HCV RNA QUALITATIVE ASSAY

Intended Use
The VERSANT® HCV RNA Qualitative Assay is an in vitro nucleic acid amplification assay for the detection of hepatitis C virus (HCV) RNA in human plasma (EDTA, sodium heparin, sodium citrate, and AGD) or serum. The VERSANT® HCV RNA Qualitative Assay is indicated for use with fresh or frozen specimens from the following populations: individuals with antibody evidence of HCV infection with evidence of liver disease, and individuals suspected to be actively infected with HCV with antibody evidence, and individuals at risk for HCV infection with antibodies to HCV. Detection of HCV RNA is evidence of active infection.

Detection of HCV RNA does not discriminate between acute and chronic states of infection. A negative result does not exclude active HCV replication. It is not known if performance is affected by the state of HCV infection (acute or chronic) or by the presence or absence of liver disease. Performance has not been demonstrated for monitoring HCV infected patients.

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

For In Vitro Diagnostic Use.

Summary and Explanation
HCV is a blood-borne pathogen that presents a worldwide public health problem. HCV is the causative agent for most blood-borne non-A, non-B hepatitis (NANB). Studies indicate that HCV is transmitted through contaminated blood and blood products, other close personal contact, and intravenous drug use. Due to the high prevalence of asymptomatic disease early after HCV infection, early detection is essential to strategies aimed at controlling the spread of the virus. Additionally, early detection allows for early intervention, which may improve the effectiveness of antiviral therapies for HCV.

In the majority of cases, HCV infection is mild or asymptomatic and may be characterized by elevated alanine aminotransferase (ALT) levels. Approximately 50% of the patients infected with HCV develop chronic liver disease, with 20% of these patients developing chronic active hepatitis or cirrhosis. Chronic infection with HCV may also be associated with increased risk for developing hepatocellular carcinoma.

HCV is a positive-stranded RNA virus with a genome of approximately 9400 nucleotides and is comprised of a core, an envelope, and five nonstructural regions. It is classified in the family Flaviviridae and is closely related in structure to the genus Flavivirus. Of the six major HCV genotypes identified to date, the S' untranslated region (S'-UTR) and a portion of the core are the most highly conserved regions of the genome.

Testing for HCV infection involves serologic screening of individuals for antibodies to HCV (anti-HCV) using enzyme immunoassays (EIA), followed by confirmation using an immunoblot assay. However, these antibody-based assays are not able to differentiate a resolved from a current infection. Recent, published studies have reported on the usefulness of nucleic acid amplification tests for HCV RNA in the detection of HCV infection. In high-risk patients or instances where hepatitis C infection is suspected due to HCV antibody positive results, a positive qualitative HCV RNA test result may be used to differentiate active from resolved infection in HCV-negative individuals. As well, CDC guidelines endorse either NAT testing or RIBA as a confirmatory test following a positive HCV EIA result. In addition to diagnosis of HCV infection, qualitative HCV RNA testing may be used for identifying the endpoint of infection following antiviral treatment.

Assay Principles
The VERSANT HCV RNA Qualitative Assay is a target amplification-based nucleic acid probe test that detects HCV RNA in human plasma and serum. The VERSANT HCV RNA Qualitative Assay utilizes Target Amplification Mediated Amplification (TAMA™) to amplify conserved regions with the S'-UTR of the HCV genome. TAMA utilizes Moloney Murine Leukemia Virus (MMLV) reverse transcriptase (RT) and T7 RNA polymerase to generate multiple RNA copies from the viral nucleic acid template. Assay performance is monitored by means of an internal nucleic acid control that is added to each specimen with the Target Capture Reagent. The VERSANT HCV RNA Qualitative Assay has three main steps, all of which are performed within a single tube: sample preparation, target amplification, and amplicon detection.

Materials Provided
Each kit contains sufficient reagents to perform a total of 100 tests or 4 to 6 runs. The reagents in this kit are components of a master kit; therefore, component expiration dates in any individual kit box may differ from the kit expiration date.

<table>
<thead>
<tr>
<th>BOX 1</th>
<th>Component</th>
<th>Quantity</th>
<th>Description</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Internal Control Reagent</td>
<td>1 x 1 mL</td>
<td>RNA transcript in HEPES buffer with T7 RNA polymerase in HEPES buffer</td>
<td>-15°C to -35°C</td>
<td></td>
</tr>
<tr>
<td>Amplification Reagent</td>
<td>1 x 8.5 mL</td>
<td>primers, dNTPs, NTPs, and cofactors in TRIS buffer with preservatives</td>
<td>-15°C to -35°C</td>
<td></td>
</tr>
<tr>
<td>Enzyme Reagent</td>
<td>1 x 2.8 mL</td>
<td>MMLV Reverse Transcriptase and T7 RNA Polymerase in HEPES buffer with sodium azide (0.05%)</td>
<td>unopened</td>
<td></td>
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<tr>
<td>HCV Probe Reagent</td>
<td>1 x 14 mL</td>
<td>Chemiluminescent oligonucleotide probes in succinate buffer with detergent</td>
<td>-15°C to -35°C</td>
<td></td>
</tr>
<tr>
<td>HCV Positive Calibrator</td>
<td>4 x 2 mL</td>
<td>inactivate HCV-positive plasma in 4x2 mL with gentamicin and sodium azide (0.2%)</td>
<td>unopened</td>
<td></td>
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<tr>
<td>HCV Negative Calibrator</td>
<td>4 x 2 mL</td>
<td>Defibrinated normal human plasma with gentamicin and sodium azide (0.2%)</td>
<td>unopened</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>BOX 2</th>
<th>Component</th>
<th>Quantity</th>
<th>Description</th>
<th>Storage</th>
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</thead>
<tbody>
<tr>
<td>Target Capture Reagent</td>
<td>1 x 50 mL</td>
<td>capture oligonucleotides and magnetic microparticles in HEPES buffer with detergent</td>
<td>2°C to 8°C</td>
<td></td>
</tr>
<tr>
<td>Selection Reagent</td>
<td>1 x 30 mL</td>
<td>borate buffer with surfactant</td>
<td>15°C to 30°C</td>
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<tr>
<td>Wash Solution</td>
<td>1 x 400 mL</td>
<td>HEPES buffer with detergent and preservatives</td>
<td>15°C to 30°C</td>
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<tr>
<td>Oil Reagent</td>
<td>1 x 24 mL</td>
<td>silicone oil</td>
<td>15°C to 30°C</td>
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<tr>
<td>Buffer for Deactivation Fluid</td>
<td>1 x 400 mL</td>
<td>sodium bicarbonate buffer, pH 9.2 to 9.4</td>
<td>15°C to 30°C</td>
<td></td>
</tr>
</tbody>
</table>

