Hepatitis B pregenomic RNA: Performance of an Automated Real-Time Quantitative Assay

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Introduction

Nucleos(t)ide analogue (NA) treatment suppresses HBV DNA synthesis for chronic hepatitis B (CHB) patients but does not affect synthesis of HBV pregenomic RNA (pgRNA). Since hepatitis B virus pgRNA is detectable in serum during NA treatment, it has been proposed as a potential biomarker of HBV covalently closed circular DNA (cccDNA) activity within the infected hepatocyte. Therefore, monitoring of serum HBV pgRNA during NA treatment of CHB patients may be valuable to clinical decisions regarding treatment.

Herein, we present the analytical performance of a new assay for the detection and quantitation of hepatitis B pregenomic RNA in CHB patients. The HBV pgRNA Research Use Only (RUO) assay utilizes a dual-target real-time quantitative PCR approach on the Abbott *m*2000 RealTi*me* system. With an LoQ (20%CV)/LoD (95% detection) at 1.65 Log U/mL, HBV pgRNA detection is calibrated against a secondary standard to the WHO HBV DNA standard such that 1 U of pgRNA = 1 IU of HBV DNA.[1]

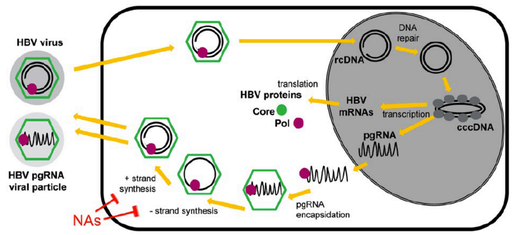

Figure 1: HBV life cycle. HBV is uncoated upon entry and the partially double-stranded rcDNA enters the nucleus. Host DNA repair machinery completes the partial strand. Host histones (gray circles) associate with cccDNA. HBV mRNAs and pgRNA are transcribed from cccDNA and are sent to the cytoplasm. HBV proteins, including Core (green) and Pol (mauve), are translated from mRNAs. Pol associates with the pgRNA; the complex is encapsidated by Core. Within the Core capsid, Pol reverse transcribes the minus (−) strand DNA from pgRNA, then the plus (+) strand. The completed virion is enveloped (not shown) and exits the cell. It is unknown how pgRNA-containing encapsidated particles exit. NAs block Pol activity (red text).[2]

Methods

RNA was isolated from 0.2mL of sample using the Abbott *m*2000 RNA selective chemistry on the Abbott *m*2000*sp* instrument. As a procedural control for each reaction, Abbott Molecular's proprietary armored RNA internal control (IC) was added during extraction for parallel amplification and detection. Amplification with concurrent detection of dual HBV RNA targets was performed on the Abbott *m2000rt* instrument. A proprietary data reduction (Abbott Diagnostics) was utilized to calculate a HBV pgRNA result. Precision/reproducibility, accuracy, and linearity were evaluated by analysis of diluted clinical samples as outlined below:

- **Precision/Reproducibility:** 3 samples of varying viral load values (low, mid, and high viral load) were tested in triplicate on 3 separate runs (N = 27 total). Intra and inter-assay performance analyses were conducted for the 27 samples.
- **Accuracy:** A panel of 17 specimens representing both on treatment (n=12) and untreated (n=5) HBV+ subjects that were previously analyzed by Abbott Diagnostics (ADD) were analyzed at bioMONTR Labs using the HBV pgRNA assay.
- **Linearity:** A HBV+ specimen with a HBV RNA value at approximately 6.5 Log U/mL was serially diluted in normal human plasma to yield sample dilutions of 1:10, 1:100, 1:1,000, 1:10,000 and 1:100,000. The neat specimen and each dilution were tested in triplicate on a single run.

Additionally, a retrospective longitudinal study utilizing specimens from 20 HBV+ subjects with chronic HBV infection who were initiating antiviral therapy were tested for HBV DNA viral load and HBV pgRNA. Samples were collected and tested at Baseline and at Weeks 12, 24, and 48 on treatment. All testing was conducted at bioMONTR Labs utilizing the Abbott RealTi*me* HBV Assay (0.5 mL protocol) for HBV DNA viral load and the RESEARCH USE ONLY HBV pgRNA assay (0.2 mL protocol) for pgRNA quantitation.

