Abstract
The measurement of anti-HCV antibodies using immunological methods and the confirmation of viral
nucleic acid based on molecular methods is important in diagnosis and follow-up of the HCV
infection. 

Objectives: In this study, we aimed to analyse HCV core antigen positivity among anti-HCV antibody positive sera to
determine the significance of testing of HCV core Ag for the laboratory diagnosis of HCV infection, by considering
the correlation between serum HCV core Ag and HCV RNA levels.

Methods: 115 patients suspected of having hepatitis C and who were positive for anti-HCV antibody were investigated
using chemiluminescent and molecular methods. Anti-HCV antibody, HCV core Ag and HCV RNA levels were detected
by the Vitros ECIQ immunodiagnostic system, Architect i2000 system and RT-PCR, respectively.

Results: The sensitivity, specificity, positive and negative predictive values and accuracy rate of HCV core Antigen assay
were detected as 86.5%(83/96), 100%(19/19), 100%(83/83), 59.4%(19/32), 88.7%(102/115) respectively.

Conclusion: HCV core Ag assay could be used for diagnosis of HCV infection as it is easy to perform, cost-effective, has
high specificity and positive predictive value. However, it should be kept in mind that it may have lack of sensitivity
and negative predictive value.

Key Words: HCV, anti-HCV antibody, HCV core Ag, HCV RNA

Introduction
The measurement of antibodies against hepatitis c virus (HCV) using immunological methods and the confirma-
tion of viral nucleic acid based on molecular methodsare important in diagnosis and follow-up of HCV
infection. The most widely used virological test for the diagnosis of HCV infection is the measurement of anti-
HCV antibody in serum, by using chemiluminescent immunoassay (CLIA) or enzyme immunoassay (EIA)
method. Sometimes there is a long seronegative pe-
riod in the course of HCV infection before an anti-HCV antibody can be found in the serum. It has
been reported that immunosuppression can also be a reason for an insufficient antibody response in a large
number of patients. Thus, anti-HCV assay results that show values under the critical value designated by EIA
or CLIA must be confirmed by an additional confirmatory
test, such as the HCV ribonucleic acid (RNA) test, or
with the preconfirmatory HCV core antigen(Ag) as-
say. Nucleic acid testing (NAT) for the detection of
HCV RNA remains the gold standard for diagnosing active HCV infections. However, in comparison with HCV core Ag and anti-
HCV antibody tests, the need for experienced staff, special laboratory conditions and equipment and
the need for standardisation are drawbacks of HCV RNA
assays. Furthermore, depending on exposure to the virus, detection of HCV RNA shows differences
in patients with no antibody found. In the last decade, several HCV core Ag assays have been developed, due to problems associated
with HCV RNA assays. The results of recent studies indi-
cated that measurements of HCV core Ag in serum or plasma can be used as indirect markers of HCV replica-
tion. The majority of the previously used enzyme-linked
immunosorbent assays (ELISAs) or EIA detecting HCV
core Ag may have required time and skill to conduct. However, a fully automated CLIA with higher
sensitivity has been developed to overcome the shortcomings of the conventional core Ag assays.

In this study, we aimed to determine the significance
of testing of HCV core Ag in laboratory diagnosis of
HCV infection, to compare HCV core Ag, anti-HCV antibody and HCV RNA levels, and to investigate the
correlation between serum HCV core Ag levels and HCV RNA levels for the diagnosis of HCV infection.

Materials and methods Serum samples
The study was carried out at Clinical Microbiology
Laboratory of Suleyman Demirel University Medical
Faculty between September 2011 and June 2012. Se-
rum samples which have been detected to be posi-
tive for anti-HCV antibody of 115 patients who had
a prediagnosis of HCV infection were investigated for
the presence of HCV core Ag and HCV RNA using
chemiluminescent and molecular methods. HCV RNA
results were accepted as the gold standard in perform-
ing the comparisons.

Ethical approval
All patients had given informed consent about the study. Ethical approval was provided by the Ethics
Committee of Medical School, Suleyman Demirel Uni-
versity (Isparta, Turkey).

Serological tests
Anti-HCV antibody, HCV core Ag and HCV RNA
levels were detected by the Vitros ECIQ immunodiag-
nostic system (Ortho-Clinical Diagnostics, Raritan,
NJ, USA), Architect i2000 system (Abbott Laboratories,
Abbott Park, IL, USA) and real time polymerase chain
reaction (RT-PCR) (Anatolia Diagnostics and Biotech-
nology Products Inc.), respectively.

Interpretation of the tests
Anti-HCV antibody test results of ≥ 1.00 signal-to-
cutoff (s/co) were considered reactive, while results of
<0.90 s/co were considered non-reactive and results of
≥ 1.00 s/co and <1.00 s/co were considered bor-
deline according to the manufacturers’ instructions.
HCV core Ag test results of <3.00 femtomole/liter (fmol/L) were considered nonreactive, and results of
≥3.00 fmol/L were considered reactive according to
the manufacturers’ instructions. Values between ≥3.00
fmol/L and <10.00 fmol/L were retested in duplicate.

For the calculation of sensitivity and specificity, low
viremia group was included in the positive group.