Materials Required But Not Provided
- Auto Detect Set, Bayer 130277D (1 x 240 mL Auto Detect 1, 1 x 240 mL Auto Detect 2)
- 500 tests
- 1.0 mL aerosol resistant tips
- 1.0 mL, 5.0 mL, and 25.0 mL sterile polypropylene pipettes
- 1.25 mL, 5.0 mL, and 5.0 mL sterile Eppendorf Repeat Pipette Comb/tips or equivalent
- 4.0 mL, 10.0 mL, and 5.0 mL sterile polypropylene pipettes
- 236°C oil bath
- Melting block
- 1°C, 1 x 41.5°C, Bayer 122982 (120V) or 1°C, 236°C oil bath
- Bayer 122982 (120V) or 122983 (240V) at 120V or 240V at 120°C to 240°C (equivalent, with internal dimensions 38 cm x 30 cm x 19 cm (15 in x 15 in x 7.5 in) in water bath to hold two TTA racks
- Disposable laboratory bench covers
- Microwaveable dish covers
- Eppendorf P 1000 pipette or equivalent (1)
- Eppendorf Pipette Tips or equivalent (3)
- Eppendorf Pipette Tips or equivalent (3)
- Exposaclean P1000 pipette or equivalent (1)
- Eppendorf Pipette Tips or equivalent (3)
- H-Low Vacuum Pump, capable of sustaining greater than 400 KPa (86 cm or 30 in Hg, Bayer 122979 (120V) or 122860 (240V) or equivalent
- Bayer Luminometer HC+, Bayer 120500 (120V)
- Bayer HC+ printer cable, Bayer 122907
- Eppendorf printer cable, Bayer 122907
- TMA Data Reduction Software, Bayer 131772
- TMA Workstation, Bayer 131773
- TMA Workstation, Bayer 131773
- TMA Workstation, Bayer 131773
Warnings and Precautions
For In Vitro Diagnostic Use.

POTENTIAL BIOHAZARD: Some components of this product contain human plasma or other human source material. All products manufactured using human source material should be handled as potentially infectious according to established laboratory practices and universal precautions. All control materials have been assayed for hepatitis B surface antigen (HBsAg). human immunodeficiency virus type I (HIV-I) p24Ag, and antibodies to HIV-1, HIV-2, and HCV. The negative control and calibrator have been assayed by FDA-approved tests and found nonreactive for antibodies to HCV. The HCV positive control and calibrator contain human plasma that is anti-HCV reactive and has been heat-inactivated. Use in laboratories where HIV and HCV infections are common. Only personnel adequately trained in handling infectious materials should be permitted to perform this type of procedure.

Use unique precautions when performing the assay. Samples may be infectious. Proper handling and disposal methods should be established according to local, state, and federal regulations. Only personnel adequately trained in handling infectious materials should be permitted to perform this type of procedure. To help prevent laboratory areas from becoming contaminated with ampicillin, arrange a work area that is not to be used for the previous step. Personnel should not move back into previous work areas without first performing the appropriate anti-contamination safeguards.

Do not eat, drink, smoke, or apply cosmetics in areas where reagents or samples are handled.
Do not use components beyond recommended storage dates.
Do not mix reagents from different kits.

Sample Collection and Handling
Handle all samples as if capable of transmitting infection.
Collect blood in sterile tubes containing K2EDTA, sodium heparin, sodium citrate, ACD or as directed by Becton-Dickinson EDTA Plasma Preparation Tubes (PPT) or Becton-Dickinson Vacutainer Serum Collection (red top) tubes.
Whole blood may be held at room temperature up to 24 hours. Do not freeze.
Remove cells from samples by centrifugation at 1000 x g for 10 to 15 minutes.
Serum or plasma may be held at 2 to 8°C for up to 48 hours or for longer periods at or below -20°C.
When processed samples were subjected to up to three freeze-thaw cycles, no qualitative differences were observed in assay performance.

Assay Procedure
PROCEDURAL NOTES:
Decontaminate work surfaces, pipettes, and other equipment regularly and spurs promptly using a 0.5% sodium hypochlorite solution. (Dilute bleach with water) Prepare bleach solution daily. Handle contaminated materials as biohazardous.
Wear personal protective apparel, including disposable gloves, throughout the assay procedure and when handling kit reagents. Thoroughly wash hands after removing gloves; dispose of gloves as biohazardous waste.
Use only supplied or specified disposable laboratory ware.
Three dedicated repeat pipettors are required: one for sample preparation, one for target amplification, and one for detection.
Three dedicated capping water baths are required: one for sample preparation (60°C), one for target amplification (41.5°C), and one for detection (60°C).
To minimize ampicillin contamination, perform the detection steps in a dedicated area on a bench separate from the sample preparation and target amplification areas.
Do not interchange vial or bottle caps as cross-contamination may occur.
Avoid microbial and ribonuclease contamination of reagents when removing aliquots from reagent bottles. Use sterile disposable pipettes and pipette tips.
Take care to avoid cross-contamination during the sample handling steps. For example, discard used material without passing over open tubes.
Use a new sealing card for each reagent and when handling kit reagents. Thoroughly wash hands after removing gloves; dispose of gloves as biohazardous waste.

Seal up the laboratory using a unidirectional workflow.
Clean all pipettes and the benches with a 0.5% sodium hypochlorite solution. Allow the bleach to contact surfaces and pipettes for at least 15 minutes and then rinse with water.
Do not use Deactivation Fluid on surfaces.
When using repeat pipettors to add reagents, avoid touching the tube with the pipette tip to minimize the chance of carryover from one tube to another.
Slowly increase the speed of the vortex mixer until the reaction mixture reaches and is maintained within the upper half of the tubes, but does not touch the sealing card. Adjust the speed of the vortex mixer so the reaction mixture is thoroughly mixed.

Prepare all reagents before starting the sample preparation procedure.
Each reagent may be aliquoted for a given run size. Use care aliquoting the Enzyme Reagent as it is very viscous. All aliquoting must be performed after reagent preparation using sterile, polycarbonate conical tubes with sealing caps in an area that is template and ampicillin free. The aliquoting area must be wiped down with diluted bleach (0.5% sodium hypochlorite plus water) before and after the aliquoting process. The aliquoted reagents must be used the same day the aliquoting was performed. Do not store reagents in the conical aliquot tubes.
Add all reagents using an Eppendorf Repeat Pipettor (or equivalent) capable of delivering specified volumes ± 1%. Accuracy and precision, otherwise indicated.
Perform sample preparation steps in an ampicillin-free area.
Add reagent to the bottom of the tube without inserting the tip into the tube or touching the tip to the rim of the tube. Unless instructed otherwise, position the pipette tip at an angle to the side of the tubes and dispense the reagent to avoid splashing.
Do not freeze the Target Capture Reagent, Wash Solution, Oil Reagent, Auto Detect 1, Auto Detect 2, or Buffer for Deactivation Fluid. The performance of the assay may be affected by use of improperly stored reagents.
Do not refrigerate or freeze the Selection Reagent. The performance of the assay may be affected by use of improperly stored Selection Reagent.
Store the HCV Probe Reagent away from light when not in use.
Do not use reagents if they appear turbid or cloudy after bringing them to the specified temperature except as noted in the procedure.
Do not refreeze thawed reagents.
Use thawed calibrators within 4 hours.
Sodium azide in the reagents can react with copper and lead plumbing to form explosive metal azides. On disposal, flush drains with a large volume of water to prevent the buildup of metal azides; if disposal is done in compliance with federal, state, and local requirements.
Refer to the operator's manuals for the Bayer Luminometer HCt and the Target Capture System for information about preparing and using the systems.

Preparing the Reagents
NOTE: Prepare the reagents in an area that is free of ampicillin.

