Original Article

Clinical Evaluation of a Newly Developed Chemiluminescent Enzyme Immunoassay for Hepatitis C Virus Core Antigen in Japan

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SUMMARY: An advanced and fully automated chemiluminescent enzyme immunoassay for the hepatitis C virus core antigen (HCVcAg) was recently developed in Japan. We aimed to evaluate its clinical utility. The new Fujirebio assay (Lumipulse Presto HCVcAg [LP-Presto]) was compared with 2 conventional assays (Lumipulse Ortho HCVcAg [LP-Ortho] and Abbott's Architect HCVcAg). Basic assessments of LP-Presto (reproducibility, stability, range of quantitation, and specificity) were performed on 220 frozen sera (83 positive and 137 negative by LP-Ortho) and 206 fresh sera (all negative by LP-Ortho). Correlation analysis was performed and the rates of concordance for each assay were determined. Additionally, the frozen sera of 42 hyperimmunoglobulinemia patients, including 3 unmeasurable by LP-Ortho, were tested by LP-Presto. All the basic assessments of LP-Presto were consistent with the results of LP-Ortho and Architect. The concordance rate between LP-Presto and LP-Ortho for the 220 frozen sera was 99.5% (219/220), and that between LP-Presto and Architect was 99.1% (218/220). LP-Presto (HCVcAg cut-off value; 20 fmol/L) was fully consistent with LP-Ortho (100%), which found 343 sera negative for HCVcAg. All 42 hyperimmunoglobulinemic sera were measurable using LP-Presto. In conclusion, the performance of LP-Presto was rapid and reliable, and nonspecific test results due to hyperimmunoglobulinemia were reduced when LP-Presto was used. Therefore, LP-Presto is a high-quality HCVcAg assay that shows promising applications.

INTRODUCTION

Hepatitis C virus (HCV) affects approximately 120–130 million people worldwide (1), and HCV infection is a major risk factor for cirrhosis and hepatocellular carcinoma (HCC) (2). Over the last few years, the management of chronic hepatitis C (CHC) has improved dramatically due to treatment with direct acting antivirals (DAAs), and the time to eradicate HCV has been reduced (3,4). Presently, the rate of HCV eradication by DAA treatment is greater than 95% (5,6). Although DAAs provide an extraordinary opportunity for successful HCV eradication (7), feasible diagnostic and treatment monitoring methods are essential to facilitate the global elimination of HCV by 2030 (8). Therefore, cost-effective and easy-to-use methods for detecting HCV are required to achieve HCV eradication (7,9).

Since HCV infection is indicated by the presence of HCV RNA in serum, to detect its presence, an HCV RNA polymerase chain reaction (PCR) assay has been used in clinical practice due to its high sensitivity, specificity, and reproducibility (10). However, the

HCV RNA assay is costly and the results are not available rapidly, making it unsuitable for use under some circumstances. In contrast, measuring the HCV core antigen (HCVcAg) is less costly, the results are available sooner, and the reagents are stable under various environmental conditions; therefore making it a better option for detecting HCV infection (11,12). HCVcAg has been reported to be a surrogate marker for HCV replication, and HCVcAg assays display a sensitivity similar to that of the HCV RNA assay (13) and can detect various HCV genotypes (14). Along with being a marker for diagnosing HCV infection, HCVcAg can also be used to evaluate the response of a patient to antiviral therapy (11), even in developing countries.

Several commercially available HCVcAg assays have demonstrated high sensitivity and specificity for diagnosing active HCV infection (15). However, we have noted that conventional HCVcAg assays produce nonspecific reactions (false-positive results), particularly for patients with hyperimmunoglobulinemia, as reported in the package inserts of the assays. Furthermore, the assays are ineffective if thermocoagulation occurs during pretreatment of the specimens.

An advanced and fully automated chemiluminescent enzyme immunoassay (CLEIA) for HCVcAg was recently developed in Japan. In the present study, we compared the diagnostic utility of the new HCVcAg assay with that of conventional assays, and assessed its clinical efficacy. This report demonstrates the broad applications of this new HCVcAg assay, with its simplified approach for the screening, diagnosis, and monitoring of patients with HCV infection in various

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environments, including in developing countries. We predict the new HCVcAg assay to have great potential as a major tool for diagnostics in the future.

MATERIALS AND METHODS

Ethical standards: Written informed consent was obtained from each patient. The study protocol was approved by the Institutional Review Board of Nagoya City University, according to the Declaration of Helsinki 2013 (acceptance number: 00000657-3).

