

HCV RNA 3.0

Assay (bDNA)

BOX	Cat. No. / REF
1	02553870
2	02554338

02616244 Rev. A, 2003-04

Intended Use

The VERSANT® HCV RNA 3.0 Assay (bDNA) is a signal amplification nucleic acid probe assay for the quantitation of human hepatitis C viral RNA (HCV RNA) in the serum or plasma (EDTA and ACD) of HCV-infected individuals using the Bayer System 340 bDNA Analyzer. Specimens containing HCV genotypes 1-6 have been validated for quantitation in the assay.

The VERSANT HCV RNA 3.0 Assay (bDNA) is intended for use as an aid in the management of HCV-infected patients undergoing anti-viral therapy. The assay measures HCV RNA levels at baseline and during treatment and is useful in predicting non-sustained virological response to HCV therapy.

The results from the VERSANT HCV RNA 3.0 Assay (bDNA) must be interpreted within the context of all relevant clinical and laboratory findings.

Assay performance characteristics have been established only for individuals treated with interferon alfa-2b plus ribavirin. No information is available on the assay's predictive values when other therapies are used.

WARNINGS:

The positive predictive values of HCV RNA levels (i.e. prediction of sustained virological response) are not intended for use in guiding therapy.

The VERSANT HCV RNA 3.0 Assay (bDNA) is not intended for use in the diagnosis or confirmation of HCV infection.

This assay has not been FDA approved for the screening of blood or plasma donors.

Summary and Explanation

The hepatitis C virus is a serious public health problem. Because HCV infections are so widespread, with an estimated 150 million people infected worldwide, early detection is essential to help control the disease.¹ HCV is the causative agent for most blood-borne non-A, non-B hepatitis (NANB)^{2,3} and is transmitted primarily through contaminated blood and blood products, intravenous drug use, and to some extent by other close personal contact.¹

HCV infection is asymptomatic or mildly symptomatic in the majority of cases and is characterized primarily by elevated alanine aminotransferase (ALT) levels.^{1,4} Approximately half the patients infected with HCV develop chronic liver disease and 20% of these develop chronic active hepatitis or cirrhosis. HCV is also a causative factor in the development of hepatocellular carcinoma.⁵

HCV is a positive-stranded RNA virus of approximately 9400 nucleotides coding for a core, an envelope, and five nonstructural domains.⁶ HCV is closely related in structure to the flaviviruses. Of the six major HCV genotypes for which coding sequences have been identified, the 5' untranslated region (5'-UTR) and a portion of the core region are most highly conserved.⁶⁻⁸

Detection of HCV is usually based on serologic screening for anti-viral antibodies with enzyme-linked immunosorbent assays (ELISA) or enzyme immunoassays (EIA). Confirmatory tests include an immunoblot assay and HCV RNA tests.^{1,9} Because the ELISA and other anti-HCV detection tests rely on antibody production, these tests are inherently less sensitive early in the disease or when antibody production is low, as observed in hemodialysis or immunocompromised patients.^{9,10} Nucleic acid based tests for HCV RNA have been shown to detect HCV an average of 50 days earlier than antibody-based tests.¹¹ Researchers have found that nearly 100% of serum samples from seropositive symptomatic patients and from seropositive blood donors with elevated ALT levels have detectable levels of HCV RNA.¹²⁻¹⁵

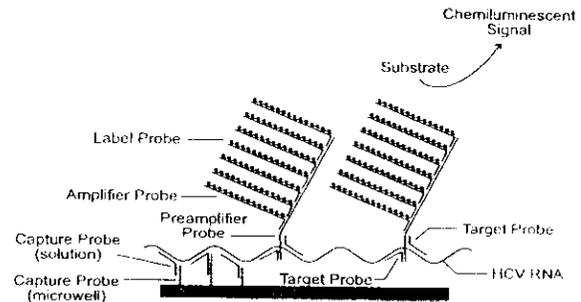
There have been recent advances in the understanding of HCV disease and therapy options available. Combination therapy with interferon plus ribavirin has been shown in several studies to be more effective than interferon monotherapy.^{9,16,17} However, patients with high pretreatment levels of HCV RNA and patients with HCV genotype 1 respond less well to treatment.¹⁸⁻²¹ In patients who respond to therapy, HCV RNA levels decline and the initial rate of RNA decline may be predictive of the long term response to therapy.²² These observations and others suggest that quantitation of HCV RNA can contribute significantly to evaluating HCV disease prognosis, selecting appropriate treatment regimens, and monitoring therapy.

Assay Principles

The VERSANT HCV RNA 3.0 Assay (bDNA) is a sandwich nucleic acid hybridization procedure for the direct quantitation of HCV RNA in human serum and plasma. After HCV genomic RNA is released from the virions, the RNA is captured onto a microwell by a set of specific, synthetic oligonucleotide capture probes. A set of target probes hybridizes to both the viral RNA and the pre-amplifier probes. The capture probes and the target probes bind to the 5' untranslated and core regions of the HCV genome. The amplifier probe subsequently hybridizes to the pre-amplifier forming a branched DNA (bDNA) complex.

Multiple copies of an alkaline phosphatase (AP) labeled probe are then hybridized to this immobilized complex. Detection is achieved by incubating the AP-bound complex with a chemiluminescent substrate. Light emission is directly related to the amount of HCV RNA present in each sample, and results are recorded as relative light units (RLUs) by the Bayer System 340

bDNA Analyzer. A standard curve is defined by light emission from standards containing known concentrations of recombinant single-stranded phage DNA. Concentrations of HCV RNA in specimens are determined from this standard curve.



Materials Provided

Understanding the Symbols

The following symbols may appear on the labeling and packaging.

Symbol	Definition
	<i>In vitro</i> diagnostic device
	Catalog Number
	Manufactured by
	Authorized Representative
	CE Mark with notified body
	Temperature limitation (2°-8°C)
	Contents sufficient for (n) tests
	Batch code
	Use by
2003-03-24	Date format (year-month-day)
2003-03	Date format (year-month)
	Store upright

Kit contains sufficient reagents and materials to perform either one complete or four partial plate assays.

BOX 1

Component	Quantity	Description	Storage
Lysis Diluent	1 x 15 mL	buffered protein solution with sodium azide (< 0.1%) and other preservatives	2° to 8°C
Lysis Reagent	1 x 2.2 mL	proteinase K solution with sodium azide (< 0.1%) and other preservatives	2° to 8°C
Capture Probes	1 x 120 µL	synthetic oligonucleotides in buffered solution with sodium azide (< 0.1%) and other preservatives	2° to 8°C
Target Probes	1 x 120 µL	synthetic oligonucleotides in buffered solution with sodium azide (< 0.1%) and other preservatives	2° to 8°C
Wash A	2 x 440 mL	buffered solution with sodium azide (< 0.1%) and other preservatives	2° to 30°C
Wash B	2 x 320 mL	buffered solution with sodium azide (< 0.1%) and other preservatives	2° to 39°C
Capture Wells	8 strips/12 wells each	polystyrene microwells coated with synthetic oligonucleotides	2° to 8°C
Barrier Film	1 x 6 sheets	high density polyethylene sheets	2° to 30°C
Pre-Amplifier/Amplifier Diluent	1 x 28 mL	buffered protein solution with sodium azide (< 0.1%) and other preservatives	2° to 8°C
Label Diluent	1 x 15 mL	buffered protein solution with sodium azide (< 0.1%) and other preservatives	2° to 8°C
Dextran Sulfate	1 x 14 mL	40% dextran sulfate solution with sodium azide (< 0.1%) and other preservatives	2° to 8°C
Pre-Amplifier Probes	1 x 130 µL	synthetic oligonucleotides in buffered solution with sodium azide (< 0.1%) and other preservatives	2° to 8°C
Amplifier Probe	1 x 130 µL	synthetic oligonucleotides in buffered solution with sodium azide (< 0.1%) and other preservatives	2° to 8°C
Substrate	1 x 12 mL	chemiluminescent substrate (Lumi-Phos® Plus)	2° to 8°C
Substrate Enhancer	1 x 12 mL	aqueous solution with sodium azide (< 0.1%) and other preservatives	2° to 8°C

BOX 2

Component	Quantity	Description	Storage
Label Probe	1 x 140 µL	enzyme-labeled synthetic oligonucleotides in buffered solution with sodium azide (< 0.1%) and other preservatives	-60° to -80°C
High Positive Control	4 x 105 µL	human plasma containing recombinant single-stranded phage DNA with sodium azide (< 0.1%) and other preservatives	-60° to -80°C
Low Positive Control	4 x 105 µL	human plasma containing BPL-treated HCV with sodium azide (< 0.1%) and other preservatives	-60° to -80°C
Negative Control	4 x 105 µL	human plasma nonreactive for HCV with sodium azide (< 0.1%) and other preservatives	-60° to -80°C
Standards A, D	1 x 440 µL/level	human plasma containing recombinant single-stranded phage DNA with sodium azide (< 0.1%) and other preservatives	-60° to -80°C
Standards B, C	1 x 220 µL/level	human plasma containing recombinant single-stranded phage DNA with sodium azide (< 0.1%) and other preservatives	-60° to -80°C
Standard E	1 x 660 µL	human plasma containing recombinant single-stranded phage DNA with sodium azide (< 0.1%) and other preservatives	-60° to -80°C

Materials Required But Not Provided

- 10 mL sterile-packaged serological pipettes
- 12 channel pipette capable of dispensing 50 to 200 µL (accuracy less than ± 5%) and 50 mL sterile-packaged, disposable reservoirs
- Positive displacement pipette capable of dispensing 1 to 7 mL
- Precision pipettes capable of dispensing 25 to 1000 µL (accuracy less than ± 5%) and sterile-packaged, aerosol-resistant, disposable tips
- 15 and 50 mL sterile-packaged, disposable polypropylene tubes
- Blank microwells, black (Cat No. 112022)
- Bleach, unscented (5.25% sodium hypochlorite)
- Bayer System 340 bDNA Analyzer with Data Management Software (DMS), version 7.0.0 or greater, for the VERSANT HCV RNA 3.0 Assay (bDNA)
- Vortex mixer
- Water bath (37° ± 2.5°C)
- 70% Ethanol

Warnings and Precautions

For In Vitro Diagnostic Use.