Target Capture Reagent with Internal Control
NOTE: Target Capture Reagent microparticles must be completely resuspended prior to use.
1. Bring Target Capture and Internal Control Reagents to room temperature. If precipitates or a gel have formed in the Target Capture Reagent during storage, perform the following:
   a. Mix the Target Capture Reagent vigorously by inverting at least ten times.
   b. Place the bottle at room temperature or in a warm water bath (< 30°C) and shake the bottle approximately every 10 minutes until the precipitates are dissolved. Do not vortex.
   c. Place the bottle in a warm water bath (< 30°C) until the gel dissolves completely, then mix the Target Capture Reagent vigorously by inverting at least ten times.
2. Invert the Target Capture and Internal Control Reagents to mix thoroughly.
3. Using a serological pipettor, add 1 mL of Internal Control Reagent into the Target Capture Reagent bottle.
4. Invert the combined solution to mix thoroughly.
5. On the label of the Target Capture Reagent bottle, record the lot number of the Internal Control Reagent and date of addition in the space provided.

The Target Capture Reagent with Internal Control added is stable when stored at 2 to 8°C for 60 days and at room temperature for up to 4 hours per 24 hours while in use.

Wash Solution
Precipitates may form in the Wash Solution during shipment or storage when temperatures fell below 15°C. If precipitates are visible, perform the following:
1. Place the Wash Solution in a water bath (> 30°C) to dissolve the precipitates.
2. Shake to mix thoroughly.
3. Ensure that precipitates are dissolved prior to use.

Amplification and HCV Probe Reagents
If precipitates formed in these reagents during storage, perform the following:
NOTE: At room temperature, the HCV Probe Reagent may take up to 4 hours with periodic mixing to completely dissolve precipitates.
1. Thaw the reagents at room temperature, at 2 to 8°C, or in a warm water bath (< 30°C).
2. Mix using a vortex mixer.
3. Ensure that the reagents are at room temperature and all precipitates are dissolved prior to use.
4. On the reagent labels, record the date the bottles were opened.

Enzyme Reagent
1. Thaw the Enzyme Reagent at room temperature or at 2 to 8°C.
NOTE: Handle the Enzyme Reagent gently to avoid excessive foaming.
2. Gently mix the reagent by inverting the vial.
3. On the vial label, record the date the vial was opened.
The Enzyme Reagent is stable for 60 days at 2 to 8°C. Do not refreeze.

The Amplification and HCV Probe Reagents are stable at 2 to 8°C. Do not refreeze.

The Amplification and HCV Probe Reagents are stable for 60 days at 2 to 8°C. Do not refreeze.
Preparation of the Solution:

1. When all precipitates are in solution, place the bottle in a room temperature water bath and allow the bottle to equilibrate for at least 1 hour.

Deactivation Fluid:

To prepare Deactivation Fluid, mix one part Buffer for Deactivation Fluid with one part bleach (5% sodium hypochlorite). The Deactivation Fluid is stable for 14 days at room temperature.

Calibrators:

The calibrators are supplied as single-use vials.

1. Thaw one vial each of positive and negative calibrators at room temperature.

Preparing the Sample:

CAUTION: To avoid contamination, do not use the repeat pipettor dedicated to sample preparation in other steps.

1. Bring the Target Capture Reagent with Internal Control to room temperature.

2. Prepare the worklist file by recording the identification information for each TTU and its sample and placing the TTU in a TTU rack.

3. Add 400 μL of the Target Capture Reagent with Internal Control into each tube.

4. Add 500 μL of sample or calibrator into the appropriate tube by inserting the pipette tip parallel to the sides of the tube and dispensing the reagent.

5. Gently press a sealing card over the TTUs and ensure that the sealing card completely covers all tubes.

6. Vortex the rack of TTUs for 10 to 20 seconds until the mixture is homogenous.

7. Incubate the rack in a 60°C ± 1°C water bath for 20 ± 1 minutes.

8. Place the rack in the laboratory bench in the sample preparation area for 15 ± 1 minutes.

9. Place the rack in the Target Capture System test tube bay.

10. After 10 ± 1 minutes, carefully remove and discard the sealing card.

Wash the contents of each tube as follows:

CAUTION: At the Target Capture System, keep the vacuum pump running following each aspiration until the tubing is dry. Otherwise, assay results may be affected.

1. Aspirate the solution from each tube using the Target Capture System.

2. Add 1 mL of Wash Solution to each tube.

3. Gently press a new sealing card over the TTUs and ensure that the sealing card completely covers each tube.

4. Remove the rack from the Target Capture System test tube bay.

5. Vortex the rack for 10 to 20 seconds. Visually verify that the microparticle pellets in each tube are completely resuspended. Repeat vortex if necessary.

6. Place the rack in the Target Capture System test tube bay.

7. After 5 ± 1 minutes, carefully remove the sealing card and discard.

8. Repeat steps a through g one time.

9. Aspirate the solution from each tube.

CAUTION: Assay results may be affected if the Wash Solution remains in the tube. Visually verify that the Wash Solution is removed from each tube.

10. Remove the rack from the Target Capture System.

11. Add 75 μL of Amplification Reagent to each tube using the dedicated repeat pipettor.

12. Add 200 μL of Oil Reagent to each tube using the dedicated repeat pipettor.

13. Gently press a new sealing card over the TTUs and ensure that the sealing card completely covers each tube.

14. Vortex the rack for 10 to 20 seconds until the microparticle pellets are completely resuspended.

15. Incubate the rack in a 60°C ± 1°C water bath for 10 ± 1 minutes.

Amplifying the Target:

CAUTION: To avoid contamination, do not use the repeat pipettor dedicated to target amplification in other steps.

1. Immediately move the rack to a 41.5°C ± 1°C water bath for 10 ± 1 minutes.

2. After incubation and while the rack is in the water bath, carefully remove and discard the sealing card.

CAUTION: Take care to dispense the Enzyme Reagent to the bottom of each tube. Enzyme Reagent caught on the sides of the tube may affect assay results.

3. Immediately add 25 μL of Enzyme Reagent to each tube. Hold the pipettor so that the tip is parallel to the sides of the tube and dispense the reagent.

4. Gently press a new sealing card over the TTUs and ensure that the sealing card completely covers each tube.

NOTE: Do not use the vortex mixer. Minimize the time the rack is out of the water bath for mixing.

5. Remove the rack from the water bath and manually shake to mix. Visually verify that the mixture is homogenous; repeat shaking if necessary.

6. Return the rack to a 41.5°C ± 1°C water bath and incubate for 60 ± 5 minutes.

7. Remove the rack from the water bath and carefully transfer the rack to the detection area. The rack may remain at room temperature for 30 minutes.

Detection—the Hybridization Protection Assay (HPA):

CAUTION: To avoid contamination, do not use the repeat pipettor dedicated to detection in other steps and do not remove any amphotols from any of the tubes.

NOTE: Prepare a 19°C ± 2°C container of water for use in the Dual Kinetic Assay detection step.

1. Carefully remove the sealing cards.

2. Add 100 μL of Probe Reagent to each tube using a repeat pipettor.

3. Gently press a new sealing card over the TTUs and ensure that the sealing card completely covers each tube.

4. Vortex the rack for 10 to 20 seconds until the mixture is homogeneous.

5. Place the rack in a 60°C ± 1°C water bath for 15 ± 1 minutes.

6. Remove the rack from the water bath and carefully remove the sealing cards.

7. Add 250 μL of Selection Reagent to each tube using a repeat pipettor.

8. Gently press a new sealing card over the TTUs and ensure that the sealing card completely covers each tube.

9. Vortex the rack for 10 to 20 seconds until the mixture is homogeneous.

10. Return the rack to a 60°C ± 1°C water bath for 10 ± 1 minutes.

Detection—the Dual Kinetic Assay:

CAUTION: Ensure that the laboratory temperature in the area where the detection step is performed is 21°C to 27°C.

