GEN-PROBE

Bayer

versant®

HCV RNA
QUALITATIVE ASSAY

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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 ACD) 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 HCV 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).[1,2] Studies indicate that HCV is transmitted through contaminated blood and blood products, other close personal contact, and intravenous drug use.[3] 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.[3,4] Approximately 50% of the patients infected with HCV develop chronic liver disease, with 20% of these patients developing chronic active hepatitis or cirrhosis.[3] Chronic infection with HCV may also be associated with increased risk for developing hepatocellular carcinoma.[5]

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 domains.[2,6] 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 5' untranslated region (5'-UTR) and a portion of the core are the most highly conserved regions of the genome.[6,7,8]

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.[3,9] 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.[9,10,11] 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-antibody positive individuals. As well, CDC guidelines endorse either NAT testing or RIBA as a confirmatory test following a postive HCV EIA result.[12] In addition to diagnosis of HCV infection, qualitative HCV RNA testing may be used for identifying the endpoint of infection following antiviral treatment.[13,14,15]

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 Transcription-Mediated Amplification (TMA)[16] to amplify conserved regions within the 5'-UTR of the HCV genome. TMA 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.

Sample preparation involves detergent lysis of the virus followed by hybridization of the free viral nucleic acid with capture oligonucleotides complementary to the 5'-UTR. This hybridization step takes place in solution and the internal control is hybridized in a similar manner. The hybridized targets are then captured onto magnetic microparticles and separated from the remaining specimen components using a magnet. Use of a magnetic field to firmly hold the captured targets in place allows for thorough washing to remove potential inhibitory substances.

TMA starts with the addition of primers, nucleotides, RT and T7 RNA polymerase to the captured, purified nucleic acid. The first primer, which contains a T7 promoter region, anneals to the captured RNA and is extended by RT to form an RNA:DNA intermediate. The RNA strand is degraded by the RNase H activity of RT. A second primer anneals to the DNA strand and the DNA polymerase function of RT generates a double-stranded DNA template. This double-stranded DNA includes the T7 promoter region that was part of the first primer. T7 RNA polymerase binds to the promoter region and transcribes the DNA to yield 100 to 1000 RNA copies. These copies are minus sense RNA and are antisense when compared to the original viral genome or the internal control template. The second primer anneals to each of these antisense RNA copies and is extended by RT to form an RNA:DNA duplex. The RNA strand is then degraded by RNase H and the first primer, containing the T7 promoter region, binds to the DNA strand. The strand is extended by RT to produce a double-stranded DNA template with a T7 promoter. Additional RNA copies are made and the process continues autocatalytically, resulting in the production of up to 10 billion amplicons within one hour.

Detection of HCV and internal control RNA amplicons produced by the TMA reaction is accomplished using hybridization protection.[17] Single-stranded nucleic acid probes that are complementary to the amplicons are labeled with chemiluminescent acridinium esters. Two different acridinium esters with different chemiluminescent properties are used: one on the probes complementary to the HCV amplicon and one on the probes complementary to the internal control amplicons. The labeled probes hybridize to the RNA amplicons, resulting in protection of the acridinium ester from hydrolysis by the Selection Reagent. Free or nonspecifically bound labeled probes are hydrolyzed by the addition of the Selection Reagent. Addition of the Auto Detect reagents causes the protected acridinium esters on the annealed probes to emit a chemiluminescent signal, which is measured in a luminometer and expressed numerically in relative light units (RLU). The different chemical modifications to the acridinium esters found on HCV and internal control probes cause them to have different light-off kinetics, resulting in different times and durations of light emission.[18] The dual kinetic nature of these acridinium esters results in a short and intense flash of light — the flasher signal — from the probes bound to the internal control and a slower emission — the glower signal — from the HCV-specific probes. The luminometer measures the virus- and internal control-specific signals for each specimen and the results are reported by the TMA data reduction software.

Materials Provided

Each kit contains sufficient reagents to perform a total of 100 tests or up to 4 runs.

The reagents in this kit are components of a master lot; therefore, component expiration dates in any individual kit box may differ from the kit lot expiration date.

BOX 1

Component	Quantity	Description	Storage
Internal Control Reagent	1 x 1 mL	RNA transcript in HEPES buffer with detergent	-15° to -35°C unopened
Amplification Reagent	1 x 8.5 mL	primers, dNTPs, NTPs, and co-factors in TRIS buffer with preservative	-15° to -35°C unopened
Enzyme Reagent	1 x 2.8 mL	MMLV Reverse Transcriptase and T7 RNA Polymerase in HEPES/TRIS buffer with sodium azide (0.05%)	-15° to -35°C unopened
HCV Probe Reagent	1 x 14 mL	Chemiluminescent oligonucleotide probes in succinate buffer with detergent	-15° to -35°C unopened
HCV Positive Calibrator	4 x 2 mL	inactivated HCV positive plasma in defibrinated normal human plasma with gentamicin and sodium azide (0.2%)	-15° to -35°C
HCV Negative Calibrator	4 x 2 mL	Defibrinated normal human plasma with gentamicin and sodium azide (0.2%)	-15° to -35°C
BOX 2			
Target Capture Reagent	1 x 50 mL	capture oligonucleotides and magnetic microparticles in HEPES buffer with detergent	2° to 8°C
BOX 3			
Selection Reagent	1 x 30 mL	borate buffer with surfactant	15° to 30°C
Wash Solution	1 x 400 mL	HEPES buffer with detergent and preservatives	15° to 30°C
Oil Reagent	1 x 24 mL	silicone oil	15° to 30°C
Buffer for Deactivation Fluid	1 x 400 mL	sodium bicarbonate buffer, pH 9.2 to 9.4	15° to 30°C

