Evaluation of the Siemens VERSANT HCV Genotype 2.0 Assay (LiPA)

Introduction

The VERSANT HCV Genotype 2.0 Assay (LiPA) is a line probe assay for the identification of HCV genotypes and differentiation of HCV subtypes in HCV RNA positive human serum or EDTA plasma specimens. The assay is intended to be used to guide the selection of treatment type and duration for individuals being considered for antiviral treatment and who are chronically infected with HCV. The kit was evaluated by the NRL for registration as a reference test in the category of “Monitoring and Management”.

Methods

Reproducibility

HCV RNA from each of 10 replicates of a high viral load (17,926 IU/ml) or a low viral load (4,930 IU/ml) specimen was extracted using the QIAamp DSP Virus Kit. Following extraction of viral RNA reverse-transcription and PCR amplification was performed using the VERSANT HCV Amplification Kit (LiPA). Each amplified product from each replicate was then tested once in the Genotype assay.

Sensitivity

A total of 52 HCV RNA positive samples, representing common HCV genotypes and subtypes, were tested as above in the Genotype assay. All specimens were of clinical origin and had been previously assigned a genotype by nucleic acid (NA) sequencing of either the 5’UTR or Core regions of the HCV genome.

Specimens for which an interpretable band pattern was produced were analysed for genotype determination. The genotype and subtype determined for each specimen by the Genotype assay was compared with that previously determined by nucleic acid (NA) sequencing to determine the concordance of genotype results.

Results

Reproducibility

High viral load specimen

The Genotype assay was able to provide genotype and subtype results concordant with NA sequencing results for each of 10 replicates of the high viral load specimen (Table 1).

Table 1. Genotyping and subtyping reproducibility in replicates of a single specimen of high viral load.

	Genotype Assigned by:		Subtype Assigned by:	
	NA Sequencing	**LiPA 2.0**	**NA Sequencing**	**LiPA 2.0**
Specimen	**1**	**1**	**a**	**a**
Number of replicates	10	10	10	10

Low viral load specimen

The Genotype assay was able to provide genotype and subtype results concordant with NA sequencing results for 9 of the 10 replicates of the low viral load specimen (Table 2). The single instance of a lack of concordance arose because one strip showed a pattern of bands that was uninterpretable owing to the presence of a very faint band observed at position 25; but for the presence of this band a correct determination of both genotype and subtype would have been achieved. The faint band at position 25 was not observed in any of the other 9 replicates. Moreover, when the specimen used in this reproducibility study was subsequently analysed on two separate occasions, genotypes and subtypes concordant with NA sequencing were determined.

Table 2. Genotyping and subtyping reproducibility in replicates of a single specimen of low viral load.

	Genotype Assigned by:		Subtype Assigned by:	
	NA Sequencing	**LiPA 2.0**	**NA Sequencing**	**LiPA 2.0**
Specimen	**6**	**6**	**d**	**c-l**
Number of replicates	10	9	10	9

Sensitivity

A total of 52 specimens were tested using the Genotype assay. Most specimens gave interpretable band patterns and in each such case interpretation was possible at both the genotype and subtype level (Table 3). A total of 5 specimens gave uninterpretable band patterns. On closer inspection it was observed that for 3 of these specimens the band pattern was such that if a single faint band was ignored on each strip an interpretable pattern could be obtained (at both genotype and subtype level for two specimens). The band patterns of the remaining two specimens did not match any of the patterns shown in the Genotype assay interpretation chart.

Table 3. The proportion of 52 specimens tested for which interpretable genotype or subtype band patterns were generated in the Genotype assay.

Typing level	Number of specimens:		
	tested	having ***interpretable*** band patterns	having ***uninterpretable*** band patterns
Genotype	52	47	5
Subtype	52	47	-

The extent to which the Genotype assay was able to correctly determine both the genotype and subtype of specimens was assessed by comparison with existing NA sequencing assignments. Of the 47 specimens with interpretable banding patterns, the Genotype assay gave one determination that was discordant with NA sequencing (Table 4).

A total of 5 specimens had previously been assigned a genotype by NA sequencing, but not a subtype. Consequently, although the Genotype assay was able to provide a determination for the subtype of each of these 5 specimens, it is not possible to assess the veracity of these determinations. Since subtype differentiation is unlikely to be as clinically relevant as genotype differentiation, the lack of NA sequencing subtype information for these specimens has not been pursued. Of the remaining specimens for which verifiable subtype information was determined, the Genotype assay gave one discordant result. The Genotype assay determined this specimen to be 6c-l. However, NA sequencing of this specimen gave a result of 6n. Although clearly discordant at the subtype level, it should be noted that: subtype ‘n’ is not included as an option in the Genotype assay interpretation chart and in any case the determination of 6c-l provides a distinction from a ‘non-6a/b’ subtype.

Table 4. The concordance between the Genotype assay and NA sequencing at both genotype and subtype levels.

Typing level	**Number of results:**		
	concordant	**discordant**	**total**
Genotype	46	1	47*
Genotype and subtype	41	1	42**

** Specimens with uninterpretable band patterns were not included in the calculation of genotype concordance; **At the time NA sequencing was performed, 5 specimens had been assigned a genotype but not a subtype. These 5 specimens were not included in the calculation of subtype concordance.*

The assay has since been registered on the Australian Register of Therapeutic Goods.