NOTE: Read all tubes in the luminometer within 10 to 75 minutes after completion of the HPA.

1. Transfer the rack from the water bath into a container of water (19°C to 27°C) for at least 10 minutes.

2. Prepare the luminometer, ensuring that there are sufficient volumes of Auto Detect 1 and Auto Detect 2 for the assay.

3. Ensure the worklist files are available on the computer with the TMA Data Reduction Software.

4. Start the TMA Data Reduction Software. Refer to the Bayer Luminometer HCV+ Operator's Manual for more information.

5. Remove the rack from the container and place the rack on absorbent tissue.

6. Carefully remove the sealing cards.

7. Wipe the outside of the TTUs with an absorbent tissue dampened with deionized water or equivalent.

8. Transfer the TTUs to the luminometer and start the run.

9. Remove the TTUs when the analysis is complete.

NOTE: Addition of the Deactivation Fluid helps prevent contamination of the laboratory equipment with amplification.

10. After removing the TTUs from the luminometer, add 1 mL Deactivation Fluid to each tube and keep at room temperature for at least 30 minutes before disposing of the contents of the tubes.

11. Decontaminate the rack by immersing it in a 0.5% sodium hypochlorite solution for at least 15 minutes. Rinse with water and air or wipe dry.

Quality Control Results and Acceptability:

The VERSANT HCV RNA Qualitative Assay kit contains an HCV Negative Calibrator and an HCV Positive Calibrator. The calibrators are used to determine run validity and analyte and internal control cutoffs. Three (3) replicates each of the kit Negative Calibrator and Positive Calibrator are required with each assay run.

The Negative Calibrator must be placed in the first three (3) tubes of the first TTU (i.e., TTU 1, positions 1, 2, 3). The Positive Calibrator must be placed in the second three (3) tubes of the first TTU (i.e., TTU 1, positions 4, 5, 6).

Good laboratory practice recommends the use of positive and negative controls to ensure functionality of reagents and proper performance of assay procedure. Controls are not included in the assay kit. External controls selected by the user may be run at any position in the assay rack following the Negative and Positive Calibrators (see above). These controls should be tested at least once with each new test kit opened. If desired, controls developed for use with the VERSANT HCV RNA Qualitative Assay are available; see the Optional Materials section for ordering information.

Quality control requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to NClLs EP12A-anc 42 CFR 493.1202(c) for guidance on appropriate Quality Control practices.

Negative Calibrator Acceptance Criteria:

- Each Negative Calibrator must have an Analyte signal that is between 0 RLU and 40,000 RLU, inclusive.
- Each Negative Calibrator must have an IC signal that is between 75,000 RLU and 300,000 RLU, inclusive.
- If one Negative Calibrator value is invalid or has an Analyte signal outside the expected range, the software recalculates the mean of the Negative Calibrator for the Analyte signal (NC, (Analyte)) using the two acceptable values for the Negative Calibrator.
- The run is invalid and must be repeated if two Negative Calibrator values are invalid or have Analyte signals outside the expected range.
- The software calculates the mean of the Negative Calibrator values for the Internal Control signal as follows:

\[ NC, (IC) = \frac{\text{Sum of IC signals for Negative Calibrators}}{\text{Number of Negative Calibrators}} \]

The software calculates the mean of the Negative Calibrator values for the Analyte signal as follows:

\[ NC, (Analyte) = \frac{\text{Sum of Analyte signals for Negative Calibrators}}{\text{Number of Negative Calibrators}} \]

Positive Calibrator Acceptance Criteria:

- Each Positive Calibrator must have an Analyte signal between 400,000 RLU and 2,700,000 RLU, inclusive.
- Each Positive Calibrator must have an IC signal that is between 475,000 RLU and 2,000,000 RLU, inclusive.
- If one Positive Calibrator value is invalid or has an Analyte signal outside the expected range, the software recalculates the mean of the Positive Calibrator for the Analyte signal (NC, (Analyte)) using the two acceptable values for the Positive Calibrator.
- The run is invalid and must be repeated if two Positive Calibrator values are invalid or have Analyte signals outside the expected range.
- The software calculates the mean of the Positive Calibrator values for the Analyte signal as follows:

\[ PC, (Analyte) = \frac{\text{Sum of Analyte signals for Positive Calibrators}}{\text{Number of Positive Calibrators}} \]
Analyte

As described above, the assay software automatically evaluates the calibrator values according to the earlier cited criteria. If the criteria are not met, the run will be reported as "invalid." As well, an invalid run, all sample results are automatically reported as "invalid." Invalid runs are not to be reported and all samples in the run must be rejected.

Run Status Codes

Tube status codes are reported on the run report for each sample. A key to the status codes is printed on the run report and is also available in the Operator's Manual.

Interpreting the Results

During the detection step, the luminescence measures the RLU for the target or of Analyte (the greater signal) and for the Internal Control (the flasher signal). The TMA Data Reduction Software then calculates two cutoffs for the assay: Analyte Cutoff (Analyte CO) for the Analyte signal and an Internal Control Cutoff (IC Cutoff) for the Internal Control signal.

The cutoff values are determined as follows:

- Internal Control Cutoff Value = NC(AIC) + (0.04 x PC(AIC))
- Analyte Cutoff Value = NC(Analyte) + (0.04 x PC(Analyte))

Where NC(AIC) is the mean of the Negative Calibrator values for the Internal Control signal, NC(Analyte) is the mean of the Negative Calibrator values for the Analyte signal, and PC(Analyte) is the mean of the Positive Calibrator values for the Analyte signal.

Cutoff Calculations:

- Analyte Cutoff: [Negative Calibrator Analyte Mean RLU] + [0.04 x Positive Calibrator Analyte Mean RLU]
- Internal Control Cutoff: 0.5 x (Negative Calibrator Internal Control Mean RLU)

Sample Validity Criteria

The validity of a sample is determined using the ratio of the Analyte signal to the Analyte Cutoff (Analyte SC/GD) and the value of the Internal Control signal relative to the IC Cutoff. For Positive Calibrators or samples that are reactive for Analyte, the Internal Control signal is not used to validate the result.

- The sample result is valid and considered nonreactive when the sample has an Analyte signal less than the Analyte Cutoff (i.e., Analyte SC < 1) and an Internal Control signal greater than or equal to the Internal Control Cutoff (IC Cutoff).
- The sample result is invalid when the sample has an Analyte signal less than the Analyte Cutoff (i.e., Analyte SC < 1) but Internal Control signal is less than the Internal Control Cutoff.
- The sample result is considered reactive when the sample has an Analyte signal greater than or equal to the Analyte Cutoff (i.e., Analyte SC > 1) and the Internal Control signal is less than or equal to 475,000 RLU.

Patient Test Results

Only results from valid runs can be reported.

The assay has not been approved for samples from patients with absence of liver disease or with absence of antibody evidence of HCV infection or for monitoring the progress of hepatitis C, including response to treatment (see Intended Use). Test interpretations are as follows:

- "Reactive" indicates that HCV RNA was detected.
- "Nonreactive" indicates that HCV RNA was not detected. Note that a nonreactive result does not preclude the presence of HCV RNA because results are dependent on adequate specimen collection, absence of inhibitors, and sufficient RNA to be detected.
- "Invalid" indicates that the sample must be retested. If the same result is generated in repeat testing, the interpretation remains "invalid."

