Quantification by COBAS HCV TaqMan offers greater than a tenfold increase in sensitivity—at 25 IU/mL (greater than one log)—and exceeds the 50 IU/mL sensitivity of the standard qualitative PCR method.

### Quick Facts

#### The HCV RNA Real-Time PCR Quantification Test:

- ▶ Provides reliable quantification of HCV RNA from 25 to 25,000,000 IU/mL.
- ▶ Confirms positive antibody screens and detects active infection.
- ▶ Replaces Hepatitis C Virus RNA Quantitative by PCR (discontinued order code: HCVTPC) and Hepatitis C Virus RNA Qualitative by PCR (discontinued order code: HCVLPC).

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## Methodology

The COBAS TaqMan PCR test comprises the extraction and purification of HCV RNA from serum or plasma, the amplification of RNA by reverse transcription PCR, and the detection of amplified products using an HCV-specific probe labeled with a fluorescent dye. The accumulation of HCV-specific amplified product is monitored in real time (in contrast to the older PCR-endpoint evaluation) with cycle-to-cycle measurements of fluorescence. The point at which fluorescence exceeds background levels inversely relates to the viral RNA concentration in the sample.

## Test Information

<b>DESCRIPTION</b>	<b>HCV RNA REAL-TIME PCR QUANTIFICATION TEST BY COBAS HCV TAQMAN</b>
<b>METHOD</b>	Real-time PCR, TaqMan
<b>ORDER CODE</b>	HCVRTP / HCVRTP
<b>CPT CODE</b>	87522
<b>SAMPLE</b>	5 mL frozen EDTA plasma (lavender-top tube) or serum (SST tube). Separate from cells within 6 hours of collection by centrifugation at 800–1600 × g for 20 minutes at room temperature. Transfer to a sterile polypropylene tube. Store and transport frozen. <b>Critical frozen.</b>
<b>COMMENTS</b>	<i>Minimum amount:</i> 2.0 mL Plasma collected by PPT tube is acceptable. <i>Stability:</i> Plasma or serum is stable 72 hours refrigerated, indefinitely if frozen at -20°C or colder. Unstable at room temperature. <b>Avoid freeze-thaw cycles.</b> Whole blood, heparinized or unfrozen samples are unacceptable. All orders for HCVLPC or HCVTPC will be cancelled and replaced with HCVRTP.
<b>SCHEDULE</b>	Monday – Saturday
<b>TURNAROUND</b>	2-6 days
<b>RANGES</b>	Not detected

## References

1. Alter MJ, et al. MMWR, Vol. 52(RR-3), February 7, 2003.
2. Fried MW, et al. N Engl J Med, Vol. 347(13), September 26, 2002.
3. NIH Consensus Final Statement on Management of Hepatitis C, September 12, 2002.