R42/43, R36/37/38, S26, S36, S23
Harmful/Irritant! May cause sensitization by inhalation and skin contact. Irritating to eyes, respiratory system and skin. In case of contact with eyes, nose immediately with plenty of water and seek medical advice. Wear suitable protective clothing. Do not breathe spray. **Contains:** Protease K, Lysis Reagent



CAUTION! POTENTIAL BIOHAZARD. This product contains human plasma or other human source material. The low positive control contains human plasma and beta propiolactone (BPL)-treated HCV viral material. Handle this product according to established good laboratory practices and universal precautions.²³⁻²⁵

- The human source components were tested and found negative for anti-HIV (Types 1 and 2), anti-HCV, and HBsAg by FDA recommended (approved/licensed) tests.
- Because no test method can offer complete assurance that laboratory specimens do not contain HIV, hepatitis B virus, or other infectious agents, specimens should be handled at the BSL 2 as recommended for any potentially infectious human serum or blood specimen in the CDC-NIH manual *Biosafety in Microbiological and Biomedical Laboratories*, 3rd Edition, 1993 and NCCLS Approved Guideline M29-A, Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue
- Disposal.** Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner, and in compliance with all federal, state, and local requirements.
- CAUTION** Sodium azide in the reagents can react with copper and lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of metal azides, if disposal into a drain is in compliance with federal, state, and local requirements
- Perform the procedure using universal precautions.
- Disinfect spills promptly using a 0.5% sodium hypochlorite solution (1:10 v/v household bleach) or equivalent disinfectant. Handle contaminated materials as biohazardous.
- Wear personal protective apparel, including disposable gloves, throughout the assay procedure. Thoroughly wash hands after removing gloves, and dispose of gloves as biohazardous waste.
- Do not eat, drink, smoke, or apply cosmetics in areas where reagents or specimens are handled
- Avoid the use of sharp objects wherever possible
- If skin or mucous membrane exposure occurs, immediately wash the area with copious amounts of water. Seek medical advice
- Do not pipette by mouth
- Use all pipetting devices and instruments with care and follow the manufacturers' instructions for calibration and quality control.
- Use aerosol-resistant pipette tips and use a new tip every time a volume is dispensed.
- Do not use reagents if precipitate is visible after bringing to specified temperature.
- Do not use components beyond the expiration date printed on the kit boxes

- Do not mix components from lot to lot.
- Use all kit components within 4 weeks after opening any component.
- Return all components to the appropriate storage condition after preparing the working reagents.
- The controls (Negative, Low Positive, and High Positive) are supplied as single use vials.
- For stability of the working reagents, refer to the *Assay Procedure* section.
- Do not interchange vial or bottle caps as cross-contamination may occur.
- Do not let the plate stand dry after any incubation. Prepare the next reagent before the plates are ejected. Add the next reagent to the plate and press **Start** within 4 minutes of ejection. If more than 4 minutes elapses, then the assay run may be compromised and the Bayer System 340 enters a warning message into the event log.

Specimen Collection and Handling

No special preparation of the individual is required prior to specimen collection. However, proper specimen handling is very important to protect the RNA from degradation.

- This assay requires 50 µL of sample for a single determination.
- Collect blood observing universal precautions for venipuncture
- For serum samples, collect blood in sterile tubes with no anticoagulants or in serum separator tubes. Allow blood to clot at room temperature and centrifuge within 4 hours to separate serum from cells. Use standard laboratory procedures to remove serum aseptically from the clot within 4 hours of collection.
- For plasma samples, collect blood in sterile tubes containing EDTA (K₂) or ACD. Store whole blood at room temperature. Do not refrigerate. Remove plasma from cells within 4 hours of collection. Separate by centrifugation at 1000 x g for 10 to 15 minutes and use standard laboratory procedures to remove the plasma.
- Do not clarify specimens by filtration or further centrifugation
- Store specimens at -60° to -80°C in sterile, screw-capped tubes. Specimens may also be stored at 2° to 8°C for up to 48 hours or at -20°C in a non-frost free freezer for up to 72 hours prior to freezing at -60° to -80°C.
- Avoid repeated freeze/thaw of the samples.*
- Handle all specimens as if capable of transmitting infection.
- If necessary to ship, ship specimens frozen on dry ice. Package and label in compliance with federal and international regulations covering the transport of clinical samples and etiological agents.

* For data see section Multiple Freeze/Thaw below.

Assay Procedure

The procedure consists of two major activities: hybridizing the probes and measuring the light output

PROCEDURAL NOTES:

- The Data Management Software reports HCV results in copies/mL and IU/mL (World Health Organization or WHO units).²⁶ See the Product Insert Supplement for the conversion factor
- One plate contains 8 strips (96 wells) with the capacity for 84 samples.
- Room temperature is 18° to 30°C
- Clean all pipettors and the benchtop with 70% ethanol before starting the procedure.
- Refer to the operator's manual for the Bayer System 340 bDNA Analyzer for more information about preparing and using the analyzer and to the operator's manual for the DMS for information about printing a plate map and analyzing assay data.

Preparing the Bayer System 340 bDNA Analyzer

Refer to *Analyzing Samples* in the operator's manual for more information.

- Ensure that all maintenance has been performed. If required, update the maintenance log
- Check the levels of rinse and cleaning solutions on the analyzer. Add solution as necessary.
- Wearing clean gloves, place a new barrier film on the frame.
- Place a silicone layer over the barrier film to complete the Sealing Pad.
- Select **Test** from the Main Menu.
- Select **Automated**. Follow the instructions to select **HCV RNA 3.0 Assay**.
NOTE: Always add samples to the left plate first.
- Select the number of strips per plate. If only the left plate has samples, select **0 Strips** for the right plate.
NOTE: If 8 strips or less are run, use blank, black microwells as necessary to fill the left and right plates.

Day 1 Procedure Hybridizing the Capture and Target Probes

NOTE: Bring the pouch containing the Capture Wells to room temperature before opening.

- Create a Sample ID file using the DMS.
- Place the Lysis Diluent in a 37°C water bath for 10 to 20 minutes or until any visible crystals dissolve and mix.
- Place the Lysis Reagent, Capture Probes, and Target Probes on the bench top and bring to room temperature.
- Place the specimens, standards (A, B, C, D, and E) and one vial of each of the controls (Negative, Low Positive, and High Positive) in cold water to thaw, then keep on ice
- Prepare the Lysis Working Reagent in a 50 mL polypropylene tube. Use 15 mL polypropylene tube for smaller volumes.
 - Add the volume appropriate to the number of strips used in the assay:

Capture Well Strips	Specimen Samples	Lysis Diluent	Lysis Reagent	Capture Probes	Target Probes
8	84	10.5 mL	1.44 mL	100 µL	100 µL
6	60	7.88 mL	1.08 mL	75 µL	75 µL
5	48	6.56 mL	0.90 mL	63 µL	63 µL
4	36	5.25 mL	0.72 mL	50 µL	50 µL
3	24	3.94 mL	0.54 mL	38 µL	38 µL
2	12	2.63 mL	0.36 mL	25 µL	25 µL

- b. Cap, then invert tube 10 times, vortex 5 seconds, and invert another 10 times to mix. Store at room temperature and let foam settle. Discard if not used within 2.5 hours.
6. Wearing clean gloves, place capture well strips in a plate. Press the capture well strips firmly into the plate until the tops of the strips are level. Remove the labs. Immediately return unused strips to the pouch, seal, date, and store at 2° to 8°C.
7. Use blank, black microwells as necessary to fill the plate.
- NOTE:** Ensure that the plate notches are on the left.
8. Place each assay plate in the Sample Orientation Tool as described in the operator's manual for the Bayer System 340 bDNA Analyzer.
9. Add 100 µL of Lysis Working Reagent to each well. Use a twelve-channel pipette and fill the plate from row A to row H.
10. Vortex the specimens, standards, and controls to mix.
11. Using a 25 to 200 µL variable or a 50 µL fixed pipette and a new tip for each sample, transfer 50 µL from each specimen, standard, or control into the appropriate capture well according to the plate map:

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std A	Std A	Std B	Std C	Std D	Std D	Std E	Std E	Std E	N-Ctl	L-PCtl	H-PCtl
B												
C												
D												
E												
F												
G												
H												

Std is Standard A through E, N-Ctl is Negative Control, L-PCtl and H-PCtl are Low and High Positive Controls, respectively.

