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Evaluation of Analytical Sensitivity and Workflow of the VERSANT Hepatitis C Virus Genotype 2.0 Assay (LiPA)

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Abstract

Background: The VERSANT® HCV Genotype 2.0 Assay (LiPA) is a reverse hybridization line probe assay that uses sequence information from both the 5′ untranslated region (UTR) and the core region to accurately distinguish between HCV genotypes 1 to 6 and subtypes 1a and 1b. Prior studies have shown that the assay can genotype 96% of HCV samples with 99.4% accuracy.[1] Assay steps have been automated to improve efficiency and decreased time to results. This study evaluates assay workflow and analytical sensitivity.

Methods: The VERSANT HCV Genotype 2.0 Assay (LiPA) is run in three steps: extraction, amplification, and genotyping. Viral RNA is extracted from plasma or serum using the VERSANT Sample Preparation 1.0 Reagents. The 5′ UTR and core regions of HCV are amplified using RT-PCR and the VERSANT HCV Amplification 2.0 Kit (LiPA). Biotinylated amplicons are hybridized to immobilized oligonucleotide probes on nitrocellulose strips and visualized using reagents in the VERSANT HCV Genotype 2.0 Assay (LiPA) Kit. Processed strips are interpreted using the optional LiPA Scan software to yield the HCV genotype. Assay intermediates from each step can either be processed immediately or stored at defined conditions. Analytical sensitivity was evaluated using one specimen for each genotype (1a, 1b, 2, 3, 4, 5, and 6) diluted separately in serum and plasma. Dilution series were prepared at concentrations ranging from 50 to 2000 IU/mL, and each target concentration was tested in multiple replicates and runs with multiple reagent lots on different days. These data are analyzed using a regression method with probit link function.

Results: Automation of extraction and strip processing allows for simultaneous processing of 94 samples. Extraction and loading of the PCR plate have been optimized with the VERSANT kPCR Sample Preparation module, a fully automated instrument for isolation and purification of nucleic acids using magnetic-bead extraction technology. Genotyping has been optimized on the automated Auto-LiPA 48 Genotyping Instrument (Strip Processor), which can process up to 46 samples and 2 controls per run. Assay times for 94 samples are 3.5 hours for extraction, 4 hours for amplification, and 4 hours for genotyping (with two Auto-LiPA 48 processors), which includes a hands-on time of 2 hours. Initial assessment of analytical sensitivity, measured as the limit of detection for individual genotypes/subtypes, was less than or equal to 500 IU/mL. Further assessments are underway to confirm the analytical sensitivity.

Conclusion: The VERSANT HCV Genotype 2.0 Assay (LiPA) is a sensitive and reliable HCV genotyping assay. Automation of the VERSANT HCV Genotype 2.0 Assay (LiPA) workflow results in higher throughput, improved efficiency, and decreased time to results.

Introduction

- The VERSANT® HCV Genotype 2.0 Assay (LiPA) is a line probe assay that identifies hepatitis C virus (HCV) genotypes 1 to 6 and subtypes 1a and 1b in human serum and plasma samples
- The assay uses sequence motifs from the core region and the 5′ untranslated region (UTR) of the hepatitis C virus (HCV) genome. The 5′ UTR provides genotyping information for genotypes 1 to 5 and some genotype 6 subtypes. The core region (in addition to the 5′ UTR) is used to distinguish other genotype 6 subtypes from genotype 1 samples and to identify subtypes a and b of genotype 1.
- Prior studies[1] have shown that the assay can genotype 96% of HCV samples with 99.4% accuracy.
- In this study, we report automation of steps of the VERSANT HCV Genotype 2.0 Assay (LiPA) and evaluation of its analytical sensitivity.

