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Analytical Performance Evaluation of the Anti-Hepatitis B Surface Antigen assay on the Atellica CI Analyzer

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Background

Hepatitis B virus (HBV) causes major liver disease and despite widespread vaccination, HBV infection, often asymptomatic, remains a global health issue.[1] Qualitative and quantitative measurement of total antibodies to hepatitis B surface antigen (anti-HBs) in human serum and plasma samples is performed in clinical laboratories to evaluate the susceptibility to HBV infection in individuals (neonates, infants, children, adolescents, and adults) prior to or following HBV vaccination or where vaccination status is unknown. Anti-HBs assays are also part of a triple serological panel (HBsAg, hepatitis B surface antigen; anti-HBs, antibody to HBsAg; anti-HBc total, total antibody (IgM and IgG) to hepatitis B core antigen) to assess current or past exposure to HBV. Routine testing with this panel is recommended by Centers for Disease Control and Prevention (CDC) in all adults aged ≥18 years at least once during a lifetime to identify most people living with HBV infection, reduce HBV prevalence and support the goal of worldwide viral hepatitis eradication.[2]

Previously, one assay was developed and commercialized for use on the Atellica IM Analyzer: the Atellica IM Anti-Hepatitis B surface Antigen 2 (aHBs2) assay.[3] For this assay, results are reported quantitatively using mIU/mL values and qualitatively interpreted. Samples are classified as reactive (≥10.0 mIU/mL) or nonreactive (<10.0 mIU/mL) based on the established 10.0 mIU/mL cutoff for anti-HBs serum, which serves as an indicator of immune status.

For over three years, the Atellica CI Analyzer (Figure 1) has been part of the Atellica Solution portfolio, offering a reduced footprint of 1.9 square meters. It is an integrated clinical chemistry and immunoassay analyzer designed for low- to mid-volume laboratories and features the same reagents,*consumables,* and sophisticated user interface as the Atellica IM Analyzer.[4]

To evaluate the analytical performance of the Atellica IM assays using this recent analyzer, precision, method comparison (MC), limit of blank, detection, quantitation (LoB, LoD, LoQ), and linearity studies were assessed as performance indicators for the Atellica IM aHBs2 assay on the Atellica CI Analyzer.

Figure 1. The Atellica CI Analyzer

Material and Methods

Precision (CLSI EP05-A3)

- Sample types: native and contrived human serum pools spiked with human aHBs-positive serum samples, and plasma quality control (QC) samples.
- One aliquot/sample; tested in duplicate; two runs/day >2 hours apart for 20 days.
- One reagent lot; two analyzers; total n = 80 replicates for each system/lot combination.
- One representative system/lot combination result across all lot and system combinations tested is shown (Table 1).
- Each testing day, new frozen aliquots were thawed and used for each run. Calibrators and QC materials were handled according to the manufacturer's instructions; two calibration events for 20-day-precision study.

Method comparison (MC)

- MC was evaluated using individual native serum samples tested on the Atellica CI Analyzer, the ADVIA Centaur XP system (parent analyzer), and the Atellica IM Analyzer using three reagent lots.
- MC was completed in 7 days using a single calibration event.
- One representative system/lot combination result across all lot and system combinations tested is presented.

MC for qualitative assay (CLSI EP12-A2)

- One replicate processed per sample except for samples falling in the retest zone (≥8.0 and <12.0 mIU/mL) repeated in duplicate in accordance with the instructions for use.
- Samples were classified as reactive (≥10.0 mIU/mL) or nonreactive (<10.0 mIU/mL) based on the established 10.0 mIU/mL cutoff. For retested samples, 2 out of 3 results approach was used for final interpretation.
- Relative sensitivity, relative specificity, and overall agreement are reported and were calculated as followed:

		Atellica IM (or ADVIA Centaur XP) Result	
		Reactive	Nonreactive
Atellica CI Result	Reactive	A	B
	Nonreactive	C	D

Relative sensitivity = 100 × A / (A + C)

Relative specificity = 100 × D / (B + D)

Overall Percent Agreement = 100 × (A + D) / (A + B + C + D)

MC for quantitative assay (CLSI EP09C-ED3)

- One initial replicate result was used for data analysis.
- Samples with results outside the assay measuring interval (3.1–1000.0 mIU/mL) were excluded from the analysis.
- Slope and intercept were calculated using weighted Deming regression analysis.

