

DEVELOPMENT OF A FLEXIBLE AND SENSITIVE IN-HOUSE REAL-TIME PCR ASSAY FOR THE QUANTIFICATION OF HBV DNA (P-217)

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BACKGROUND

HBV-DNA quantification has been utilized in clinical practice for monitoring response to therapy, evaluating indications for treatment, identifying emergence of resistance and for the assessment of occult HBV infection. Improved sensitivity of HBV-DNA tests is of critical importance for the management of HBV infection

AIMS

To modify a previously developed in-house real-time PCR assay (Paraskevis et al, 2002) into a flexible system for HBV-DNA quantification according to the WHO 1st International Standards (IU/mL).

METHODS

Previously used HBV-DNA standards were calibrated against the WHO 1st International Standard for HBV DNA (OptiQuant® HBV DNA Quantification Panel, Accrometrix Europe B.V.). The 95% and 50% HBV-DNA detection limits were calculated by probit analysis.

RESULTS

According to the calibration results, 1 IU/mL = 2.8 copies/mL. Consequently, the assigned values can be reported as International Units per milliliter (IU/mL). The 95% and 50% detection end-point of the assay were 25 and 10 IU/mL. Most importantly, the sensitivity of the assay can be further enhanced by using larger volumes of plasma (up to 5 mL) instead of the standard quantity of 0.5 mL. HBV-DNA isolation from large volumes has been accomplished by a concentration step performed before the standard procedure. Consecutive quantification of HBV-DNA isolated from > 0.5 mL showed a very good correlation with the standard procedure. Finally using this flexible platform, the quantification of HBV-DNA can range from < 10 to > 1010 IU/mL.

CONCLUSIONS

We developed an ultra sensitive in-house real-time PCR assay for HBV-DNA quantification. The analytical sensitivity of the standard module can be further enhanced (< 10 IU/mL) by utilizing larger volumes of plasma.

References

D. Paraskevis, C. Haida, N. Tassopoulos, M. Raptopoulou, D. Tsantoulas, H. Papachristou, V. Sypsa, A. Hatzakis. Development and Assessment of a Novel Real-Time PCR Assay for the Quantitation of HBV-DNA. *J Virol Methods* 2002; 103:201-212.