Su1334 Increasing the performance of a fully automated quantitative assay for the detection of circulating HBV pregenomic RNA

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1. 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. HBV RNA is of particular interest in patients who are on nucleos(t)ide analog therapy as HBV DNA is generally low or undetectable in these patients [1]. An increasing number of studies [2-4] have shown 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 an automated dual-target, quantitative assay for the measurement of HBV pgRNA (v1.0) with a lower limit of quantitation (LLOQ) of 1.65 log U/mL (~152 copies/mL) [5]. Here we report on a modified assay (v2.0) with increased overall precision, sensitivity (15-fold), and a limit of detection of 10-11 HBV RNA copies/mL. Quantitated HBV RNA levels with the v2.0 assay were indistinguishable from v1.0. We also show storage and freeze/thaw stability for HBV RNA.

2. Methods

- A Research Use Only (RUO) fully automated real-time PCR assay for the detection and quantitation of HBV RNA (v1.0) was developed for the Abbott m2000 (Abbott Molecular Diagnostics, Des Plaines, IL, USA) platform and previously described [5]. 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 (0.6 mL) which improve analytical performance. Performance (linearity, sensitivity, standard deviation, and concordance) of the new assay (v2.0) was compared to v1.0. 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 both assays. Longitudinal samples from 3 on-therapy patients were also tested.

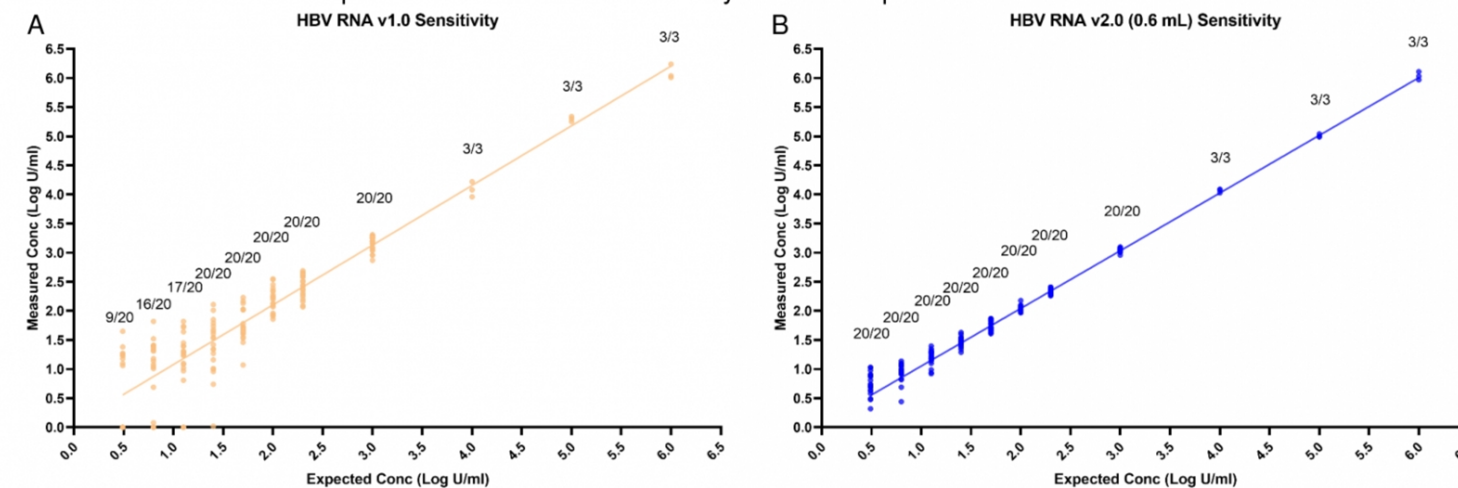
Table 1: Comparison of precision and reported results with v1.0 and v2.0

Expected Concentration (log U/mL)	v1.0 Stdev (log U/mL)	v2.0 Stdev (log U/mL)	Δv1.0-v2.0 Reported Result (log U/mL)
6.00	0.13	0.07	0.05
5.00	0.04	0.03	0.25
4.00	0.13	0.04	-0.05
3.00	0.13	0.03	0.01
2.30	0.20	0.04	-0.05
2.00	0.21	0.06	0.00
1.70	0.28	0.09	-0.14

