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# Multicenter clinical evaluation of alinity m HBV assay performance

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ABSTRACT

*Background:* Accurate molecular methods to detect and quantify hepatitis B virus (HBV) DNA are essential to diagnose chronic infections, guide treatment decisions, assess response to treatment, and determine risk of HBV-related complications. New generations of real-time HBV DNA assay platforms provide results in less than 2 – 3 h, with continuous loading of specimens and true random-access capability.
*Objectives:* We examined the clinical performance of the new Alinity m HBV assay, run on the fully automated, continuous, random-access Alinity m platform, to accurately detect and quantify HBV DNA in a large series of patient samples infected with different HBV genotypes frequently encountered in clinical practice.
*Study design:* This international, multisite study assessed the precision and reproducibility of the Alinity m HBV assay and compared its performance to four HBV assays currently in clinical use.
*Results:* The Alinity m HBV assay demonstrated linear quantitation of HBV DNA in plasma samples, with high precision (coefficient of variation 4.1 %–8.8 %) and reproducibility. The Alinity m HBV assay showed excellent correlation (correlation coefficients ≥ 0.947) with comparator HBV assays, with an overall observed bias ranging from −0.07 to 0.17 $Log_{10}$ IU/mL. 97 % of quantifiable patient results were < 1 $Log_{10}$ IU/mL different than the respective comparator assays, with comparable results across HBV genotypes.
*Conclusions:* The newly developed real-time PCR-based Alinity m HBV assay is sensitive, reproducible, and accurately quantifies HBV DNA levels from HBsAg-positive patients across the full dynamic range of quantification.

## 1. Introduction

Hepatitis B virus infection affected approximately 257 million people worldwide in 2015 [1]. Chronic HBV infection is a major cause of liver disease including cirrhosis and decompensated liver failure, as well as hepatocellular carcinoma (HCC), one of the most frequent cancers in areas of high HBV endemicity, such as sub-Saharan Africa and Asia. Globally, viral hepatitis accounted for 1.34 million deaths in 2015, a toll as high as that of tuberculosis and higher than those of HIV infection and malaria [1]. Despite the availability of an effective

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vaccine and potent antiviral drugs, chronic HBV infection remains a significant public health concern.

For initial diagnosis and for life-long monitoring of treatment efficacy, accurate and sensitive assays based on nucleic acid technology (NAT) are recommended by the international Clinical Practice Guidelines for HBV DNA detection and quantification [2,3]. Current HBV assays and analyzer platforms have a broad range of linear quantification and a limit of detection on the order of 10 IU/mL or less. However, these assays are typically run in batches and require at least one full day to complete analyses and report results. The Abbott RealTi*m*e™ HBV assay (Abbott Molecular, Des Plaines, IL, USA) and the cobas® AmpliPrep/cobas Taqman HBV assay, version 2.0 (CAP/CTM HBV 2.0; Roche Molecular Systems, Pleasanton, CA, USA) are the most widely used HBV assays in clinical practice. They show satisfactory performance for HBV DNA detection and quantification [4–6]. Abbott Molecular (Abbott Molecular, Des Plaines, IL, USA) recently introduced the Alinity™ m HBV assay, a polymerase chain reaction (PCR)-based dual-probe assay that is run on the fully automated Alinity m analyzer. The Alinity m system uses a consolidated, continuous, random-access workflow that eliminates the need for batch processing of samples and automates all aspects of nucleic acid testing in a single step. The Alinity m system reports results for the first 12 samples in less than 2 h, with results for each successive 12 samples reported every 16 min thereafter. Here, we examined the performance of the Alinity m HBV assay compared to several different commercially available HBV assays to accurately detect and quantify HBV DNA in a large series of samples from patients infected with various HBV genotypes.

## 2. Materials and methods

### 2.1. Clinical specimens and study sites

Serum (n = 120) and plasma (n = 1422) samples from patients with chronic HBV infection were collected at nine health care sites in Europe (Hôpital Universitaire Henri Mondor, Créteil, France; Medizinisches Infektiologiezentrum Berlin, Germany; PZB Aachen, Germany; Azienda Ospedaliera di Padova, Padua, Italy; Hospital Universitario Ramón y Cajal, Madrid, Spain; Laboratori de Referència de Catalunya, El Prat de Llobregat/Barcelona, Spain) and United Kingdom (West of Scotland Specialist Virology Center, Glasgow, UK), Australia (Victorian Infectious Diseases Reference Laboratory, Melbourne, Australia); and Africa (Lancet Laboratories, Johannesburg, South Africa).