12. Immediately return remaining specimens and standards to -60° to -80°C. Standards and specimens may be frozen and thawed up to four times. Discard remaining thawed Negative, Low Positive, and High Positive control material.
13. When prompted place the plates on the loading tray of the analyzer. If 8 strips or less are run, use blank, black microwells to fill the left and right plates as necessary.
14. Place the assembled Sealing Pad on each plate. Close the analyzer access door and press **Start**.
NOTE: The overnight incubation consists of a 1 hour incubation, followed by a cool cycle, and then a 14 hour incubation. The analyzer displays the time remaining for each incubation.
15. Incubate 15 to 18 hours (overnight). After 15 hours, the analyzer enters a hold mode until you proceed with the next step.

Day 2 Procedure Hybridizing the Pre-Amplifier and Amplifier Probes

NOTE: If Wash A and Wash B are stored at 2° to 8°C, allow 2 hours to bring them to room temperature, and mix by inverting each bottle several times before adding to the analyzer bottles.

NOTE: Each cool/wash cycle takes approximately 20 minutes depending on the room temperature. The instrument washes and aspirates reagent from the wells, ejects the plates, then beeps at the end of each cycle. Leave the plates on the analyzer heater tray while adding the next reagent.

CAUTION: Do not let the plate stand dry. Prepare the next reagent before the plates are ejected. Add the next reagent to the plate and press **Start** within 4 minutes of ejection. If more than 4 minutes elapses, then the assay may be compromised and the Bayer System 340 bDNA Analyzer enters a warning message into the event log.

1. Ensure Wash A and Wash B bottles have enough solution for the number of strips used in the assay. Add solution as necessary.

Capture Well Strips	Specimen Samples	Wash A	Wash B
8	84	440 mL (1 Bottle)	320 mL (1 Bottle)
6	60	440 mL	320 mL
5	48	440 mL	320 mL
4	36	440 mL	320 mL
3	24	220 mL	200 mL
2	12	220 mL	160 mL

2. Place the Pre-Amplifier/Amplifier Diluent and Dextran Sulfate in a 37°C water bath for at least 10 minutes. Swirl the bottle with Dextran Sulfate and invert the diluent several times to mix until homogeneous. Keep at 37°C.
3. Prepare the Pre-Amplifier/Amplifier Working Diluent in a 50 mL polypropylene tube. Use a 15 mL polypropylene tube for smaller volumes.
 - a. Add the volume appropriate to the number of strips used in the assay. Add Dextran Sulfate using a positive displacement pipette.

Capture Well Strips	Specimen Samples	Pre-Amplifier/Amplifier Diluent	Dextran Sulfate
8	84	21.0 mL	7.0 mL
6	60	18.0 mL	6.0 mL
5	48	15.0 mL	5.0 mL
4	36	12.0 mL	4.0 mL
3	24	9.0 mL	3.0 mL
2	12	6.0 mL	2.0 mL

- b. Cap tube, then invert 10 times and vortex 20 seconds to mix. Repeat until diluent is thoroughly mixed. Working diluent may be very foamy. Place in a 37°C water bath and let foam dissipate. Discard if not used within 2 hours.

4. Place the Pre-Amplifier Probes on the benchtop and bring to room temperature.
5. Prepare the Pre-Amplifier Working Reagent in a 15 mL polypropylene tube.
 - a. Add the volume appropriate to the number of strips used in the assay:

Capture Well Strips	Specimen Samples	Pre-Amplifier/Amplifier Working Diluent	Pre-Amplifier Probes
8	84	13.0 mL	100 µL
6	60	9.8 mL	75 µL
5	48	8.4 mL	65 µL
4	36	7.2 mL	55 µL
3	24	5.1 mL	39 µL
2	12	3.9 mL	30 µL

- b. Cap tube, then invert 10 times and vortex 5 to 20 seconds to mix. Repeat until reagent is thoroughly mixed. Place in a 37°C water bath and let foam dissipate. Discard if not used within 30 minutes.

6. When the overnight incubation is completed, press **Start** on the analyzer to cool and wash the plates.

The cool/wash cycle takes about 20 minutes.

7. When the plates are ejected, immediately add 100 µL of Pre-Amplifier Working Reagent to each well. Use a twelve-channel pipette and fill the plate from row A to row H.

8. Press **Start**.

The incubation takes 30 minutes and is followed by a cool/wash cycle. Prepare the Amplifier Working Reagent when the cool/wash cycle starts.

9. Place the Amplifier Probe on the bench top and bring to room temperature.

10. Prepare the Amplifier Working Reagent in a 15 mL polypropylene tube.

- a. Add the volume appropriate to the number of strips used in the assay:

Capture Well Strips	Specimen Samples	Pre-Amplifier/Amplifier Working Diluent	Amplifier Probe
8	84	13.0 mL	100 µL
6	60	9.8 mL	75 µL
5	48	8.4 mL	65 µL
4	36	7.2 mL	55 µL
3	24	5.1 mL	39 µL
2	12	3.9 mL	30 µL

- b. Cap tube, then invert 10 times and vortex 5 to 20 seconds to mix. Repeat until reagent is thoroughly mixed. Place in 37°C water bath and let foam dissipate. Discard if not used within 30 minutes.

11. When the plates are ejected, immediately add 100 µL of Amplifier Working Reagent to each well. Use a twelve-channel pipette and fill the plate from row A to row H.

12. Press **Start**.

The incubation takes 30 minutes and is followed by a cool/wash cycle.

Hybridizing the Label Probe

1. Place the Label Diluent in a 37°C water bath for at least 10 minutes. Mix by inverting the bottle several times.

2. Thaw the Label Probe in cold water (4 to 15°C) just before using.

NOTE: Immediately return any unused Label Probe to -60° to -80°C freezer.

3. When the cool/wash cycle starts, prepare the Label Working Reagent in a 15 mL polypropylene tube.

- a. Add the volume appropriate to the number of strips used in the assay:

Capture Well Strips	Specimen Samples	Label Diluent	Label Probe
8	84	12.0 mL	100 µL
6	60	9.0 mL	75 µL
5	48	7.5 mL	63 µL
4	36	6.0 mL	50 µL
3	24	5.0 mL	41 µL
2	12	3.6 mL	30 µL

- b. Cap tube, then invert 10 times. Store at room temperature and let foam dissipate. Discard if not used within 30 minutes.

4. When the plates are ejected, immediately add 100 µL of Label Working Reagent to each well. Use a twelve-channel pipette and fill the plate from row A to row H.

5. Press **Start**.

The incubation takes 45 minutes and is followed by a cool/wash cycle.

6. At the beginning of the Label Probe incubation, place the Substrate and Substrate Enhancer on the bench top and bring to room temperature.

Measuring the Light Output

1. When the wash cycle starts, prepare the Substrate Working Reagent in a 15 mL polypropylene tube.

- a. Add the volume appropriate to the number of strips used in the assay:

Capture Well Strips	Specimen Samples	Substrate	Substrate Enhancer
8	84	11.0 mL	1.0 mL
6	60	8.3 mL	0.75 mL
5	48	6.9 mL	0.63 mL
4	36	5.5 mL	0.50 mL
3	24	4.1 mL	0.38 mL
2	12	2.8 mL	0.25 mL

- b. Cap tube and invert 10 times to mix. Store at room temperature. Discard if not used within 30 minutes.

- When the plates are ejected, immediately add 80 µL of Substrate Working Reagent to each well. Use a twelve-channel pipette and fill the plate from row A to row H.
- Press **Start**
The incubation takes 30 minutes

Analyzing the Data

During the substrate incubation, prepare the DMS to receive the data from the analyzer.

The analyzer automatically reads the light units in each of the wells of the plates and transfers the data to the DMS for analysis.

Calibrating the Assay

Run the five kit standards with each assay to generate a standard curve. Refer to the plate map in *Hybridizing the Capture and Target Probes* for placement. The standard curve is used to determine the viral load in each sample.

Quality Control

To monitor assay performance, three levels of control material must be included with every assay. Treat all control samples the same as specimens.

The assay is considered valid if all the following conditions occur:

- The values determined for the HCV positive controls are within the specified range
- The relative light units (RLUs) for the standards are geometric mean RLU Std A > RLU Std B > RLU Std C > geometric mean RLU Std D > geometric mean RLU Std E. The geometric means are calculated by the DMS software.
- The HCV Negative Control has a value ≤ 3200 copies/mL.
- If any of the above conditions are not met, do not report patient results from the affected run. Determine the cause for quality control failure and repeat run.

If the assay must be repeated, then do the following:

- Review these instructions to ensure that the assay is performed according to the procedure recommended by Bayer Diagnostics.
- Verify that the standards and controls are in the appropriate location specified by the plate map.
- Verify that the materials are not expired.
- Verify that the required Bayer System 340 bDNA Analyzer maintenance was performed.

Standardization

The VERSANT HCV 3.0 RNA Assay (bDNA) is standardized in copies/mL using an RNA transcript derived from the 5' untranslated and core regions of the HCV genome as a reference. The quantity of the RNA in the standard is determined by three independent analytical methods.²⁷ The correlation between results in IU/mL and copies/mL was determined by quantitating the WHO HCV Standard in the VERSANT HCV 3.0 RNA Assay (bDNA) using the RNA transcript reference material. The WHO HCV Standard lot number and conversion factor used to convert the result from copies/mL to IU/mL may be found in the Product Insert Supplement.

