SIEMENS

# *VERSANT ® HCV Genotype 2.0 Assay (LiPA)* **RxOnly**

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| **Current Revision and Date** | 26036 Rev. 1, 2017-10 | |
| **Product Name** | VERSANT® HCV Genotype 2.0 Assay (LiPA) (40 Test) | REF 10492004 |
| | VERSANT® HCV Genotype 2.0 Assay (LiPA) (100 Test) | REF 10492005 |
| **Specimen Type** | Amplified Biotinylated DNA (PCR product) derived from VERSANT HCV Amplification 2.0 Kit (LiPA) | |
| **Sample Volume** | 10 µL Amplified Biotinylated DNA (PCR product) | |

## *Intended Use*

The VERSANT® HCV Genotype 2.0 Assay (LiPA) is a line probe assay, for *in vitro* diagnostic use, which identifies Hepatitis C virus (HCV) genotypes 1 to 6 and subtypes 1a and 1b in human serum or plasma ($K_2$EDTA, ACD-A CPD, and CPDA) samples.

The VERSANT® HCV Genotype 2.0 Assay (LiPA) is intended to be used as an aid in the management of patients with chronic HCV infection to guide the selection of antiviral treatment.

The VERSANT HCV Genotype 2.0 Assay (LiPA) is not approved as a donor screening test for HCV or as a diagnostic test to confirm the presence of HCV.

For Professional Use.

## *Summary and Explanation*

The Hepatitis C viral genome is highly variable. Based on a consensus proposal in 2005, the hepatitis virus is classified into 6 genotype groups based on phylogenetic analyses of the genomic sequence. These genotypes differ by 31% to 34% of their nucleotide sequences and about 30% of their amino acid sequences.[1] Each genotype contains one or more HCV subtypes or variants. Subtypes of a specific HCV genotype, of which there are a total of 67, differ by 20% to 25% at the nucleotide sequence level from other members of the genotype.[2]

In the United States, genotype 1 is the predominant HCV genotype (75.8%) followed by genotype 2 (12%), genotype 3 (10.4%), and genotype 4 (1.2%).[3] Genotypes 5 and 6, at less than 1% each, are rarely seen in the United States.[3]

The HCV genome consists of a core (C), envelope (E), nonstructural (NS) domains and 5'- and 3'-untranslated regions (also referred to as the noncoding region or NCR).[4,5] The NCR including the 5' untranslated region (UTR) is highly conserved while the envelope region is variable.[4] The 5' UTR contains multiple genotype-specific motifs that can provide accurate genotyping information for genotypes 1 to 5 and genotype 6, subtypes a and b. The core region contains genotype and subtype-specific motifs that can

help distinguish genotype 1 from genotype 6 (subtypes c to l), and can also help distinguish between subtype 1a and subtype 1b.

Determination of the HCV genotype is important to prescribe the appropriate HCV treatment regimen and to predict response to antiviral treatment. Until 2011, pegylated interferon (IFN) with or without ribavirin (RBV) was the standard of care with overall SVR rates of 50%-70% depending on the HCV genotype.[6,7] Since then, several direct active antivirals (DAAs) have been approved as therapies for HCV. These newer drugs are more effective treatments compared to IFN with or without RBV with SVR rates of 60%-100%. Even with these improved therapies, the HCV genotype is the mainstay for guiding selection of the most appropriate antiviral regimen.[6,7]

Both the American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver (EASL) base their optimal treatment recommendations on the HCV genotype.[6,7] At the subtype level, treatment recommendations are typically different for subtypes 1a and 1b. For all other HCV genotypes, there is no clinical relevance to knowing the subtype. Therefore, currently, it is most important and clinically relevant to determine the HCV subtype for genotype 1.

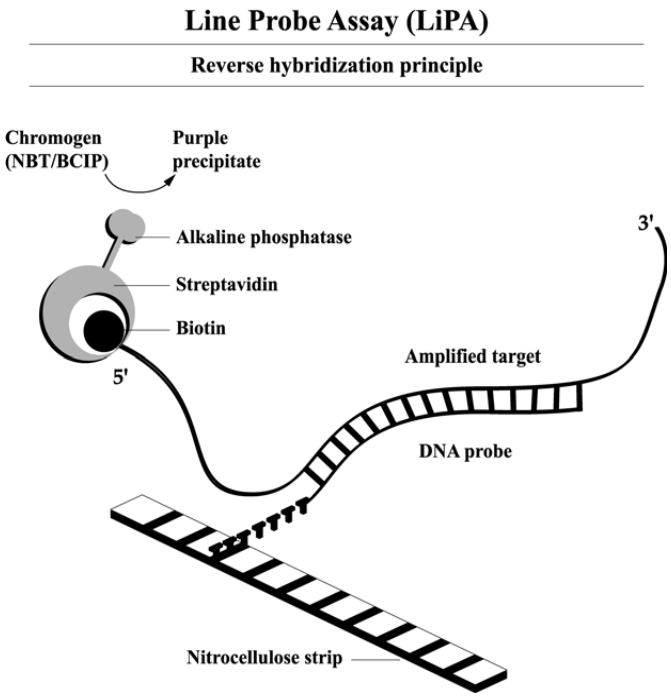
# *Principles of the Procedure*

The VERSANT HCV Genotype 2.0 Assay (LiPA) utilizes reverse hybridization technology to identify HCV genotypes (**Figure 1**). Using the VERSANT HCV Amplification 2.0 Kit, the 5’ UTR and core region of the HCV RNA are amplified by RT-PCR. This biotinylated DNA PCR product is hybridized to immobilized oligonucleotide probes.

The probes, which are bound to a nitrocellulose strip by a poly(T) tail, are specific for the 5' UTR and the core region of different HCV genotypes.

After the hybridization step, unhybridized PCR product is washed from the strip, and alkaline phosphatase-labeled streptavidin (conjugate) is bound to the biotinylated hybrid. BCIP/NBT chromogen (substrate) reacts with the streptavidin-alkaline phosphatase complex forming a purple/brown precipitate, which results in a visible banding pattern on the strip.

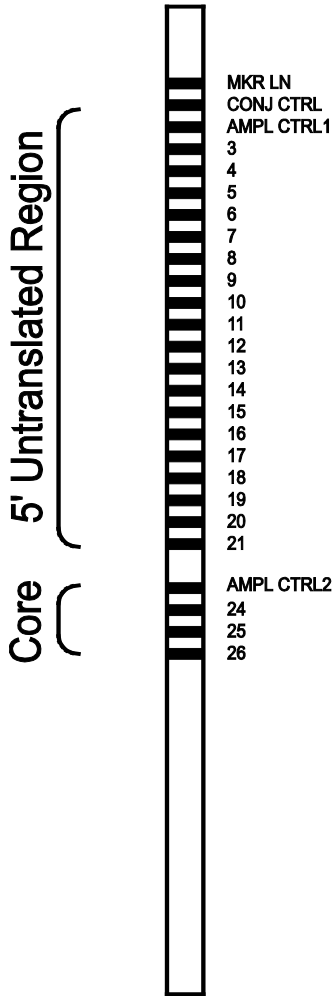
**Figure 1**




The VERSANT HCV Genotype 2.0 Assay (LiPA) strips have 3 control lines and 22 parallel DNA probe lines (**Figure 2**) containing sequences specific for HCV genotypes 1 to 6. The conjugate control (CONJ CTRL) line monitors the color development reaction. The amplification control (AMPL CTRL 1) at line 2 contains universal probes that hybridize to the PCR product from the 5' UTR. The amplification control (AMPL CTRL 2) at line 23 contains universal probes that hybridize to the PCR product from the core region.

HCV genotypes are determined by aligning the assay strips with the VERSANT HCV Genotype 2.0 Assay (LiPA) Reading Card and comparing the line patterns from the assay strips with the patterns shown on the VERSANT HCV Genotype 2.0 Assay (LiPA) Interpretation Chart. Automated reading and interpretation of the VERSANT HCV Genotype 2.0 Assay is available using the LiPA-Scan HCV IVD Software (US Only) version 1.00 or higher. For more information, refer to the LiPA-Scan HCV Software User's Manual.

**Figure 2**


5' Untranslated Region
Core
MKR LN
CONJ CTRL
AMPL CTRL1
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
AMPL CTRL2
24
25
26

# Reagents

Two VERSANT HCV Genotype 2.0 Assay (LiPA) configurations are available: the standard kit and the large kit. The standard kit contains sufficient reagents and materials to perform 40 tests including samples and controls in as many as four partial runs. The large kit contains sufficient reagents and materials to perform 100 tests including samples and controls in as many as five partial runs.

## VERSANT HCV Genotype 2.0 Assay (LiPA)

The VERSANT HCV Genotype 2.0 Assay (LiPA) contains the following components:

| Component | Standard Kit Quantity | Large Kit Quantity | Description | Storage |
|---|---|---|---|---|
| STRIPS | 2 x 20 | 5 x 20 | Nitrocellulose membranes coated with oligonucleotide probes. Identified by a green marker line | 2° to 8°C |
| CONJ 100X | 1 x 1.5 mL | 2 x 1.5 mL | Concentrated conjugate containing streptavidin-labeled with alkaline phosphatase with protein stabilizers and preservatives. | 2° to 8°C |
| CONJ DIL | 1 X 150 mL | 2 X 150 mL | Conjugate diluent containing phosphate buffer with 0.1% 2-Chloroacetamide, detergents, protein stabilizers and other preservatives | 2° to 8°C |
| DENAT SOLN | 1 x 1 mL | 2 x 1 mL | Denaturation solution containing 1.7% sodium hydroxide | 2° to 8°C |
| HYB SW SOLN | 2 x 220 mL | 5 x 220 mL | Hybridization and stringent wash solution containing sodium chloride, and sodium citrate buffer with detergent and preservatives | 2° to 8°C |
| RINSE SOLN 5X | 1 x 150 mL | 2 x 150 mL | Concentrated rinse solution containing phosphate buffer with 0.5% 2-Chloroacetamide, sodium chloride, detergent and other preservatives | 2° to 8°C |
| SUBS BUF | 2 x 180 mL | 3 x 180 mL | Substrate buffer containing TRIS buffer with 0.1% 2-Chloroacetamide, magnesium chloride, sodium chloride, and other preservatives | 2° to 8°C |
| SUBS BCIP/NBT 100X | 1 x 1.5 mL | 2 x 1.5 mL | Substrate containing 1.6% 5-Bromo-4-chloro-3-indolyl-phosphate and 4-Nitroblue tetrazolium in 83% dimethylformamide | 2° to 8°C |
| **READING CARD** | 1 | 1 | Used to identify positive bands on a strip | |
| **DATA REPORTING SHEETS** | 4 | 10 | Pages used for reporting and storing the results of an assay | |
| **INTERPRETATION CHART** | 1 | 1 | Provides banding patterns for identification of genotypes | |

All opened and unopened test reagents, including the coated test strips, are stable until the expiration date if kept refrigerated (2° to 8°C).