During testing of the VERSANT HCV RNA Qualitative Assay, results with low SCD values (approximately 1-4) were not observed. Caution should be used in interpreting results with these low SCD values. Results from the VERSANT HCV RNA Qualitative Assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician.

Multiple myeloma is characterized by the presence of elevated levels of malignant plasma cells and immunoglobulins in the blood of patients. Testing of specimens from myeloma patients in the VERSANT HCV RNA Qualitative Assay may result in interference for some samples. Such samples have been noted to yield diffuse, smudged pellets during the target capture step of the assay. Therefore, the appearance of diffuse, smudged pellets during the target capture step should be noted and the results from such specimens should be treated with caution.

Use of this product should be limited to personnel who have been trained in the procedure.

Performance Characteristics: Nonclinical Studies

Specificity

The specificity of the VERSANT HCV RNA Qualitative Assay was determined using 1000 serum and 1514 EDTA plasma specimens from anti-HCV negative or nonreactive in the VERSANT HCV RNA Qualitative Assay, yielding a specificity of 99.6%.

Analytical Sensitivity

Limit of Detection

The Limit of Detection (LOD) for the VERSANT HCV RNA Qualitative Assay was determined by testing serial dilutions of the WHO International Standard for HCV genotype 1 RNA (NIBSC code 96/790). The following table shows the percent detection of each panel member. Each panel member was tested in replicates ranging from 60 to 240.

<table>
<thead>
<tr>
<th>Concentration (IU/mL)</th>
<th>Reactive</th>
<th>Non-Reactive</th>
<th>Invalid</th>
<th>Total</th>
<th>Percent Valid</th>
<th>Detected</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>1</td>
<td>59</td>
<td>0</td>
<td>60</td>
<td>1.7</td>
<td>0.3 - 8.9</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>23</td>
<td>36</td>
<td>1</td>
<td>90</td>
<td>35.0</td>
<td>27.6 - 45.7</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>178</td>
<td>61</td>
<td>1</td>
<td>240</td>
<td>74.5</td>
<td>68.7 - 75.7</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>220</td>
<td>19</td>
<td>1</td>
<td>240</td>
<td>92.1</td>
<td>87.9 - 94.9</td>
<td></td>
</tr>
<tr>
<td>7.5</td>
<td>236</td>
<td>2</td>
<td>2</td>
<td>240</td>
<td>99.2</td>
<td>97.0 - 99.8</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>60</td>
<td>0</td>
<td>0</td>
<td>60</td>
<td>100</td>
<td>94.0 - 100</td>
<td></td>
</tr>
<tr>
<td>18.5</td>
<td>59</td>
<td>0</td>
<td>1</td>
<td>60</td>
<td>100</td>
<td>94.0 - 100</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>60</td>
<td>0</td>
<td>0</td>
<td>60</td>
<td>100</td>
<td>94.0 - 100</td>
<td></td>
</tr>
</tbody>
</table>

* Confidence Interval

NOTE: Inadequate results were not included in the statistical calculations. Percent Values Detected:

Serial dilutions of the WHO International Standard for HCV genotype 1 RNA were detected ≥95% of the time as low as 7.5 IU/mL. Linear regression analysis determined 5.3 IU/mL (95% probability) as the limit of detection for the VERSANT HCV RNA Qualitative Assay.

Detection of HCV Genotypes Using Transcripts

Transcripts of HCV genotypes 1, 2a, 2b, 3a, 4a, 5a, and 6a made from the 5' untranslated region of the HCV genome were tested using the VERSANT HCV RNA Qualitative Assay. All transcripts were quantitated using photometric analysis and confirmed using hyperchomucity and OD500. The copies/mL were converted to IU/mL using an in-house conversion factor of 5.2 copies/mL = 1 IU/mL.

Dilutions of each transcript were tested at 9.6 IU/mL (50 copies/mL) for genotypes 1, 2a, 3a, 4a, 5a, and 6a. Each transcript was tested in replicates ranging from 30 to 720. The results are shown in the table below:

<table>
<thead>
<tr>
<th>Genotype (copies/mL)</th>
<th>Total Reactive</th>
<th>Non-Reactive</th>
<th>Invalid</th>
<th>Detected</th>
<th>Percent Valid</th>
<th>Detected</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>9.6 (50)</td>
<td>210</td>
<td>0</td>
<td>230</td>
<td>99.1</td>
<td>95.6 - 99.7</td>
<td></td>
</tr>
<tr>
<td>3a</td>
<td>9.6 (50)</td>
<td>210</td>
<td>0</td>
<td>230</td>
<td>99.1</td>
<td>95.6 - 99.7</td>
<td></td>
</tr>
<tr>
<td>4a</td>
<td>9.6 (50)</td>
<td>210</td>
<td>0</td>
<td>230</td>
<td>99.1</td>
<td>95.6 - 99.7</td>
<td></td>
</tr>
<tr>
<td>5a</td>
<td>9.6 (50)</td>
<td>210</td>
<td>0</td>
<td>230</td>
<td>99.1</td>
<td>95.6 - 99.7</td>
<td></td>
</tr>
<tr>
<td>6a</td>
<td>9.6 (50)</td>
<td>210</td>
<td>0</td>
<td>230</td>
<td>99.1</td>
<td>95.6 - 99.7</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Inadequate results were not included in the data calculations for Percent Valid Detected.

With the exception of genotype 2b, all transcript sequences were detected ≥95% of the time at 9.6 IU/mL (50 copies/mL). Genotype 2b was detected ≥95% of the time at 14.4 IU/mL (75 copies/mL).

Detection of HCV Genotypes Using Clinical Specimens

Clinical specimens representing HCV genotypes 1 to 6 at different concentrations were used to determine the percent detection of the VERSANT HCV RNA Qualitative Assay. The specimens were quantitated using the VERSANT HCV RNA 3.0 Assay (COVA). The genotypes of the specimens were provided by the specimen vendor and confirmed using the VERSANT HCV Genotype Assay (LIPA) and sequencing. The table below shows the percent detected:

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Concentration (IU/mL)</th>
<th>Total Reactive</th>
<th>Non-Reactive</th>
<th>Invalid</th>
<th>Detected</th>
<th>Percent Valid</th>
<th>Detected</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.8 (25)</td>
<td>50</td>
<td>87</td>
<td>3</td>
<td>96.7</td>
<td>90.7 - 98.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9.6 (50)</td>
<td>495</td>
<td>490</td>
<td>4</td>
<td>99.2</td>
<td>97.9 - 99.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>14.4 (75)</td>
<td>60</td>
<td>59</td>
<td>1</td>
<td>98.3</td>
<td>91.1 - 99.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>19.2 (100)</td>
<td>90</td>
<td>90</td>
<td>0</td>
<td>100</td>
<td>95.9 - 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>96.2 (500)</td>
<td>60</td>
<td>60</td>
<td>0</td>
<td>100</td>
<td>94.0 - 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9,815 (50,000)</td>
<td>30</td>
<td>30</td>
<td>0</td>
<td>100</td>
<td>88.7 - 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2a</td>
<td>9.6 (50)</td>
<td>210</td>
<td>200</td>
<td>10</td>
<td>95.2</td>
<td>91.5 - 97.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Inadequate results were not included in the data calculations for Percent Valid Detected.
**Nonclinical Specimen Studies**

**Anticoagulant Specimens**
HCV-negative specimens and specimens spiked with HCV 1 at 9.6 IU/mL (50 copies/mL) were collected in serum separator tubes (SST PLUS, plastic), K3 EDTA (PLUS, plastic), K3 EDTA (FIT), sodium citrate (glass, 4%), ACD-solution A (glass) and sodium heparin (PLUS, plastic 60 USP units/mL). None of the anticoagulants tested affected the sensitivity and specificity of the VERSANT HCV RNA Qualitative Assay.

