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Clinical evaluation of a newly developed chemiluminescent enzyme immunoassay in Japan for hepatitis C virus core antigen

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Running title: A newly developed CLEIA for HCV core antigen.

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Summary

An advanced and fully automated chemiluminescent enzyme immunoassay for 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 negative by LP-Ortho. Correlation analysis was performed. Concordance rates between each assay were determined. Additionally, 42 frozen sera of patients with hyperimmunoglobulinemia, including 3 unmeasurable by LP-Ortho, were tested by LP-Presto.

The basic assessments of LP-Presto were all consistent with LP-Ortho and Architect. Concordance rates between LP-Presto and LP-Ortho for the 220 frozen sera were 98.8% (219/220), and between LP-Presto and Architect were 97.6% (218/220). LP-Presto (HCVcAg cut-off value; 20 fmol/L) was 100% consistent with LP-Ortho, which found a total of 343 sera negative for HCVcAg. All 42 hyperimmunoglobulinemic sera were measurable by LP-Presto.

In conclusion, LP-Presto shows rapid, reliable performance. Nonspecific testing results due to hyperimmunoglobulinemia were reduced by LP-Presto. LP-Presto is a high-quality HCVcAg assay that shows promise for various 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 several years, the management of chronic hepatitis C (CHC) has improved dramatically because of the use of direct acting antivirals (DAAs) for treatment, and the time to HCV eradication has been reduced (3, 4). The rate of HCV eradication by DAA treatment is greater than 95% (5, 6). While DAAs provide an extraordinary opportunity for successful HCV eradication (7), feasible diagnostic and treatment monitoring methods are essential for facilitating global elimination of HCV by 2030 (8). That is, cost-effective and easy-to-use methods for detecting HCV are needed for achieving HCV eradication (7, 9).

HCV infection is indicated by the presence of HCV RNA in serum. Because of its high sensitivity, specificity, and reproducibility, an HCV RNA polymerase chain reaction (PCR) assay has been used in clinical practice (10). However, under some circumstances, an HCV RNA assay is not useful because it is costly, and the results are not rapidly available. In contrast, measurement of the HCV core antigen (HCVcAg) provides a better option because it is less costly and the results are available sooner (11). In addition, the reagents are stable under various environmental conditions (12). HCVcAg has been reported to be a surrogate marker for HCV replication, and the assays show a sensitivity similar to that of the HCV RNA assay (13) and detect various HCV genotypes (14). Along with being a marker that diagnoses HCV infection, HCVcAg can also be used to evaluate response to antiviral therapy (11), even in developing countries.

Several available commercial HCVcAg assays have demonstrated high sensitivity and specificity for the diagnosis of active HCV infection (15). We have pointed out, however, that the conventional HCVcAg assays have produced nonspecific reactions (false-positive results), especially for patients with hyperimmunoglobulinemia, which is
reported in the package inserts of the assays. Furthermore, the assays are ineffective if thermocoagulation occurs during the pretreatment of specimens.

An advanced and fully automated chemiluminescent enzyme immunoassay (CLEIA) for HCVcAg was recently developed in Japan. In this study, we aimed to compare the diagnostic utility of the new HCVcAg assay with that of conventional assays, and to assess its clinical efficacy. This report shows the broad applications of this HCVcAg assay, with its new simplified approach for the screening, diagnosis, and monitoring of patients with HCV infection in various environments, including developing countries.

We consider the new HCVcAg assay to have potential as a major tool for future diagnostics.

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 anti-HCV antibody and satisfied the following enrollment criteria of the study: 1) the diagnosis of CHC was based on serum positivity for HCV RNA at least one time, as described in the next paragraph; 2) the patients had or had not received IFN-based therapy and/or DAA treatment; 3) the patients did or did not achieve a sustained virological response. The patients were enrolled at Nagoya City University Hospital from November 2012 to August 2015.

Anti-HCV serological test, HCV RNA assessment, and HCV genotype
Serum anti-HCV antibody were performed by a fully automated CLEIA (Lumipulse Presto Ortho HCV; Ortho Clinical Diagnostics K.K., Tokyo, Japan). Those samples showing a cut-off index (C.O.I.) > 1.0 were considered positive for anti-HCV antibody.

