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COMPARISON OF HEPATITIS B VIRUS INNO-LiPA GENOTYPING BETWEEN FROZEN PLASMA AND SampleTanker™ A DRIED TRANSPORTATION MATRIX

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BACKGROUND

Hepatitis B Virus (HBV) is a partially double-stranded DNA virus with approximately 350 million chronically infected patients worldwide[1]. HBV is a major causative agent for chronic hepatitis infection, cirrhosis and the development of hepatocellular carcinoma[1,2]. Viral-load and drug-resistance testing are routinely used to monitor patient antiviral treatment[2]. Reference laboratories and clinical trial studies require shipment to testing facilities under frozen conditions, which is expensive and cumbersome. We have previously demonstrated the utility of SampleTanker™ (Research Think Tank, Inc.) as an inexpensive dried transportation alternative for RNA viruses[3,4]. Here we describe a retrospective analysis of archived HBV samples under ambient dried storage conditions using SampleTanker*.

This study presents an evaluation and direct comparison of frozen plasma to a novel dried ambient transportation matrix in combination with 3 different reverse hybridization line-probe kits for analysis of HBV sequences. The study also compares the utility of the method in relation to ease of use and speed to results.

METHODS

Twenty blinded archived HBV positive plasma samples collected for routine testing in the United States were obtained for this study. Samples were packaged and shipped next day frozen on dry ice to the testing facility. Viral load values and treatment history were not available at the time of testing.

Plasma specimens were thawed and 0.5 mL was loaded onto a SampleTanker matrix for each specimen and 0.5 mL was refrozen for comparison. The SampleTanker specimens were allowed to dry in a circulating bio-safety cabinet for a minimum of 5 hours prior to capping in the storage and shipping vessel. The SampleTanker specimens were stored under ambient conditions for 2 days prior to reconstitution and recovery. The SampleTanker specimens were recovered in 0.5 mL total volume.

For each extraction method, a total of 200 µL of plasma and reconstituted SampleTanker specimen was used. All specimens were extracted in parallel using the NucliSens® MiniMAG Extraction System (bioMerieux) and QIAamp DNA Blood Mini Kit (Qiagen), respectively. The elution for each extraction method was standardized to 200 µL for each specimen type.

For both extraction methods, 10 µL of extracted viral DNA was used in an outer PCR amplification reaction for each of the three assays: INNO-LiPA HBV DR, INNO-LiPA HBV PreCore and INNO-LiPA HBV Genotyping kits (Innogenetics). Resultant outer PCR amplicons were subsequently used in a inner (*nested)* PCR amplification following kit manufacturers protocols. The *nested* PCR amplicons were directly loaded onto an *Auto*-LiPA instrument (Innogenetics) following the standard protocols for the reverse hybridization reaction, detection and analysis.

After the reverse hybridization reaction was completed on the *Auto*-LiPA instrument, the strips were air dried and placed on an internally developed grid (Research Think Tank, Inc) using analysis standards provided by the manufacturer for band determination and reporting.

RESULTS

A total of 240 tests were run for this analysis. Reverse hybridization results for the SampleTanker specimens were obtained, in first round analysis, for 75% (15 of 20) of the INNO-LiPA HBV DR Kit, for 85% (17 of 20) of the INNO-LiPA Pre-Core Kit and for 75% (15 of 20) of the INNO-LiPA Genotyping Kit using the NucliSens MiniMAG Extraction System. Reverse hybridization results for the SampleTanker specimens were obtained, in first round analysis, for 70% (14 of 20) of the INNO-LiPA -LiPA HBV DR Kit, for 70% (14 of 20) of the INNO-LiPA Pre-Core Kit and for 70% (14 of 20) of the INNO-LiPA Genotyping Kit for the QIAamp DNA Blood Mini Kit extractions (**Table 1**).

Table 1: Plasma and SampleTanker Comparisons.

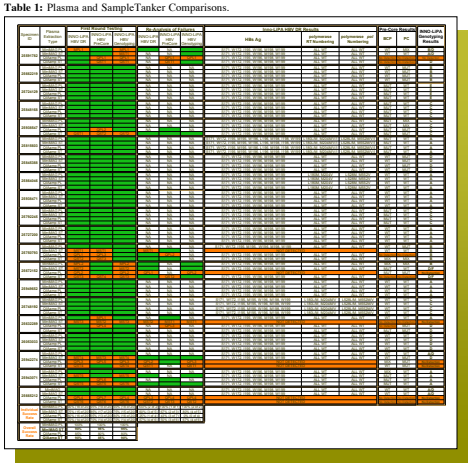
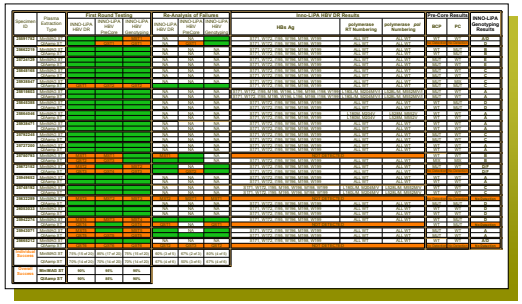


Table 2: SampleTanker Comparisons.



Samples deemed failed or lacking positive control bands on the Inno-LiPA strips were re-analyzed in a reflux secondary testing and the combined overall success rate was 90% (18 of 20) for the INNO-LiPA HBV DR Kit, 95% (19 of 20) for the INNO-LiPA Pre-Core Kit, and 95% (19 of 20) for the INNO-LiPA Genotyping Kit for the NucliSens MiniMAG Extraction System and 90% (18 of 20) for the INNO-LiPA HBV DR Kit, 85% (17 of 20) for the INNO-LiPA Pre-Core Kit, and 90% (18 of 20) for INNO-LiPA Genotyping Kit for the QIAamp DNA Blood Mini Kit extractions (**Table 1**). For comparative results obtained from plasma see poster TP24 titled Evaluation of MiniMAG Extraction as a Rapid Method of Isolating Hepatitis B Viral Nucleic Acid for Analysis Using the INNO-LiPA Line-Probe Assay.

The average success rate for all three INNO-LiPA kits between the plasma and SampleTanker for the MiniMAG extraction was 100% and 93.3% and for the QIAamp extraction was 81.6% and 88.3%, respectively. Results from matched extracted specimens of plasma to SampleTanker were highly concordant for all three INNO-LiPA HBV kits (**Table 1**). Reproducibility of the extraction methods using SampleTanker and INNO-LiPA assays were also similar (**Table 2**).

CONCLUSIONS

- The SampleTanker dried plasma specimens were stable during ambient storage conditions.
- Accuracy and reproducibility of results of the SampleTanker specimens were comparable to INNO-LiPA HBV product insert specifications.
- The HBV INNO-LiPA assay success rate was highest for all sample types using the MiniMAG Extraction System.
- An increased success rate with the three INNO-LiPA assays was observed for all SampleTanker extractions when compared to plasma QIAamp extraction.
- SampleTanker is a potential cost-effective alternative to the conventional shipping of frozen or refrigerated samples, with potential utility in cooperative studies or in routine testing of blood collected in remote places in developing countries.

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*The SampleTanker technology is covered by U.S. patent (U.S.S.N. 60/561,037) and by international patents.

*For Research Use Only. Not For Use In Diagnostic Procedures.