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PERFORMANCE CHARACTERISTICS OF THE QUALITATIVE AND QUANTITATIVE TARGET-CAPTURE PCR HBV NAT ASSAY

V. Shyamala, P. Arcangel, J. Cottrell, D. Coit, A. Medina-Selby, C. McCoin, D. Chien, B. Phelps*

Chiron Corporation, Blood Testing Division, Emeryville, CA 94608

Background:

The application of nucleic-acid amplification (NAT) technology has dramatically improved the detection of HIV-1 and HCV at earlier stages of infection. The next anticipated NAT assay includes testing for HBV DNA. Here we describe the development of a Target-Capture PCR technology based NAT assay for HBV.

Methods:

A magnetic bead-based protocol was used for isolation of HBV DNA target along with a related internal control in a single tube from 0.5 mL plasma. The Region X on the isolated target on the beads was amplified by TaqMan technology. The qualitative assay was validated for analytical and clinical sensitivity, specificity, and reproducibility. The quantitative assay utilizes a 12 member panel of standards tested in triplicate in the range of 50-105 IU/mL. A blinded panel of HBV samples by QCMD were tested for detection and quantitation.

Results:

The analytical sensitivity of the qualitative Target-Capture PCR HBV assay determined with WHO HBV standard indicated >95% positivity at 15 IU/mL. The assay detects all known genotypes, has very high specificity and tolerates a variety of anti-coagulants, interfering substances, and plasma from pathological conditions. The quantitative assay estimates HBV in the range of 10²-10⁹ IU/mL for unknowns. The QCMD HBV results indicate the detection of the lowest copy member and also very close estimation of IU/mL for the A genotype.

Conclusions:

Using a combination of magnetic bead based target capture and TaqMan amplification-detection a rapid, sensitive, user-friendly, and accurate assay for HBV DNA detection and quantitation has been developed.