

# Lab Facets

## Hepatitis C Virus RNA by PCR: NGI HCV QUANTASURE™

### Introduction

Hepatitis C virus (HCV) infection is the most common chronic blood-borne infection in the United States. The CDC estimates that during the 1980s, approximately 230,000 new infections occurred annually.<sup>1</sup> An estimated 3.9 million (1.8%) Americans are chronically infected with the hepatitis C virus.<sup>2</sup> Although the incidence of new HCV infections has declined during the past decade, approximately 30,000 new cases of acute HCV occur each year, and fewer than one-third of these are diagnosed. The consequences of HCV infection are estimated to be associated with 10,000 deaths every year.<sup>3</sup> HCV is transmitted primarily through large or repeated direct percutaneous exposures to blood.

Recent studies have demonstrated that injecting-drug use currently accounts for 60% of cases of HCV transmission in the United States.<sup>1</sup> Other risk groups include persons receiving a blood transfusion prior to 1990, persons involved in high-risk sexual activity, individuals in the health care profession with on-the-job exposure to blood, and those receiving kidney dialysis treatment. Most patients with acute HCV infection are asymptomatic. HCV resolves without treatment in 15% to 25% of cases. Six months after initial infection, HCV remains in 75% to 85% of those infected. These individuals develop chronic hepatitis C.

Chronic infection can lead to chronic liver disease or cirrhosis and its complications. Most studies have reported that cirrhosis develops in as many as 20% of individuals with chronic HCV over a period of 20 to 30 years.<sup>1</sup> In the United States, HCV is a leading cause of chronic liver disease, the most common indication for liver transplantation.<sup>2</sup> Chronic infection with HCV is also associated with an increased risk of hepatocellular carcinoma, or liver cancer.<sup>2</sup> Treatment for HCV is aimed at reducing or eliminating viral burden and decreasing or eliminating the progression of liver damage. Current treatment strategies include interferon- $\alpha$ -based therapies in combination with ribavirin. Depending on their characteristics, with these therapies 40% to 60% of patients can achieve a sustained virologic response, which is durable and associated with long-term histologic benefit.<sup>4</sup>

### Laboratory Methods

Initial diagnosis of HCV infections is commonly achieved through the use of serological assays such as enzyme immunoassays (EIA) that detect antibodies to HCV (anti-HCV). These tests detect anti-HCV in 97% of infected patients; however, they do not distinguish between acute, chronic, or resolved infection.<sup>1</sup> More sophisticated tests, based on nucleic acid detection, are available and widely used for HCV diagnosis. These tests help resolve false-positive antibody results and may also provide better assessment of treatment effectiveness.<sup>3</sup>

### Clinical Utility of HCV RT-PCR Assays

Detection of HCV RNA in patient serum or plasma by highly sensitive nucleic acid tests, such as reverse transcription polymerase chain reaction (RT-PCR), is important for confirming the diagnosis of hepatitis C and for assessing the antiviral response to therapy.<sup>5</sup> The quantitative assessment provided by some of these RT-PCR assays of HCV RNA levels in patients before, during, and after therapy has tremendous potential for improving the clinical management of chronic hepatitis C. Measuring patient viral load levels before treatment is important and can aid in determining the duration of therapy.<sup>6</sup> After initial therapy, a drop in HCV levels is helpful as a predictor of sustained response.<sup>7</sup>

### HCV RNA Quantitation by NGI HCV QUANTASURE™

The NGI HCV QuantaSure™ detection and quantitation assay has been shown to be an extremely sensitive, specific, and reproducible method for measuring HCV RNA levels in serum or plasma. In fact, NGI HCV QuantaSure™ is the most sensitive quantitative RT-PCR assay available. Viral nucleic acids are extracted and isolated from patient plasma or serum. Then RNA is converted to cDNA by reverse transcription, and a conserved segment of the HCV genome is targeted and amplified. Multiple primer pairs are used to ensure detection of different HCV genotypes. Amplified cDNA is detected by hybridization with an internal digoxin-labeled probe followed by a colorimetric immunoassay. QuantaSure™ uses the best

of NGI's proprietary PCR technology to ensure the highest degree of sensitivity and a broad dynamic range for measuring hepatitis C RNA levels (2 IU/mL to 2,000,000 IU/mL or 5 copies/mL to 5,000,000 copies/mL). The assay employs a dilution series of known copy number standards and multiple PCR reactions that terminate at various cycle numbers for quantitation at optimized points of linearity on established standard curves. Multiple PCR reactions terminated at higher cycle numbers and extraction techniques that concentrate nucleic acids are also used to ensure the highest degree of sensitivity.

**Hepatitis C Virus NGI QuantaSure™ by PCR, Quantitative**  
 ..... **140639**

**CPT** 87522

**Synonyms** HCV NGI QuantaSure™; NGI HCV QuantaSure™; QuantaSure™ HCV

**Special Instructions** Submit a separate test request form for each specimen.

**Specimen** Serum or plasma, **frozen**

**Volume** 2.5 mL

**Container** Plasma preparation tube (PPT), red-stopper tube, serum-separator tube, yellow-stopper (ACD plasma) tube, or lavender-stopper (EDTA plasma) tube. Do **not** use green-stopper (heparin tubes)

**Collection** Centrifuge specimen within six hours of collection, remove plasma or serum, and transfer specimen to a screw-cap cryo tube. Ship frozen on dry ice. **Note:** If PPT tubes are used, do **not** transfer plasma. Centrifuge specimen and ship frozen on dry ice.

**Storage Instructions** **Freeze.** (PPT tubes can be stored at room temperature for 72 hours prior to shipping.)

**Causes for Rejection** Hemolysis, green-stopper (heparin) tube; specimen not frozen (not applicable for PPT tubes); PPT not centrifuged

**Limitations** The NGI QuantaSure™ assay has a quantitative range of 2 IU/mL to 2,000,000 IU/mL or 5 copies/mL to 5,000,000 copies/mL.

**Methodology** Polymerase chain reaction (PCR) amplification and detection

**References**

- Centers for Disease Control and Prevention (CDC). Recommendations for prevention and control of hepatitis C virus (HCV) infection and HCV-related chronic disease. *MMWR*. 1998; 47(N° RR-19):1-32.
- Carithers RL. Liver transplantation. *Liver Transpl*. 2000; 6(1):122-135.
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- Alter H. To C or not to C: These are the questions. *Blood*. 1995; 85:1681-1695.
- Pawlotsky JM, Bouvier-Alias M, Hezode C, Darthuy F, Remire J, Dhumeaux D. Standardization of hepatitis C virus RNA quantification. *Hepatol*. 2000; 32(3):654-659.
- Zeuzem S, Lee JH, Franke A, et al. Quantification of the initial decline of serum hepatitis C virus RNA and response to interferon alfa. *Hepatol*. 1998; 27:1149-1156.

**Assay Range:**  
**2 IU/mL — 2,000,000 IU/mL**  
**5 copies/mL — 5,000,000 copies/mL**

**For additional information regarding  
 NGI HCV QUANTA**SURE**™ or HCV QUANTA**SURE**™ *PLUS*  
 testing, contact your local LabCorp representative  
 or your nearest LabCorp facility, or call the  
 Center for Molecular Biology and Pathology  
 at 800-533-0567.**



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