Detection capability (CLSI EP17-A2)

LoB: Highest measurement result that is likely to be observed on a blank sample with a probability of 95%.

- Five undetectable analyte level samples; six replicates/sample; two runs/day, 5 days, two instruments, three reagent lots: total of 300 measurements per reagent lot. LoB was calculated non-parametrically as the 95th percentile ranked position of all blank samples using the following equation: Rank Position = 0.5 + (n x 0.95), where n is the total number of replicates. The maximum of all lots was taken as the final estimated value.

LoD: lowest concentration of aHBs detectable with a probability of 95%.

- Four low analyte level samples; six replicates/sample; two runs/day, 5 days, two instruments, three reagent lots: total of 240 measurements per reagent lot. LoD was analyzed non-parametrically. For each lot, the 5th percentile value per sample was calculated. The lowest median of a sample where the 5th percentile was ≥LoB was taken as the LoD for the lot. The largest LoD of all lots was the final estimated LoD value.

LoQ: lowest amount of measurand that can be accurately quantified with a total error which satisfies the total error acceptance criteria specific for the assay.

- Five low analyte level samples; six replicates/sample; two runs/day, 5 days, two instruments, three reagent lots: total of 300 measurements per reagent lot. Total error was calculated using the Westgard model (TE=|bias| + 2*SD). LoQ was determined as the lowest analyte concentration at which the total error is ≤ 30% or the LoD, whichever was greater.
- Prior to the start of each study, LoB, LoD and LoQ samples were prepared and frozen in aliquots. On each testing day, fresh aliquots were thawed.
- When the estimated LoB, LoD and LoQ were lower than the design requirement goal for the assay, a conservative value for LoB, LoD and LoQ were set and reported for the assay (Table 4).

Linearity (CLSI EP06-ED2)

- A dilution series composed of 16 levels prepared by mixing high and low analyte samples in a known mathematical relationship; five replicates/level; one instrument, three reagent lots
- Expected values were calculated for each level from the measurand concentrations of the low and high samples. A best-fitted straight-line regression was fit through the mean observed values versus the expected values. Bias was calculated for each level as the difference between the mean observed value and the value predicted by the linear regression model. These biases were converted into % bias values, with respect to the predicted value for each sample, and compared to the acceptance criteria (allowable deviation from linearity) for the assay.

Results

Precision

Table 1. Precision for the Atellica IM aHBs2 assay on the Atellica CI Analyzer

Specimen Type	Mean (n=80) (mIU/mL)	Repeatability		Within-laboratory Precision	
		SD (mIU/mL)	CV (%)	SD (mIU/mL)	CV (%)
Serum	5.0	0.19	3.8	0.39	7.8
Serum	9.9	0.44	4.4	0.60	6.1
Serum	17.7	0.21	1.2	0.42	2.4
Serum	118.4	1.45	1.2	2.02	1.7
Serum	275.7	3.76	1.4	4.73	1.7
Serum	511.8	5.98	1.2	7.28	1.4
Serum	775.5	7.17	0.9	10.25	1.3
Plasma QC	113.7	1.82	1.6	2.31	2.0

All 80 negative QC results are not reported in this summary table because all results are below LoQ (3.1 mIU/mL).

The Atellica IM aHBs2 assay on the Atellica CI Analyzer demonstrated ≤4.4% repeatability CV and ≤7.8% within-laboratory precision CV across the sample interval.