Serum HCV RNA levels were measured by 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 $1.0 \times 10^8$ 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. LP-Presto was provided by Fujirebio, Inc., and LP-Ortho and Architect were used routinely by the hospital laboratory. The detection limits ranged from 20 to 50,000 fmol/L for LP-Presto, from 50 to 50,000 fmol/L for LP-Ortho, and from 3 to 20,000 fmol/L for Architect. 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 each patient’s gender and age, were collected. A total of 220 frozen sera (83 positive and 137 negative for HCVcAg by LP-Ortho), and a total of 206 fresh sera obtained on the day the assay was performed and were negative by LP-Ortho, were studied. Of 83 frozen sera positive for HCVcAg by LP-Ortho, 65 were obtained from CHC patients with HCV genotype 1, and 18 were 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 of LP-Presto to obtain data on reproducibility, sample stability, range of quantitation, and specificity. The reproducibility assessment consisted of 6 replicate measurements of each of 3 sera positive for HCV genotype 1. The mean value and standard deviation (SD) and the coefficient of variation (CV) were calculated for each of the samples. The sample stability assessment consisted of storing 7 clinical samples positive for HCV genotype 1 at 10°C for 0, 1, 4, 8, and 15 days and measuring the samples at each time point. The percent differences between pre- and poststorage values were calculated (prestorage values/poststorage values [%]). The range of quantitation consisted of measuring 7 samples positive for HCV genotype 1 with low HCVCag concentrations 20 times. The approximate curve was constructed based on the relationship between the mean value and CV (%). By the approximate curve, the concentration with CV20% was defined as a 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 concordance rates were determined between LP-Presto, LP-Ortho, and Architect.

The effects of hyperimmunoglobulinemia on the results of the HCVCag assay

A total of 42 frozen sera from patients with hyperimmunoglobulinemia (immunoglobulin [Ig]A ≥ 1,000 mg/dL, n = 5; IgM ≥ 500 mg/dL, n = 11; and IgG ≥ 3,000 mg/dL, n = 26) that were negative for HCVCag by LP-Ortho were measured by LP-Presto. The levels of IgA, IgM, and IgG had been measured in the sera at the time the study began.
Results

Reproducibility, Sample stability, and range of quantitation of LP-Presto for HCVcAg

Reproducibility testing demonstrated the following mean values ± SD of 3 positive samples with low, medium, and high concentrations of HCVcAg: 207.7 ± 8.0 fmol/L, 1,951.5 ± 53.0 fmol/L, and 39,348.7 ± 1,034.5 fmol/L, respectively. The CVs were 3.8%, 2.7%, and 2.6%, respectively. The details are shown in Table 2a.

Sample stability testing found that the mean percent differences between pre- and poststorage values of 7 samples ranged from 85% to 109%. The details are shown in Table 2b.

The range of quantitation assessment shows the following respective mean values with 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 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 all negative (< 20 fmol/L) by LP-Presto, with a cut-off value of 20 fmol/L. Meanwhile, there was only 1 discrepancy between these assays below the cut-off value of 10 fmol/L by LP-Presto. The discrepancy was from a patient with multiple myeloma, and was measured by LP-Presto to have an HCVcAg level of 15.8 fmol/L. 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 negative results on LP-Presto was found to be better than LP-Ortho, which was 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 the LP-Presto to LP-Ortho assay or to the Architect assay were ≥ 2 or ≤ 0.5, respectively) were found (No.’s 1-4). The results of No.’s 1, 2, and 3 are shown in the graphs. Because the HCVcAg concentration in No. 4 was very low, the result could not be shown on the graph. The details are summarized in Table 3.

Concordance rates between LP-Presto, LP-Ortho, and Architect for HCVcAg

Table 4 shows the details on the concordance between LP-Presto, LP-Ortho, and Architect. Overall, the rates of concordance between LP-Presto and LP-Ortho and between LP-Presto and Architect were 99.5% (219/220) and 99.1% (218/220), respectively.

We had 2 discrepancies; No. 5 was negative for LP-Presto and positive for both LP-Ortho and Architect, and No. 6 was negative for both LP-Presto and LP-Ortho and positive for Architect (Table 4). No. 5 was positive for HCV genotype 2, indicating patient No. 5 had viremia (Table 4c). No. 6 was negative for HCV-RNA, indicating patient No. 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 measured: 41 specimens were negative and 1 specimen (IgG 7,125 mg/dL) was false-positive by LP-Presto. Two and 3 specimens could not be measured by Architect and LP-Ortho, respectively. The details are shown in Table 5.
Discussion

Although the HCV eradication rate by DAAs is probably 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 HCV elimination, aiming for a 90% reduction in prevalence and 65% reduction in HCV-related mortality by 2030 (16). HCV RNA assays have been used in clinical practice because of their high specificity, sensitivity, and reproducibility, although their costs restrict their use in various locations (10). Therefore, reliable, easy-to-use, and inexpensive assays are needed to detect HCV and confirm the success or failure of HCV eradication. In this study, we introduced a newly developed, fully automated HCVcAg assay that should fulfill the desired criteria and will be available soon in Japan. The findings of this study suggest that an automated HCVcAg assay is an appropriate tool for screening and diagnosing HCV infections in large numbers of CHC patients, particularly in a population estimated to have a high prevalence of HCV infection, i.e., residents of developing countries.

