## Poster Abstract P13

### Increasing the performance of a fully automated, quantitative, assay for the detection of circulating HBV pregenomic RNA

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**Background:** Hepatitis B virus pregenomic RNA (HBV pgRNA) has been proposed as a potential circulating biomarker for the activity of covalently closed circular DNA (cccDNA) that is present in infected hepatocytes of HBV patients. There are an increasing number of studies showing the utility of HBV RNA quantitation in monitoring the effectiveness of both experimental and standard of care therapies and it is being investigated as an endpoint for clinical trial effectiveness and therapy removal. We have previously reported on the development of a fully automated dual-target, quantitative assay for the measurement of HBV pgRNA with a lower limit of quantitation (LLOQ) of 1.65 log U/mL (~152 copies/mL). Here we report on a modified assay v2.0 with increased overall sensitivity by 7-15-fold.

**Methods:** A Research Use Only (RUO) fully automated real-time PCR assay was developed for the Abbott *m*2000 (Abbott Molecular Diagnostics, Des Plaines, IL, USA) platform and previously described (v1.0). Briefly, targets in conserved regions of the HBV x and core genes are used to ensure robust detection in the presence of mutations, and the assay is standardized against a WHO secondary DNA standard. An internal control is included to detect PCR interference. Assay LLOQ was measured by Probit analysis to be 1.65 log U/mL (~152 copies/mL) using a 0.2 mL sample volume input and 95% detection threshold. Modifications were made to the reagent formulation, cycling parameters, and sample input volumes which improve analytical performance. Performance (linearity, sensitivity, and standard deviations) of the new assay (v2.0) was compared with v1.0 using serial dilution panels from selected HBV clinical samples. A patient sample with high levels of HBV RNA was selected from which a panel of 11 serial dilutions into negative human plasma was made. Target HBV RNA concentrations ranged from 1.00E6 log U/mL (~3.41E6 copies/mL) down to 3.13 U/mL (~10-11 copies/mL) and either 3 or 20 replicates at each dilution were tested with the v1.0 and v2.0 assays.

**Results:** Overall sensitivity of the v1.0, defined as 95% detection was determined to be 25 U/mL (~86 copies/mL). The v2.0 assay with a 0.2 mL input volume showed enhanced sensitivity down to 6.3 U/ml (~22 copies/mL). Increasing the sample input volume to 0.6 mL further increased the overall sensitivity to 3.1 U/mL (~10-11 copies/mL). Linear regression analysis showed a highly linear relationship between expected and quantitated HBV RNA levels (R^2 >0.998) using the v2.0 assays. Standard deviations in quantitation between replicates were also lower with the v2.0 assays, suggesting further enhancements were made with regards to run-to-run variability. Lastly, quantitated values of samples above the v1.0 assay LLOQ were indistinguishable from quantitated values from the v2.0 assays, confirming the ability to compare results between the different assay versions.

**Conclusions:** Here we have shown increased sensitivity with the HBV RNA v2.0 assay resulting in a 7-15-fold increase in sensitivity over the first-generation assay and reducing the limit of detection to ~10-11 copies/mL. Importantly, this increased sensitivity does not impact quantitation at higher HBV RNA levels compared to the v1.0 assay, allowing for future studies to compare results with those that were run on the v1.0. Future studies will be conducted to determine if improved HBV RNA sensitivity yields additional clinical insights regarding therapy effectiveness and/or off therapy outcomes.