EVALUATION OF THE VERSANT® HBV DNA 3.0 ASSAY (bDNA)+ AND COMPARISON WITH THE COBAS™ HBV TAQMAN ANALYTE SPECIFIC REAGENT ASSAY

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Abstract:

1.) To evaluate the performance characteristics of the Versant ® HBV DNA 3.0 assay (bDNA)+ (Versant, Bayer Healthcare LLC, Tarrytown, NY). 2.) To compare the Versant assay to the COBAS HBV TaqMan Analyte Specific Reagent assay (TaqMan) (Roche Molecular Systems, Pleasanton, CA) using random and defined patient populations.

Methods: Linearity and reproducibility were evaluated using 8 serial dilutions of 2 high titer HBV DNA patient samples tested in replicate. Accuracy of the Versant and TaqMan assays was assessed by 5 serial dilutions of World Health Organization (WHO) HBV DNA international standard (96/746 I). Correlation between Versant and TaqMan was assessed using Deming regression analysis on 3 groups of HBV DNA positive samples with HBV DNA titers ranging from 200 to >100,000,000 HBV DNA copies/mL: 168 random HBV DNA positive samples, 100 HBe antigen (Ag)-/HBe antibody (Ab)+ HBV DNA positive samples, and 100 HBeAg+/HBeAb- HBV DNA positive samples. All samples used in the evaluation were deidentified.

Results: The relationship of the Versant assay to expected concentration of serially diluted high-titer samples was: log observed = (0.983 × log expected) + 0.0054. Within-run percent coefficient of variance (CV) ranged from 0.06% to 2.40% at 5.62 and 4.16 log HBV DNA copies/mL, respectively. Between-run percent CV ranged from 0.35% to 1.46% at 7.96 and 4.20 log HBV DNA copies/mL respectively. Both the Versant and TaqMan assays provided accurate estimation of HBV DNA copies/mL concentration of the WHO International Standard (96/746 I). Observed vs. expected equations for Versant were: observed = 1.07 expected – 0.39, R^2 = 0.996, and for TaqMan: observed = 1.10 expected – 0.47, R^2 = 0.995. Correlation studies demonstrated significant non-agreement between the Versant and TaqMan assays. Deming regression equations were as follows: for the randomly selected positive samples, y = 0.915x + 0.840 (R^2 = 0.944, Standard Error of Estimates (SEE) = 0.485), for the 100 HBeAg+/ HBe Ab – samples, y = 0.927x + 0.796, (R^2 = 0.836, SEE = 0.644), and for the 98 HBeAg-/ HBeAb+ samples y = 1.104x-0.405, (R^2 = 0.935, SEE = 0.374).

Conclusion: The Versant assay performs well with respect to within-run and between-run precision, and displays excellent linearity between the range of 2,000 to 100,000,000 HBV DNA copies/mL. WHO International standard (96/746 I) dilutions indicated that both assays were accurate with respect to the international standard. All clinical correlations displayed inconsistent bias between the assays. Clinical sample comparisons and large SEEs indicate that results from the 2 assays would not be interchangeable for a given clinical sample.

Introduction:

Quantitative HBV DNA measurements not only play a critical role in the management of patients undergoing anti-viral therapy, but they also contribute to the prognostics of HBV infection. Recent treatment guidelines refer to specific HBV DNA threshold levels for initiation of therapy, although these levels have been chosen somewhat arbitrarily (1). Baseline HBV DNA levels are used as prognostic indicators prior to treatment initiation in the chronically infected patient and in those with hepatocellular carcinoma secondary to HBV infection (2). Decreases in HBV DNA concentration are used to assess treatment efficacy in chronically infected HBV patients (3, 4) and in those with recurrent HBV infection post transplant (5, 6).

