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Hepatitis C virus core antigen: Analytical performances, correlation with viremia and potential applications of a quantitative, automated immunoassay

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ABSTRACT

Background: Testing for hepatitis C virus core antigen (HCV Ag) may represent a complementary tool to anti-HCV and HCV-RNA in the diagnosis and monitoring of HCV infection.
Objective: To evaluate the performance characteristics of the automated Abbott ARCHITECT HCV Ag assay.
Study design: Five sites analyzed over 3000 routine serum samples from populations at different risk, comparing HCV Ag results with anti-HCV screening and supplemental assay results and with HCV-RNA.
Results: The HCV Ag assay showed a specificity of 100%, a good precision (CV < 10%) and excellent dilution linearity ($r > 0.999$). The sensitivity (3 fmol/L) corresponds to 700–1100 IU/mL of HCV-RNA. A non-linear correlation with HCV-RNA was found: $r = 0.713$ vs. Siemens bDNA (523 specimens), $r = 0.736$ vs. Roche Cobas TaqMan (356 specimens) and $r = 0.870$ vs. Abbott Real-Time PCR (273 specimens). HCV Ag quantitation was equally effective on different HCV genoypes (239 for genotype 1/1a/1b/1c, 108 for genotype 2/2a/2c, 86 for genotype 3/3a, 50 for genotype 4/4a/4c/4d). Testing of subjects at high risk for HCV and with potential or actual impairment of the immune system identified 2 cases negative for anti-HCV and positive for HCV Ag on 361 hemodialyzed (0.6%) and 7 cases on 97 (7.2%) among transplant recipients. HCV Ag positivity anticipated anti-HCV seroconversion in all three cases of acute hepatitis C.
Conclusions: HCV Ag may be used as reflex testing on anti-HCV positive individuals to confirm or exclude an active infection, and on subjects with acute hepatitis or belonging to high risk groups.

1. Background

Testing for viral antigens is established in clinical virology and has been successfully applied for the diagnosis of hepatitis B virus infection since almost 40 years. In hepatits C virus (HCV) infection, this strategy has been hampered by difficulties in expressing surface antigens and to produce monoclonal or polyclonal antibodies able to detect these antigens.[1] The attention was then focused on the core gene products which have been demonstrated to circulate in the bloodstream of infected individuals.[2] A first assay for HCV core antigen (HCV Ag) was developed in 1999[3] and several others followed in the next few years.[4–8] These assays showed a reasonable correlation with HCV RNA[4,7,8] and their use for the diagnosis of acute hepatitis C as well as to evaluate disease progression and/or response to treatment in chronic infections was prospected.[7–12] However, the analytical sensitivity was still significantly lower than that of HCV RNA assays, which became increasingly sensitive over time.[13–16] Furthermore, as the detection of HCV Ag during all phases of infection requires fractionation of HCV particles[3] and dissolution of immune complexes, the sample pre-tretment steps reduced the assay throughput, hampering the adoption of HCV Ag testing in routine settings.

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2. Objective

A new quantitative fully automated assay for HCV Ag on the ARCHITECT platform (Abbott Diagnostics, Wiesbaden, Germany) has become recently available. Our purpose was to evaluate the performance characteristics of this assay in routine laboratory practice and to prospect its use for valuable clinical applications.

3. Study design

Five clinical microbiology or virology laboratories located in Italy (University Hospitals of Ancona, Bologna, Brescia and Parma) and Spain (Hospital 12 de Octubre, Madrid) carried out this study according to local policies for *in vitro* studies, employing only anonymous surplus specimens.

The ARCHITECT HCV core Ag assay was used according to the manufacturer's indications.[6] The main features of this assay are the automated on-board pretreatment step, which releases the antigen both from viral particles and from immune complexes, an enhanced sensitivity with a detection limit at 3 femtomoles per liter (fmol/L), or 0.06 picograms per milliter (pg/mL) of recombinant c11 Ag (residues 1–160) of the HCV genotype 2a isolate (GenBank accession no. I49748),[6] and a dynamic range extended to 180,000 fmol/L with an automated 1:9 dilution.

Third-generation automated assays for anti-HCV detection on the Abbott ARCHITECT and J&J Ortho (Raritan, NJ) Vitros Eci platforms were employed in three and two sites, respectively. The Ortho-Chiron RIBA-3 has been used as a supplemental assay in all sites. The concordance between anti-HCV and HCV Ag was evaluated by testing for HCV Ag 420 anti-HCV negative and 1196 anti-HCV reactive samples; of the latter, 640 were also been tested by RIBA-3.

