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Evaluation of the Analytical Performance of the Hepatitis A Total Antibodies Assay on the Atellica CI Analyzer

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Background

Hepatitis A infection, caused by the hepatitis A virus (HAV), is part of viral hepatitis global health concern. Transmission typically occurs through the fecal-oral route, either via direct person-to-person contact or through the consumption of contaminated food or water. Although most HAV infections result in lifelong immunity or can be prevented through vaccination, a small percentage of cases still lead to hepatic complications and fatalities worldwide. To diagnose HAV infection, clinical laboratories rely on serological testing, which detect serum immunoglobulin (Ig)M and/or IgG antibodies specific to HAV (anti-HAV).[1]

The Atellica IM Hepatitis A Total (aHAVT) assay was previously developed and commercialized for use on the Atellica IM Analyzer.[2] This assay allows the quantitative determination of total anti-HAV (IgM and IgG). Results are reported using both numerical mIU/mL values and qualitative interpretation. Samples are classified as reactive (≥20 mIU/mL) or nonreactive (<20 mIU/mL) based on a clinically verified 20 mIU/mL cutoff for anti-HAV serum, which serves as an indicator of susceptibility or immune status following previous or ongoing HAV infection, or after HAV vaccination.

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.[3]

To evaluate the analytical performance of the Atellica IM assays using this new 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 aHAVT assay on the Atellica CI Analyzer.

Figure 1. The Atellica CI Analyzer

Material and Methods

Precision (CLSI EP05-A3)

- Sample types: native and pooled human serum samples, and quality control (QC) sample.
- 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 (CLSI EP12-A2)

- MC was evaluated using individual native human 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 at least 3 nonconsecutive days using a single calibration event.
- One representative system/lot combination result across all lot and system combinations tested is presented (Table 2).
- One replicate processed per sample.
- Samples were classified as reactive (≥20.0 mIU/mL) or nonreactive (<20.0 mIU/mL) based on the 20 mIU/mL cutoff.
- 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)

Detection capability (CLSI EP17-A2)

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

- Four undetectable analyte level samples; five replicates/sample; two runs/day, 3 days, one instrument, three reagent lots: total of 120 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 anti-HAV detectable with a probability of 95%.

- Five low analyte level samples; five replicates/sample; two runs/day, 6 days, one instrument, three reagent lots: total of 300 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 in a sample at which the within-lab coefficient of variation (CV) is ≤20%.

- Eight low analyte level samples; five replicates/sample; two runs/day, 5 days, one instrument, two reagent lots: total of 400 measurements per reagent lot. For each lot, the within-laboratory precision for each sample was plotted against the mean concentration of each sample and fitted using a power function to give a precision profile. LoQ for each reagent lot was determined as the analyte concentration corresponding to 20% within-lab CV or the LoD, whichever is greater. The largest LoQ across all lots tested was taken as the LoQ for the assay.
- 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 3).

Linearity (CLSI EP06-ED2)

- A dilution series composed of 9 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 aHAVT 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	14.86	0.921	6.2	1.192	8.0
Serum	24.46	0.727	3.0	0.969	4.0
Serum	29.33	0.866	3.0	1.098	3.7
Serum	44.06	0.809	1.8	1.208	2.7
Serum	59.09	1.316	2.2	1.863	3.2
Serum	79.87	1.062	1.3	1.593	2.0
Positive QC	39.15	1.082	2.8	1.238	3.2

The Atellica IM aHAVT assay on the Atellica CI Analyzer demonstrated ≤6.2% repeatability CV and ≤8.0% within-laboratory precision CV across the sample interval.

Method Comparison

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

20.0 mIU/mL Cutoff		Atellica IM aHAVT on the Atellica IM Analyzer		
		Reactive	Nonreactive	Total
Atellica IM aHAVT on the Atellica CI Analyzer	Reactive	175	1	176
	Nonreactive	0	111	111
	Total	175	112	287

Relative sensitivity: 100% (175/175);
95% confidence interval: 97.85–100%
Relative specificity: 99.11% (111/112);
95% confidence interval: 95.12–99.84%
Overall agreement: 99.65% (286/287);
95% confidence interval: 98.05–99.94%

The design requirements for method comparison were met for the aHAVT assay with a relative specificity ≥ 98% and a relative sensitivity ≥ 98%. One discordant sample was a borderline sample resulting at 20.57 mIU/mL (reactive) on Atellica CI, and at19.17 mIU/mL (nonreactive) on Atellica IM. Similar relative sensitivity and specificity results were obtained when comparing the Atellica IM aHAVT assay using the Atellica CI Analyzer to the ADVIA Centaur aHAVT assay using the ADVIA Centaur XP system (specificity = 99.09% (95% CI, 95.03–99.84; n = 110), and sensitivity = 98.80% (95% CI, 95.71–99.67; n = 166).

Detection Capability

Table 3. LoB, LoD and LoQ for the Atellica IM aHAVT assay on the Atellica CI Analyzer

Specimen Type	Assay	Total Replicates per Reagent Lot	LoB Reported	LoD and LoQ Reported
Serum	Atellica IM aHAVT	120 (LoB) 300 (LoD) 400 (LoQ)	2.00 mIU/mL	7.00 mIU/mL

Linearity

Table 4. Linear interval for the Atellica IM aHAVT assay on the Atellica CI Analyzer

Specimen Type	Assay	# of Sample Combinations Tested	Linearity Reported
Serum	Atellica IM aHAVT	9	7.00–100.00 mIU/mL

The Atellica IM aHAVT assays is linear on the Atellica CI Analyzer across the interval indicated in Table 4. The lower limit of the linear interval is defined by the analytical sensitivity (LoQ) estimated to be 7.00 mIU/mL for this assay.

Conclusion

All results indicate that the Atellica IM aHAVT assay demonstrated comparable analytical performance for the serological determination of total anti-HAV antibodies when tested on the Atellica CI Analyzer. In addition, strong qualitative 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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