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Comparative Effectiveness of Dried-Plasma Hepatitis B Virus Viral Load (VL) Testing in Three Different VL Commercial Platforms Using ViveST for Sample Collection

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Ninety-six samples from hepatitis B virus (HBV)-infected individuals were used to compare ViveST samples to frozen samples in COBAS TaqMan, RealArt, and VERSANT. Correlation (*r*) between ViveST samples and frozen samples was 0.99 in all three platforms. Correlations among tests using frozen samples were 0.96 for COBAS and RealArt, 0.94 for COBAS and VERSANT, and 0.97 for VERSANT and RealArt. The results indicate that ViveST may be useful in clinical practice. Different HBV-VL platforms correlated well with one another.

Current commercial hepatitis B virus (HBV) viral load (VL) platforms require plasma separation and freezing within several hours of collection to ensure the integrity of nucleic acid, which limits their utility and accessibility, especially in developing countries. ViveST is a dried specimen storage transportation system that allows the shipment of samples at ambient temperature and increases the yield of plasma volume compared to that of the dried blood spot test, which has been validated for HIV and HCV VL testing (1, 3). In this study, we aimed to (i) describe the precision and reproducibility of using ViveST for HBV-VL testing and (ii) compare the performance of three different commercial HBV-VL platforms.

The study was approved by ethics committee at the Federal University of Sao Paulo/Brazil, and samples were obtained from HBV-infected patients without antiviral treatment and from non-HBV-infected controls on the same day in this institution.

Frozen samples from 96 HBV-infected and 42 uninfected individuals were selected and evaluated using three platforms: the Cobas TaqMan HBV test V2.0 (Roche, Branchburg, NJ), the RealArt HBV PCR kit (Abbott Laboratories, Chicago, IL), and the Versant HBV bDNA test, 3.0 (Siemens, Tarrytown, NY). The dynamic detection range of the Roche platform varied from 6 to 110,000,000 IU/ml, whereas that of the Abbott platform varied from 15 to 1,000,000,000 IU/ml, and that of the Siemens platform varied from 357 to 17,857,100 IU/ml. One milliliter of each sample was applied to the ViveST device, placed in a driDOC drying platform, and dried for 12 h, and the remaining samples were frozen in −20°C to be processed after 10 days. Dried samples were stored at room temperature for 10 days prior to the reconstitution process, using 1.0 ml of reconstitution buffer. Statistical analysis included *t* tests, Bland-Altman plot, and Pearson correlation.

Two samples with HBV VLs greater than 9 $\log_{10}$ IU/ml were used to generate triplicate samples of 8 $\log_{10}$, 7 $\log_{10}$, 6 $\log_{10}$, 5 $\log_{10}$, 4 $\log_{10}$, 3 $\log_{10}$, and 2 $\log_{10}$ IU/ml. Dilutions were analyzed in triplicate in ViveST samples and as liquid plasma, which demonstrated mean intra-assay variances among replicates of 0.07 $\log_{10}$ and 0.09 $\log_{10}$ IU/ml ($P = 0.3880$) for frozen and ViveST-dried plasma, respectively, using the Roche platform; 0.21 and 0.11 $\log_{10}$ IU/ml ($P = 0.3535$) using the Abbott platform; and 0.10 and 0.12 $\log_{10}$ IU/ml ($P = 0.7128$) using the Siemens platform.