Three commercial target or signal nucleic acid amplification methods have been used for quantitative HCV-RNA detection. Two sites used the Cobas TaqMan assay (CTM) with automated extraction (CAP/CTM) or manual extraction (HP/CTM) (Roche Diagnostics GmbH, Mannheim,Germany); one site used the HCV RealTi*m*e (Abbott Laboratories. Abbott Park, IL); two sites employed the Versant HCV RNA assay 3.0 (bDNA; Siemens Healthcare Diagnostics, Deerfield, IL) (6, 14, 23, 28). The limit of quantification is 15 and 12 international units per milliliter (IU/mL) for the Roche and Abbott real-time assays and 615 IU/mL for bDNA assay. HCV genotypes were determined by the Versant HCV genotype LiPA assay or by sequencing of the 5′ non-coding region. The concordance and correlation between HCV Ag and HCV-RNA were checked on 1480 samples (617 in comparison with bDNA, 537 and 326 in comparison with Roche and Abbott PCRs).

The clinical sensitivity of the HCV Ag assay was assessed on sequential samples from three cases of acute hepatitis C observed at two sites during the study period. Additionally, samples from 361 adults treated by hemodialysis for chronic renal insufficiency and from 97 solid organ or bone marrow transplant recipients were also tested.

3.1. Data analysis

The *r* correlation coefficient was calculated by Pearson's least squares method. The difference between positivity rates was evaluated by chi-square and chi-square for linear trend.

4. Results

4.1. Analytical evaluation

The ARCHITECT HCV Ag intra-assay reproducibility was evaluated on a sample with high levels of HCV Ag (undiluted, 1:10 and 1:100); the coefficients of variation (CVs) were 3.33%, 3.84% and 2.88%, respectively. The total imprecision was checked at three sites on 45 replicates of the low and high positive controls over six months with two different lots of reagents: total CVs were 8.58% and 9.14%, respectively.

None of the 420 anti-HCV negative specimens was positive for HCV Ag (specificity 100%, 95% confidence limits 99.4–100%); the mean and median values were 0.12 and 0.00 fmol/L respectively. Additionally, all samples from 22 patients with acute non-C viral hepatitis (15 hepatitis A, 6 hepatitis B, 1 hepatitis delta) were negative for HCV Ag; mean and median values were 0.44 and 0.00 fmol/L, respectively.

Assay linearity was verified on five samples with HCV Ag >10,000 fmol/L, serially diluted and tested in duplicate. On all samples the linearity was maintained through the entire dynamic range and the *r* coefficient between expected and observed values ranged between 0.9997 and 0.9998.

Table 1
Positivity rates for HCV Ag according to anti-HCV antibody levels, expressed as sample/cutoff (S/CO) values, in 596 subjects positive for anti-HCV by Ortho Vitros Eci and in 600 subjects positive for anti-HCV by Abbott ARCHITECT. In the disaggregation used for S/CO ranges, samples with a S/CO from 1 to 1.99 are low-level reactives by both antibosy assays, while the other two groups are not directly comparable.

Assay	S/CO range	N. samples	HCVAg+	% HCV Ag+
Vitros	1.00–1.99	140	1	0.7%
	2.00–7.99	271	9	3.3%
	≥8	185	110	59.5%
ARCHITECT	1.00–1.99	170	1	0.6%
	2.00–9.99	217	64	29.5%
	≥10	213	178	83.6%

4.2. Concordance with anti-HCV and clinical evaluation in patients at risk

Of 596 sera reactive by the Vitros Eci assay, 120 (20.1%) were positive for HCV Ag, whereas of 600 anti-HCV reactives by ARCHITECT anti-HCV 243 were HCV Ag positives (40.5%). Relating the positivity rates to the sample/cutoff ratio (S/CO) of the anti-HCV assays, HCV Ag was detected in 0.7% of specimens with a S/CO between 1.00 and 1.99 and positivity increased to 59.5% and 83.6% of specimens with high S/CO values (≥8 by Vitros and ≥10 by ARCHITECT) as defined according to the CDC recommendations[17] for performing anti-HCV supplemental testing (Table 1). Similar results were obtained comparing HCV Ag with RIBA-3: a positivity was recorded in 1/122 negative samples (0.8%), in 17/353 indeterminates (4.8%; all reactive for anti-core or anti-NS3) and in 99/165 positives (60.0%; chi square for linear trend: $p < 0.01$).