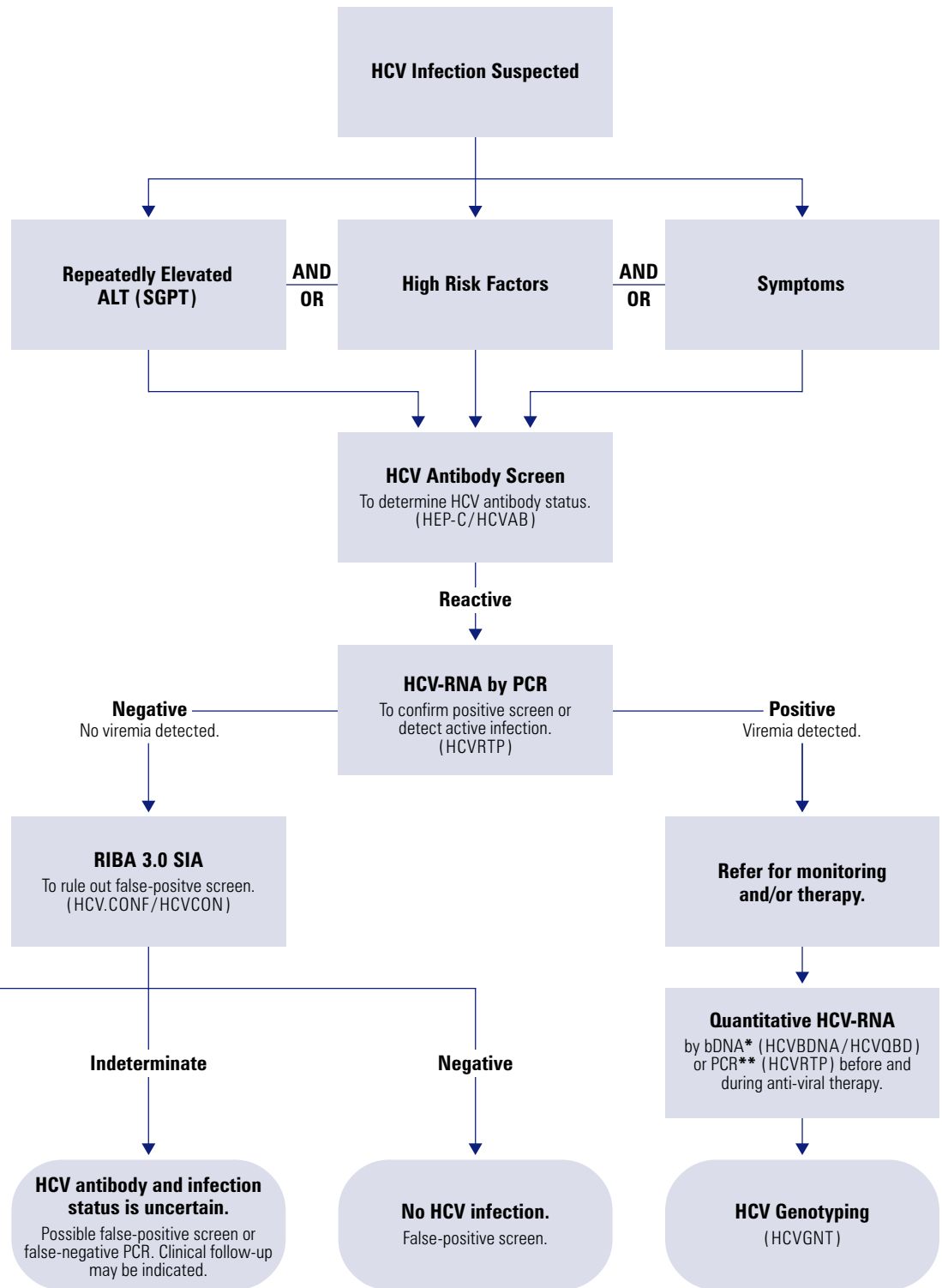
### The following tests are being discontinued:

Discontinued Order Codes	Description
HCVLPC / HCVLPC	HCV by PCR, Qualitative
HCVQNR / QTQLR	HCV Quant PCR, Qual Reflex
HCVTPC / HCVTPC	HCV by PCR, Quantitative
HCVLNG / QLQTGR	HCV Qual PCR, Quant / Geno Reflex
HCVQLR / QLQTR	HCV Qual PCR, Quant Reflex
HEPCCN / HEPCCN	HCV Antibody with Confirmation
HCVQTR / HCVQTR	HCV bDNA with Reflex to PCR

The following panels are being discontinued because positive HCV antibody screens are no longer reflexing to RIBA. Panels with the same tests, excluding the RIBA reflex, can be ordered using the new order codes:

Discontinued Order Codes	Description	New Order Code
ABCHPR / ABCHPR	Hepatitis A, B, C + Reflex C	ABCHEP
HEPACR / HEPACR	Hepatitis Panel, Acute + Reflex C	HEPACU
HEPCRR / HEPCRR	Hepatitis Panel, Chronic + Reflex C	HEPCHR

# Hepatitis C Virus Testing Algorithm



## Reportable Ranges

\* bDNA: 615–7,700,000 IU/mL

\*\* PCR: 25–25,000,000 IU/mL

For more information, please call PAML Client Services at 1-800-349-8586 or (509) 927-6299.

