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Performance Evaluation of the Atellica IM aHCV Assay*†

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Performance Evaluation of the Atellica IM aHCV Assay*†

Abstract

Introduction
The ADVIA Centaur® Hepatitis C Virus IgG Antibody (HCV) assay† is an in vitro diagnostic immunoassay for the qualitative determination of immunoglobulin G (IgG) antibodies to hepatitis C virus in serum or plasma. The assay may be used in conjunction with other serological and clinical information to aid in the diagnosis of individuals with symptoms of hepatitis and in individuals at risk for hepatitis C infection.

The primary objective of this study was to demonstrate the analytical performance of a similar aHCV assay*† for use on the Atellica® Immunoassay (IM) Analyzer, an automated high-throughput immunoassay analyzer by Siemens Healthineers.

Methods
The Atellica® IM aHCV Assay*† uses the same reagent and calibrator formulation as the ADVIA Centaur HCV assay. The assay uses two HCV recombinant antigens (c200 and NS5) and one synthetic HCV core (c22) peptide contained in the assay's solid phase. The c200 protein is derived from both the NS3 and NS4 sequences. The c22 peptide is derived from the core region of the genome and contains the major HCV core epitope.The Lite Reagent contains monoclonal anti–human IgG labeled with acridinium ester and is used to detect anti-HCV IgG in the sample.

Precision of the Atellica IM aHCV Assay was evaluated according to CLSI protocol EP05-A3, and the method comparison of the Atellica IM aHCV and ADVIA Centaur HCV assays was performed according to CLSI protocol EP12-A2.

Results
The observed repeatability for the Atellica IM aHCV Assay ranged from 3.4% to 6.3% CV (from 0.80 to 8.61 Index), and within-lab precision ranged from 7.2% to 11.5% CV. The method comparison between the Atellica IM aHCV and ADVIA Centaur HCV assays yielded 100% total agreement (126 negative and a minimum of 106 positive) with native patient samples.

Conclusion
The Atellica IM aHCV Assay has demonstrated analytical performance capable of measuring IgG antibodies to hepatitis C virus with good accuracy and precision.

Background

Hepatitis C is caused by the hepatitis C virus (HCV), which is a small, positive-sense RNA virus belonging to the *Flaviviridae* family.[1] Infection is endemic throughout the world and poses a serious health problem. It is estimated that approximately 170 million individuals worldwide are chronically infected with HCV and that chronic infection is one of the major causes of liver damage, cirrhosis, and hepatocellular carcinoma.[2,3] The presence of antibodies to HCV indicates that an individual is or has been infected with HCV.[4] Antibodies to HCV can generally be detected within 6 to 12 weeks of infection, and titer can continue elevating for several months or years.[5]

Principle of the Assay
The Atellica IM aHCV Assay is an indirect, two-wash sandwich immunoassay used for the detection of IgG antibody to hepatitis C virus (HCV) in human serum or plasma. The sample is incubated with solid phase containing recombinant and synthetic peptide HCV antigens. Antigen–antibody complexes will form if anti-HCV antibody is present in the sample. Lite Reagent containing monoclonal anti–human IgG labeled with acridinium ester is used to detect anti-HCV IgG in the sample.

The Atellica IM aHCV Assay uses two recombinant HCV-encoded (c200 and NS5) antigens and one synthetic HCV-encoded core (c22) peptide. The c200 protein is derived from both the NS3 and NS4 sequences. At least two major epitopes are located within the NS3 and NS4 regions. These two specific epitopes have been extensively studied and shown to be critical for the detection of antibodies in individuals infected with HCV. The NS5 antigen is derived from the putative RNA polymerase portion of the HCV genome. A significant number of individuals infected with HCV develop an immunologic response to NS5. The c22 peptide contains the major HCV core epitope. An immunologic response to the core protein is often an early indicator of infection by HCV.[1,6]

Methods

Precision (repeatability)
Protocol: CLSI EP05-A3

- Two reagent lots, two Atellica IM Analyzers; Atellica IM Analyzers and ADVIA Centaur XP systems use the same reagent formulation.
- 20 test days, samples in duplicate, two runs/day on two systems; 2 hours (minimum) between runs.
- n = 80 for each system/lot combination.
- Sample types:
  - Native and contrived (normal serum spiked with high native samples) patient samples (Samples 1–5).
  - High (control 1) and low (control 2) ADVIA Centaur controls.
- Calibration was performed on days 1 and 10.

Comparison of the Atellica IM aHCV Assay to the ADVIA Centaur HCV assay
Protocol: CLSI EP09-A3

- Two reagent lots (1, 2) on one Atellica IM Analyzer and one ADVIA Centaur XP system.
- Samples from outside vendors:
  - 126 negative, 108 positive in singlicate on each system.
  - Samples falling in the equivocal zone (≥ 0.8 to < 1.0) were repeated in duplicate.

Seroconversion study

- Two reagent lots (1, 2) on one Atellica IM Analyzer and one ADVIA Centaur XP system.
- Six seroconversion panels (ZeptoMetrix, Franklin, MA, U.S.).
- Two calibrator lots (1, 2).

Onboard stability (OBS)
Protocol: CLSI EP25-A3

- Two reagent lots, one Atellica IM Analyzer.
- Six samples, including one nonreactive and one reactive native human serum sample, calibrators, and controls, were frozen in aliquots prior to the start of the study.
- New aliquots were thawed each testing day (0, 7, 14, 21, 28, 35, 41, and 42) and assayed in triplicate.
- Open and fresh static packs from each reagent lot were used and stored on the system throughout the study.
- Sample types:
  - One high-titer human serum sample (HHS) and one low-titer human serum sample (LHS).
  - High and low human plasma-based ADVIA Centaur calibrators (HCal, LCal).
  - One negative ADVIA Centaur control (Ctrl–) and one positive ADVIA Centaur control (Ctrl+).
- Calibration was performed at the beginning of the study.

