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Analytical Performance Characteristics and Clinical Utility of a Novel Assay for Total Hepatitis C Virus Core Antigen Quantification▽

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The detection and quantification of hepatitis C virus (HCV) core antigen in serum or plasma by the use of different assay formats have previously been shown to represent useful markers of viral replication. In the present study, the intrinsic performance characteristics and the potential clinical utility of a novel assay for the quantification of total HCV core antigen were comprehensively assessed by using clinical serum samples and specimens contained in various evaluation panels. The Architect HCV Ag assay showed a specificity of 100%. The intra- and interassay coefficients of variation ranged from 3.6 to 8.0% and from 4.7 to 9.5%, respectively. Except for HCV genotype 2 isolates, the analytical sensitivity was always less than 10 fmol core antigen/liter, corresponding to approximately 500 to 3,000 IU of HCV RNA/ml. Linearity was guaranteed throughout the dynamic range (10 to 20,000 fmol/liter). When seroconversion panels were tested, the assay was not inferior to HCV RNA detection and reduced the preseroconversion period by 4 to 16 days. The results obtained by core antigen and HCV RNA quantification for 385 clinical specimens were correlated by regression analysis ($r = 0.857$), but the calculated conversion equation differed significantly from the line of identity. Monitoring of viral kinetics by use of either core antigen or RNA concentrations in 38 HCV-infected patients undergoing antiviral combination therapy resulted in very similarly shaped curves in all cases. Finally, the Architect HCV Ag assay was also shown to enable high-throughput screening of *in vitro* HCV RNA replication. With these results taken together, the Architect HCV Ag assay proved to be a specific, reproducible, highly sensitive, and clinically applicable test format which will find its future place in the context of virological HCV diagnostics.

The virological diagnosis of infection with the hepatitis C virus (HCV) is based on the detection of specific anti-HCV antibodies. Since anti-HCV immunoassays, however, cannot distinguish between acute, past, and persistent infections, screening for HCV RNA is currently regarded as the method of choice for the confirmation of an active infection in both immunocompetent patients who are anti-HCV positive and immunocompromised individuals who may not mount an adequate antibody response (9, 27, 35, 41).

Assays for the amplification of HCV RNA are expensive and time-consuming and require sophisticated technical equipment and highly trained personnel. These constraints, however, do not apply to the detection of HCV core antigen, which is easy to perform in an immunoassay format, provides results in a comparably short time frame, and, theoretically, is less prone to sample carryover and, hence, contamination than assays based on nucleic acid amplification (17). During the past decade, therefore, several HCV core antigen tests were developed as potential alternatives to HCV RNA testing (1, 4, 43). The use of these assays in clinical laboratory settings documented that HCV core antigen can be detected in the serum of individuals during the window period of acute infection (26, 30, 44). Furthermore, it was conclusively shown that core antigen levels correlate well with HCV RNA concentrations and that quantification of the HCV core protein may consequently be a marker of disease progression (10, 15) or could be used to monitor the response to antiviral therapy in chronically infected patients (4, 13, 23, 34, 42). Given the generally favorable performance characteristics of HCV core antigen assays, it seemed reasonable to further refine this analytical format. One step in this direction was the design of a chemiluminescent magnetic particle-based assay by Abbott Diagnostics (Wiesbaden, Germany) which allows the highly sensitive detection of free HCV core antigen prior to the formation of anti-HCV antibodies (20). Addition of an immune complex-dissociating reaction finally resulted in a test for the quantification of both free and previously antibody-bound, i.e., total, HCV core antigen (24, 25).

The aims of the present study conducted in our laboratory with the Abbott HCV Ag assay were (i) to establish comprehensively its intrinsic analytical performance characteristics, including the correlation of HCV core antigen and HCV RNA concentrations; (ii) to determine its potential clinical utility in the management of HCV-infected patients; and (iii) to demonstrate its general usefulness for the analysis of HCV replication in cell culture systems.

MATERIALS AND METHODS

Determination of anti-HCV and HCV RNA, quantification of HCV RNA, and typing of HCV isolates. Antibodies to HCV were detected in sera from selected seroconversion panels by the Architect anti-HCV assay (Abbott Diagnostics, Wiesbaden, Germany) and, additionally, by the Monolisa HCV Ag-Ab Ultra assay (Bio-Rad, Munich, Germany) (19). The Versant HCV RNA qualitative test (a transcription-mediated amplification [TMA] assay) (36) and the Versant HCV RNA 3.0 assay (a branched DNA [bDNA] assay) (37) (both available from

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Siemens Diagnostics, Eschborn, Germany) were used to amplify HCV RNA isothermally and to establish the HCV RNA concentration by use of the bDNA technology, respectively. Typing of the HCV isolates was achieved by CLIP sequencing of a core fragment and, subsequently, the automated assignment of genotypes and subtypes, as previously described in full detail (38).