As previously described, HCVcAg assays used instead of RNA assays have shown complete clinical equivalence with the latter and substantial savings for the diagnosis of acute hepatitis C (17) or 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 hour instead of the up to 7 hours needed for HCV RNA assays (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. Generally, the cost of an HCV RNA assay is several-fold that of an HCVcAg assay (8, 21); therefore, an HCVcAg assay instead of an HCV RNA assay might lead to decreases in the cost of diagnosis and time to result, 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 an HCV RNA assay remains the gold standard for the diagnosis of an active HCV infection, but an HCVcAg assay can be used when PCR is not available (22).

This study found 4 samples that 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 HCVcAg values determined by some HCVcAg quantification kits (23, 24, 25). Although the sequences of the core regions of HCV isolates from these 4 samples were not examined, we infer that amino acid polymorphisms in the HCV core region might have led to divergence in the values obtained by the assays.

Additionally, we found 2 discrepancies; No. 5 was positive for HCV genotype 2, suggesting that the negative result by LP-Presto was false-negative. No. 6 was negative for HCVcAg by both LP-assays and negative for HCV-RNA, suggesting that the positive result by Architect was false-positive. Focusing on No. 5, we think that the discrepant results between LP-Presto and LP-Ortho occurred because the antibodies to HCVcAg used in the LP-Ortho and LP-Presto assays are different. Regarding factors that cause the different results by the LP-Ortho and LP-Presto assays, it may be possible to be polymorphisms of HCVcAg, although the sample was not examined this time. Furthermore, because LP-Presto is fully automated, its method of sample treatment and the compositions of the reagents are different from those of LP-Ortho, which might also account for the discrepant result for No. 5.

Although the gold standard for diagnosing HCV infection remains the HCV RNA assay, an HCVcAg assay has been recommended (A1 level) as an alternative to PCR when PCR is not available or is too expensive (26). An HCVcAg assay is also recommended by guidelines as a method for identifying patients with active HCV infection among individuals positive for anti-HCV antibody. Recently, WHO has also promoted the same approach that updates 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 schedule used for HCV RNA assays; namely, 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; the assays 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, and particularly for difficult-to-reach populations (31). Assays for HCVcAg from DBS’s indicated that the HCVcAg on DBS’s was stable. The results showed 76.1% sensitivity and 97.3% specificity. The performance of an HCVcAg assay of samples from DBS’s was not affected by HIV co-infection or varied HCV genotypes. Although an HCVcAg assay of samples from DBS’s has reduced sensitivity, because of its specificity, it can be used in resource-restricted locations (31). Another study evaluated HCVcAg detection in plasma samples and samples from DBS’s. Paired samples of plasma and DBS were prepared from remnant samples used for diagnosis, and plasma HCV RNA levels and HCVcAg levels were quantified. For diagnosing plasma samples with HCV RNA levels ≥ 3000 IU/mL, the sensitivity and specificity for detecting HCVcAg in plasma were 97.7% and 100%, respectively. The sensitivity and specificity of HCVcAg detection from DBS’s were 88.6% and 97%, respectively (32). Although these results demonstrate that the level of HCVcAg quantified in plasma was higher than that in DBS’s, the specificity of an HCVcAg assay for antigen on DBS’s is sufficient for qualitative analysis.

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

In conclusion, the new LP-Presto assay for HCVcAg is a reliable, easy-to-use, and reasonable cost- and time-saving method for diagnosing HCV infection and monitoring patients during HCV eradication therapy. Because the nonspecific results caused by hyperimmunoglobulinemia were reduced by LP-Presto, it shows potential as a high-quality HCVcAg assay for various applications. The roles of LP-Presto for HCVcAg used in centralized testing facilities for various types of samples, including DBS’s from isolated locations, need further study.

Acknowledgments
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Conflicts 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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Figure legends

Fig. 1. 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.

Abbreviations: HCVcAg, Hepatitis C virus core antigen; LP-Presto, Lumipulse Presto HCVcAg; LP-Ortho, Lumipulse Ortho HCVcAg; Architect, Architect HCVcAg assay.
### Table 1. The features of each HCVcAg assay

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

Abbreviations: 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.
### Table 2. Reproducibility testing of the LP-Presto (a), and sample stability testing of the LP-Presto (b)