Genotyping based on phylogenetic analysis of a portion of the overlapping S gene was available for 148 clinical specimens, which included 29 genotype A, 29 genotype B, 17 genotype C, 51 genotype D, and 22 genotype E samples.

The study was performed in accordance with the principles of Good Clinical Practice and conducted in adherence with the Declaration of Helsinki. Only residual samples for routine HBV testing were used. All samples were anonymized prior to the study, with an identification number containing no patient identifiers assigned to each sample.

### 2.2. HBV DNA detection and quantification

Each clinical specimen (fresh, refrigerated or frozen) was tested using two different HBV DNA assays: fresh (n = 154), refrigerated (n = 102) or frozen (n = 1286) samples on the Alinity m HBV assay and fresh (n = 239) or frozen (n = 1303) samples on a commercially available HBV assay routinely used at each study site. Comparator assays were either based on real-time PCR: RealTi*m*e HBV assay (Abbott Molecular, Des Plaines, IL, USA), CAP/CTM HBV v2.0 test (Roche Molecular Systems, Pleasanton, CA, USA), or cobas 6800 HBV test (Roche Molecular Systems, Pleasanton, CA, USA); or transcription-mediated amplification (TMA): Aptima HBV Quant assay (Hologic Inc., San Diego, CA, USA). Remnant samples were either fresh, stored refrigerated (2 − 8 °C) for less than 72 h or frozen ( − 20 °C or colder) for less than 72 months prior to being tested with the Alinity m HBV assay. Fresh samples which were used for comparison between Alinity m HBV and Aptima HBV quant assays were split into two aliquots prior to same day testing on the respective platforms. Frozen samples were processed with minimal freeze-thaw cycle. Results were compared to the test result obtained by one of the four commercially available HBV assay.

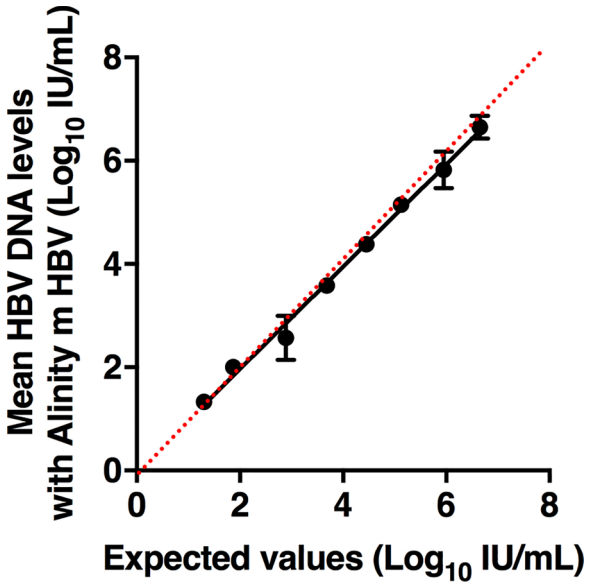


**Fig. 1.** Alinity m HBV assay linearity. HBV DNA quantification by Alinity m HBV assay of undiluted and one-fifth serial dilutions of a clinical specimen containing $1.7 \times 10^7$ HBV DNA IU/mL (7.2 $Log_{10}$ IU/mL). The dotted line is the equality line.

Alinity m HBV assay: HBV DNA was isolated from 300 µL of sample per the manufacturer's instructions. The data were analyzed by the system software, version 1.2 or later. The dynamic range of quantification of the Alinity m HBV assay is 10 to $10^9$ IU/mL (1.0–9.0 $Log_{10}$ IU/mL). The limit of detection (LOD) is 6.72 IU/mL in plasma and 9.62 IU/mL in serum and the lower limit of quantification (LLOQ) is 10 IU/mL. The Alinity m HBV assay primers and probes target the viral surface (S) gene.

