

Evaluation of the Siemens VERSANT HCV Amplification 2.0 Kit (LiPA)

Introduction

The Siemens VERSANT HCV Amplification 2.0 Kit (LiPA) is designed for use in reverse transcription and amplification of the 5' untranslated region (5'UTR) and core region of the Hepatitis C Virus (HCV) genome. Amplified products generated with this kit can be analysed using the Siemens Versant HCV Genotype 2.0 Assay (LiPA). The kit was evaluated by the NRL for registration as a reference test in the category of "Monitoring and Management".

Methods

Reproducibility

Two specimens, of high (17,926 IU/ml) and low (4,930 IU/ml) viral load, were tested ten times each. Viral RNA was extracted using the QIAamp DSP Virus Kit. The presence of amplified products of 240 and/or 270 base pairs was determined using agarose gel electrophoresis. The size of visible bands was estimated with reference to a 100 basepair DNA ladder.

Sensitivity

A total of 52 specimens of different genotype and origin were tested once each. The presence of amplified products of either 240 and/or 270 base pairs was determined as above.

Results

Reproducibility

High viral load specimen

All 10 replicates of specimens with either high or low viral load were successfully amplified, each producing both 240 and 270 basepair amplicons (Table 1).

Table 1. Amplification reproducibility in replicates of a single specimen of high viral load.

Viral load	Number of replicate amplifications	Number of 240 basepair amplicons	Number of 270 basepair amplicons
High	10	10	10
Low	10	10	10

Sensitivity

All of the 52 specimens tested showed either one or both of the 240 and 270 basepair amplicons (Table 2). While the 240 basepair amplicon was observed for all specimens, for three of these specimens the 270 base pair amplicon was not observed. Most specimens showed clear, distinct bands corresponding to only the 270 and/or 240 basepair amplicons. In a small number of specimens several faint bands were observed below the 240 basepair amplicon.

Table 2. Amplification sensitivity in 52 clinical specimens.

Number of amplifications	Number of 240 basepair amplicons	Number of 270 basepair amplicons
52	52	49

The 270 basepair amplicon represents the Core region of the HCV genome. Of the three specimens for which the 270 basepair amplicon was not observed, one specimen subsequently generated 2 'core' lines (lines 23 and 25), sufficient to allow a correct genotype to be interpreted, in the VERSANT HCV Genotype 2.0 Assay. Of the remaining two specimens, although a core control line (line 23) was present in each, the absence of other lines in the 'core' region of these strips meant that the genotypes were interpreted by using interpretable line patterns in the 5'UTR region of these strips.

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