The unique aspects of HBV biology and lack of standardization among HBV DNA assays have made HBV quantification challenging. HBV has a large potential range of viral concentrations (7), and tests used to quantify HBV DNA require an analytical measurement range (AMR) of 7 logs or greater. Active HBV replication is frequently marked by the presence of HBV e antigen (HBeAg) while the presence of anti-HBe and the loss of HBeAg are surrogate markers for resolution of HBV infection. Patients who are positive for HBeAg have, on average, higher HBV DNA titers than those without the antigen. However, many HBV infected patients do not have HBeAg, are anti-HBe positive, and have mutations in the core and precore regions. These mutations may impact quantitative HBV DNA tests such as the COBAS HCV Monitor and COBAS TaqMan ASR assays whose primers are targeted to these regions, leading to incorrect quantification of HBV DNA. Finally, the lack of standardization between assays has made it difficult for physicians to use results from different methods interchangeably. The introduction of the World Health Organization (WHO) international standard for HBV DNA (97/746) (8) allows for increased standardization of quantitative HBV DNA assays, similar to that now available for HCV RNA (9). Unfortunately, manufacturers and laboratories have been slow to adopt the WHO HBV DNA standard, which offers the potential for increased concordance between disparate quantitative HBV DNA methodologies. To meet clinical needs quantitative HBV DNA assays should have a large AMR, be insensitive to mutations in HBV DNA, and be standardized to WHO reference material.

We have evaluated some of the critical performance characteristics of the Versant HBV DNA 3.0 assay (bDNA) (Versant) and COBAS HBV TaqMan ASR (TaqMan) assays. We have evaluated the linearity and precision of the Versant assay using replicates of diluted clinical samples. We have also assessed the concordance of the TaqMan and Versant assays in HBeAg positive (anti-HBe Negative), HBeAg negative (anti-HBe positive), and random patient samples. We have further compared the performance of the TaqMan and Versant assays with respect to the WHO standard for HBV DNA (97/746).

Materials and Methods:

Sample Selection:
Samples used in this study were clinical samples submitted to ARUP for HBV DNA testing by the COBAS HBV TaqMan ASR (TaqMan) testing and were de-identified prior to testing in this study. The Institutional Review Board at the University of Utah approved the use of samples for the purpose of this study.

COBAS HBV TaqMan Analyte Specific Reagent (ASR) Assay
Calibration:
A single high titer HBV DNA containing sample was serially diluted in normal human serum (NHS) to create 8 calibrators. Each calibrator was run in 8 replicates concurrently with 4 replicates of the WHO HBV international standard diluted 1:4 in the COBAS HBV TaqMan ASR (TaqMan) assay using the Qiagen BioRobot 9604 with the QIAamp 96 Virus kit and Qiagen biorobot 9604 for sample preparation and loading of K-tubes. Two-hundred uL of patient sample were processed using the QIAamp virus kit following the manufacturer's guidelines, with a calium chloride buffer used to reconstitute the Qiagen protease instead of protease solvent and used at ½ the normal volume (5.1mL). Samples were amplified in the COBAS TaqMan Analyzer using the cycling conditions given in the HBV TaqMan ASR master mix Technical Bulletin. Results were normalized to HBV WHO standard and IU to copy ratio was previously determined to be 1:5. Calibration coefficients were calculated using Roche ASR External Calibration Software Version 2.0 and copy/mL values. The calibration coefficients are considered valid for a single lot of reagents only.

Versant HBV DNA 3.0 assay (bDNA) +
The Versant HBV DNA 3.0 Assay (bDNA,Versant) assay was performed as per the manufacturer's instructions. Fifty (50) uL of patient sample were processed.

Study Design:
Accuracy:
One vial of the WHO international standard for HBV DNA (97/746) was reconstituted as recommended and serially diluted in HBV DNA negative normal human plasma (NHP) through 5 levels (1:4, 1:8, 1:40, 1:400, and 1:800 dilutions). Each dilution was tested in duplicate in both the TaqMan and Versant assays. Observed vs. expected was determined for each assay and plotted. Correlation of results between assays was determined through Deming Regression analysis.

Linearity/ Reproducibility:
Two (2) high-titer HBV DNA samples were diluted through 8 dilution levels covering the range of the Versant assay. Each dilution member was run in triplicate over 4 separate Versant runs.

Within-run and Between-run precision:
Data from the linearity study was used to estimate the within run precision of the Versant assay

Clinical Correlation:
One-hundred eighty-four (184) samples previously tested by TaqMan and spanning the range of the TaqMan assay were selected and tested in Versant assay. Log HBV DNA copies/mL results were compared between assays using Deming Regression analysis. Samples with results greater than the upper limit of the Versant assay were diluted and retested.