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, 10.0 mL, and 25.0 mL serological pipettes
- 1.25 mL, 5.0 mL, 12.5 mL sterile Eppendorf Repeat Pipettor Combitips or equivalent
- 4.0 mL, 10.0 mL, and 50.0 mL sterile polypropylene tubes
- Bleach, unscented (5 % sodium hypochlorite)
- Biohazard waste container
- Circulating water baths (2 x 60° ± 1°C, 1 x 41.5° ± 1°C), Bayer 122982 (120V) or 122983 (240V) or equivalent, with internal dimensions 38 cm W x 30 cm D x 19 cm H (15 in W x 11.8 in D x 7.5 in H) with water bath spacers to hold two TTU racks
- Disposable laboratory bench covers
- Laboratory coats (2)
- Eppendorf P1000 pipette or equivalent (1)
- Eppendorf Repeat Pipettors or equivalent (3)
- High-Flow Vacuum Pump, capable of sustaining greater than or ecual to 93 kPag (66 cm or 26 in Hg), Bayer 122979 (120V) or 122980 (240V) or equivalent
- Bayer Luminometer HC+, Bayer 129293 (115V)
- Bayer HC+ computer system with monitor, Bayer 122975
- Bayer HC+ printer, Bayer 122976
- Bayer HC+ printer cable, Bayer 122977
- TMA Data Reduction Software, Bayer 131772
- TMA Worklist Editor, Bayer 131773

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- Assay Protocol diskette, Bayer 131774
- Multi-tube vortex mixers (2), VWR Multitube Vortexer or equivalent
- Pipette bulb or aid
- Sealing Cards, Bayer 122987
- Bayer Target Capture System, Bayer 129897
- Ten Tip Cassettes (TTCs), Bayer 122989
- Ten Tube Units (TTUs), Bayer 122988
- Tub or container 45 cm W x 60 cm D (17.7 in W x 23.6 in D)
- Uninterruptible Power Supply
- Water resistant thermometers (4)

Optional Materials

- Control Set, Bayer 130277E (4 x 1 mL Positive Control, 4 x 1 mL Negative Control)
- Barcode Reader, wedge kit, Bayer 122978

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 good laboratory practices and universal precautions. All control materials have been assayed for hepatitis B surface antigen (HBsAg), human immunodeficiency virus type 1 (HIV-1) 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-treated to inactivate the virus. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents.
- Use universal 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 amplicon, arrange a unidirectional workflow in the laboratory. Proceed from the sample preparation to the target amplification and then to the detection areas. Do not return samples, equipment, or reagents to the area where you performed the previous step. Personnel should not move back into previous work areas wihout 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 lots.

Sample Collection and Handling

- Handle all samples as if capable of transmitting infection.
- Collect blood in sterile tubes containing K_2EDTA, sodium heparin, sodium citrate, ACD or in 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 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 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 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 circulating 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 amplicon 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 step.
- Room temperature is 15° to 30°C. The temperature for the detection area must be 21° to 27°C to ensure consistent light emission kinetics.
- Set up the laboratory using a unidirectional workflow.
- Clean all pipettors and the benchtops with a 0.5% sodium hypochlorite solution. Allow the bleach to contact surfaces and pipettors 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.
- Equilibrate the water baths to 60° ± 1°C and 41.5° ± 1°C.
- Bring all reagents to room temperature and mix thoroughly prior to use. Ensure that precipitates are dissolved.
- 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. Aliquoting must be performed after reagent preparation using sterile, polypropylene conical tubes with sealing caps in an area that is template and amplicon free. The aliquoting area must be wiped down with diluted bleach (0.5% sodium hypochlorite in water) before and after the aliquoting process. The aliquotec 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 with ± 5% accuracy and ± 5% precision, unless otherwise indicated.
- Perform sample preparation steps in an amplicon-free area.
- Add reagent to the bottom of the tube without inserting the pipette 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 into a drain is in compliance with federal, state, and local requirements.
- Refer to the operator's manuals for the Bayer Luminometer HC+ 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 amplicon.

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:

   For Precipitates:

   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.

   For a gel:

   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 fall 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 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.

CAUTION: Store the HCV Probe Reagent away from light when not in use.

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

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 reagent label, record the date the vial was opened.

The Enzyme Reagent is stable for 60 days at 2° to 8°C. Do not refreeze.

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Selection Reagent

If precipitates formed in the Selection Reagent during shipment or storage, perform the following:

1. Place the reagent in a 60° ± 1°C water bath for 45 minutes, shaking the bottle every 5 to 10 minutes.
2. 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.

Do not use the Selection Reagent until it has equilibrated.

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.
2. Invert the vials to mix thoroughly.

Once thawed, use calibrators within 4 hours.

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 samples and place 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 tubes by inserting the pipette tip containing the sample or calibrator into the reagent in the tube. Dispense the material and hold the pipette plunger down until you remove the tip from the solution.
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 homogeneous.
7. Incubate the rack in a 60° ± 1°C water bath for 20 ± 1 minutes.
8. Place the rack on a 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.
11. 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.

    a. Aspirate the solution from each tube using the Target Capture System.
    b. Add 1 mL of Wash Solution to each tube.
    c. Gently press a new sealing card over the TTUs and ensure that the sealing card completely covers each tube.
    d. Remove the rack from the Target Capture System test tube bay.
    e. Vortex the rack for 10 to 20 seconds. Visually verify that the microparticle pellets in each tube are completely resuspended; repeat vortex if necessary.
    f. Place the rack in the Target Capture System test tube bay.
    g. After 5 ± 1 minutes, carefully remove the sealing card and discard.
    h. Repeat steps a through g one time.
    i. 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.
12. Remove the rack from the Target Capture System.
13. Add 75 µL of Amplification Reagent to each tube using the dedicated repeat pipettor.
14. Add 200 µL of Oil Reagent to each tube using the dedicated repeat pipettor.
15. Gently press a new sealing card over the TTUs and ensure that the sealing card completely covers each tube.
16. Vortex the rack for 10 to 20 seconds until the microparticle pellets are completely resuspended.
17. Incubate the rack in a 60° ± 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° ± 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° ± 1°C water bath and incubate for 60 ± 5 minutes.
7. Remove the rack from the water bath and 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 amplicon from any of the tubes.

