SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: Genotyping assay for hepatitis C virus

Device Trade Name: VERSANT® Sample Preparation 1.0 Reagents
VERSANT® HCV Amplification 2.0 Kit (LiPA)
VERSANT® HCV Genotype 2.0 Assay (LiPA)
VERSANT® HCV Control 2.0 Kit (LiPA)

Device Procode: OBF

Applicant's Name and Address: Siemens Healthcare Diagnostics
725 Potter Street
Berkeley, CA 94710

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P160016

Date of FDA Notice of Approval: March 14, 2017

II. INDICATIONS FOR USE

The genotyping device for hepatitis C virus (LiPA) consists of the following 4 separate kits:

- VERSANT® HCV Genotype 2.0 Assay (LiPA)
- VERSANT® Sample Preparation 1.0 Reagents
- VERSANT® HCV Amplification 2.0 Kit (LiPA)
- VERSANT® HCV Control 2.0 Kit (LiPA)

VERSANT® HCV Genotype 2.0 Assay (LiPA)

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 for use as a donor screening test for HCV or as a diagnostic test to confirm the presence of HCV.

VERSANT® Sample Preparation 1.0 Reagents

The VERSANT® Sample Preparation 1.0 Reagents kit is intended to be used, along with the VERSANT® kPCR Molecular System Sample Preparation module (SP Module), for the isolation and purification of nucleic acids for in vitro diagnostic

applications. The product is intended for use by laboratory professionals trained in molecular biology techniques.

VERSANT® HCV Amplification 2.0 Kit (LiPA)

The VERSANT® HCV Amplification 2.0 Kit (LiPA) is designed for use with the VERSANT® HCV Genotype 2.0 Assay (LiPA) and the VERSANT® HCV Control 2.0 Kit (LiPA) products. The HCV Amplification 2.0 kit is for *in vitro* diagnostic use for reverse transcription and amplification of the 5' untranslated region (5'UTR) and core region of the Hepatitis C Virus (HCV) genome.

VERSANT® Control 2.0 Kit (LiPA)

The VERSANT® HCV Control 2.0 Kit (LiPA) is designed for use with the VERSANT® HCV Amplification 2.0 Kit (LiPA) and the VERSANT® HCV Genotype 2.0 Assay (LiPA) products.

The VERSANT® HCV Control 2.0 Kit (LiPA) is for *in vitro* diagnostic use to monitor the performance of all steps of the VERSANT® HCV Genotype 2.0 assay.

All four kits are required to extract nucleic acids from patient samples, serve as external controls, amplify specific regions of the HCV sequence, hybridize the amplified material to probes and identify the HCV genotype.

III. CONTRAINDICATIONS

None

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the labeling for the VERSANT® Sample Preparation 1.0 Reagents, VERSANT® HCV Amplification 2.0 Kit (LiPA), VERSANT® HCV Genotype 2.0 Assay (LiPA) and VERSANT® Control 2.0 Kit (LiPA).

V. DEVICE DESCRIPTION

Four VERSANT® reagent kits are required to obtain an HCV genotype from a serum or plasma sample from a patient infected with HCV using the VERSANT® HCV Genotype 2.0 Assay (LiPA):

1 . Extraction and Purification of Viral RNA
The VERSANT® kPCR Molecular System SP is a fully automated sample preparation instrument that utilizes VERSANT® Sample Preparation 1.0 Reagent for the isolation and purification of nucleic acids using magnetic bead extraction technology. The VERSANT® Sample Preparation 1.0 Reagent consists of Box 1 that contains buffers, beads and wash solutions and Box 2 (SMN 10286027) that contains the enzyme to lyse cells.

2. Reverse Transcription – Polymerase Chain Reaction

Amplification of the HCV RNA is performed using the VERSANT® HCV Amplification 2.0 Kit (LiPA). The kit contains 4 or 10 tubes of enzyme mix for the 40 test and 100 test kits, respectively, as well as either 1 or 2 tubes of amplification mix for the 40 test and 100 test kits, respectively. The enzyme mix consists of reverse transcriptases, a DNA polymerase and Uracil-N-Glycosylase. The amplification mix contains synthetic oligonucleotides in buffer with dNTP/dUTP and $MgCl_2$.

3. Genotyping with the VERSANT® HCV Genotype 2.0 Assay (LiPA)

The detection and identification of HCV genotypes using the VERSANT® HCV Genotype 2.0 Assay (LiPA) (SMN 10492004/40 tests and 10492005/100 tests) is based upon reverse hybridization of the RT-PCR (Reverse Transcriptase Polymerase chain reaction) amplified nucleic acid to HCV sequence specific oligonucleotides (probes) bound to a membrane strip. The VERSANT® HCV Genotype 2.0Assay (LiPA) Kit contains the reagents listed in the table below.

Table 1: Components in the VERSANT® HCV Genotype 2.0 Assay (LiPA)

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
100X Conjugate	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
Conjugate Dilution Buffer	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
Denaturation Solution	1 x 1 mL	2 x 1 mL	Denaturation solution containing 1.7% sodium hydroxide	2° to 8°C
Hybridization and Stringent Wash Solution	2 x 220 mL	5 x 220 mL	Hybridization and stringent wash solution containing sodium chloride, sodium citrate buffer with detergent and preservatives	2° to 8°C
5X Rinse Solution	1 x 150 mL	2 x 150 mL	Concentrated rinse solution containing phosphate buffer with 0.5% 2-Chloroacetamide, NaCl, detergent and other preservatives	2° to 8°C
Substrate Buffer	2 x 180 mL	3 x 180 mL	Substrate buffer containing TRIS buffer with 0.1% 2- Chloroacetamide, $MgCl_2$, NaCl and other preservatives.	2° to 8°C
100X BCIP/NBT Substrate	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	

4. Controls

The VERSANT® HCV Control 2.0 Kit (LiPA) (SMN 10719668) contains 2 tubes of negative control (human plasma, nonreactive for HCV) and 2 tubes of positive control (Armored RNA construct containing HCV 5' UTR (untranslated region) and Core sequences for Genotype 3 in human plasma that is nonreactive for HCV). The positive and negative controls require the same isolation and purification steps to extract the nucleic acids from the plasma matrix, as the test samples.

