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COMPARISON OF HEPATITIS B VIRUS INNO-LIPA GENOTYPING BETWEEN FROZEN PLASMA AND Sample Tanker TM 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. 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?. Reference laboratories and clinical trial studies require shipment to testing acilities under frozen conditions, which is expensive and cumbersome. We have previously demonstrated the utility of SampleTankerTM (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 SampleTankerTM.

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 HBS 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 μ L 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 list (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)

*The SampleTanker technology is covered by U.S. patent (U.S.S.N. 60/561.037) and by international patents.

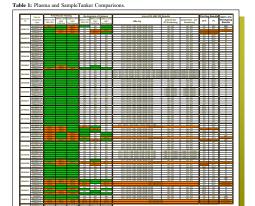


Table 2: SampleTanker Comparisons.

_	Type	HEVER	PreCore	Genotyping	HEVER	PreCore	Genotyping		RT Numbering	Numbering			Res
	Oldano 17				30	565		\$171, W172, FIRE, W18E, M18E, W18E \$171, W172, FIRE, W18E, M18E, W18E	E1 107	ALL WT	WT		Е
28662219					- 55	NA.	50	\$171, W172, C86, 9786, 9786, W186	61.07	ALLWY	WY		
	GAMES IT				- 20	100		\$171, W172, CRE, W186, W186, W186	2010	21.07	- 07		
	Medical ST				200	100	- 20	\$171, W172, 198, W186, W186, W189	ALT:	ALL WIT	901		
								\$171, W172, 1981, W186, W186, W189		ALL WT			
	Medical II				-	- 00	- 60	\$171, W72, 788, W36, W38, W38	27.07	21.01	-		
	Mindows ST				- 22	NA.	30	STEE WITH THE STREET MADE WATER		ALLWY	10.7		
20010003	Medical ST					195			CHECK SECURITY		100		
ALTERNATION IN	Medical II				-	100	- 12	STEEL SCORE FOR SPINS STORE STORE	21.07	211.007	10.7	-	
	CEAnno NT				300	NA.	50	3171 W172 CR6, N/396 M198, W199	81.107	ALL WY	10.7	14.77	
******	Mindold ST				70.0	500	50	STEE WITH THE STREE MINE WINE	11809/3000/	LATEN MARTY	10.7	9.7	
28038471	Ministra 17												
	Michael ST												
	Mindold 17						AA.				WT		
								\$171, W172, Citt. M786, M186, W186		ALL WT	MX		
	Medicks 17					565		\$171, W172, Clab, 16756, M184, W186	ALIT	ALLWY	1607		
	OtAamo 37							\$171, W172, CSS, N/396, M198, W199	ALIT	ALLWT			ľ
	Micholog 37					365	55	3171, W172, CSS, W196, M198, W199		ALLWT	WT		
HIVATING.	QAiny 11				36	195		\$171, W172, 1785, 9736, 9158, W185	ALW	XLWI	WT		
					365	198	NA.		LIBERT DOCUMENT		WT		
28022289	GiAamo 17				250	NA.	ASA.	STEE, WITZ, CHE MISS, WISS, SCIES, SCIES			WT		
	CEAnno TT				MATO	MA	Mars		B 81 107		No. of		100
28842274 28842274 28842271					- 00	100	- 55	8171 W172 CHR W786 M186 W188 8171 W172 CHR W786 W188 W188	819	ALL WT		907	_
	GAINS 17				764	164	NA.	\$171, W172, 198, W196, W198, W199 \$171, W172, 198, W196, W198, W199	AL III	ALL WT	WT		
			10000		-00	- 86	-	\$171, W172, 198, W186, W188, W188		ALL WIT			_
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		10000	_	2011		90		STEE WITH THE STEE MINE WINE		ALLWAY	10.7		_
	CEAsing ST	0.075	-		_	- 85		3171, W172, C86, W186, W186, W189 3171, W172, C86, W186, W186, W189	61.07	ALL WT	10.7		_
	Medical 17	-	COLUM	0000	765	195	505	\$171, W172, 1785, W186, W186, W186	20.01	201.00		- 81	_
	GAme 11	2000	2000	2000	7007	200	erin.	AND ADDRESS OF THE PARTY OF THE		100 771	-271	107	
				_	_		_						-
Zattess	Ministra 17	785-75-420	80% (17 of 20)	75% (16 of 20)	60% (2 of 5)	674 (2 of 3)	80% (6 w/0)						
	QiAanp 11	705-74 #20	70% (16 of 20)	70% (14 = 120)	675 (6415)	MN (SWE)	675 (646)						
Section 1	MINIMAD ST	90%	10%	105									
	Qifama 31	10%	HS	10%									

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).

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 Genotyping Kit for the NucliSens MiniMAG Extraction System and 90% (18 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) of the INNO-LiPA Genotyping Kit for the QLamp 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 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 OIAamp 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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