PERFORMANCE OF A NOVEL AUTOMATED ASSAY FOR THE DETECTION AND QUANTIFICATION OF HBV PREGENOMIC RNA / CIRCULATING RNAs IN CHRONIC HBV PATIENTS

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INTRODUCTION

Quantification of HBV circulating RNAs is a promising biomarker for the evaluation of target engagement of new HBV antivirals and the definition of new endpoints of treatment since circulating pregenomic RNA has been proposed to reflect HBV covalently closed circular DNA (cccDNA) activity within infected hepatocytes. In the context of emerging antiviral molecules, high sensitivity and a broad linear range of quantification are crucial.[1]

AIM

To evaluate a Roche HBV RNA investigational assay (IA).

MATERIAL & METHODS

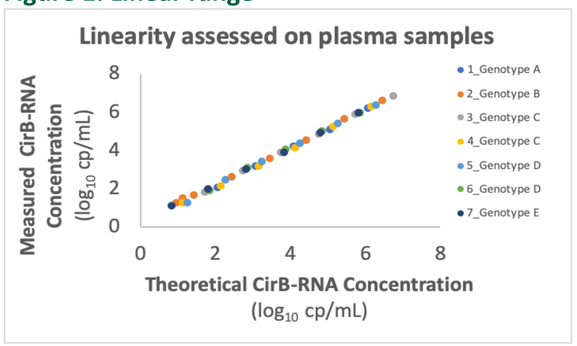
Circulating HBV RNA was quantified by real-time PCR using the Roche HBV RNA investigational assay (IA) for use on the cobas® 6800/8800 Systems (Roche Diagnostics, Pleasanton, CA, USA). The HBV RNA primers and probes were designed to be genotype inclusive and the assay has an internal quantitation standard. The HBV RNA assay is a quantitative nucleic acid test with a lower level of quantification (LLoQ) of 10 copies/mL (cp/mL) that enables the detection and quantification of HBV RNAs in EDTA plasma or serum. All tests were performed by trained operators in accordance with the manufacturers' specifications. Runs were considered valid if internal controls were valid and no protocol deviations or incidents occurred that might affect the validity of the data.

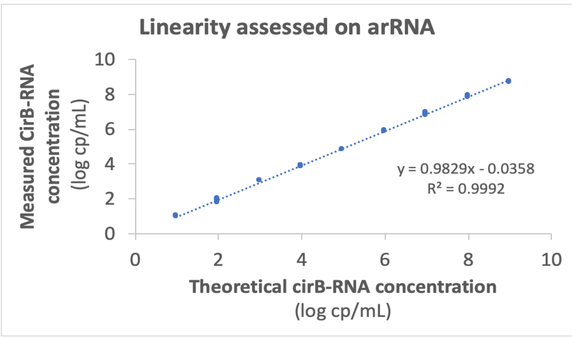
The performance characteristics (sensitivity, linearity, specificity, and tolerance of DNA) have been assessed using armored RNA (arRNA) and selected plasma and serum samples of HBV infected and negative patients stored at -80°C.

The performance was also compared to that of a manual workflow using cfRNA extraction (Roche) and RT-PCR using the same RT-PCR mix on a LC480.

RESULTS

Figure 1. Linear range





Sample	Genotype	HBV DNA (log IU/mL)	Disease phase	HBeAg status	Treatement	Correlation curve	R2
1	A	8.8	CH	+	No	y=0.9964x-0.0145	0.9984
2	B	8.3	CI	+	No	y=0.9677x+0.172	0.9991
3	C	1.7	CH	+	Yes	y=1.0114x-0.1171	0.9991
4	C	8.3	CH	+	No	y=1.0052x-0.0253	0.9995
5	D	4.5	CH	+	Yes	y=1.0087x-0.0413	0.9991
6	D	8.2	CI	+	No	Y=1.0055x-0.0076	0.9980
7	E	8.7	CI	+	No	y=0.9705x+0.1418	0.9996

Linearity was assessed for genotypes A through E using clinical samples (see table for characteristics) and ranged, independently of HBV DNA viral loads, from 10 to 10^7 cp/mL for plasma samples and up to 10^9 copies/mL for synthetic armored (ar)RNA. The IA does not report titers below the LLOQ of 10 cp/mL, however we detected templates down to 1 cp/mL.