RealTi*m*e HBV assay: HBV DNA was processed from 500 µL of sample using the *m*2000*sp*™ and the *m*2000*rt* analyzers for automated sample extraction and real-time PCR amplification and detection, according to the manufacturer's instructions. The data were analyzed with Abbott RealTi*m*e *m*2000*rt* software, version 8. The dynamic range of quantification of the RealTi*m*e HBV assay is 10 to $10^9$ IU/mL (1.0–9.0 $Log_{10}$ IU/mL), with an LOD of 6.4 IU/mL in plasma and 3.8 IU/mL in serum and an LLOQ of 10 IU/mL. The viral region targeted by RealTi*m*e HBV assay primers and probes is the viral S gene.

CAP/CTM HBV v2.0 assay: HBV DNA was processed from 650 µL of sample using the cobas AmpliPrep and cobas TaqMan 96 analyzer for automated sample extraction and real-time PCR amplification and detection, according to the manufacturer's instructions. The dynamic range of quantification of HBV DNA for the CAP/CTM HBV v2.0 assay is 20 to $1.7 \times 10^8$ IU/mL (1.3–8.2 $Log_{10}$ IU/mL), with an LOD of 9.0 IU/mL in plasma and 19.0 IU/mL in serum and an LLOQ of 20 IU/mL. The CAP/CTM HBV v2.0 assay primers and probes target the HBV pre-Core/Core region (preC-C) gene.

cobas 6800 HBV assay: HBV DNA was extracted, purified, and amplified from 500 µL of samples on the cobas 6800 analyzer system according to the manufacturer's instructions. The dynamic range of quantification is 10 to $10^9$ IU/mL (1.0–9.0 $Log_{10}$ IU/mL), with an LOD of 6.6 IU/mL in plasma and 3.5 IU/mL in serum and an LLOD of 10 IU/mL. The viral region targeted by the cobas 6800 HBV assay primers and

**Table 1**
(a) Precision, (b) Detection Rate, and (c) Reproducibility of the Alinity m HBV Assay.

| | Panel Member (Genotype) | Target Concentration ($Log_{10}$ IU/mL) | No. of Replicates | Mean of Measured HBV DNA ($Log_{10}$ IU/mL) | SD ($Log_{10}$ IU/mL) | CV (%) |
|---|---|---|---|---|---|---|
| (1a) Precision | GT A-1 | 3.30 | 59 | 3.29 | 0.13 | 4.1 |
| | GT A-2 | 2.00 | 63 | 1.69 | 0.15 | 8.8 |
| | GT C-1 | 3.30 | 62 | 3.06 | 0.13 | 4.2 |
| | GT C-2 | 2.00 | 61 | 1.56 | 0.13 | 8.5 |

| | Panel Member (Genotype) | Target Concentration ($Log_{10}$ IU/mL) | No. of Replicates | No. of Replicates Detected | Detection Rate (%) |
|---|---|---|---|---|---|
| (1b) Detection Rate | GT A-3 | 1.0 | 63 | 56 | 88.9 |
| | GT C-3 | 1.0 | 62 | 61 | 98.4 |

| | Control[a] | Target Concentration ($Log_{10}$ IU/mL) | No. of Replicates | Mean of Measured HBV DNA ($Log_{10}$ IU/mL) | SD ($Log_{10}$ IU/mL) | CV (%) |
|---|---|---|---|---|---|---|
| (1c) Reproducibility | LPC | 2.52 – 2.77 | 292 | 2.7 | 0.18 | 6.7 |
| | HPC | 4.48 – 4.87 | 292 | 4.6 | 0.23 | 5.0 |

SD: standard deviation; CV: coefficient of variation; LPC: low positive control; HPC: high positive control.
[a] Target concentration for LPC and HPC obtained from assigned QC concentrations across lots used in the study.