Results

Precision (repeatability)
The standard deviation (SD) and coefficient of variation (CV) observed for all samples, calibrators, and controls demonstrated acceptable results. The observed repeatability for the Atellica IM aHCV Assay ranged from 2.8% to 6.3% CV (from 0.79 to 9.53 Index), and within-lab precision ranged from 3.7% to 11.5% CV, indicating that variability between sample runs is low and that the assay demonstrates acceptable precision. Results shown are for one reagent lot on one Atellica IM Analyzer and are representative of data for both lots and systems.

Sample	n	Mean Index	Repeatability		Within-lab Precision	
			SD[a] Index	CV[b] (%)	SD[a] Index	CV[b] (%)
Sample 1	80	0.37	0.02	5.5	0.07	NA
Sample 2	80	0.80	0.03	3.4	0.09	11.5
Sample 3	80	1.35	0.05	3.9	0.12	9.2
Sample 4	80	3.97	0.18	4.7	0.29	7.2
Sample 5	80	8.61	0.55	6.3	0.62	7.2
Negative Control	80	0.07	0.00	NA	0.06	NA
Positive Control	80	2.98	0.19	6.4	0.27	9.1

[a]SD: standard deviation. [b]CV: coefficient of variation.

Comparison of the Atellica IM aHCV Assay to the ADVIA Centaur HCV Assay
Serum samples were run on the ADVIA Centaur XP system and Atellica IM Analyzer using two reagent lots. For lot 1, 126 HCV-negative and 106 HCV-positive serum samples were used in the sensitivity and specificity calculations. For lot 2, 126 HCV-negative and 108 HCV-positive serum samples were used in the sensitivity and specificity calculations.

Relative specificity
A total of 126 HCV-nonreactive samples were tested using the Atellica IM aHCV Assay. The performance of the Atellica IM aHCV Assay is shown in the following tables:

Lot 1

Number	Nonreactive	Equivocal	Reactive	Relative Specificity
126	126	0	0	100% (126/126)

Lot 2

Number	Nonreactive	Equivocal	Reactive	Relative Specificity
126	126	0	0	100% (126/126)

The relative specificity of the Atellica IM aHCV Assay was 100% (126/126) with a 95% confidence interval of 97.0% to 100%.

Relative sensitivity
A set of 106 HCV-reactive samples was tested with lot 1 using the Atellica IM aHCV Assay. A total of 108 samples was tested with lot 2. The performance of the Atellica IM aHCV Assay is shown in the following tables:

Lot 1

Number	Nonreactive	Equivocal	Reactive	Relative Sensitivity
106	0	0	106	100% (106/106)

Lot 2

Number	Nonreactive	Equivocal	Reactive	Relative Sensitivity
108	0	0	108	100% (108/108)

The relative sensitivity of the Atellica IM aHCV Assay was 100% (106/106) with a 95% confidence interval of 96.5% to 100% for lot 1 and 100% (108/108) with a 95% confidence interval of 96.6% to 100% for lot 2.

Seroconversion study
The seroconversion results indicated that the Atellica IM aHCV Assay on the Atellica IM Analyzer detects reactivity on the same bleed day as does the assay on the ADVIA Centaur XP system, indicating high concordance between the two systems.

Reagent Lot	Panel ID	1st Reactive Bleed Day of Total Sample Collection Days		Difference in 1st Reactive Bleed Day
		ADVIA Centaur XP	Atellica IM	
1	1	9th of 12	9th of 12	0
	2	5th of 8	5th of 8	0
	3	5th of 8	5th of 8	0
	4	7th of 10	7th of 10	0
	5	12th of 13	12th of 13	0
	6	4th of 5	4th of 5	0
2	1	9th of 12	9th of 12	0
	2	5th of 8	5th of 8	0
	3	5th of 8	5th of 8	0
	4	7th of 10	7th of 10	0
	5	12th of 13	12th of 13	0
	6	4th of 5	4th of 5	0

Onboard stability (OBS)
The table shows results for two reagent lots used to assess the repeatability of six samples over 42 days. OBS is determined by an insignificant slope (p value ≥0.05) over the testing interval or by the time point at which the analyte drift limit of ±10% or ±2 SD of within-lab precision (whichever is greater) is exceeded.

Reagent Lot	Sample	Onboard Stability (Days)
1	HHS	42
	LHS	42
	Ctrl+	42
	Ctrl–	42
	HCal	42
	LCal	42
2	HHS	42
	LHS	42
	Ctrl+	42
	Ctrl–	42
	HCal	42
	LCal	42

Reagent onboard stability claim for Atellica IM aHCV Assay: 41 days.

Conclusions

- The Atellica IM aHCV Assay on the Atellica IM Analyzer demonstrated good precision for results across the range of 0.80 to 8.61 Index:
  - Repeatability: 3.4%–6.3% CV.
  - Within-lab precision: 7.2%–11.5% CV.
- Assay results on the Atellica IM Analyzer and the predicate ADVIA Centaur XP system showed high concordance:
  - Relative specificity: 100%.
  - Relative sensitivity: 100%.
- Seroconversion results were identical for 6/6 panels.
- Reagents are stable onboard for up to 41 days.

The foregoing data indicate that the Atellica IM aHCV Assay demonstrates analytical performance capable of qualitative determination of IgG antibodies to hepatitis C virus in serum or plasma.