# *Warnings and Precautions*

**Danger!**

**SUBS BCIP/NBT 100X**: **H312+H332** Harmful in contact with skin. Harmful if inhaled. **H319** Causes serious eye irritation. **H360D** May damage the unborn child. **P201** Obtain special instructions before use. **P280** Wear protective gloves. Wear eye/face protection. Wear protective clothing. **P305+P351+P338** IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. **P308+P313** IF exposed or concerned: Get medical advice/attention. **P501** Dispose of contents and container in accordance with all local, regional, national and international regulations.
**Contains:** n,n-dimethylformamide

**Danger!**

**DENAT SOLN: H290** May be corrosive to metals. **H314** Causes severe skin burns and eye damage. **P280** Wear protective gloves. Wear eye/face protection. Wear protective clothing. **P301+P310+P331** IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician. Do NOT induce vomiting. **P303+ P361+P353+P310** IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. Immediately call a POISON CENTER or doctor/physician. **P305+P351+P338** IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. **P501** Dispose of contents and container in accordance with all local, regional, national and international regulations.
**Contains**: sodium hydroxide

**Warning!**

**SUBS BUF** and **RINSE SOLN 5X**: **H317** May cause an allergic skin reaction. **P280** Wear protective gloves. Wear eye/face protection. Wear protective clothing. **P272** Contaminated work clothing should not be allowed out of the workplace. **P302+P352** IF ON SKIN: Wash with plenty of soap and water. **P333+P313** If skin irritation or rash occurs get medical advice/attention. **P363** Wash contaminated clothing before reuse. **P501** Dispose of contents and container in accordance with all local, regional, national and international regulations.
**Contains**: 2-chloracetamide

- Use universal precautions when performing the assay. Handle samples with care to prevent sample contamination. Refer to *Appendix A* for more information about contamination control.
- 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 samples are handled.
- Avoid the use of sharp objects wherever possible.
- If skin or mucous membrane exposure occurs, immediately wash the area with copious amounts of water. Seek medical advice.
- Do not pipette by mouth.
- Do not use components beyond their expiration date.

- Store the kit away from any source of contaminating DNA, especially amplified nucleic acid.
- Alterations in the physical appearance of kit components may indicate instability or deterioration.
- Avoid microbial contamination of reagents when removing measured volumes from reagent bottles. Use sterile disposable pipettes and sterile packaged, aerosol resistant pipette tips.
- For additional guidance on contamination control, please refer to the Appendix A in this insert and CLSI MM3-A2, Molecular diagnostic methods for infectious diseases; Approved Guideline.[8]
- Use a new, sterile packaged, aerosol resistant pipette tip for each dispense of the sample.
- Use sterile disposable laboratory materials.
- Do not wash and reuse trays or other disposable materials.
- Do not mix reagents from different lots.
- Use all pipetting devices and instruments with care and follow the manufacturer's instructions for calibration and quality control.

# *Specimen Collection and Handling*

The VERSANT HCV Genotype 2.0 Assay (LiPA) uses PCR products specific to the 5' UTR and the core region of the HCV genome. These PCR products are produced using the VERSANT HCV Amplification 2.0 Kit (LiPA) after extraction of viral RNA from human serum or plasma using the VERSANT kPCR Molecular System SP and the VERSANT Sample Preparation 1.0 Reagents kit.

- This assay requires 10-µL of PCR product.
- After performing the denaturation step of the assay, store the remainder of the PCR product immediately (within 30 minutes) at -20° ± 5°C.
- Store the PCR product up to 4 months at -20°± 5°C. Do not exceed 4 freeze/thaw cycles of the PCR product.

# *Procedure*

## *Materials Provided*

| REF | Contents | Number of Tests |
|---|---|---|
| 10492004 | VERSANT HCV Genotype 2.0 Assay (LiPA) Standard Kit | 40 |
| 10492005 | VERSANT HCV Genotype 2.0 Assay (LiPA) Large Kit | 100 |

## Materials Required But Not Provided

### Sample Preparation Area

| REF | Description |
|---|---|
| 06635740 (10282928) | VERSANT kPCR Molecular System SP (VERSANT kPCR Sample Prep) |
| 10286026 | VERSANT Sample Preparation 1.0 Reagents Kit Box 1* |
| 10286027 | VERSANT Sample Preparation 1.0 Reagents Kit Box 2* |
| 10489008 | Large reagent troughs and small reagent troughs |
| 10282929 | 1000-µL pipette tips |
| 10282930 | 300-µL pipette tips |
| 10283255 | 96-well, 2-mL nuclease free, deep well plates |
| 10282998 | Barcoded 96-well semi-skirted polypropylene plates for PCR |
| 10361381 | Microcide SQ |
| | 70% ethanol (ethyl alcohol) |
| | Bleach, unscented (0.5% sodium hypochlorite) |
| | Deionized water |

### Amplification Area

| REF | Description |
|---|---|
| 10719668 | VERSANT HCV Control 2.0 Kit (LiPA)* |
| 10719666 | VERSANT HCV Amplification 2.0 Kit (LiPA) 40 Test* |
| 10719667 | VERSANT HCV Amplification 2.0 Kit (LiPA) 100 Test* |
| | Thermal cycler capable of a temperature range of 25° to 95°C and cycling at 1 degree/second and 2 degrees/second |
| | Dedicated pipette, adjustable for dispensing 1 to 20 µL, 20 to 200 µL, and 200 to 1000 µL for amplification steps |
| | Sterile-packaged, disposable pipette tips with aerosol-resistant barriers |
| | Sterile screw-cap polypropylene tubes (optional for storing eluate after extraction) |
| | PCR plates or sterile tubes according to the thermal cycler's instruction manual |
| | 70% ethanol (ethyl alcohol) |
| | Bleach, unscented (0.5% sodium hypochlorite) |

* Required materials for the extraction of HCV nucleic acids and generation of PCR product is a prerequisite to performing this assay. These required products for those steps are not included as part of the VERSANT HCV Genotype 2.0 Assay (LiPA).

### *Genotyping Area*

| REF | Description |
|---|---|
| 10315618 | AutoBlot 3000H Strip Processor* |
| 10315381 | AutoBlot 3000H Incubation Tray (25 pack)* |
| 10313066 | *Auto*-LiPA 48 Strip Processor* |
| 10325628 | *Auto*-LiPA 48 Incubation Tray (25 pack)* |
| | Distilled or deionized water |
| | Disposable gloves |
| | Plastic forceps |
| | Graduated cylinders to measure reagent volumes in Tables 1 and 2 |
| | One dedicated set of pipettes, adjustable for dispensing 1 to 20 µL, 20 to 200 µL, and 200 to 1000 µL to be used during both the conjugation and color development steps |
| | Sterile-packaged, aerosol-resistant, disposable pipette tips |
| | Timer (2 hours ± 1 minute) |
| | Stir bar (supplied with strip processor) |
| | Vortex mixer or equivalent |

***** Only one strip processor is required (AutoBlot3000H or *Auto*-LiPA 48)

## *Optional Materials*

| REF | Description |
|---|---|
| 10322207 | LiPA-Scan HCV Software verified 110 volt flat-bed scanner<br>Contact your technical applications specialist for information |
| 10329226 | LiPA-Scan Reading Template (25 pack), |
| 10492006 | LiPA-Scan HCV IVD Software version 1.00 or higher (US Only) |
| | A computer that meets the following specifications:<br>• Microsoft Windows XP or Microsoft Windows 7<br>• 1 GHz processor or faster<br>• 500 MB free Hard Disk Space (recommended: 2 GB or more)<br>• 2 GB RAM (recommended: 4 GB or more RAM)<br>• Minimum Screen Resolution: 800 by 600 pixels<br>• USB 2.0 is recommended |
| | Dispensing multipipettor (Multipipette, Eppendorf, or equivalent) |
| | Water bath temperature adjustable to minimum 50° ± 1°C |

## *Assay Procedure*

The procedure consists of these major activities: denaturing the PCR product, hybridizing the samples, washing the strips, and developing the color. The following sections describe the assay procedure using the AutoBlot 3000H or *Auto*-LiPA 48 Strip Processor.

### Procedural Notes

- Bring all VERSANT HCV Genotyping 2.0 Assay (LiPA) reagents and test strips to 20° to 25°C approximately 60 minutes before use and return to the refrigerator immediately after use.
- Perform in a location with room temperature at 20° to 25°C.
- Use clean forceps to handle strips. Do not touch strips with your hands as the oils from your hands could interfere with hybridization and color development.
- Use only pencil to write on the strips. The assay reagents may remove ink from the strips.
- To help prevent laboratory areas from becoming contaminated, maximize the physical separation of the pre- and post-amplification steps.
- Do not return PCR product, equipment, or reagents to the area where you performed the previous step. If you need to return to a previous work area, first perform the appropriate anti-contamination safeguards.
- Keep each strip in the same trough throughout the procedure.
- Refer to *Appendix A* for more information about contamination control.