Methods and Results

Workflow

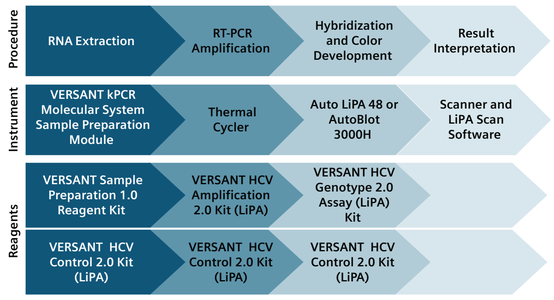
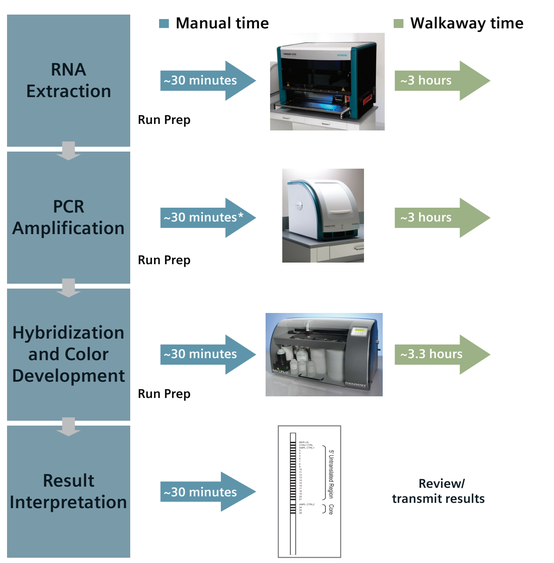


Figure 1. Workflow of the VERSANT HCV Genotype 2.0 Assay (LiPA).

- The VERSANT HCV Genotype 2.0 Assay (LiPA) is run in three steps: extraction, amplification and genotyping.
  - **RNA Extraction:** HCV RNA is extracted from patient serum or plasma samples using the VERSANT Sample Preparation 1.0 Reagent Kit in conjunction with the VERSANT kPCR Molecular System Sample Preparation module.
  - **RT-PCR Amplification:** Amplified, biotinylated DNA is generated from the purified viral RNA in a combined reverse transcriptase-polymerase chain reaction amplification using reagents in the VERSANT HCV Amplification 2.0 Kit (LiPA) and a thermal cycler.
  - **Genotyping (Hybridization and Color Development):** Biotinylated DNA PCR product is hybridized to immobilized oligonucleotide probes on a nitrocellulose strip specific for the 5′ UTR and core region of different HCV genotypes. Alkaline phosphatase-labeled streptavidin is bound to the biotinylated hybrid, and BCI/NBT chromogen reacts with the streptavidin-alkaline phosphatase complex, forming a purple/brown precipitate resulting in a visible banding pattern on the strip.
  - **Result Interpretation:** The patient's HCV genotype is identified by semiautomated interpretation by the LiPA-Scan software and/or by manual interpretation using the interpretation chart to interpret the pattern of positive bands on the developed strip.
- The VERSANT HCV 2.0 Control Kit (LiPA) contains positive and negative run controls that are used for run validation at all steps of the assay.
- Sample intermediates from RNA extraction and amplification can either be immediately processed or stored at appropriate conditions (-60°C to -80°C for RNA and -20°C for amplified biotinylated DNA) and used at a later time.



*With Automated PCR set-up, the manual time for PCR amplification can be reduced to 5 minutes.

Figure 2. VERSANT HCV Genotype 2.0 assay times for 94 assays.

- The VERSANT kPCR Assay steps have been automated to provide higher throughput, improve efficiency, and decrease time to results.
- The VERSANT kPCR Molecular System Sample Preparation module is a fully automated instrument for isolation and purification of nucleic acids using magnetic-bead extraction technology.
- Hybridization and color development has been optimized on the automated Auto-LiPA 48 strip processor. Each Auto-LiPA 48 can process up to 46 samples and 2 controls per run.
- Assay times for 94 samples are 3.5 hours for extraction, 4 hours for amplification, and 4 hours for genotyping (with two Auto-LiPA 48 processors) including a total hands-on time of 2 hours.
- Assay times are shorter when processing fewer samples. Processing of 46 samples reduces assay time for extraction to 2.5 hours and assay time for amplification to 3.5 hours. The total hands-on time is also reduced to 1.5 hours.

