Comparison of a Newly Developed Automated and Quantitative Hepatitis C Virus (HCV) Core Antigen Test with the HCV RNA Assay for Clinical Usefulness in Confirming Anti-HCV Results

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Hepatitis C virus (HCV) is a global health care problem. Diagnosis of HCV infection is mainly based on the detection of anti-HCV antibodies as a screening test with serum samples. Recombinant immunoblot assays are used as supplemental tests and for the final detection and quantification of HCV RNA in confirmatory tests. In this study, we aimed to compare the HCV core antigen test with the HCV RNA assay for confirming anti-HCV results to determine whether the HCV core antigen test may be used as an alternative confirmatory test to the HCV RNA test and to assess the diagnostic values of the total HCV core antigen test by determining the diagnostic specificity and sensitivity rates compared with the HCV RNA test. Sera from a total of 212 treatment-naive patients were analyzed for anti-HCV and HCV core antigen both with the Abbott Architect test and with the molecular HCV RNA assay consisting of a reverse transcription-PCR method as a confirmatory test. The diagnostic sensitivity, specificity, and positive and negative predictive values of the HCV core antigen assay compared to the HCV RNA test were 96.3%, 100%, 100%, and 89.7%, respectively. The levels of HCV core antigen showed a good correlation with those from the HCV RNA quantification (r = 0.907). In conclusion, the Architect HCV antigen assay is highly specific, sensitive, reliable, easy to perform, reproducible, cost-effective, and applicable as a screening, supplemental, and preconfirmatory test for anti-HCV assays used in laboratory procedures for the diagnosis of hepatitis C virus infection.

Hepatitis C virus (HCV) was first recognized in 1974 as a non-A, non-B hepatitis virus (NANBH) and first identified in 1989 using molecular methods, but to date, the virus has never been visualized or grown in cell culture (7, 22). HCV is a positive-strand RNA virus that belongs to the family Flaviviridae (14). HCV is a global health care problem, and the World Health Organization (WHO) estimates that at least 170 million people (3% of the world’s population) are infected with HCV worldwide (30).

Diagnosis of HCV infection is mainly based on the detection of anti-HCV antibodies by the enzyme immunoassay (EIA) or chemiluminescence immunoassay (CLIA) of serum samples. The anti-HCV assay is used as a screening test. Recombinant immunoblot assays are used as supplemental tests and for the final detection and quantification of HCV RNA in confirmatory tests. Three different generations of anti-HCV test kits have been developed. The first-generation HCV EIA detects only antibodies against the nonstructural region 4 (NS4) with recombinant antigen c100-3 (12). With the development of second-generation tests, additional antigens from the core region (c22-3), the NS3 region (c33c), and a part of c100-3 (5-1-1) from the NS4 region can be used (8). The third-generation EIA anti-HCV test currently used includes an additional antigen from the NS5 region and a reconfiguration of the core and NS3 antigens (29).

Anti-HCV assays have several disadvantages, such as a high rate of false positivity, a lack of sensitivity of detection in the early window period of 45 to 68 days after infection, the inability to distinguish between acute (ongoing active, viremic), past (recovered), and persistent (chronic) infections, and a possibility of false negativity with samples from immunocompromised patients, who may not have an adequate antibody response (9, 17, 19, 21). Recombinant immunoblot assays, types of EIAs, also have several disadvantages, such as being difficult to perform and having a high percentage of indeterminate results and a high cost. Therefore, these anti-HCV assays are not often used in developing countries or in routine diagnostic laboratory procedures (10). The HCV RNA assay is a reliable method but needs technical skill and may also result in false positivity because of contamination, and it is time intensive and more expensive (16).

In this study, we aimed to compare the HCV core antigen (HCV Ag) test with the HCV RNA assay for confirming anti-HCV results to determine whether the HCV Ag test may be used as an alternative confirmatory test to the HCV RNA test and to assess the diagnostic values of the total

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the Department of Microbiology, Konya Education and Research Hospital, Selcuk University (Konya, Turkey), and the HCV RNA assay was performed in Research, Sydney, Australia). The RNA was extracted from serum samples using PCR (RT-PCR) method using Rotor Gene 6000 real-time analysis (Corbett many) with a lower detection limit of 20 IU/ml which uses a reverse transcription-

specimen is considered repeatedly reactive (16, 23).

for HCV Ag. If one or both of the duplicates have values of

indicate that the specimen is nonreactive, the specimen is considered nonreactive

fmol/liter and

quantification of the HCV Ag in human serum or plasma samples. The sample

uses a two-step chemiluminescent microparticle immunoassay technology for the

Architect HCV Ag assay was

approximately 36 to 40 min. The cutoff value is 3.00 fmol/liter (0.06 pg/ml); thus, samples

of

3.00 fmol/liter are considered reactive, and samples with values of

10.00 fmol/liter are retested in duplicate. If both retest values

Table 2. Performances of the HCV Ag and anti-HCV tests compared to the HCV-RNA assay

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV Ag (Abbott)</td>
<td>96.3 (93.3–99.2)</td>
<td>100 (100–100)</td>
<td>100 (100–100)</td>
<td>89.7 (81.8–97.5)</td>
<td>97.2 (93.4–100)</td>
</tr>
<tr>
<td>Anti-HCV (Abbott)</td>
<td>96.3 (93.3–99.2)</td>
<td>26.9 (14.9–38.9)</td>
<td>80.2 (74.6–85.8)</td>
<td>70.0 (49.9–90.1)</td>
<td>79.2 (74.9–82.4)</td>
</tr>
</tbody>
</table>

* Values in parentheses are ranges. PPV, positive predictive value; NPV, negative predictive value.
grams per milliliter, and \( x \) is the concentration of HCV RNA in \( \log_{10} \) international units per milliliter. This analysis indicated that the relationship between the concentration of HCV Ag and HCV RNA was consistent throughout the common dynamic ranges of the assays.