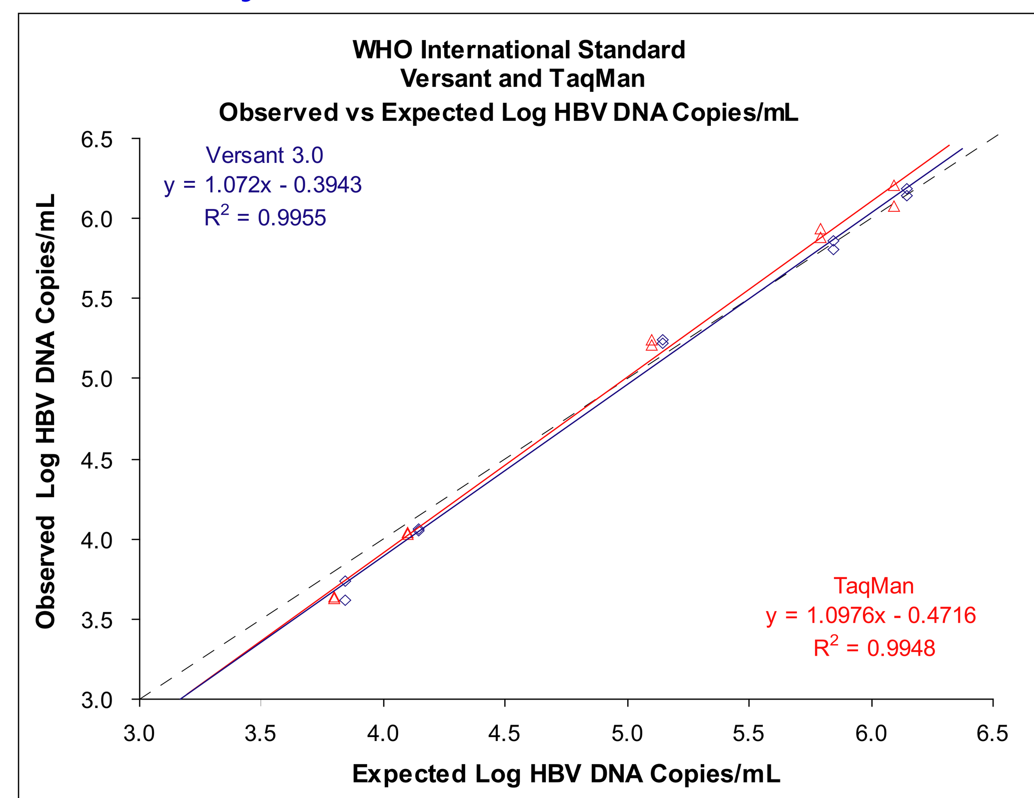
Prospective Clinical Correlation:
Samples with HBeAg, HBeAb and HBV DNA status known were collected and tested in the Versant assay. HBV DNA levels in the TaqMan and Versant assays were compared using 100 patient samples with the following serology status:

1.)HBeAg positive & HBeAb negative (100 Samples)
2.)HBeAg negative & HBeAb positive (100 Samples)

Log HBV DNA copies were compared using Deming Regression analysis. Samples with results greater than the upper limits of quantiation in either assay were diluted and re-tested. Overall bias between Versant and TaqMan was evaluated using the paired Student T statistic

