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 international quantification 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 (aRNA) 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 cRNA extraction (Roche) and RT-PCR using the same RT-PCR mix on a LC480.

RESULTS

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Figure 1. Linear range

Linearity was assessed for genotypes A through E using clinical samples (see table for characteristics) and ranged, independent of HBV DNA viral loads, from 10 to 10⁷ copies/ml for plasma samples and up to 10⁸ copies/ml for synthetic armored (a)RNA. The IA does not report titer 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 log₁₀ cp/ml) and <0.7% for higher RNA loads. Reproducibility showed a variability below 0.09 log₁₀ cp/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 representation (A through G), and clinical phases according to EASL guidelines: HBeAg negative (-)/positive (+) chronic hepatitis (CH) or chronic infection (CI) (see Table for repartition).

In untreated patients, all HBeAg-positive patients were cirB-RNA positive, all with quantitative 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-RNA were detected in 10/18 NUC-treated patients (treatment duration 3-260 months), with higher levels than HBV DNA.

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 (R²=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.

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REFERENCES

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

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