References:
1. E. Butler, J. Gersch, A. McNamara, K. Luk, V. Holzmayer, M. Medina, E. Schiff, M. Kuhns, and G. Cloherty (2018). Hepatitis B Virus Serum DNA and RNA Levels in Nucleos(t)ide Analog-Treated or Untreated Patients During Chronic and Acute Infection. *Hepatology*, Vol. 68 (6), 2106-2117.
2. G. Cloherty, E. Butler, and M. Kuhns (2018). Serum Hepatitis B virus RNA as a Potential Diagnostic Biomarker During Chronic Hepatitis B Virus Infection. *Clinical Liver Disease*, Vol. 13 (3), 90-92.

Results: Analytical Performance

Precision/Reproducibility:

Precision results are summarized in Table 1. The standard deviations were ≤0.25 Log U/mL (intra-assay) and ≤0.18 Log U/mL (inter-assay). The 95% CI were <±0.28 (intra-assay) and <±0.12 (inter-assay).

Table 1: Summary of Intra-Assay and Inter-Assay Precision (mean values)

Abbott RealTime HBV pgRNA_Intra-assay and Inter-assay Precision												
	Intra-Assay Precision									Inter-Assay Precision		
Concentration	Low			Mid			High			Low	Mid	High
Run #	1	2	3	1	2	3	1	2	3			
Replicates	3	3	3	3	3	3	3	3	3	9	9	9
Mean (Log U/mL)	1.90	1.79	1.75	3.84	3.72	3.65	5.72	5.65	5.58	1.81	3.73	5.65
Std Dev (Log U/mL)	0.23	0.13	0.19	0.25	0.09	0.08	0.09	0.13	0.17	0.18	0.16	0.13
95% CI	0.26	0.15	0.22	0.28	0.10	0.09	0.11	0.14	0.19	0.12	0.11	0.09

Accuracy:

A regression analysis of bioMONTR Labs' results (test method) and ADD's results (comparator method) shows agreement with an R^2 >0.95 (Figure 2).

A Bland-Altman plot shows a 95% confidence agreement interval spanning a narrow range (1.1 to -0.65 Log U/mL difference between the two methods) with a 0.23 bias when compared to the comparator method (Figure 3).

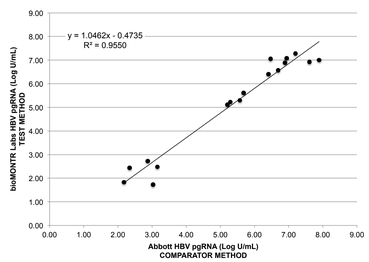

Figure 2: Regression analysis: bioMONTR Labs (test method) versus ADD (comparator method).

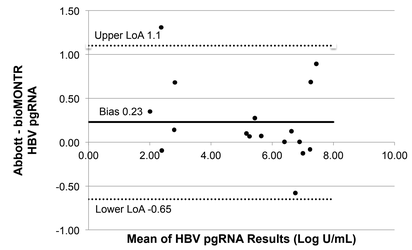

Figure 3: Bland-Altman plot: bioMONTR Labs (test method) versus ADD (comparator method). Limits of agreement are defined by 95% CI (dashed lines). Bias indicated by the black line.

Linearity:

Linear amplification demonstrated a directly proportional trend ranging from 1.5 to 6.6 Log U/mL HBV pgRNA (R^2 >0.99, see Figure 4).

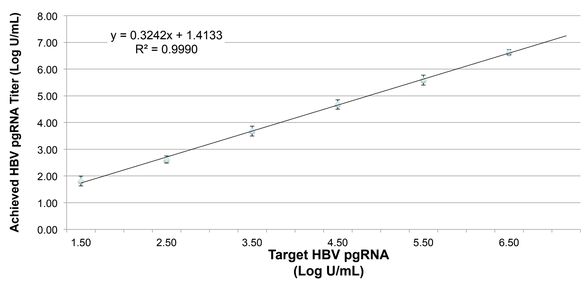

Figure 4: Linear regression analysis.