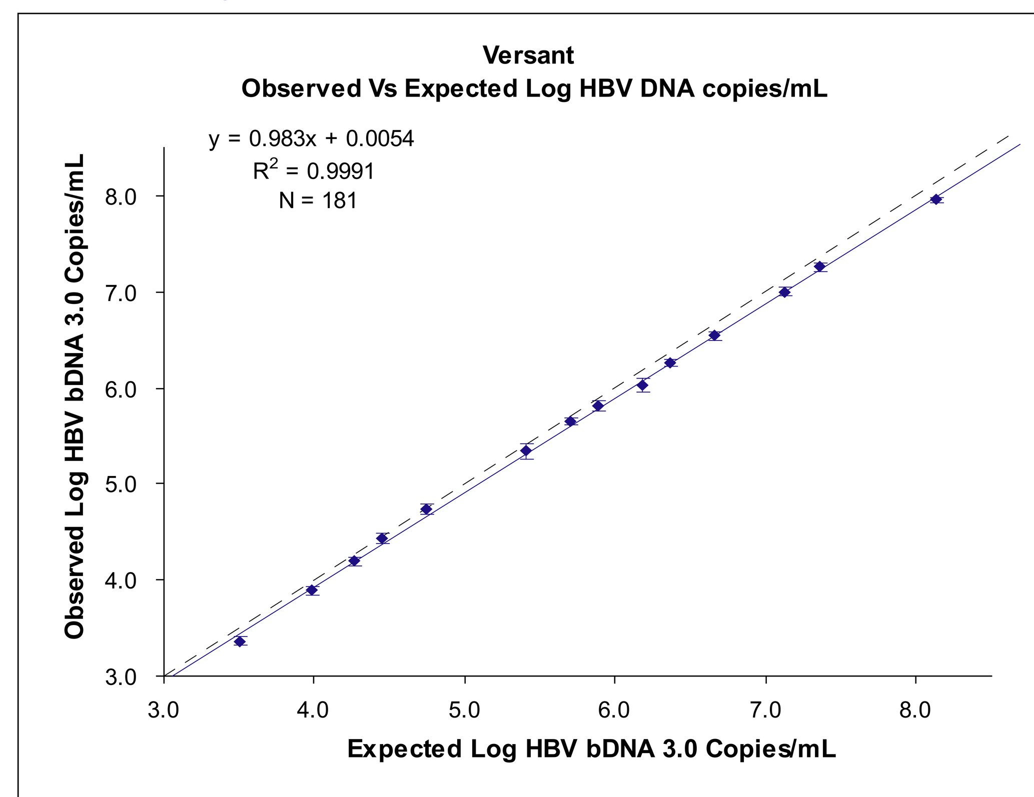
Results:

Accuracy



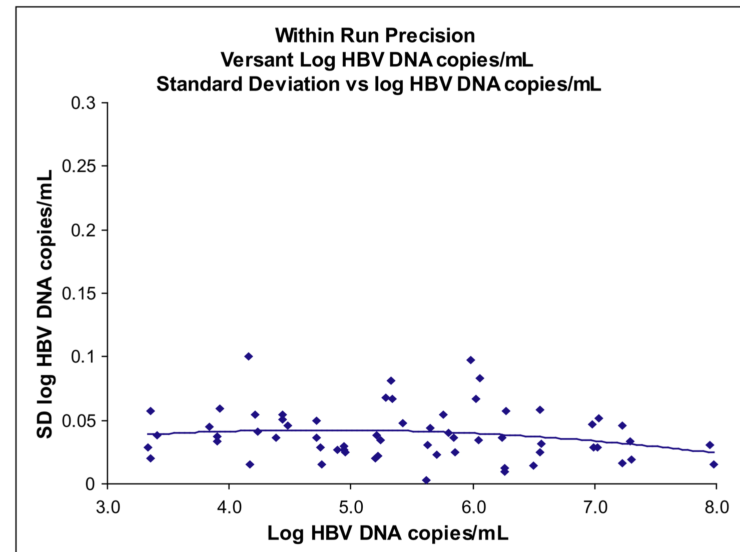
One vial of the WHO international standard for HBV DNA (97/746) was reconstituted as recommended, and serially diluted in HBV DNA negative normal human plasma (NHP) through 5 levels (1:4, 1:8, 1:40, 1:400, and 1:800). Each dilution was tested in duplicate in both the TaqMan and Versant assays. Observed vs. expected results were determined for each assay using HBV DNA copies/mL. The dashed line represents unity.