Sample Preparation

The VERSANT® kPCR Molecular System SP is a fully automated liquid handling workstation used in conjunction with VERSANT® Sample Preparation 1.0 Reagents to isolate nucleic acids. The VERSANT® kPCR Molecular System consists of the bar code reader, instrument and software. Assay parameters specific to the VERSANT® HCV Genotype 2.0 Assay (LiPA) assay are contained in an assay specific test definition file. The magnetic silica technology is a nonspecific capture method and is target independent; it captures any DNA or RNA present in the sample. Due to their extremely small size and homogeneous shape, the magnetic beads can be fully dispersed in solution, allowing thorough nucleic acid binding, washing and elution.

The VERSANT® Sample Preparation technology employs chaotropic high salt conditions to disrupt cells and release nucleic acids, as well as protect them from cellular nucleases. The nucleic acids are captured on silica coated beads. The beads are separated by a magnetic field and washed to remove proteins, nucleases, and other cellular impurities. The nucleic acids are eluted in a small volume of elution buffer, ready for subsequent analysis.

Two hundred and fifty microliters (250 µL) of patient sample, or Positive or Negative controls are processed using the automated VERSANT® kPCR Molecular System SP. The Positive and Negative controls from the VERSANT® Control HCV 2.0 Kit (LiPA) are processed along with the patient samples and can be used for run validation at all steps of the assay.

The VERSANT® kPCR Molecular System SP produces 50 µL of eluate and a volume of 20 µL is needed for the amplification step. The remainder of the eluate can be stored at -80 ºC for further testing. Additionally, depending on time constraints, the user can continue with the next step or store the eluate at -80 ºC until the next day.

Reverse Transcription and Amplification

Twenty microliters (20 µL) of extracted RNA is added to the RT-PCR mastermix prepared from reagents in the VERSANT® HCV Amplification 2.0 Kit (LiPA). All reagents needed for both reactions are added prior to initiation of the reverse transcription step so that the tube does not need to be manipulated or opened until the PCR amplification reactions are complete.

The genomic HCV RNA is reversed transcribed into cDNA using a mixture of 2 reverse transcriptases enzymes, an HCV specific primer, and an excess of a mixture of four deoxynucleoside triphosphates (dNTP) and deoxyuridine triphosphate (dUTP). Reverse

transcriptase extends the primer along the target RNA, thus copying the RNA into its exact DNA complement (cDNA).

The mixture is then heated to inactivate the reverse transcriptases and activate the Taq polymerase. The reaction uses two pairs of biotinylated primers, and a thermostable Taq DNA polymerase to co-amplify two distinct biotinylated DNA fragments specific for the 5' UTR and core regions of HCV. These primers are designed to permit amplification of two regions of the genome which are used to determine the different genotypes. These primers are labeled with biotin to facilitate visualization of the biotinylated DNA end product in the subsequent LiPA genotyping procedure.

PCR Product Contamination Control

PCR amplification is performed in the presence of dUTP to generate amplification products which can be selectively degraded by treatment with uracil-N- glycosylase (UNG) and heat. UNG cleaves the glycosidic bond between the uracil base and the deoxyribose ring, creating a basic site that is susceptible to heat. The ENZ MIX component of the VERSANT® HCV Amplification 2.0 Kit (LiPA) contains a heat-labile UNG enzyme. Following the addition of the purified viral RNA to the master mix and prior to the initiation of the RT step, the reaction mixture is allowed to incubate at room temperature for 10 minutes. This step removes the uracil base from any contaminating amplification product made with dUTP. The UNG is inactivated when the temperature is increased to 50 ºC to initiate the RT reaction, preventing the removal of uracil from the newly synthesized cDNA. As the temperature is further increased during the first PCR step, any abasic amplification products are completely degraded.

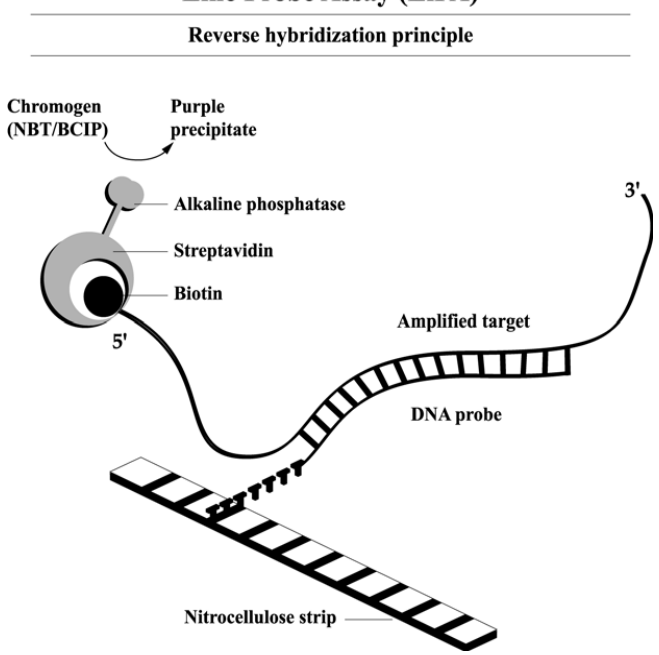
Genotyping with the VERSANT® HCV Genotype Assay (LiPA)

The detection and identification of HCV genotypes using the VERSANT® HCV Genotype 2.0 Assay (LiPA) is based upon reverse hybridization of the RT-PCR amplified nucleic acid to HCV sequence specific oligonucleotides (probes) bound to a membrane strip.

The genotyping regions chosen for use in the assay are the 5'UTR and core regions of the Hepatitis C virus. The 5'UTR region is optimal for genotyping due to the high conservation within—but increased variability between—genotypes. Probes specific for the core region have been added to allow improved discrimination between subtypes a and b of genotype 1 and to enable the differentiation of the rare genotype 6, subtypes c-l, from genotype 1.