**Table 2**
Comparison of HBV DNA Levels Using the Alinity m HBV and RealTi*m*e HBV Assays[a].

| | | RealTi*m*e HBV (n) | | |
|---|---|---|---|---|
| | | Not detected | < 10 IU/mL | > 10 IU/mL |
| Alinity m HBV (n) | Not detected | 129 | 69 | 12[b] |
| | < 10 IU/mL | 14 | 51 | 27[c] |
| | > 10 IU/mL | 13[d] | 8[e] | 459 |

[a] Six additional samples were reported as > Upper Limit of Quantitation (ULQ) by both methods; 3 additional samples were reported as > ULQ by Alinity m HBV assay and quantitated by RealTi*m*e HBV assay.
[b] RealTi*m*e results ranged from 1.28 to 1.53 $Log_{10}$ IU/mL.
[c] RealTi*m*e results ranged from 1.08 to 1.39 $Log_{10}$ IU/mL.
[d] Alinity m results ranged from 1.32 to 1.60 $Log_{10}$ IU/mL.
[e] Alinity m results ranged from 1.27 to 1.39 $Log_{10}$ IU/mL.

probes is the preC-C gene.

Aptima HBV Quant assay: On the Panther® analyzer system, HBV DNA was isolated from 500 µL of sample according to the manufacturer's instructions. The dynamic range of quantification of Aptima HBV Quant assay is 10 to $10^9$ IU/mL (1.0–9.0 $Log_{10}$ IU/mL), with an LOD of 5.6 IU/mL in plasma and 4.3 IU/mL in serum and an LLOQ of 10 IU/mL. The Aptima HBV Quant assay dual primers and probe target the viral S gene.

### 2.3. Analytical analysis – precision and detectability

Assay precision and detectability were evaluated across four testing sites using a panel consisting of different HBV DNA concentrations obtained by diluting clinical samples in normal human plasma (Exact Diagnostics, Fort Worth, TX, USA). HBV DNA in each panel sample was value assigned based on the RealTi*m*e HBV DNA assay. Each panel contained samples of HBV genotype A or genotype C at target concentrations of $2 \times 10^3$ IU/mL (3.30 $Log_{10}$ IU/mL), $1 \times 10^2$ IU/mL (2.00 $Log_{10}$ IU/mL), and 10 IU/mL (1.00 $Log_{10}$ IU/mL). Mean, standard deviation (SD), and percent coefficient of deviation (% CV) were evaluated at concentrations > 10 IU/mL and detectability was evaluated at 10 IU/mL.

### 2.4. Analytical analysis – reproducibility

Reproducibility was assessed by evaluating assay controls from multiple reagent lots across testing sites. Mean, SD, and %CV of each assay's quality controls (QC) were evaluated at each participating study site along with the overall QC mean and SD from all sites.

### 2.5. Statistical analysis

Descriptive statistics are reported as means ± SD. Relationships between quantitative variables were studied by means of Deming regression. Bland Altman analysis was used to evaluate the differences in quantification between the assays. All statistical analyses were performed using PC SAS version 9.3 (SAS, Cary, NC, USA).

## 3. Results

### 3.1. The Alinity m HBV assay demonstrates linear quantification of HBV DNA in plasma

The linearity of the Alinity m HBV assay was assessed by testing a plasma specimen containing $1.7 \times 10^7$ IU/mL (7.2 $Log_{10}$ IU/mL) HBV DNA, undiluted and after one-fifth serial dilutions to 1.7 IU/mL (0.2 $Log_{10}$ IU/mL). Each dilution was tested in triplicate in the same run, and the means and ranges were compared with the expected HBV DNA levels. The expected difference for one-fifth dilution was 0.70 $Log_{10}$ IU/mL. The differences between the undiluted sample and one-fifth serially diluted samples were comparable and compatible with the expected values (0.83 ± 0.36 $Log_{10}$ IU/mL; 0.67 ± 0.07 $Log_{10}$ IU/mL; 0.77 ± 0.07 $Log_{10}$ IU/mL; 0.80 ± 0.05 $Log_{10}$ IU/mL; 1.02 ± 0.42 $Log_{10}$ IU/mL; 0.57 ± 0.05 $Log_{10}$ IU/mL; 0.67 ± 0.11 $Log_{10}$ IU/mL). Fig. 1 shows the measured values for the one-fifth dilution series samples (correlation coefficient $r = 0.99$, linear regression equation, $y = 0.9911x - 0.01629$).