NOTE: Prepare a 19° to 27°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° ± 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° ± 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° 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° 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 HC+ Operator's Manual for more information.
5. Remove the rack from the container and place the rack on absorbent material.
6. Carefully remove the sealing cards.
7. Wipe the outside of the TTUs using an absorbent tissue dampened with deionized water or equivalent.

   This removes residue from the outside of the tubes and reduces static electricity that might affect luminometer readings.
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 amplicon.

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., TTU1, 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 NCCLS EP12-A and 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_x(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_x(IC) = Sum of IC signals for Negative Calibrators / Number of Negative Calibrators

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

NC_x (Analyte) = Sum of Analyte signals for Negative Calibrators / 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.
- Internal Control signal value must be less than or equal to 475,000 RLU.
- If one Positive Calibrator value is outside the range, the software recalculates the Positive Calibrator mean (PC_x(Analyte)) using the two acceptable Positive Calibrator values.
- The run is invalid and must be repeated if two Positive Calibrator values are outside the expected range.

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

PC_x (Analyte) = Sum of the Analyte signals for Positive Calibrators / Number of Positive Calibrators

Summary of Calibrator Acceptance Criteria

Negative Calibrator:	
Analyte	≥ 0 and ≤ 40,000 RLU
Internal Control	≥ 75,000 and ≤ 300,000 RLU

Positive Calibrator:	
Analyte	≥ 400,000 and ≤ 2,700,000 RLU
Internal Control	≤ 475,000 RLU

Run Validity Criteria

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, in 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 repeated.

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 also is available in the Operator's Manual.

Interpreting the Results

During the detection step, the luminometer measures the RLU for the target or Analyte (the glower signal) and for the Internal Control (the flasher signal). The TMA Data Reduction Software then calculates two cutoffs for the assay: an 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 = 0.5 x [NC_x(IC)]

Analyte Cutoff Value = NC_x(Analyte) + [0.04 x PC_x(Analyte)]

Where NC_x(IC) is the mean of the Negative Calibrator values for the Internal Control signal, NC_x(Analyte) is the mean of the Negative Calibrator values for the Analyte signal, and PC_x(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 S/CO) 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 S/CO < 1) and an Internal Control (IC) 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 S/CO < 1) and the Internal Control (IC) 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 S/CO ≥ 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 verified 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 S/CO values (approximately 1-4) were not observed. Caution should be used in interpreting results with these low S/CO values. Results from the VERSANT HCV RNA Qualitative Assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician.

Limitations

Reliable results are dependent on adequate specimen collection, transport, storage and processing procedures.

HCV RNA detection is dependent on the amount of virus present in the specimen and may be affected by specimen collection methods, patient factors and/or state of infection.

Although RNA representing all recognized HCV genotypes 1-6 can be detected with this test, sensitivity and other performance characteristics have not been determined for all HCV genotypes using clinical specimens.

The effect of therapeutic drugs for bacterial and fungal infections on the VERSANT HCV RNA Qualitative Assay has not been determined.

The effect of elevated concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT) on the VERSANT HCV RNA Qualitative Assay has not been determined..

Inhibition by specimen factors may occur and yield false negative results. Incorporation of target capture technology is designed to eliminate specimen inhibitors, but specimen inhibition may still occur. An internal control (IC) has been added to identify specimens that may be inhibitory in the VERSANT HCV RNA Qualitative Assay.

Multiple myeloma is characterized by the presence of elevated levels of malignant plasma cells and immunoglobulins in the blood of patients.[19] 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 1504 EDTA plasma specimens from anti-HCV negative volunteer blood donors. The specimens were negative for antibodies to HCV using FDA-approved methods. Of the total samples tested, 2495/2504 were 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).[20] The following table shows the percent detection of each panel member. Each panel member was tested in replicates ranging from 60 to 240.

Concentration (IU/mL)	Reactive	Non-Reactive	Invalid	Total	Percent Valids Detected	95% CI*
0.1	1	59	0	60	1.7	0.3 – 8.9
1	23	36	1	60	39.0	27.6 – 51.7
2.5	178	61	1	240	74.5	68.7 – 79.7
5	220	19	1	240	92.1	87.9 – 94.9
7.5	236	2	2	240	99.2	97.0 – 99.8
10	60	0	0	60	100	94.0 – 100
18.5	59	0	1	60	100	94.0 – 100
50	60	0	0	60	100	94.0 – 100

* Confidence Interval

NOTE: Invalid results were not included in the data calculations for Percent Valids 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 phosphate analysis and confirmed using hyperchromicity and OD_{260}. The copies/mL were converted to IU/mL using an in-house conversion factor 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 360 to 720. The results are shown in the table below:

HCV Genotype	Concentration IU/mL (copies/mL)	Reactive	Non-Reactive	Invalid	Total	Percent Valids Detected	95% CI
1	9.6 (50)	716	3	1	720	99.6	98.8 – 99.9
2a	9.6 (50)	357	3	0	360	99.2	97.6 – 99.7
2b	14.4 (75)	350	10	0	360	97.2	95.0 – 98.5
3a	9.6 (50)	356	4	0	360	98.9	97.2 – 99.6
4a	9.6 (50)	357	1	2	360	99.7	98.4 – 99.9
5a	9.6 (50)	356	4	0	360	98.9	97.2 – 99.6
6a	9.6 (50)	685	35	0	720	95.1	93.3 – 96.5

NOTE: Invalid results were not included in the data calculations for Percent Valids Detected.