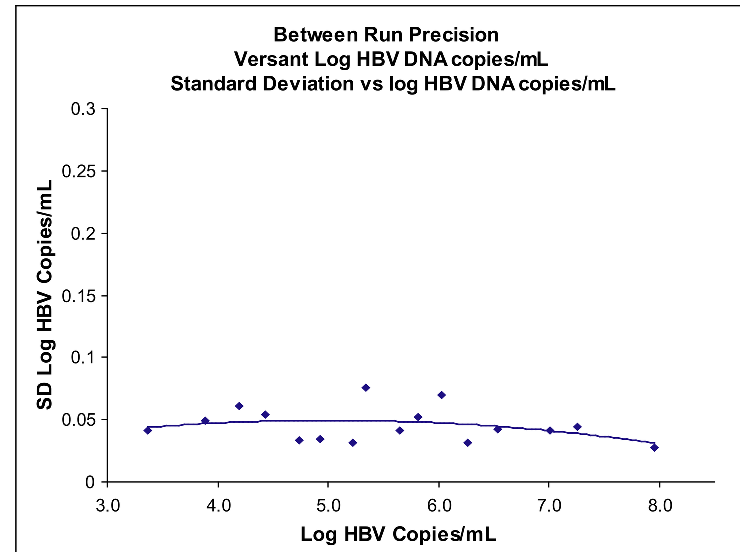
Linearity Studies



Two (2) high-titer HBV DNA samples were diluted through 8 dilutions covering the range of the Versant assay. Each dilution member was run in triplicate on each of 4 separate Versant runs. Observed vs. expected titers were calculated using a dilution in the middle of the range of the assay with the lowest CV to back calculate the titers of the remaining dilutions. Expected concentration ranged from 3,116 to 135 million HBV DNA copies/mL. Seven (7) replicates of the highest titer dilutions in one sample, and four (4) of the lowest titer dilutions in the second sample were unable to be quantitated because the results were greater than and less than the analytical measurement range (AMR) of the assay, respectively. Error bars represent one standard deviation of all results determined across all runs, and the black dashed line represents unity.