### 3.2. The Alinity m HBV assay demonstrates high precision and reproducibility across study sites

Precision was evaluated across four participating sites by testing dilutions of clinical samples containing $2 \times 10^3$ IU/mL (3.30 $Log_{10}$ IU/mL), $10^2$ IU/mL (2.00 $Log_{10}$ IU/mL), and 10 IU/mL (1.00 $Log_{10}$ IU/mL) of genotype A or genotype C HBV DNA. As shown in Table 1a, the coefficients of variation ranged from 4.1 %–8.8 %. At a concentration near the LLOD, the Alinity m HBV assay exhibited a high level of detectability (≥88.9 %; Table 1b). Reproducibility was characterized by a coefficient of variation of 6.7 % for the low positive control (LPC) and of 5.0 % for the high positive control (HPC; Table 1c).

### 3.3. The Alinity m HBV assay shows comparable performance to routine HBV assays used in clinical practice

In total, 1542 samples from HBsAg-positive individuals were tested using the Alinity m HBV assay and one of four comparator assays at the nine participating study sites: the RealTi*m*e HBV assay (n = 791), CAP/

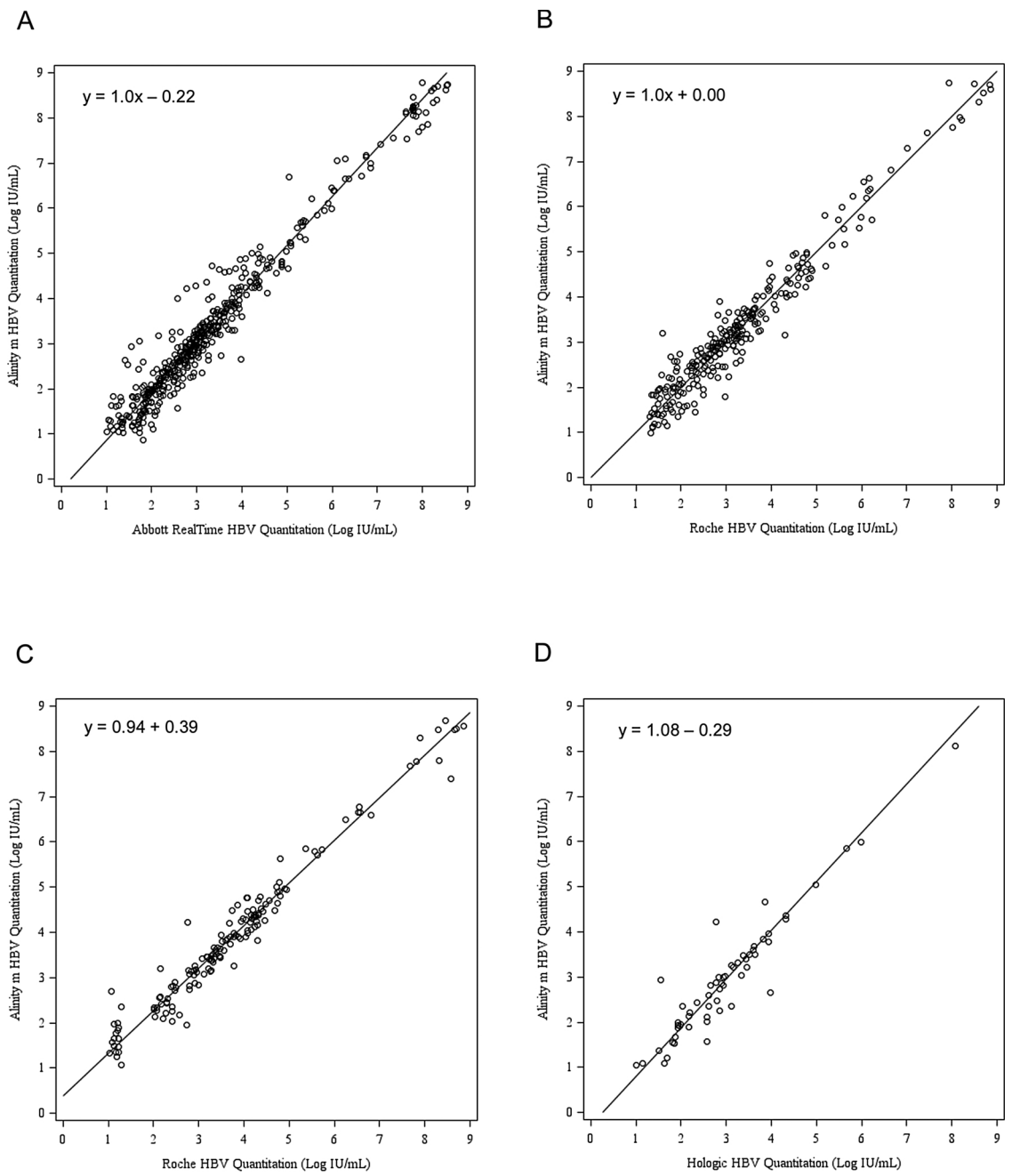


**Fig. 2.** Alinity m HBV assay performance. Deming regression of HBV DNA levels showing correlation between Alinity m HBV assay and (A) RealTi*m*e HBV assay, (B) CAP/CTM HBV v2.0 assay, (C) cobas 6800 HBV assay, and (D) Aptima HBV Quant assay.

**Table 3**
Performance Comparison of Alinity m HBV and Comparator HBV Assays.

| Comparator Assay | Samples (n) | Correlation Coefficient (r) | Mean of Bias $Log_{10}$ IU/mL | SD of Bias $Log_{10}$ IU/mL |
|---|---|---|---|---|
| Abbott RealTi*m*e HBV assay | 459 | 0.979 | 0.05 | 0.377 |
| CAP/CTM HBV assay v. 2.0 | 265 | 0.973 | 0.01 | 0.357 |