Patients: Serum specimens were obtained from patients with CHC who were positive for the anti-HCV antibody and satisfied the following study enrollment criteria: i) CHC was diagnosed based on serum positivity for HCV RNA at least once, as described in the next paragraph; ii) patients had or had not received IFN-based therapy and/or DAA treatment; iii) the patients did or did not achieve a sustained virological response. All the patients enrolled in this study were admitted to Nagoya City University Hospital between November 2012 and August 2015.

Anti-HCV serological test, HCV RNA assessment, and HCV genotype: Serum anti-HCV antibodies were analyzed using a fully automated CLEIA (Lumipulse Presto Ortho HCV; Ortho Clinical Diagnostics K.K., Tokyo, Japan). Samples with a cut-off index > 1.0 were considered positive for the anti-HCV antibody.

Serum HCV RNA levels were measured using the Cobas AmpliPrep/Cobas TaqMan HCV Test, v2.0 (Roche Diagnostics K.K., Tokyo, Japan) according to the manufacturer's instructions. The lower and upper limits of quantitation were 15 IU/mL and 1.0×10^8 IU/mL, respectively.

HCV genotypes were identified based on the nucleotide sequences of HCV core regions, as determined by SRL Inc. (Tokyo, Japan).

HCVcAg assay: The new HCVcAg assay (Lumipulse Presto HCVcAg [LP-Presto]; Fujirebio, Inc., Tokyo, Japan) was compared with a conventional assay (Lumipulse Ortho HCVcAg [LP-Ortho]) and a control assay (Architect HCVcAg assay [Architect]; Abbott Japan Co., Ltd., Tokyo, Japan), according to the manufacturers' instructions of each assay. LP-Presto was provided by Fujirebio, Inc., whilst LP-Ortho and Architect were used routinely by the hospital laboratory. The detection limits ranged between 20 and 50,000 fmol/L, 50 and 50,000 fmol/L, and 3 and 20,000 fmol/L for LP-Presto, LP-Ortho, and Architect, respectively. The features of each HCVcAg assay are shown in Table 1.

Study design: All frozen sera were stored at -80°C until use. Data, including the gender and age of each patient, were collected. A total of 220 frozen sera (83 positive and 137 negative for HCVcAg by LP-Ortho) and 206 fresh sera (obtained on the day the assay was performed and negative by LP-Ortho) were studied. Of the 83 frozen sera positive for HCVcAg by LP-Ortho, 65 were obtained from CHC patients with HCV genotype 1, and 18 were obtained from CHC patients with HCV genotype 2. According to the data from the reagent provider, we confirmed that LP-Ortho and LP-Presto showed equivalent reactivity to HCV genotypes 1a, 1b, 2a, and 2b.

We performed basic diagnostic assessments on LP-Presto regarding reproducibility, sample stability, range of quantitation, and specificity. The reproducibility assessment consisted of 6 replicate measurements each on 3 sera positive for HCV genotype 1. The mean value, standard deviation (SD), and the coefficient of variation (CV) were calculated for each sample. For the sample stability assessment, 7 clinical samples positive for HCV genotype 1 were stored at 10°C for 0, 1, 4, 8, and 15 days, with the samples measured at each time point. The percentage difference between pre- and post-storage values was calculated (pre-storage values/post-storage values [%]). For the range of quantitation, 7 samples positive for HCV genotype 1 with low HCVcAg concentrations were each measured 20 times. An approximate curve was constructed based on the relationship between the mean value and CV (%). Using the approximate curve, the concentration with a CV of 20 % was defined as the quantity limit value. In this report, the calculated quantity limit value is shown as a representative result. The specificity assessment consisted of measuring 343 sera (206 fresh and 137 frozen sera) that were negative for HCVcAg by LP-Ortho.

Correlation analysis was performed and the rates of concordance were determined between LP-Presto, LP-Ortho, and Architect.

Effects of hyperimmunoglobulinemia on HCVcAg assay results: A total of 42 frozen sera from patients with hyperimmunoglobulinemia (immunoglobulin [Ig] A $\geq 1,000$ mg/dL, $n = 5$; IgM ≥ 500 mg/dL, $n = 11$; and IgG $\geq 3,000$ mg/dL, $n = 26$) that were negative for HCVcAg by LP-Ortho were analyzed using LP-Presto. The serum IgA, IgM, and IgG levels were measured at the start of the study.