Establishment of performance characteristics of Abbott Architect HCV Ag assay. The Architect HCV Ag assay (Abbott Diagnostics) is a chemiluminescent microparticle immunoassay for the quantitative determination of HCV core antigen in human serum and plasma. After the disruption of immunocomplexes, HCV core antigen is captured on the surface of paramagnetic microparticles coated with three different monoclonal antibodies. The addition of another two acridinium-labeled anti-HCV core monoclonal antibodies, as well as pretrigger and trigger solutions, to the reagent mixture results in a chemiluminescent reaction which is measured in relative light units. The quantification of HCV core antigen concentrations (in fmol/liter) is possible by the use of a standard curve that is generated by running six calibrators in duplicate (20, 25).

To establish the specificity of the Architect HCV Ag assay, specimens from 100 healthy individuals with no history of hepatitis virus infection were tested. All of these sera were negative for antibodies to HCV by the Architect anti-HCV assay.

The intra- and interrun reproducibilities were determined by analyzing sera from three patients whose mean HCV core antigen concentrations ranged from 88 to 7,087 fmol/liter. These materials were used for quintuple determinations in five separate runs.

The analytical sensitivity of the Architect HCV Ag assay was assessed by serial dilution of samples from a well-characterized HCV genotype panel (38). The specimens were obtained from patients chronically infected with HCV and contained isolates belonging to subtypes 1a, 1b, 2a, 3a, 4a, 5a, and 6f. The specimens were tested in duplicate in at least three different runs. Calculation of the analytical sensitivity, i.e., the concentration of HCV core antigen which could be detected with a probability of 95%, was achieved by probit analysis.

Three HCV seroconversion panels (BBI Diagnostics, West Bridgewater, MA) were used to ascertain the diagnostic sensitivity (29). The specimens of these panels contained HCV subtype 1a (panel PHV905), 2b (panel PHV914), and 3a (panel PHV921) isolates, respectively, and were analyzed by the following assays: the Architect anti-HCV, Monolisa HCV Ag-Ab Ultra, Architect HCV Ag, and Versant HCV RNA qualitative (TMA) assays.

The linear range of core antigen quantification was determined by use of a series of nine dilutions of two high-titer samples (which contained HCV isolates of subtypes 1a and 1b) that went off-scale in the assay. Each dilution was analyzed in triplicate in three runs. According to a protocol of the Societé Française de Biologie Clinique, the means ± standard deviations (SDs) were calculated from the observed HCV core antigen concentrations, and a relevant deviation from linearity was assumed when these ranges did not intersect with the line of identity between the expected and the observed values (2, 45).

Possible carryover was studied by measuring the amount of HCV core antigen in samples containing low (L) and high (H) concentrations. The sequence $H_1H_2L_1L_2L_3$ was repeated 10 times. The results for L_1 were compared with the results for L_3 and were checked for statistically significant differences at the 1% level by the Wilcoxon signed-rank test for paired samples (28).

To compare the HCV core antigen and the HCV RNA concentrations, 385 HCV RNA-positive samples from 266 HCV-infected patients (147 men, 119 women; mean age, 53 years) were analyzed by both the Architect HCV Ag and the Versant HCV RNA 3.0 (bDNA) assays. Correlations between the results obtained and the conversion equations were calculated by Passing/Bablok regression analysis. This robust, nonparametric procedure was explicitly designed for method comparison in laboratory medicine and, unlike linear regression analysis, does not require special assumptions with regard to the type of distribution of the sample-related values or the error terms (32).

Furthermore, samples from 90 HCV RNA-positive subjects on hemodialysis treatment (45 men, 45 women; mean age, 61 years) were tested. These materials were available from a former study performed by our group (40) and allowed comparison of the HCV core antigen determinations by the Architect assay with those previously determined by the trak-C test (Johnson & Johnson, Ortho Clinical Diagnostics, Rochester, NY).

To evaluate the potential suitability of the Architect HCV Ag assay for monitoring individuals undergoing antiviral combination treatment with pegylated alpha interferon and the nucleoside analogue compound ribavirin, 221 follow-up serum samples from 38 patients (20 men, 18 women; mean age, 45 years) were subjected to quantification by the core antigen and bDNA assays, respectively. According to the outcome of treatment, patients were classified as sustained virological responders, relapsers, and nonresponders. Predictive values of the virological response to therapy were calculated on the basis of the total HCV core antigen and HCV RNA concentrations obtained at week 12 after the initiation of antiviral treatment. The positive predictive value in this context represented the proportion of patients achieving a sustained virological response among subjects with a ≥2-log decrease in HCV RNA and core antigen concentrations at week 12. Conversely, the negative predictive value was defined as the percentage of relapsers or nonresponders among those who did not develop a ≥2-log drop in both the HCV RNA and the core antigen concentrations at that time point (34).