### Reagents, Solutions and Strips Stability

- The diluted conjugate and substrate solutions are stable for 24 hours at 20° to 25°C when stored in the dark.
- The diluted rinse solution is stable for 24 hours at 20° to 25°C.
- All opened and unopened test reagents, including the coated test strips, are stable until the expiration date if kept at the appropriate storage conditions.
- Store developed dry strips in the dark at 18° to 25°C for up to 6 months.

# *Processing Strips on AutoBlot 3000H*

## Preparing the System

1. Remove the VERSANT HCV Genotype 2.0 Assay Kit from the refrigerator.
2. Allow all reagents to reach room temperature (20° to 25°C) before use.

   **NOTE:** Allow at least one hour to equilibrate to room temperature and refer to Steps 4 and 11 for additional pre-warming instructions for Hybridization/Stringent Wash Solution (HYB/SW SOLN).
3. Determine the volume of the HYB/SW SOLN needed to complete a run. Refer to **Table 1**. The volumes shown are sufficient to allow for priming the pump tubing.

   **NOTE:** If using more than one bottle, pre-warm and mix each bottle before combining contents.

**Table 1: AutoBlot 3000H Recommended Reagent Volumes**

| | Dilute Rinse Solution | | Dilute Conjugate | | Dilute Substrate | | Substrate Buffer | Hybridization and Stringent Wash Solution |
|---|---|---|---|---|---|---|---|---|
| No. of Strips | DI $H_2O$ (mL) | RINSE SOLN 5X (mL) | CONJ DIL (mL) | CONJ 100X (µL) | SUBS BUF (mL) | SUBS BCIP/NBT 100X (µL) | SUBS BUF (mL) | HYB/SW SOLN (mL) |
| 5 | 64 | 16 | 20 | 200 | 20 | 200 | 20 | 74 |
| 6 | 76 | 19 | 22 | 220 | 22 | 220 | 22 | 82 |
| 7 | 84 | 21 | 24 | 240 | 24 | 240 | 24 | 90 |
| 8 | 96 | 24 | 26 | 260 | 26 | 260 | 26 | 98 |
| 9 | 104 | 26 | 28 | 280 | 28 | 280 | 28 | 106 |
| 10 | 112 | 28 | 30 | 300 | 30 | 300 | 30 | 106 |
| 11 | 124 | 31 | 32 | 320 | 32 | 320 | 32 | 114 |
| 12 | 132 | 33 | 34 | 340 | 34 | 340 | 34 | 122 |
| 13 | 144 | 36 | 36 | 360 | 36 | 360 | 36 | 130 |
| 14 | 152 | 38 | 38 | 380 | 38 | 380 | 38 | 138 |
| 15 | 160 | 40 | 40 | 400 | 40 | 400 | 40 | 146 |
| 16 | 172 | 43 | 42 | 420 | 42 | 420 | 42 | 154 |
| 17 | 180 | 45 | 44 | 440 | 44 | 440 | 44 | 162 |
| 18 | 192 | 48 | 46 | 460 | 46 | 460 | 46 | 170 |
| 19 | 200 | 50 | 48 | 480 | 48 | 480 | 48 | 178 |
| 20 | 208 | 52 | 50 | 500 | 50 | 500 | 50 | 178 |

4. Pre-warm the HYB/SW SOLN in a water bath to 37° to 50°C for 60 minutes (optional) or refer to step 11 for pre-warming on the on the AutoBlot 3000H heated bottle plate.
5. Turn the AutoBlot 3000H on by pressing the ON/OFF switch on the back of the unit.
6. At the Ready for a New Test prompt, press **YES**.
7. At the Run Assay prompt, press **ENTER**.
8. Follow the screen prompt to latch pump pads, then press **YES**.
9. At the Preheat system prompt, press **YES**.
10. Follow the screen prompt to close the tray cover, then press **ENTER**.

    The system starts pre-heating the tray platform and the bottle plate.

    **NOTE:** During the preheat cycle, keep the aspirate cover closed.
11. While the system is preheating, prepare the reagents and strips.

    **NOTES:**

    - Preheat the system for 30 minutes if you pre-warmed the HYB/SW SOLN in a water bath.
    - Preheat the system for 60 minutes to warm both the system and the HYB/SW SOLN on AutoBlot 3000H heated bottle plate.
    - When preheating is complete, mix the HYB/SW SOLN well before use to dissolve all crystals.

## Preparing the Reagents

Refer to **Table 1** to determine the volume of each reagent needed to complete a run.

**NOTES:**

- The volumes shown are sufficient to allow for priming the pump tubing.
- Diluent volumes are rounded to the nearest 1 mL.

1. Prepare Dilute Rinse, Dilute Conjugate, and Dilute Substrate solutions using the following dilutions for each reagent:
    a. To make the Dilute Rinse solution, dilute the concentrated Rinse Solution (RINSE SOLN 5X) 1:5 (1 part + 4 parts) in distilled or deionized water.
    b. To make the Dilute Conjugate solution, dilute the concentrated Conjugate (CONJ 100X) 1:100 (1 part + 99 parts) in Conjugate Diluent (CONJ DIL).
    c. To make the Dilute Substrate solution, dilute the concentrated Substrate (SUBS BCIP/NBT 100X) 1:100 (1 part + 99 parts) in Substrate Buffer (SUBS BUF).

    **NOTES:**

    - Cover the Substrate vial with foil to protect it from light.
    - Mix reagents gently by inversion or swirling.
2. Fill the bottles with the correct reagents.
3. Place reagents in the AutoBlot 3000H reagent holder at the back of the system in the following order from left to right:
    a. Diluted Rinse solution
    b. SUBS BUF
    c. Diluted Substrate Solution
    d. Diluted Conjugate

4. Add a stir bar to the preheated HYB/SW SOLN and place it on the heated bottle plate, using the appropriate-size heat transfer ring.

## Preparing the PCR Product

1. Lay a clean bench liner or equivalent down on a clean bench top.
2. Allow PCR product to reach room temperature.
3. Write sample identifications and the kit lot numbers on LiPA-Scan Reading Templates.
4. Using clean forceps, remove the appropriate number of Strips (1 strip per sample) from the tube by gripping each above the green marker line.

   **NOTE:** Do not touch the Strips with your hands. Use clean forceps only.
5. Using a pencil only, label the top of each strip (above the green marker line) with an identification number.

   **NOTES:**

   - Use each trough only once.
   - Load Strips 1–10 beginning in the trough immediately to the right of the center trough (do not place strips in the center trough).
   - Load Strips 11–20 beginning in the trough immediately to the left of the center trough.
   - Place the negative control Strip in a trough between two troughs where sample strips will be processed.
6. Place the labeled strips in the upper portion of the trough (1 trough per strip) with the marker lines facing up at the top of the tray.

   **NOTES:**

   - If the strips curl, it is difficult to lay them in the trough.
     - To uncurl strips, pre-wet each strip with warmed Hybridization/Stringent Wash (HYB/SW).
     - Using clean forceps, dip each curled strip into HYB/SW reagent.
     - Wetting the strips with the HYB/SW reagent does not impact the performance of the assay.
   - When the system preheat cycle is completed, the system emits a beep to indicate the preheat cycle is done. Reagents warmed on the system may require additional time depending on the volume.
7. Follow the screen prompt to insert the straws into the reagent bottles.
8. Insert the Hybridization and the Stringent Wash straws into the HYB/SW bottle.
9. Prime the reagent pumps by dispensing two times for each line.
10. After a pump is primed, press **NO** to move onto the next pump to prime.
11. Enter the number of Strips to run (1–20), and then press **ENTER**.
12. At the Start Assay prompt, press **YES.**

## Denaturing the PCR Product and Starting the Run

1. Pipet 10 µL Denaturation Solution (DENAT SOLN) into the lower end of each trough starting from the trough to the right of the center trough, and working right.

   **NOTES:**

   - The Denaturation Solution must not come into contact with the strip.
   - If loading more than ten samples, start sample eleven to the left of the center trough and continue working left.
   - Close the vial immediately after use.

2. Add 10 µL of the appropriate PCR product directly into the DENAT SOLN in the trough, and mix by pipetting up and down approximately 3 times.

   **NOTES:**

   - The PCR product must not come into contact with the strip.
   - Change tips for each sample.

3. Incubate the tray at room temperature for 5 minutes to denature the PCR product.
4. Press **Enter,** then open the aspirate lid and quickly load the tray.
5. Close the aspirate lid and then press **Enter**.

   The automatic processing of the HCV protocol begins.

   **NOTES:**

   - During each incubation, the strips should be submerged and move freely in the troughs. Strips may flip over during the reagent addition steps; however, the assay performance is not affected as long as the strips are submerged and move freely.
   - Recommend the final aspiration is completed within 20 hours of the start of the run.

## Completing the Assay

1. At the Aspirate Now prompt, press **YES**.
2. At the Purge Tubing Now prompt, press **YES**.
3. Using clean forceps remove each strip from its trough and place the strip with the marker line facing up on absorbent paper.
4. Dry strips completely before reading.
5. Store the developed and dried strips in the dark at 18° to 25°C for up to 6 months.

## Cleaning the AutoBlot 3000H

1. Refer to the AutoBlot 3000H operator's guide for information on cleaning and maintaining the system.
2. Discard all materials in a safe and acceptable manner, and in accordance with proper laboratory practices and local environmental regulations.

## *Processing Strips on Auto-LiPA 48*

### Preparing the System

**1.** Remove the VERSANT HCV Genotype 2.0 Assay Kit from the refrigerator.

**2.** Allow all test materials to reach room temperature (20° to 25°C) before use.

**NOTE:** Allow at least two hours for the Hybridization/Stringent Wash Solution (HYB/SW SOLN) to equilibrate to room temperature and refer to Steps 4 and 20 for additional pre-warming instructions.