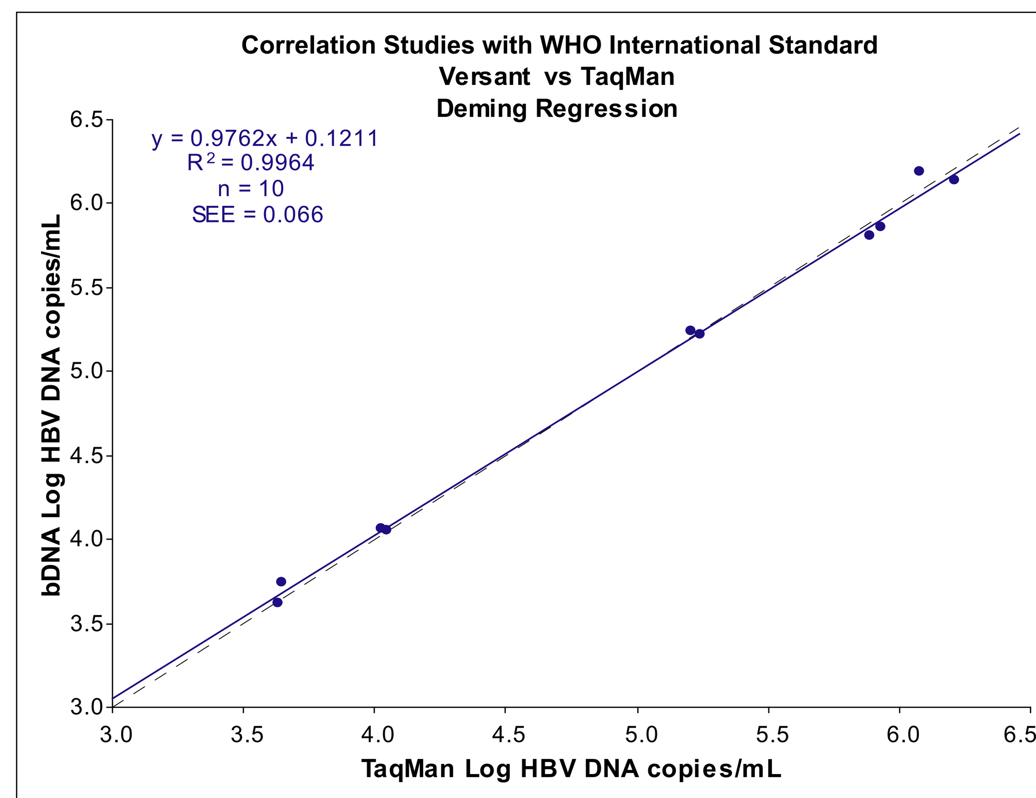
Precision



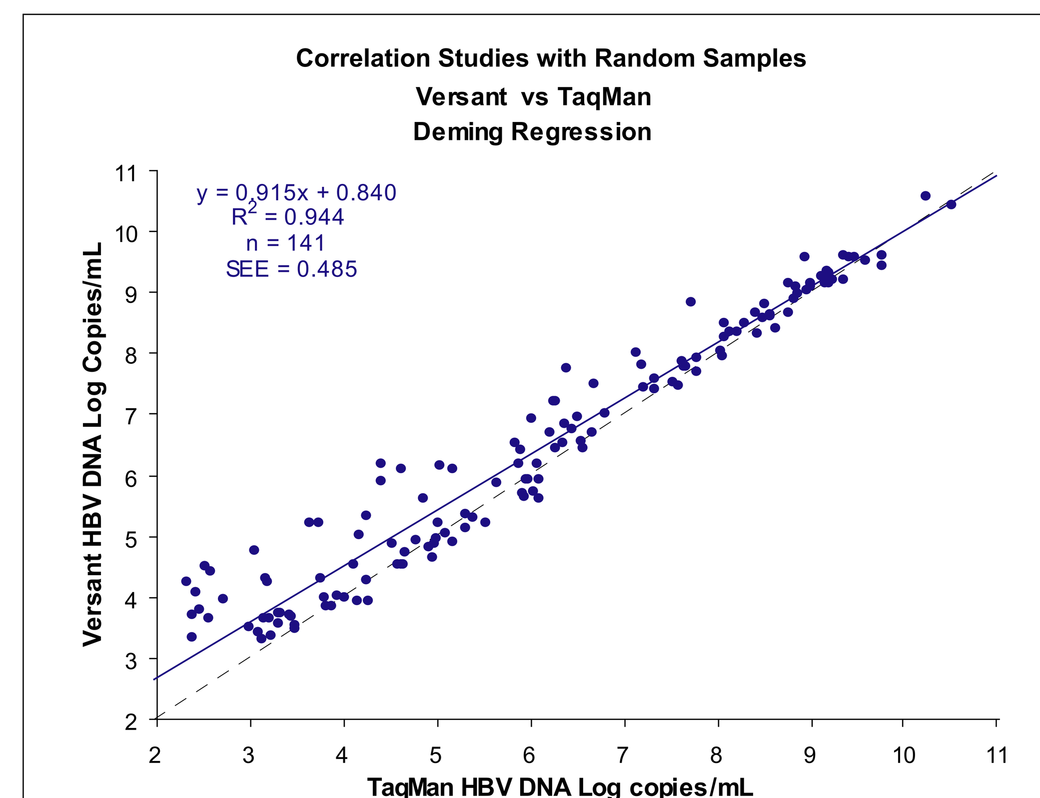


Two (2) high-titer HBV DNA samples were diluted through 8 dilution levels covering the range of the Versant assay. Each dilution member was run in triplicate on each of 4 separate Versant runs. Within-run precision ranged from 0.06% at 5.62 log HBV DNA copies/mL to 2.40% at 4.16 log HBV DNA copies/mL. Between-run precision ranged from 0.35% at 7.96 log HBV DNA copies/mL to 1.46% at 4.20 log HBV DNA copies/mL. Between sample standard deviation was 0.068 (15.7% CV), between-run standard deviation was 0.018 logs (4.1% CV), within-run standard deviation was 0.062 logs (14.3% CV), and total run-to-run standard deviation was 0.064 logs (14.9% CV).