Finally, the Architect HCV Ag assay was used to monitor HCV replication in the cellular replicon system (21). For this purpose, Huh 7 cells which had been stably transfected with a bicistronic HCV AD78-based full-length construct (31) were treated for 48 h either with 0.001 to 100 IU of alpha interferon/ml (Roferon A; Roche Pharma, Grenzach-Whylen, Germany) or with 1.2 to 100 IU of the protease inhibitor BILN 2061/ml (kindly provided by J. Timm, Essen, Germany) (14, 18). The cells were then lysed with nondenaturing buffer (Abcam, Cambridge, MA) at 4°C for 30 min and centrifuged at 12,000 rpm for 20 min. The supernatants obtained were diluted 1:10 with HCV RNA-negative human serum and were subsequently subjected to HCV core antigen testing and HCV RNA quantification.

Statistical analyses. Data were $\log_{10}$ transformed and analyzed by use of the SPSS software package (version 14.0 for Windows; SPSS Inc., Chicago, IL) and the Evaluation Program for Method Comparison (EVAPAK for Windows, version 3.1; Roche Diagnostics, Mannheim, Germany).

TABLE 1. Impact of HCV genotypic variability on analytical sensitivity of Architect HCV Ag assay

HCV subtype	Analytical sensitivity (fmol/liter)[a]	95% CI (fmol/liter)
1a	5.3	3.8–8.3
1b	3.5	2.5–5.3
2a	13.5	9.9–20.2
3a	3.9	2.8–6.0
4a	4.2	3.1–6.5
5a	7.0	4.9–11.0
6f	7.0	4.9–11.0

[a] Calculation of the analytical sensitivity, i.e., the concentration of HCV core antigen which could be detected with a probability of 95%, was achieved by probit analysis.

RESULTS

Analytical performance characteristics of Architect HCV Ag assay. Analysis of 100 anti-HCV-negative specimens from healthy individuals revealed that the test had an analytical specificity of 100% (95% confidence interval [CI], 96.4% to 100%).

Assessment of the intrarun reproducibility by repeat testing of sera from three patients (mean HCV core antigen concentrations, 88, 853, and 7,087 fmol/liter) resulted in coefficients of variation (CVs) of 3.6%, 4.2%, and 8.0%, respectively. The corresponding CVs for between-run imprecision ranged from 4.7% to 9.5%.

Table 1 summarizes the results of analyses of the analytical sensitivity of the Architect HCV Ag assay. HCV isolates belonging to genotypes 1, 3, 4, 5, and 6 could be detected with a probability of 95% at concentrations of less than 10 fmol/liter. Analysis of different samples containing HCV subtype 2a variants, however, resulted in a slightly higher detection limit. Thus, the assay generally meets the manufacturer's claims, i.e., that specimens with core antigen concentrations of ≥10 fmol/liter be judged reactive and that those samples yielding concentrations of ≥3.0 to <10.0 fmol/liter must be retested in duplicate and be considered repeatedly reactive for HCV core antigen if the result of at least one of the duplicate determinations is ≥3.0 fmol/liter.

TABLE 2. Performance of Architect HCV Ag assay with serum samples from seroconversion panels

Panel	HCV subtype	Day on which the following was first detected:			
		Ab[a]	Ag-Ab[b]	Core Ag[c]	RNA[d]
PHV905	1a	11	11	0[e]	0
PHV914	2b	16	12	0	0
PHV921	3a	7	4	0	0

[a] Antibody (Ab) determined by the Architect anti-HCV assay (Abbott Diagnostics).
[b] Antigen and antibody (Ag-Ab) determined by the Monolisa HCV Ag-Ab Ultra assay (Bio-Rad).
[c] Core antigen (Ag) determined by the Architect HCV Ag assay (Abbott Diagnostics).
[d] HCV RNA determined by the Versant HCV RNA qualitative (TMA) test (Siemens Diagnostics).
[e] Zero represents the first sample from the panel.

The diagnostic sensitivity of the Architect HCV Ag assay was evaluated by utilizing three commercially available panels composed of sequential samples from patients who seroconverted in tests for the detection of anti-HCV antibodies. These samples contained three different genotypes of HCV and had RNA titers that ranged from 1,295 IU/ml (panel PHV905, sample 7) to 1,640,570 (panel PHV921, sample 2). The results obtained by the Architect HCV Ag assay were compared to the findings obtained by the Architect anti-HCV assay, the Monolisa HCV Ag-Ab Ultra assay, and the Versant HCV RNA qualitative (TMA) test (Table 2). In each of the seroconversion panels analyzed, HCV core antigen was detected earlier than anti-HCV or the simultaneous presence of HCV antigen and antibody by use of the Monolisa test kit. Consequently, detection of the HCV core antigen resulted in a reduction of 4 to 16 days in the window period. On the other hand, testing for HCV core antigen provided a detection ability equivalent to that of HCV RNA amplification by the isothermal TMA assay.

