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


Abstract

Background: The VERSANT® HCV Genotype 2.0 Assay (LiPA) is a reverse hybridization line probe assay that uses sequence information from 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 at each target concentration was tested in multiple replicates and runs with multiple reagent lots on different days. 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 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-UPA 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 Auto-UPA 48 processors), which includes a hands-on time of 2 hours. Initial assessment of analytical sensitivity, measured as the limit of detection for the VERSANT HCV Genotype 2.0 Assay (LiPA) to detect each genotype/subtype, was less than or equal to 50 IU/mL.

Conclusions: 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.

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 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 HCV 5' UTR and core region of different HCV genotypes. Alkaline phosphatase-labeled streptavidin is bound to the biotinylated hybrid, and BCNIP chromogen reacts with the streptavidin-alkaline phosphatase complex, forming a purple/pink 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 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 (-20°C to -80°C for RNA and -20°C to -25°C for immobilized biotinylated DNA) and used at a later time.

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

<table>
<thead>
<tr>
<th>Genotype/Subtype</th>
<th>Plasma</th>
<th>Serum</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>LoD Estimate (95% Confidence Limits)</td>
<td>LoD Estimate (95% Confidence Limits)</td>
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<tr>
<td></td>
<td>(IU/mL)</td>
<td>(IU/mL)</td>
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<tr>
<td>1a</td>
<td>195 (152–239)</td>
<td>211 (170–300)</td>
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<tr>
<td>1b</td>
<td>356 (291–489)</td>
<td>437 (287–467)</td>
</tr>
<tr>
<td>2a</td>
<td>289 (236–401)</td>
<td>301 (245–464)</td>
</tr>
<tr>
<td>2b</td>
<td>203 (196–330)</td>
<td>291 (218–516)</td>
</tr>
<tr>
<td>3a</td>
<td>131 (98–308)</td>
<td>150 (119–266)</td>
</tr>
<tr>
<td>3b</td>
<td>266 (199–468)</td>
<td>255 (203–364)</td>
</tr>
<tr>
<td>4a</td>
<td>292 (232–431)</td>
<td>301 (267–722)</td>
</tr>
<tr>
<td>4b</td>
<td>312 (258–419)</td>
<td>310 (274–457)</td>
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</tbody>
</table>

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

1* Limit of detection for the VERSANT HCV Genotype 2.0 Assay (LiPA) for each genotype/subtype along with 95% confidence limits is shown in Table 2.

2 The Limit of detection for each genotype/subtype was u400 IU/mL in plasma and u650 IU/mL in serum.

3 The Limit of detection 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


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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