Hepatitis C virus (HCV) is a global pathogen, infecting more than 185 million individuals, with global seroprevalence estimated at 2.8% (95% uncertainty interval [UI], 2.6 to 3.1%) and more than 1 million new cases reported annually (1). Previous studies on the detection of HCV core antigen (HCVcAg) demonstrated that this antigen (Ag) represents a robust stable marker of HCV replication (2). Quantification of HCVcAg can be performed using an automated, highly reproducible, chemiluminescent, microparticle immunoassay with a shorter turnaround time for robustness (3). Measurement of HCVcAg and HCV RNA measurements is more robust for GT1 and GT4 than for GT3 (4), with greater variance of HCVcAg and RNA measurements for GT3 (5, 6), with greater variance for positive GT3 samples than for samples of other GTs (7). However, the cause of those discordances was not explored in those studies. The present study was undertaken to investigate the discordance between HCVcAg and RNA measurements in GT3a HCV-infected individuals.

Plasma/serum samples from HCV-infected individuals (n = 511) that were referred to the National Virus Reference Laboratory (NVRL) for HCV investigations were included to correlate HCVcAg and RNA measurements for GT1a, GT1b, and GT3. False-negative HCVcAg cases were defined as cases with undetectable HCVcAg but HCV RNA levels of ≥4 log_{10} IU/ml, which is above the lower limit of detection of the HCVcAg assay, based on previous studies (7–9) and our own analyses. The controls were samples in which both HCVcAg and RNA were detectable. HCVcAg was quantified on the Architect HCV Ag platform (Abbott Diagnostics, Wiesbaden, Germany). The assay cutoff threshold for a positive result is ≥3 fmol/liter, whereas values of 3 to 10 fmol/liter and >10 fmol/liter are reported as weak positive and positive, respectively. HCV viral loads were determined using the Abbott Molecular m2000 RealTime System (Abbott Diagnostics, Wiesbaden, Germany). HCV genotyping was performed with the Innogenetics Versant HCV GT2.0 assay (Siemens Healthcare, Milan, Italy) or the RealTime HCV Genotype II assay (Abbott, Wiesbaden, Germany). Employing a previously described method (10), a 1,256-bp fragment encompassing the entire HCV core gene from the 5′ untranslated region (UTR) to the E1 gene was amplified for bidirectional sequencing, which was performed on the 3500 Dx platform (Applied Biosystems, Foster City, CA) using BigDye Terminator chemistry (version 3.1). Sequence chromatograms were investigated manually using sequence analysis software (SeqMan Pro version 11.2.1; DNASTar). The consensus sequences were aligned by using ClustalW in Bioedit (version 7.0.5). Fisher’s exact test (categorical variables), the Mann-Whitney U test (continuous variables), and Spearman’s rank correlation coefficient tests were performed using MedCalc version 14.8.1.

HCVcAg- and RNA-positive samples for GT1a (n = 261), GT1b (n = 79), and GT3 (n = 171) were evaluated. Strong positive correlations between HCVcAg and RNA levels (P < 0.0001) were observed for all three GTs (Fig. 1). However, the correlation coefficient for the correlation of HCVcAg and RNA levels was lower for GT3 (r = 0.79) than for GT1a (r = 0.87) and GT1b (r = 0.86). Greater variance for GT3, compared to GT1a and GT1b, was apparent (Fig. 1). Furthermore, the HCVcAg/RNA ratio for GT3 was significantly lower than those for GT1a and GT1b (P < 0.0001), while there was no significant difference in the HCVcAg/RNA ratios for GT1a and GT1b (P = 0.44), indicating the underquantification of HCVcAg, relative to the corresponding viral loads, for GT3 samples.

Genetic characterization of the core gene in GT3a HCV false-negative serological results for genotype 3a core antigen.
negative cases and controls determined amino acid substitutions associated with the underquantification of HCVcAg. A48T was found in 5.5% of controls (2/36 samples) versus 42.9% of cases (3/7 samples) (P < 0.05). T49A/P was found in 8.3% of controls (3/36 samples) versus 42.9% of cases (3/7 samples) (P < 0.05).

The alignment of 160 amino acid residues in the core region of 36 controls and seven cases is shown in Fig. 2. For case 7, substitutions at residues 48 and 49 were absent, but we noted the presence of L44M; this mutation was not seen in other sequences from either cases or controls (Fig. 2). In the Los Alamos database, L44 predominates in all HCV GTs (range, 98.58 to 100%) and is present in 100% of GT3a sequences, which indicates the relative rarity of L44M. Consequently, this mutation may also affect the ability of the monoclonal antibodies in the assay to detect HCVcAg.

Additionally, five controls with substitutions in either residue 48 (n = 2) or residue 49 (n = 3) (Fig. 1) deviated significantly from the trend of the majority of GT3 samples, and their HCVcAg/RNA ratios were significantly lower than those for the other controls, without these substitutions (P = 0.0009). The characteristics of controls and cases containing significant substitutions are shown in Table 1. These data suggest that underquantification of HCVcAg levels can occur when substitutions at residues 48 and 49 are present in the HCV core protein.