| *cobas* 6800 HBV assay | 153 | 0.981 | 0.17 | 0.341 |
| Aptima HBV Quant assay | 59 | 0.947 | −0.07 | 0.419 |

CTM HBV v2.0 assay (n = 402), cobas 6800 HBV assay (n = 195), and Aptima HBV Quant assay (n = 154).

Of 791 samples tested with the Alinity m and Abbott RealTi*m*e HBV assays, 459 fell within the dynamic range of quantification of both assays (Table 2). Fig. 2A shows the relationships between HBV DNA levels measured, with a strong correlation between the two assays (r = 0.98, Deming regression equation, y = 1.0x – 0.22; Table 3). Bland Altman analysis demonstrated a weak bias across the quantitative range of the two assays (0.05 ± 0.38 $Log_{10}$ IU/mL; Table 3). No major difference in quantification was observed based on the HBV DNA levels. In 94.5 % (434/459) of the samples, the difference between the assays was within 1.96 times the SD of bias. In the 5.5 % (25/459) of samples

**Table 4**
Comparison of HBV DNA Levels Using the Alinity m and CAP/CTM v2.0 HBV Assays[a].

| | | CAP/CTM HBV v2.0 (n) | | |
|---|---|---|---|---|
| | | Not detected | < 20 IU/mL | > 20 IU/mL |
| Alinity m HBV (n) | Not detected | 65 | 11 | 3[b] |
| | < 10 IU/mL | 6 | 17 | 4[c] |
| | > 10 IU/mL | 3[d] | 19[e] | 265 |

[a] Two additional samples were reported as > Upper Limit of Quantitation (ULQ) by both methods; 7 additional samples was reported as > ULQ by CAP/CTM HBV v2.0 assay and quantitated by Alinity m HBV assay.
[b] CAP/CTM results ranged from 1.32 to 1.92 $Log_{10}$ IU/mL.
[c] CAP/CTM results ranged from 1.3 to 2.38 $Log_{10}$ IU/mL.
[d] Alinity m results ranged from 1.07 to 1.22 $Log_{10}$ IU/mL.
[e] Alinity m results ranged from 1.01 to 1.87 $Log_{10}$ IU/mL.