With the exception of genotype 2b, all genotype transcripts 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 (bDNA). 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:

HCV Genotype	Concentration IU/mL (copies/mL)	Total	Reactive	Non-Reactive	Invalid	Percent Valids Detected	95% CI
1	4.8 (25)	90	87	3	0	96.7	90.7 – 98.9
1	9.6 (50)	495	490	4	1	99.2	97.9 – 99.7
1	14.4 (75)	60	59	1	0	98.3	91.1 – 99.7
1	19.2 (100)	90	90	0	0	100	95.9 – 100
1	96.2 (500)	60	60	0	0	100	94.0 – 100
1	9,615.4 (50,000)	30	30	0	0	100	88.7 – 100
2b	9.6 (50)	210	200	10	0	95.2	91.5 – 97.4

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HCV Genotype	Concentration IU/mL (copies/mL)	Total	Reactive	Non-Reactive	Invalid	Percent Valids Detected	95% CI
2b	14.4 (75)	176	173	3	0	98.3	95.1 – 99.4
2b	19.2 (100)	120	120	0	0	100	96.9 – 100
2b	28.8 (150)	30	29	0	1	100	88.3 – 100
2b	38.5 (200)	90	90	0	0	100	95.9 – 100
2b	57.7 (300)	10	10	0	0	100	72.3 – 100
3a	9.6 (50)	120	120	0	0	100	96.7 – 100
3a	19.2 (100)	60	60	0	0	100	94.0 – 100
3a	38.5 (200)	40	40	0	0	100	91.2 – 100
4/4a	9.6 (50)	120	114	6	0	95.0	89.5 – 97.7
4/4a	14.4 (75)	30	30	0	0	100	88.7 – 100
5a	9.6 (50)	120	118	0	2	100	96.9 – 100
6a	9.6 (50)	120	114	3	3	97.4	92.7 – 99.1
6a	14.4 (75)	30	30	0	0	100	88.7 – 100

NOTE: Invalid results were not included in the data calculations for Percent Valids Detected.

The overall percent detected across all HCV genotypes tested was ≥ 95% at 9.6 IU/mL (50 copies/mL).

In a supplemental study, 61 clinical specimens representing genotypes 1 to 6 were tested at 1,000, 300 and 100 c/mL; no testing was performed at 50 c/mL. With the exception of specimens representing HCV genotype 2, all specimens were detected at all levels. All clinical specimens containing HCV genotype 2 were detected at 300 c/mL. In summary, 75 specimens representing HCV genotypes 1 to 6 showed reactivity in the VERSANT HCV RNA Qualitative Assay. These included 8 specimens in the transcript testing, 6 in the clinical specimen testing, and 61 in the supplemental testing.

Analytical Specificity

Cross-Contamination Frequency

The potential cross-contamination frequency was determined by testing replicates of a high titer HCV genotype 1 positive specimen and replicates of an HCV negative specimen. HCV positive samples (1 x 10^6 copies/mL) were alternated with HCV negative samples using a "checkerboard" pattern. Forty-five (45) replicates each of HCV negative and HCV positive samples were tested in each of five runs, for a total of 225 replicates of the HCV positive samples and 225 replicates of the HCV negative samples. No false results were obtained; two negative samples were invalid. Combined results across all runs yielded a cross-contamination frequency of 0% (0/223).

Microorganisms and Viruses

The potential cross-reaction and interference of other microorganisms and viruses was evaluated by adding selected microorganisms and viruses to HCV negative specimens and specimens spiked with HCV genotype 1 at 9.6 IU/mL (50 copies/mL); skin flora microorganisms that may contaminate a blood sample or microorganisms and viruses that can co-infect individuals with HCV infection were tested. The microorganisms and viruses were pooled and tested at final concentrations of 5 x 10^4 CFU/mL or 5 x 10^4 copies/mL, respectively. Pool 1 contained *E. coli, P. aeruginosa, K. pneumoniae, H. influenzae,* and cytomegalovirus (CMV) (Towne). Pool 2 contained *E. cloacae, P. fluorescens, S. aureus, S. marcescens,* and *S. pneumoniae*. Pool 3 contained *S. epidermidis,* Streptococcus group B, *C. albicans,* hepatitis B virus (HBV), and HIV-1 B. Pool 4 contained HIV-1 A, HIV-1 C, and HIV-1 D. Pool 5 contained HIV-1 E, HIV-1 F, HIV-1 O and *P. acnes*. Hepatitis G virus (HGV) also was tested both in the presence and absence of spiked HCV 1a at 9.6 IU/mL (50 copies/mL) using five HGV-positive specimens. HGV titers were not known due to the lack of an HGV quantitative assay. For all microorganisms and viruses tested, no cross-reactions or interference were observed in the VERSANT HCV RNA Qualitative Assay.

Potentially Interfering Substances

Endogenous Substances

Potentially interfering endogenous substances were tested by adding these substances to HCV negative specimens and specimens spiked with HCV 1 at 9.6 IU/mL (50 copies/mL). The concentrations of potentially interfering endogenous substances were tested according to NCCLS Document EP7-P.[21]

The following endogenous substances were tested: 500 mg/dL hemoglobin, 60 mg/dL bilirubin (conjugated), 60 mg/dL bilirubin (unconjugated), 3,000 mg/dL triglycerides, and 8 g/dL protein. None of the endogenous substances tested interfered with the sensitivity and specificity of the VERSANT HCV RNA Qualitative Assay.

Therapeutic Drugs

The potential interference of commonly prescribed drugs to treat HCV or other viral diseases was tested by adding these substances to HCV negative specimens and specimens spiked with HCV 1 at 9.6 IU/mL (50 copies/mL). The drugs were pooled and tested at final concentrations five times the reported peak serum or plasma concentrations in the therapeutic range. Pool 1 contained Intron A, Ribavirin, and Azathioprine. Pool 2 contained Cyclosporine, Aldactone, and Prednisone. Pool 3 contained Roferon A, Tacrolimus, and Amantadine HCl. Pool 4 contained Fluoxetine HCl, Peginterferon Alfa-2b, and Azidothymidine. Pool 5 contained Ganciclovir and Dideoxycytidine. Pool 6 contained Didanosine and Didehydrodeoxythymidine. None of the drugs tested interfered with the sensitivity and specificity of the VERSANT HCV RNA Qualitative Assay.

