

## INTERPRETATION OF HBV NAT RESULTS IN HEALTHY SUBJECTS (P-112)

G. Cambie, A. Degiuli, S. Conca, E. Parabiaghi, L. Vignati, M.L. Nardella, M. D'Agostino, F. D'Agostino  
Azienda Ospedaliera di Lodi, Lodi, Italy

**Background:** NAT tests are presently considered the most suitable method for blood donor selection (also in HBsAg negative chronic phase of infection) in case of intermediate or high HBV endemicity, mainly in consideration of the unacceptable rate of donor exclusion related to anti-HBc based screening in such areas. However discordant results obtained on the same sample by NATs of different format sometimes make data interpretation difficult.

**Aims:** Our aim was to evaluate HBV DNA reactivity by PCR amplification of multiple HBV ORFs in a group of HBsAg negative, anti-HBc highly reactive healthy subjects.

**Methods:** 40 subjects were selected from our laboratory routine for the following features: HBsAg negative by four different ELISA tests (AxSYM Abbott, Enzygnost Behring, Monolisa Pasteur, Medical Systems), anti-HBc strongly positive (> 96% of inhibition), anti-HBe and anti-HBs positive at different levels, anti-HCV, anti-HDV and anti-HIV negative at AxSYM Abbott, ALT in the normal range. After adequate information, they were followed for at least 6 months through serial bleeds (mean 4, range 3-5). On every sample HBsAg, anti-HBc, ALT and HBV DNA were carried out and on the last one the whole initial serologic panel was repeated. DNA was extracted from 200ul (QIAGEN) and amplified by in house PCR with primer pairs specific for S, Pol, X, pre-C/C, C region of HBV genome and the amplified products were detected in agarose gel electrophoresis, with proper controls. Every DNA test was performed in duplicate in at least two different analytical sessions. Sensitivity (7.8-11.7 U.I. 95% detection probability), specificity, repeatability and robustness of the method had been previously validated.

**Results:** In most of the cases only a fraction of the researched viral sequences was amplified: at least one in 28/40, two in 27, three in 24. HBV genome was apparently complete (amplification of X, pre-C/C, Core, S, Pol) in 2/40. Core reactivity was the most frequent (27/40), the less frequent was S, detected in 4 cases, 2 of which apparently complete. In each subject serologic pattern, ALT level and DNA reactivity profile remained unchanged throughout the study period.

**Conclusions:** The subjects pre-selection, PCR analytical sensitivity and peculiar design of the present study led to a high HBV DNA prevalence but only in a limited proportion of reactive cases the whole panel of HBV sequences was amplified. Discrepancies in PCR results obtained with different HBV primer pairs in the same subject hasn't an univocal interpretation yet. In this study S region appears a quite reliable serum marker of genome integrity and probable productive infection. On the contrary, detection in blood of sequences like pre-C/Core, mostly not associated to one or more of the other regions, might better correlate with highly mutated, defective viruses or integrated forms. Theoretically, even circulating 'defective' genomes could indicate persistence of intracellular, episomic viruses exhibiting minimal replication and/or reactivation potential. Nevertheless NATs capable of amplifying sequences in a single HBV ORF, particularly Core in this study, cannot give conclusive informations about the existence of productive infection nor the actual, prevalent DNA molecular form.