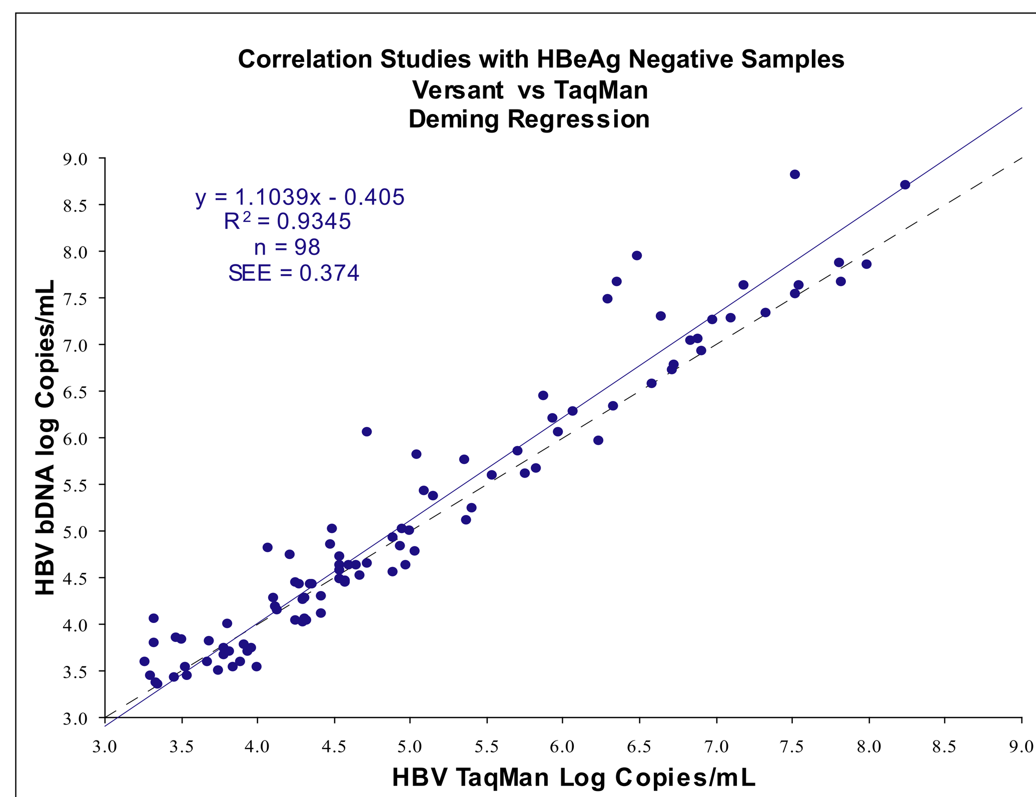
Correlation Studies



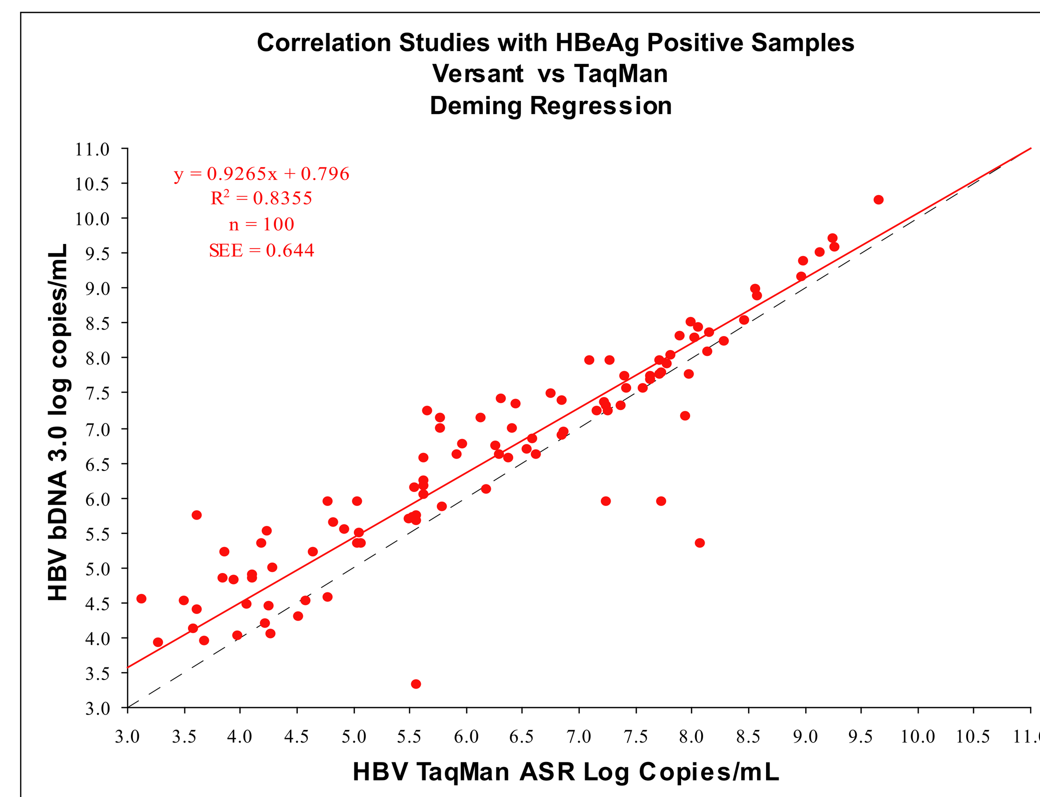
One vial of the WHO international standard for HBV DNA (97/746) was reconstituted as recommended, and serially diluted in HBV DNA negative normal human plasma (NHP) through 5 levels (1:4, 1:8, 1:40, 1:400, and 1:800 dilutions). Each dilution was tested in duplicate in both the TaqMan and Versant assays. Log HBV DNA copies/mL for each assay are plotted for a given dilution. The Deming regression Versant and TaqMan HBV DNA copies/mL is: y = 0.9762x + 0.1211, R^2 = 0.9964, n = 10, SEE = 0.066. The dashed line represents unity.