To evaluate the degree of conservation of the HCV core protein, all available core sequences (n = 5,623) were downloaded from the Los Alamos database (http://hcv.lanl.gov/content/index) and aligned with the Web-based Jalview (11). At position 48, alanine is the most common residue, while A48T is present in 0.35 to 6.70% of all GTs. At position 49, threonine predominates; however, T49A was seen in all GTs (range, 0.1 to 4.5%). T49P is also present in GT1a, GT1b, GT2, and GT4 (range, 0.4 to 15.7%). Overall, in GT3a, A48T was seen in 2 (0.71%) of 282 deposited sequences; these two sequences originated in Sweden and India. T49A was seen in 12 (4.26%) of 282 GT3a sequences; six of these sequences originated in Pakistan and Thailand, and the United States, and the other three had no information on the country of origin.

In 2000, Tokita et al. identified 4% of GT1b HCV samples (4/107 samples) with relatively low HCVcAg levels, as measured with a fluorescence enzyme immunoassay, compared to corresponding HCV RNA values (12). All four samples showed a point mutation (T49P) in comparison with eight controls in which this substitution was not found. In 2012, a German group reported one GT3 HCV case with an HCVcAg level of only 5.16 fmol/liter, as detected with the Abbott Architect assay, despite a viral load of 5.63 log₁₀ IU/ml (B. Schulte, S. Susser, B. Ritter, C. Sarrazin, A. Heim, and B. Wolk, presented at an Abbott-sponsored symposium, 2012). Two mutations in an epitope targeted by monoclonal antibodies in the HCVcAg assay were identified; however, neither the epitope nor the substitution was specified in the presentation.

FIG 1 Scatter plots depicting the correlation between HCVcAg (log₁₀ fmol/liter) and HCV RNA (log₁₀ IU/ml) measurements. Arrows, samples in which A48T (n = 2) and T49A (n = 3) were identified in the GT3a HCV core protein.

FIG 2 Amino acid sequence alignment (160 residues) of the mature GT3a HCV core protein, showing comparison of sequences derived from controls (n = 36) and cases (n = 7). Amino acid positions in the HCV core protein are numbered, and sequence identity is represented by single dots. Red box, region including residues 48 and 49, which were significantly associated with compromised HCVcAg measurements. X, presence of more than one amino acid.
The authors stated that the core sequence analysis from the European Hepatitis C Virus Database showed that the coincidence of these mutations was infrequent. In another study, Murayama et al. evaluated the correlation of HCV RNA and HCVcAg measurements with five different commercial HCVcAg assays, using a reference panel of GT1 and GT2 samples (13). Twelve GT1b or GT2 samples exhibited the polymorphisms R47G, A48T, and T49A/P, which correlated with HCVcAg underquantification with multiple HVCcAg assays. Those results showed that the Architect assay exhibited the highest sensitivity; however, generally the sensitivity of all of the commercially available HVCcAg assays was still insufficient to detect low-titer HCV infections (13). The false-negative HVCcAg results in the present study were associated with low to medium viral loads (range, 4.35 to 5.42 log_{10} IU/ml), while our data suggested that 42.8% of HCV-positive samples (219/511 samples) had high viral loads (≥6 log_{10} IU/ml). The Architect HVCcAg assay has proved invaluable in the diagnosis and management of HCV infections. The test facilitates the detection of viremic individuals without the need for HCV RNA investigations, allowing more rapid referral and risk management. The test is also useful for the identification of individuals with spontaneously resolved HCV infections. While a false-negative HVCcAg result could indicate erroneously that an infection had resolved, current best practices would ensure HCV RNA testing to confirm this interpretation. Finally, the HVCcAg test has provided a logistically easier method to screen for the presence of acute HCV infections in the settings of dialysis units, organ donor assessments, percutaneous injuries, or relapses following HCV treatment. In these scenarios, it is probable that HCV viral loads would be sufficiently high for the HVCcAg assay to be employed for HCV detection.

Notably, after GT1, GT3 is second most prevalent GT worldwide (54.3 million cases [30.1% of the total]), and its seroprevalence is highest in southern Asia (14). This distribution has been attributed potentially to the association of GT3 with persons who inject drugs (15) and recent migrations from India and Pakistan, where GT3a predominates (15–17). Therefore, the endemic nature of GT3 HCV in southern Asia might have implications for the utilization of the HVCcAg assay for detection of acute infections. The inclusion of confirmatory protocols in the management of HCV infections is indicated, particularly in low-income regions in which access to qPCR assays is limited and GT3a HCV is endemic.

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**Nucleotide sequence accession numbers.** The sequences determined were submitted to GenBank with the following accession numbers: KP797837 to KP797872 (controls) and KP797873 to KP797879 (cases).

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