Table 1. The features of each HCVcAg assay

	New assay	Conventional assay	Control assay
Assay	LP-Presto	LP-Ortho	Architect
Measuring instrument	LP-Presto II	LP-G1200	Architect Plus
Pretreatment	- (fully automatic)	+	- (fully automatic)
Measurement principle	CLEIA	CLEIA	CLIA
Required amount of serum (μL)	20	80	108
Measurement time (min)	26.5	55	36

HCVcAg, hepatitis C virus core antigen; LP-Presto, Lumipulse Presto HCVcAg; LP-Ortho, Lumipulse Ortho HCVcAg; Architect, Architect HCVcAg assay; LP-Presto II, Lumipulse Presto II; LP-G1200, Lumipulse G1200; CLEIA, chemiluminescent enzyme immunoassay; CLIA, chemiluminescent immunoassay; min, minutes.

A Newly Developed CLEIA for HCV Core Antigen

Table 2. Reproducibility testing of the LP-Presto (A) and sample stability testing of the LP-Presto (B)

(A)						
Sample name			Serum L	Serum M	Serum H	
HCVcAg values (fmol/L)	Measurement times	1	223.1	1,943.8	39,885.9	
		2	200.7	1,954.1	40,486.0	
		3	205.6	2,039.3	39,944.4	
		4	206.5	1,882.5	38,696.9	
		5	202.6	1,971.4	39,455.9	
		6	207.7	1,917.8	37,623.0	
	Mean	207.7	1,951.5	39,348.7		
	SD (fmol/L)	8.0	53.0	1,034.5		
CV (%)	3.8	2.7	2.6			
HCVcAg, hepatitis C virus core antigen; SD, standard deviation; CV, coefficient of variation; Serum L, positive sample with low concentration; Serum M, positive sample with medium concentration; Serum H, Positive sample with high concentration; LP-Presto, Lumipulse Presto HCVcAg assay.						
(B)						
Parameters measured	Sample No.	Storage time				
		0 day	1 day	4 days	8 days	15 days
HCVcAg values (fmol/L)	73	6,200.0	6,014.7	5,986.9	6,019.5	6,065.3
	85	587.4	555.1	562.1	551.1	554.4
	89	4,406.1	4,332.8	4,279.3	4,301.8	4,400.1
	90	87.2	82.2	82.0	82.0	80.8
	91	21,639.4	20,350.6	19,337.7	18,668.0	18,396.3
	102	7,925.9	7,767.3	7,877.6	7,772.2	8,133.5
	113	27,535.2	29,056.0	28,658.3	29,961.7	28,697.0
Percent differences between pre- and poststorage values (%)	73		97	97	97	98
	85		95	96	94	94
	89		98	97	98	100
	90		94	94	94	93
	91		94	89	86	85
	102		98	99	98	103
	113		106	104	109	104
HCVcAg, hepatitis C virus core antigen; LP-Presto, Lumipulse Presto HCVcAg assay.						

HCVcAg, hepatitis C virus core antigen; LP-Presto, Lumipulse Presto HCVcAg assay.

RESULTS

Reproducibility, sample stability, and range of quantitation of LP-Presto for HCVcAg: The reproducibility testing of 3 positive samples with low, medium, and high concentrations of HCVcAg gave the following mean values $\pm$ SD: 207.7 $\pm$ 8.0 fmol/L, 1,951.5 $\pm$ 53.0 fmol/L, and 39,348.7 $\pm$ 1,034.5 fmol/L, respectively. The CVs were 3.8%, 2.7%, and 2.6%, respectively, as shown in Table 2A.

Sample stability testing found that the mean percentage difference between the pre- and post-storage values of 7 samples ranged between 85%–109%, as shown in Table 2B.

The range of quantitation assessment gave the following respective mean values and CVs: 4.9 fmol/L, 29.9%; 9.6 fmol/L, 13.8%; 14.5 fmol/L, 10.7%; 19.6 fmol/L, 8.7%; 30.3 fmol/L, 6.2%; 49.7 fmol/L, 4.8%; and 102.0 fmol/L, 4.0%. From the approximate curve, the quantity limit value was calculated to be 6.3 fmol/L.