**3.** Determine the volume of the Hybridization/Stringent Wash Solution (HYB/SW SOLN) needed to complete a run. Refer to **Table 2**.

The volumes shown are sufficient to allow for priming the pump tubing.

**NOTE:** If using more than one bottle, pre-warm and mix each bottle before combining contents.

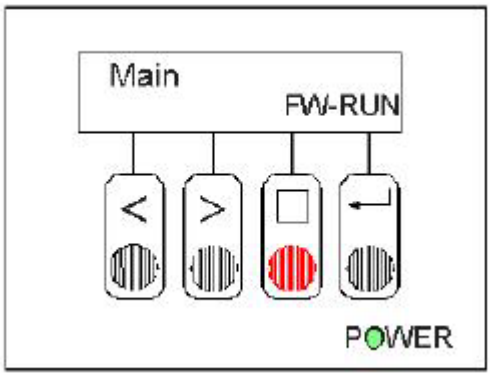
**Table 2: *Auto*-LiPA 48 Recommended Reagent Volumes**

| | Dilut Rinse Solution | | Dilute Conjugate | | Dilute Substrate | | Substrate Buffer | Hybridization and Stringent Wash Solution |
|---|---|---|---|---|---|---|---|---|
| No. of Strips | DI $H_2O$ (mL) | RINSE SOLN 5X (mL) | CONJ DIL (mL) | CONJ 100X (µL) | SUBS BUF (mL) | SUBS BCIP/NBT 100X (µL) | SUBS BUF (mL) | HYB/SW SOLN (mL) |
| 3 | 48 | 12 | 16 | 160 | 16 | 160 | 16 | 44 |
| 6 | 76 | 19 | 22 | 220 | 22 | 220 | 22 | 68 |
| 9 | 104 | 26 | 28 | 280 | 28 | 280 | 28 | 92 |
| 12 | 132 | 33 | 34 | 340 | 34 | 340 | 34 | 116 |
| 15 | 160 | 40 | 40 | 400 | 40 | 400 | 40 | 140 |
| 18 | 192 | 48 | 46 | 460 | 46 | 460 | 46 | 164 |
| 21 | 220 | 55 | 52 | 520 | 52 | 520 | 52 | 188 |
| 24 | 248 | 62 | 58 | 580 | 58 | 580 | 58 | 212 |
| 27 | 276 | 69 | 64 | 640 | 64 | 640 | 64 | 236 |
| 30 | 304 | 76 | 70 | 700 | 70 | 700 | 70 | 260 |
| 33 | 336 | 84 | 76 | 760 | 76 | 760 | 76 | 284 |
| 36 | 364 | 91 | 82 | 820 | 82 | 820 | 82 | 308 |
| 39 | 392 | 98 | 88 | 880 | 88 | 880 | 88 | 332 |
| 42 | 420 | 105 | 94 | 940 | 94 | 940 | 94 | 356 |
| 45 | 448 | 112 | 100 | 1000 | 100 | 1000 | 100 | 380 |
| 48 | 480 | 120 | 106 | 1060 | 106 | 1060 | 106 | 404 |

**4.** Pre-warm the Hybridization/Stringent Wash Solution (HYB/SW SOLN) reagent bottle in a water bath (optional) at 37° to 50°C (approximately 60 minutes) and mix well before use to dissolve crystals and refer to Preparing the Reagents, Steps 3 and 4. Alternatively, place the HYB/SW SOLN bottle in an appropriate-size heat transfer shell, add a magnetic stir bar to the bottle, and place it on the incubator unit of the *Auto*-LiPA 48 to pre-warm.

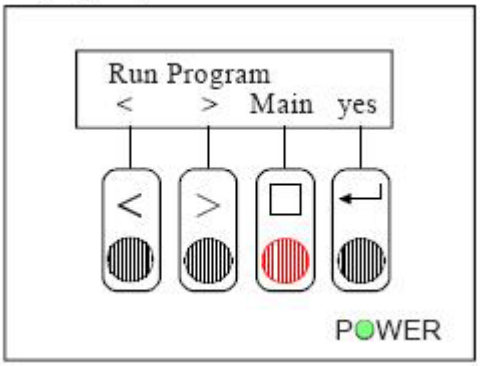
**5.** Use the ON/OFF switch on the back panel of the system to turn the system on.

**6.** Wait until the system proceeds to the Main Mode and displays the following message:




**7.** Press the **RUN** key to enter the Standby Mode.

Run Program displays.




The *Auto*-LiPA 48 is in the Run Program menu and ready to start a test.

**8.** Remove and clean metal sieves (filters) from the heating/cooling system distribution tubes before each use.

**NOTE:** If sieves are dirty, clean them under running water.

**9.** Fill the 2 bottles of the heating/cooling system with distilled water.

Fill the hot water bottle to the upper portion of the red marker line and the cold water bottle to its broadest upper portion.

**10.** Insert these bottles in the Styrofoam holders.

**11.** Place the holders in the system.

**12.** Insert the system distribution tubes.

**13.** Place the covers securely over the Styrofoam holders.

**14.** Confirm that the waste tubes are properly inserted:

- The tube with the black label is inserted into the Waste bottle.
- The tube without a label is inserted into the Hazardous Waste Bottle.

**15.** On the Run Program display (standby mode), select **YES**.

**16.** To confirm, WASTE BOTTLE OK, select **YES**.

The message "Check Sieves" displays.

**17.** If the sieves were checked and cleaned as described in step 8, select **YES**.

**18.** Using the + and – keys, scroll through the assay menu and select **LiPAv2**.

**19.** To confirm your selection, select **YES**.

**20.** To accept the hybridization temperature of 50.0°C, select **YES**.

The instrument's heating system starts to come to the specified temperature.

**NOTES:**

- Confirm that the magnetic stir bars are working properly by picking up the metal holder and verifying the change in sound.
- Preheat for approximately 45 to 60 minutes to warm both the system and the HYB/SW SOLN on the incubator unit of the *Auto*-LiPA 48.

## Preparing the Reagents

Refer to **Table 2** to determine the volume of each reagent needed to complete a run.

**NOTES:**

- The volumes shown are sufficient to allow for priming the pump tubing.
- Diluent volumes are rounded to the nearest 1 mL.

**1.** Prepare Dilute Rinse, Dilute Conjugate, and Dilute Substrate solutions using the following dilutions for each reagent:

a. To make the Dilute Rinse solution, dilute the concentrated Rinse Solution (RINSE SOLN 5X) 1:5 (1 part + 4 parts) in distilled or deionized water.

b. To make the Dilute Conjugate solution, dilute the concentrated Conjugate (CONJ 100X) 1:100 (1 part + 99 parts) in Conjugate Diluent (CONJ DIL).

c. To make the Dilute Substrate solution, dilute the concentrated Substrate (SUBS BCIP/NBT 100X) 1:100 (1 part + 99 parts) in Substrate Buffer (SUBS BUF).

**2.** Fill the bottles with the correct reagents.

**3.** Place the bottles in the system on the corresponding colored spots as shown in **Table 3**.

**NOTE:** Verify reagent bottles are more than 1 inch away from the heated water bottle and the bottle incubator.

**Table 3: Color Coding for Reagent Bottles**

| VERSANT HCV Genotype Assay (LiPA) Reagent | Tube Number | Tube Color Code |
|---|---|---|
| HYB/SW SOLN (Stringent Wash bottle) | 1 and 2 (bundle of 3 tubes) | Green and Blue |
| RINSE SOLN (Diluted) | 3 (bundle of 3 tubes) | Orange |
| CONJ (Diluted) | 4 | Red |
| SUBS BUF | 5 | White |
| SUBS BCIP/NBT (Diluted) | 6 | Yellow |

4. Insert the color-coded tubes into the corresponding bottles as described in **Table 3**. Make sure the tubes reach the bottom of the bottles.

   **NOTE:** Stringent Wash (2) and Rinse Solution (3) are bundles of 3 tubes.

## Preparing the PCR Product

1. Lay a clean bench liner or equivalent down on a clean bench top.
2. Allow the PCR product to reach room temperature.
3. Write sample identifications and the kit lot numbers on LiPA-scan reading templates.
4. Using clean forceps, remove the appropriate number of Strips (1 strip per sample) from the tube by gripping each above the green marker line.

   **NOTE:** Do not touch the Strips with your hands. Use clean forceps only.
5. Using a pencil only, label the top of each strip (above the green marker line) with an identification number.

   **NOTES:**
   - Place the negative control Strip in a trough between two troughs where sample Strips will be processed.
   - Use each trough only once.
6. Place the labeled strips in the upper portion of the tray (1 trough per Strip) with the marker lines facing up at the top of the tray.

   **NOTES:**
   - If the strips curl, it is difficult to lay them in the trough.
     - To uncurl strips, pre-wet each strip with warmed Hybridization/Stringent Wash (HYB/SW).
     - Using clean forceps, dip each curled strip into HYB/SW reagent.
     - Wetting the strips with the HYB/SW reagent does not impact the performance of the assay.
   - Wait until the system displays “Insert Tray” to prepare your samples.

## Denaturing the PCR Product and Starting the Run

1. Pipet 10 µL Denaturation Solution (DENAT SOLN) into the lower end of each trough.

   **NOTES:**

   - The Denaturation Solution must not come into contact with the strip.
   - Close the vial immediately after use.

2. Add 10 µL of PCR product directly into DENAT SOLN in the trough, and mix by pipetting up and down approximately 3 times.

   **NOTES:**

   - The PCR product must not come into contact with the strip.
   - Change tips for each sample.

3. Load the tray into the *Auto*-LiPA 48.
4. Ensure that the tray is completely pushed to the back of the tray support and positioned in the center.

   The tray should fit completely over the seals of the tray support.

