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Determination of anti-HCV signal to cut-off value in patients with hepatitis C virus infection and the variety of antibody responses

Murat Ocal, Mehmet Emin Bulut

Department of Medical Microbiology, University of Health Sciences, Şişli Hamidiye Etfal Training and Research Hospital, İstanbul, Turkey

ABSTRACT

Objectives: The diagnosis of hepatitis C virus (HCV) infection starts with the detection of antibodies against recombinant or synthetic HCV proteins by Enzyme Immunoassay (EIA). Although EIA tests are highly sensitive, false positivity rates are not low. Positive anti-HCV results are generally confirmed with complementary tests such as Nucleic Acid Amplification Tests (NAAT), or Western Blot modifications.

Methods: The anti-HCV results of 199,516 individuals referred from various clinics between 2015 and 2019 were evaluated retrospectively at University of Health Sciences, Şişli Hamidiye Etfal Training and Research Hospital, Medical Microbiology Laboratory. From the 2039 samples, of which EIA tests resulted borderline and reactive, 1419 samples having Line Immunoassay (LIA) confirmatory test results were included in the study.

Results: LIA tests yielded positive, negative and indeterminate for 820 (57.8%), 519 (36.6%) and 80 (5.6%) of 1419 samples, respectively. The optimal threshold point for EIA anti-HCV signal to cut-off (S/Co) according to LIA was found to be 15.85 corresponded to diagnostic sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of 94.9%, 94.8%, 96.6%, 92.1%, 94.9%, respectively. The most common proteins detected in LIA positive samples were C1 96.3%, C2 90.4%, and NS3 93.2%.

Conclusions: To prevent false positivities, confirmatory tests must be used for samples with low S/Co ratios. The use of S/Co value will make significant contribution to reducing both false-positive results and the LIA confirmatory test consumption. There was no correlation between the number of bands and EIA index values in LIA positive samples, while the relationship between the number of 3+ bands and index values was remarkable.

Keywords: Hepatitis C virus, signal to cut-off, enzyme immunoassay, line immunoassay, confirmatory test

Hepatitis C virus (HCV) is 40-50 nm in diameter, enveloped, single-stranded RNA virus classified in *Flaviviridae* family [1]. HCV is a chronic hepatitis agent with worldwide distribution which infects more than 170 million people posing a serious public health threat, yet has no protective vaccine [2, 3]. It is estimated that 3% of the world population is infected with HCV, and the prevalence is between 0.5-1% in Turkey [4]. Although hepatitis C is usually in form of asymptomatic infection, it also becomes chronic at a rate of

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Address for correspondence: *Mehmet Emin Bulut, MD., University of Health Sciences, Şişli Hamidiye Etfal Training and Research Hospital, Department of Medical Microbiology, İstanbul, Turkey. E-mail: eminbulut212@gmail.com, GSM: +90 532 353 77 41*

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75-85%. Cirrhosis develops in approximately 20-30% of patients with HCV infection within 20 years and total of 1-4% of patients develop hepatocellular carcinoma [5]. Although a meta-analysis study showed that the global incidence of HCV infection decreased, mathematical models show that deaths due to secondary liver disease in HCV infection will continue to increase [6].

Laboratory diagnosis of HCV infection needs to be reliable [7]. False positivity is an important issue in terms of time, cost and patient's psychological state [7, 8]. Since there are no neutralizing antibodies against HCV, anti-HCV can be detected not only in chronic hepatitis C patients, but also in most HCV patients who recover. For this reason, it cannot be clearly differentiated whether anti-HCV positivity indicates current or past infection [8]. A positive anti-HCV result can indicate active (acute or chronic) or past HCV infection, as well as false positivity [8, 9]. The healthy carriers of HCV infection may show a specific antibody response to HCV antigens, which may play role in disease control. Detecting these antibodies may allow this response to be fully characterized, which can identify specific antibodies that have potential clinical value [10].