Specificity of LP-Presto for HCVcAg: A total of 206 fresh and 137 frozen sera negative for HCVcAg by LP-Ortho were found to be negative (< 20 fmol/L) by LP-Presto, with a cut-off value of 20 fmol/L. Meanwhile, there was only one discrepancy between these assays below the 10 fmol/L cut-off value of LP-Presto, which

was from a patient with multiple myeloma and had an HCVcAg level of 15.8 fmol/L when measured by LP-Presto. The sample was negative for anti-HCV antibody and showed hyperimmunoglobulinemia (IgG, 6,792 mg/dL).

Correlations between LP-Presto, LP-Ortho, and Architect for HCVcAg: Among the 137 specimens negative for HCVcAg by LP-Ortho, the distribution of LP-Presto negative results was found to be better than that of LP-Ortho, which showed a convergence to 5 fmol/L. Among the 83 specimens positive for HCVcAg by LP-Ortho, correlation analysis between LP-Presto and LP-Ortho yielded the following values: $y = 1.023x + 131.5$, $r = 0.967$ (Fig. 1A). Correlation analysis between LP-Presto and Architect yielded the following values: $y = 1.381x - 56.76$, $r = 0.941$ (Fig. 1B). Four divergent samples (divergence ratios of LP-Presto to LP-Ortho or to Architect were ≥ 2 or ≤ 0.5 , respectively) were found (Samples 1–4). The results of samples 1, 2, and 3 are indicated on the graphs. Because the HCVcAg concentration of sample 4 was very low, the result could not be shown on the graph. These details are summarized in Table 3.

Concordance rates between LP-Presto, LP-Ortho, and Architect for HCVcAg: Details of the concordance between LP-Presto, LP-Ortho, and Architect are shown

Fig. 1. (Color online) Correlations between LP-Presto assay for HCVcAg and conventional assays. Four divergent samples were found (No.1–4). No.'s 1, 2, and 3 are shown in the graphs. Because the concentration of HCVcAg in No. 4 was very low, No.4 is not shown. **A)** Correlations between LP-Presto and LP-Ortho assays. Because the values of LP-Presto and LP-Ortho assay were sufficiently correlated, this graph cannot show No.1. **B)** Correlations between LP-Presto and Architect. HCVcAg, hepatitis C virus core antigen; LP-Presto, lumipulse presto HCVcAg; LP-Ortho, lumipulse ortho HCVcAg; Architect, architect HCVcAg assay.

Table 3. Divergent samples between LP-Presto, LP-Ortho, and Architect

Divergent sample No.	HCVcAg values (fmol/L)			Age	Gender	HCV genotype	Clinical stage
	LP-Presto	LP-Ortho	Architect				
1	7,141.8	6,174.7	1,220.0	51	M	1	Cirrhosis
2	4,502.4	18,053.4	13,900.0	41	M	1	Chronic hepatitis
3	1,614.4	5,639.8	5,050.0	37	M	2	Chronic hepatitis
4	828.5	404.0	685.0	82	F	2	Chronic hepatitis

HCVcAg, hepatitis C virus core antigen; LP-Presto, Lumipulse Presto HCVcAg; LP-Ortho, Lumipulse Ortho HCVcAg; Architect, Architect HCVcAg assay; M, male; F, female.

Table 4. Concordance rates between LP-Presto and LP-Ortho (A) and between LP-Presto and Architect (B), and discrepancies between LP-Presto, LP-Ortho, and Architect (C)

(A)

	LP-Ortho		Total
	+	-	
LP-Presto	82	0	82
	1 (Case No. 5)	137	138
Total	83	137	220

(B)

	Architect		Total
	+	-	
LP-Presto	82	0	82
	2 (Case Nos. 5 and 6)	136	138
Total	84	136	220

(C)

Case No.	HCVcAg values (fmol/L)			Age	Gender	Anti-HCV	HCV-RNA (log IU/mL)	HCV genotype	Primary disease
	LP-Presto	LP-Ortho	Architect						
5	13.6	53.0	48.3	54	F	Positive	4.1–6.6	2	Chronic hepatitis
6	0.1	2.6	3.9	84	M	Positive	Negative	Not available	Leukemia (after treatment)

HCVcAg, hepatitis C virus core antigen; LP-Presto, Lumipulse Presto HCVcAg; LP-Ortho, Lumipulse Ortho HCVcAg; Architect, Architect HCVcAg assay; F, female; M, male.

in Table 4. The rates of concordance between LP-Presto and LP-Ortho or Architect were 99.5% (219/220) and 99.1% (218/220), respectively.