# Hepatitis C Virus Testing

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TO	Determine antibody status	Confirm antibody reactivity, identify active infection, and quantitate HCV RNA	Confirm antibody reactivity (following negative PCR)	Quantitate HCV RNA	Determine HCV genotype
TEST NAME	Hepatitis C Antibody Screen	HCV RNA Quantification	HCV Antibody Confirmation	HCV RNA Quantification	HCV Genotyping by PCR and Probe
DESCRIPTION	HCV Antibody Detection	HCV RNA by PCR, Quantitative	HCV AB Confirmation	HCV RNA by bDNA, Quantitative	HCV Genotyping
METHOD	ICMA	Real-Time PCR (Roche COBAS HCV TaqMan)	Recombinant Immunoblot (CHIRON RIBA HCV 3.0)	bDNA (Bayer HCV bDNA 3.0)	RT-PCR and Line Probe DNA Hybridization Assay
FDA APPROVAL	FDA approved	This test was developed and its performance characteristics determined by Sacred Heart Medical Center. It has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes. This laboratory is certified under CLIA '88 as qualified to perform high-complexity clinical testing.	FDA approved	FDA approved	This test has not yet received FDA approval and is considered for research only.  A positive HCV antibody test does not discriminate between past and active HCV infection. Active HCV infection should be established by HCV RNA by PCR (test code: HCV RTP) before or concurrent with ordering this test.
ORDER CODE	HEP-C	HCV RTP	HCV.CONF	HCVBDNA	HCVGNT
CPT CODE	86803	87522	86804	87522	87902
SAMPLE	<b>1 mL serum</b> (brick-top tube, SST or Corvac). Separate serum from cells and place in separate plastic tube. Store and transport refrigerated.	<b>5 mL frozen plasma</b> (EDTA). Plasma must be separated from cells within 6 hours of collection by centrifugation at 800-1600 × g for 20 minutes at room temperature. Transfer to a sterile polypropylene tube and freeze. Store and transport frozen.	<b>1 mL serum</b> (brick-top tube, SST or Corvac). Separate serum from cells and place in separate plastic tube. Store and transport refrigerated.	<b>1 mL frozen serum</b> (brick-top tube, SST or Corvac). Separate serum from cells aseptically within 4 to 6 hours of collection, place in sterile plastic tube, and freeze. Store and transport frozen.	<b>2 mL frozen plasma</b> (EDTA). Plasma must be separated from cells within 6 hours of collection by centrifugation at 800-1600 × g for 20 minutes at room temperature. Transfer to a sterile polypropylene tube and freeze. Store and transport frozen.
REPORTABLE RANGE	Reactive or Nonreactive	25 to 25,000,000 IU/mL	Reactive, Indeterminate, or Nonreactive	615 to 7,700,000 IU/mL (3,200 to 40,000,000 copies/mL)	Not applicable
COMMENTS	Minimum amount: 0.5 mL Other acceptable samples: plasma. Stability: 8 hours at room temperature, 7 days refrigerated, 3 months frozen at -20°C.	Minimum amount: 2.0 mL Other acceptable samples: ACD plasma, serum (SST tube), and PPT tubes. Stability: CRITICAL FROZEN—Store and transport frozen. <b>Avoid repeated freeze-thaw cycles.</b>	Minimum amount: 0.5 mL Other acceptable samples: plasma. Stability: 8 hours at room temperature, 14 days refrigerated, 3 months frozen at -20°C.	Minimum amount: 0.3 mL Other acceptable samples: EDTA plasma. Stability: 48 hours refrigerated, 72 hours frozen at -20°C, stable if frozen at -70°C. <b>Avoid repeated freeze-thaw cycles.</b>	Minimum amount: 1.0 mL Other acceptable samples: ACD plasma, serum (SST tube), and PPT tubes. Stability: CRITICAL FROZEN—Store and transport frozen. <b>Avoid repeated freeze-thaw cycles.</b>
APPLICATIONS	Initial antibody screening test to detect past or present HCV infection.  Note: This test does not discriminate between past and present infection.	To confirm positive antibody screen. To determine active HCV infection.  To detect HCV RNA within 1 to 3 weeks post-exposure in the absence of antibody activity. To determine pre-therapy baseline HCV RNA levels. To monitor therapeutic efficacy.	To confirm positive antibody screen when HCV RNA by PCR is negative.  Order only on HCV Antibody Screen-reactive/HCV RNA by PCR-negative samples. Note: This test does not discriminate between past and present infection.	To determine pre-therapy baseline HCV RNA levels.  To monitor therapeutic efficacy.  Order only on patient with known active HCV infection.	Determine HCV genotype after active infection is established by HCV Qualitative PCR.  Use in combination with baseline plasma or serum HCV RNA levels to estimate probability of sustained therapeutic response and to determine optimal duration of therapy.
SCHEDULE	Sunday through Friday nights	Monday through Saturday	Tuesday and Friday	Wednesday days, with overnight incubation	Tuesday and Thursday
TURNAROUND	24–48 hours	2–6 days	4–6 days	2–9 days	2–6 days