Calculation of Results

The data output from the Bayer System 340 bDNA Analyzer are reported as relative light units (RLUs). The light emitted is directly related to the number of HCV RNA copies/mL. A 4-parameter logistic curve is used to model the logarithm of the RLUs as a function of the logarithm of HCV RNA concentration.

The DMS uses the observed RLUs and the concentrations of the five standard curve points to determine the best curve fit to plot the standard curve and calculate the concentration of HCV RNA for each control and patient sample. The standard curve is calculated from nine wells of standards (one well each of Standards B and C, two wells each of Standards A and D, and three wells of Standard E). The results of each assay are printed in a report.

The Data Management Software (DMS) reports test results in copies/mL and the World Health Organization (WHO) International Units (IU/mL).²⁶ Refer to the VERSANT HCV RNA 3.0 Assay (bDNA) Product Insert Supplement for the conversion factor.

Results presented in International Units per milliliter (IU/mL) were obtained using a conversion factor of 5.2 copies/IU. This conversion factor was determined by characterizing the VERSANT HCV RNA 3.0 Assay (bDNA) standardization system using WHO HCV Standard Lot No. 96/790.

Interpretation of Results

Quantitation Limits

The following quantitation limits are defined for the VERSANT HCV RNA 3.0 Assay (bDNA):

- The Limit of Detection (LoD) is the lowest concentration of virus that yields an assay result at or above the Detection Cutoff in 95% of replicate determinations. The LoD for this assay is 1,000 IU/mL (5,200 HCV RNA copies/mL).
- The Detection Cutoff (DC) is the point on the assay quantitation scale such that 95% of negative specimens produce results below this cutoff with 95% confidence. The DC for this assay is 615 IU/mL (3,200 HCV RNA copies/mL).
- The Upper Quantitation Limit (UQL) for this assay has been determined to be 7,690,000 IU/mL (40,000,000 HCV RNA copies/mL).
- The quantitation range of the assay is from 615 IU/mL (3,200 HCV RNA copies/mL) to 7,690,000 IU/mL (40,000,000 HCV RNA copies/mL).

CAUTION:

Results reported between DC (615 IU/mL) and LoD (1,000 IU/mL) must be interpreted with caution because the detection rate of the assay gradually decreases from 95% at 1,000 IU/mL to 39.5% at 615 IU/mL, and the variability of the assay increases from 26.6% CV at 1,000 IU/mL to 32.4% CV at 615 IU/mL.

Establishment of Cutoff and Threshold Values

The Detection Cutoff (DC) was established in preclinical trials and confirmed in the clinical trial using specimens from 999 HCV-seronegative volunteer blood donors.

The Limit of Detection (LoD) was established by interpolation between the two reproducibility panel members that had perfect detection results that bracketed 95% detection. Each panel member had 216 determinations.

The optimal threshold values at Weeks 4, 8, 12, and 24 for prediction of non-sustained virological response were chosen based on the viral load, or log drop in viral load from baseline, with the largest number of predicted non-sustained virological responders and a negative predictive value (NPV) of ≥ 95%. The 2-log drop in viral load from baseline established for Weeks 8, 12, and 24 in this study is consistent with the log drop in viral load reported in the 2002 NIH HCV Consensus Statement.²⁸

Assay results are reported as follows:

- Values below the DC are reported as < 3200* HCV RNA copies/mL and < 615* IU/mL
- Values between the DC and the UQL are reported as a quantitation, i.e. numbers.
- Values above the UQL are reported as > 40000000* HCV RNA copies/mL and > 7692310* IU/mL.

Interpretation of Clinical Results

Prediction Rules Using Viral Load and Log Drop Thresholds

The performance of the VERSANT HCV RNA 3.0 Assay (bDNA) was evaluated for prediction of virological response in 351 chronic HCV-infected subjects who had undergone standard treatment with interferon alpha-2b plus ribavirin (REBETRON®) for up to 48 weeks (see the Performance Characteristics section for more detail on the clinical study design and results). Prediction rules at Weeks 4, 8, 12 and 24 are shown in Table 1. Categorization of non-sustained virological responders (NSVR) and sustained virological responders (SVR) is based on a positive or negative test result, respectively, using a sensitive qualitative HCV RNA test > 6 months after the end of treatment.

NOTE: In the following tables, the definition of predicted sustained virological responders is provided for information only. The prediction of SVR is not sufficiently robust to be used for making decisions regarding continuation or termination of treatment.

Table 1. Definition of Prediction Rules

Week	Type of Rule	Prediction Rule	Predicted Non-sustained Virological Responders	Predicted Sustained Virological Responders
4	Viral Load Threshold	100,000 IU/mL ²	≥ 100,000 IU/mL	< 100,000 IU/mL
	Log Drop ¹	1 Log Drop	< 1 log and ≥ 1,000 IU/mL ²	> 1 log or < 1,000 IU/mL
8	Viral Load Threshold	10,000 IU/mL ²	≥ 10,000 IU/mL	< 10,000 IU/mL
	Log Drop	2 Log Drop	≤ 2 log and > 1,000 IU/mL	> 2 log or < 1,000 IU/mL
12	Viral Load Threshold	10,000 IU/mL	≥ 10,000 IU/mL	< 10,000 IU/mL
	Log Drop	2 Log Drop	≤ 2 log and ≥ 1,000 IU/mL	> 2 log or < 1,000 IU/mL
24	Viral Load Threshold	10,000 IU/mL	≥ 10,000 IU/mL	< 10,000 IU/mL
	Log Drop	2 Log Drop	≤ 2 log and ≥ 1,000 IU/mL	> 2 log or < 1,000 IU/mL

- Log drops are calculated relative to pretreatment baseline viral load
- 100,000 IU/mL = 520,000 HCV RNA copies/mL, 10,000 IU/mL = 52,000 HCV RNA copies/mL, 1,000 IU/mL = 5,200 HCV RNA copies/mL

Table 2 shows the predictive values of each of the prediction rules at Weeks 4, 8, 12, and 24; for example, failure to achieve a 2-log decrease (100-fold drop) in HCV RNA or a viral load < 1,000 IU/mL by 8 or 12 weeks predicted non-sustained response in 97.5% and 97.4% of treated patients, respectively. Failure to decrease viral load to less than 10,000 IU/mL (52,000 HCV RNA copies/mL) by 8 or 12 weeks predicted non-sustained response in 98.7% and 97.1% of treated patients, respectively.

Table 2. Predictors of Non-Sustained and Sustained Virological Response at Weeks 4, 8, 12, and 24¹ of Therapy

Week	Prediction Rules	Negative Predictive Value (NPV) ²		Positive Predictive Value (PPV) ³	
		% (95% CI)	N	% (95% CI)	N
4	100,000 IU/mL	96.6 (88.3-99.6)	57/59	55.5 (49.2-61.7)	142/256
	1 Log Drop ⁴	94.0 (86.7-98.0)	79/84	60.2 (53.5-66.5)	139/231
8	10,000 IU/mL	98.7 (92.8-100.0)	74/75	66.5 (59.6-73.0)	135/203
	2 Log Drop	97.5 (91.2-99.7)	77/79	67.3 (60.3-73.8)	134/199
12	10,000 IU/mL	97.1 (90.1-99.7)	68/70	59.1 (52.6-65.3)	143/242
	2 Log Drop	97.4 (90.9-99.7)	75/77	60.9 (54.3-67.1)	143/235
24 ⁵	10,000 IU/mL	100.0 (81.5-100.0)	18/18	64.5 (54.6-73.5)	69/107
	2 Log Drop	100.0 (82.4-100.0)	19/19	65.1 (55.2-74.1)	69/106

- Data shown are from the Bayer Clinical Study
- NPV = Percent of non-sustained virological responders out of the patients who are predicted non-sustained virological responders, i.e. HCV RNA positive 6 months post-therapy
- The positive predictive value (PPV) of HCV RNA levels is not intended to be used to guide therapy. PPV = Percent of sustained virological responders out of the patients who are predicted sustained virological responders, i.e. HCV RNA negative 6 months post-therapy
- Log drops are calculated relative to pretreatment baseline viral load.
- For subjects receiving 48 weeks of treatment

The NPV of the log drop rule at Week 12 as shown in the Bayer clinical study is consistent with the results for studies of HCV-infected patients receiving pegylated interferon plus ribavirin reported in the 2002 NIH HCV Consensus Statement²⁶. Although the positive predictive values were lower than the negative predictive values, they are provided in Table 2 as additional information for the treating physician and the patients undergoing antiviral therapy.

Using the Viral Load Threshold Prediction Rule

HCV RNA viral load results that fall near the prediction thresholds cannot be interpreted with absolute certainty. Because of individual variability in response to treatment there is a chance that a patient whose viral load measurement remains above a predictive threshold while on therapy may still achieve a sustained virological response (HCV RNA negative at 6 months post-therapy). Alternatively, a patient whose HCV RNA levels fall below a decision threshold may not achieve a sustained virological response. This may be due, in part, to individual biological variation in HCV RNA levels, where maximum changes of 0.75 to 3.1 log have been reported in untreated patients.^{29,30}

Using the Log Drop Prediction Rule

The biological variability reported in the Within Subject Variability section of this package insert (see Performance Characteristics: Clinical Studies) and in other published papers^{29,30} indicates that a small number of individuals chronically infected with HCV and not on anti-HCV treatment may have fluctuations in viral load over time of up to 3.1 log. In the current study, the largest ratio between the minimum and maximum viral load was 1.6 log over a 2-month period, and the median viral load change for the patients was 0.27 log. Due to individual biological variability in HCV RNA viral levels at baseline there is a chance that a patient who does not achieve a 2-log drop and is > 1,000 IU/mL while on therapy might still achieve a sustained virological response (HCV RNA negative 6 months post-therapy). Alternatively, a patient who achieves a greater than 2-log drop or is < 1,000 IU/mL may not achieve a sustained virological response.