Other Potentially Interfering Substances

The effect of other potentially interfering substances was determined by testing HCV negative specimens and specimens spiked with HCV 1 at 9.6 IU/mL (50 copies/mL). The disease categories tested were: myeloma IgG (n=12) positive specimens, anti-nuclear antibody positive specimens (n=10), anti-doublestranded DNA positive specimens (n=6), rheumatoid factor positive specimens (n=19), and specimens from subjects with systemic lupus erythematous (n=10).

With the exception of a subset of the myeloma specimens, none of the tested samples from subjects with HCV-like disease states interfered with the performance of the VERSANT HCV RNA Qualitative Assay. Refer to *Limitations* for information on myeloma specimens.

Nonclinical Specimen Studies

Anticoagulant Studies

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), K_2 EDTA (PLUS, plastic), K_2 EDTA (PPT), sodium citrate (glass, 4%), ACD-solution A (glass) and sodium heparin (PLUS, plastic 60 USP units) tubes. None of the anticoagulants tested affected the sensitivity and specificity of the VERSANT HCV RNA Qualitative Assay.

Specimen Storage Studies

Samples from the anticoagulant study (see above) were used to evaluate the effect of storing whole blood samples for up to 24 hours at room temperature and the subsequently processed specimens (serum and plasma) for up to 48 hours at 2° 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 specimens can be stored at 2° 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 multi-center study at four geographically diverse clinical sites. The study evaluated serum or plasma specimens from 1,511 subjects enrolled in hepatology 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 anti-HCV serology and 741 (49.0%) 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 (49.0%) subjects and 112 (7.4%) 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 (36.0%) 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, 689 (45.6%); Black, Non-Hispanic, 588 (38.9%); White, Hispanic, 149 (9.9%); 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 needle-stick accidents or another occupational exposure, blood or blood product transfusion, past or current injection-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 within 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 are shown combined.

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, ten (10) anti-HCV serology results were indeterminate. Of the remaining 1,501 VERSANT HCV RNA Qualitative Assay and conclusive anti-HCV results available in this population, 93.6% were in agreement between the two assays. The VERSANT HCV RNA Qualitative Assay detected HCV RNA in 930 of 1,014 (91.7% Positive Agreement) anti-HCV serology positive specimens, but not in 475 of 486 (97.7% Negative Agreement) anti-HCV serology negative specimens. Sixteen (16) results were RIBA indeterminate or negative, however, five (5) of the 16 were PCR positive. Therefore, these five (5) subjects were infected. The VERSANT HCV RNA Qualitative Assay agreed with all 5 of these results (100%).

Of 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) 368 (98.7%) of 373 specimens from subjects with elevated ALT and liver histopathology, (b) 92 (74.8%) of 123 specimens from subjects with normal ALT and liver histopathology, and (c) 26 (100%) of 26 specimens from subjects with elevated ALT and no liver histopathology. Of the 129 specimens collected from subjects without anti-HCV with evidence 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, three (3) of the six (6) were PCR positive. Therefore, these three (3) subjects were infected. The VERSANT HCV RNA Qualitative Assay agreed with all three (3) 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 (92.1%) anti-HCV serology positive specimens, but not in 328 of 336 (97.6%) anti-HCV serology negative specimens. Nine (9) results were RIBA indeterminate or negative; however, PCR was positive for four (4) of the nine (9) subjects, indicating that they were infected. The VERSANT HCV RNA Qualitative Assay agreed with all four (4) of these results (100%).

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Table X. Agreement of the VERSANT HCV RNA Qualitative Assay and Anti-HCV Serology for Each Study Population

	N	VERS+ Ser+	VERS+ Ser-	VERS- Ser+	VERS- Ser-	% Neg Agree	95% C.I.	% Pos Agree	95% C.I.
Total	1511	935	11	84	481	97.8	96.0-98.9	91.8	89.9-93.4
Anti-HCV Serology	1511	935	11	84	481	97.8	96.0-98.9	91.8	89.9-93.4
EIA R / RIBA Pos	1014	930	0	84	0	N/A	N/A	91.7	89.8-93.3
EIA R / RIBA Neg	6	1[1]	0	0	5	100	47.8- 100	100	2.5-100
EIA R / RIBA Ind	10	4[1]	0	0	6[3]	100	54.1- 100	100	39.8-100
EIA NR[2]	481	0	11	0	470	97.7	95.9-98.9	N/A	N/A
Anti-HCV Serology, ALT, Liver Histological Findings									
Total	658	490	2	37	129	98.5	94.6-99.8	93.0	90.5-95.0
Elevated ALT and Liver Histopathology	425	371	0	5	49	100	92.7- 100	98.7	96.9-99.6
EIA R / RIBA Pos	373	368	0	5	0	N/A	N/A	98.7	96.9-99.6
EIA R / RIBA Neg	3	1[1]	0	0	2	100	15.8- 100	100	2.5-100
EIA R / RIBA Ind	3	2[1]	0	0	1[3]	100	2.5- 100	100	15.8-100
EIA NR[2]	46	0	0	0	46	100	92.3-100	N/A	N/A
Normal ALT and Liver Histopathology	205	92	2	31	80	97.6	91.5-99.7	74.8	66.2-82.2
EIA R / RIBA Pos	123	92	0	31	0	N/A	N/A	74.8	66.2-82.2
EIA R / RIBA Neg	1	0	0	0	1	100	2.5-100	N/A	N/A
EIA R / RIBA Ind	1	0	0	0	1[3]	100	2.5- 100	N/A	N/A
EIA NR[2]	80	0	2	0	78	97.5	91.3-99.7	N/A	N/A
Elevated ALT and No Liver Histopathology	26	26	0	0	0	N/A	N/A	100	86.8-100
EIA R / RIBA Pos	26	26	0	0	0	N/A	N/A	100	86.8-100
EIA R / RIBA Neg	0	0	0	0	0	N/A	N/A	N/A	N/A
EIA R / RIBA Ind	0	0	0	0	0	N/A	N/A	N/A	N/A
EIA NR[2]	0	0	0	0	0	N/A	N/A	N/A	N/A
Normal ALT and No Liver Histopathology	2	1	0	1	0	N/A	N/A	50.0	1.3-98.7
EIA R / RIBA Pos	2	1	0	1	0	N/A	N/A	50.0	1.3-98.7
EIA R / RIBA Neg	0	0	0	0	0	N/A	N/A	N/A	N/A
EIA R / RIBA Ind	0	0	0	0	0	N/A	N/A	N/A	N/A
EIA NR[2]	0	0	0	0	0	N/A	N/A	N/A	N/A
At Risk									
Total	1175	769	8	66	332	97.6	95.4-99.0	92.1	90.1-93.8
EIA R / RIBA Pos	831	765	0	66	0	N/A	N/A	92.1	90.0-93.8
EIA R / RIBA Neg	2	1[1]	0	0	1	100	2.5- 100	100	2.5- 100
EIA R / RIBA Ind	7	3[1]	0	0	4[3]	100	39.8- 100	100	29.2-100
EIA NR[2]	335	0	8	0	327	97.6	95.3-99.0	N/A	N/A