As shown in Fig. 1, the results obtained with serial dilutions of two high-titer samples (which contained HCV isolates of subtypes 1a and 1b) covering the whole reported range of quantification by the Architect HCV Ag assay yielded excellent agreement between the observed and the expected values. This was reflected not only by coefficients of correlation of 1.0 but also by the equations of the regression lines, both of which were very close to the respective lines of identity. Since the ranges formed by the means ± SDs of the values observed in all nine dilutions constantly intersected with the lines of identity, the linearity of HCV core antigen quantification could be assumed to be between 10 fmol/liter and 20,000 fmol/liter, thereby justifying the manufacturer's claims.

Analyses of 10 series of samples, each of which consisted of two materials with high HCV core antigen concentrations (H_1, H_2), followed by three specimens that initially tested negative for HCV core antigen (L_1 to L_3), led to borderline results for L_1 in 9 of 10 runs. This finding, suggestive of potential specimen-dependent carryover, was reported to the manufacturer of the assay for further clarification.

The testing of 385 specimens for both total HCV core antigen and HCV RNA concentrations (Fig. 2) revealed that the results obtained by the two assays were correlated. However, the regression equation varied significantly from the line of identity: $\log_{10}$ HCV core antigen (fmol/liter) = 1.0 $\log_{10}$ HCV RNA (IU/ml) − 2.2 (95% CIs for slope and intercept, 0.966 to 1.026 and −2.343 to −2.012, respectively). Outliers occurred only very rarely and were recorded, e.g., for two samples which showed HCV RNA concentrations of about $\log_{10}$ 2.8 IU/ml, whereas the core antigen contents were $\log_{10}$ 3.4 fmol/liter and $\log_{10}$ 3.75 fmol/liter, respectively. These two specimens, which harbored HCV subtypes 2a and 2b were obtained from a 68-year-old woman on maintenance hemodialysis treatment and a 44-year-old male outpatient for whom no detailed clinical information was available. The reasons for the analytical discrepancies observed remain unknown.

To assess whether the total HCV core antigen/HCV RNA ratio was influenced by HCV genotypic variability, separate regression lines were calculated for isolates belonging to HCV types/subtypes 1a, 1b, 2, and 3. Whereas the slopes of the equations varied insignificantly from the lines of identity, intercepts ranging from −2.085 (subtype 1b variants) to −2.571 (genotype 3 strains) were calculated. On the basis of these equations, it was delineated that 1 fmol/liter of HCV core

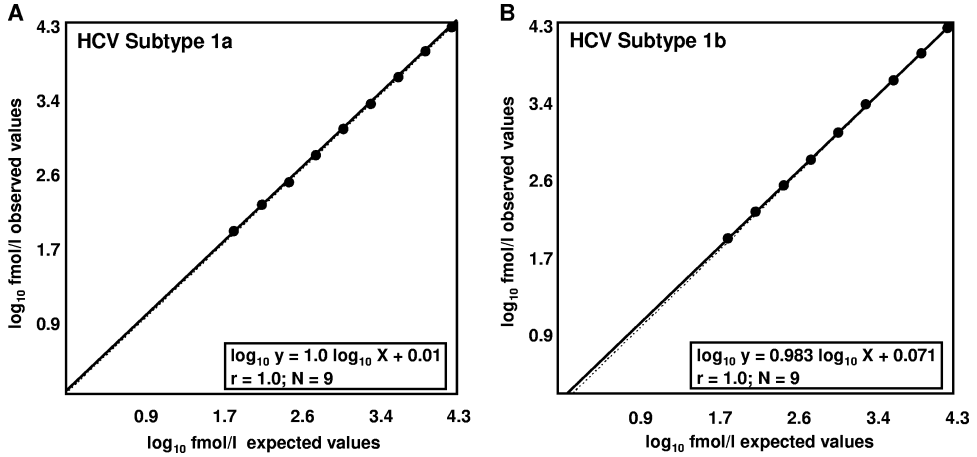

FIG. 1. Linear range of quantification by the Architect HCV Ag assay. Nine serial dilutions of two high-titer samples containing either HCV subtype 1a (A) or 1b (B) isolates were tested in triplicate in three separate runs. The observed mean values (filled circles) were plotted against the expected values (dotted lines) by using logarithmic scales.

