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

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Introduction

Nucleos(t)ide analog (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 covertly 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 m2000 RealTime system. With an LOQ (20% CV)/LoD (95% detection) at 1.65 Log U/mL, HBV pgRNA detection was calibrated against a secondary standard to the WHO HBV DNA standard such that 1 U of pgRNA = 1 IU of HBV DNA.

Methods

RNA was isolated from 0.2 mL of sample using the Abbott m2000 RNA selective chemistry on the Abbott m2000sp 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, high) 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 RealTime 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.

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>
<thead>
<tr>
<th>Concentration</th>
<th>Low</th>
<th>High</th>
<th>Intra-Assay Precision</th>
<th>Inter-Assay Precision</th>
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<tr>
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<td>18</td>
<td>19</td>
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</table>

**Accuracy:**

A regression analysis of bioMONTR Labs’ results (test method) and ADD’s results (comparator method) shows agreement with an R² >0.95 (Figure 2).

**Linearity:**

Linear amplification demonstrated a directly proportional trend ranging from 1.5 to 6.0 Log U/mL HBV pgRNA (R² >0.99, see Figure 4).

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 LoD in all but 3/20 samples (Figure 5D).

Conclusions

- Quantification of HBV pgRNA on the Abbott m2000 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.

References:


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Figure 1: HBV lifecycle. HBV is uncoated upon entry into the cell, where it replicates to form a virion that is enveloped (not shown) and exits the cell. NAs block Pol activity (red line).

Figure 2: Regression analysis of bioMONTR Labs’ test method versus ADD comparator method.

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

Figure 4: Linear regression analysis.

Figure 5: 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).

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