5. Close the Tray cover using the three tray-clamping bar clips and the two lid screws.
6. Close the system front cover.

   **NOTE:** The run will not proceed if the system cover is not closed.

7. The *Auto*-LiPA 48 displays "Insert tray, press any key."
8. Press any key.
9. Select the specific starting position.
10. Enter the number of STRIPS to run (1–48).
11. When asked if the last aspiration step should be done, select **NO**.

    The first step of the procedure displays "Proc. 1 : INC."

12. To start the run, select **YES**.

    At the end of the assay, the *Auto*-LiPA 48 displays "Pause Cont." to allow the final aspiration.

    **NOTES:**

    - During each incubation, the strips should be submerged and move freely in the troughs. Strips may flip over during the reagent addition steps; however, the assay performance is not affected as long as the strips are submerged and move freely.
    - Recommend the final aspiration is completed within 20 hours of the start of the run.

13. Press the button under "Cont."

    The *Auto*-LiPA 48 aspirates the final rinse solution.
    The message "You should clean, press any key" displays.

14. Press any key to end the program.

## Completing the Assay

1. Remove the tray with samples and place it next to the system.
2. Discard leftover reagents and waste materials in a safe and acceptable manner, and in accordance with proper laboratory practices and local environmental regulations.

   **NOTE:** Remember to remove the magnetic stir bar from the HYB/SW SOLN.

3. Insert an empty tray into the *Auto*-LiPA 48 and close the cover to prevent dust from falling into the tray holder.

4. Use clean forceps to remove each strip from its trough and place the strip with the marker line facing up on absorbent paper.
5. Dry strips completely before reading.
6. Store the developed and dried strips in the dark at 18° to 25°C for up to 6 months.

### Cleaning the *Auto*-LiPA 48

1. Refer to the *Auto*-LiPA 48 operator's manual for information on cleaning and maintaining the system.
2. Discard all materials in a safe and acceptable manner, and in accordance with proper laboratory practices and local environmental regulations.

## *Quality Control*

1. Include one HCV negative control and one HCV positive control in each run.

   **Procedural Note:** Process the controls along with the samples through the extraction and amplification steps. The VERSANT HCV Control 2.0 Kit (LiPA) includes plasma based control samples specifically designed for use in the VERSANT HCV Genotype 2.0 Assay (LiPA), starting from extraction.

   - The VERSANT negative HCV control sample (CONTROL **-**) should have only a positive CONJ CTRL (Line 1). There should be no apparent signal for any other line on the strip.
   - The VERSANT positive HCV control sample (CONTROL **+**) should give positive results on the following lines: CONJ CTRL (Line 1), AMPL CTRL 1 (Line 2), 13, 14, 15, AMPL CTRL 2 (Line 23), and 24.

   If either control gives a pattern other than the one specified for the control, the run is invalid and must be repeated.
2. Line 1 of the strip is the CONJ CTRL.

   **Procedural Note:** This line is always positive when the strip was processed correctly. The intensity of this line should be similar to each strip in the same assay run.

   **NOTE:** If the CONJ CTRL (Line 1) line is negative, the strip is invalid and you must repeat the LiPA assay for that sample.
3. Line 2 of the strip is the AMPL CTRL 1 line.

   **NOTE:** This line is positive when PCR product specific to the HCV 5' UTR is present.
   - The presence of positive CONJ CTRL (Line 1) and AMPL CTRL 1 (Line 2) lines on a positive control or a patient sample qualify the results for further interpretation.
   - A positive CONJ CTRL (Line 1) line and a negative AMPL CTRL 1 (Line 2) line on a strip indicate that the strip was processed correctly, but PCR product specific to the HCV 5' UTR was not present in the reaction trough.
   - If AMPL CTRL 1 (Line 2) is not present then the strip cannot be interpreted.

4. Line 23 of the strip is the AMPL CTRL 2 line.

   **NOTE:** This line is positive when PCR product specific to the HCV core region is present.
   When this line is negative, PCR product specific to the HCV core region was not present in the reaction trough.

# Results

## Interpreting Results

### A. Semi-Automated Interpretation

1. Attach strips to the LiPA-Scan Reading Template.
   **NOTE:** Carefully align the conjugate control line (CONJ CTRL) of the strips with the pre-printed black alignment bar on the reading template.
2. Refer to the *LiPA-Scan HCV Software User's Manual* for additional instructions on preparing and reading the strips using the LiPA Scan Software.

**NOTE:** Before scanning results, ensure that the scanner is configured and calibrated.

3. When using the LiPA-Scan software for determining the HCV genotype of a sample, the operator must perform the following steps to ensure the interpretation is correct:
   - The operator verifies that strip alignment is correct for the software to correctly call the reactive bands.
   - The operator verifies that the software did not inappropriately call a band that is not visible (i.e., the operator should confirm that the software is calling bands and not extraneous materials on the strip)
   - The operator verifies that the software did not miss a band that is visible to the eye.
   - The operator edits the file if the band calling is not correct based on the observations above.

### B. Manual Interpretation

**NOTES:**

- Part A of the Interpretation Chart is used to interpret genotype 1, subtypes a and b of genotype 1, and the genotype 6 patterns that are identified using both the 5' UTR and the core region.
- Part B of the Interpretation Chart is used to interpret genotypes 2 to 5, and those genotype 6 patterns that are distinguishable from genotype 1 using the 5' UTR only.

1. Attach the strips to a Data Reporting Sheet.
2. Place the Reading Card over the strip being read.

**NOTE**: Ensure that the green marker line on the strip aligns with MKR LN on the Reading Card.

**Procedural Note:** A line is considered positive when a clear purple/brown band appears on the strip, at the end of the color development procedure. Color intensities between lines on a strip may differ from one line to the next.

3. Record on the Data Reporting Sheet all line numbers that are positive on each strip.
4. For each qualified sample, compare the banding pattern of lines 1 to 21 to the 5' UTR sections of the Interpretation Chart.
5. For all samples with a pattern shown in the 5' UTR section of Part A of the Interpretation Chart:
   - If the AMPL CTRL 2 (line 23) is positive, compare the banding pattern of lines 23 to 26 to the patterns shown in the core region of the Interpretation Chart and report the result associated with the core pattern.
   - If information from the core region is unavailable or inconclusive, you will not be able to differentiate genotype 1 and genotype 6. In this case, the result from the 5' UTR section may be reported with a note that information from the core region is not available, and the possibility of genotype 6 cannot be excluded. The correct interpretation of subtypes 1a and 1b requires the

presence of the core region.

**NOTE:** The band pattern 1, 2, 7 is indeterminate when the core lines 23 and/or 24 are negative.

- If the AMPL CTRL 2 (Line 23) is positive, but the pattern of lines 23 to 26 does not match any of the patterns shown in the core region section of the Interpretation Chart, the result cannot be interpreted.

**6.** Part B of the Interpretation Chart shows patterns which are interpreted using only the 5' UTR section of the chart. Positive reactivity on core lines 23 to 26 may be observed, but these lines are not considered in the interpretation of the results of these genotypes.

**7.** If the pattern does not match any of the patterns shown in the 5' UTR sections of the Interpretation Chart, the results cannot be interpreted.

# *Limitations*

- The reagents and procedures for extraction, amplification and genotype identification steps should only be used by personnel trained in molecular biology techniques.
- These reagents and procedures have been designed to allow identification of the sample's HCV genotype even at low viral titers. Like any highly sensitive method that utilizes amplification methodology, extreme care must be taken when performing this assay, because the amplification kit is prone to carryover of trace amounts of contamination (from HCV positive samples or HCV amplicons) that may interfere with interpretation and accurate HCV genotyping. Therefore, it is essential that such contamination is avoided by careful adherence to the workflow and cleaning procedures specified in these instructions. Good laboratory practices and careful performance of the procedures are required for correct results. Refer to Appendix A for more information about contamination control.
- If information from the HCV core region is not available, it is not possible to differentiate genotype 6 from genotype 1. Furthermore, HCV subtypes 1a and 1b are more accurately identified using information from the core region.
- In rare cases, unidentifiable patterns may be produced. These may be due to the sequence heterogeneity of the HCV genome, mixed infections or cross contamination. In these cases, it is recommended that an alternate method of HCV genotyping be used.
- Recently, recombinant HCV isolates have been identified[9-13]. The prevalence of recombinant strains, though relatively rare, varies in different regions of the world[14-15]. The performance characteristics have not been established for this assay using recombinant strains. Depending on the site of recombination in hepatitis C virus, the assay may give an uninterpretable result or a genotype for one of the parent strains of the recombinant virus.
- This kit uses viral RNA extracted from human serum or plasma. The VERSANT Sample Preparation 1.0 Reagents Kit used with the VERSANT kPCR Molecular System SP have been validated for use with this kit. No other reagents, procedures, and methods have been validated for use with this kit.

# *Performance Characteristics*

## Analytical Sensitivity

### Limit of Detection (LoD) by Genotype

The VERSANT HCV Genotype 2.0 Assay (LiPA) Limit of Detection (LoD) was estimated separately in serum and plasma for HCV genotypes 1a, 1b, 2, 3, 4, 5, and 6. A single HCV specimen from each genotype was diluted to target concentrations ranging from 25 IU/mL to 1000 IU/mL. Four (4) lots of the VERSANT Sample Preparation 1.0 Reagent Kits and the VERSANT HCV Control 2.0 Kit (LiPA); and 7 lots of the VERSANT HCV Amplification 2.0 Kit (LiPA) and the VERSANT HCV Genotype 2.0 Assay (LiPA) Kit were used to estimate and confirm the LoD. Each target concentration level was tested with a minimum of 2 replicates per run, with 2 runs per day, for at least 4 days, for a total of at least 20 measurements.

The LoD estimates for genotypes 1-6 in plasma and serum are shown in **Table 4**.

