Comparison of the Aptima HBV Quant assay in the automated Panther® System with the Abbott Realtime HBV assay in the quantification of Hepatitis B Virus.

D. Orta, N. d'Empaire, E. Carmenatis, and R. Guevara
Biocollections Worldwide Inc. 5735 NE 2nd Ave. Miami, Fl 33137

ABSTRACT

Background: Hepatitis B virus (HBV) is one of the most common infectious diseases affecting more than 350 million people worldwide. HBV is a major cause of chronic liver disease with an increased risk of cirrhosis and hepatocellular carcinoma. Monitoring HBV DNA levels, prior to and during treatment, aids in assessing response to treatment and the emergence of resistance. Nowadays there are multiple assays and platforms available to detect and quantify HBV. The objective of this study was to compare the Aptima HBV Quant Assay, released by Hologic, Inc®, with the Abbott Molecular Realtime HBV assay. The Aptima HBV Quant assay is an in vitro dual-target nucleic acid amplification test for the quantitation of HBV DNA in human plasma and serum on the Panther™ System. The Abbott Realtime HBV assay is an RT-PCR test run on the Abbott m2000 System.

Methods: To evaluate sensitivity and specificity performance of the Aptima HBV Quant Assay, results from one hundred twenty-nine plasma specimens, previously tested on the Abbott m2000 platform, were compared. In addition, to corroborate specificity, some of the HBV negative specimens used were positive for Human Immunodeficiency Virus, Hepatitis C Virus, Hepatitis A Virus and *Toxoplasma gondii*. One hundred and ten specimens, with known viral loads, were used to test the quantitative performance. Precision was tested using an HBV positive specimen repeated fifteen times in different runs. The specimens were assayed using the Aptima HBV Quant Assay on the Panther System following the manufacturer's instructions. Results obtained were compared using the EP Evaluator program.

Results: HBV Viral Loads were analyzed through both methods to determine whether the methods were equivalent within the allowable total error of 1 $\log_{10}$ IU/mL. One hundred and ten positive specimens were compared over a range of 1.00 to 9.00 $\log_{10}$ IU/ml, the test passed with a correlation of 0.98. The difference between the methods was within allowable error for 105 of the 110 specimens (95.5%) tested. For the qualitative performance, results showed a positive agreement of 98.2% and a negative agreement of 100%. Two out of 112 specimens showed not detected results on the Panther System. Both samples had detectable, but not quantifiable viral loads (<10 IU/mL) on the Abbott m2000 System. This can be explained because 10 IU/mL is within the allowable error at low viral load ranges for the Panther system. Specificity of the Aptima HBV Quant assay showed a not detected result for all specimens, which represents a 100% agreement. For the precision study the EP Evaluator results showed a mean of 6.126 $\log_{10}$ IU/mL with a standard deviation of 0.051. These results were within 2 SD ranges.

Conclusions: The Aptima HBV Quant assay demonstrated high efficiency, accuracy and reproducibility for the detection and quantification of HBV, with a performance equal to that of the Abbott Realtime HBV assay. The random access to the Panther system along with the full automation, the ease of use, and the reduction in hands-on time, makes the Aptima HBV Quant assay a good option to detect and quantify HBV.

METHODS & PROCEDURES

❑ In this study one hundred twenty-nine frozen plasma specimens, previously tested on the Abbott m2000 platform, were used.

❑ The specificity performance was tested using seventeen HBV negative specimens, some of which were positive for Human Immunodeficiency Virus, Hepatitis C Virus, Hepatitis A Virus and Toxoplasma gondii.

❑ The sensitivity was evaluated using one hundred and twelve positive specimens.

❑ Precision was tested using an HBV positive specimen repeated fifteen times in different runs.

❑ The quantitative performance was assessed using one hundred and ten specimens, with known viral loads.

❑ The specimens were assayed using the Aptima HBV Quant Assay on the Panther System following the manufacturer instructions.

❑ Results were evaluated in the EP Evaluator program using the qualitative method comparison, simple precision, and two methods comparison.