Standard deviation is lower with v2.0 and reported results are within v1.0 standard deviation

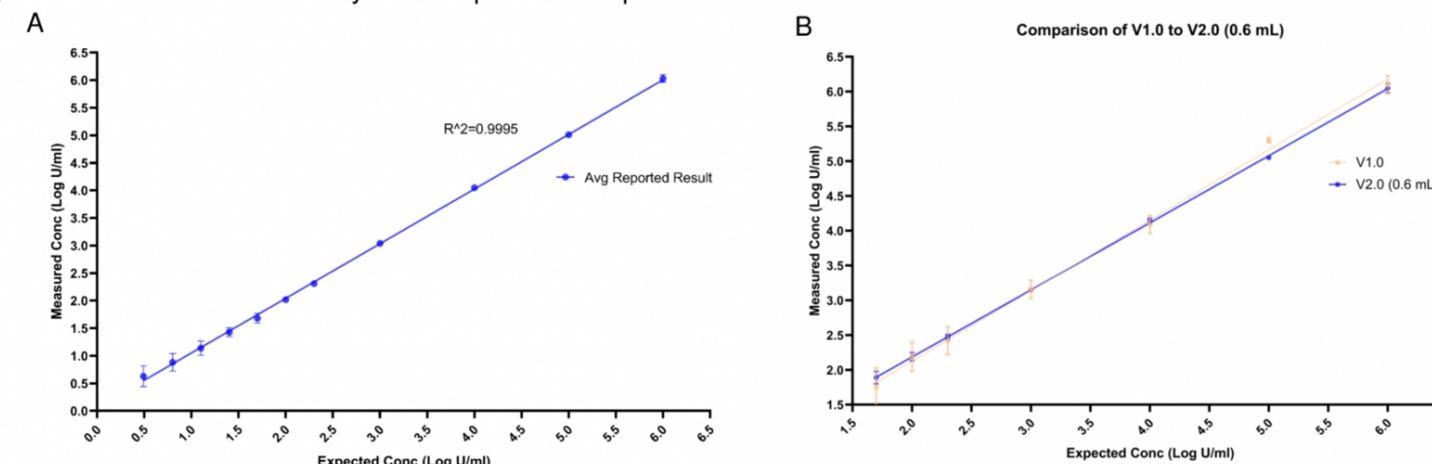
3. Results

Figure 1. HBV RNA serial dilution panel shows increased sensitivity of v2.0 compared to v1.0



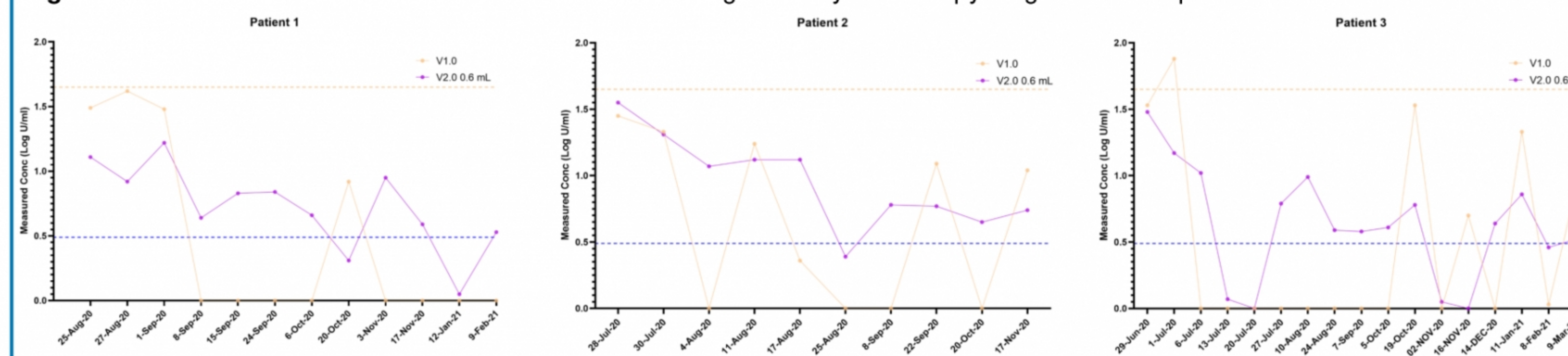
An HBV clinical sample was serially diluted to the expected concentrations shown on the x-axis and tested with (A) HBV RNA v1.0 and (B) HBV RNA v2.0 with the measured concentrations reported on the y-axis. Numbers above each concentration indicate the number of positive detections at each tested concentration.

