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# Prediction of Hepatitis C Virus Viremia and Determination of Signal-to-cut-off Value Using Roche Elecsys Anti-HCV Screening Test

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## ABSTRACT

**Objective:** For the diagnosis of hepatitis C virus (HCV), the detection of HCV antibodies by serological tests is primarily performed using enzyme immunoassays and chemiluminescence-based methods, and positive results are confirmed by HCV ribonucleic acid (RNA) testing. This study aimed to evaluate the performance of the anti-HCV test and to determine the optimal signal-to-cut-off (S/CO) ratio for predicting viremia.

**Methods:** Anti-HCV levels in serum samples were analyzed using the electrochemiluminescent immunoassay method, while HCV RNA was detected in plasma samples using real-time polymerase chain reaction.

**Results:** A total of 1,010 anti-HCV-reactive patients (474 males, 536 females) were included. The median age was 52 years for males and 62 years for females ($p<0.001$). HCV RNA positivity was detected in 16.6% (168/1,1010) of the patients. The median anti-HCV S/CO value was 48.70 in HCV-RNA-positive individuals and 37.65 in HCV-RNA-negative individuals ($p<0.001$). All patients with an S/CO ratio <1.25 were HCV RNA negative, whereas those with an S/CO ratio >293 were HCV RNA positive. Receiver operating characteristic analysis yielded an area under the curve (AUC) of 0.59 (95% confidence interval: 0.55-0.82), with an optimal S/CO of 8.23 yielding 95.2% sensitivity and 36% specificity.

**Conclusion:** The optimal S/CO value yielding the highest sum of sensitivity and specificity was determined to be 8.23. Although an S/CO of 8.23 showed promise for detecting viremia in our cohort, the relatively low AUC of 0.59 suggests that its utility may be limited and that it should be interpreted cautiously, particularly in low-prevalence populations.

**Keywords:** Hepatitis C virus, immunoassay, polymerase chain reaction, ROC curve, sensitivity

## INTRODUCTION

Hepatitis C virus (HCV) belongs to the *Flaviviridae* family and has a positive-sense, single-stranded ribonucleic acid (RNA) genome (1). This virus, transmitted primarily through parenteral routes, is common among hemodialysis patients and intravenous drug users (2). HCV infection is recognized as an important cause of liver-related health problems worldwide (3,4). According to the World Health Organization data, the Eastern Mediterranean and Europe report the highest HCV infection rates, at 2.3% and 1.5%, respectively. In other regions, the frequency of HCV infection is estimated to range between 0.5% and 1% (5). In Türkiye, a study conducted in 2012 reported that the prevalence of anti-HCV was between 0.5% and 1% (6).

HCV diagnosis typically commences with detection of antibodies using enzyme immunoassay (EIA) (7). HCV antibody reactivity is assessed by the signal-to-cut-off (S/CO) ratio, calculated as the optical density of the test sample divided by the cut-off value (8). While these tests exhibit high sensitivity and specificity, false-positive results are frequently encountered in populations where anti-HCV prevalence is below 10% (9,10). To confirm the presence of an active infection, HCV RNA testing is recommended for individuals with a positive anti-HCV antibody result (9). However, a positive anti-HCV test does not always indicate an active infection; it may reflect ongoing viremia, a previously resolved infection, or a false-positive result (4).

False-positive results may occur, especially in cases with low-titer HCV antibody reactivity. These results pose diagnostic challenges for low-risk groups, immunocompromised individuals, and populations without liver disease. Reported false-positive rates range from 15% to 60% among healthcare workers, blood donors, individuals with sexually transmitted infections, and those with healthy immune systems (11,12).