The results from the VERSANT HCV RNA 3.0 Assay (bDNA) must be interpreted within the context of all relevant clinical and laboratory findings.

Baseline Characteristics Predictive of SVR

Table 3 shows the percent of sustained virological responders and odds ratios for prediction of SVR using baseline characteristics and treatment duration variables from the Bayer clinical study. The variables that were the most predictive of SVR (i.e., had the highest odds ratio) were genotype and baseline HCV RNA with a threshold of 100,000 (10⁵) IU/mL (520,000 HCV RNA copies/mL).

Table 3. Percent Response and Odds Ratios for Prediction of SVR Using Baseline Characteristics¹

Predictor	Variable	N	Percent with SVR	Odds Ratio (95% Confidence Interval)
Genotype	Non-1	112	70.5%	4.3 (2.6, 7.1)
	1	239	36.0%	
Baseline HCV RNA	≤ 10 ⁵ IU/mL	63	66.7%	2.7 (1.5, 5.0)
	> 10 ⁵ IU/mL	288	42.7%	
Treatment Duration	48 Weeks	189	54.0%	1.8 (1.2, 2.9)
	24 Weeks	162	38.9%	
Liver Disease Stage ²	NC	288	49.3%	1.7 (0.9, 3.1)
	C	63	36.5%	
Age	< 40	77	57.1%	1.7 (1.0, 2.9)
	≥ 40	274	44.2%	
Previous Treatment	Naive	275	48.7%	1.4 (0.8, 2.4)
	Experienced	76	40.8%	
Gender ³	Female	113	49.6%	1.2 (0.7, 1.9)
	Male	238	45.8%	

1 Data shown are from the Bayer Clinical Study

2 NC = non-cirrhotic, C = cirrhotic

3 Early studies^{20,31} have reported that gender was a statistically significant predictor of SVR. However, later studies have shown that gender and body weight are confounded^{32,33} and, when ribavirin dose is based on body weight, gender is no longer predictive of SVR³⁴.

Reporting Results

It is recommended that the following be included in the results report:

The VERSANT HCV RNA 3.0 Assay (bDNA) measures HCV RNA levels at baseline and during treatment and is useful in predicting non-sustained response to HCV therapy. This test is not intended for use in the diagnosis or confirmation of HCV infection. Treatment may be assessed using absolute viral load levels, or by comparing the observed decrease in viral load compared with pretreatment baseline levels. The rules are only a guide and must be interpreted within the context of all relevant clinical and laboratory findings.

Interpretation rules using absolute viral load:

- At week 4, if HCV RNA is ≥ 100,000 IU/mL, then there is a 97% chance of treatment failure.
- At week 8, if HCV RNA is ≥ 10,000 IU/mL, then there is a 99% chance of treatment failure.
- At week 12, if HCV RNA is ≥ 10,000 IU/mL, then there is a 97% chance of treatment failure.
- At week 24, if HCV RNA is ≥ 10,000 IU/mL, then there is a 100% chance of treatment failure.

Interpretation rules using decrease in viral load compared with pretreatment baseline level:

- At week 4, if HCV RNA is ≥ 10% (≤ 1 log drop) of its pretreatment baseline level and ≥ 1,000 IU/mL, then there is a 94% chance of treatment failure.
- At week 8, if HCV RNA is ≥ 1% (≤ 2 log drop) of its pretreatment baseline level and > 1,000 IU/mL, then there is a 97% chance of treatment failure.
- At week 12, if HCV RNA is ≥ 1% (≤ 2 log drop) of its pretreatment baseline level and ≥ 1,000 IU/mL, then there is a 97% chance of treatment failure.
- At week 24, if HCV RNA is ≥ 1% (≤ 2 log drop) of its pretreatment baseline level and ≥ 1,000 IU/mL, then there is a 100% chance of treatment failure.

The following information is not intended for use in guiding therapy but is provided as additional information for treating physicians and their patients undergoing antiviral therapy. Patients whose results do not meet the above interpretation rules have a slightly higher chance of sustained virological response (ranging on average from 55% to 67%) than expected at baseline (47%) when other factors, e.g., HCV genotype, baseline viral load, are not taken into consideration.

The following results were obtained using Bayer's VERSANT® HCV RNA 3.0 Assay (bDNA). Values obtained with other manufacturers' assay methods may not be used interchangeably.

Limitations and Precautions

The number of African-Americans studied was too low to adequately establish appropriate assay performance for this group. The results of this assay when applied to African-Americans must be cautiously interpreted.

The assay is limited to the quantitation of HCV RNA in human serum or plasma. Total protein level > 9 g/dL and unconjugated bilirubin levels > 10 mg/dL in specimens may reduce the quantitation of HCV RNA.

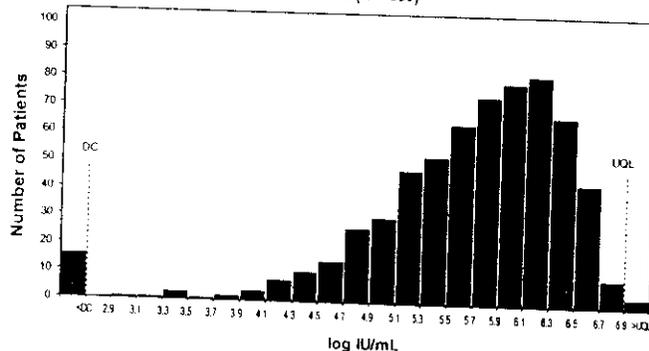
Performance Characteristics: Clinical Studies

Expected HCV RNA Results at Baseline

Viral loads were measured by the Bayer VERSANT® HCV RNA (bDNA) Assay in 650 chronically infected patients who were not undergoing therapy at the time of specimen collection. In 431 patients, quantification values were obtained from baseline specimens just before the initiation of anti-HCV therapy; the remaining 219 patients were not subsequently put on therapy.

Figure 1 shows the distribution of HCV RNA log quantitations in IU/mL for the combined population of subjects with chronic HCV infection. The median quantitation was 5.8 log IU/mL with 5th and 95th percentiles of 4.5 log IU/mL and 6.6 log IU/mL in the log scale, or a median of 694,566 IU/mL with 5th and 95th percentiles of 33,570 IU/mL and 3,681,828 IU/mL.

Figure 1. Distribution of log HCV RNA in IU/mL (N = 650)



Prediction of Anti-HCV Treatment Response Using Results from Specimens Collected at Baseline and Early in Treatment

The objectives of this study were to determine if HCV RNA quantitations or changes in quantitations were predictive of sustained virological response (SVR) and non-sustained virological response (NSVR) to interferon alfa-2b plus ribavirin (REBETRON) at post-treatment follow-up (PTF). Retrospectively collected specimens from 351 subjects treated with interferon alfa-2b plus ribavirin were assayed across 6 sites in North America (5) and Europe (1). Specimens were collected at baseline, Weeks 4, 8, 12, 24 (for subjects who had 48 weeks of treatment), end of treatment, and PTF. SVR was defined as having no detectable HCV RNA at PTF using a sensitive qualitative HCV RNA test. The population included previously treated and treatment-naive subjects. Table 4 shows a description of the Bayer study population.

Table 4. Description of Study Population

Characteristic	Number of Subjects (N = 351)	Percent of Subjects
Race		
Asian	14	4.0% (14/351)
African-American	4	1.1% (4/351)
Caucasian	235	67.0% (235/351)
Hispanic	16	4.6% (16/351)
Other ¹	82	23.4% (82/351)
Age Range		
20-24	2	0.6% (2/351)
25-34	29	8.3% (29/351)
35-44	135	38.5% (135/351)
45-54	130	37.0% (130/351)
55-59	28	8.0% (28/351)
60-64	16	4.6% (16/351)
65-74	11	3.1% (11/351)
Genotype²		
1	239	68.1% (239/351)
2	50	14.2% (50/351)
3	58	16.5% (58/351)
4	6	1.7% (6/351)
5	0	0.0% (0/0)
6	2	0.6% (2/351)
6-Month Treatment Duration	162	46.1% (162/351)
Genotype 1	85	52.5% (85/162)
Genotype non-1	77	47.5% (77/162)
1-Year Treatment Duration	189	53.8% (189/351)
Genotype 1	154	81.5% (154/189)
Genotype non-1	35	18.5% (35/189)

Characteristic	Number of Subjects (N = 351)	Percent of Subjects
Previous Therapy	76	21.6% (76/351)
Males	54	71.0% (54/76)
Females	22	29.0% (22/76)
No Previous Therapy	275	78.4% (275/351)
Males	184	66.9% (184/275)
Females	91	33.1% (91/275)

- Subjects treated and tested at the site in France did not have race recorded for legal reasons.
- Percentages add up to greater than 100% because 4 subjects had mixed infections.