Analytical Sensitivity

- Limit of detection was evaluated using one specimen for each genotype/subtype (1a, 1b, 2, 3, 4, 5 and 6) diluted separately in serum and plasma.
- Dilution series were prepared at concentrations ranging from 25 to 1000 IU/mL.
- At least 20 replicates were tested at each target concentration using 4 lots of reagents on different days.
- Detection rates were calculated and the data were analyzed using a regression method with probit link function.

Table 1. Detection rates for each genotype/subtype at target concentrations in serum and plasma.

Genotype/ Subtype	Concentration Tested (IU/mL)	Detection Rate Plasma Percentage (Ratio) [95% Confidence Limits]	Detection Rate Serum Percentage (Ratio) [95% Confidence Limits]
1a	50	20 (4/20) [8.1–41.6]	20 (4/20) [8.1–41.6]
	100	60 (12/20) [38.7–78.1]	40 (8/20) [21.9–61.3]
	200	65 (13/20) [43.3 -81.9]	80 (16/20) [58.4–91.9]
	300	85 (17/20) [64.0–94.8]	90 (18/20) [69.9–97.2]
	400	100 (20/20) [83.9–100.0]	95 (19/20) [76.4–99.7]
1b	50	30 (6/20) [14.6–51.9]	30 (6/20) [14.6–51.9]
	100	50 (10/20) [29.9–70.1]	70 (14/20) [48.1–85.5]
	200	80 (16/20) [58.4–91.9]	90 (18/20) [69.9–97.2]
	300	95 (19/20) [76.4–99.7]	95 (19/20) [76.4–99.7]
	400	100 (20/20) [83.9–100.0]	95 (19/20) [76.4–99.7]
1*	50	65 (26/40) [49.5–77.9]	52.5 (21/40) [37.5–67.1]
	100	87.5 (35/40) [73.9–94.5]	85 (34/40) [70.9–92.9]
	200	97.5 (39/40) [87.1–99.9]	95 (38/40) [87.1–98.6]
	300	97.5 (39/40) [87.1–99.9]	97.5 (39/40) [87.1–99.9]
	400	100 (40/40) [91.2–100.0]	100 (40/40) [91.2–100.0]
2	25	35 (7/20) [18.1–56.7]	Not Tested
	50	50 (10/20) [29.9–70.1]	50 (10/20) [29.9–70.1]
	75	70 (14/20) [48.1–85.5]	Not Tested
	100	85 (17/20) [64.0–94.8]	85 (17/20) [64.0–94.8]
	200	100 (20/20) [83.9–100.0]	90 (18/20) [69.9–97.2]
	300	95 (19/20) [76.4–99.7]	90 (18/20) [69.9–97.2]
	400	100 (20/20) [83.9–100.0]	100 (20/20) [83.9–100.0]
3	25	55 (11/20) [34.2–74.2]	50 (10/20) [29.9–70.1]
	50	75 (15/20) [53.1–88.8]	55 (11/20) [34.2–74.2]
	100	85 (17/20) [64.0–94.8]	60 (12/20) [38.7–78.1]
	150	Not Tested	95 (19/20) [76.4–99.7]
	200	100 (20/20) [83.9–100.0]	100 (20/20) [83.9–100.0]
4	25	Not Tested	40 (8/20) [21.9–61.3]
	50	60 (12/20) [38.7–78.1]	50 (10/20) [29.9–70.1]
	75	Not Tested	70 (14/20) [48.1–85.5]
	100	75 (15/20) [53.1–88.8]	75 (15/20) [53.1–88.8]
	200	90 (18/20) [69.9–97.2]	95 (19/20) [76.4–99.7]
	300	95 (19/20) [76.4–99.7]	95 (38/40) [87.1–98.6]
	400	100 (20/20) [83.9–100.0]	100 (20/20) [83.9–100.0]
5	50	35 (7/20) [18.1–56.7]	0 (0/20) [0–16.1]
	100	65 (13/20) [43.2–81.9]	10 (2/20) [2.8–30.1]
	200	90 (18/20) [69.9–97.2]	20 (4/20) [8.1–41.6]
	300	90 (18/20) [69.9–97.2]	25 (5/20) [11.2–46.9]
	400	100 (20/20) [83.9–100.0]	42.5 (17/40) [28.5–57.8]
	500	Not Tested	100 (20/20) [83.9–100.0]
	600	Not Tested	100 (20/20) [83.9–100.0]
	700	Not Tested	95 (19/20) [76.4–99.7]
	800	Not Tested	100 (20/20) [83.9–100.0]
6	50	25 (5/20) [11.2–46.9]	40 (8/20) [21.9–61.3]
	100	40 (8/20) [21.9–61.3]	30 (6/20) [14.6–51.9]
	200	80 (16/20) [58.4–91.9]	75 (15/20) [53.1–88.8]
	300	90 (18/20) [69.9–97.2]	90 (18/20) [69.9–97.2]
	400	100 (20/20) [83.9–100.0]	100 (20/20) [83.9–100.0]