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Anti-HCV antibodies are typically detected in viremic patients and in individuals with resolved infections (7). The critical role of anti-HCV antibody S/CO in predicting viremia has been reported in the literature (6). Researchers have investigated estimates of viremia produced by various commercial immunoassay kits. S/CO cut-offs reported for predicting viremia across these kits include: Abbott HCV EIA 2.0 (≥3.8), Ortho HCV version 3.0 EIA (≥3.8), Vitros anti-HCV (≥8), AxSYM anti-HCV (≥10.0), Architect anti-HCV (≥5.0), and Advia Centaur HCV (≥11.0). All these cut-offs demonstrate a positive predictive value (PPV) of ≥95% (13). High anti-HCV S/CO values are associated with HCV RNA positivity, suggesting that the S/CO value can be a predictor of viremia. Therefore, determining the optimum S/CO values for diagnosing HCV infection remains important (2,5,13).

This study aims to determine the optimal S/CO ratio of a commercial immunoassay kit for predicting true antibody positivity and HCV RNA viremia among anti-HCV-reactive patients.

## METHODS

Ethical approval for this study was obtained from the University of Health Sciences Türkiye, Ankara Etlik City Hospital Ethics Committee (decision no: AEŞH-BADEK-2024-806, date: 28.08.2024). The study was conducted in accordance with the Declaration of Helsinki.

Retrospective analysis was conducted on results from patients whose serum samples processed by the University of Health Sciences Türkiye, Ankara Etlik City Hospital Hospital Microbiology Virology Laboratory showed anti-HCV reactivity between October 2022 and January 2024 and for whom concurrent HCV RNA testing was performed. During this period, all individuals who presented to our hospital with a reactive Roche Elecsys anti-HCV screening test result were included in the study.

The primary inclusion criteria were patients identified as positive or reactive by the Roche Elecsys anti-HCV screening test who had undergone an HCV RNA polymerase chain reaction test within the same timeframe as the anti-HCV reactivity detection (preferably within 30 days).

During the study period, patients with missing HCV RNA results, those with results obtained outside the study period, or those who underwent more than one anti-HCV or HCV RNA test were excluded to ensure uniformity in data evaluation. However, since HCV RNA testing was not universally performed for all anti-HCV-reactive patients, a degree of selection bias may have affected the observed viremic rate.

Due to the retrospective nature of our study, it was not possible to systematically access detailed clinical information such as patients' immunosuppressive status, prior HCV treatment history, or chronic comorbidities, nor to comprehensively evaluate the potential effects of these factors on anti-HCV antibody levels. This was acknowledged as a fundamental limitation of the study and was taken into account when interpreting the results.

Anti-HCV testing in serum samples was performed using the electrochemiluminescence immunoassay method on a Cobas® 8000 analyzer with the Elecsys anti-HCV kit (both from Roche Diagnostics GmbH, Germany). This test kit uses recombinant antigens derived from the structural core protein and the non-structural NS3 and NS4 proteins encoded by the HCV genome. According to the Elecsys anti-HCV test criteria, samples with a cut-off index below 0.9 were considered non-reactive, those between 0.9 and 1.0 were considered borderline, and those with values of 1.0 or higher were considered reactive. All samples with borderline results were reanalyzed in accordance with the manufacturer's instructions. The study group consisted of patients who were anti-HCV reactive and had HCV RNA test results available.

HCV RNA detection was carried out using the Roche Cobas® 8800 system, which integrates both viral RNA isolation and amplification processes. RNA extraction and quantitative assessment of HCV RNA were performed using the RNA Isolation Kit and the HCV Quantitative Nucleic Acid Test Kit (Roche Diagnostics GmbH, Germany).

Patient results were retrospectively analyzed using the laboratory information management system.

### Statistical Analysis

Data analysis was conducted using SPSS version 25 (SPSS Inc., Chicago, IL, USA). The normality of variable distributions was assessed by both visual inspection and the Kolmogorov-Smirnov test. To compare median values between two groups, the Mann-Whitney U test was applied, whereas the Kruskal-Wallis test was employed for comparisons across multiple age categories. The association between categorical variables was examined using the Pearson's chi-square test.

