

# Sensitive Quantitation of HBV Genotypes A - H

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## Background and Objectives

The worldwide prevalence of chronic HBV individuals at elevated risk for development of serious liver disease requires high performance diagnostics. The measurement of HBV DNA levels in serum and plasma has become the most direct and reliable method used for monitoring HBV. This approach can be used to aid in the management of HBV infection by identifying individuals with high levels of viral replication who might benefit from antiviral therapy, monitoring patients on therapy, predicting the success of antiviral therapy, and identifying the development of resistance to therapeutic agents.

To meet the growing clinical demand for a sensitive, quantitative assay with a broad quantitative range, affigene® HBV trender has been developed for measuring HBV DNA in serum and plasma.

## Method

affigene® HBV trender is based on the Scorpion™ Technology (see fig 4). This technology provides a sensitive and rapid method for real-time quantitation of HBV DNA. An internal control (IC) is included in each sample to indicate for the effects of inhibition, and control for sample preparation, amplification and detection.

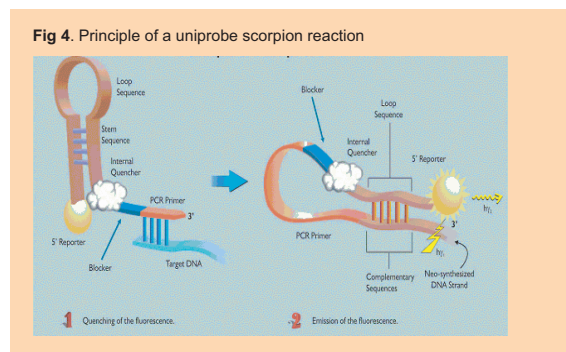
The quantitation efficiency of the kit was evaluated by quantitating the eight different HBV genotypes (A to H) cloned into plasmids. Each genotype was quantitated in three different concentrations.

The LOD was determined by analysis of dilutions of the WHO International Standard for HBV NAT assays 97/746. The sample was diluted to six levels in HBV negative plasma and serum, respectively. Each dilution was prepared in 24 replicates. Probit analysis was used to calculate the LOD concentration.

The precision was determined by analysis of two different dilutions of the PeliSpy VQC HBV (genotype A). The precision was determined at 40 occasions.

The quantitative range was determined by analysis of dilutions of the PeliSpy VQC HBV (genotype A). The sample was parallelly diluted in HBV negative plasma and DNA was extracted and analysed.

All data were generated using affigene® DNA extraction and Mx3000P™ (Stratagene).



## Results

### Inclusivity of HBV genotypes A-H

All genotypes were quantitated with equal efficiency (see fig 1).

### Limit of detection (LOD)

LOD was defined as the titer level where 95% of all replicates of an HBV containing sample are determined positive.

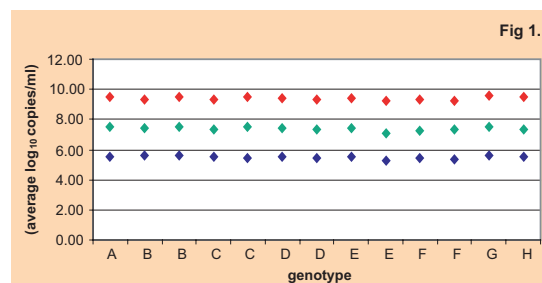
The LOD using 1000 µl plasma was determined to 3.4 IU/ml (95% conf interval: 2.4-13 IU/ml). The LOD using 200 µl plasma was determined to 51 IU/ml (95% conf interval: 31-151 IU/ml). The LOD using 200 µl of serum was determined to 89 IU/ml (95% conf interval 56-183 IU/ml).

### Precision

The precision is shown in table 1.

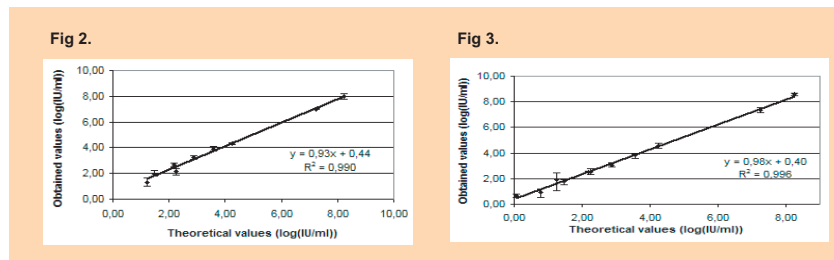
### Quantitative range

The quantitative range was 172-1.72x10<sup>8</sup> IU/ml using either 200 ml and 1000 µl of plasma. The precision has not been determined below 172 IU/ml (see figs 2 and 3).



**Table 1.**

| Sample: | Expected titre (IU/ml) | Obtained titre (IU/ml) | Log titre (log <sub>10</sub> (IU/ml)) | Total imprecision (log <sub>10</sub> (IU/ml)) |
|---------|------------------------|------------------------|---------------------------------------|-----------------------------------------------|
| High:   | 1.72x10 <sup>7</sup>   | 1.1x10 <sup>7</sup>    | 7.1                                   | 0.23                                          |
| Low:    | 1.72x10 <sup>2</sup>   | 3.3x10 <sup>2</sup>    | 2.5                                   | 0.25                                          |



### QCMD panel

Correlation between titers generated with affigene® HBV trender and the 2003 QCMD/VQC sample panels is excellent (see table 2)

### Diagnostic specificity

The diagnostic specificity of the assay was 100% as none of 105 HBV negative blood donor samples (determined by serology assay) was determined positive.

**Table 2.**

| Panel member | Expected Titre log <sub>10</sub> (c/ml) | affigene titre log <sub>10</sub> (c/ml) |
|--------------|-----------------------------------------|-----------------------------------------|
| #1           | 6.0                                     | 6.6                                     |
| #2           | 3.0                                     | 3.3                                     |
| #3           | neg                                     | neg                                     |
| #4           | 5.0                                     | 4.9                                     |
| #5           | 4.0                                     | 4.1                                     |
| #6           | 3.5                                     | 3.8                                     |
| #7           | 2.3                                     | 4.2                                     |
| #8           | 5.0                                     | 5.4                                     |

## Conclusion

affigene® HBV trender provides a robust and sensitive method for measuring pre- and post-treatment HBV DNA levels in all patients regardless of genotype. The assay performance is well suited for monitoring patients being treated with the new efficacious therapies for HBV and for studies designed to determine the relationships between HBV viral load and the disease state of the emergence of drug resistance.

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