In all three acute hepatitis C cases the first available sample was positive for HCV Ag and HCV-RNA, while anti-HCV appeared 2 to 4 weeks later. A sharp, parallel decrease of HCV Ag and HCV-RNA levels was observed in one subject treated with antivirals 3 months after onset (Fig. 1).

Of the 361 hemodialyzed patients, 277 (76.8%) were anti-HCV negative, and two of them (0.6%) were positive for HCV Ag, while of the 84 subjects positive for anti-HCV, 62 were positive and 22 negative for HCV Ag (Fig. 2). Of the 44 patients tested also for HCV-RNA the concordance with HCV Ag was 100% (34 negatives and 10 positives). Also among transplant recipients the majority of patients (82/97, or 84.5%) were negative for anti-HCV, but 7 of them (7.2%) were positive for HCV Ag and HCV-RNA was negative in two (Fig. 2). The concordance between HCV RNA and HCV Ag was 98% (76 negatives and 19 positives). The frequency of active HCV infection was higher than among hemodyalized, as 14 of the 15 anti-HCV positives were positive for HCV Ag.

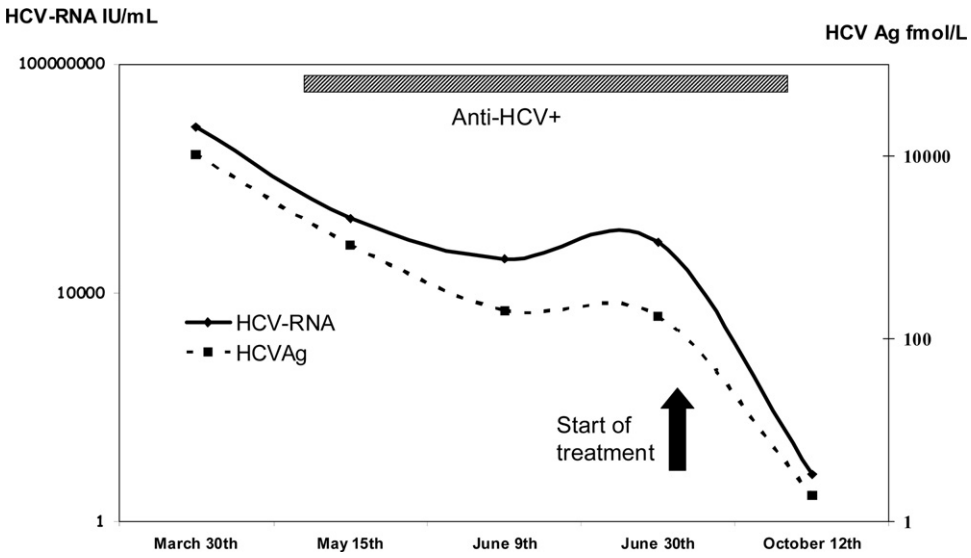

Fig. 1. Dynamics of HCV-RNA, HCV Ag and anti-HCV in one case of acute HCV infection. Anti-HCV was detectable 45 days after the positivity for RNA and Ag. Both HCV-RNA and HCV Ag show a sharp decrease following the administration of antiviral therapy, that was started 13 weeks after onset.

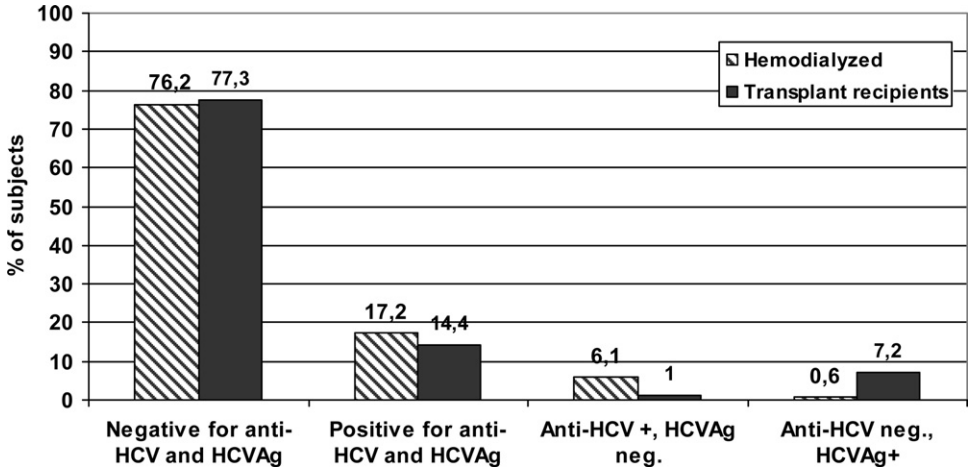

Fig. 2. Serological markers of HCV infection among 361 hemodialyzed patients and 97 transplant recipients. The positivity rates for anti-HCV + HCV Ag between the two groups were not significantly different, whereas the differences among cases positive only for anti-HCV or only for HCV Ag were significant ($p < 0.05$).