The amplified, biotinylated DNA derived from a patient specimen (the target nucleic acid) is hybridized to a set of sequence-specific oligonucleotide probes that are immobilized on a nitrocellulose membrane. The probes are attached to the nitrocellulose support by a poly(T) tail and are specific for the 5'UTR or core region of the different HCV genotypes. Each type of probe is applied as a different line, and thus the test is referred to as a line probe assay or LiPA. Under well-defined hybridization conditions, including pH and temperature, such probes allow the specific capture of matching DNA fragments from the patient specimen in a single reaction and subsequent visual detection as described below.

Line Probe Assay Test Principle



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Once the hybridization procedure is completed, a wash step is performed to remove any unbound or weakly bound material. An alkaline phosphatase-labeled streptavidin conjugate is then added, which binds to the biotinylated hybrid. Upon addition of the chromogen substrate, 5-bromo-4-chloro- 3-indolyl-phosphate and 4-nitro blue tetrazolium (BCIP/NBT), a purple brown precipitate is formed that reacts with the streptavidin-alkaline phosphatase complex. This results in a pattern of positive bands on the strip that are indicative of the patient's genotype. Due to small differences in probe affinity, reaction conditions, or amount of amplified material, the color intensity of the different lines may vary. Because the assay is a qualitative test, lines are read as positive or negative based on both visual detection and calibrated optical density cut-off values. The HCV genotype of the specimen is interpreted from the resulting pattern of probe lines on the strip.

Genotype Interpretation

The LiPA strips contain three control lines that assure specific steps of the assay are successfully performed:

The conjugate control line (line 1) is designed to ensure that the color development reaction has taken place. The absence of conjugate control line indicates that an error occurred during the detection step of the assay, such as inappropriate amount of a reagent, the wrong reagent added during strip processing or a reagent was improperly prepared. Strips that do not develop a conjugate control line are considered invalid and the assay will need to be repeated.

Two amplification control lines have been included on the strip, one specific for the 5'UTR region (line 2) and the other for the core region of the HCV genome (line 3). These lines are designed to confirm the presence of the resulting amplicon. If the 5'UTR control line on the LiPA strip is negative, it may indicate that the RT-PCR reaction was inhibited, a low

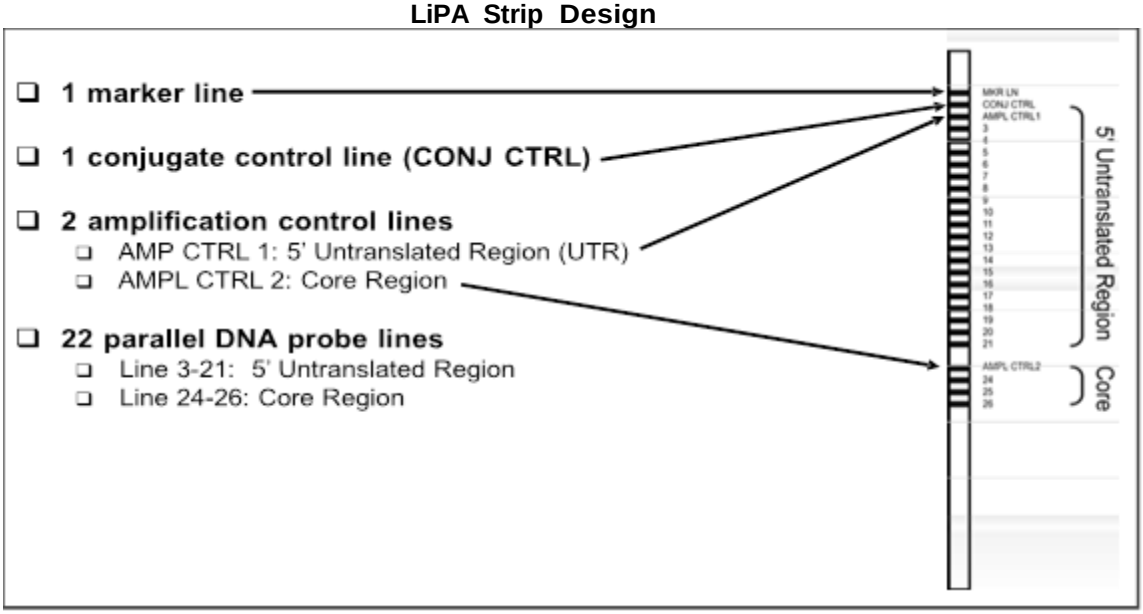
concentration of HCV RNA was present in the sample, the sample was from an HCV negative patient, or an operator error occurred. If the core amplification control line is negative, it is not possible to differentiate genotype 6 subtypes c-l from genotype 1, and it is not possible to differentiate subtypes a and b of genotype 1. If the amplification control lines give the appropriate positive results, the positive bands for each specimen strip can be used to interpret the genotype.

The pattern of bands determines the genotype identity. HCV genotypes and subtypes react in distinct patterns, each of which is genotype and/or subtype- specific.

There are two methods to identify the genotype of a patient:
1. manually using the reading card and interpretation chart
2. semi-automated using the LiPA-Scan Software

Manual HCV Genotype Interpretation
In order to identify the genotype of a patient, a plastic reading card is placed over the strip. HCV genotype is determined by aligning the assay strips with the reading card and recording the line numbers corresponding to the positively colored bands that have appeared. The genotype corresponding to the banding patterns can then be derived using the reading card and the interpretation chart.

LiPA Strip Design



Semi-automated HCV Genotype Interpretation
The developed strips are scanned and analyzed with LiPA-Scan software. The software interprets the band pattern and reports a genotype. The operator confirms that the results are accurate.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

Currently, there is one other FDA approved medical device for determining the HCV genotype(s) in plasma or serum, the Abbott RealTi*m*e HCV Genotype II assay. The only currently available conventional alternative methodology for this indication is

nucleic acid sequencing. Each alternative has its own advantages and disadvantages. A patient should fully discuss the alternatives with his/her physician to select the method that best meets expectations and lifestyle.

VII. MARKETING HISTORY

The VERSANT® Sample Preparation Reagent 1.0 Kit (Box 1 (SMN 10286026 and Box 2 SMN 10286027) has been on sale outside the US since November 2008.