Method Comparison

Table 2. Qualitative method comparison for the Atellica IM aHBs2 assay on the Atellica IM and Atellica CI Analyzers

10.0 mIU/mL Cutoff		Atellica IM aHBs2 on the Atellica IM Analyzer		
		Reactive	Nonreactive	Total
Atellica IM aHBs2 on the Atellica CI Analyzer	Reactive	118	0	118
	Nonreactive	0	122	122
	Total	118	122	240

Relative sensitivity: 100% (118/118); 95% confidence interval: 96.85–100%

Relative specificity: 100% (122/122); 95% confidence interval: 96.95–100%

Overall agreement: 100% (240/240); 95% confidence interval: 98.43–100%

The design requirements for method comparison were met for the aHBs2 assay with a relative specificity ≥ 98% and a relative sensitivity ≥ 98%. Identical sensitivity and specificity results were obtained when comparing the Atellica IM aHBs2 assay using the Atellica CI Analyzer to the ADVIA Centaur aHBs2 assay using the ADVIA Centaur XP system. No discordant results were observed at cutoff between the compared devices.

Table 3. Quantitative method comparison for the aHBs2 assay on the Atellica IM Analyzer, Atellica CI Analyzer and ADVIA Centaur XP system

Specimen Type	Assay	Comparison Analyzer (x)	n	r	Regression Equation	Sample Range
Serum	Atellica IM aHBs2	Atellica IM	100	0.999	y = 0.97x – 0.2 mIU/mL	4.5–850.5 mIU/mL
	ADVIA Centaur aHBs2	ADVIA Centaur XP		0.993	y = 1.09x + 0.1 mIU/mL	3.9–809.9 mIU/mL

The design requirements for method comparison were met for the aHBs2 assay. When analyzed by regression, Atellica IM aHBs2 assay on the Atellica CI Analyzer recovered samples spanning the measuring interval, with a slope of 1.00 ± 0.10 and a correlation coefficient ≥ 0.95 (r) compared to the Atellica IM Analyzer as well as the ADVIA Centaur aHBs2 assay on the ADVIA Centaur XP system.

One representative weighted Deming fit and percent difference plots on the Atellica CI Analyzer for samples ranges indicated in Table 3 are shown for the Atellica IM aHBs2 assay (Figure 2).

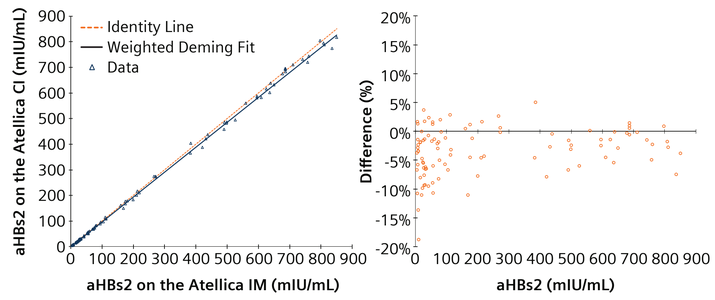

Figure 2. Weighted Deming linear regression and difference plots for the Atellica IM aHBs2 assay on the Atellica IM and Atellica CI Analyzers.

Detection Capability

Table 4. LoB, LoD and LoQ for the Atellica IM aHBs2 assay on the Atellica CI Analyzer

Specimen Type	Assay	Total Replicates per Reagent Lot	LoB Reported	LoD and LoQ Reported
Serum	Atellica IM aHBs2	300 (LoB) 240 (LoD) 300 (LoQ)	1.7 mIU/mL	3.1 mIU/mL

For the aHBs2 assay, the reported LoB was 1.7 mIU/mL and the reported LoD and LoQ were 3.1 mIU/mL, respectively.

Linearity

Table 5. Linear interval for the Atellica IM aHbs2 assay on the Atellica CI Analyzer

Specimen Type	Assay	# of Sample Combinations Tested	Linearity Reported
Serum	Atellica IM aHBs2	16	3.1–1000.0 mIU/mL

The Atellica IM aHBs2 assays is linear on the Atellica CI Analyzer across the interval indicated in Table 5. The lower limit of the linear interval is defined by the analytical sensitivity (LoQ) estimated to be 3.1 mIU/mL for this assay.

Conclusion

All results indicate that the Atellica IM aHBs2 assay demonstrated comparable analytical performance for the serological determination of anti-HBs when tested on the Atellica CI Analyzer. In addition, strong qualitative and quantitative agreement was observed between the assay on the Atellica CI Analyzer and the Atellica IM Analyzer. Altogether, these results support that the Atellica CI Analyzer has comparable performance capability to the Atellica IM Analyzer.

References

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