VERS = VERSANT Ser = Serology R = Reactive NR = Nonreactive Pos = Positive Neg = Negative Agree = Agreement N/A = 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 indeterminant and PCR was negative.

Agreement with PCR

As shown in Table Y, 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 (99.7% positive Agreement) and not in 83 specimens (91.2% Negative Agreement). The VERSANT HCV RNA Qualitative Assay was in 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 6 EIA nonreactive specimens, but not in 459 EIA nonreactive specimens.

Of the 522 VERSANT HCV RNA Qualitative Assay and PCR test results for subjects with anti-HCV and biochemical or histological evidence of liver disease, 520 (99.6%) were in agreement between the two assays: (a) 373 (100%) of 373 specimens from subjects with elevated ALT and liver histopathology, (b) 121 (98.4%) of 123 specimens from subjects with normal ALT and liver histopathology, and (c) 26 (100%) of 26 specimens from subjects with elevated ALT and no liver histopathology. Both assays were in 100% agreement for specimens with indeterminate or negative RIBA results. The HCV TMA Assay and the PCR test detected HCV RNA in 2 of 4 specimens with indeterminate RIBA results and in 1 of 4 specimens with RIBA negative results. Of the 126 specimens collected from subjects without anti-HCV, 125 (99.2%) were in agreement. Both assays detected HCV RNA in 1 EIA nonreactive specimen, but not in 124 EIA nonreactive specimens.

Of the 830 VERSANT HCV RNA Qualitative Assay and PCR test results for subjects at risk for HCV infection with anti-HCV, 824 (99.3%) were in agreement between the two assays. Both assays detected HCV RNA in 759 specimens, but not in 65 specimens. Furthermore, both assays were in 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 325 specimens collected from subjects at risk for HCV infection without anti-HCV, 321 (98.8%) 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 nonreactive specimens.

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

	N	VERS+ PCR+	VERS+ PCR-	VERS- PCR+	VERS- PCR-	% Neg Agree	95% C.I.	% Pos Agree	95% C.I.
Total	1500[1]	932	12	3	553	97.9	96.3-98.9	99.7	99.1-99.9
Anti-HCV Serology	1500	932	12	3	553	97.9	96.3-98.9	99.7	99.1-99.9
EIA R / RIBA Pos	1013	921	8	1	83	91.2	83.4-96.1	99.9	99.4-100
EIA R / RIBA Neg	6	1	0	0	5	100	47.8-100	100	2.5-100
EIA R / RIBA Ind	10	4	0	0	6	100	54.1-100	100	39.8-100
EIA NR[2]	471	6	4	2	459	99.1	97.8-99.8	75.0	34.9-96.8
Anti-HCV Serology, ALT, Liver Histological Findings									
Total	658	489	3	0	166	98.2	94.9-99.6	100	99.2-100
Elevated ALT and Liver Histopathology	425	371	0	0	54	100	93.4-100	100	99.0-100
EIA R / RIBA Pos	373	368	0	0	5	100	47.8-100	100	99.0-100
EIA R / RIBA Neg	3	1	0	0	2	100	15.8-100	100	2.5-100
EIA R / RIBA Ind	3	2	0	0	1	100	2.5-100	100	15.8-100
EIA NR[2]	46	0	0	0	46	100	92.3-100	N/A	N/A
Normal ALT and Liver Histopathology	205	91	3	0	111	97.4	92.5-99.5	100	96.0-100
EIA R / RIBA Pos	123	90	2	0	31	93.9	79.8-99.3	100	96.0-100
EIA R / RIBA Neg	1	0	0	0	1	100	2.5-100	N/A	N/A
EIA R / RIBA Ind	1	0	0	0	1	100	2.5-100	N/A	N/A
EIA NR[2]	80	1	1	0	78	98.7	93.1-100	100	2.5-100
Elevated ALT and No Liver Histopathology	26	26	0	0	0	N/A	N/A	100	86.8-100
EIA R / RIBA Pos	26	26	0	0	0	N/A	N/A	100	86.8-100
EIA R / RIBA Neg	0	0	0	0	0	N/A	N/A	N/A	N/A
EIA R / RIBA Ind	0	0	0	0	0	N/A	N/A	N/A	N/A
EIA NR[2]	0	0	0	0	0	N/A	N/A	N/A	N/A
Normal ALT and No Liver Histopathology	2	1	0	0	1	100	2.5-100	100	2.5-100
EIA R / RIBA Pos	2	1	0	0	1	100	2.5-100	100	2.5-100
EIA R / RIBA Neg	0	0	0	0	0	N/A	N/A	N/A	N/A
EIA R / RIBA Ind	0	0	0	0	0	N/A	N/A	N/A	N/A
EIA NR[2]	0	0	0	0	0	N/A	N/A	N/A	N/A
At Risk									
Total	1164	768	7	3	386	98.2	96.4-99.3	99.6	98.9-99.9
EIA R / RIBA Pos	830	759	5	1	65	92.9	84.1-97.6	99.9	99.3-100
EIA R / RIBA Neg	2	1	0	0	1	100	2.5-100	100	2.5-100
EIA R / RIBA Ind	7	3	0	0	4	100	39.8-100	100	29.2-100
EIA NR[2]	325	5	2	2	316	99.4	97.7-99.9	71.4	29.0-96.3