The VERSANT® Sample Preparation Reagent 1.0 Kit is sold in the following countries: Algeria, Australia, Austria, Belgium, Canada, Costa Rica, Croatia, Cyprus, Czech Republic, Denmark, Egypt, El Salvador, Finland, France, Germany, Greece, Honduras, Hungary, Iceland, Ireland, Italy, Jordan, Korea, Liechtenstein, Lithuania, Luxembourg, Malta, Mexico, Netherlands, New Zealand, Nicaragua, Norway, Peru, Philippines, Portugal, Serbia, Slovakia, Slovenia, South Africa, Spain, Sweden, Switzerland, Syria, Tunsia, Turkey, United Kingdom, United States, Vietnam.

The VERSANT® HCV Genotype 2.0 Assay composed of the following:

- VERSANT® HCV Amplification 2.0 Kit (LiPA)
- VERSANT® HCV Genotype 2.0 Assay (LiPA)
- VERSANT® Control 2.0 Kit (LiPA)
- VERSANT® Sample Preparation 1.0 Reagents

has been sold in the following countries: Algeria, Argentina, Australia, Austria, Belgium, Brazil, Canada, Canary Islands, Chile, China, Croatia, Czech Republic, Denmark, France, Georgia, Germany, Great Britain, Greece, Hong Kong, Hungary, Indonesia, Ireland, Israel, Italy, Kuwait, Lithuania, Luxemborg, Malaysia, Mexico, Morocco, Netherlands, New Zealand, Norway, Oman, Poland, Portugal, Qatar, Republic of Korea, Saudi Arabia, Singapore, Slovakia, South Africa, Spain, Sweden, Switzerland, Taiwan, Thailand.

This product has not been withdrawn from the market from any country related to safety or effectiveness, or for any other reasons.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

When used according to the instructions in the package insert, there are no known direct adverse effects of this device on the health of the user.

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 individuals being considered for antiviral treatment. Therefore, patients have already been diagnosed as HCV positive by another method eliminating the possibility of false negative samples.

Direct acting antivirals (DAA's) can act on more than one HCV genotype. For example, Sofosbuvir can be used in the treatment of HCV genotypes 1, 2, 3 and 4. Nevertheless, it is still recommended to determine the genotype prior to initiating treatment. If a patient is assigned an incorrect genotype, the patient would not respond optimally to the treatment provided. This would result in a second test to determine the correct genotype. Hence, incorrect genotypes result in a delay of optimal treatment.

IX. SUMMARY OF NONCLINICAL STUDIES

Nonclinical studies were performed at Siemens Healthcare Diagnostics, Berkeley, CA to evaluate the performance characteristics of the VERSANT® HCV Genotype 2.0 assay (LiPA). The studies are described below. All of the nonclinical studies were performed using the four reagent kits that comprise this assay, multiple VERSANT® Molecular Systems SP, three different thermal cyclers and two different auto strip processors.

A. Laboratory Studies

1. Limit of Detection (LoD)

The purpose of this study was to establish the Limit of Detection (LoD) of the VERSANT® HCV Genotype 2.0 Assay (LiPA) in serum and plasma for each genotype 1a, 1b, 2, 3, 4, 5, and 6. The experimental design followed the CLSI guideline EP17-A2. Four (4) lots of the VERSANT® Sample Preparation 1.0 Reagent Kit, 4 lots of the VERSANT® HCV Control 2.0 Kit (LiPA), 7 lots of the VERSANT® HCV Amplification 2.0 Kit (LiPA) and 7 lots of the VERSANT® HCV Genotype 2.0 Assay (LiPA) Kit were used for this study.

The study was conducted in two phases. The goal of Phase 1 was to establish the Limit of Detection of the VERSANT® HCV Genotype 2.0 Assay (LiPA) for each genotype/subtype separately in serum and plasma. At least twenty replicates at target concentrations ranging from 25 IU/mL to 1000 IU/mL of HCV genotypes 1-6 in serum and plasma were tested. Each target concentration was tested with a minimum of 2 replicates per run, with 2 runs per day for at least 4 days. Using probit analysis, the LoD for each genotype was estimated separately for serum and plasma and separately for the semi-automated results and for the manually interpreted results.

The goal of Phase 2 was to verify the Limit of Detection of the VERSANT® HCV Genotype 2.0 Assay (LiPA) for each genotype separately in serum and plasma. The LoD for plasma was verified separately in K_2EDTA plasma, ACD plasma, and CPD plasma. A single concentration at the LoD claim for each genotype/subtype obtained in Phase 1 was tested in 20 replicates each for serum and plasma (K_2EDTA, ACD and CPD). The acceptance criteria stated that each genotype/subtype provides at least 95% detection rate at the concentration tested.

The LoD claim for genotype 1-6 in plasma and serum are shown in the table below. Detection rate at the LoD claim in serum and plasma was ≥ 95% for each genotype.

Table 2: Limit of Detection in Plasma and Serum

Limit of Detection for HCV genotype/subtype in plasma and serum							
	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 LoD claim for the VERSANT® HCV Genotype 2.0 Assay (LiPA) is 400 IU/mL in plasma and 650 IU/mL in serum.

2. Analytical Specificity

Potentially Interfering Substances

Two independent studies were conducted to address potentially interfering substances:

- Interference by antiviral drugs
- Interference by endogenous substances

Both studies followed CLSI guideline EP7-A2, *Interference Testing in Clinical Chemistry; Approved Guideline – Second Edition.* Studies were performed with HCV genotypes 1a, 1b, 2, and 3 at the concentrations shown below.

Table 3: 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

These genotypes were chosen because they resemble the current genotype distribution in the US. All studies passed the acceptance criteria.

Endogenous Substances

Panels were prepared by adding one specimen each of HCV genotypes 1a, 1b, 2 and 3 to pooled HCV negative human plasma. Potentially interfering endogenous substances were then added into HCV samples at the concentrations listed in the table below. Potential effects on genotyping rate and accuracy were tested using these samples.

Table 4 Endogenous Substances Tested

Endogenous Substance	Concentrations Tested
Hemoglobin	500 mg/dL
Triglycerides (Intralipid)	3000 mg/dL
Conjugated bilirubin	20 mg/dL
Unconjugated bilirubin	20 mg/dL
Protein (Albumin solution)	9 g/dL

Three (3) replicates were prepared for each combination of endogenous substance and HCV genotype to yield a total sixty (60) test samples. These samples were tested alongside two (2) replicates of each HCV genotype sample (no endogenous substance) as positive controls and two (2) replicates of each endogenous substance (no HCV) as specificity controls.