**Specimen Storage Conditions**
Samples from the anticoagulant study (see above) were used to evaluate the effect of storing whole blood specimens for up to 24 hours at room temperature and the subsequently processed specimens (serum and plasma) for up to 48 hours at 2°C to 8°C. No adverse effects on sensitivity or specificity of the VERSANT HCV RNA Qualitative Assay were observed for the whole blood or processed specimens under the storage conditions tested. Whole blood collected in the tested tube types can be stored at room temperature for up to 24 hours and subsequently processed serum and plasma samples can be stored at 2°C to 8°C for up to 48 hours prior to testing in the VERSANT HCV RNA Qualitative Assay.

**Multiple Freeze-Thaw Cycles Studies**
The effects of one, two, and three freeze-thaw cycles on processed specimens were tested in HCV negative specimens and specimens spiked with HCV 1 at 9.6 IU/mL (50 copies/mL). Up to three freeze-thaw cycles of HCV negative and HCV positive processed specimens had no effect on the performance of the VERSANT HCV RNA Qualitative Assay.

**Performance Characteristics: Clinical Studies**
Performance characteristics for the VERSANT HCV RNA Qualitative Assay were established in a multicenter study at four geographically diverse clinical sites. The study evaluated serum or plasma specimens from 1,511 subjects enrolled in hepatitis clinics, intravenous drug abuse clinics, transfusion centers and AIDS clinics. The study population included 938 (62.1%) subjects with a medical history of liver disease, or positive HCV and 673 (44.8%) subjects diagnosed with chronic HCV hepatitis. A history of one or more risk factors was reported by 1,175 (77.8%) subjects. Symptoms associated with HCV infection were reported by 741 (49.5%) subjects and 172 (11.7%) subjects were infected with HIV or another hepatitis virus. No patients were on anti-viral therapy at the time of enrollment into the study.

Of the 1,511 total subjects, 544 (35.9%) were female and 967 (64.0%) were male. Subject age ranged from 17 years to 89 years with a mean of 47 years. Ethnicity representation included: White, Non-Hispanic, 688 (45.6%); Black, Non-Hispanic, 586 (38.6%); White, Hispanic, 145 (9.5%); Asian/Pacific Islander, 23 (1.5%); Black Hispanic, 19 (1.3%); Native American, Alaskan, 8 (0.5%); and unknown or other, 35 (2.3%).

**Clinical Study Results**
A total of 5,542 EIA, RIBA, PCR and VERSANT HCV RNA Qualitative Assay results were used in the clinical data analysis. Performance characteristics were based on calculations of Positive and Negative Percent Agreement and 95% Confidence Intervals of VERSANT HCV RNA Qualitative Assay results compared to anti-HCV serology results and to PCR results in three different populations: subjects with or without anti-HCV, subjects with anti-HCV with or without biochemical (i.e., elevated ALT) or histological evidence of liver disease, and subjects at risk for HCV with or without anti-HCV. Liver histopathology was characterized by cirrhosis, fibrosis, hepatocellular carcinoma, or other histopathological diagnosis. Subjects were classified as ‘at risk’ for HCV if they were exposed to a high-risk activity, which included occupational exposure, blood or blood product transfusion, or past or current drug use or use of shared drug tools, multiple sex partners, sex with an HCV-positive partner, men having sex with men, dialysis, or a history of a sexually transmitted disease (STD).

For assay comparisons made with each population, performance of the VERSANT HCV RNA Qualitative Assay was similar across the four study sites and for each specimen type. Summary data are provided in Tables X and Y for each population and overall. Serum and plasma data were shown.

**Agreement with Anti-HCV Serology**
Performance of the VERSANT HCV RNA Qualitative Assay compared to anti-HCV serology was similar for each population and overall as shown in Table X. Of the 1,511 VERSANT HCV RNA Qualitative Assay and anti-HCV results available in subjects with or without evidence of HCV, 1,014 (67.1%) were in agreement between the two assays. The VERSANT HCV RNA Qualitative Assay detected HCV RNA in 930 of 1,014 (91.7%) positive specimens. The VERSANT HCV RNA Qualitative Assay agreed with all 5 of these results (100%).

**Table X:**
<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Positive Agreement</th>
<th>Negative Agreement</th>
<th>Percent Valid Detected</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>138 (93.6%)</td>
<td>5 (92.7%)</td>
<td>95.0</td>
<td>94.3</td>
</tr>
<tr>
<td>Plasma</td>
<td>133 (93.6%)</td>
<td>5 (92.7%)</td>
<td>95.0</td>
<td>94.3</td>
</tr>
</tbody>
</table>

For the 522 specimens collected from subjects with anti-HCV and biochemical or histological evidence of liver disease, the VERSANT HCV RNA Qualitative Assay detected HCV RNA in 486 (93.1%) specimens. HCV RNA was detected in: (a) 358 (68.7%) of 533 specimens from subjects with elevated ALT and liver histopathology, (b) 92 (74.3%) of 123 subjects from specimens with normal ALT and liver histopathology, and (c) 26 (100%) of 26 specimens from subjects with elevated ALT and normal liver histopathology. Of the 126 specimens collected from subjects without HCV or evidence of liver disease, the VERSANT HCV RNA Qualitative Assay did not detect HCV RNA in 127 (98.4%) specimens. Six (6) results were RIBA indeterminate or negative, however, five (5) of the 16 were PCR positive. Therefore, these three (3) subjects were infected. The VERSANT HCV RNA Qualitative Assay agreed with all 5 of these results (100%).

Of the 1,175 subjects at risk for HCV, the VERSANT HCV RNA Qualitative Assay detected HCV RNA in 765 of 831 (92.0%) anti-HCV positive specimens, but mt
Table X. Agreement of the VERSANT HCV RNA Qualitative Assay and Anti-HCV Serology for Each Study Population

<table>
<thead>
<tr>
<th>VERSANT</th>
<th>Serology</th>
<th>Anti-HCV</th>
<th>ALT</th>
<th>Liver Histological Findings</th>
<th>Total</th>
<th>EIA R / RIBA Pos</th>
<th>EIA R / RIBA Neg</th>
<th>EIA R / RIBA Ind</th>
<th>EIA NR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal ALT and Liver Histology</td>
<td>205</td>
<td>92</td>
<td>2</td>
<td>31</td>
<td>80</td>
<td>97.6</td>
<td>91.5-99.7</td>
<td>74.8</td>
<td>66.2-82.2</td>
</tr>
<tr>
<td>Elevated ALT and No Liver Histology</td>
<td>26</td>
<td>28</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>100</td>
</tr>
<tr>
<td>Normal ALT and No Liver Histology</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>100</td>
</tr>
<tr>
<td>At Risk</td>
<td>1175</td>
<td>769</td>
<td>8</td>
<td>66</td>
<td>332</td>
<td>97.6</td>
<td>95.4-95.0</td>
<td>92.1</td>
<td>90.1-93.8</td>
</tr>
<tr>
<td></td>
<td>EIA R / RIBA Pos</td>
<td>831</td>
<td>755</td>
<td>0</td>
<td>66</td>
<td>0</td>
<td>N/A</td>
<td>92.1</td>
<td>90.1-93.8</td>
</tr>
<tr>
<td></td>
<td>EIA R / RIBA Neg</td>
<td>2</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>100</td>
<td>2.5-100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>EIA R / RIBA Ind</td>
<td>7</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>41</td>
<td>100</td>
<td>100</td>
<td>29.2-100</td>
</tr>
<tr>
<td></td>
<td>EIA NR</td>
<td>335</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>327</td>
<td>97.6</td>
<td>95.3-95.0</td>
<td>91.0-92.0</td>
</tr>
</tbody>
</table>