Statistical analysis
Statistical analyses were performed using IBM SPSS
Statistics version 15.0 (SPSS Inc., Chicago, IL, United
States). Descriptive variables were presented as num-
bers and percentages. Sensitivity was accepted as the probability of be-
ting test positive with the presence of the disease and calculated as (true positive) / (true positive + false
negative). Specificity was accepted as the probability
of being test negative with the absence of the dis-
case and calculated as (true negative) / (true negative
+ false positive). Positive predictive value was accepted as the probability having disease when test is positive
and calculated as (true positive) / (true positive + false
positive). Negative predictive value was accepted as the
probability of not having disease when test is negative
and calculated as (true negative) / (false negative + true
negative).

A receiver operating characteristic (ROC) curve analysis
was performed to determine a cut-off HCV Ag value
in order to justify the cut-off of the manufacturer. A
p < 0.05 was taken (considered) to indicate statistical
significance.

Results
Serum samples were provided from a total of 115 pa-
tients (56.5 %) women and 50 (43.5 %) men. The patients’ ages ranged from 16 to 86 years (57.9 ± 14.5
years). Of the 115 patients with anti-HCV antibody posi-
tivity, 83 were determined as positive, 32 were negative
for HCV core Ag, and 84 were positive for HCV RNA.
In addition, low viremia levels were detected among 12
samples and 19 samples were detected as negative (95%
CI 5.7-17.2 s/co). HCV core Ag and HCV RNA results of
the 115 samples with anti-HCV antibody positivity
are summarized in table 1.
Table 1: The comparison of HCV Ag and HCV RNA results in 115 patients with positive Anti HCV.

<table>
<thead>
<tr>
<th>HCV Ag</th>
<th>In terms of positivity and negativity</th>
<th>In terms of viral load</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10^10</td>
<td>Positive**</td>
<td>PPV*: 100%</td>
</tr>
<tr>
<td>Non determined</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Reactive</td>
<td>83</td>
<td>PPV*: 100%</td>
</tr>
<tr>
<td>Non-reactive</td>
<td>19</td>
<td>NPV*: 59.4%</td>
</tr>
<tr>
<td>Total</td>
<td>115</td>
<td>Accuracy*: 88.7%</td>
</tr>
</tbody>
</table>

**Positive** = Positive result for HCV Ag; **Non determined** = Non determined result for HCV Ag; **Reactive** = Reactive result for HCV Ag; **Non-reactive** = Non-reactive result for HCV Ag.

Comparing the total of 115 anti-HCV antibody positive serum samples with the test results of the HCV core Ag and HCV RNA assays, the sensitivity, specificity and positive and negative predictive values and accuracy rate of HCV core Ag assay were detected as 86.5% (83/96), 100% (19/19), 100% (83/83), 90.4% (19/21) and 88.7% (102/115) respectively.

The sensitivity of the test used in our study was approximately 0.06 pg/ml. The sensitivity of the HCV core Ag assay was 3.00 fmol/l (i.e. 0.06 pg/ml), based on the e11 recombinant Ag.

The ROC curve analysis showed exactly the same sensitivity and specificity rates with our results if HCV core Ag ≥5.455 fmol/l was accepted as a cut-off value. This finding was close to the manufacturer’s cut-off, however we considered that the small difference might be due to the low number of negative patients.

Previous studies have shown that detection of HCV core Ag assay in serum or plasma is useful as an indirect marker of HCV replication due to the good correlation between HCV core Ag and HCV RNA levels. Our specificity and positive predictive values were found as 100% and similar results were obtained in comparison with the other studies showing that there were no false-positive results. However, our sensitivity (86.5%) and negative predictive value (59.4%) were a little bit lower than those of the other studies. Leary et al. demonstrated that the HCV core Ag was detected prior to the appearance of anti-HCV antibody in the patients’ sera and this phenomenon may have resulted in a reduction of the window period by 23 days or even longer. However, since we conducted this study with anti-HCV antibody positive serum samples, we didn’t have any sample with a result like HCV core Ag-reactive and anti-HCV antibody negative, so we were not able to consider whether the early HCV infection without antibodies could be detected using the HCV core Ag assay.

In a study using 152 serum samples to compare HCV RNA with HCV core Ag, Koroglu et al. found that sensitivity, specificity and positive and negative predictive values were 96.9%, 100% and 99.1%, respectively. Furthermore, in a similar comparison of 212 serum samples with anti-HCV antibody positivity, Keski et al. found that sensitivity, specificity and positive and negative predictive values were 96.3%, 100%, 100% and 89.7%, respectively. In addition, Park et al. obtained similar results comparing HCV RNA with HCV core Ag in 282 serum samples; sensitivity, specificity and positive and negative predictive values were determined to be 90.2%, 100%, 100% and 86.4%, respectively.

Consequently, all positive results found by the HCV core Ag assay were also positive with the HCV RNA assay. However, all negative results found by the HCV core Ag assay were not negative with the HCV RNA assay. Thus, it can be concluded that the positive results of the HCV core Ag assay can be reported as positive. However, when there is a serum sample showing anti-HCV antibody positivity, the negative results found by the HCV core Ag assay should be also confirmed by a HCV RNA assay.
References