The structural proteins of HCV processed by cellular proteases are core proteins (C), envelope glycoproteins E1, E2, and non-structural NS2, NS3, NS4A, NS4B, NS5A, NS5B proteins, which are processed by viral proteases [11]. The diagnosis of HCV infection starts with the detection of antibodies against recombinant or synthetic HCV proteins by Enzyme Immunoassay (EIA) [12]. First-generation EIA tests are intended to detect the antibodies against the c100-3 epitopes from the NS4 region. Core and NS3 regions were added to the second generation tests, and NS5 region epitopes were added to the third generation EIA tests in addition to the core and NS3 regions. With the use of third-generation EIA anti-HCV test, the HCV infection has become detectable at 7-8th weeks after transmission [7, 8, 13].

Although EIA tests are highly sensitive, false positivity rates are not low. False positivity is more likely to occur in populations with low prevalence [14]. Positive anti-HCV results are generally confirmed with complementary tests [12]. Among the confirmatory or complementary tests, the most widely used tests are Nucleic Acid Amplification Tests (NAAT), or Western Blot modifications, ie. Recombinant Immunoblot Assay (RIBA) and Line Immunoassay (LIA) tests [3, 15]. The U.S. Centers for Disease Control and Prevention (CDC) recommended a diagnostic algorithm in 2003, which included RIBA and NAAT to determine positivity in anti-HCV screening tests at signal to cut-off (S/Co) rates [16]. In 2013, an updated algorithm was released that included testing anti-HCV positive samples using only NAAT (9). A negative RIBA result usually indicates false positive anti-HCV screening test, except for the early stage of acute infection and immunosuppression, while a positive RIBA result indicates current or past infection [17]. Determining HCV-RNA with Polymerase Chain Reaction (PCR) is considered as the gold standard method to evaluate viremia in patients during antiviral treatment and follow-up and is used to confirm the diagnosis of HCV infection [14]. However, using only the NAAT testing, may lead to challenges in the diagnosis in patients with recovered/past HCV infection [8]. While both NAAT and antibody confirmatory tests (RIBA and LIA) are used in Europe as complementary tests, there are no Food and Drug Administration (FDA) approved confirmatory antibody test commercially available in United States (US) [18].

When anti-HCV S/Co ratios are evaluated, it has been suggested that low levels may be associated with false positive results while high levels may reflect actual infection status and can be used to predict HCV viremia [19, 20]. However, the optimal S/Co values showing the actual infection status might vary from one manufacturer to another. For this reason, during HCV diagnosis, differences in S/Co values that are found by using various commercial tests should be taken into account [14].

The purpose of the present study was to determine S/Co ratios in anti-HCV reactive individuals, to evaluate the predictive performance of EIA with LIA testing, and examine the diversity of antibody responses against various HCV proteins in individuals infected with HCV.

METHODS

Patients and Samples

The anti-HCV results of 199,516 individuals referred from various clinics between 2015 and 2019 were

evaluated retrospectively at University of Health Sciences, Şişli Hamidiye Etfal Training and Research Hospital, Medical Microbiology Laboratory. From the 2039 samples, of which EIA tests resulted borderline and reactive, 1419 samples having LIA confirmatory test results were included in the study, after exclusion of samples that were previously confirmed.

Serological Diagnosis

Enzyme Immunoassay (EIA) Method (Elecsys Anti-HCV II, Roche Diagnostics, Germany) was used for anti-HCV screening test. For EIA, in line with the manufacturer's instructions, the samples with S/Co index value < 0.9 were considered nonreactive, those with ≥ 0.9 to < 1 were considered borderline, and ≥ 1 as reactive. The borderline or reactive results were retested with the same test kit. Confirmatory test was performed for the samples with S/Co index value ≥ 0.9.

The confirmatory test was performed by using the Semi-Quantitative LIA (INNO-LIA HCV Score, Innogenetics-Belgium). The INNO-LIA HCV Score Test is intended for detecting the antibodies from C1, C2 from the core area, E2 from the hypervariable region (HVR), NS3 from the helicase region, and NS4, NS5 region antigens. The band reactivity was evaluated with visual calibration against IgG control bands on each strip. The bands were evaluated as negative (no bands), +/-, level 1+, level 3+. The LIA results were interpreted as follows:

1-A sample is NEGATIVE for HCV antibodies:

- if all HCV antigen lines have a negative reactivity rating,
- if only one HCV antigen line has a reactivity of ±, except when the reactivity is observed for NS3.