4.3. Comparison with HCV-RNA

(a) bDNA; in 82 of the 617 specimens HCV-RNA was <615 IU/mL, and 16 of them (19.5%) were positive for HCV Ag with levels from 3 to 16 fmol/L. Of the 535 HCV-RNA positive specimens, 523 (97.8%) were HCV Ag positive for a total concordance between the two assays of 95.5%; (b) Roche TaqMan; in 98 of the 537 HCV-RNA was <15 IU/mL, and 4 of them (4.1%) were positive for HCV Ag with levels from 4.8 to 41 fmol/L. Of the 439 HCV-RNA positives, 356 (81.1%) were HCV Ag positive for a total concordance between the two assays of 67.0%; (c) Abbott Real-Time PCR; in 32 of the 326 specimens HCV-RNA was <12 IU/mL, and 7 of them (21.9%) were positive for HCV Ag with levels from 6.7 to 140 fmol/L. Of the 294 HCV-RNA positives, 273 (92.9%) were HCV Ag positive, for a total concordance between the two assays of 85.9%. The positivity rates for HCV Ag according to HCV-RNA levels are reported in Table 2.

By linear regression analysis of 15 samples with HCV-RNA values between 500 and 2500 IU/mL, the detection threshold of HCV Ag corresponded to 700–1100 IU/mL of HCV-RNA. We then considered as true discordants between HCV-RNA and HCV Ag the eleven specimens with HCV-RNA below the detection limit of the two real-time PCRs that were reactive for HCV Ag and the ten specimens with HCV-RNA >10,000 IU/mL that were negative for HCV Ag. The *r* coefficient of HCV Ag was 0.713 vs. bDNA assay (Fig. 3A), 0.743 vs. Roche TaqMan (Fig. 3B) and 0.870 vs. Abbott Real-Time (Fig. 3C), and the relationship was non-linear vs. all three quantitative HCV-RNA assays. Genotype-independent efficiency of the HCV Ag assay was determined on 483 specimens

Table 2
HCV Ag positivity rates according to increasing levels of HCV-RNA as determined by one signal amplification method (Siemens bDNA) and two target amplification methods (Roche and Abbott Real-Time PCRs). HCV-RNA values are expressed in international units per milliliter (IU/mL) on a logarithmic (log) scale. The first group includes 212 samples with no detectable HCV-RNA.

HCV RNA log IU/mL	N. samples	N. HCVAg+	% HCV Ag+	Median HCV Ag (fmol/L)
<3	319	63	19.7%	7
3–3.99	193	158	81.9%	16
4–4.99	276	268	97.1%	121
5–5.99	371	370	99.7%	1392
≥6	321	320	99.7%	7343
Total	1480	1179	79.7%	805