The overall Pearson coefficients among paired frozen and ViveST-prepared samples were 0.9988 (Roche platform), 0.9979 (Abbott platform), and 0.9971 (Siemens platform) ($P < 0.0001$). Bland-Altman plot showed that mean $\log_{10}$ difference between values that were obtained from ViveST and frozen plasma samples were 0.14 $\log_{10}$ (standard variation, −0.26 to 0.54 $\log_{10}$), 0.14 $\log_{10}$ (−0.37 to 0.65 $\log_{10}$), and 0.11 $\log_{10}$ (−0.36 to 0.58 $\log_{10}$) for the Roche, Abbott, and Siemens platforms, respectively. Overall, differences in VL values greater than 0.5 $\log_{10}$ IU/ml between ViveST and plasma samples were seen for 6 of 96 (6.2%) specimens (Roche platform), 7 of 96 (7.3%) (Abbott platform), and 4 of 96 (4.2%) (Siemens platform). Three of 96 frozen samples from HBV-infected individuals were negative in all three platforms. There were 84 positive samples in the Roche platform, 89 in the Abbott platform, and 57 in the Siemens platform when frozen specimens were used (there were 4 invalid results using the Roche platform). Among the 84 samples that were positive when tested as frozen specimens, 81 were positive using ViveST in the Roche platform. In the Abbott platform, 89 and 87 samples were positive as frozen and ViveST specimens, respectively. In the Siemens platform, 57 and 48 samples were positive as frozen and ViveST specimens, respectively. Overall, differences greater than 0.5 $\log_{10}$ between VL values for ViveST samples and those for frozen samples were seen in 17 of 288 (6%) specimens.

The overall Pearson coefficient value was 0.9596 ($P < 0.0001$) for the Roche versus the Abbott platform, 0.9424 ($P < 0.0001$) for the Roche versus the Siemens platform, and 0.9673 ($P < 0.0001$) for the Abbott versus the Siemens platform (Fig. 1B). Five samples (5.2%) were negative in the Roche platform and positive in either the Abbott or Siemens platform, four (4.2%) were negative in the Abbott platform and positive in the Roche and Siemens platforms, and 35 were negative in the Siemens platform and positive in the Roche and Abbott platforms. The higher number of negative re-

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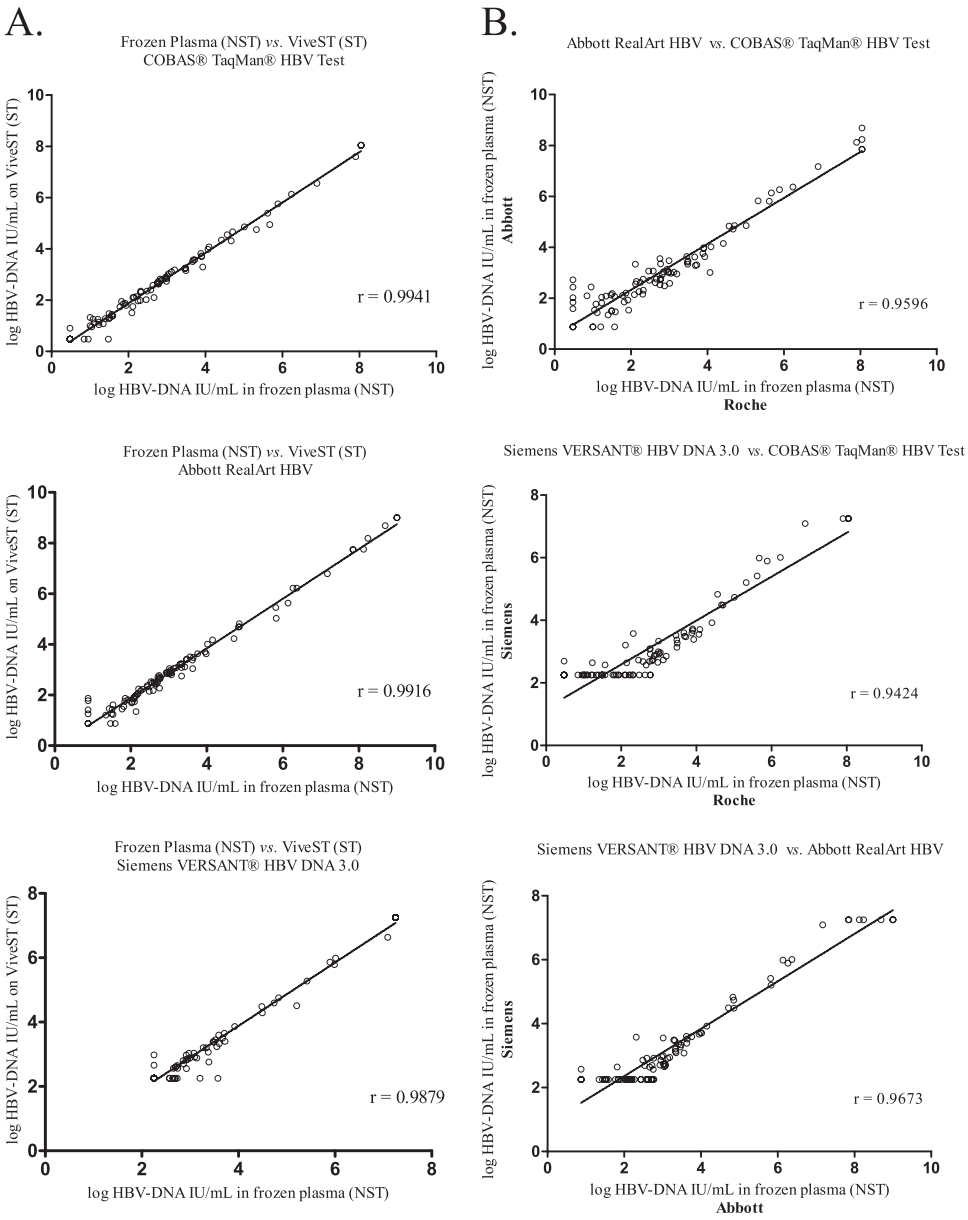