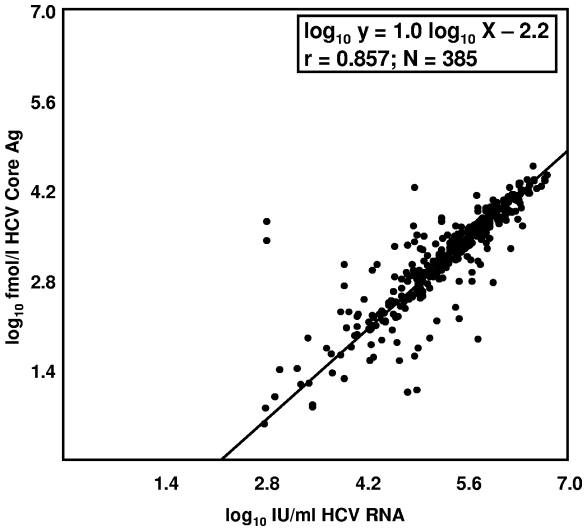

FIG. 2. Scatter plot of $\log_{10}$ core antigen concentration (fmol/liter) and RNA concentration (IU/ml) determined in 385 samples by the Architect HCV Ag and Versant HCV RNA 3.0 (bDNA) assays, respectively. The correlation coefficient and conversion equation given in the inset were calculated by nonparametric Passing/Bablok regression analysis.

antigen corresponded to the following HCV RNA concentrations: 169 IU/ml (subtype 1a), 135 IU/ml (subtype 1b), 200 IU/ml (genotype 2), and 257 IU/ml (genotype 3).

Use of the Architect HCV Ag assay to monitor patients on hemodialysis treatment and individuals undergoing antiviral therapy. When sera obtained from 90 HCV RNA-positive patients on maintenance hemodialysis were analyzed by both the Ortho trak-C and the Architect HCV Ag assays, 80 specimens were reactive by the trak-C test, whereas 10 sample materials did not provide positive results. The mean HCV RNA concentration in the 10 serum samples was 1,152 IU/ml. By using the Abbott kit, it was also possible to detect HCV core antigen in these 10 samples with very low levels of viremia, resulting in a diagnostic sensitivity of 100% in this particular context.

Among the 38 patients undergoing antiviral combination therapy who had been included in this study, 29 were infected with isolates belonging to HCV genotype 1 or 4 and 9 harbored type 2 or 3 variants. Sixteen individuals (42%) developed a sustained virological response to therapy, 12 (32%) were negative for HCV RNA at the end of treatment but experienced a relapse thereafter, and 10 (26%) subjects failed to respond to the administration of pegylated alpha interferon and ribavirin. As illustrated in Fig. 3, monitoring of the viral kinetics by determination of either the HCV RNA or the core antigen concentration resulted in very similarly shaped curves. This good agreement was particularly well demonstrated by calculating the predictive values of the sustained virological response to treatment on the basis of at least 2-log decreases in the total HCV core antigen and HCV RNA concentrations at week 12 after the initiation of antiviral therapy (Table 3). In patients infected with HCV type 1 or 4, positive and negative predictive values of 45% and 100%, respectively, were established for both assays. The corresponding values for subjects harboring type 2 or 3 strains were 87.5% and 100%, respectively. Consequently, monitoring of viral kinetics during therapy by measurement of the HCV RNA or the core antigen concentration led to identical predictions of the outcome, at least when these forecasts were based on the 2-log-drop criterion at the end of week 12 of treatment.

Use of the Architect HCV Ag assay to monitor HCV replication in cell culture systems. To validate the assay for use for monitoring antiviral efficacy *in vitro*, Huh 7 cells stably transfected with an AD78-based HCV full-length construct were treated with alpha interferon or the protease inhibitor BILN 2061 for 48 h prior to cell lysis and processing through the Architect HCV Ag and Versant HCV RNA 3.0 assays. Figure 4 depicts the typical dose-response of HCV RNA replication and core protein expression following the administration of 0.001 to 100 IU of alpha interferon/ml or 1.2 to 100 nmol of