VERSANT = VERSANT Serology | Reactive NR = Nonreactive Pos = Positive Neg = Negative Agree = Agreement NIA = Insufficient data for meaningful result Ind = Indeterminate

1 Subject was designated infected per CDC guidelines if RIBA was indeterminate or negative, and PCR was positive.
2 Patients who had anti-HCV EIA non-reactive results were studied for approximating the specificity of the AMPLICOR HCV Test, v2.0, but these data do not imply performance for testing of anti-HCV EIA non-infected individuals.
3 Subject was designated as having uncertain infection per CDC guidelines if RIBA was indeterminate or negative, and PCR was negative.

Agreement with PCR

As shown in Table X, the performance of the VERSANT HCV RNA Qualitative Assay compared to an FDA-cleared PCR test was similar for each population and overall. Of the 1,013 anti-HCV serology positive specimens, HCV RNA was detected in 921 specimens by both assays (91.7% positive agreement) and not in 83 specimens (91.2% negative agreement). The VERSANT HCV RNA Qualitative Assay was 100% agreement with PCR for specimens with indeterminate or negative RIBA results. The VERSANT HCV RNA Qualitative Assay and the PCR test detected HCV RNA in 4 of 10 specimens with indeterminate RIBA results and in 1 of 6 specimens with RIBA negative results. Of the 471 anti-HCV serology negative specimens, both assays detected HCV RNA in 8 EIA nonreactive specimens, but not in 459 EIA reactive specimens.

Of the 830 VERSANT HCV RNA Qualitative Assay and PCR test results for subjects at risk for HCV infection with anti-HCV, 62 (9.9%) were in agreement between the two assays. Both assays detected HCV RNA in 759 specimens, but not in 65 specimens. Furthermore, both assays were 100% agreement for specimens with indeterminate or negative RIBA results. The VERSANT HCV RNA Qualitative Assay and the PCR test detected HCV RNA in 3 of 7 specimens with indeterminate RIBA results and in 1 of 2 specimens with RIBA negative results. Of the 320 specimens collected from subjects at risk for HCV infection without anti-HCV, 97 (48.8%) were in agreement. Of the 325 anti-HCV serology negative specimens from subjects at risk for HCV infection, both assays detected HCV RNA in 5 EIA nonreactive specimens, but not in 316 EIA reactive specimens.

Table Y. Agreement of the VERSANT HCV RNA Qualitative Assay and PCR for Each Study Population

<table>
<thead>
<tr>
<th>VERSANT</th>
<th>Serology</th>
<th>EIA R / RIBA Pos</th>
<th>EIA R / RIBA Neg</th>
<th>EIA R / RIBA Ind</th>
<th>EIA NR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal ALT and Liver Histology</td>
<td>205</td>
<td>91</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Elevated ALT and No Liver Histology</td>
<td>26</td>
<td>28</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Normal ALT and No Liver Histology</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

VERSANT = VERSANT Serology | Reactive NR = Nonreactive Pos = Positive Neg = Negative Agree = Agreement NIA = Insufficient data for meaningful result Ind = Indeterminate

1 Eleven inconclusive results due to repeatedly invalid or equivocal PCR test results are not included in any calculations.
2 Patients who had anti-HCV EIA non-reactive results were studied for approximating the specificity of the AMPLICOR HCV Test, v2.0, but these data do not imply performance for testing of anti-HCV EIA non-infected individuals.

Clinical Specimen Storage Study

Specimen storage conditions were evaluated for their effects on assay performance. Specimens from each of 72 subjects were separated into two aliquots. One aliquot was stored at 2 to 8°C and tested within 48 hours. The other aliquot was stored frozen at -20°C or below for up to 64 days. Data indicated no difference in the detection of HCV RNA in specimens stored in the two conditions.

Signal Analysis

A summary of the VERSANT HCV RNA Qualitative Assay signal analysis for the assay calibrators and controls and the internal control in plasma and serum specimens is shown in the table below.

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>Mean RLU (x 1000)</th>
<th>SD (x 1000)</th>
<th>Min RLU (x 1000)</th>
<th>Max RLU (x 1000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>111</td>
<td>1,265</td>
<td>102</td>
<td>8.0</td>
</tr>
<tr>
<td>Negative</td>
<td>110</td>
<td>5.6</td>
<td>3.0</td>
<td>53.2</td>
</tr>
</tbody>
</table>
# Control N Mean S/C0 SD % CV Min S/C0 Max S/C0
Positive 54 22.5 2.1 9.5 11.2 27.3
Negative 54 0.07 0.04 55.1 0.01 0.2

<table>
<thead>
<tr>
<th>Control</th>
<th>SD</th>
<th>%CV</th>
<th>Min S/C0</th>
<th>Max S/C0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>210 2.1</td>
<td>0.09</td>
<td>4.3</td>
<td>1.7</td>
</tr>
<tr>
<td>Plasma</td>
<td>355 2.1</td>
<td>0.1</td>
<td>6.1</td>
<td>1.0</td>
</tr>
</tbody>
</table>

## Reproducibility
Reproducibility testing was performed at three laboratories (A, B, C) to obtain measures of repeatability and reproducibility during the clinical trial. Two of the sites were outside laboratories and one was in-house. Testing was also conducted in-house during the preclinical phase (D). In the clinical testing, the three sites were provided with six identical panels of eight samples containing 0 to 9.615 IU/mL (0 to 50,000 copies/mL) genotype 1 or 0 to 57.7 IU/mL (0 to 3,000 copies/mL) genotype 2b in serum or plasma. In the preclinical phase testing, six member serum or plasma panels for genotype 1 at 0 to 14.4 IU/mL (0 to 75 copies/mL) and genotype 2b at 0 to 69.2 IU/mL (0 to 360 copies/mL) were tested. At sites A, B, and C, each of two operators performed two days of testing with each of three kit lots on a total of six days of testing. At site D, three operators tested the genotype 1 panel with each of three kit lots on six separate days. Similarly, site D tested the genotype 2b panel with each of three kit lots on each of five separate days. Reproducibility testing at or near the assay's limit of detection was not done with genotypes other than genotype 1.