The HCV RNA test was considered the reference (gold standard) method, and the effectiveness of the anti-HCV test in detecting viremia was assessed by receiver operating characteristic (ROC) curve analysis. Key cut-off values were identified using ROC analysis, and the sensitivity, specificity, PPV, and negative predictive value (NPV) were calculated accordingly, along with their 95% confidence intervals (CI). No outlier exclusion or data transformation was applied to S/CO values prior to ROC analysis; all analyses used raw S/CO values. Statistical significance was defined as $p<0.05$.

## RESULTS

A total of 1,010 patients with anti-HCV reactivity [474 males (46.9%) and 536 females (53.1%)] were included in the study. The median age was 52 years (range: 2-92 years) for male patients and 62 years (range: 2-101 years) for female patients; this difference was statistically significant ($p<0.001$). The median anti-HCV S/CO values were 47.1 for males and 35.5 for females ($p=0.01$).

HCV RNA positivity was observed in 16.6% (168/1,010) of anti-HCV-reactive patients. The median ages of HCV RNA-positive male and

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female patients were 36 and 62 years, respectively (p=0.002). The median anti-HCV S/CO values were 67.17 (range, 1.25-379) in HCV RNA-positive individuals and 37.65 (range, 1.25-293) in HCV RNA-negative individuals; this difference was statistically significant (p<0.001; Table 1). All 49 patients with an anti-HCV S/CO ratio <1.25, including those with low S/CO values above the reactive cut-off (≥1.0), were HCV RNA negative, whereas all 9 patients with an S/CO ratio >293 were HCV RNA positive.

The lowest HCV RNA positivity rate (2.4%) was observed in the group with S/CO values between 1 and 4, whereas the highest positivity rate (23.8%) was observed in the group with S/CO values ≥15 (p<0.001) (Table 2). According to the ROC analysis, the sensitivity, specificity, PPV, and NPV at an S/CO threshold of 8.23 were 95.2%, 36%, 22.9%, and 97.4%, respectively (Table 3). The area under the curve (AUC) was 0.59 (95% CI: 0.55-0.82); this was statistically significant (p<0.001).

The ROC curve illustrating the predictive performance of the anti-HCV screening test for HCV viremia is presented in Figure 1.

**Table 1. Basic features of patients according to HCV RNA levels**

| | All patients (n=1010) | Viremia group (n=168) | Non-viremia group (n=842) | p-value† |
|---|---|---|---|---|
| Male, n (%) | 474 (46.9) | 93 (55.4) | 381 (45.2) | 0.017 |
| Female, n (%) | 536 (53.1) | 75 (44.6) | 461 (54.8) | |
| Age*, year | 58 | 46.5 | 59 | 0.007 |
| Age range | 2-101 | 11-97 | 2-101 | |
| Anti-HCV S/CO* (S/CO§ range) | 48.70 (1.01-379) | 67.17 (1.25-379) | 37.65 (1.01-293) | <0.001 |

*: Median, §: S/CO: Signal-to-cut-off, †: Pearson's chi-square (comparison by gender), Mann-Withney U test (comparison by age and anti-HCV S/CO values), HCV: Hepatitis C virus, RNA: Ribonucleic acid

**Table 2. HCV RNA positivity dispersion of anti-HCV reactive patients**

| HCV RNA | | | | |
|---|---|---|---|---|
| **Anti-HCV S/CO*** | **Positive, n (%)** | **Negative, n (%)** | **All, n (%)** | **p-value†** |
| 1-4 | 6 (2.4) | 242 (97.6) | 248 (100) | |
| 5-9 | 7 (7.3) | 89 (92.7) | 96 (100) | |
| 10-14 | 2 (8.7) | 21 (91.3) | 23 (100) | <0.001 |
| ≥15 | 153 (23.8) | 490 (76.2) | 643 (100) | |
| ≥1 (All reactive results) | 168 (16.6) | 842 (83.4) | 1010 (100) | |

*: S/CO: Signal-to-cut-off value, †: Pearson's chi-square, HCV: Hepatitis C virus, RNA: Ribonucleic acid