VERS = VERSANT R = Reactive NR = Nonreactive Pos = Positive Neg = Negative Agree = Agreement N/A = Insufficient data for meaningful result Ind = Indeterminate N/A = Insufficient data for meaningful result

[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 44 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:

Calibrator	N	Mean RLU (x 1000)	SD (x 1000)	% CV	Min RLU (x 1000)	Max RLU (x 1000)
Positive	111	1,265	102	8.0	795	1493
Negative	110	5.6	3.0	53.2	0	13.1

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Control	N	Mean S/CO	SD	% CV	Min S/CO	Max S/CO
Positive	54	22.5	2.1	9.5	11.2	27.3
Negative	54	0.07	0.04	55.1	0.01	0.2

Internal Control	N	Mean IC S/CO	SD	%CV	Min S/CO	Max S/CO
Serum	355	2.1	0.1	6.1	1.0	3.0
Plasma	210	2.1	0.09	4.3	1.7	2.2

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 577 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 for 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

	HCV RNA				
Genotype	IU/mL	Copies/mL	N	% Valid Agreement	95% CI
1	4.8	25	217	89.9	85.1 – 93.2
1	9.6	50	214	96.3	92.8 – 98.1
1	14.4	75	216	98.6	96.0 – 99.5
2b	46.1	240	219	98.2	95.4 – 99.3
2b	57.6	300	224	97.8	94.9 – 99.0
2b	69.2	360	218	99.1	96.7 – 99.8
1	96	500	108	100	96.6 – 100
2b	192	1,000	108	100	96.6 – 100
1	9,615	50,000	108	100	96.6 – 100
Negative Serum	0	0	218	95.9	92.3 – 97.8
Negative Serum	0	0	225	99.6	97.5 – 99.9
Negative Serum	0	0	108	100	96.6 – 100

Overall Reproducibility for EDTA Plasma Panel Members

	HCV RNA				
Genotype	IU/mL	Copies/mL	N	% Valid Agreement	95% CI
1	9.6	50	216	96.8	93.5 – 98.4
1	96	500	107	100	96.6 – 100
2b	57.6	300	219	96.8	93.6 – 98.4
2b	192	1,000	106	97.2	92.0 – 99.4
2b	577	3,000	107	99.1	94.9 – 100
Negative Serum	0	0	213	98.1	95.3 – 99.3
Negative Serum	0	0	221	99.5	97.5 – 99.9
Negative Serum	0	0	108	98.1	93.5 – 99.8

Frequencies of Serum Results for Samples with and without HCV RNA

	HCV RNA		Site-to-Site			Lot-to-Lot			Day-to-Day		
Geno-type	IU/mL	Copies/mL	Site	N correct/ N tested	% Valid Agree	Lot	N correct/ N tested	% Valid Agree	Day	N correct/ N tested	% Valid Agree
1	9.6	50	D	206/214	96.3	6	68/72	94.4	1	33/35	94.3
						5A	68/70	97.1	2	34/36	94.4
						5B	70/72	97.2	3	35/36	97.2
									4	35/35	100
									5	34/36	94.4
									6	35/36	97.2
1	96	500	A	36/36	100	3	36/36	100	1	18/18	100
			B	36/36	100	4	36/36	100	2	18/18	100
			C	36/36	100	5	36/36	100	3	18/18	100
									4	18/18	100
									5	18/18	100
									6	18/18	100
2b	57.6	300	D	219/224	97.8	6	75/75	100	1	42/44	95.5
						3	73/75	97.3	2	44/45	97.8
						4	71/74	95.9	3	45/45	100
									4	44/45	97.8
									5	45/45	100

Frequencies of Serum Results for Samples with and without HCV RNA

	HCV RNA		Site-to-Site			Lot-to-Lot			Day-to-Day		
	IU/mL	Copies/mL	Site	N correct/ N tested	% Valid Agree	Lot	N correct/ N tested	% Valid Agree	Day	N correct/ N tested	% Valid Agree
2b	192	1,000	A	36/36	100	3	36/36	100	1	18/18	100
			B	36/36	100	4	36/36	100	2	18/18	100
			C	36/36	100	5	36/36	100	3	18/18	100
									4	18/18	100
									5	18/18	100
									6	18/18	100
1	9,615	50,000	A	36/36	100	3	36/36	100	1	18/18	100
			B	36/36	100	4	36/36	100	2	18/18	100
			C	36/36	100	5	36/36	100	3	18/18	100
									4	18/18	100
									5	18/18	100
									6	18/18	100
Negative Serum	0	0	A	36/36	100	3	36/36	100	1	18/18	100
			B	36/36	100	4	36/36	100	2	18/18	100
			C	36/36	100	5	36/36	100	3	18/18	100
									4	18/18	100
									5	18/18	100
									6	18/18	100
Negative Serum	0	0	D	209/218	95.9	6	71/72	98.6	1	36/36	100
						5A	67/72	93.1	2	36/36	100
						5B	71/74	95.9	3	35/38	92.1
									4	31/36	86.1
									5	35/36	97.2
									6	36/36	100
Negative Serum	0	0	D	219/220	99.5	6	72/73	98.6	1	44/44	100
						3	74/74	100	2	44/44	100
						4	73/73	100	3	43/44	97.7
									4	43/43	100
									5	45/45	100

