

affigene® HBV trender in WHO Collaborative Study 2009

“Collaborative Study to Establish a World Health Organization International Genotype Panel for Hepatitis B Virus Nucleic Acid Amplification Technique (NAT) – Based Assays”¹

Background

A Collaborative Study was designed by the World Health Organisation (WHO) and the Paul-Ehrlich-Institut (PEI), aimed to evaluate a panel of lyophilized plasma samples containing different Hepatitis B Virus (HBV) genotypes, for use with Nucleic acid Amplification Technique (NAT)-based diagnostic testing. 17 laboratories from 12 different countries participated in the study. Of the 19 datasets that were submitted to the PEI in total, 16 were quantitative NAT, two qualitative NAT and one sequence- and genotype analysis. 14 of the quantitative datasets were analysed with commercial real-time PCR instruments. The study includes the parallel testing of the 2nd International Standard for HBV DNA (97/750, genotype A2). This report presents the result of the quantitative analysis using affigene® HBV trender.

Material and methods

The HBV panel (5086/08) consisted of 15 samples of varying geographical origin representing the following HBV genotypes: A (3 samples), B (3), C (3), D (3), E (1), F (1) and G (1). The corresponding genotypes for each sample were unknown to the participants at the time of testing.

The HBV panel and the 97/750 standard were prepared as duplicates using affigene® DNA extraction, at three separate test occasions. Subsequently, the samples were analysed as single replicates using affigene® HBV trender on the Mx3000P® instrument (Stratagene, La Jolla, CA). Two different kit lots of affigene® HBV trender were used in the testing. The results from all three tests were reported to the PEI, who compiled the data from all participants.

Results and discussion

The 15 samples and the 97/750 standard had expected viral loads ranging from 1×10^4 to 1×10^6 copies/ml. Figure 1 displays the quantitative results of the HBV panel, where the viral load is plotted versus sample. The genotype and geographical origin is indicated for each sample.

The figure shows the expected viral load, the reported mean of viral loads reported from the 16 quantitative datasets and the obtained mean from the three affigene® HBV trender runs.

All present genotypes were detected by affigene® HBV trender, with good accuracy in quantitation and small run to run variation. The quantitative results are all within one standard deviation (SD) from the Total Mean (TM) of all of all reported datasets, with the exception of the D3 genotype sample from South Africa and the reference sample. For these two samples, the result was slightly higher than one SD from the reported mean, however within the criteria set for precision for affigene® HBV trender ($\pm 0,3 \log_{10}$).

¹M. Chudy et. al, “Collaborative Study to Establish a World Health Organization International Genotype Panel for Hepatitis B Virus Nucleic Acid Amplification Technique (NAT) – Based Assays”, WHO/BS/09.2121 report, World Health Organisation, 2009

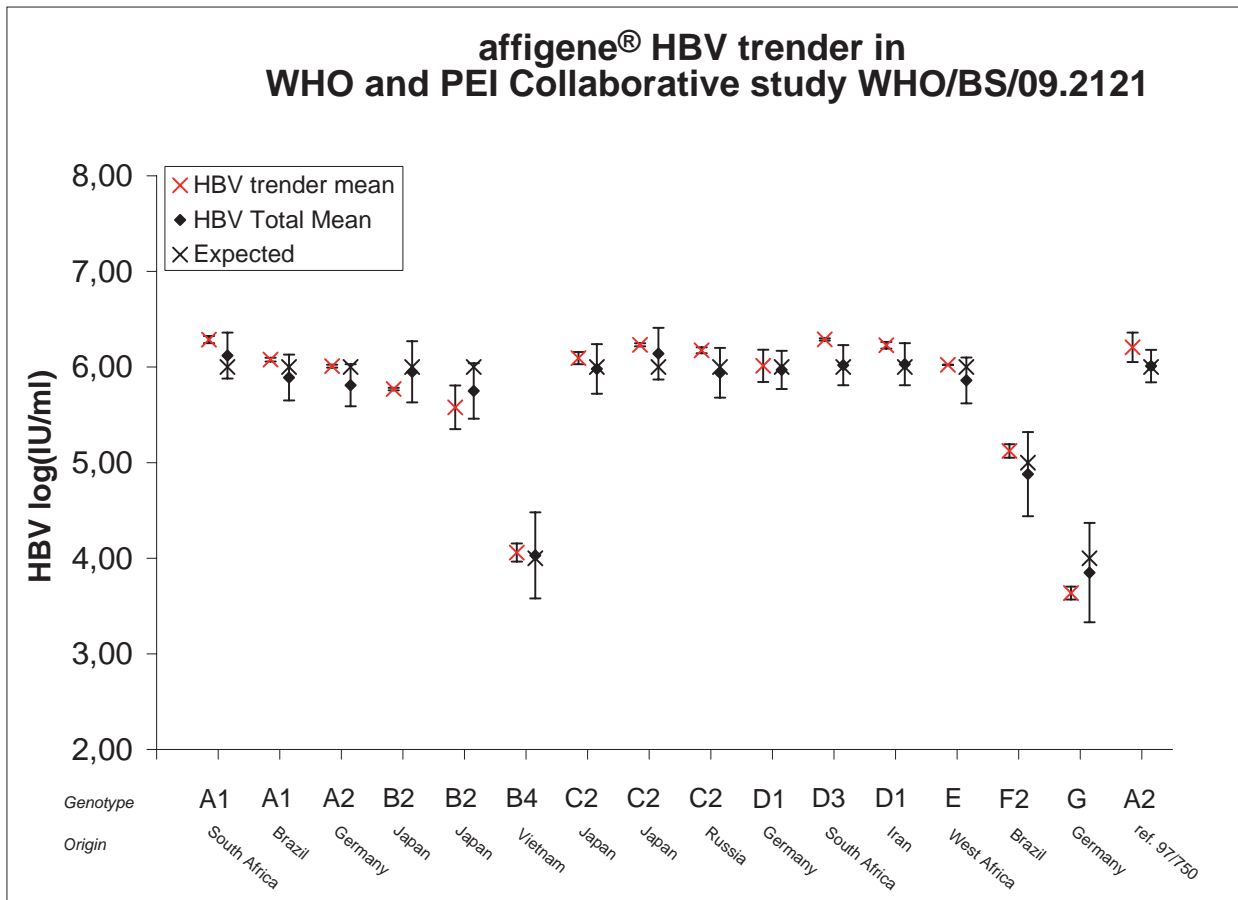


Figure 1. Results of the 2009 HBV panel WHO/BS/09.2121

The expected viral load is displayed as a cross (X). The reported mean viral load from the 16 quantitative datasets is shown as a diamond (◆), with error bars (shown as ± 1 SD of the log mean of the 16 datasets). The three affigene® HBV trender datasets are displayed as the mean (X), with error bars indicating ± 1 SD of the log mean.

Conclusion

- The affigene® HBV trender assay performed well in the Collaborative Study, detecting all genotypes A-G.
- The affigene® HBV trender results are consistent with small run to run variation.
- The quantitative results are all within one SD from the mean of all reported data, except for two samples. The D3 genotype sample and the reference are slightly higher, however within the criteria for precision set for affigene® HBV trender ($\pm 0,3 \log_{10}$).