Prediction of SVR and NSVR

Table 5 shows the percent of sustained virological responders and odds ratios for prediction of SVR using baseline characteristics and treatment duration variables. The variables that were the most predictive of SVR (i.e., had the highest odds ratio) were genotype and baseline HCV RNA with a threshold of 100,000 (10⁵) IU/mL (520,000 HCV RNA copies/mL).

Table 5. Percent Response and Odds Ratios for Prediction of SVR Using Baseline Characteristics¹

Predictor	Variable	N	Percent with SVR	Odds Ratio (95% Confidence Interval)
Genotype	Non-1	112	70.5%	4.3 (2.6, 7.1)
	1	239	36.0%	
Baseline HCV RNA	≤ 10 ⁵ IU/mL	63	66.7%	2.7 (1.5, 5.0)
	> 10 ⁵ IU/mL	288	42.7%	
Treatment Duration	48 Weeks	189	54.0%	1.8 (1.2, 2.9)
	24 Weeks	162	38.9%	
Liver Disease Stage ²	NC	288	49.3%	1.7 (0.9, 3.1)
	C	63	36.5%	
Age	< 40	77	57.1%	1.7 (1.0, 2.9)
	≥ 40	274	44.2%	
Previous Treatment	Naive	275	48.7%	1.4 (0.8, 2.4)
	Experienced	76	40.8%	
Gender ³	Female	113	49.6%	1.2 (0.7, 1.9)
	Male	238	45.8%	

- Data shown are from the Bayer Clinical Study
- NC = non-cirrhotic, C = cirrhotic
- Early studies^{20,31} have reported that gender was a statistically significant predictor of SVR. However, later studies have shown that gender and body weight are confounded^{32,33} and, when ribavirin dose is based on body weight, gender is no longer predictive of SVR³⁴

Viral load decision thresholds ranging from 615 to 400,000 IU/mL and log drop in viral load (baseline to Week 4, 8, 12, or 24) thresholds ranging from 1 to 3 logs were evaluated for their ability to predict SVR and NSVR. Optimal viral load and log drop thresholds were selected at each timepoint based on the largest number of predicted NSVR and negative predictive values (NPV) ≥ 95%. The selected prediction rules are shown in Table 6.

NOTE: In the following tables, definition of predicted sustained virological responders is provided for information only. The prediction of SVR is not sufficiently robust to be used for making decisions regarding continuation or termination of treatment.

Table 6. Definition of Prediction Rules

Week	Type of Rule	Prediction Rule	Predicted Non-sustained Virological Responders	Predicted Sustained Virological Responders ¹
4	Viral Load Threshold	100,000 IU/mL ²	≥ 100,000 IU/mL	< 100,000 IU/mL
	Log Drop ¹	1 Log Drop	≤ 1 log and ≥ 1,000 IU/mL ²	> 1 log or < 1,000 IU/mL
8, 12 and 24	Viral Load Threshold	10,000 IU/mL ²	≥ 10,000 IU/mL	< 10,000 IU/mL
	Log Drop	2 Log Drop	≤ 2 log and ≥ 1,000 IU/mL	> 2 log or < 1000 IU/mL

- Log drops are calculated relative to pretreatment baseline viral load
- 100,000 IU/mL = 520,000 HCV RNA copies/mL, 10,000 IU/mL = 52,000 HCV RNA copies/mL, 1,000 IU/mL = 5,200 HCV RNA copies/mL

Table 7 shows the NPV and positive predictive values (PPV) of the viral load and log drop rules for Weeks 4, 8, 12, and 24 (for subjects receiving 48 weeks of treatment) and the corresponding adjusted and unadjusted odds ratios for SVR.

The adjusted odds ratios are based on multivariate logistic regression models. The final model selected adjusted for the following variables: genotype, liver disease stage, length of therapy, previous treatment, and age.

Table 7. Predictors of Sustained and Non-Sustained Virological Response at Weeks 4, 8, 12, and 24 and Corresponding Odds Ratios for SVR

Week	Prediction Rules	Negative Predictive Value (NPV) ¹		Positive Predictive Value (PPV) ²		Odds Ratio ³	
		% (95% CI)	N	% (95% CI)	N	Unadjusted	Adjusted
4	100,000 IU/mL	96.6 (88.3-99.6)	57/59	55.5 (49.2-61.7)	142/256	35.5	26.4
	1 Log Drop ⁴	94.0 (86.7-98.0)	79/84	60.2 (53.5-66.5)	139/231	23.9	22.3
8	10,000 IU/mL	98.7 (92.8-100.0)	74/75	66.5 (59.6-73.0)	135/203	146.9	117.8
	2 Log Drop	97.5 (91.2-99.7)	77/79	67.3 (60.3-73.8)	134/199	79.4	69.1
12	10,000 IU/mL	97.1 (90.1-99.7)	68/70	59.1 (52.6-65.3)	143/242	49.1	29.8
	2 Log Drop	97.4 (90.9-99.7)	75/77	60.9 (54.3-67.1)	143/235	58.3	43.1
24 ⁵	10,000 IU/mL	100.0 (81.5-100.0)	18/18	64.5 (54.6-73.5)	69/107	NA	NA
	2 Log Drop	100.0 (82.4-100.0)	19/19	65.1 (55.2-74.1)	69/106	NA	NA

- NPV = Percent of non-sustained virological responders out of the patients who are predicted non-sustained virological responders; i.e. HCV RNA positive 6 months post-therapy.
- The positive predictive value (PPV) of HCV RNA levels is not intended to be used to guide therapy. PPV = Percent of sustained virological responders out of the patients who are predicted sustained virological responders; i.e. HCV RNA negative 6 months post-therapy.
- Odds ratios cannot be calculated if the NPV is 100%. Those cases are indicated by NA. All odds ratios were significant (p < .001).
- None of the log drop rules at Week 4 attained an NPV of 95%. The 1-log drop had the highest NPV.
- For subjects receiving 48 weeks of treatment

The NPVs for the viral load rules ranged from 96.6% to 100%. The NPVs for the log drop rules for Weeks 8, 12, and 24 ranged from 97.4% to 100%. The highest NPV at Week 4 using a log drop threshold was 94%.

Tables 8 and 9 show the NPVs and PPVs of the viral load and log drop rules for Weeks 4, 8, 12, and 24 (for subjects receiving 48 weeks of treatment) for subjects infected with HCV genotype 1 and HCV genotype non-1, respectively.

Table 8. HCV Genotype 1: Predictors of SVR and NSVR at Weeks 4, 8, 12, and 24

Week	Prediction Rules	Negative Predictive Value (NPV) ¹		Positive Predictive Value (PPV) ²	
		% (95% CI)	N	% (95% CI)	N
4	100,000 IU/mL	96.5 (87.9-99.6)	55/57	46.6 (38.7-54.6)	75/161
	1 Log Drop ³	93.3 (85.1-97.8)	70/75	50.3 (41.9-58.8)	72/143
8	10,000 IU/mL	98.5 (92.1-100.0)	67/68	57.6 (48.2-66.7)	68/118
	2 Log Drop	97.2 (90.3-99.7)	70/72	58.8 (49.2-67.9)	67/114
12	10,000 IU/mL	96.9 (89.3-99.6)	63/65	49.0 (40.9-57.2)	75/153
	2 Log Drop	97.1 (90.1-99.7)	68/70	50.7 (42.3-59.0)	75/148
24 ⁴	10,000 IU/mL	100.0 (81.5-100.0)	18/18	59.3 (48.2-69.8)	51/86
	2 Log Drop	100.0 (82.4-100.0)	19/19	60.0 (48.8-70.5)	51/85

- NPV = Percent of non-sustained virological responders out of the patients who are predicted non-sustained virological responders; i.e. HCV RNA positive 6 months post-therapy.
- The positive predictive value (PPV) of HCV RNA levels is not intended to be used to guide therapy. PPV = Percent of sustained virological responders out of the patients who are predicted sustained virological responders; i.e. HCV RNA negative 6 months post-therapy.
- None of the log drop rules at Week 4 attained an NPV of 95%. The 1-log drop had the highest NPV.
- For subjects receiving 48 weeks of treatment

For Table 8, the NPVs for the viral load rules ranged from 96.5% to 100% in subjects infected with genotype 1. The NPVs for the log drop rule for Weeks 8, 12 and 24 ranged from 97.1% to 100%. The highest NPV at Week 4 using a log drop threshold was 93.3%.