**Table 4: VERSANT HCV Genotype 2.0 Assay (LiPA)**

**Limit of Detection Estimates (IU/mL)**

| | 1a | 1b | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| **Plasma (IU/mL)** | 400 | 300 | 200 | 150 | 300 | 300 | 350 |
| **Serum (IU/mL)** | 350 | 350 | 300 | 200 | 300 | 650 | 350 |

The limit of detection (LoD) for the VERSANT HCV Genotype 2.0 Assay (LiPA) is 400 IU/mL in plasma and 650 IU/mL in serum.

### Upper Assay Limit

Assay performance was evaluated in 30 HCV specimens with high viral loads representing HCV genotypes 1a, 1b, 2, 3, 4, 5 and 6. The viral loads ranged from 1,204,177 IU/mL to 7,692,310 IU/mL. All 30 specimens (100%) produced the correct HCV genotype with the VERSANT HCV Genotype 2.0 Assay (LiPA) demonstrating an upper assay limit of 7.7 million IU/mL.

## Analytical Specificity

### Analytical Carryover Specificity

Carryover specificity for the VERSANT HCV Genotype 2.0 Assay (LiPA) was evaluated using a high titer HCV genotype 1a sample (1,289,253 IU/mL) and HCV negative human plasma. Seventy two (72) replicates of the HCV positive sample were arranged in a checkboard pattern with 72 replicates of the HCV negative sample to maximize the potential for cross-contamination during nucleic acid extraction and PCR amplification. No amplification was observed in any of the HCV-negative samples. Carryover specificity was 100% (72/72) with 95% CI: 94.93% to 100%, with three thermocyclers: the GeneAmp PCR System 9700, the Agilent Mx3005P, and the Biorad PTC-200 and with both the *Auto*-LiPA 48 and the AutoBlot 3000H strip processors.

## Potential Interfering Substances

Potential interference of endogenous substances and common anti-viral drugs on the performance of the VERSANT HCV Genotype 2.0 Assay (LiPA) was evaluated using a single HCV specimen from genotype 1a, 1b, 2 and 3 diluted to the target concentrations shown in **Table 5** and with HCV negative human plasma as specificity controls.

**Table 5: Target HCV Concentrations Used for Testing Interference**

| HCV Genotype | Endogenous Substance Interference Target HCV Concentration (IU/mL) | Antiviral Drug Interference Target HCV Concentration (IU/mL) |
|---|---|---|
| 1a | 1300 | 714 |
| 1b | 5000 | 6360 |
| 2 | 600 | 738 |
| 3 | 600 | 378 |

## Endogenous Substances

Potential interference from hemoglobin, triglycerides, protein and conjugated and unconjugated bilirubin was evaluated at the elevated levels shown in **Table 6.**

**Table 6: Endogenous Substances Tested**

| Endogenous Substance | Concentrations Tested |
|---|---|
| Hemoglobin | 500 mg/dL |
| Triglycerides | 3000 mg/dL |
| Conjugated bilirubin | 20 mg/dL |
| Unconjugated bilirubin | 20 mg/dL |
| Protein | 9 g/dL |

No interference in the performance of the VERSANT HCV Genotype 2.0 Assay (LiPA) was observed in the presence of endogenous substances for all HCV positive and negative samples tested.

## Commonly Prescribed Anti-Viral Drugs Study

Potential interference in assay performance was evaluated for commonly prescribed anti-viral drugs listed in **Table 7**. The drugs were pooled into 5 groups based on their solubility properties and tested at three times their Cmax concentrations.

**Table 7: Drugs Evaluated for Potential Interference**

| Drug Class | Drug Name |
|---|---|
| Nucleoside HIV RT inhibitors | Abacavir sulfate (ABC)<br>Didanosine (ddI)<br>Emtricitabine (FTC)<br>Lamivudine (3TC)<br>Stavudine (d4T)<br>Zidovudine (AZT) |
| Nucleotide DNA Polymerase Inhibitors | Tenofovir (TNV)<br>Adefovir dipivoxil |
| HIV and HCV Protease Inhibitors | Atazanavir<br>Boceprievir<br>Darunavir<br>Fosamprenavir<br>Indinavir<br>Lopinavir<br>Nelfinavir<br>Ritonavir |

| Drug Class | Drug Name |
|---|---|
| | Saquinavir<br>Telaprevir<br>Tipranavir |
| Non-Nucleoside RT Inhibitors | Efavirenz<br>Nevirapine |
| Fusion inhibitor | Enfuvirtide<br>Maraviroc |
| HIV Integrases | Raltegrivir |
| HCV and HBV antivirals | Acyclovir<br>Entecavir<br>Ganciclovir<br>Ribavirin<br>Telbivudine<br>Valganciclovir hydrochloride |
| Immune Moderators | Interferon alfacon-1<br>Interferon alpha-2b<br>Peginterferon alpha-2a<br>Peginterferon alpha-2b |

No interference in the performance of the VERSANT HCV Genotype 2.0 Assay (LiPA) was observed in the presence of anti-viral drugs for all HCV positive and negative samples tested.

## Cross Reactivity

Potential cross-reactivity with the VERSANT HCV Genotype 2.0 Assay (LiPA) was evaluated using a single HCV specimen each from genotype 1a, 1b, 2 and 3 diluted to the target concentrations shown in **Table 8**. HCV negative human plasma and serum were also tested as specificity controls.

**Table 8: Target HCV Concentrations Used for Testing Cross Reactivity**

| HCV Genotype | Target HCV Concentration (IU/mL) |
|---|---|
| 1a | 1300 |
| 1b | 450 |
| 2 | 600 |
| 3 | 600 |

Ten (10) unique patient samples for each of the non-HCV disease states listed in **Table 9** were tested for cross-reactivity. The VERSANT HCV Genotype 2.0 Assay (LiPA) gave accurate HCV genotype results in the presence of these disease states for all HCV positive and negative samples tested.

**Table 9: Non- HCV Disease States Evaluated for Cross-Reactivity**

| Non- HCV Disease States |
|---|
| Antinuclear Antibody Disease State (ANA) |
| Rheumatoid Arthritis |
| Systemic Lupus Erythematosus (SLE) |

The viruses (HIV, CMV, EBV and HBV), fungus and bacteria listed in **Table 10** were tested for cross-reactivity with the VERSANT HCV Genotype 2.0 Assay (LiPA) at medically relevant concentrations. Ten (10) unique hepatitis A patient specimens were also tested. Samples containing the listed microorganisms gave accurate HCV genotype results with the VERSANT HCV Genotype 2.0 Assay (LiPA) for all HCV positive and negative samples tested.

**Table 10: Microorganisms Evaluated for Potential Interference**

| Viruses | Fungus and Bacteria |
|---|---|
| Hepatitis A Virus | Candida albicans |
| Hepatitis B Virus | Enterobacter cloacae |
| Human Immunodeficiency Virus | Escherichia coli |
| Epstein-Barr Virus | Haemophilus influenzae |
| Cytomegalovirus | Klebsiella pneumoniae |
| | Pseudomonas aeruginosa |
| | Pseudomonas fluorescens |
| | Serratia marcescens |
| | Streptococcus agalactiae |
| | Staphylococcus aureus |
| | Staphylococcus epidermidis |
| | Streptococcus pneumonia |

## Specimen Types and Stability

Specimen Stability was evaluated by diluting one specimen each from HCV Genotypes 1a, 1b, 2 and 3 in $K_2$EDTA Plasma, Plasma from PPT tubes, Serum and Serum from SST tubes at the target concentrations shown in **Table 11**.

**Table 11: Target HCV Concentrations Used to Test Specimen Stability in K2EDTA Plasma, PPT tubes, Serum and SST tubes**

| HCV Genotype | Target HCV Concentration (IU/mL) |
|---|---|
| 1a | 1300 |
| 1b | 450 |
| 2 | 600 |
| 3 | 600 |

Specimens in $K_2$EDTA Plasma, Plasma from PPT tubes, Serum and Serum from SST tubes may be stored at 2 to 8°C for 48h, -20±5 °C for 72 h or at -60 to -80 °C for long term storage for use with the VERSANT HCV Genotype 2.0 Assay (LiPA). Specimens should not exceed four freeze-thaw cycles prior to use with the VERSANT HCV Genotype 2.0 Assay (LiPA).

ACD, CPD and CPDA plasma were also validated for use with the VERSANT HCV Genotype 2.0 Assay (LiPA) by diluting 30 independent specimens of HCV Genotypes 1a, 1b, 2, 3, 4, 5 and 6 into ACD, CPD and CPDA plasma at 1.5X the Limit of Detection (refer to **Table 4**). All three anticoagulants have acceptable performance with the VERSANT HCV Genotype 2.0 Assay (LiPA).

## Reproducibility

Reproducibility of the VERSANT HCV Genotype 2.0 Assay (LiPA) was evaluated using a 14-member reproducibility panel. A single specimen from HCV genotypes 1a, 1b, 2, 3, 4, 5 and 6 were each diluted in HCV negative human plasma to a low target and a high target HCV concentration as shown in **Table 12**.

**Table 12: Target HCV Concentrations Used for Evaluating Reproducibility**

| HCV Genotype | Low Target HCV Concentration (IU/mL) | High Target HCV Concentration (IU/mL) |
|---|---|---|
| 1a | 714 | 100,000 |
| 1b | 6099 | 100,000 |
| 2 | 738 | 100,000 |
| 3 | 378 | 100,000 |
| 4 | 3531 | 100,000 |
| 5 | 579 | 100,000 |
| 6 | 378 | 100,000 |

A total of 3 lots each of the VERSANT Sample Preparation 1.0 Reagent Kit, the VERSANT HCV Control 2.0 Kit (LiPA), the VERSANT HCV Amplification 2.0 Kit (LiPA) and the VERSANT HCV Genotype 2.0 Assay (LiPA) Kit were used to process 54 replicates of each panel member at each of 3 sites.