**Table 3. The performance of anti-HCV ECLIA test for prediction of HCV viremia**

| Parameters | Anti-HCV S/CO ratios and test performances, (%) | | | | |
|---|---|---|---|---|---|
| S/CO | 1.24 | 7.54 | 8.23 | 15.01 | 100.10 |
| Sensitivity (%) | 100 | 95.2 | 95.2 | 91.1 | 82.7 |
| Specificity (%) | 4.5 | 35.2 | 36.0 | 41.8 | 26.7 |
| PPV (%) | 17.3 | 22.7 | 22.9 | 23.8 | 18.4 |
| NPV (%) | 100 | 97.4 | 97.4 | 95.9 | 88.6 |

S/CO: Signal-to-cut-off, PPV: Positive predictive value, NPV: Negative predictive value, ECLIA: Electrochemiluminescence immunoassay, HCV: Hepatitis C virus

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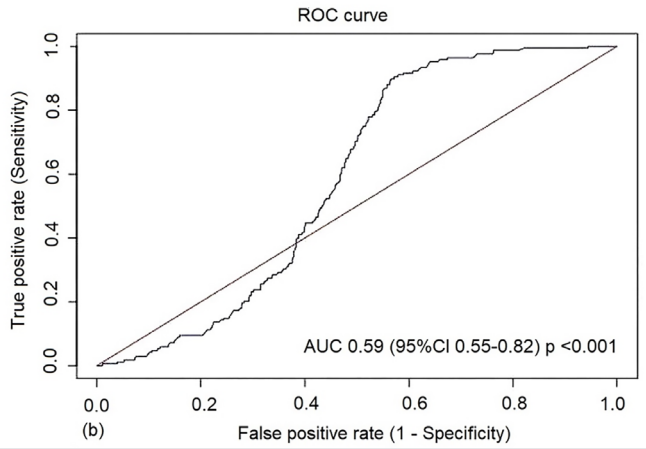


**Figure 1.** Diagnostic performance of the anti-HCV screening test
*AUC: Area under the curve, ROC: Receiver operating characteristic, HCV: Hepatitis C virus*

## DISCUSSION

The accuracy of EIA tests in diagnosing HCV has improved markedly since anti-HCV screening was first introduced in 1989. Although these tests are reported to have an accuracy exceeding 99%, false-positive rates remain high in low-prevalence populations (13). Several factors can contribute to false-positive anti-HCV results, including elevated gamma globulin levels (e.g., individuals of African descent, patients with multiple myeloma, or individuals with rheumatoid factor), liver diseases, autoimmune disorders, viral infections such as human immunodeficiency virus or hepatitis B virus, previous vaccinations, prolonged serum storage, and temperature fluctuations (14).

In 2003, the CDC proposed a diagnostic algorithm that included the S/CO ratio, nucleic acid amplification test (NAAT), and recombinant immunoblot assay (RIBA). However, in 2013, RIBA was removed from the algorithm, and only NAAT was retained as a confirmatory method. As a result, distinguishing between false-positive anti-HCV results and past infections became more challenging (15). Anti-HCV reactivity may indicate viremia, a past infection, or a false-positive result (4). False-positive results not only complicate diagnosis but also lead to unnecessary additional testing and patient anxiety, underscoring the importance of improved predictive cut-offs (8,14,16).

In this study, no viremia was detected in patients with anti-HCV S/CO ≤1.24, while HCV RNA positivity was found in 23.8% of those with S/CO ≥15. Production of anti-HCV antibodies results from antigenic stimulation due to viral replication, and higher anti-HCV antibody levels are often correlated with increased viral stimulation (17). The optimal S/CO cut-off was determined to be 8.23, yielding 95.2% sensitivity and 36% specificity. However, the ROC AUC was only 0.59, indicating limited diagnostic performance. This high sensitivity underscores the test's potential clinical value, especially for early detection of HCV infection in large-scale screening programs and resource-limited settings. Furthermore, it may reduce the need for more costly HCV RNA testing.