One-hundred sixty-eight (168) samples previously tested by TaqMan and spanning the range of the TaqMan assay (200 to 1,000,000,000 HBV DNA copies/mL) were selected and tested in Versant assay. Log HBV DNA copies/mL results were compared between assays using Deming Regression analysis. Thirty-nine (39) samples with results greater than the upper limit of the Versant assay required dilution to achieve quantitative results. Eighteen (18) samples tested in TaqMan required dilution to obtain quantitative results. Twenty-seven (27) samples with quantitative results in TaqMan (range: 212 to 5,270 copies/mL) were undetectable in Versant. The Deming regression is: y = 0.915x + 0.840 R^2 = 0.944, n = 141, SEE = 0.485. A slope that was significantly different from 1.0 indicated non-linearity between assays. Versant and TaqMan showed statistically significant non-linearity of quantitation with respect to each other. The black dashed line represents unity.



HBeAg negative & HBeAb positive Clinical Sample Correlation
One-hundred (100) samples submitted to ARUP for HBV DNA, HBeAg, and HBeAb testing were selected (TaqMan quantitation range: 1,860 to 176 million copies/mL) and tested in the Versant assay. Two samples returned results of <2,000 HBV DNA copies/mL (TaqMan quantitation: 2,390 and 2,540 copies/mL) and 2 samples required dilution to obtain quantitative results in the Versant assay. Deming regression is y = 1.1039x - 0.405, R^2 = 0.9345, n = 98, SEE = 0.374. Versant and TaqMan showed statistically significant non-linearity with respect to each other. The black dashed line represents unity.



HBeAg positive & HBeAb negative Clinical Sample Correlation
One-hundred (100) samples submitted to ARUP for HBV DNA, HBeAg, and HBeAb testing were selected (TaqMan results ranged from 1,340 to 4.5 billion HBV DNA copies/mL) and tested in the Versant assay. Thirteen (13) samples required dilution to obtain quantitative results in both the Versant and TaqMan assays. Deming regression is y = 0.927x + 0.796, R^2 = 0.8355, n = 100, SEE = 0.644. Versant and TaqMan showed statistically significant non-linearity with respect to each other. Versant results were on average 0.419 logs higher than TaqMan. However due to non-linearity between assays, bias was not constant across the quantitation range. The black dashed line represents unity.

Conclusions:

- The Versant and TaqMan assays perform linearly with respect to the WHO international standard for HBV DNA (97/746) and are highly correlated between methods.
- The Versant assay is linear from 2,000 to 100,000,000 HBV DNA copies/mL. The TaqMan assay appears to be more sensitive than the Versant assay and has a greater AMR
- The Versant assay displays excellent within and between run precision
- Correlation studies demonstrated significant non-agreement between the bDNA and TaqMan assays
- Variation between samples tested in TaqMan and Versant indicate that the same assay should be used to monitor patients being considered for or undergoing therapy for HBV infection.

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Not for diagnostic procedures in the United States