Table 9. HCV Genotype Non-1: Predictors of SVR and NSVR at Weeks 4, 8, 12, and 24

Week	Prediction Rules	Negative Predictive Value (NPV) ¹		Positive Predictive Value (PPV) ²	
		% (95% CI)	N	% (95% CI)	N
4	100,000 IU/mL	100.0 (15.8-100.0)	2/2	70.5 (60.3-79.4)	67/95
	1 Log Drop	100.0 (66.4-100.0)	9/9	76.1 (65.9-84.6)	67/88
8	10,000 IU/mL	100.0 (59.0-100.0)	7/7	78.8 (68.6-86.9)	67/85
	2 Log Drop	100.0 (59.0-100.0)	7/7	78.8 (68.6-86.9)	67/85
12	10,000 IU/mL	100.0 (47.8-100.0)	5/5	76.4 (66.2-84.8)	68/89
	2 Log Drop	100.0 (59.0-100.0)	7/7	78.2 (68.0-86.3)	68/87
24 ³	10,000 IU/mL	NA	NA	85.7 (63.7-97.0)	18/21
	2 Log Drop	NA	NA	85.7 (63.7-97.0)	18/21

- NPV = Percent of non-sustained virological responders out of the patients who are predicted non-sustained virological responders; i.e. HCV RNA positive 6 months post-therapy.
- The positive predictive value (PPV) of HCV RNA levels is not intended to be used to guide therapy. PPV = Percent of sustained virological responders out of the patients who are predicted sustained virological responders; i.e. HCV RNA negative 6 months post-therapy.
- For subjects receiving 48 weeks of treatment

For Table 9, the NPVs for genotype non-1 for both viral load and log-drop rules were 100% at Weeks 4, 8, and 12 but sample sizes for subjects with NSVR were small, ranging from 2 to 9 patients. The lower sample sizes for subjects with NSVR reflect the fact that subjects infected with non-1 genotypes have a better response to therapy than subjects infected with genotype 1.

Summary of NSVR and SVR Results

The selected viral load rules were 100,000 IU/mL (520,000 HCV RNA copies/mL) at Week 4 and 10,000 IU/mL (52,000 HCV RNA copies/mL) at Weeks 8, 12, and 24. The NPVs of the viral load prediction rules were > 95% at all four timepoints, independent of genotype.

The highest NPV of the log drop in viral load rule was 33.3% at Week 4, using a 1-log drop. The selected log drop in viral load rule was 2 logs at Weeks 8, 12, and 24. The NPVs of this log drop in viral load rule were > 95% at all three timepoints, independent of genotype.

While the PPVs were lower than the NPVs, ranging on average from 55% to 67% for both viral load threshold and log drop, they were slightly higher than the expected percent of SVR of 47% at baseline where other predictive factors, e.g., HCV genotype, baseline viral load, were not taken into consideration.

The data from this study using a 2-log drop rule are consistent with those reported in the National Institutes of Health Consensus Development Conference Statement, Management of Hepatitis C: 2002.²⁸ The NIH statement was based primarily on study populations treated with pegylated-interferon plus ribavirin.

Clinical Specificity

Serum specimens were collected from 90 HCV negative subjects who had been diagnosed with the following infections, who had the following antibodies/antigens, or who had a histological/clinical diagnosis of the following liver pathologies: hepatitis B virus (HBV), human immunodeficiency virus (HIV-1), anti-nuclear antigen (ANA), rheumatoid factor (RF), primary biliary cirrhosis (PBC), autoimmune hepatitis (AIH), alcoholic cirrhosis (AC), NASH (non-alcoholic steatohepatitis) and hepatitis A virus (HAV). The results of the disease state specimens were compared to those of 100 HCV negative blood donors. Specimens collected from subjects infected with other liver disease pathogens or who had non-viral liver diseases had a specificity equivalent to that of the specificity of the blood donors. Both populations yielded observed specificities of 100%.

Within Subject Variability

Twenty-nine subjects (16 males and 13 females) with clinically stable, chronic HCV infection were enrolled in this study. Serum specimens were drawn from subjects weekly for 6 to 8 weeks with an average of 7 weeks. Enrollment and continued eligibility criteria for the study were 1) No antiviral therapy or clinical events within the 3 months prior to the start of the study and 2) No initiation of antiviral therapy or clinical events during the 8-week study period.

The ultimate objective of the study was to determine the minimum fold-change (ratio) between two successive measurements that were unlikely to occur by chance. The minimum fold-change was determined using the estimated total variability which is composed of assay and biologic variability. The fold-change for the VERSANT HCV RNA 3.0 Assay (bDNA) was estimated to be 2.6-fold or 0.41 log. The biologic variability alone was 2.1 fold. The largest ratio between the minimum and maximum viral load within any subject was 1.6 log and the median change across all subjects was 0.27 log.

Performance Characteristics: Nonclinical Studies

Analytical Specificity

The specificity of the VERSANT HCV RNA 3.0 Assay (bDNA) was determined using 999 anti-HCV negative serum specimens from men and women of which the majority were repeat volunteer blood donors. The specimens were determined to be negative for antibodies to HCV using an FDA approved HCV ELISA assay. Samples were tested across 4 testing sites using 3 kit lots of the VERSANT HCV RNA 3.0 Assay (bDNA). Of the 999 samples tested, 992 (99.3%) produced results below the LoD (< 1,000 IU/mL or 5,200 HCV RNA copies/mL) with a lower 95% confidence limit of 98.7%. Alternatively, of the 999 samples tested, 987 (98.8%) produced results below the detection cutoff (< 615 IU/mL or 3,200 HCV RNA copies/mL) with a lower 95% confidence limit of 98.1%.

Limit of Detection (LoD)

The Limit of Detection (LoD) is the lowest concentration of virus that yields an assay result at or above the detection cutoff 95% of the time. The LoD was determined by examining the percent of the reproducibility panel members (shown in Table 10) with values at or above the DC. Panel members QC1 through QC6 were detected 100% of the time. Member QC7 was detected 72.7% of the time. The HCV RNA concentration that would be detected 95% of the time was obtained by interpolation of the percentiles. The point estimate for the LoD across the 3 clinical lots was 988 IU/mL (5,137 HCV RNA copies/mL). The upper 95% confidence limit of this estimate was 1,234 IU/mL (6,415 HCV RNA copies/mL). Table 10 shows the percent detection for panel members bracketing the LoD.

Table 10. Percent Detection for Panel Member Bracketing the LoD

Panel Member	Concentration IU/mL (copies/mL)	Percent Detection
QC6	1,519 (7,901)	100.0%
LoD (Interpolated Result)	988 (5,137)	95.0%
QC7	760 (3,950)	72.7%
DC	615 (3,200)	39.5%
QC8	380 (1,975)	19.4%

Reproducibility

The performance characteristics of the VERSANT HCV RNA 3.0 Assay (bDNA) were assessed using a two-part, eight-member panel with HCV target concentrations between 481 and 7,690,000 IU/mL (2,500 and 40,000,000 HCV RNA copies/mL). Two panel members, QC1 and QC3, were made by diluting recombinant single-stranded bacteriophage DNA into HCV negative human plasma. For the remaining panel members, beta-propiolactone (BPL)-treated HCV-positive patient specimens were diluted into negative human plasma. For both recombinant single-stranded bacteriophage DNA and virus-containing panel members, the lower concentration panel members were created from the higher concentration panel members. Gravimetric determinations were made for each dilution to obtain the precise dilution factor.

Each panel member was tested 3 times per assay plate. Two operators at each of 3 testing sites performed 2 assay plates per day on 2 separate days using each of the 3 kit lots, for a total of 12 assay plates per operator (4 per kit lot). Data were analyzed using a nested analysis of variance. Table 11 shows the concentration, the total log standard deviation (SD), within-plate %CV and the total %CV for each of the panel members.

Table 11. Total logSD, Within-Plate, and Total Coefficient of Variation (%CV) for Reproducibility Panel Members

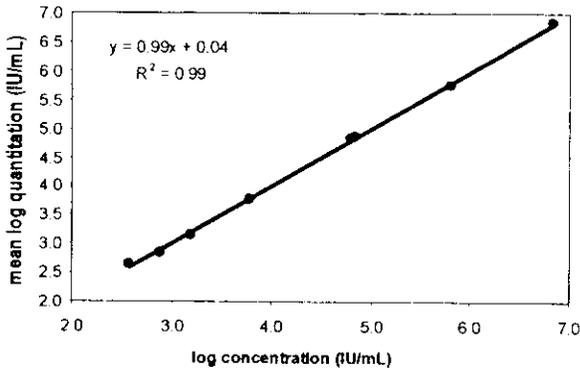
Panel Member	N	Expected Concentration IU/mL (copies/mL)	Total log SD	Within-Plate %CV	Total %CV
QC1	216	6,820,720 (35,467,746)	0.07	13.9%	17.0%
QC2	216	607,756 (3,160,333)	0.07	9.1%	17.0%
DT ¹	NA ²	100,000 (520,000)	0.07	NA	17.0%
QC3	216	68,207 (354,677)	0.06	10.2%	15.0%
QC4	216	60,776 (316,033)	0.07	9.3%	16.4%
DT	NA	10,000 (52,000)	0.09	NA	21.0%
QC5	216	6,078 (31,603)	0.08	10.9%	18.8%
QC6	216	1,519 (7,901)	0.10	18.9%	24.2%
QC7	216	760 (3,950)	0.12	24.2%	28.2%
DC ³	NA	615 (3,200)	0.14	26.9%	32.4%
QC8	216	380 (1,975)	0.18	33.1%	42.2%

- DT = Decision Threshold Data from this reproducibility study were combined with data from reproducibility studies across specimen matrices and genotypes to obtain an estimate of the assay precision profile. A quadratic model was fitted to the precision profile to determine total reproducibility across sites, lots, operators, matrices, and genotypes at the decision thresholds.
- NA = Not applicable
- For the Detection Cutoff (DC), the within-plate, total %CV and LogSD were obtained by interpolation.