The sensitivity of the HCV Ag assay was 96.3% (range, 93.3 to 99.2%), and there were no false-positive results or cross-reactivity. The within-run coefficient of variation (CV) was 10%. The levels of HCV Ag showed a good correlation with those from the HCV RNA quantification (\( r = 0.907 \)). The HCV Ag assay showed excellent linearity over the range of 0.5 to 12,000 fmol/liter (Fig. 2).

**DISCUSSION**

Anti-HCV tests based on CLIA or EIA methods of measuring anti-HCV antibodies in sera are the virologic test methods that are the most commonly used in routine laboratory procedures for the diagnosis of HCV infection worldwide (4, 29). The most common problem in the laboratory-screening anti-HCV assay is the false positivity of samples with low titers. Among immunocompetent populations with an anti-HCV prevalence below 10% (e.g., volunteer blood donors, military personnel, general population, health care workers, or clients attending sexually transmitted diseases clinics), the proportion of false-positive results is approximately 35% (range, 15% to 60%) (14, 21, 24).

Anti-HCV tests have several disadvantages, such as the prolonged duration of the window period between the time of infection and the detection of HCV antibodies: approximately 45 to 68 days. Because anti-HCV tests are based on detection of antibodies that are markers of the immune response, there are many false-negative results in immunocompromised patients because of inadequate observation or a lack of observation of response. Immunoassays also cannot distinguish acute, past, or persistent infections from each other. Patients who have recovered from infection may be found to be seropositive due to the persistence of antibodies (anti-HCV IgG) in serum for a long period and even for life. The anti-HCV test does not distinguish the individuals who have resolved HCV infection from the patients with active/ongoing HCV infection. As a consequence, anti-HCV assay results with values under the critical value used by EIA or CLIA need to be confirmed by an additional confirmatory test, such as the HCV RNA test, or with the preconfirmatory HCV Ag assay (5, 10, 14, 17, 25).

The HCV RNA test is extensively used to confirm antibody-based screening test results. Amplification methods (target amplification by RT-PCR, transmission-mediated amplification [TMA], and signal amplification by branched DNA) are the most expensive methods ($45 to $50 per test for real-time PCR, $10 to $12 per test for HCV Ag CLIA, and $5 to $6 per test for anti-HCV CLIA) compared with anti-HCV and HCV Ag tests and require sophisticated technical equipment and highly trained personnel. One specific problem with the HCV RNA assay is that HCV RNA can be temporarily undetectable because of the transient, partial control of viral replication by the immune response. Patients in a period of
nonviremia may be found to be anti-HCV positive and HCV RNA negative. In this situation, the HCV RNA test should be repeated a few weeks later with a new sample. This need for retesting is a disadvantage of the HCV RNA test. In addition, nucleic acid amplifications are labor-intensive and time-consuming methods and have the risk of laboratory contamination; for these reasons, amplification methods are not suitable for widespread use in most laboratories, especially in developing countries (1, 4, 11, 23, 27). Therefore, the HCV Ag assay is needed as a supplemental or preconfirmatory test to preconfirm anti-HCV results and distinguish false-positive results from the accurate ones because it is easy to perform and reliable, has high specificity and sensitivity rates, is cost-effective, is able to shorten the duration of the time to diagnosis of infection in patients during the window period, and has a lower risk of laboratory contamination than assays based on nucleic acid amplification technology (31, 28).

During the past decade, several HCV Ag tests have been developed as potential alternatives to the HCV RNA assay (3). The first was developed by Tanaka et al. (28) in 1995, and then Aoyagi et al. (2) developed a new and 100-fold more sensitive test in 1999. In previously reported studies, HCV Ag was detected 1 day later than HCV RNA in patients undergoing seroconversion (5, 6, 20).

In this study, the sensitivity, specificity, and positive and negative predictive values of the HCV Ag Abbott CLIA were found to be 96.3%, 100%, 100%, and 89.7%, respectively. In different studies performed with the same test, equal or nearly equal values were found: Park et al. (18) found 100% specificity, Ross et al. (23) found 100% specificity, Morota et al. (16) found 99.8% specificity, Miedouge et al. (15) found 99.2% specificity, Leary et al. (13) found 99% specificity, Song et al. (26) found 97.2% sensitivity, and Park et al. (18) found 90.2% sensitivity. In our study, the diagnostic specificity rate (100%) is equal to the rates from two recent studies performed by Park et al. (18) and Ross et al. (23), almost equal to the value in the study performed by Morota et al. (16) (99.8%), and very near the values from two studies by Miedouge et al. (15) (99.2%) and Leary et al. (13) (99%). The diagnostic sensitivity rate of this study (96.3%) is very close to the rate found by Song et al. (26) (97.2%) and higher than the value found by Park et al. (18) (90.2%).

In conclusion, the Architect HCV Ag assay is highly specific, sensitive, reliable, easy to perform, reproducible, cost-effective, and applicable as a screening, supplemental, and preconfirmatory test for anti-HCV assays in the laboratory procedures used for the diagnosis of hepatitis C virus infection.
REFERENCES


