Performance Evaluations of the Abbott Alinity m Assay in Comparison with the Abbott m2000 Assay for Hepatitis B and Hepatitis C Viruses

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Background: The quantification of the hepatitis B virus (HBV) or hepatitis C virus (HCV) is critical for the diagnosis and prognostic follow-up of the viral infection. The Alinity m assay is a recently developed, fully automated “random-access” system for quantitative molecular assays. The aim of this study was to verify the validity of the Alinity m assay by comparing its performance in HBV and HCV quantifications with the established Abbott m2000 HBV and HCV assays.

Methods: The precision, linearity, limit of detection (LOD), correlation with the Abbott m2000 assay, and interference were evaluated.

Results: The within-laboratory standard deviation ranged from 0.106 to 0.137 log IU/mL for HBV and from 0.073 to 0.097 log IU/mL for HCV, which was lower than the manufacturer's specification of 0.25 log IU/mL, indicating good precision. Linearity was observed from 1.14 to 8.14 log IU/mL for the HBV assay and from 1.09 to 7.09 log IU/mL for the HCV assay. The LODs of HBV and HCV were 10 and 6.39 IU/mL, respectively, which were equivalent to or better than those claimed by the manufacturer. For comparative evaluation between Alinity m and m2000 assays, 142 HBV and 70 HCV samples were tested. The correlation test revealed a strong correlation for both markers, and the Passing-Bablok regression analysis did not reveal any significant deviation.

Conclusions: The Alinity m assay demonstrated excellent performance for HBV and HCV quantifications with reduced hands-on time and a random-access format.

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Key Words Hepatitis B virus, Hepatitis C virus, DNA, RNA, Quantification test

INTRODUCTION

The diagnoses of hepatitis B virus (HBV) and hepatitis C virus (HCV) infections rely on viral nucleic acid testing (NAT) [1]. The quantifications of HBV DNA and HCV RNA are critical for the diagnosis, establishing the prognosis of the viral infection, and monitoring the virologic response

to anti-viral therapy [2]. International clinical practice guidelines recommend the use of sensitive NAT for HBV DNA and HCV RNA detections.

Many automated molecular quantification systems have been developed and are in use in laboratories. However, most molecular systems operate generally on a batch testing of multiple samples, resulting in little flexibility,

delayed results, and increased costs. The Alinity m (Abbott Molecular Inc., Des Plaines, IL, USA) is a recently developed fully automated “random-access” system for quantitative molecular assays. It provides the first result of less than 115 minutes and a throughput of up to 300 samples in 8 hours. The Alinity m system allows samples to be assayed as soon as they arrive in the laboratory, throughout the day, reducing the turnaround time and hands-on time of medical technicians.

Here, we evaluated the analytical performance of Alinity m HBV and HCV quantification assays with the established Abbott m2000 HBV and HCV assays (Abbott Molecular Inc.). The study protocol was approved by the Institutional Review Board of the Catholic University of Korea (OC19DISI0178).

MATERIALS AND METHODS

Ethylenediamine tetraacetic acid blood samples received for viral quantification were tested between December 2019 and January 2020. Blood samples were centrifuged, and aliquots of plasma were stored at -80°C for several days. Each aliquot was thawed out once and centrifuged before processing on the Alinity m or m2000 assay. Assays were calibrated to international standards. The precision, linearity, lower limit of detection (LOD), correlation, and interference were evaluated. Comparisons were conducted for 142 clinical samples of HBV and 70 clinical samples of HCV. MedCalc ver. 19.0 (MedCalc, Ostend, Belgium) was used for the statistical analysis, and $P < 0.05$ was considered statistically significant.

To assess the precision, a set of commercial controls and calibrator consisting of three different levels (2, 4, and 6 log IU/mL) of HBV (Abbott RealTime HBV Control, Abbott Molecular Inc.; Abbott RealTime HBV Calibrator, Abbott Molecular Inc.) and HCV (Abbott RealTime HCV Control, Abbott Molecular Inc.; Abbott RealTime HCV Calibrator, Abbott Molecular Inc.) were assayed in duplicate, twice a day for 5 consecutive days according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [3]. Package inserts specify that Alinity m HBV and HCV were designed to achieve a within-laboratory standard

deviation (SD) of less than or equal to 0.25 log IU/mL.