The results met the acceptance criteria for genotyping rate (≥ 90%) and genotyping accuracy (≥ 99%) for each HCV genotype tested.

Anti-viral Drugs

The drugs evaluated included eight (8) different types of medications: nucleoside HIV RT inhibitors, nucleotide DNA polymerase inhibitors, HIV and HCV protease inhibitors, non-nucleoside RT inhibitors, fusion inhibitors, HIV integrase inhibitors, HCV and HBV antivirals, and immune modulators (see table below). The drugs were pooled based on their solubility properties into 5 groups and spiked into negative human serum at three times their C_{max} concentrations. Aliquots were then individually spiked with HCV genotypes 1a, 1b, 2 and 3. For each drug pool tested, there were a total of 12 replicates, 3 replicates each from each genotype.

Table 5: List of Anti-Viral Drugs Tested

Drug	Drug Name
Nucleoside HIV RT Inhibitors	Abacavir sulfate (ABC) Didanosine (ddI) Emtricitabine (FTC) Lamivudine (3TC) Stavudine (d4T) Zidovudine (AZT)
Nucleotide DNA Polymerase Inhibitors	Tenofovir Adefovir dipivoxil

Drug	Drug Name
HIV and HCV Protease Inhibitors	Atazanavir, Boceprievir, Darunavir, Fosamprenavir, Indinavir, Lopinavir, Nelfinavir, Ritonavir, Saquinavir, Telaprevir, Tipranavir
Non-Nucleoside RT Inhibitors	Efavirenz, Nevirapine
Fusion Inhibitor	Enfuvirtide, Maraviroc
HIV Integrase	Raltegrivir
HCV and HBV antivirals	Acyclovir, Entecavir, Ganciclovir, Ribavirin, Telbivudie Valganciclovir
Immune Moderators	Interferon alfacon-1, Interferon alpha-2b, Peginterferon alpha-2a, Peginterferon alpha-2b

Results:
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.

3. Cross-reactivity with non-HCV Disease States and Other Blood-borne Microorganisms

The purpose of this study was to evaluate potential cross-reactivity with three non-HCV disease states and seventeen common microorganisms on the genotyping rate and genotyping accuracy of HCV samples using the VERSANT® HCV Genotype 2.0 Assay (LiPA). The table below lists the non-HCV diseases and microorganisms that were tested.

Table 6: List of Non-HCV Disease States/Microorganisms Tested

	Disease State/ Microorganism
non-HCV Disease	Anti-nuclear antigen
	Rheumatoid factor
	Systemic Lupus Erythematosus
	Cytomegalovirus
	Epstein-Barr Virus
	Hepatitis A Virus
	Hepatitis B Virus
	Human Immunodeficiency Virus
	Candida albicans

	Disease State/ Microorganism
Microorganism	Enterobacter cloacae
	Escherichia coli
	Haemophilus influenzae
	Klebsiella pneumoniae
	Pseudomonas aeruginosa
	Pseudomonas fluorescens
	Serratia marcescens
	Streptococcus agalactiae
	Staphylococcus aureus
	Staphylococcus epidermidis
	Streptococcus pneumonia

Protocol for Non-HCV Disease:
Patient serum samples from 3 different non-HCV disease conditions were spiked with HCV positive plasma and/or serum samples of HCV genotype 1a, 1b, 2, or 3. Each HCV genotype was spiked into two patient serum samples from each disease state (i.e., two patient samples with Rheumatoid factor were spiked with HCV genotype 1a while another two patient samples with Rheumatoid factor were spiked with HCV genotype 1b, etc.).

Protocol for Microorganisms:
Potentially interfering microorganisms were tested for

- Cross reactivity – the microorganism does not produce a false positive result for an HCV genotype
- Interference – the microorganism does not prevent proper genotyping of HCV genotypes tested at 1.5 X LoD

Each microorganism was spiked into serum sample tubes except for Hepatitis A virus (HAV). In this case, HAV positive patient sample tubes were spiked with a HCV genotype/subtype.

Results:
Two hundred and ninety (290) samples were analyzed in this study. All cross reactivity samples that contained potentially interfering microorganisms were negative. At least 9 of the 10 replicates of each of the non-HCV disease states and Hepatitis A virus samples and at least 11 of the 12 replicates of each of the potentially interfering organism samples gave the correct genotype result. Thus, the samples from three different non-HCV disease states or samples containing 17 different potentially interfering microorganisms yielded results that met the requirements for ≥ 90% genotyping rate and ≥99% genotyping accuracy with the VERSANT® HCV Genotype 2.0 Assay (LiPA).

4. Analytical Carryover

This study was designed to demonstrate that strips processed from HCV RNA-negative samples are not contaminated by samples with high HCV viral concentrations when tested simultaneously with the VERSANT® HCV Genotype 2.0 Assay (LiPA).

A high titer HCV genotype 1a stock (1.2 x 10^6 IU/mL) was used as the positive sample. Pooled human K_2EDTA plasma that was pre-qualified to be HCV RNA negative using an FDA approved Transcript Mediated Amplification (TMA) Assay (Hologic, Marlborough, MA) was used as the HCV negative test sample. Seventy two (72) replicates of each of the positive and negative samples were arranged in a checkerboard pattern to maximize cross contamination during nucleic acid extraction and PCR amplification.

Samples were processed using three thermocyclers: the GeneAmp PCR System 9700, the Agilent Mx3005P or the Biorad PTC-200, and with the Auto-LiPA 48 or the AutoBlot 3000H in order to test for potential contamination across all equipment used in the assay.

Results demonstrate that analyte carryover specificity was 100%. These results demonstrate that the VERSANT® HCV Genotype 2.0 Assay (LiPA) met acceptance criteria and is able to process HCV negative and high HCV viral load samples simultaneously without cross-contamination.

5. Specimen Stability

The purpose of this study was to evaluate HCV specimen stability in serum and plasma. Specimen stability was evaluated by diluting one specimen each from HCV Genotypes 1a, 1b, 2 and 3 in K_2EDTA plasma, plasma from PPT tubes, serum and serum from SST tubes at the target concentrations shown in Table 7.