2-A sample is POSITIVE for HCV antibodies:

- if at least two HCV antigen lines have a reactivity of ± minimum or higher.

3-A sample is considered INDETERMINATE for HCV antibodies:

- if one HCV antigen line has a reactivity rating of 1+ or higher,
- if the NS3 line reacts with a reactivity of ± or higher and all other antigen lines are negative.

Statistical Analysis

SPSS 15.0 for Windows was used for statistical analysis. The descriptive statistics were given as numbers and percentages for categorical variables; and as mean values, standard deviations, and minimum-maximum values for numeric variables. In independent groups, the comparisons of numeric variables were made with the Kruskal Wallis Test in more than two group comparisons since the normal distribution condition was not met. The subgroup analyses were made with the Mann Whitney U-test. The cut-off point was selected with the analysis of the "Received Operator Characteristic (ROC)" Curve. The sensitivity, specificity, positive predictive value, and negative predictive value calculations were made for the resulting cut-off value. The statistical alpha significance level was taken as $p < 0.05$.

RESULTS

From the 2039 samples, of which EIA tests resulted in borderline and reactive, 1419 samples were included in the study, after exclusion of samples that were previously confirmed. LIA tests yielded positive, negative and indeterminate for 820 (57.8%), 519 (36.6%) and 80 (5.6%) of the 1419 samples, respectively (Table 1).

Table 1. Numerical data of EIA index value and LIA

		Mean ± SD (Min - Max)	Median (IQR)
EIA Index Value		36.3 ± 39.4 (0.91-216.4)	26 (3.18-52)
		n (%)	
EIA	S/Co 0.9 - 1.0	31 (2.2)	
	S/Co ≥ 1	1388 (97.8)	
LIA	Indeterminate	80 (5.6)	
	Negative	519 (36.6)	
	Positive	820 (57.8)	

EIA = Enzyme Immunoassay, LIA = Line Immunoassay, IQR = Interquartile range, S/co= signal to cut-off, SD = standard deviation, Min = minimum, Max = maximum

Table 2. The EIA S/Co values relative to LIA results

		EIA	
		Mean ± SD (Min-Max)	**Median (IQR)**
LIA	Indeterminate	26.3 ± 22.9 (2.8-117.5)	20.3 (7.3-40.1)
	Negative	4.39 ± 6.46 (0.91-59.69)	1.99 (1.29-4.23)
	Positive	57.4 ± 38.8 (3.1-216.4)	45.4 (30.4-71.8)
	p value	**< 0.001**	
All subgroup comparisons $p < 0.001$			

EIA = Enzyme Immunoassay, LIA = Line Immunoassay, IQR = Interquartile range, SD = standard deviation, Min = minimum, Max = maximum

A total of 465 (56.7%) of the individuals who were found positive with LIA were female and 355 (43.3%) were male while the mean age of patients was 56.1 ± 18.3 years.

According to the confirmation test results, the mean S/Co value of the positive group was found to be significantly higher compared to the indeterminate and negative groups while mean value of the indeterminate group was significantly higher compared to the negative group (all comparisons $p < 0.001$) (Table 2). EIA test minimum, maximum, 25-75% percentile, and median index values are shown in Fig. 1 along with LIA results.

Indeterminate results were excluded from the study, and the ROC curve was drawn to determine the optimal screening test cut-off value. The Area Under the Curve was 0.987 (SE:0.003) (Fig. 2). The optimal threshold point for EIA anti-HCV S/Co according to