Figure 2. HBV RNA v2.0 Linearity and comparison of reported results from v1.0 and v2.0



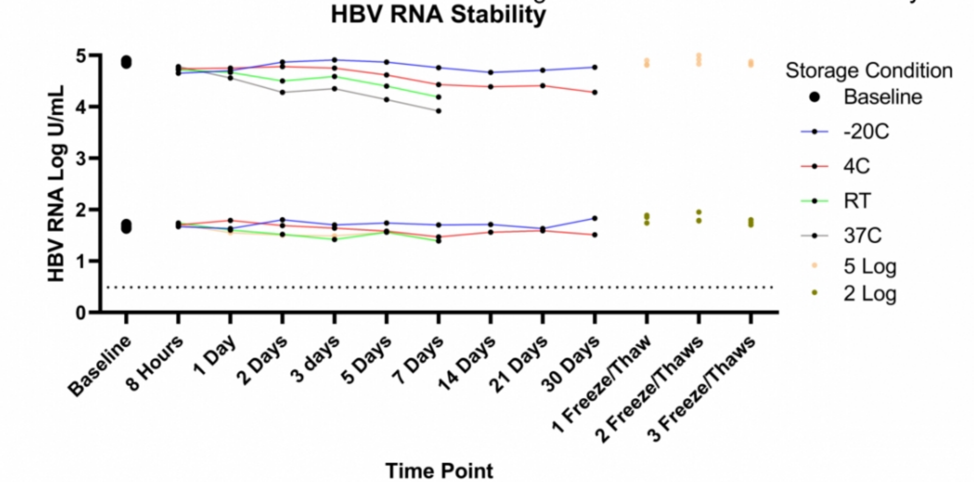
(A) Reported HBV RNA v2.0 results show strong linearity across the dynamic range of the assay. (B) Comparison of v1.0 and v2.0 quantitated HBV RNA results showing reported results are indistinguishable between the two versions.

Figure 3. HBV RNA v2.0 enhances detection and increases granularity in low copy longitudinal samples



Longitudinal HBV RNA results from 3 individual patients who were tested with HBV RNA v1.0 (green) and v2.0 (blue). Horizontal lines indicate v1.0 and v2.0 limits of detection.

Figure 4. HBV RNA is stable under various storage conditions and freeze/thaw cycles



An HBV clinical sample was serially diluted to two concentrations (5 and 2 Log U/mL) and stored under various conditions for the indicated amount of time (x-axis) before testing with HBV RNA v2.0. Baseline and Freeze/Thaw samples were stored at -80.

4. Conclusions

- HBV RNA v2.0 is ~15 fold more sensitive than v1.0 and detected 100% (20/20) of tested replicates at 10 copies/mL concentration.
- Reported results are linear (R^2=0.9995) across the dynamic range of the v2.0 assay.
- Results reported by v2.0 are within the standard deviation of those reported by v1.0 showing good concordance between assay versions.
- Increased sensitivity of the HBV RNA v2.0 (0.6 mL) assay yields tangible increases in detected and quantifiable results in low RNA copy samples from patients on therapy.
- HBV RNA shows good short-term stability under various storage conditions with recovered signal dropping less than 1 Log U/mL after 7 days at RT or 37C.
- HBV RNA showed no loss in recovered signal after 3 freeze/thaw cycles.

References

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Affiliations and Financial Disclosures

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