Table 7: Target HCV Concentrations Used to Test Specimen Stability in K_2EDTA Plasma, PPT tubes, Serum and SST Tubes

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

The study demonstrated that specimens in K_2EDTA plasma, plasma from PPT tubes, serum and serum from SST tubes may be stored at 2 to 8ºC for 48h, -20 ±5ºC for 72 h or at -60 to -80ºC 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).

6. Reagent Stability

The purpose of this study was to determine the stability of the three VERSANT® HCV Genotype 2.0 Assay (LiPA) reagent kits and the Sample Preparation 1.0 Reagent Kit together. The four kits were tested as a unit for their ability to genotype clinical samples of HCV genotypes 1a, 1b, 2, 3, 4, 5 and 6 diluted to 3 X LoD in pooled K_2EDTA plasma. At each stability time point, 76 replicates were tested; 11 replicates of each genotype except for genotype 6 which was tested 10 times.

Baseline, 13-month and end of shelf life real-time stability testing of unopened kits was assessed for three lots of each of the four reagent kits. Genotyping rate and genotyping accuracy at all test points passed all acceptance criteria. Data from the baseline real-time stability test point verify that reagents kits sequestered for stability testing of the VERSANT® HCV Genotype 2.0 Assay (LiPA) had acceptable product performance at the start of the study. Data from 13-month real-time stability test point verifies that reagents kits still had acceptable performance. Data from the end of shelf life testing verifies the expiration dating for all four reagent kits included in the product labeling.

Based on the stability data, the shelf life for each of the four VERSANT® HCV Genotype 2.0 Assay (LiPA) reagent kits are as follows.

Table 8: Shelf Life for the Four VERSANT® HCV Genotype 2.0 Assay (LiPA) Reagent Kits

Kit Description	Storage Temp	Shelf Life
VERSANT® Sample Preparation 1.0 Reagent Kit	15-30°C (Box1) 2-8°C (Box2)	24 months
VERSANT® HCV	2-8°C	24 months
VERSANT® HCV Amplification 2.0 Kit	-20 ± 5°C	29 months
VERSANT® HCV Genotype 2.0 Assay (LiPA) Kit	2-8°C	18 months

Shipping stability, which was assessed concurrently with real-time stability, also passed all acceptance criteria. This verifies that product performance is acceptable when shipping conditions are met.

7. Reproducibility

Reproducibility of the VERSANT® HCV Genotype 2.0 Assay (LiPA) was evaluated using a 14 member reproducibility panel. Single specimens containing 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 9.

Table 9: 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	6,099	100,000
2	738	100,000
3	378	100,000
4	3,531	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 clinical test 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.

Table 10: 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 with the reference method results.

Assay performance, measured by genotyping rate and genotyping accuracy, at Site 3 was significantly different from the other two sites (p-values ≤ 0.05) as shown in Table 11. No significant difference in assay performance was observed between Site 1 and Site 2. The difference at Site 3 was attributable to one operator (Table 12). 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 13.

Table 11: Effect of Clinical Trial Site on Assay Performance

Clinical Site	Manual Method of Interpretation				Semi-Automated Method of Interpretation			
	Genotyping Rate[a]	Genotyping Rate	Genotyping Accuracy[b]	Genotyping Accuracy	Genotyping Rate[a]	Genotyping Rate	Genotyping Accuracy[b]	Genotyping Accuracy
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 12: Assay Performance at Site 3 Stratified by Operators

Operator at Site 3[a]	Manual Method of Interpretation				Semi-Automated Method of Interpretation			
	Genotyping Rate[b]	Genotyping Rate	Genotyping Accuracy[c]	Genotyping Accuracy	Genotyping Rate [Fraction]	Genotyping Rate [Percent]	Genotyping Accuracy [Fraction]	Genotyping Accuracy [Percent]
Operator 1	352/378	93.1	348/352	98.9	369/378	97.6	368/369	99.7
Operator 2	373/378	98.7	371/373	99.5	347/378	91.8	343/347	98.8

[a] For a given run, the semi-automated method of interpretation was conducted by one operator, while a second operator at the site interpreted the processed assay strips for each run using the manual method of interpretation. Hence, the results from the manual method of interpretation for operator 1 correspond to the results from the semi-automated method of interpretation for operator 2. These samples were processed by the same operator.
[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.

Table 13: Effect of Clinical Trial Site on Assay Performance with a Subset of Specimens used to Evaluate Assay Accuracy

Clinical Site	Manual Method of Interpretation				Semi-Automated Method of Interpretation			
	Genotyping Rate [Fraction]	Genotyping Rate [Percent]	Genotyping Accuracy [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

Results of Reproducibility Studies:

There were no significant differences in assay performance between kit lots for both manual and semi-automatic interpretation method.

There were no significant differences in assay performance between the manual method of interpretation and the semi-automated method of interpretation.

Assay performance at one clinical trial site was shown to be significantly different compared to the other two sites. Further investigation determined that operator laboratory skills were the cause of this discrepancy. Following further training, assay performance was the same at all three sites.

Conclusions Drawn from Non Clinical Studies

The VERSANT® HCV Genotype 2.0 Assay (LiPA) was evaluated to demonstrate performance claims for specificity, accuracy, endogenous substances interference, carryover, reproducibility, mixed infections, limit of detection and stability. These performance characteristics were measured using genotyping accuracy and genotyping rate. Together with the clinical trial results, these data support the intended use claims of the VERSANT® HCV Genotype 2.0 Assay (LiPA).

X. SUMMARY OF PRIMARY CLINICAL STUDIES

A. Study Design

Clinical Validity

The overall objective of the Clinical Validity Study was to show a relationship between the HCV genotype obtained by the VERSANT® HCV Genotype 2.0 Assay (LiPA) System and the probability of achieving sustained virologic response (SVR_{12}) for individuals receiving HCV treatment. HCV genotypes 1a, 1b, 2, 3, 4, 5, and 6 collected from individuals with chronic HCV infection who had known HCV treatment outcome were tested.