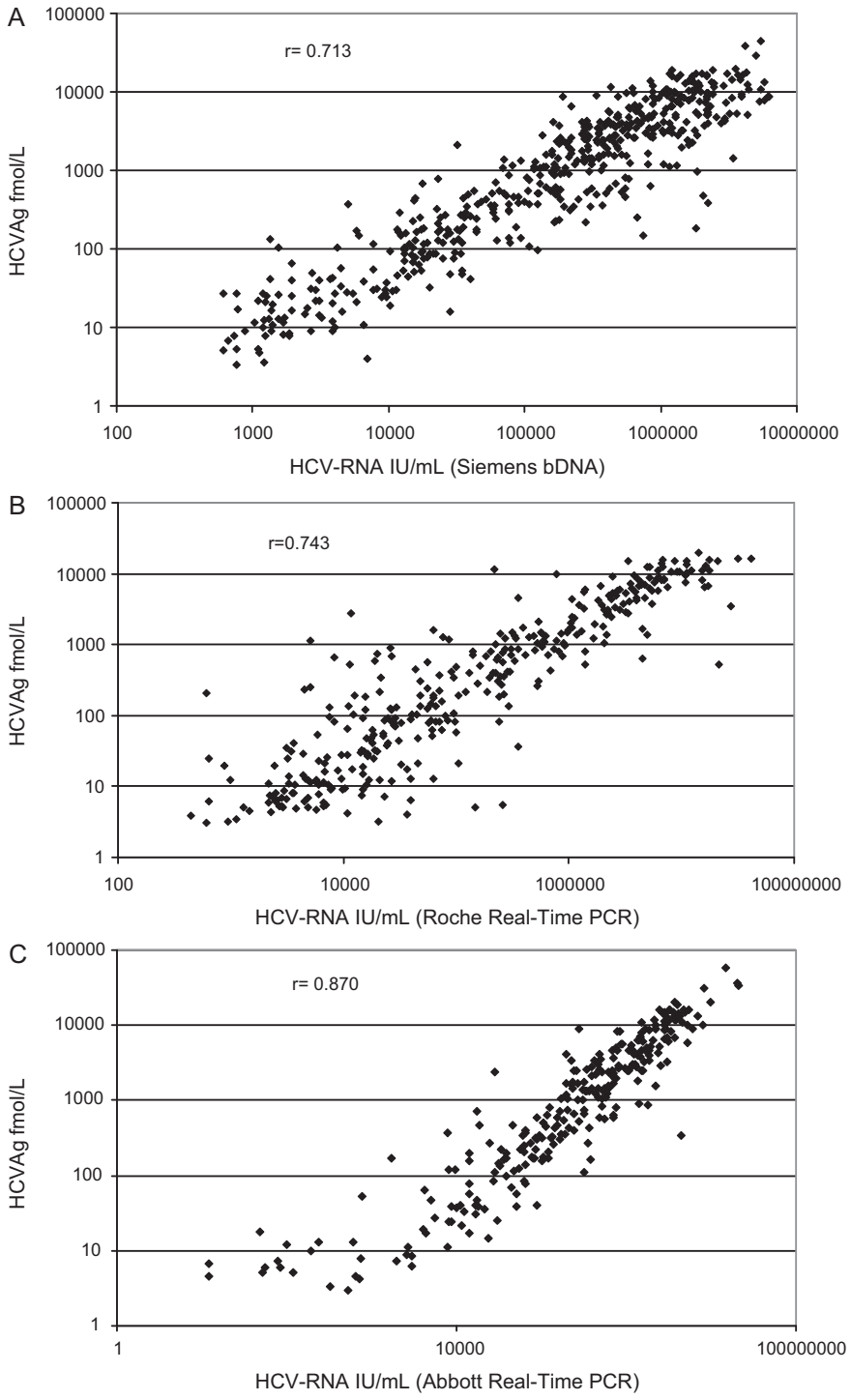

Fig. 3. Correlation between HCV Ag and HCV-RNA detected by three different nucleic acid amplification methods. (A) Siemens branched DNA (523 samples); (B) Roche TaqMan (356 samples); (C) Abbott Real-T*ime* PCR (273 samples). The correlation coefficients by least-squares method (*r*) indicate a general agreement between serological and virological results, though the relationship is non-linear.

infected by the 4 major HCV genotypes (239 by 1/1a/1b/1c, 108 by 2/2a/2c, 86 by 3/3a, and 50 by 4/4a/4c/4d). The overall positivity rate for HCV Ag was 95.7% and the median HCV-RNA value on HCV Ag negative samples was 944 IU/mL. The efficiency on genotype 3 samples was lower (91.9%) probably because of the lower viremia levels (median: 69,000 IU/mL vs. 170,000 for the other genotypes).

5. Discussion

The persistence of HCV after an acute, often undiagnosed, infection does not lead necessarily to a lifelong infection that is still a major cause of chronic hepatitis, cirrhosis and mortality. The spontaneous clearance of HCV in 17% of untreated subjects found positive for anti-HCV and HCV RNA in an open population study

has ben reported[18] and cross-sectional surveys on HCV infection indicate that only a proportion of anti-HCV positive subjects show signs of viral replication.[18–20] This is relevant also in high-risk populations, since it has been shown that a previous humoral response towards HCV, albeit not protective against reinfections, may lead to a shorter course of active infection with clearance of RNA within 150 days in the majority of reinfected subjects.[21] Determining the presence of an active HCV infection is clinically relevant, and in this respect the automated assay for HCV Ag evaluated in this study is more suitable than HCV-RNA methods, that are more expensive and require a dedicated sample and skilled personnel. The results obtained on anti-HCV positives indicate that almost 50% are negative for HCV antigen, thus excluding the presence of active HCV replication.[20] A potential bias is that HCV Ag is less sensitive than HCV-RNA: in this study the sensivitity of the new HCV Ag assay corresponds to about 1000 IU/mL of HCV RNA, in accord with the estimated equivalency between pg/mL of HCV Ag and IU/mL of HCV-RNA[9,22] and with another recent experience on the ARCHITECT assay that reported an HCV Ag threshold corresponding to 428–2700 IU/mL of HCV-RNA.[11] This has limited relevance in untreated, chronically infected subjects with HCV-RNA levels usually ranging from 10^4 to 10^7 IU/mL.[4,23,24]