### Overall Reproducibility for Serum Panel Members

<table>
<thead>
<tr>
<th>HCV RNA</th>
<th>Genotype</th>
<th>IU/mL</th>
<th>Copies/mL</th>
<th>N</th>
<th>% Valid Agreement</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype 1</td>
<td>1</td>
<td>9.6 50 216 96.6 93.5 - 98.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotype 1</td>
<td>1</td>
<td>96 500 100 96.6 96.6 - 100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotype 2b</td>
<td>2b</td>
<td>57.6 300 219 98.6 93.4 - 99.5</td>
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<tr>
<td>Genotype 2b</td>
<td>2b</td>
<td>192 1,000 100 96.6 96.6 - 100</td>
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<tr>
<td>Negative Serum</td>
<td>0</td>
<td>0</td>
<td>218 95.9 92.3 - 97.8</td>
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<tr>
<td>Negative Serum</td>
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<td>0</td>
<td>225 99.6 97.5 - 99.9</td>
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</tr>
<tr>
<td>Negative Serum</td>
<td>0</td>
<td>0</td>
<td>108 98.8 95.6 - 100</td>
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<td></td>
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</tbody>
</table>

### Overall Reproducibility for EDTA Plasma Panel Members

<table>
<thead>
<tr>
<th>HCV RNA</th>
<th>Genotype</th>
<th>IU/mL</th>
<th>Copies/mL</th>
<th>N</th>
<th>% Valid Agreement</th>
<th>95% CI</th>
</tr>
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<tbody>
<tr>
<td>Genotype 1</td>
<td>1</td>
<td>9.6 50 216 96.6 93.5 - 98.4</td>
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<tr>
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<tr>
<td>Genotype 2b</td>
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<td>57.6 300 219 98.6 93.4 - 99.5</td>
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<td>Genotype 2b</td>
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<td>192 1,000 100 96.6 96.6 - 100</td>
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<td>Negative Serum</td>
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<td>0</td>
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<td>Negative Serum</td>
<td>0</td>
<td>0</td>
<td>108 98.8 95.6 - 100</td>
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</tbody>
</table>

## Frequencies of Serum Results for Samples with and without HCV RNA

### Frequencies of Serum Results for Samples with and without HCV RNA

<table>
<thead>
<tr>
<th>HCV RNA</th>
<th>IU/mL</th>
<th>Copies/mL</th>
<th>Site-to-Site N correct/ % Valid tested</th>
<th>Lot-to-Lot N correct/ % Valid tested</th>
<th>Day-to-Day N correct/ % Valid tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype 1</td>
<td>1</td>
<td>96 500 100</td>
<td>36/36 100</td>
<td>1 18/18 100</td>
<td>1 18/18 100</td>
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<tr>
<td>Genotype 1</td>
<td>1</td>
<td>96 500 100</td>
<td>36/36 100</td>
<td>1 18/18 100</td>
<td>1 18/18 100</td>
</tr>
<tr>
<td>Genotype 1</td>
<td>1</td>
<td>96 500 100</td>
<td>36/36 100</td>
<td>1 18/18 100</td>
<td>1 18/18 100</td>
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<td>Genotype 1</td>
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<td>96 500 100</td>
<td>36/36 100</td>
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<td>36/36 100</td>
<td>1 18/18 100</td>
<td>1 18/18 100</td>
</tr>
</tbody>
</table>

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

## References
AUTO DETECT SET

Cat. No: 130277D 130654 Rev. A, 2002-11

Intended Use
The VERSANT™ Auto Detect Set is to be used with the Bayer™ Luminometer HC+ and the LEADER HC Luminometer.

For In Vitro Diagnostic Use

Materials Provided

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Description</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auto Detect 1</td>
<td>1 x 240 mL</td>
<td>0.1% hydrogen peroxide in 1 mM nitric acid</td>
<td>15°C to 30°C</td>
</tr>
<tr>
<td>Auto Detect 2</td>
<td>1 x 240 mL</td>
<td>1.6N sodium hydroxide</td>
<td>15°C to 30°C</td>
</tr>
</tbody>
</table>

Warnings and Precautions

- For In Vitro Diagnostic Use.
  - 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.
  - Do not pipet by mouth.
  - If skin or mucous membrane exposure occurs, immediately wash the area with copious amounts of water. Seek medical advice.
  - Dilute spills with water before wiping the surface dry.
  - Do not use components beyond recommended storage dates.

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.

Technical Assistance

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

GP P/N IN0080 Rev. A

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HCV RNA QUALITATIVE ASSAY CONTROL SET
Cat. No. 130277E 130555 Rev. A, 2002-11

Intended Use
The VERSANT® HCV RNA Qualitative Assay Control Set is to be used for monitoring assay run performance in laboratory procedures. The controls are formulated for use with the VERSANT HCV RNA Qualitative Assay.

For In Vitro Diagnostic Use.

If desired, the VERSANT HCV RNA Qualitative Assay Control Set may be used in order to comply with local, state and/or federal requirements or accreditation requirements and the user's standard laboratory Quality Control procedures. It is recommended that the user refer to NCCLS EP-12A and 42 CFR 493.12020 for guidance on appropriate quality control practices.

Materials Provided

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Description</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>4 x 1 mL</td>
<td>Defibrinated normal human plasma containing gentamicin and 0.2% sodium azide</td>
<td>-15°C to -35°C</td>
</tr>
<tr>
<td>HCV Positive Control</td>
<td>4 x 1 mL</td>
<td>Defibrinated normal human plasma with inactivated HCV containing gentamicin and 0.2% sodium azide</td>
<td>-15°C to -35°C</td>
</tr>
</tbody>
</table>

Materials Required But Not Provided

- VERSANT HCV RNA Qualitative Assay

Warnings and Precautions

For In Vitro Diagnostic Use.

- POTENTIAL BIOHAZARD: This product contains human plasma or other human source material and biological source material of non-human origin. All products manufactured using human or non-human biological source material should be handled as potentially infectious. Each human plasma donor unit used in the manufacture of this product was tested and found non-reactive for hepatitis B surface antigen (HBsAg), human immunodeficiency virus type 1 (HIV-1) p24Ag, antibodies to HIV-1 and HIV-2, and antibodies to hepatitis C virus (HCV) by FDA-approved methods. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents.

- Handle this product according to established good laboratory practices and universal precautions.

- POTENTIAL BIOHAZARD: The positive control contains human plasma and heat-inactivated HCV.

- 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.

- Disinfect spills promptly using a 0.5% sodium hypochlorite solution. (Dilute bleach with water.) Prepare bleach solution daily. 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.

- If skin or mucous membrane exposure occurs, immediately wash the area with copious amounts of water. Seek medical advice.

- Do not pipet by mouth.

- Do not use components beyond recommended storage dates.

- Avoid microbial and ribonuclease contamination of reagents when removing aliquots from reagent bottles. Use sterile disposable pipettes and pipette tips.

Procedure

NOTE: Used thawed reagents within 4 hours.

Prepare the samples:

1. Thaw the controls at room temperature (15°C to 30°C).
2. Shake gently or vortex to mix thoroughly.
3. Proceed with Preparing the Sample in the Assay Procedure section in the VERSANT HCV RNA Qualitative Assay package insert.

Expected Results

If controls fail to meet the expected results, the assay run is invalid. Refer to the operator’s manuals for troubleshooting.

<table>
<thead>
<tr>
<th>Control</th>
<th>Target RNA Concentration (copies/mL)</th>
<th>HCV Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>0</td>
<td>Non-reactive</td>
</tr>
<tr>
<td>Positive</td>
<td>300</td>
<td>Reactive</td>
</tr>
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</table>

Limitations

The Controls are for monitoring assay performance and cannot be substituted for the calibrators or a primary reference in the assay.

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.

Technical Assistance

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

References


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