Note: 1* data are pooled from subtypes 1a and 1b.

- Detection rates at each target concentration along with 95% confidence limits are shown in Table 1.
- Detection rate is the proportion of samples that yield a genotype/subtype result that is accurate among the total number of valid samples.
- One specimen each for HCV subtypes 1a and 1b and genotypes 2, 3, 4, 5, and 6 were used to generate a dilution series separately in serum and plasma.
- HCV genotypes of test samples were determined by sequencing the NS5b region of the viral genome using the HCV NS5b genotype characterization by BigDye® Terminator (BDT) sequencing Assay performed at Siemens Clinical Laboratory, Berkeley.
- Viral concentration of HCV stocks were determined via the FDA-approved VERSANT HCV RNA 3.0 Assay (bDNA).
- At least five target concentrations were tested, and at each target concentration, at least 20 replicates were tested.
- For each genotype, at least three target concentrations had a detection rate between 10 and 90, inclusive, and at least one target concentration had a detection rate of ≥95%.
- Limit of detection for each genotype/subtype is the lowest HCV RNA concentration expressed in IU/mL that yields at least 95% detection rate.
- Limit of detection for each genotype/subtype was calculated by fitting the detection rates from at least five levels against their concentrations using a regression model with a probit link function.

Table 2. Limit of detection of the VERSANT HCV Genotype 2.0 Assay (LiPA) in serum and plasma.

Genotype/ Subtype	Plasma		Serum	
	LoD Estimate [95% Confidence Limits] (IU/mL)	LoD (IU/mL)	LoD Estimate [95% Confidence Limits] (IU/mL)	LoD (IU/mL)
1*	195 [152–299]	200	211 [170–300]	250
1a	356 [291–489]	400	347 [287–467]	350
1b	289 [236–401]	300	310 [245–464]	350
2	203 [156–330]	250	291 [218–516]	300
3	133 [96–304]	150	175 [139–260]	200
4	266 [199–468]	300	255 [203–364]	300
5	292 [232–431]	300	627 [566–722]	650
6	312 [258–419]	350	334 [274–457]	350

Note: LoD is the LoD Estimate rounded up to the nearest 50.

- Limit of detection for the VERSANT HCV Genotype 2.0 Assay (LiPA) for each genotype/subtype along with 95% confidence limits are shown in Table 2.
- The Limit of detection for each genotype/subtype was ≤400 IU/mL in plasma and ≤650 IU/mL in serum.
- The Limit of detection was lowest for genotype 3 in plasma (150 IU/mL) and highest for genotype 5 in serum (650 IU/mL).

Conclusions

- Automation of the VERSANT HCV Genotype 2.0 Assay (LiPA) workflow results in higher throughput, improved efficiency and decreased time to results.
- The VERSANT HCV Genotype 2.0 Assay is a sensitive and reliable HCV genotyping assay.

References

1. Verbeeck J, Stanley MJ, Shieh J, Celis L, Huyck, E, Wollants E, Morimoto J, Farrior A, Sablon E, Jankowski-Hennig M, Schaper C, Johnson P, Ranst MV, Brussel MV. J Clin Microbiol. 2008;1901.

VERSANT HCV Genotype 2.0 Assay (LiPA) is CE-marked in Europe and for research use only (RUO) in the U.S (currently under FDA review for PMA approval).

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