**Table 5**
Comparison of HBV DNA Levels Using the Alinity m and cobas 6800 HBV Assays.

| | | cobas 6800 HBV (n) | | |
|---|---|---|---|---|
| | | Not detected | < 10 IU/mL | > 10 IU/mL |
| Alinity m HBV (n) | Not detected | 31 | 0 | 1[a] |
| | < 10 IU/mL | 4 | 0 | 3[b] |
| | > 10 IU/mL | 3[c] | 0 | 153 |

[a] *cobas* 6800 sample quantitated at 1.3 $Log_{10}$ IU/mL.
[b] *cobas* 6800 quantitation ranged from 1.05 to 1.18 $Log_{10}$ IU/mL.
[c] Alinity m quantitation ranged from 1.06 to 1.25 $Log_{10}$ IU/mL.

**Table 6**
Comparison of HBV DNA Levels Using the Alinity m and Aptima HBV Assays.

| | | Aptima HBV Quant (n)[a] | | |
|---|---|---|---|---|
| | | Not detected | < 10 IU/mL | > 10 IU/mL |
| Alinity m HBV (n) | Not detected | 0 | 45 | 6[b] |
| | < 10 IU/mL | 0 | 28 | 8[c] |
| | > 10 IU/mL | 0 | 5[d] | 59 |

[a] Two additional samples were reported as > Upper Limit of Quantitation (ULQ) by both methods; 1 additional sample was reported as > ULQ by Alinity m HBV assay and quantitated by Aptima Panther HBV assay.
[b] Panther quantitation for 5/6 samples ranged from 1.0 to 1.67 $Log_{10}$ IU/mL; 1 sample was 4.2 $Log_{10}$ IU/mL.
[c] Panther quantitation ranged from 1.0 to 1.26 $Log_{10}$ IU/mL.
[d] Alinity m quantitation ranged from 1.10 to 1.42 $Log_{10}$ IU/mL.

where the difference exceeded 1.96 times the SD of bias, 3.9 % (18/459) of results were higher and 1.5 % (7/459) were lower with the Alinity m HBV assay compared to the Abbott RealTi*m*e HBV assay.

Of 402 samples tested using the Alinity m HBV and CAP/CTM HBV v2.0 assays, 265 fell within the dynamic range of both assays (Table 4). A strong correlation between the two assays was found (r = 0.97; Deming regression equation, y = 1.0x + 0.00; Fig. 2B; Table 3). Bland Altman analysis demonstrated a weak bias across the quantitative range of the two assays (0.01 ± 0.36 $Log_{10}$ IU/mL; Table 3). No major difference in quantification was observed according to the HBV DNA level. In 95.1 % (252/265) of the samples, the difference between the assays was within 1.96 times the SD of bias. In the 4.9 % (13/265) of samples where the difference exceeded 1.96 times the SD of bias, 3% (8/265) of results were higher and 1.9 % (5/265) were lower with the Alinity m HBV assay compared to the CAP/CTM HBV v2.0 assay.

Of 195 samples tested with the Alinity m HBV and cobas 6800 assays, 153 fell within the dynamic range of both assays (Table 5). A strong correlation between the two assays was found (r = 0.98; Deming regression equation, y = 0.94 + 0.39; Fig. 2C; Table 3). Bland Altman analysis demonstrated a modest bias across the quantitative range of the two assays (0.17 ± 0.34 $Log_{10}$ IU/mL; Table 3). No major difference in quantification was observed according to the HBV DNA level. In 94.8 % (145/153) of the samples, the difference between the assays was within 1.96 times the SD of bias. In the 5.2 % (8/153) of samples where the difference exceeded 1.96 times the SD of bias, 2.6 % (4/153) of the results were higher and 2.6 % (4/153) were lower with the Alinity m HBV assay compared to the cobas 6800 HBV assay.

Finally, of 154 samples tested using the Alinity m HBV and Aptima HBV Quant assays, 59 fell within the dynamic range of both assays (Table 6). A strong correlation between the two assays was found (r = 0.95; Deming regression equation, y = 1.08 – 0.29; Fig. 2D; Table 3). A weak bias of HBV DNA level was observed (-0.07 ± 0.42 $Log_{10}$ IU/mL; Table 3). No major difference in quantification was observed according to the HBV DNA level. In 93 % (55/59) of the samples, the difference between the assays was within 1.96 times the SD of bias. In the 7% (4/59) of samples where the difference exceeded the 1.96 time the SD of bias, 3.4 % (2/59) of the results were higher and 3.4 % (2/59) were lower with the Alinity m HBV assay compared to the Aptima HBV Quant assay.