Technical Assistance

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

References

1. Choo Q-L, Weiner AJ, Overby, LR, et al. Hepatitis C virus: the major causative agent of viral non-A, non-B hepatitis. Br Med Bull 1990;46:423–41.
2. Choo Q-L, Kuo G, Weiner AJ, et al. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 1989;244:359–61.
3. EASL international consensus conference on hepatitis C. Consensus Statement. J Hepatol. 1999;30:956–61.
4. Alter HJ. Chronic consequences of non-A, non-B hepatitis. In: Current perspectives in hepatology. *Edited by LB Seef and JH Lewis. New York:Plenum Press, 1989;83–97.*
5. Colombo M, Rumi MG, Donato MF, et al. Hepatitis C antibody in patients with chronic liver disease and hepatocellular carcinoma. Dig Dis Sci 1991;36:1130–3.
6. Houghton M, Weiner A, Han J, et al. Molecular biology of the hepatitis C viruses: implications for diagnosis, development and control of viral disease. Hepatol 1991;14:381-8.
7. Cha TA, Beall E, Irvine B, et al. At least five related, but distinct, hepatitis C genotypes exist. Proc Natl Acad Sci USA 1992;89:7144–8.
8. Simmonds P, Holmes EC, Cha TA, et al. Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenic analysis of the NS-5 region. J Gen Virol 1993;74:2391–9.
9. Schiff ER, de Medina M, Kahn RS. New perspectives in the diagnosis of hepatitis C. Seminars in Liver Disease. 1999;19 (suppl 1):3–15.
10. *Sarrazin C, Teuber G, Kokka R, et al.* Detection of residual Hepatitis C virus RNA by transcription-mediated amplification in patients with complete virologic response according to polymerase chain reaction-based assays. Hepatol. 2000;32 (4):818–23.
11. Di Bisceglie AM. Hepatitis C. Lancet. 1998;351:351–5.
12. Recommendations for prevention and control of hepatitis C virus (HCV) infection and HCV-related chronic disease. MMWR. 1998; Vol. 47/No. RR-19:1-39
13. Beld M, Penning M, van Putten M, et al. Low levels of hepatitis C virus RNA in serum, plasma, and peripheral blood mononuclear cells of injecting drug users during long antibody-undetectable periods before seroconversion. Blood. 1999;94:1183–91.
14. Di Bisceglie AM, Bacon BR. The unmet challenges of hepatitis C. Sci Am 1999;80–85.
15. Poynard T, Marcellin P, Lee SS, et al. Randomised trial of interferon alfa2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alfa2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. Lancet. 1998;352:1426–32.
16. Kacian DL, Fultz TJ. Nucleic acid sequence amplification methods. 1995. U.S. Patent 5,399,491. European Patent EP 0 408 295.
17. Arnold LJ, Hammond PW, Wiese WA, Nelson NC. Assay formats involving acridinium-ester-labeled DNA probes. Clin Chem. 1989;35:1588–94.
18. Nelson NC, BenCheikh A, Matsuda E, Becker M. Simultaneous detection of multiple nucleic acid targets in a homogenous format. Biochem 1996;35:8429–38.
19. Bataille R, Harousseau JL Multiple myeloma. *New Engl J Med 1997;333:1657–64.*
20. Saldanha J, Lelie N, Heath A. Establishment of the first international standard for nucleic acid amplification technology (NAT) assays for HCV RNA. WHO Collaborative Study Group. Vox Sang. 1999;76(3):149–58.
21. National Committee for Clinical Laboratory Standards. Interference testing in clinical chemistry. Proposed Guideline. NCCLS document EP7-P. Wayne (PA):NCCLS;1986.

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GP P/N IN0079, Rev. A.

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GEN-PROBE

Bayer

versant®

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

Component	Quantity	Description	Storage
Auto Detect 1	1 x 240 mL	0.1% hydrogen peroxide in 1 mM nitric acid	15° to 30°C
Auto Detect 2	1 x 240 mL	1.6N sodium hydroxide	15° to 30°C

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

130655 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 493.1202© for guidance on appropriate quality control practices.

Materials Provided

Component	Quantity	Description	Storage
Negative Control	4 x 1 mL	Defibrinated normal human plasma containing gentamicin and 0.2% sodium azide	-15° to -35°C
HCV Positive Control	4 x 1 mL	Defibrinated normal human plasma with inactivated HCV containing gentamicin and 0.2% sodium azide	-15° to -35°C

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.[1-3]
- **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° 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.

Control	Target RNA Concentration (copies/mL)	HCV Interpretation
Negative	0	Non-reactive
Positive	300	Reactive

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

1. *Centers for Disease Control. Update: Universal precautions for prevention of transmission of* human immunodeficiency virus, hepatitis B virus and other bloodborne pathogens in healthcare settings MMWR. 1988;37:377–82, 387–8.
2. National Committee for Clinical Laboratory Standards. Protection of laboratory workers from instrument biohazards and infectious disease transmitted by blood, body fluids, and tissue; approved guideline. NCCLS Document M29-A. Wayne (PA):NCCLS;1997 Dec. 90p.
3. Federal Occupational Safety and Health Administration. Bloodborne Pathogens Standard, 29 CFR 1910.1030.

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