RESULTS

Method Comparison

A total of 129 specimens where used to assess performance of the test. Figures 1 and 2 show a positive agreement of 98.2% (sensitivity) and a negative agreement of 100% (specificity) for an overall agreement of 98.4%. Two out of 112 specimens showed not detected results on the Panther System. Both samples had detectable, but not quantifiable viral loads (<10 IU/mL) on the Abbott m2000 System. This can be explained because 10 IU/mL is within the allowable error at low viral load ranges for the Panther System.
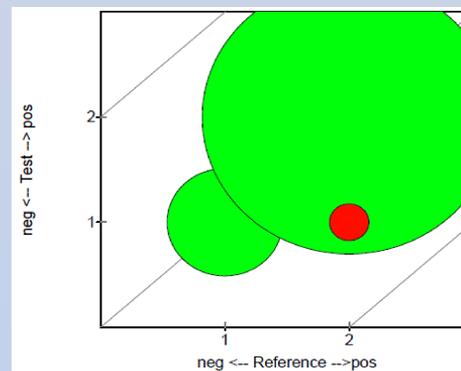

Figure 1: EP Evaluator HBV results for the comparison of 129 specimens using Abbott m2000 and Panther Hologic methods.

Statistical Summary

	Negative Reference	Positive Reference	Total
Negative Test	17	2	19
Positive Test	--	110	110
Total	17	112	129

Number excluded or missing: 0

Figure 2: Statistical summary of the Abbott m2000 System and Panther Hologic comparison based on positive and negative results.

One hundred and ten specimens with known genotypes and HBV viral loads, were used to test the quantitative performance of the Aptima HBV Quant Assay to evaluate accuracy. The specimens were compared over a range of 1.00 to 9.00 $\log_{10}$ IU/ml, the test passed with a correlation of 0.98, showing a high concordance between the two assays (Figure 3). The difference between the methods was within allowable error (1 $\log_{10}$ IU/mL) for 105 of the 110 specimens (95.5%) tested.
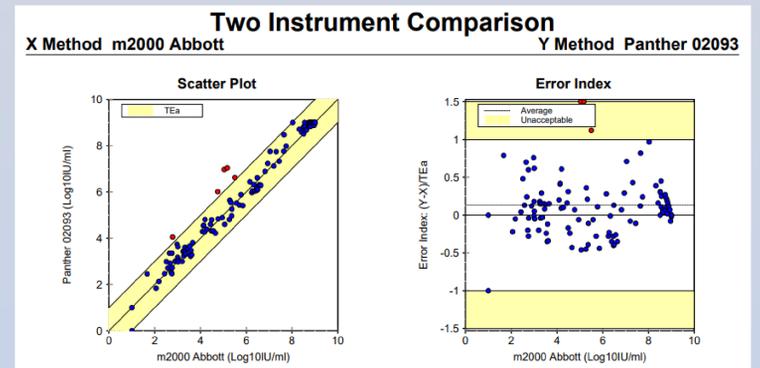

Figure 3: EP Evaluator HBV results for the quantitative comparison of 110 specimens using Abbott m2000 and Panther Hologic methods.

Precision

For the precision study the EP Evaluator results showed a mean of 6.126 $\log_{10}$ IU/mL with a standard deviation of 0.051. These results were within 2 SD ranges as required for repeatability (Figures 4 and 5).
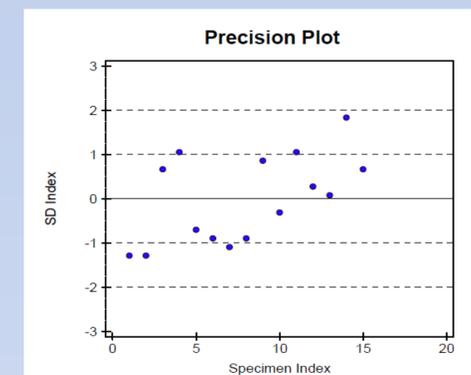

Figure 4: Simple precision experiment showing data from three different runs.

Precision Statistics

Obs Standard Dev (SD)	0.051
Pass/Fail/Uncertain	Yes
95% Confidence for Obs SD	0.038 to 0.081
Obs Coef of Variation (CV)	0.8%
Obs Mean	6.126 Log10IU/ml
Number of Specimens (N)	15 of 15
95% CI for Obs Mean	6.098 to 6.154
Obs 2 SD Range	6.024 to 6.228

Goal: 0.07 Log10IU/ml

Figure 5: Statistics for simple precision study.

CONCLUSIONS

❑ The results obtained show that the Aptima HBV Quant assay has excellent accuracy, sensitivity and reproducibility with an analytical performance comparable to that of the Abbott Realtime HBV assay.

❑ The Aptima HBV Quant assay is a faster assay with only 2.5 hrs to first result, improving productivity with a considerable reduction in operating time.

❑ The random sample loading without the need for batching samples, together with the continuous access to change reagents and consumables, brings about high flexibility and reduces hands-on time.

❑ All of this makes the Aptima HBV Quant assay on the fully automated Panther System an excellent option for HBV quantification.