### *3.4. The Alinity m HBV assay performance is comparable across HBV genotypes*

To assess the influence of the HBV genotype on HBV DNA quantification, plasma samples from 148 patients with chronic infection with HBV genotype A to E were tested in parallel with the Alinity m HBV assay, RealTi*m*e HBV assay, and CAP/CTM HBV v2.0 assay. A significant correlation was found between HBV DNA levels measured in the same plasma specimens with Alinity m HBV assay and RealTi*m*e HBV or CAP/CTM HBV v2.0, regardless of the HBV genotype (data not shown).

## 4. Discussion

In this international, multicenter study, which included a large number of clinical specimens from patients infected with various HBV genotypes, the new Alinity m HBV assay accurately and reproducibly detected and quantified HBV DNA levels, with performance comparable to that of several HBV assays that are currently widely used in clinical practice. An excellent correlation (correlation coefficients ≥0.95) was seen between the Alinity m HBV assay and the four comparator HBV assays. Only a modest difference in HBV DNA levels with the Alinity m HBV assay was observed, with an overall bias ranging from -0.07 to 0.17 $Log_{10}$ IU/mL and 97 % of quantifiable patient results < 1 $Log_{10}$ IU/mL different than the respective comparator assay (data not shown). A high proportion of patient results fell within 1.96 times the SD of bias of the method comparison (93–95 %). The difference in performance was independent of the HBV genotype and most likely will have no implications for clinical practice. A substantial number of specimens was observed with detectable HBV DNA by either RealTi*m*e HBV or Aptima HBV Quant compared to undetectable HBV DNA results with Alinity m HBV (Tables 2 and 6). Majority of the RealTi*m*e HBV samples had an additional freeze-thaw cycle prior to testing with Alinity m HBV. Comparison between Alinity m HBV and Aptima Holigic quant assays was performed using fresh specimens, the observed differences in this detectability could potentially be attributed to slight variances in the respective LOD values as well as intrinsic assay variability at this very low level.

This study has several limitations. The precision study was performed with a panel containing only genotypes A and C. Thus, although our results strongly suggest that a good performance of the Alinity m HBV assay across all HBV genotypes, further studies will be needed covering those seen throughout the world including genotypes F to H. Samples were not tested on each platform with the same freeze-thaw

cycle. Additionally, age of samples could have also contributed to differences in performance especially on the low end of the dynamic range where greater assay variability exists. In addition, HBV DNA monitoring in patients receiving nucleoside or nucleotide analogue therapy is missing from our analysis; the performance of the Alinity m HBV assay for monitoring will need to be evaluated in future longitudinal studies.

This is the first field evaluation of the Alinity m HBV assay. The newly developed real-time PCR-based Alinity m HBV assay is sensitive, reproducible, and accurately quantifies HBV DNA levels in serum and plasma samples from HBsAg-positive patients. Quantification is linear over the full dynamic range of quantification that covers values observed in untreated and treated patients with chronic HBV infection. In addition, the Alinity m HBV assay is run on the Alinity m platform, a fully automated, continuous, random-access molecular diagnostic analyzer. The Alinity m analyzer has a processing capacity of 300 samples per 8 h shift, enabling same-day reporting of HBV test results to shorten the time between diagnosis and treatment, which may improve patient care and outcomes. We conclude that the Alinity m HBV assay will be useful for rapid detection and quantification of HBV DNA in clinical practice.

## Author contribution

SB and FO contributed equally to this study.

## Disclosure statement

AV, AG, EG, FO, GN, MK, SB, KJ, RE, RG, LM and MP have no conflicts of interest.

HK has received speaker honorariums and/or travel grants from AbbVie, GILEAD, Hexal,

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JCG has received speaker honorariums from Hologic and Gilead, research grants from Roche and AbbVie, and travel grants from Siemens and Abbott.

LMG has received a speaker honorarium from Abbott and a travel grant from Hologic.

MO has received speaker honorariums from Roche, Hologic, Cepheid, and Siemens; received travel grants from Roche, Abbott, Hologic, and Vela Diagnostics; and acted as advisor for Cepheid, Siemens, and Abbott.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jcv.2020.104514.

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