The linearities of the Alinity m HBV and HCV were verified in the claimed linear range for each DNA and RNA level according to CLSI guidelines [4]. The claimed measuring interval ranged from 1.0 to 9.0 log IU/mL for the HBV assay and from 1.0 to 8.0 log IU/mL for the HCV assay. Ten serially diluted pooled plasma samples ranging from 1.14 to 8.14 log IU/mL were measured in duplicate for HBV, and seven serially diluted plasma samples ranging from 1.09 to 7.09 log IU/mL were measured for HCV. The linear fit, second order, and third order polynomial regression analyses were performed. The regression equation was obtained (y , measured concentration; x , expected concentration) using a linear-fit model.

LODs of HBV and HCV were evaluated using samples including levels of LOD claimed by the manufacturer according to CLSI guidelines [5]. The LOD in the HBV assay was estimated by testing 15 replicates of two levels of the Abbott m2000 HBV-positive control (10 and 20 IU/mL). For the LOD of HCV, the positive control was diluted to approximately 6, 9, and 12 IU/mL and tested 14 times for 6 IU/mL while 12 times for 9 and 12 IU/mL. The probit analysis was used for the HCV LOD estimation.

For correlation evaluation between Alinity m and m2000 assays, 142 HBV and 70 HCV samples ranging from 0 to 8.71 log IU/mL for HBV and from 0 to 6.95 log IU/mL for HCV (determined using the m2000 assay) were sequentially chosen at random. Method comparisons were evaluated with the Passing-Bablok regression with quantifiable results (HBV: 105/142; HCV: 28/70) according to CLSI guidelines [6].

Common potentially interfering factors, hemoglobin, triglycerides, and bilirubin were studied by adding these substances to ten HBV-positive, ten HCV-positive, and three negative samples, respectively. Test results were compared before and after the addition of the substances.

RESULTS

1. Precision

Table 1 shows the within-run and within-laboratory

SDs of HBV and HCV tests. Both HBV and HCV had within-laboratory SDs lower than the manufacturer's specification (0.25 log IU/mL). The results suggest a good precision at all clinically meaningful levels.

2. Linearity

Linearity was observed from 2.14 to 8.14 log IU/mL in HBV and from 2.09 to 7.09 log IU/mL in HCV (Fig. 1). The regression equation was obtained (y, measured concentration; x, expected concentration) using a linear-fit model: $y=0.9897+0.8223x$, $r=0.995$ (recovery rate, 96%–187%) for HBV, and $y=-0.6187+1.081x$, $r=0.998$ (recovery rate, 49%–100%) for HCV. The expected bias for non-linearity between the linear fit and the best non-linear (second order polynomial) fit in HBV was 21.4% (95% confidence interval [CI], 13.6% to 29.1%), 1.6% (95% CI,

1.0% to 2.2%), –3.3% (95% CI, –4.5% to –2.1%), –4.2% (95% CI, –5.7% to –2.7%), –3.4% (95% CI, –4.6 to –2.2%), –1.7% (95% CI, –2.3 to –1.1%), 0.5% (95% CI, 0.3 to 0.7%), and 3.0% (95% CI, 1.9% to 4.1%) at 1.14, 2.14, 3.14, 4.14, 5.14, 6.14, 7.14, and 8.14 log IU/mL, respectively. No second- or third-order polynomial fit is statistically better than a linear fit at the 5% significance level in HCV.

3. Limit of detection verification

All 30 HBV samples (10 and 20 IU/mL) were detected by Alinity m HBV. The claimed LOD of HBV by the manufacturer was 10 IU/mL, equivalent to our result. The Alinity m HCV assay detected all the 12- and 9-IU/mL samples but missed two 6-IU/mL samples. The LOD of HCV was calculated as 6.39 IU/mL by the probit analysis. The result was better than the claimed LOD (12 IU/mL)

Table 1. Precision results of the Alinity m HBV and HCV assays

Variable	HBV			HCV		
	High*	Medium	Low	High	Medium	Low
Within-run SD	0.074	0.116	0.125	0.089	0.054	0.076
Within-run CV (%)	1.2	2.6	5.2	1.5	1.1	2.8
Within-lab SD	0.106	0.137	0.126	0.097	0.073	0.076
Within-lab CV (%)	1.6	3.1	5.2	1.6	1.5	2.8

Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; SD, standard deviation; CV, coefficient of variation.

*High, 6 log IU/mL; medium, 4 log IU/mL; low, 2 log IU/mL.

Fig. 1. Linearity graph of Alinity m HBV (A) and HCV (B) assays. Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus.

Table 2. Quantitative concordance of viral load results by the Alinity m and m2000 assays

Abbott Alinity m	Abbott m2000					
	HBV			HCV		
	Quantified	Detected <LOD	Not detected	Quantified	Detected <LOD	Not detected
Quantified	105	0	0	28	1	1
Detected <LOD	2	6	4	1	4	1
Not detected	0	2	23	0	1	33

HBV concordance: 94.4% (134/142); HCV concordance: 92.9% (65/70).

Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; LOD, limit of detection.

Fig. 2. Quantitative agreement between Alinity m HBV, HCV, and m2000 assays. (A) The Passing-Bablok analysis for HBV results, with correlation coefficient r of 0.986. (B) Difference plot (Bland-Altman analysis) for HBV results showing a mean difference of 0.1 log IU/mL between assays. (C) The Passing-Bablok analysis for HCV results, with correlation coefficient r of 0.980. (D) Difference plot (Bland-Altman analysis) for HCV results showing a mean difference of 0.17 log IU/mL between assays. Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus.

according to the manufacturer.

4. Correlation

Among the 142 HBV samples, 105 showed quantified results both in Alinity m and m2000 (Table 2). For HCV, among 70 samples, 28 showed quantified results. Two samples showed “not detected” in Alinity m HBV that turned detected (<10 IU/mL) in the m2000 assay, whereas four samples showed the opposite pattern. Among 70 HCV samples, two were detected (261 and <12 IU/mL, respectively) in the Alinity m HCV assay and turned “not detected” in the m2000 assay, and one “not detected” in Alinity m HCV turned detected (<12 IU/mL) in the m2000 assay. The overall concordance between the Alinity m and m2000 analyses was 94.4% (134/142) for HBV and 95.7% (67/70) for HCV.

The correlation test with quantified tests showed a strong correlation for both markers (Fig. 2). The Passing-Bablok regression analysis did not reveal any significant deviation from linearity between the two assays, and 97.14% of HBV samples (102/105) and 92.86% of HCV samples (26/28) showed delta differences less than 0.5 log IU/mL. The mean difference in viral DNA levels between the two assays was 0.1 log IU/mL for HBV and 0.17 log IU/mL for HCV.

5. Interference

Hemoglobin, triglycerides, or bilirubin showed no interferences with the HBV test results.

DISCUSSION

The clinical performances of the novel Alinity m assays for HBV and HCV quantifications were compared to those of m2000 assays. We showed that the Alinity m assay has an excellent precision and analytic sensitivity, with an estimated LOD equivalent to that claimed by the manufacturer. Quantitative HBV and HCV results showed a strong correlation and estimated to be equivalent within allowable differences with m2000 assays.

Alinity m systematically showed higher concentrations for both HBV and HCV compared to m2000 (0.08 log IU/

mL for HBV and 0.10 log IU/mL for HCV), consistent with another comparison study [7]. The target sequences and LODs for HBV and HCV remained unchanged in both assays, but a second probe was added. The over-quantification might have resulted from the improved performance in Alinity m, and since it avoids false-negative results, it is not a disadvantage.

Qualitative discrepancies were observed both in HBV (8/142, 5.6%) and HCV (5/70, 7.1%) tests (Table 2). All discrepancies were observed in the low tittered samples, and the lower precision in NAT assays at low viremic levels could lead to such qualitative discrepancies. In HBV, Alinity m detected four samples that were not detected in the m2000 assay, and other two samples showed the opposite pattern. The HCV assays showed a similar tendency. Alinity m HCV detected two samples that were negative in the m2000 assay, and only one sample showed the opposite pattern. It suggests that Alinity m is more sensitive compared to m2000, and Bland-Altman graphs show the same results (Fig. 2). The mean of Alinity m-m2000 was +0.1 log IU/mL for HBV and +0.17 log IU/mL for HCV. The more sensitive performance of Alinity m could support its use in clinical practice more precisely.

The analytical turnaround time and hands-on time are significantly decreased in the Alinity m assay. Ready-to-use reagents, random access, and 30 minutes of hands-on time of the Alinity m assay improved the laboratory workflow and technicians' availability dramatically. Without the need to work in a batch mode, Alinity m provides the results on a daily basis, and it helps physicians to decide patients' management in time.

A limitation of our study is that different HBV genotypes were not tested on HBV DNA quantification. Most HBV genotypes in Korea are genotype C2 or a mixed pattern of genotypes B and C, and other genotypes rarely occur [8,9]. Although only minor differences were observed among genotypes A, B, C, D, and E in a study [10], in the study of Braun et al. [11], the genotype affected in quantification showed higher results than expected for genotype D and lower results than expected for genotype A.

In conclusion, the Alinity m assays demonstrated a performance similar to the m2000 assay for HBV and

HCV quantifications. With a reduced hands-on time and random-access format, it is suitable for high-throughput HBV DNA and HCV RNA monitoring in large hospital laboratories.

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