Two discrepancies were found; sample 5 was negative

for LP-Presto and positive for both LP-Ortho and Architect, while sample 6 was negative for both LP-Presto and LP-Ortho and positive for Architect (Table 4). Sample 5 was positive for HCV genotype 2, indicating

Table 5. Results of HCVcAg assays of samples from patients with hyperimmunoglobulinemia

Immunoglobulin	Immunoglobulin level (mg/dL)	Number of cases	LP-Presto		LP-Ortho		Architect	
			False positive	Unmeasurable	False positive	Unmeasurable	False positive	Unmeasurable
IgA	1,000–2,000	4	0	0	0	0	0	0
	2,000–3,000	0	–	–	–	–	–	–
	3,000–4,000	1	0	0	0	1	0	1
	500–2,000	8	0	0	0	0	0	0
IgM	2,000–4,000	2	0	0	0	0	0	0
	4,000–6,000	1	0	0	0	0	0	0
	3,000–5,000	23	0	0	0	0	7	0
IgG	5,000–7,000	1	0	0	0	1	0	0
	7,000–9,000	2	1	0	0	1	0	1

HCVcAg, hepatitis C virus core antigen; LP-Presto, Lumipulse Presto HCVcAg; LP-Ortho, Lumipulse Ortho HCVcAg; Architect, Architect HCVcAg assay; IgA, immunoglobulin A; IgM, immunoglobulin M; IgG, immunoglobulin G.

that patient 5 had viremia (Table 4C). Sample 6 was negative for HCV-RNA, indicating that patient 6 did not have viremia (Table 4C).

Effects of hyperimmunoglobulinemia on HCVcAg assays: Of the 42 frozen sera with hyperimmunoglobulinemia that were negative for HCVcAg by LP-Ortho, 42 samples could be analyzed, of which 41 were negative and 1 (IgG 7,125 mg/dL) was false-positive by LP-Presto. Two and 3 specimens could not be analyzed by Architect and LP-Ortho, respectively. These details are shown in Table 5.

DISCUSSION

Although the HCV eradication rate by DAAs is likely to be higher than 95% (5,6), HCV infection remains a major worldwide risk factor for cirrhosis and HCC (2). The World Health Organization (WHO) recently called for the elimination of HCV, aiming for a 90% reduction in prevalence and a 65% reduction in HCV-related mortality by 2030 (16). HCV RNA assays have been used in clinical practice due to their high specificity, sensitivity, and reproducibility; however, their cost restricts their use in some areas (10). Therefore, reliable, easy-to-use, and inexpensive assays are needed to detect HCV and confirm the success or failure of HCV eradication. In the present study, we introduced a novel, fully automated HCVcAg assay that should fulfill the aforementioned criteria and will soon be available in Japan. The findings of this study suggest that an automated HCVcAg assay is appropriate for screening and diagnosing HCV infections in large numbers of CHC patients, particularly in populations estimated to have a high prevalence of HCV infection such as developing countries.

As described previously, HCVcAg assays have shown complete clinical equivalence with RNA assays and incur substantial savings for the diagnosis of acute hepatitis C (17) and for monitoring dialysis patients (18). Additionally, HCVcAg assays are less expensive than HCV RNA assays for monitoring patients receiving HCV eradication treatment (17,19), and for detecting HCV relapse after DAA therapy in patients living in developing countries. HCVcAg assays do not require specialized technicians and have a much faster turnaround time, with results available in 1 h instead of up to 7 h needed for HCV RNA assay results (19,20).

The new LP-Presto HCVcAg assay provides results within 30 minutes. In Japan, the costs of HCVcAg and HCV RNA assays are ¥1,110 and ¥4,370, respectively. Since, the cost of an HCV RNA assay is generally several-fold that of an HCVcAg assay (8,21); therefore, using HCVcAg assays instead of HCV RNA assays might reduce the cost of diagnosis and the time taken to obtain results, and improve patient follow-up in certain environments. In fact, the results of a study that compared an HCV RNA assay with an HCVcAg assay for the detection and quantification of HCV viremia in Egyptian participants led the authors to conclude that while the HCV RNA assay remains the gold standard for the diagnosis of an active HCV infection, an HCVcAg assay can be used when PCR is not available (22).