Specimens from 217 individuals infected with HCV genotype 1a, 1b, 2, 3, 4, 5 or 6 as determined by the NS5b HCV genotyping reference method were tested at three clinical trial sites. The specimens were obtained from individuals prior to initiating treatment with Direct-Acting Antivirals (DAAs). All specimens from the particular genotype were treated with the identical DAA regime. Samples were collected retrospectively for the clinical trial. Inclusion and exclusion criteria are listed below:

1. Clinical Inclusion and Exclusion Criteria

   Inclusion Criteria:

   - At least 18 years of age
   - Specimen available from a time point prior to or during HCV treatment
   - Specimen collected from subjects infected with the same HCV genotype who have also received the same HCV treatment.
   - HCV RNA viral load > 2000 IU/mL
   - Documented HCV treatment outcome available

Exclusion Criteria:

- Subject quit HCV treatment and viral load was not documented upon exiting the study

Specimens of genotype 4 and subtype 1a and 1b were collected from patients treated with Sofosbuvir (SOF), Pegylated Interferon (peg-IFN) and Ribavirin (RBV). Genotype 2 and 3 specimens were collected from patients treated with SOF and RBV, and genotype 5 and 6 specimens were collected from patients treated with SOF and Ledipasvir (LDV). Tables 16a and 16b show the demography of the patients, the genotype distribution and the treatment received. Individuals were enrolled in clinical trials[1] and were treated according to the standard of care or until treatment was discontinued. Individuals enrolled in the study attained SVR_{12} or NSVR (Non-Sustained Virologic Response) as documented at a follow-up visit after 12 weeks of treatment.

2. Follow-up Schedule:

   All studies were retrospective using banked samples. Follow-up did not apply.

3. Endpoints:

   Results were compared to sequencing for the rate and accuracy study and to treatment outcome for the clinical validity study to determine accuracy, as noted above.

Table 14: Distribution of HCV Genotype Specimens in the Clinical Validity Study

HCV Genotype (NS5b)	Quantity (% of total specimens)	Treatment Received
1a	40 (18.2%)	SOF+peg-IFN+RBV
1b	40 (18.2%)	SOF+peg-IFN+RBV
2	40 (18.2%)	SOF+RBV
3	40 (18.2%)	SOF+RBV
4	20 (9.1%)	SOF+peg-IFN+RBV
5	20 (9.1%)	SOF+LDV
6	20 (9.1%)	SOF+LDV
Total	**220**	
SOF: Sofosbuvir; peg-IFN: Pegylated Interferon; RBV: Ribavirin; LDV: Ledipasvir		

B. Accountability of PMA Cohort

Two hundred and twenty samples from patients with known treatment outcomes were used in the clinical validity study. Of these, 217 gave evaluable results and three samples were invalid and therefore not included in the calculations.

C. Study Population Demographics and Baseline Parameters

Table 15: Demography of HCV Infected Subjects Enrolled in the Clinical Validity Study

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	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	29	36.25
	≥ 3E6 IU/mL	51	63.75
Baseline HCV RNA (Genotype Non 1a & b)	< 3E6 IU/mL	91	65
	≥ 3E6 IU/mL	49	35

D. Safety and Effectiveness Results

1. Safety Results

   Since the study utilized retrospective patient samples there were no adverse effects, and safety analysis of the study is not applicable.

2. Effectiveness Results

   The results from the Clinical Validity Study for genotyping rate and genotyping accuracy are summarized in Tables 16 and 17 below.

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)]	
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)]	
1a	140	136/140	97.1	(92.88, 98.88)	135/136	99.26	(95.95, 99.87)
1b	140	138/140[d]	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.

Rate of SVR_{12} in Response to HCV Treatment for each Genotype

Viral load was measured 12 weeks after completion of treatment for patients enrolled in clinical trials. Refer to Tables 18a, 18b, and 18c for SVR/non-SVR distributions based on genotype results obtained with the NS5b, and the VERSANT® HCV Genotype 2.0 Assay (LiPA) System manual, and semi- automated methods of interpretation.

Table 18a: Summary of the SVR_{12} Results Using the Reference Method NS5b to Determine Genotype

Genotype (NS5b)	# SVR12	# Non-SVR	Total
1a	35	5	40
1b	29	11	40
2	40	0	40
3	32	8	40
4	19	1	20
5	20	0	20
6	19	1	20
Total	**194**	**26**	**220**

Table 18b: Summary of the SVR_{12} Results Using the VERSANT® HCV Genotype 2.0 Assay (LiPA) System Manual Method of Interpretation to Determine Genotype

Genotype (Manual)	# SVR_{12}	# Non-SVR	Total
1a	35	4	39
1b	29	11	40
2	40	0	40
3	32	8	40
4	19	1	20
5	18	0	18
6	19	1	20
Total	**192**	**25**	**217**

Table 18c: Summary of the SVR Results Using the VERSANT® HCV Genotype 2.0 Assay (LiPA) System Semi-Automated Method of Interpretation to Determine Genotype

Genotype (Semi-Auto)	# SVR12	# Non-SVR	Total
1a	34	4	38
1b	29	11	40
2	40	0	40
3	32	8	40
4	19	1	20
5	19	0	19
6	19	1	20
Total	**192**	**25**	**217**

The clinical utility of the VERSANT® Genotype 2.0 Assay (LiPA) was assessed by evaluating the association between HCV genotype and the probability of achieving SVR_{12}. For individual genotypes/subtypes, the observed SVR_{12} rates ranged from 72.5% to 100%. Patients with subtype 1b had the lowest SVR_{12} rate at 72.5% (29/40), while genotypes 2 and 5 had the highest rate, with 100% SVR_{12}.

Analysis of the SVR_{12} rate was performed to compare SVR_{12} rates between genotype 1 (pooled 1a and 1b) and the other non-1 genotypes by calculating the SVR_{12} rate ratios. The odds ratios for SVR_{12} between genotype 1 (pooled 1a and 1b) and the other non-1 genotypes were calculated to assess the relative odds for SVR_{12} between the genotypes. The results for the analysis are summarized in Tables 19a and 19b for the VERSANT® HCV Genotype 2.0 Assay (LiPA) System manual and semi-automated methods of interpretation, respectively.