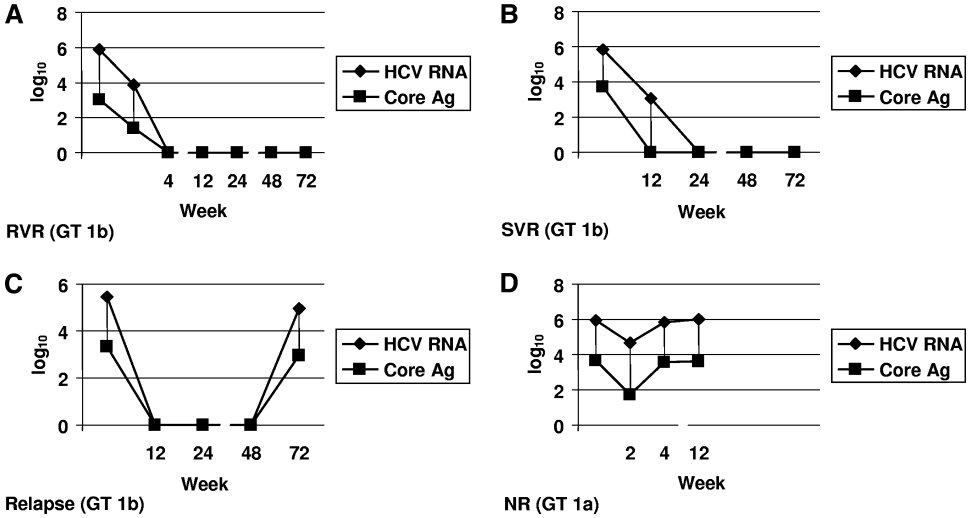

FIG. 3. Viral kinetics estimated by testing for HCV core antigen (■) and RNA (◆) in four individuals receiving antiviral combination therapy with pegylated alpha interferon and ribavirin. (A) Rapid virological response (RVR); (B) sustained virological response (SVR); (C) results for a patient who developed a relapse after the cessation of antiviral treatment; (D) results for a subject not responding to therapy. GT, HCV genotype.

TABLE 3. Predictive values of sustained virological response to pegylated alpha interferon-ribavirin on the basis of a 2-log decrease in total HCV core antigen and HCV RNA concentrations at week 12 after initiation of antiviral treatment

Assay target and HCV genotype	No. of specimens with the following concn changes at wk 12:		PPV[a]		NPV[b]	
	<2 log	≥2 log	Ratio	%	Ratio	%
HCV core antigen						
GT[c]1/4 (n = 29)	9	20	9/20	45.0	9/9	100
GT 2/3 (n = 9)	1	8	7/8	87.5	1/1	100
HCV RNA						
GT 1/4 (n = 29)	9	20	9/20	45.0	9/9	100
GT 2/3 (n = 9)	1	8	7/8	87.5	1/1	100

[a] PPV, positive predictive value.
[b] NPV, negative predictive value.
[c] GT, HCV genotype.

BILN 2061. HCV RNA levels (relative to those in nontreated cells) decreased up to 19.4% (alpha interferon) and 4.2% (BILN 2061). These drops were paralleled by reductions in the level of HCV core protein expression to 7.9% (alpha interferon) and 29.4% (BILN 2061) of the levels for the respective controls.

DISCUSSION

The detection and quantification of HCV core antigen in the serum or plasma of infected patients by using different assay formats were previously shown to narrow the preseroconversion (window) phase of acute HCV infections (26, 30) and to be a useful marker of viral replication (4, 5, 11, 13, 23, 34, 42, 47).

In the present study, a novel test for the determination of the total HCV core antigen concentration, i.e., the Abbott HCV Ag assay (24, 25), was comprehensively evaluated in a routine clinical laboratory setting in order to establish its intrinsic analytical performance characteristics and to determine its potential utility in the clinical management of HCV-infected patients.

We found the assay to be specific, reproducible, linear over a wide dynamic range, and analytically sensitive. The specificity of 100% (95% CI, 96.4 to 100%) recorded in our investigation is in line with the results of Morota and coworkers (25), who reported a 99.98% specificity when they tested 5,394 anti-HCV-negative sera. Intra- and between-run imprecisions of 3.6% to 8.0% and 4.7% to 9.5%, respectively, must be rated as more than satisfactory, given both the fact that the assay operates down to a range of HCV core antigen concentrations of about 10^{-14} to 10^{-13} mol/liter and the observation that another previously available HCV core antigen detection kit (i.e., the trak-C assay) exhibited coefficients of variation as large as 33% (intrarun) and 27% (between run) (4). This good reproducibility of quantification by the Architect HCV Ag assay led to the manufacturer's justified recommendation to perform only single determinations instead of duplicate ones. In our hands, the assay met the manufacturer's claims in terms of linearity and proved to enable accurate measurements of from 10 to 20,000 fmol core antigen/liter. It remains to be established, however, whether the same linear range can be maintained when samples containing non-type 1 HCV isolates are analyzed. The testing of serial dilutions of specimens from a well-characterized HCV genotype panel (38) with subsequent probit analysis of the results obtained indicated 95% analytical sensitivities of between 3.5 (subtype 1b) and 13.5 (subtype 2a) fmol core antigen/liter, thereby principally confirming with clinical material the results of Morota and colleagues (25), who could constantly detect 3 fmol/liter of recombinant HCV core antigen. Thus, with the exception of HCV genotype 2 isolates, quantification of HCV core antigen with the Architect assay is not considerably affected by HCV genotypic variability. On the basis of the correlation of the HCV core antigen concentrations and the amounts of HCV RNA, as established in the present study by nonparametric regression analyses, the analytical sensitivity of the Architect HCV Ag assay corresponds to 428 (subtype 1b), 896 (subtype 1a), 2,700 (subtype 2a), and 1,002 (subtype 3a) IU of HCV RNA/ml. When these results are compared to the respective performance characteristics of,