In this study, 4 samples showed inconsistent results between the LP-Presto vs LP-Ortho and Architect assays. Amino acid polymorphisms in the core region have been reported to affect the HCVcAg values determined by some HCVcAg quantification kits (23–25). Although the core region sequences of the HCV isolates from these 4 samples were not examined, we infer that amino acid polymorphisms in the HCV core region may have caused the divergence of the obtained values.

Additionally, we found 2 discrepancies; sample 5 was positive for HCV genotype 2, suggesting that the LP-Presto negative result was a false-negative. Sample 6 was negative for HCVcAg by both LP-assays and was negative for HCV-RNA, suggesting that the positive result obtained by Architect was a false-positive. Regarding sample 5, we believe that the discrepant results between LP-Presto and LP-Ortho occurred because the HCVcAg antibodies used in the LP-Ortho and LP-Presto assays are different. It may be possible that HCVcAg polymorphisms caused the different results obtained by the LP-Ortho and LP-Presto assays, although the sample was not examined this time. Furthermore, since LP-Presto is fully automated, its method of sample treatment and reagent compositions are different from those of LP-Ortho, which may also explain the discrepant result obtained for sample 5.

Although HCV RNA assay remains as the gold standard for diagnosing HCV infection, the HCVcAg assay has been recommended (A1 level) as an alternative to PCR when PCR is not available or is too expensive (26). HCVcAg assays are also recommended

as a method for identifying patients with active HCV infection among individuals positive for the anti-HCV antibody. Recently, the WHO has also endorsed this approach, and has updated the diagnostic principles for both HBV and HCV infections (27). For patients receiving treatment for HCV eradication, the schedule for performing HCVcAg assays will be the same as that used for HCV RNA assays; consequently, the assay is performed at baseline, between weeks 2 and 4 (optional), at the end of treatment, and at post-treatment follow-ups (26). HCVcAg assays for patients treated for CHC have already been described, and were used either for patients treated with IFN-based regimens (20,28,29) or with DAAs (11,19,30).

A recent report indicated that HCVcAg quantification by Architect from a dried blood spot (DBS) is an attractive alternative to conventional HCV serology in settings with restricted resources, particularly for difficult-to-reach populations (31). HCVcAg assays performed on DBS samples indicated that the HCVcAg on DBSs was stable, and showed 76.1% sensitivity and 97.3% specificity. The performance of the HCVcAg assay on DBS samples was not affected by HIV co-infection or varied HCV genotypes. Although the HCVcAg assay has reduced sensitivity on DBS samples due to its high specificity, it can be used in resource-restricted locations (31). Another study evaluated HCVcAg detection in plasma and DBS samples. Paired plasma and DBS samples were prepared from the remnants of samples used for diagnosis, and plasma HCV RNA levels and HCVcAg levels were quantified. When diagnosing plasma samples with HCV RNA levels $\geq 3,000$ IU/mL, the sensitivity and specificity were found to be 97.7% and 100%, respectively. The sensitivity and specificity of HCVcAg detection from DBS samples were found to be 88.6% and 97%, respectively (32). Although these results demonstrate that the level of HCVcAg quantified in the plasma was higher than that in DBS samples, the specificity of the HCVcAg assay for DBS samples is sufficient for qualitative analysis.

Our study has several limitations. Firstly, our study was only performed on patients with HCV genotypes 1 or 2; therefore the HCVcAg assay must be assessed in patients infected with other genotypes and in those coinfecting with other viruses such as HIV or HBV. A previous study found that the differences between the lower limits of HCVcAg detection by Architect in samples positive for HCV RNA from genotypes 1–4 were not significant (13). Secondly, all of our study participants were positive for the anti-HCV antibody.

In conclusion, the new LP-Presto assay for HCVcAg is a reliable, easy-to-use, cost- and time-saving method for diagnosing HCV infection and monitoring patients during HCV eradication therapy. Because the number of nonspecific results caused by hyperimmunoglobulinemia was reduced by LP-Presto, the method shows potential as a high-quality HCVcAg assay for various applications. Further studies are required to determine the roles of LP-Presto for HCVcAg in centralized testing facilities for various sample types, including that of DBS from isolated locations.

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Conflict of interest Yasuhito Tanaka is currently conducting research sponsored by Chugai Pharmaceutical Co., Ltd., Bristol-Myers Squibb, Janssen Pharmaceutical K.K., AbbVie Inc. MSD K.K., and Gilead Sciences. The other authors declare no conflicts of interest. The LP-Presto assay was provided by Fujirebio, Inc in relation to joint research.

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