FIG 1 Linear regression plot HBV VL results (log_{10} copies/ml) from 96 frozen plasma samples compared to that of ViveST-prepared samples (A) and from 96 frozen plasma samples comparing the Abbott, Roche, and Siemens platforms (B).

sults obtained with the Siemens platform in general related to the fact that the Siemens test had a lower limit of detection. The 35 (36.5%) negative results obtained with the Siemens platform were from samples presenting a mean VL of 103 IU/ml and a median of 31 IU/ml (from 7 to 594 IU/ml) in the Roche platform and a mean of 173 IU/ml and a median of 122 IU/ml (from 22 to 591 IU/ml) in the Abbott platform. Three samples in the Roche platform and 7 samples in the Abbott platform presented VL above 357 IU/ml, which is lower limit of detection for the Siemens test. Results from ViveST samples regarding the proportion of false-negative results were also similar to the results described above for the frozen plasma samples among the three different platforms (data on file).

Among samples from uninfected individuals, one ViveST sample tested using the Abbott platform (17 IU/ml) and two frozen plasma samples tested using the Siemens platform (544 and 981 IU/ml) were positive, accounting for 2.4% and 4.8% of false-positive results, respectively, suggesting the need for confirmation of results for low-VL samples. False-negative results were also detected in low-VL ViveST samples (3 in the Roche platform, 2 in the Abbott platform, and 9 in the Siemens platform) and low-VL frozen samples (1 in the Roche platform, 4 in the Abbott platform, and 2 in the Siemens platform).

The percentages of HBV-positive samples were 90.3% in the Roche platform, 95.7% in the Abbott platform, and 61.3% in the Siemens platform. Further studies analyzing the performance of these commercial kits vis-à-vis the genetic diversity of HBV may be fundamental to the understanding of these results. In addition, we cannot ignore the fact that the higher lower limit of detection

of the Siemens platform (357 IU/ml) provided a high number of HBV VLs below the limit of detection of the other platforms in these clinical samples (36.5% of samples), which can be critical in treatment monitoring. HBV resistance can be selected even with low VLs under antiviral treatment (2).

We have demonstrated that HBV VL testing can be successfully accomplished with dried plasma samples from ViveST in the commercial kits that are currently available. This finding may be of particular importance in resource-limited settings, where more sophisticated lab tests are performed in centralized laboratories and most of the cost is related to the shipment of infectious materials using dry ice.

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