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VALIDATION OF ALTERNATIVE NAT HBV ASSAY: A HIGHLY SENSITIVE PCR BASED ASSAY FOR HBV DNA.

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Introduction:

The Transcription Mediated Amplification (TMA) based Procleix® Ultrio™ assay for nucleic acid detection of HCV, HIV and HBV is being developed by Gen-Probe. Nucleic acid tests by alternate technologies for all three viruses are required for clinical trials and used as supplemental assays to confirm samples positive by the Ultrio assay. We have developed and validated a Bead capture-Taqman based Alternative NAT HBV assay for confirming TMA positives HBV samples.

Materials and Methods:

In consultation with Regulatory, Validation, Quality Assurance and Biostatistics groups, with a total of 2750 samples, an exhaustive validation protocol was developed to evaluate the performance of the Alternative NAT HBV assay. The aspects of validation included Analytical and Clinical sensitivity, Specificity, and Reproducibility. Clinical sensitivity study included known HBV genotypes and naturally positive HBV samples. Specificity included testing 1000 normal donors, samples positive for other viruses, compatibility of commonly used anticoagulants including K3EDTA, Sodium Citrate, K2EDTA, ACD, PPT, Heparin, CPDA-1, CPD and CP2D. Sensitivity of the assay was tested in the presence of a variety of bacteria, pathological samples, and freeze-thaw conditions.

Results:

In the Analytical sensitivity panel comprising of 80 replicates each of serially diluted WHO HBV International standard, the Alternative NAT HBV assay had >95% positivity at 15 IU/ml. Genotypes A, B, E, and G were detected at 100 copies/ml, genotypes C and D were detected at 300 copies/ml. Fifty naturally HBV infected donors tested positive. The specificity of the Alternative NAT HBV assay was confirmed through negative results from 1000 normal donor samples and with plasma containing a variety of other viral nucleic acids. Plasma collected with different anticoagulants was compatible with this assay, as also plasma from pathological conditions, plasma containing interfering substances, plasma contaminated with bacterial cultures. The assay was sufficiently robust to detect HBV at 15 IU/ml in plasma following three freeze-thaws. The reproducibility test performed by Bayer Reference Testing Labs (BRTL) with 576 samples was 100% positive at 15 IU/ml.

Conclusions:

An Alternative NAT HBV assay has been validated, and meets the criterion for sensitivity, specificity and reproducibility to be used as a supplemental test to confirm samples positive by the Procleix dHBV assay. The testing of TMA positive HBV samples will be performed at the Bayer Reference and Testing Laboratory.