Although this cut-off is comparable to values reported in regional studies, its relatively low AUC limits general applicability. Therefore, our findings should be interpreted as context-specific data, reflecting the characteristics of our study population and the Elecsys testing platform, rather than as a universally applicable diagnostic cut-off. Regional variations are evident in the literature, potentially reflecting differences in population characteristics, genotype distribution, disease prevalence, and healthcare settings. For instance, Huang et al. (18) in Taiwan (low prevalence, predominantly genotype 1b) reported no HCV RNA detection in patients with S/CO ≤10; for values >10, sensitivity and specificity were both 81%. In Korea, Seo et al. (4) in a study with high screening coverage and mixed genotype distribution, reported 94.4% sensitivity and 97.3% specificity for an S/CO of 10.9. Tiwari et al. (19) in India (higher prevalence and genotype 3 predominance), found 95% sensitivity and 92% specificity for an S/CO ≥6. Moretti et al. (20) conducted a study in Italy and recommended cut-offs of 10.3 for 95% PPV and 3.0 for NPV. In Türkiye, Gülseren et al. (21) in Balıkesir reported anti-HCV cut-offs of 8.9 and 5.0 that were highly predictive of viremia. Similarly, Şanlıdağ et al. (22) in Manisa observed a strong correlation between anti-HCV levels (S/CO) and HCV RNA. Furthermore, Fidan et al. (23) in Ankara reported a 56.2% RNA positivity among patients with S/CO values greater than 10; no viremia was detected in any patient with S/CO values below 3.0.

Recent studies have proposed different S/CO cut-offs, varying by test system and patient demographics. In a study conducted in İstanbul, Türkiye, Sarıkaya et al. (24) determined a cut-off of 10.86 for Elecsys, with 96.1% sensitivity and 61.2% specificity. Although this is higher than the cut-off in our study, it still provides strong predictive value. Kang et al. (25) conducted a study in Korea and reported an AUC of 0.970, sensitivity of 99.7%, and specificity of 87.5% at S/CO=8; they identified S/CO=5 as a cut-off or ruling out false positives. Our value of 8.23 aligns with these findings. Nevertheless, the lower AUC in our study indicates limited discriminatory power and highlights the importance of interpreting S/CO values in relation to local population dynamics, testing protocols, and prevalence settings. Given the variability observed, each laboratory should consider establishing its own decision limits tailored to the population it serves and the test platform it uses.

Dabanlıoğlu et al. (26) reported a sensitivity of 72% and a specificity of 88% at an S/CO cut-off of 15.4. Such a high cut-off may help reduce false positives and lower costs. As shown in Table 4, the distribution of S/CO values and corresponding HCV RNA positivity rates in our cohort further illustrates this relationship, highlighting the trade-off between sensitivity, specificity, and cost-effectiveness when selecting an optimal cut-off (4,18,19,21,22,24-26).

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**Table 4. Comparison of S/CO values, sensitivity, and specificity of different immunoassay methods in predicting HCV viremia**

| Country | S/CO value | Sen. (%) | Spe. (%) | Assay | Manufacturer | Format | Ref. |
|---|---|---|---|---|---|---|---|
| Korea | 10.9 | 94.4 | 97.3 | Architect | Abbott Lab. | CLIA | (4) |
| Taiwan | 10.0 | 81.0 | 81.0 | AxSYM HCV 3.0 | Abbott Lab. | MEIA | (18) |
| India | 6.0 | 95.0 | 92.0 | Vitros® | Ortho-clinical Diag. | CLIA | (19) |
| Korea | 8.0 | 99.7 | 87.5 | Architect | Abbott Lab. | CLIA | (25) |
| Türkiye | 8.9 | 93.0 | 91.0 | Architect | Abbott Lab. | CLIA | (21) |
| Türkiye | 5.0 | 95.6 | 52.7 | Architect<br>LiaisonXL Murex | Abbott Lab.<br>DiaSorin | CLIA<br>CLIA | (22) |
| Türkiye | 10.86 | 96.1 | 61.2 | Elecsys | Roche Diag. | ECLIA | (24) |
| Türkiye | 15.4 | 72.0 | 88.0 | Architect | Abbott Lab. | CLIA | (26) |
| Türkiye* | 8.23 | 95.2 | 36.0 | Elecsys | Roche Diag. | ECLIA | - |