Repeatability led to a coefficient of variation of 4.7% (low level samples <2 $\log_{10}$ cp/mL) and <0.7% for higher RNA loads. Reproducibility showed a variability below 0.09 $\log_{10}$ copies/mL throughout the linearity range.

A specificity of 100% was achieved (verified with sera and plasma from HBV-negative patients replicating HIV, HCV, CMV, or HEV and uninfected patients).

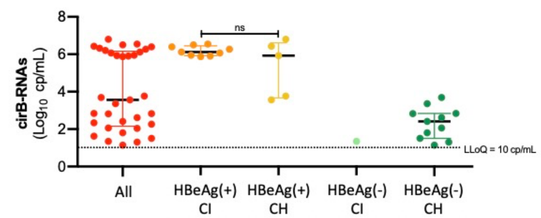
Figure 2. cirB-RNA levels

We then assessed cirB-RNA levels on 56 clinical samples including 38 untreated and 18 NUC-treated chronically infected patients selected for HBV DNA viral load diversity, genotype representativity (A through G), and clinical phases according to EASL guidelines : HBeAg negative (-)/positive (+) chronic hepatitis (CH) or chronic infection (CI) (see Table for repartition).

		HBe + (n=21)							HBe - (n=35)						
	Geno	A	B	C	D	E	F	G	ND	A	B	C	D	E	F	G	ND
Chronic hepatitis	No TT	1		1	1	2				1	1	1	7	7			
	TT	1	1	2	2	1				1	1	1	2	2	1	1	2
Chronic infection	No TT		3	1	2	3				1		1	1	1	1		2
	TT																

In untreated patients, all HBeAg(+) patients were cirB-RNA positive, all with quantifiable values, with no difference between CH and CI patients, while 64% of HBeAg(-) CH and only 14% of HBeAg(-) CI patients had quantifiable cirB-RNA. cirB-RNA levels were higher in HBeAg(+) vs HBeAg(-) patients, ~2 logs lower than HBV DNA, independently of genotype.

Finally, cirB-RNAs were detected in 10/18 NUC-treated patients (treatment duration 3-260 months), with higher levels than HBV DNA.



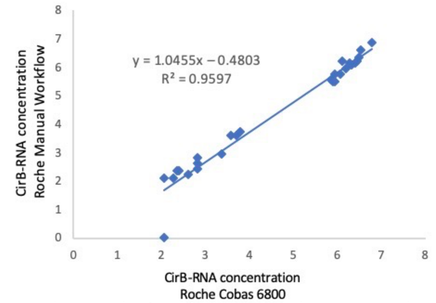
	All	HBeAg(+) CI	HBeAg(+) CH	HBeAg(-) CI	HBeAg(-) CH
Positive/total	44/56	9/9	5/5	4/7	16/17
Quantif/DNQ*/Neg	33/11/12	9/0/0	5/0/0	1/3/3	11/5/1
median (Log10 cp/mL)	3.6	6.1	5.9	1.4	2.4

Untreated only

**DNQ=detected not quantifiable (below LLoQ)*

HBeAg(+)	HBV DNA log IU/mL	HBV RNA log cp/mL	Delta DNA-RNA
No TT	8.2±0.7	6.0±0.8	2.2±0.4
TT	2.9±1.9	3.4±1.9	-1.1±2

Figure 3 Correlation of cirB-RNA levels between automated and manual workflows



A very good correlation between the manual workflow and the automated Roche HBV RNA IA was observed (R^2= 0.9597)

CONCLUSION

The Roche HBV RNA assay is a high throughput, very sensitive and genotype inclusive assay to evaluate the clinical relevance of circulating HBV RNA quantification. The manual version can be easily implemented for fundamental research purposes.
Further evaluation of the use of this assay in CHB patients can be found In poster *0722.*

ACKNOWLEDGEMENTS

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DISCLOSURES

AH, BS, AB, MH, AL, AG, MH are Roche employees, LW is a former Roche employee.

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