Linearity

Results from the reproducibility panel were also used to establish linearity of the VERSANT HCV RNA 3.0 Assay (bDNA). There was a direct proportional relationship between the dilutions tested and the number of HCV RNA copies reported. The assay was shown to be linear from 615 to 7,690,000 IU/mL (3,200 to 40,000,000 HCV RNA copies/mL). The accuracy of the assay, defined as the percent recovery (ratio of observed mean quantitation to expected concentration), was from 92% to 108% across the quantitation range. The assay is accurate throughout the quantitation range used to predict non-sustained response to therapy. In addition, assay linearity was confirmed using serial dilutions of patient specimens that spanned the range from approximately 481 to 4,800,000 IU/mL (2,500 to 25,000,000 HCV RNA copies/mL) that were collected as serum and in EDTA and ACD anticoagulants.

Figure 2. Linearity of the VERSANT HCV RNA 3.0 Assay (bDNA)



Potentially Interfering Exogenous Substances

The potential interference of commonly prescribed drugs and other pathogens that may be found in HCV-infected individuals was tested by adding these substances to HCV-negative serum specimens (N = 45) and to specimens spiked with a low concentration of HCV (N = 25). The drugs were pooled as shown in Table 12 and tested at final concentrations five times the reported peak serum or plasma concentrations in the therapeutic range, except lamivudine which was tested at a concentration of one-half C_{max}. Pathogens were also pooled as shown in Table 12. The following viruses were tested: HAV at ~10⁶ TCID₅₀, ~10⁶ HBV DNA copies/mL, ~10⁶ HIV-1 RNA copies/mL, and CMV at > 10⁵ DNA copies/mL. Bacterial and fungal pathogens were diluted to a final concentration of 10³ CFU/mL. The drugs and pathogens listed in Table 12 were found to have no effect on the VERSANT HCV RNA 3.0 Assay (bDNA) at the target concentrations.

Table 12. Prescription Drugs and Pathogens Tested

Drugs Tested	Pathogens Tested
Pool 1 prednisone, ganciclovir, indinavir, lamivudine	Pool 5 CMV, HBV, HIV-1, HAV
Pool 2 pegylated interferon alpha 2b (PEG-INTRON), cyclosporin, acyclovir, AZT, ritonavir	Pool 6 <i>E. coli</i> , <i>E. cloacae</i> , <i>P. aeruginosa</i> , <i>K. pneumoniae</i> , <i>P. fluorescens</i> , <i>Streptococcus</i> group B
Pool 3 ribavirin, alpha interferon 2b (INTRON A), tacrolimus, DDI, neftinavir, D4T	Pool 7 <i>S. aureus</i> , <i>S. epidermidis</i> , <i>H. influenzae</i> , <i>S. pneumoniae</i> , <i>S. marcescens</i> , <i>C. albicans</i>
Pool 4 mycophenolate mofetil, rapamycin, azathioprine, saquinavir, amantadine, trimethoprim/sulfamethoxazole	

Potentially Interfering Endogenous Substances

The potentially interfering endogenous substances hemoglobin, triglycerides, conjugated and unconjugated bilirubin, and protein were tested by adding these substances to HCV-negative serum specimens (N = 35 to 45, depending on which substance tested) and to specimens spiked with a low concentration of a HCV (N = 25).

Specimens from HCV-negative individuals with elevated levels of alanine aminotransferase (ALT) at twice the upper normal limit (UNL), aspartate aminotransferase (AST) at > UNL, and gamma glutamyl transferase (GGT) at > UNL were collected. The RLU_s were compared to those from a normal blood donor population.

Specimens that are ...	Demonstrate no change in results up to ...
hemolyzed	500 mg/dL of hemoglobin
lipemic	3000 mg/dL of triglycerides
icteric	40 mg/dL of conjugated bilirubin 10 mg/dL of unconjugated bilirubin
proteinemic	9 g/dL of protein
Liver Enzymes (ALT, AST, GGT) ¹	2 x UNL ² for ALT, > UNL for AST, GGT

1 ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma glutamyl transferase

2 UNL = upper limit of normal range

HCV-positive specimens with > 10 mg/dL of unconjugated bilirubin or > 9 g/dL total protein had lower quantitations than the reference specimens

Chronic hepatitis C patients with very high concentrations of unconjugated bilirubin are unlikely candidates for antiviral treatment. Whereas chronic hepatitis patients often have conjugated bilirubinemia, the unconjugated form usually occurs in patients with fulminant hepatitis, cirrhosis, rare inborn errors of metabolism (e.g. Crigler-Najjar Syndrome), or hemolytic diseases

Elevated protein concentrations may theoretically reduce HCV RNA concentrations in only a small fraction of samples. The NHANES III study demonstrated protein > 9g/dL in 0.1% of the general population and 2.6% of subjects positive for both anti-HCV antibody and HCV RNA. Clinical utility studies demonstrate the efficacy of this test despite this theoretical limitation (patients were not excluded from these studies based on serum protein results)

Two studies using 40 HCV-positive serum specimens, 50% of which contained cryoprecipitates, demonstrated that HCV RNA quantitation by the VERSANT HCV RNA 3.0 Assay (bDNA) is not affected by presence of cryoprecipitates. The first study showed that the Lysis Working Reagent (LWR) disrupts the HCV-cryoprotein complex and prevents it from re-precipitating. The second study showed that when the specimens were chilled and centrifuged to pellet the HCV-cryoprotein complex, the sum of the HCV RNA in the supernatant and in the pellet was equal to the HCV RNA level in the uncentrifuged specimen.

Multiple Freeze/Thaw Cycles

The effect of 1, 2, 3 and 4 freeze-thaw cycles frozen at -60° to -80°C was tested on 40 HCV negative and 25 HCV-positive serum specimens. Up to 4 freeze-thaw cycles on HCV-negative or HCV-positive specimens had no effect on the performance of the VERSANT HCV RNA 3.0 Assay (bDNA).

Anticoagulants and Specimen Handling

Matched patient specimens collected in serum separator tubes (SST), K₂EDTA (plastic) tubes, K₂EDTA (PPT), and ACD-solution A tubes were evaluated. All specimens were centrifuged and frozen at -60° to -80°C within 4 hours of collection except for the PPT tubes, which were centrifuged within 2 hours. All the collection devices provided statistically equivalent HCV quantitations in HCV-positive specimens and equivalent RLU_s in HCV-negative specimens. In another study, serum, EDTA and ACD specimens were either frozen immediately at -60° to -80°C after centrifugation or held at 2° to 8°C for either 8, 24, or 48 hours, then frozen. HCV quantitations at all time points were statistically equivalent to those of the serum of plasma specimens that were immediately frozen.

Table 13 summarizes the appropriate specimen collection and handling conditions for use with the VERSANT HCV RNA 3.0 Assay (bDNA).

Table 13. Summary of Anticoagulant and Specimen Handling Studies

Tube Type	Time to Centrifugation	Specimen Handling	Storage Temperature	Allowable Duration Prior to Freezing
Serum Separator Vacutainer	≤ 4 hours	Serum left on separator gel	2° - 8°C	48 hours after collection
K ₂ EDTA Vacutainer	≤ 4 hours	Plasma removed from cells	2° - 8°C	48 hours after collection
ACD (Soluton A) Vacutainer	≤ 4 hours	Plasma removed from cells	2° - 8°C	48 hours after collection
K ₂ EDTA PPT	< 2 hours	Plasma left on separator gel	2° - 8°C	48 hours after collection

An additional study was performed comparing the precision, linearity and LoD for serum, K₂EDTA and ACD solution A. Six-member panels were constructed that ranged 481 to 4,800,000 IU/mL (2,500 to ~25,000,000 HCV RNA copies/mL) for each matrix. Panels were tested by 3 operators, each using 3 kit lots. Each panel member in each matrix had 72 determinations. Reproducibility, linearity, and analytical sensitivity were equivalent for all three matrices. The largest difference in LoD was 1.2-fold, well within the precision of the assay.

HCV Genotypes

Two studies were performed to evaluate assay performance against the following HCV genotypes and subtypes: 1, 2, 3, 4, 5 and 6. The first study evaluated assay performance using RNA transcripts made from the 5' untranslated region of cloned examples of the genotypes listed above. All transcripts were quantitated using phosphor analysis and confirmed using hyperchromicity and absorbance at 260 nm.

Each transcript was tested at two concentrations: 384,615 (high) and 1,346 (low) IU/mL (2,000,000 and 7,000 HCV RNA copies/mL). For each non-1 genotype transcript at each concentration level, the relative recovery of the non-1 genotypes was estimated in relation to the 1 genotype, which is the genotype against which the assay is standardized. Two operators performed the testing, each using 3 kit lots, for a total of 18 determinations per level per genotype. All recoveries were within 1.5-fold of genotype 1.

In the second study, the precision, linearity and LoD were compared across the following genotypes: 1, 2, 3, 4, 5 and 6. A patient specimen representing each of these genotypes was serially diluted to 4 target concentrations: 384,615, 1,923, 962 and 481 IU/mL (2,000,000, 10,000, 5,000 and 2,500 HCV RNA copies/mL). Linearity and LoD were equivalent for all genotypes tested. The estimated LoDs for all non-1 genotypes were within 1.3 fold of genotype 1 LoD. For target concentration levels above the detection cutoff of 615 IU/mL (3,200 HCV RNA copies/mL), the precision of the assay was equivalent across all genotypes.

Technical Assistance

For customer support, please contact your local technical support provider or distributor.

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