No significant difference in performance of the VERSANT HCV Genotype 2.0 Assay (LiPA) was observed with different reagent kit lots using either the manual or the semi-automated method of interpretation as shown in **Table 13**.

**Table 13: Effect of Reagent Kit Lot on Assay Performance**

| | Manual Method of Interpretation | | | | Semi-Automated Method of Interpretation | | | |
|---|---|---|---|---|---|---|---|---|
| Kit Lot | Genotyping Rate[a] [Fraction] | Genotyping Rate [Percent] | Genotyping Accuracy[b] [Fraction] | Genotyping Accuracy [Percent] | Genotyping Rate [Fraction] | Genotyping Rate [Percent] | Genotyping Accuracy [Fraction] | Genotyping Accuracy [Percent] |
| Lot 1 | 740/753 | 98.27 | 735/740 | 99.32 | 736/756 | 97.74 | 732/736 | 99.46 |
| Lot 2 | 740/756 | 97.88 | 738/740 | 99.73 | 735/756 | 97.22 | 733/735 | 99.73 |
| Lot 3 | 743/759 | 97.89 | 742/743 | 99.87 | 739/759 | 97.36 | 738/739 | 99.86 |

**[a] Genotyping rate is the proportion of valid genotype results that were interpretable**

**[b] Genotyping accuracy is the proportion of interpretable results that match the reference method results**

Forty-two (42) of the 45 samples that had non-interpretable genotype results were from low target HCV concentration panel members. Among the high concentration panel members, only 3 samples could not be genotyped. All inaccurate genotypes were from the low HCV concentration panel members and half of the inaccurate results were genotype 1a samples that were reported as genotype 1 results because of missing information from the core region.

Assay performance, measured by genotyping rate and genotyping accuracy, at Site 3 was significantly different from the other two sites as shown in **Table 14**. No significant difference in assay performance was observed between Site 1 and Site 2. The difference at Site 3 was attributable to one operator. After further training the site corrected this problem, as shown by an inter-site comparison of a subset of the specimens used to evaluate assay accuracy as shown in **Table 15**.

### Table 14: Effect of Clinical Trial Site on Assay Performance

| Clinical Site | Manual Method of Interpretation | | | | Semi-Automated Method of Interpretation | | | |
|---|---|---|---|---|---|---|---|---|
| | Genotyping Rate[a] [Fraction] | Genotyping Rate [Percent] | Genotyping Accuracy[b] [Fraction] | Genotyping Accuracy [Percent] | Genotyping Rate[a] [Fraction] | Genotyping Rate [Percent] | Genotyping Accuracy[b] [Fraction] | Genotyping Accuracy [Percent] |
| Site 1 | 748/756 | 98.94 | 747/748 | 99.87 | 749/756 | 99.07 | 748/749 | 99.87 |
| Site 2 | 750/756 | 99.21 | 749/750 | 99.87 | 745/756 | 98.54 | 744/745 | 99.87 |
| Site 3 | 725/756 | 95.90 | 719/725 | 99.17 | 716/756 | 94.71 | 711/716 | 99.30 |

**[a] Genotyping rate is the proportion of valid genotype results that were interpretable**

**[b] Genotyping accuracy is the proportion of interpretable results that match with the reference method results**

**Table 15: Effect of Clinical Trial Site on Assay Performance with a Subset of Specimens used to evaluate Assay Accuracy**

| Clinical Site[a] | Manual Method of Interpretation | | | | Semi-Automated Method of Interpretation | | | |
|---|---|---|---|---|---|---|---|---|
| | Genotyping Rate[b] [Fraction] | Genotyping Rate [Percent] | Genotyping Accuracy[c] [Fraction] | Genotyping Accuracy [Percent] | Genotyping Rate [Fraction] | Genotyping Rate [Percent] | Genotyping Accuracy [Fraction] | Genotyping Accuracy [Percent] |
| Site 1 | 189/193 | 97.93 | 189/189 | 100 | 187/193 | 96.89 | 187/187 | 100 |
| Site 2 | 191/194 | 98.45 | 189/191 | 98.95 | 188/194 | 96.91 | 186/188 | 98.94 |
| Site 3 | 186/193 | 96.37 | 185/186 | 99.46 | 186/193 | 96.37 | 185/186 | 99.46 |

[a] **Site 1, 2 and 3 are the same three sites described in Table 14.**

[b] **Genotyping rate is the proportion of valid genotype results that were interpretable**

[c] **Genotyping accuracy is the proportion of interpretable results that match with the reference method results**

## Accuracy

Accuracy of the VERSANT HCV Genotype 2.0 Assay (LiPA) was evaluated in 800 unique, retrospectively collected HCV genotype 1-6 specimens including 140 specimens each from HCV Genotype 1a, 1b, 2 and 3 and 80 specimens each of HCV Genotype 4, 5 and 6. The reference method used to determine the correct genotype was nucleotide sequencing of the NS5b region.

The percentage of specimens that yielded a genotype result ranged from 90% to 100% for the individual genotypes using the manual method of interpretation as shown in **Table 16**. The percentage of correct genotypes ranged from 98.6% to 100% for the individual genotypes.

The percentage of specimens that yielded a genotype result ranged from 87.5% to 100% for the individual genotypes using the semi-automated method of interpretation as shown in **Table 17**. The percentage of correct genotypes ranged from 98.55% to 100% for the individual genotypes.

The HCV genomes of the two (2) discordant genotype 1b specimens were sequenced in their entirety and were found to be genotype 2/1b recombinants with breakpoints in the NS2 region. These recombinant samples were reported as genotype 1b by NS5b sequencing and as genotype 2 by the VERSANT HCV Genotype 2.0 Assay which targets the 5'UTR region.

**Table 16: Genotyping Rate and Genotyping Accuracy using the VERSANT HCV Genotype 2.0 Assay (LiPA) Manual Method of Interpretation**

| Genotype | Total Tested | Genotyping Rate[a] [Fraction] | Genotyping Rate [Percent (95% CI[c])] | Genotyping Accuracy[b] [Fraction] | Genotyping Accuracy [Percent (95% CI[c])] |
|---|---|---|---|---|---|
| 1a | 140 | 138/140 | 98.6 (94.6, 99.82) | 137/138 | 99.3 (96.01, 99.87) |
| 1b | 140 | 140/140 | 100 (96.30, 100) | 138/140[d] | 98.6 (94.94, 99.61) |
| 2 | 140 | 140/140 | 100 (96.30, 100) | 140/140 | 100 (97.33, 100) |
| 3 | 140 | 140/140 | 100 (96.30, 100) | 140/140 | 100 (97.33, 100) |
| 4 | 80 | 78/80 | 97.5 (88.64, 99.08) | 78/78 | 100 (95.31, 100) |
| 5 | 80 | 75/80 | 93.8 (86.30, 98.29) | 75/75 | 100 (95.13, 100) |
| 6 | 80 | 72/80 | 90.0 (75.83, 93.09) | 72/72 | 100 (94.93, 100) |

**[a] Genotyping rate is the proportion of valid genotype results that were interpretable**

**[b] Genotyping accuracy is the proportion of interpretable results that match with the reference method result**

**[c] The 95% upper and lower confidence limits**

**[d] The two discordant genotype 1b samples were recombinant genotype 2/1b specimens**

**Table 17: Genotyping Rate and Genotyping Accuracy using the VERSANT HCV Genotype 2.0 Assay (LiPA) Semi-Automated Method of Interpretation**

| Genotype | Total Tested | Genotyping Rate[a] [Fraction] | Genotyping Rate [Percent (95% CI[c])] | Genotyping Accuracy[b] [Fraction] | Genotyping Accuracy [Percent (95% CI)[c]] |
|---|---|---|---|---|---|
| 1a | 140 | 136/140 | 97.1 (92.88, 98.88) | 135/136 | 99.26 (95.95, 99.87) |
| 1b | 140 | 138/140 | 98.6 (94.94, 99.61) | 136/138[d] | 98.55 (94.87, 99.6) |
| 2 | 140 | 140/140 | 100 (97.33, 100) | 140/140 | 100 (97.33, 100) |
| 3 | 140 | 140/140 | 100 (97.33, 100) | 140/140 | 100 (97.33, 100) |
| 4 | 80 | 78/80 | 97.5 (91.34, 99.31) | 78/78 | 100 (95.31, 100) |
| 5 | 80 | 76/80 | 95 (87.84, 98.04) | 76/76 | 100 (95.19, 100) |
| 6 | 80 | 70/80 | 87.5 (78.5, 93.07) | 70/70 | 100 (94.8, 100) |

**[a] Genotyping rate is the proportion of valid genotype results that were interpretable**

**[b] Genotyping accuracy is the proportion of interpretable results that match with the reference method result**

**[c] The 95% upper and lower confidence limits**

**[d] The two discordant genotype 1b samples were recombinant genotype 2/1b specimens**

## Clinical Studies

The clinical utility of the VERSANT Genotype 2.0 Assay (LiPA) was assessed by evaluating the association between the HCV genotype, as determined by the VERSANT Genotype 2.0 Assay (LiPA), and the probability of achieving Sustained Virologic Response at 12 weeks ($SVR_{12}$) to the treatment regimens outlined in **Table 19**. Duration of the treatment was based on the pretreatment HCV genotype assignment determined by the clinical trial from which the samples were derived.

Subject demographics of the study population are summarized in **Table 18**.