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bioMONTR® Labs

Results: Clinical Sample Testing

Scatterplots of HBV DNA viral load versus HBV pgRNA results at both Baseline and Week 24 (Figures 5A & 5C, respectively).

Corresponding bar graphs demonstrate the relationship between DNA and pgRNA results among all 20 patients at Baseline and Week 24 (Figures 5B & 5D, respectively). At Week 24, HBV pgRNA is elevated relative to HBV DNA viral load in all on-treatment patients with pgRNA detectable above the LoQ in all but 3/20 samples (Figure 5D).

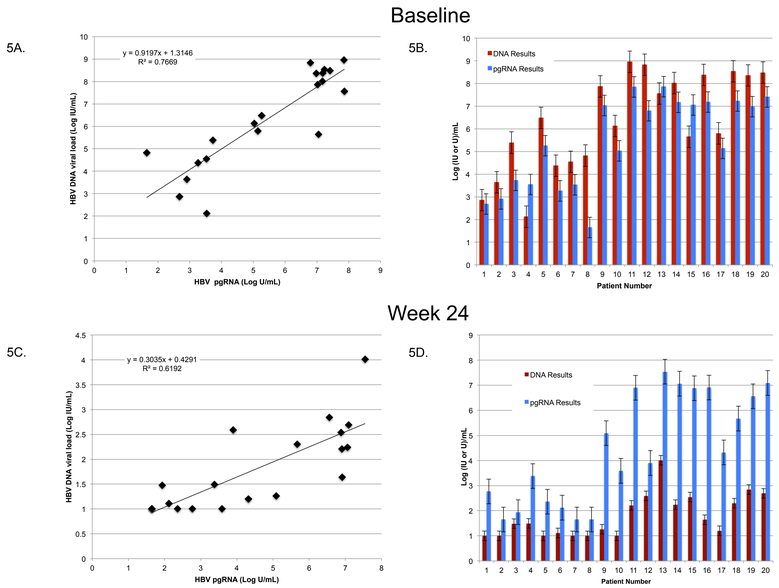

Figure 5: A. Baseline HBV pgRNA viral load vs. HBV DNA linear regression. B. Corresponding bar graph for Baseline HBV pgRNA vs. HBV DNA viral load. C. Week 24 HBV pgRNA viral load vs. HBV DNA linear regression. D. Corresponding bar graph for Week 24 HBV pgRNA vs. HBV DNA viral load.

Figure 6A is representative of a subject responding to therapy. pgRNA concentrations are greater than the DNA viral load, consistent with suppression of HBV DNA synthesis. Figure 6B represents a virologic failure with a rebound of HBV DNA at Week 48 while pgRNA values remained static.

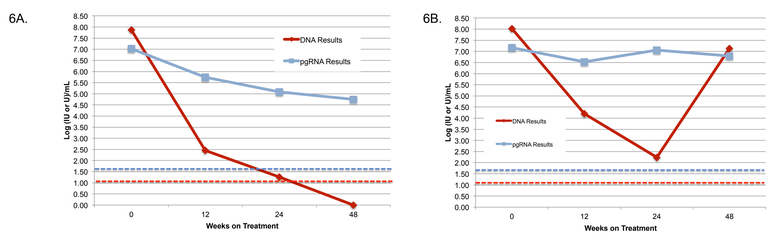

Figure 6: Line graphs demonstrating HBV DNA vs HBV pgRNA viral load for CHB subjects on antiviral therapy. Blue dashed lines represent the LoQ for pgRNA (1.65 Log U/mL) and red dashed lines represent the LoQ for DNA (1 Log IU/mL).

Conclusions

- Quantification of HBV pgRNA on the Abbott *m*2000 system provides reproducible results from plasma or serum with high sensitivity and specificity.
- This automated, high-throughput, RUO assay provides accurate quantitation of HBV pgRNA in patients on NA treatment and will be a valuable tool for monitoring HBV pgRNA viral load especially in drug development trials.