Tables 19a and 19b show that the odds for SVR_{12} for genotype 1 were generally lower relative to the other genotypes, except for genotype 1 relative to genotype 3 for which the odds ratio was about 1. In particular, the odds for SVR_{12} is statistically significantly lower for genotype 1 relative to genotype 2 (odds ratio of 0.051 for both manual and semi-automated methods of interpretation). Furthermore, as a group, genotype 1 (pooled 1a and 1b) resulted in statistically significantly lower odds for SVR_{12} compared to the group of all non-1 genotypes (pooled genotypes 2, 3, 4, 5, 6). All other odds ratios were not statistically significant as the confidence limits contain 1.

Table 19a: Ratios of SVR_{12} Rates and Odds Ratios for SVR_{12} by Genotype Based on the Manual Method of Interpretation

Genotype Comparison	SVR Rate Comparison	SVR Rate Ratio	Odds Ratio	95% CI for OR
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)

CI= 95% confidence interval (lower and upper limit, respectively)

Table 19b: Ratios of SVR_{12} Rates and Odds Ratios for SVR_{12} by Genotype Based on the Semi-Automated Method of Interpretation

Genotype Comparison	SVR Rate Comparison	SVR Rate Ratio	Odds Ratio	95% CI for OR
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)

CI= 95% confidence interval (lower and upper limits, respectively)

The LiPA results matched 100% with the NS5b reference method. Patients with HCV genotype 1 are less likely to achieve SVR_{12} than patients with genotype 2 or genotype 3. Current HCV therapy with DAA's resulted in shorter duration of therapy and high rates of SVR_{12} (89%-90%). The observed SVR_{12} rate by genotype/subtype is consistent with published results using similar treatments with DAA drugs.

3. Subgroup Analyses

   No subgroup analyses were performed.

4. Pediatric Extrapolation

   In this premarket application, existing clinical data was not leveraged to support approval of a pediatric patient population.

E. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included three investigators. None of the clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

XI. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

In accordance with the provisions of section 515(c)(3) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the FDA Microbiology Devices Advisory Panel, an FDA advisory committee, for review and recommendation.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The limit of detection was determined for each genotype in both serum and plasma. The LoD claim for the VERSANT® HCV Genotype 2.0 Assay (LiPA) is 400 IU/mL in plasma and 650 IU/mL in serum.

The VERSANT® HCV Genotype Assay 2.0 (LiPA) met requirements for reproducibility for different lots of reagents over multiple days of testing.

The genotyping rate and accuracy study used 800 unique samples. 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 17. The percentage of correct genotypes ranged from 98.6% to 100% for the individual genotypes.

Potentially interfering substances studies including endogenous substances, microorganisms and current anti-viral drugs, showed no interference with genotyping rate or accuracy.

Specimen stability was evaluated in K_2EDTA Plasma, Plasma from PPT tubes, Serum and Serum from SST tubes at the target concentrations. There was no impact to genotyping accuracy or rate.

Specimens may be stored in blood collection tubes at 2 to 8ºC for 48h, -20 ±5ºC for 72 h or at -60 to -80ºC 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).

The SVR_{12} rate for all patients (all genotypes and subtypes pooled) was 88.48% (192/217) for both the manual and semi-automated methods of interpretation. For individual genotypes/subtypes, the observed SVR_{12} rates ranged from 72.5% to 100%. Patients with subtype 1b had the lowest SVR_{12} rate at 72.5% (29/40), while genotypes 2 and 5 were the highest, with 100%

SVR_{12}.

The VERSANT® HCV Genotype 2.0 Assay (LiPA) provides an accurate diagnosis of HCV genotypes 1a, 1b, 2, 3, 4, 5, and 6 as shown by a genotyping accuracy rate of ≥ 99% as compared to the reference method. The SVR_{12} rate by genotype is consistent with published results using similar treatments with direct acting antiviral drugs as demonstrated in the clinical validity study. 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 utilizes the 5'UTR region.

B. Safety Conclusions

The risks of the device (i.e. incorrect genotype result) are based on nonclinical laboratory as well as data collected in a clinical studies conducted to support PMA approval as described above.

The genotyping rate and accuracy study included 800 unique samples. The percentage of specimens that yielded a genotype result was 97.9% for all genotypes and ranged from 90% to 100% for the individual genotypes using the manual method of interpretation as shown in Table 17. The percentage of correct genotypes was 99.6% for all genotypes and ranged from 98.6% to 100% for the individual genotypes.

The VERSANT® HCV Genotype Assay 2.0 (LiPA) has the same inherent risk to patients as any other in vitro diagnostic test that requires that blood be drawn. Therefore, common safety practices must be observed when drawing the sample. There were no significant adverse effects of the VERSANT® HCV Genotype Assay 2.0 (LiPA) assay reported during the studies.

C. Benefit-Risk Determinaton

The probable benefits of the device are based on data collected in the analytical and clinical studies conducted to support the pre-market approval as described above.

The benefit of using the VERSANT® HCV Genotype Assay 2.0 (LiPA) is that clinicians will be able to accurately determine the HCV genotype for each patient. Thus the patient will receive the correct treatment and achieve SVR without timely delays.

Additional factors to be considered in determining probable risks and benefits for the VERSANT® HCV Genotyping Assay 2.0 (LiPA) device included: inability to decipher between genotype 6 and genotypes 1a or 1b because the core region information is unavailable, inability to identify mixed infections (although this is a rare event in the USA) and recombinant HCV. In all cases, the risk to patient is a delay in treatment or incorrect DAA. Since the course of treatment for the current DAAs is short, the delay is not substantial.

1. Patient Perspectives: This submission did not include specific information on patient perspectives for this device.

In conclusion, given the available information above, the data support that, for detection of HCV genotype for a patient, the probable benefits outweigh the probable risks.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the instructions for use. Data from pre- clinical and clinical data show that the VERSANT® HCV Genotype Assay 2.0 (LiPA) is an accurate method to determine the genotype of HCV present in the patient.

XIII. CDRH DECISION

CDRH issued an approval order on March 14, 2017. The final conditions of approval cited in the approval order are described below.

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.

Post-approval Requirements and Restrictions: See approval order.