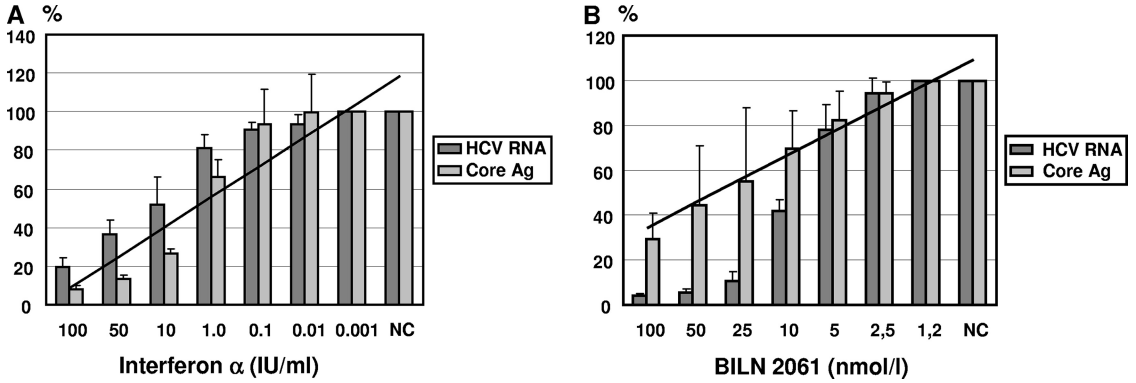

FIG. 4. Huh 7 cells stably transfected with an AD78-based HCV full-length construct were treated for 48 h with alpha interferon (A) or the protease inhibitor BILN 2061 (B). The impact of this antiviral therapy on *in vitro* HCV replication was monitored by determination of both the core antigen and the RNA concentrations. The values shown are the means ± SDs of three replicates and are expressed as a percentage relative to the results for nontreated cells.

e.g., the trak-C test, whose detection limit was assumed to range from 10,000 (22) to 27,000 (17) IU of HCV RNA/ml, the Architect HCV Ag assay achieved, on average, an 8- to 21-fold increase in analytical sensitivity.

The combination of high specificity and outstanding analytical sensitivity makes the Architect HCV Ag assay a highly suitable candidate for second-line supplemental screening for HCV in a community-based population. Whereas the trak-C test kit failed to detect up to 10% of viremic samples in such a setting (17), viral loads of less than 500 to 3,000 IU of HCV RNA/ml will hardly ever be encountered in treatment-naïve patients chronically infected with HCV (3). According to our experience, a diagnostic algorithm, in which the Architect HCV Ag assay could substitute for nucleic acid amplification as the first choice for the confirmation of active HCV infection, would obviate the need for nucleic acid amplification for approximately 75% of all anti-HCV-positive samples. Because of the current rather moderate price of HCV core antigen measurements by the Architect assay, such a strategy might also result in considerable cost savings (8, 24).

The findings of the investigations of the diagnostic sensitivity of the Architect HCV Ag assay outlined above demonstrated that in the seroconversion panels, core antigen was detected earlier than anti-HCV or the simultaneous presence of HCV antigen and antibody in a so-called fourth-generation anti-HCV immunoassay (19). Consequently, the detection of HCV core antigen led to a reduction of the preseroconversion (window) period in patients with acute HCV infections of 4 to 16 days and provided results equivalent to those of HCV RNA amplification by the isothermal TMA assay. These findings are generally in line with those reported by Leary et al. (20) for the free-antigen version of the Abbott test. Translation of the results obtained with the Architect HCV Ag assay with the samples from the seroconversion panels into the biology of acute HCV infections would mean that the test is probably able to detect HCV in all specimens from patients with infections in the plateau phase. It is also conceivable that the Architect assay may recognize HCV in a considerable portion of samples from patients in the ramp-up phase but will definitely miss HCV in specimens taken from patients in the pre-ramp-up phase of acute HCV infection (12, 30). Additional testing of blood donors for the presence of HCV core antigen will, hence, lead to improved blood safety compared to that achieved by the use of third-generation anti-HCV tests alone. This holds particularly true for countries with a low or median human development index in which the technology and the staff required to perform anti-HCV screening of blood donors are already present. According to data inferred from the Global Database on Blood Safety for the years 2001 and 2002 (39, 46), these prerequisites should apply to approximately 30.6 million blood donations.