#### (a)

<table>
<thead>
<tr>
<th>Measurement times</th>
<th>Serum L (fmol/L)</th>
<th>Serum M (fmol/L)</th>
<th>Serum H (fmol/L)</th>
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<tr>
<td>1</td>
<td>223.1</td>
<td>1943.8</td>
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<td>2</td>
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<td>3</td>
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<td>2039.3</td>
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<td>4</td>
<td>206.5</td>
<td>1882.5</td>
<td>38696.9</td>
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<td>5</td>
<td>202.6</td>
<td>1971.4</td>
<td>39455.9</td>
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<tr>
<td>6</td>
<td>207.7</td>
<td>1917.8</td>
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<tr>
<td><strong>Mean</strong></td>
<td><strong>207.7</strong></td>
<td><strong>1951.5</strong></td>
<td><strong>39348.7</strong></td>
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<tr>
<td><strong>SD (fmol/L)</strong></td>
<td><strong>8.0</strong></td>
<td><strong>53.0</strong></td>
<td><strong>1034.5</strong></td>
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<tr>
<td><strong>CV</strong></td>
<td><strong>3.8%</strong></td>
<td><strong>2.7%</strong></td>
<td><strong>2.6%</strong></td>
</tr>
</tbody>
</table>

Abbreviations: 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.
### Parameters measured

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>0 day</th>
<th>1 day</th>
<th>4 days</th>
<th>8 days</th>
<th>15 days</th>
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</table>

### Abbreviations: HCVcAg, Hepatitis C virus core antigen; LP-Presto, Lumipulse Presto HCVcAg assay.
**Table 3.** Divergent samples between LP-Presto, LP-Ortho, and Architect

<table>
<thead>
<tr>
<th>Divergent samples</th>
<th>HCVcAg values (fmol/L)</th>
<th>Age</th>
<th>Gender</th>
<th>HCV genotype</th>
<th>Clinical stage</th>
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<tbody>
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<td>M</td>
<td>1</td>
<td>Chronic hepatitis</td>
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<tr>
<td>No.3</td>
<td></td>
<td>37</td>
<td>M</td>
<td>2</td>
<td>Chronic hepatitis</td>
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<tr>
<td>No.4</td>
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<td>82</td>
<td>F</td>
<td>2</td>
<td>Chronic hepatitis</td>
</tr>
</tbody>
</table>

Abbreviations: 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)  

<table>
<thead>
<tr>
<th>LP-Presto</th>
<th>LP-Ortho</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ (≥20 fmol/L)</td>
<td>82</td>
<td>0</td>
</tr>
<tr>
<td>-</td>
<td>1 (Case No. 5)</td>
<td>137</td>
</tr>
<tr>
<td>Total</td>
<td>83</td>
<td>137</td>
</tr>
</tbody>
</table>

(b)  

<table>
<thead>
<tr>
<th>LP-Presto</th>
<th>Architect</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ (≥20 fmol/L)</td>
<td>82</td>
<td>0</td>
</tr>
<tr>
<td>-</td>
<td>2 (Case Nos. 5 and 6)</td>
<td>136</td>
</tr>
<tr>
<td>Total</td>
<td>84</td>
<td>136</td>
</tr>
<tr>
<td>Case No.</td>
<td>HCVcAg values (fmol/L)</td>
<td>Age</td>
</tr>
<tr>
<td>---------</td>
<td>------------------------</td>
<td>-----</td>
</tr>
<tr>
<td></td>
<td>LP-Presto</td>
<td>LP-Ortho</td>
</tr>
<tr>
<td>No.5</td>
<td>13.6</td>
<td>53.0</td>
</tr>
<tr>
<td></td>
<td>Chronic hepatitis</td>
<td></td>
</tr>
<tr>
<td>No.6</td>
<td>0.1</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>Not available</td>
<td>Leukemia (After treatment)</td>
</tr>
</tbody>
</table>

Abbreviations: HCVcAg, Hepatitis C virus core antigen; LP-Presto, Lumipulse Presto HCVcAg; LP-Ortho, Lumipulse Ortho HCVcAg; Architect, Architect HCVcAg assay; F, Female; M, Male.
Table 5. Results of HCVcAg assays of samples from patients with hyperimmunoglobulinemia

<table>
<thead>
<tr>
<th>Immunoglobulin</th>
<th>Immunoglobulin level (mg/dL)</th>
<th>Number of cases</th>
<th>LP-Presto</th>
<th>LP-Ortho</th>
<th>Architect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>False positive</td>
<td>Unmeasurable</td>
<td>False positive</td>
</tr>
<tr>
<td>IgA</td>
<td>1,000〜2,000</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2,000〜3,000</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>3,000〜4,000</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IgM</td>
<td>500〜2,000</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2,000〜4,000</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>4,000〜6,000</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IgG</td>
<td>3,000〜5,000</td>
<td>23</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5,000〜7,000</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>7,000〜9,000</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations: 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.
Fig. 1

(a) LP-Presto HCVcAg (fmol/L) vs. LP-Ortho HCVcAg (fmol/L)

(b) LP-Presto HCVcAg (fmol/L) vs. Architect HCVcAg (fmol/L)

No. 1
No. 2
No. 3