Another pitfall of HCV diagnosis is the occurrence of an active infection with a low-level or undetectable antibody response. This is well documented in the earlier phases of infection, as confirmed on the three cases of acute hepatitis included in this study, and has been the major factor for screening blood donations also for HCV-RNA, in Italy as well as in most industrialized countries.[15,16] In our experience, HCV Ag was identified in 0.6–0.7% of patients with a weak positive result for anti-HCV and in 0.8% of cases with a negative result by RIBA-3: this figures, albeit lower than recently reported by Seiskari et al.,[25] indicate that an active infection may occur also in patients with a weak antibody response. Furthermore, several reports indicated that an "occult" HCV infection is quite common among individuals with impairment of the immune response, such as hemodialyzed[26,27] and HIV-positive individuals,[28] and the possibility of a "healthy carrier" status for HCV without serological evidence of infection was demonstrated.[29] The data obtained on two populations at high risk for HCV infection (hemodialyzed) and with a pharmacologically induced immunosuppression (transplant recipients) confirmed that seronegative HCV infections do occur, and particularly in the latter, with 7.2% of subjects negative for anti-HCV and positive for HCV Ag. The finding of two cases negative for HCV-RNA among the HCV Ag positive subjects may represent an artifact[11] and needs to be confirmed. The clinical utility of testing for HCV Ag in transplant settings seems clear, and the results obtained in hemodialyzed confirm earlier observations obtained with less sensitive HCV Ag assays[4,26] and also with the ARCHITECT assay[30] with two cases of positivity for HCV-RNA and HCV Ag among 2752 anti-HCV negative patients. Thus, also in the dialysis setting a reflex testing for HCV Ag on anti-HCV negatives should be recommended, considering that the specificity of the automated HCV Ag assay (99.8% declared, 100% in this study) is higher than the older ones.[11]

HCV is highly variable and the performance of diagnostic assays should not be influenced by this. Earlier reports have indicated a genotype-dependent variability of HCV-RNA assays[14,31,32] and of one HCV Ag test.[33] The HCV Ag assay evaluated in the present study seems robust in this respect, as a 95.7% positivity rate was found on 483 specimens containing the four major genotypes and almost all HCV Ag negative specimens showed a low viral load.

The good correlation with three HCV-RNA assays confirm the results reported by other Authors.[10,11,30,34] In our experience only 21 specimens, or 1.4% of all samples assayed, were truly discordant between HCV Ag and HCV-RNA, and thus the consistency of serological and virological results was very good.

The relationship between HCV Ag and HCV-RNA was non linear by all three nucleic acid amplification methods. The core antigen to HCV RNA ratio may vary slightly from one infected patient to another: this may be due to the presence of HCV core antigen both incomplete virions and in RNA-free core protein structures in peripheral blood.[9,22] Other factors, such as the different dynamics of the two parameters in some individuals[26] and the variability of results generated by some nucleic acid amplification methods on different genotypes[32] shall be also taken in account when considering the discrepancies between HCV Ag and HCV-RNA. When considering the possibility of replacing quantitative HCV-RNA with HCV Ag testing in specific contexts all these factors shall be evaluated, along with the economic and operative advantages of the serological assay that costs less than half of current molecular methods, does not require a dedicated sample and allows a very rapid turnaround time.

In conclusion, our results generate solid evidence on the performance characteristics of the new assay for HCV core antigen and support the efficiency and clinical value of the new algorithm for HCV serological diagnosis that has been recently proposed,[10,25] in which reflex HCV Ag testing on anti-HCV positive individuals will enable to confirm or exclude an active infection and will be useful also on anti-HCV negative individuals at high risk of infection and in patients with an acute hepatitis of unknown origin.

Conflicts of interest

Dr. Maria Sainz and Dr. Claudio Galli are currently employed by Abbott Diagnostics as Scientific Affairs Managers in Spain and in Italy, respectively.

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