**Table 18: Demographics of HCV Infected Subjects**

| Characteristics | Category | Number of Subjects | Percentage of Total |
|---|---|---|---|
| Age | < 40 Years | 21 | 9.5 |
| | ≥ 40 Years | 199 | 90.5 |
| Gender | Female | 85 | 38.6 |
| | Male | 135 | 61.4 |
| Race/Ethnicity | White | 173 | 79 |
| | American Indian or Alaska Native | 7 | 3.2 |
| | Asian | 18 | 8.2 |
| | Black | 20 | 9.1 |
| | Native Hawaiian or Other Pacific Islander | 1 | 0.5 |
| | Other | 1 | 0.5 |
| Baseline HCV RNA (Genotype 1a & b) | < 3E6 IU/mL | 120 | 54.5 |
| | ≥ 3E6 IU/mL | 100 | 45.5 |
| Baseline HCV RNA (Genotype Non 1a & b) | < 3E6 IU/mL | 91 | 65 |
| | ≥ 3E6 IU/mL | 49 | 35 |

A total of 220 specimens with known treatment outcomes were included in the study. Of these, 217 specimens yielded a valid genotype result that was interpretable. The distribution of these 217 results by genotype along with the treatment regimens are summarized in **Table 19**.

**Table 19: $SVR_{12}$ Results using the VERSANT HCV Genotype 2.0 Assay (LiPA)**

| Genotype | Treatment Received[a] | Manual Method of Interpretation | | Semi-Automated Method | |
|---|---|---|---|---|---|
| | | $SVR_{12}$[b] (Fraction) | % SVR [Percent, (95% CI [e])] | $SVR_{12}$ [b] (Fraction) | % SVR [Percent, (95% CI [e])] |
| 1a | SOF+peg-IFN+RBV | 35/39 | 89.74 (76.42, 95.94) | 34/38 | 89.47 (75.87, 95.83) |
| 1b | SOF+peg-IFN+RBV | 29/40 | 72.50 (57.17, 83.89) | 29/40 | 72.50 (57.17, 83.89) |
| 1[c] | SOF+peg-IFN+RBV | 64/79 | 81.01 (71.01, 88.14) | 63/78 | 80.77 (70.67, 87.98) |
| 2 | SOF+RBV | 40/40 | 100 (91.24, 100) | 40/40 | 100 (91.24, 100) |
| 3 | SOF+RBV | 32/40 | 80 (65.24, 89.50) | 32/40 | 80 (65.24, 89.50) |
| 4 | SOF+peg-IFN+RBV | 19/20 | 95 (76.39, 99.11) | 19/20 | 95 (76.39, 99.11) |
| 5 | SOF+LDV | 18/18 | 100 (82.41, 100) | 19/19 | 100 (83.18, 100) |
| 6 | SOF+LDV | 19/20 | 95 (76.39, 99.11) | 19/20 | 95 (76.39, 99.11) |
| All non-1[d] | Mixed Treatments | 128/138 | 92.75 (87.17, 96.02) | 129/139 | 92.81 (87.26, 96.05) |
| **Total** | | **192/217** | **88.48** (83.55, 92.07) | **192/217** | **88.48 (83.55, 92.07)** |

**[a] SOF= Sofosbuvir; LDV=Ledipasvir; peg-IFN=Pegylated interferon; RBV=Ribavirin**

**[b] $SVR_{12}$= Sustained Virologic Response assessed at 12 weeks post initiation of treatment. $SVR_{12}$ column refers to the fraction of patients that had SVR to the indicated treatment.**

**[c] Genotype 1a and Genotype 1b results were pooled to obtain genotype 1.**

**[d] All non-1 is pooled genotypes 2, 3, 4, 5,6**

**[e] The 95% upper and lower confidence limits**

The odds for the $SVR_{12}$ rate for genotype 1 was statistically significantly lower relative to genotype 2 (odds ratio of 0.051). Furthermore, genotype 1 (pooled subtype 1a and 1b) resulted in statistically significant lower odds for $SVR_{12}$ as compared to the group of all non-1 genotypes (pooled genotypes 2, 3, 4, 5 and 6). See **Tables 20 and 21**.

**Table 20: Odds Ratios for $SVR_{12}$ by Genotype Based on the Manual Method of Interpretation**

| Genotype Comparison | $SVR_{12}$ Rate Comparison | $SVR_{12}$ Rate Ratio | Odds Ratio | Odds Ratio (95% CI[a]) |
|---|---|---|---|---|
| 1 vs 2 | 81.01% vs. 100% | 0.810 | 0.051 | (0.003, 0.882) |
| 1 vs 3 | 81.01% vs. 80% | 1.013 | 1.067 | (0.410, 2.778) |
| 1 vs 4 | 81.01% vs. 95% | 0.853 | 0.225 | (0.028, 1.812) |
| 1 vs 5 | 81.01% vs. 100% | 0.810 | 0.112 | (0.006, 1.970) |
| 1 vs 6 | 81.01% vs. 95% | 0.853 | 0.225 | (0.028, 1.812) |
| 1 vs all non-1 | 81.01% vs. 92.75% | 0.873 | 0.333 | (0.142, 0.783) |

[a] 95% lower and upper confidence interval

**Table 21: Odds Ratios for $SVR_{12}$ by Genotype Based on the Semi-Automated Method of Interpretation**

| Genotype Comparison | $SVR_{12}$ Rate Comparison | $SVR_{12}$ Rate Ratio | Odds Ratio | Odds Ratio (95% CI[a]) |
|---|---|---|---|---|
| 1 vs 2 | 80.77% vs. 100% | 0.808 | 0.051 | (0.003, 0.869) |
| 1 vs 3 | 80.77% vs. 80% | 1.010 | 1.050 | (0.403, 2.736) |
| 1 vs 4 | 80.77% vs. 95% | 0.850 | 0.221 | (0.027, 1.784) |
| 1 vs 5 | 80.77% vs. 100% | 0.808 | 0.105 | (0.006, 1.837) |
| 1 vs 6 | 80.77% vs. 95% | 0.850 | 0.221 | (0.027, 1.784) |
| 1 vs all non-1 | 80.77% vs. 92.81% | 0.870 | 0.326 | (0.138, 0.766) |

[a] 95% lower and upper confidence interval

## *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. www.siemens.com/diagnostics.

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## *Understanding the Symbols*

The following symbols may appear on the labeling and packaging:

| Symbol | Description |
|---|---|
| IVD | *In vitro* diagnostic medical device |
|  | Use by |
| REF | Catalog Number |
| LOT | Batch code |
|  | Consult instructions for use |
|  | Temperature Limitation |
|  | Manufacturer |
| **RxOnly** | Caution: Federal (USA) law restricts this device to sale by or on the order of a licensed healthcare professional. |
|  | Store Upright |



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# APPENDIX A

## Recommendation of Laboratory Design for Contamination Control

Three separate working areas are required to minimize the risk of contamination of a new sample or reagent with nucleic acid from preceding samples or amplification products (carryover contamination). Workflow in the laboratory must proceed in a unidirectional manner, beginning in the RNA extraction area (Area 1) and moving into the RT-PCR master mix preparation and addition of extracted sample to the RT-PCR reactions (Pre-amplification) area (Area 2) and then into the RT-PCR amplification and detection area (Area 3).

The three areas may be in one room, if necessary. If two rooms are available, combine Area 1 and Area 2 in one room and have Area 3 in a separate room. In this case, it is recommended that the floor is marked to indicate the various areas.

- To minimize the risk of possible carryover contamination, ensure that RNA extraction and reagent preparation are separated. For example, use separate hoods facing in opposite directions or separate rooms.
- Never perform sample handling and or RNA extraction and reagent preparation in the same hood.
- To avoid cross-contamination, have two separate hoods available in Area 2. These hoods should not face each other. Use one hood in Area 2 for RT-PCR master mix preparation. Use the other hood in Area 2 for adding the extracted RNA and prepared RT-PCR master mix to the amplification PCR plate or tubes for RT-PCR amplification.
- Dedicate all equipment, tubes, and pipette tips to the specific work area.
- Pipette tips must contain aerosol-resistant barriers.
- Remove gloves and lab coats before changing areas.

**Area 1 – RNA Extraction**: For the extraction and preparation of RNA samples.

- Do not bring amplified reaction mixtures into Area 1.
- Area 1 must be a separate area or separate room containing a certified biological safety cabinet.
- All equipment in Area 1 should be used exclusively in Area 1.
- Proceed to Area 2 after removing your laboratory coat and gloves.

**Area 2 – Reagent Preparation & Pre-Amplification:** For the preparation of RT-PCR master mix and the addition of extracted RNA to the reaction well or tube for amplification.

Dedicate separate hoods in Area 2 to preparing the RT-PCR master mix and the addition of extracted sample RNA to the reaction well or tubes for amplification. Equipment and materials (such as pipettes, tips, gloves, and tubes) are to be dedicated for each hood in Area 2 and kept separate from other work areas.

- Do not bring amplified reaction mixtures, extracted samples or clinical specimens into the RT-PCR master mix preparation hood in Area 2.
- Do not bring equipment and materials from the hood used for sample addition into the RT-PCR master mix preparation hood in Area 2.
- Do not bring amplified reaction mixtures or un-extracted clinical specimens into the hood used for adding extracted sample to the RT-PCR reaction well or tubes for amplification in Area 2. Extracted sample RNA and prepared master mix may be used in this hood.
- All equipment in Area 2 should be used exclusively in Area 2. Maintain separate supplies of materials and equipment for each hood in Area 2.

- Do not bring equipment and materials used during post amplification steps such as performing the genotype assay into this area.
- Proceed to Area 3 after removing your laboratory coat and gloves.

**Area 3 – Post RT-PCR Amplification and Detection:** For post RT-PCR Amplification and running the genotype assay.

- After entering Area 3, do not go back into Areas 1 or 2 the same day.
- If possible, hand off items for Area 3 to someone who will not enter Areas 1 or 2 that day. If handing-off is not possible, wear clean shoe covers and gloves, and a dedicated Area 3 lab coat into Area 3.
- Upon exiting Area 3 and before entering any of the other areas, decontaminate by disposing of shoe covers and gloves, and removing your lab coat, to prevent contamination of Areas 1 and 2.

To prevent cross-contamination between areas, provide disposable shoe covers at the entrance of Area 3.