*: This study, S/CO: Signal-to-cut-off, HCV: Hepatitis C virus, Sen.: Sensitivity, Spe.: Specificity, Ref: Reference, Diag.: Diagnostics, Lab: Laboratories, MEIA: Microparticle enzyme immunoassay, CLIA: Chemiluminescent immunoassay, ECLIA: Electrochemiluminescence immunoassay

Although the predictive power of S/CO values for viremia is well-supported, their use should not rely solely on a cut-off-based interpretation. Our results emphasize that specificity and diagnostic performance vary significantly based on test kit, population, and epidemiological context (24-27). Recent evidence also highlights the importance of accounting for false-positive rates and performance differences across platforms when making clinical decisions (24-27). Therefore, when constructing diagnostic algorithms based on S/CO, factors such as platform compatibility and false-positive risk must be considered.

Kang et al. (25) reported false-positive rates up to 45% in low-prevalence settings. Dabanlıoğlu et al. (26) reported a false-positive rate of 13.9%. Both studies support the incorporation of S/CO values into diagnostic workflows. Cho et al. (27) reported that Elecsys had an AUC of only 0.432 and performed worse than the Atellica and Alinity systems. These findings, along with those by Sarıkaya et al. (24), Kang et al. (25), and Dabanlıoğlu et al. (26), indicate that laboratories should establish system-specific cut-off values based on local data.

Sarıkaya et al. (24) reported S/CO values in patients with HCV genotype 1b; however, their study did not specifically examine the association between genotype and S/CO. Nonetheless, S/CO values remain potential virological markers for patient risk stratification, with low values (<3-5) indicating a low likelihood of viremia (14,24-28). Further research is needed to determine its independence from genotype.

### Study Limitations

Several limitations of this study should be acknowledged. Because it was designed as a retrospective epidemiological study, it was not possible to assess whether the patients had received treatment. Additionally, only patients with both anti-HCV reactivity and concurrent HCV RNA results were included. This inclusion criterion may have introduced selection bias, as patients who underwent HCV RNA testing could represent a subgroup with higher clinical suspicion of active infection, different demographic characteristics or comorbidity profiles, or specific risk factors compared with all anti-HCV-reactive individuals. Consequently, the observed viremic rate in our sample may be overestimated relative to the general anti-HCV-reactive population. This limitation reduces the external validity (generalizability) of our findings and should be considered when interpreting the results and when comparing them with studies that include broader or unselected populations. The modest AUC value in our dataset may also stem from patient-related variables, such as advanced age and comorbidity burden, both of which can affect the immune response and reduce the predictive performance of antibody-based assays, such as Elecsys. These variables should be taken into account when comparing diagnostic accuracy across studies.

## CONCLUSION

This study found that the diagnostic performance of anti-HCV S/CO values in detecting viremia was limited, as indicated by ROC analysis. These findings suggest that S/CO values alone are insufficient to reliably predict HCV viremia and should be interpreted in conjunction with confirmatory testing and population-specific characteristics.

### Ethics

**Ethics Committee Approval:** Ethical approval for this study was obtained from the University of Health Sciences Türkiye, Ankara Etlik City Hospital Ethics Committee (decision no: AEŞH-BADEK-2024-806, date: 28.08.2024).

**Informed Consent:** Retrospective study.

### Footnotes

**Author Contributions:** Concept - A.B., M.F.K., G.K.; Design - A.B., M.F.K., G.K.; Data Collection and/or Processing - A.B., M.F.K.; Analysis and/or Interpretation - A.B., M.F.K.; Literature Search - A.B., M.F.K., G.K.; Writing - A.B., M.F.K., G.K.

**Conflict of Interest:** The authors have no conflict of interest to declare.

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