The notion that the Architect HCV Ag assay in our evaluation study showed a tendency for carryover prompted extensive and yet unfinished investigations by the manufacturer. According to the results available so far, this phenomenon appears not to be mainly attributable to specimen-dependent effects but, rather, seems to derive from sample-independent phenomena most likely originating from the reagent used in the test concurrently implemented for anti-HCV detection. The microparticles contained in the Architect anti-HCV kit are coated with HCV core antigen, and hence, carryover of traces of this solution as a contaminant can already cause false-positive results in the Architect HCV Ag assay. The manufacturer, however, is confident that this problem will soon be solved by modifications of the existing "maintenance protocols" (Abbott Diagnostics, personal communication).

As regards the potential utility of the Architect HCV Ag assay for the management of patients infected with the virus, our findings demonstrate that the outstanding analytical sensitivity of the test resulted in an improved clinical performance when the kit was used to monitor individuals with HCV viremia on maintenance hemodialysis treatment. While the trak-C assay could not detect core antigen in 10 of 90 HCV RNA-positive samples obtained from German hemodialysis centers (38), its Architect counterpart correctly recognized all of these specimens with very low levels of HCV viremia. Therefore, use of the Abbott test might provide at least an interim solution for the yet unresolved issue and the still ongoing discussion in the dialysis community of the need for and advantage of supplemental HCV RNA testing of anti-HCV-negative patients on maintenance hemodialysis (3, 7, 9, 16, 40). Similar considerations could apply to other settings, including transplantation medicine, in which the availability of a sensitive immunoassay for HCV core antigen determination should also represent a valuable diagnostic benefit for the immediate resolution of peculiar serological constellations which are particularly problematic in the course of decision making (20).

Monitoring of viral kinetics by determination of either the HCV RNA or the core antigen concentration in 38 patients undergoing antiviral combination therapy for their chronic infections generally led to very comparably shaped curves. Of note, completely identical predictions of the therapeutic outcomes were obtained when the forecasts, independently of the diagnostic assay applied, were based on the at least 2-log-drop criterion at the end of week 12 of treatment (27). Notwithstanding our results, the principal possibility of discrepancies by HCV core antigen and HCV RNA quantification in patients under antiviral treatment should always be kept in mind (4). Furthermore, the findings of the HCV core measurements in patients who receive antiviral treatment are currently difficult to interpret since it is entirely unclear whether or not, e.g., "stop rules" similar to those already established in clinical trials for HCV RNA concentration determinations (27) will be also applicable for the quantitative determination of HCV core antigen (4, 13, 23, 34, 42, 47). Consequently, at present, it remains unlikely that the Architect HCV Ag assay will be able to find its place in monitoring the response to antiviral treatment of patients chronically infected with HCV (41).

Finally, the Architect HCV Ag assay was utilized to follow HCV replication in the replicon system (21). The treatment of Huh 7 cells stably transfected with an AD78-based full-length HCV construct (31) with various concentrations of alpha interferon and the protease inhibitor BILN 2061 (14, 18) led to concomitant decreases in both intracellular HCV core antigen and HCV RNA concentrations. In principle, these findings proved that the Architect HCV Ag assay, like the trak-C test (6), can be favorably applied as a relatively cheap and easy-to-use alternative for HCV RNA amplification in basic science settings requiring a high-throughput screening of *in vitro* HCV replication. Further studies are currently under way in our

laboratory to determine whether the Architect HCV Ag assay is also beneficial in assessing susceptible cell lines transiently transfected with HCV genomes. It can be assumed that in such a context, the Abbott assay should prove to be a particularly valuable adjunct to its various predecessors, owing to both its superior analytical sensitivity and the fact that in these situations RNA-based techniques for the detection of HCV replication are difficult to perform because of the large amounts of residual HCV input RNA still present (6, 33).

Taken together, in our evaluation studies the Architect HCV Ag assay turned out to be a robust, specific, reproducible, highly sensitive, and clinically applicable test format. Given that the carryover problem will finally be solved by modification of the existing maintenance protocols, we are confident that the Architect HCV Ag assay might find its proper place in future HCV diagnostics, e.g., as a supplemental test for confirming active HCV infection in anti-HCV-positive subjects, as a screening assay for monitoring patients who are at a known risk for acquiring HCV infections, and as an assay that provides benefits for *in vitro* high-throughput HCV replication testing in basic research settings, including testing of cell culture systems only transiently transfected with HCV constructs.

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