

**CPAL**Central Pennsylvania Alliance
Laboratory

Technical Bulletin

No. 34

February 15, 2003

New Test - HCV Viral Load by bDNA

Starting Date: March 3, 2003

Medical Use: Quantification of HCV RNA before and during treatment of combination interferon and ribavirin can provide important information on the likelihood of response to treatment. Early viral response (EVR), defined as a minimum 2 log decrease in viral load during the first 12 weeks of treatment, is predictive of sustained virological response and should be a routine part of monitoring. Patients who fail to achieve an EVR at week 12 of treatment have only a small chance of achieving a sustained virological response even if therapy is continued for a full year. Treatment needs not be extended beyond 12 weeks in these patients¹. Determination of HCV viral levels before treatment, at 12 weeks, and 24 weeks of treatment is recommended²⁻³.

Method: Bayer Versant HCV RNA 3.0 bDNA assay

Analytical Measurement Range:

In copies/mL : 3200 - 40,000,000 copies/mL
In IU/mL: 615 - 7,700,000 IU/mL

Reference Intervals: < 3200 copies/mL

Supportive Data:

Forty six patient plasma samples (from York Hospital) were tested using bDNA method at CPAL and the results agreed very well with the results from Hershey Medical Center. The linear correlation has a slope of 0.99 and intercept of -35058, $r^2 = 0.95$. Forty eight negative specimens were tested in duplicate and all had results less than the lower detection limit (615IU/mL). Within-run precision were tested at four RNA levels with each one being assayed 6 times. The CVs of these samples were between 0.5% to 2% in log unit. Linearity was verified by serial dilutions of a patient sample with a high viral load (3.7 million IU/mL). The results of the correlate well with the target value. We also compared Bayer's bDNA method with Roche's Cobas Amplicor PCR method using 116 patient specimens. We found the two methods comparable at RNA levels below 500,000 IU/mL. The slope is 1.09 and the intercept is -3662 and $r^2=0.74$.

Specimen Collection:

Issued on: January 12, 2004

For question about this, and other, information, call Central Pennsylvania Laboratory at 1-888-480-1422

No special patient preparation is required. Both serum and EDTA plasma are acceptable. However, proper specimen handling is very important to protect the RNA from degradation. The minimum sample requirement is 100 µL of sample for a single determination. We require 2 mL of specimen.

Serum: Collect blood in sterile tubes with no anticoagulants or in SST tubes. Allow blood to clot at room temperature and centrifuge within 4 hours to separate serum from cells.

Remove serum from the clot within 4 hours of collection to a sterile screw-capped tube.

Plasma: Collect 7 mL blood in **K3-EDTA** tube (K3-EDTA, 0.15% v/v final). The blood is to be stored at room temperature (do not refrigerate) for **no more than 4 hours** before the plasma is separated. Prolonged storage at room temperature will result in loss of HCV RNA from the RNAases in the blood. Whenever possible, patients should be instructed to come to the hospital's outpatient collection facility where the specimen, once collected, can be rapidly processed. The blood is to be centrifuged at **1000 x g for 15 minutes within 4hr** of collection. Do not clarify the sample by further centrifugation or filtration. Remove plasma to a sterile screw-capped tube.

Specimen Storage:

The specimen (either serum or separated plasma) is to be stored at -20⁰C in a non frost-free freezer for up to 72 hr after collection or at -60⁰C to -80⁰C for longer intervals. Rapid freezing is essential to preserve viral RNA. **Note: Whenever possible, store specimen at -60⁰C to -80⁰C in sterile screw-capped tubes.** Avoid repeated freeze-thaw of the samples.

Shipment: Ship specimens frozen on dry ice.

Cautions: This assay is limited to the quantitation of HCV RNA in human plasma or serum to monitor the change of HCV viral load. It is not for diagnosis of HCV infection. A HCV RNA level less than 3200 copies/mL (615 IU/mL) does not exclude viremia and may reflect only a transient decline in viral level below the detection limit of the assay. A follow up qualitative HCV RNA by TMA (sensitivity = 5-10 IU/mL) or by PCR (sensitivity = 50 IU/mL) should be performed to confirm the absence of active HCV replication.

- References:**
1. NIH Consensus Development Program Consensus Statement, Management of Hepatitis C: 2002.
 2. Dufour DR, Lott JA, Nolte FS, Gretch DR, Koff RS and Seeff LB, "Diagnosis and monitoring of hepatic injury. I. Performance characteristics of laboratory tests", Clinical Chemistry, 2000; 46:2027-2049.
 3. Clinical Implications: Hepatitis in the New Millennium - HCV Molecular Tools, American College of Gastroenterology, 2001

2 of 3

1803 Mt. Rose Avenue
York, Pa. 17403

***Controlled Copy, Do Not Duplicate
Only***

l:\cpal\bulletin\...\029_iPTH.doc

For Internal Use

Laboratory Lu Song, Ph.D. , Technical Director, CPAL Lab
Contact: 717-851-1422

3 of 3

1803 Mt. Rose Avenue
York, Pa. 17403

***Controlled Copy, Do Not Duplicate
Only***

l:\cpal\bulletin\...\029_iPTH.doc

For Internal Use