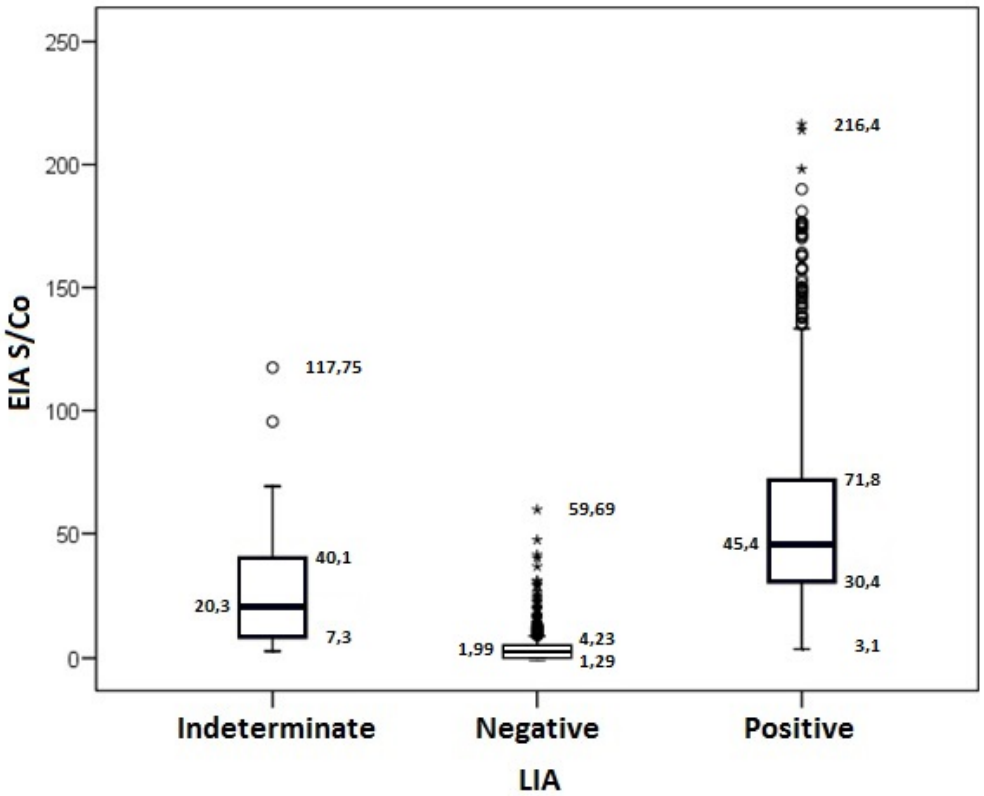

Fig. 1. Box-and-whisker plot for EIA anti-HCV S/Co levels relative to LIA test results. The horizontal line within each box represents median value, top and bottom of each box represent the 25th and 75th percentiles, respectively.

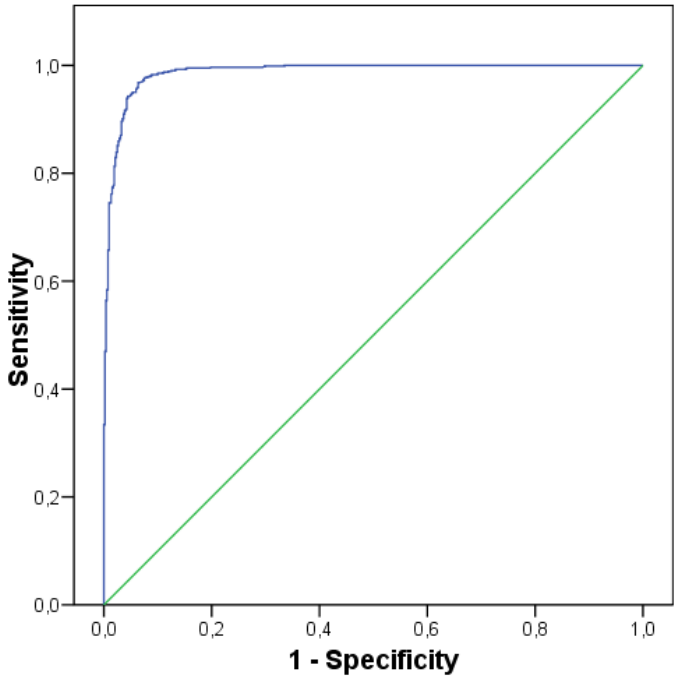

Fig. 2. LIA test ROC curve based on anti-HCV EIA results (AUC:0.987 (SE:0.003) 95% CI 0.982-0.992).

LIA was found to be 15.85 and following values were found for threshold point; sensitivity of 94.9%, specificity of 94.8%, positive predictive value (PPV) of 96.6%, negative predictive value (NPV) of 92.1%, accuracy of 94.9%.

The C1, NS3 and C2 bands were most commonly detected among the positive samples, while NS3 was the most frequently detected HCV antigen band in in-

Table 3. Protein band counts and percentages in LIA-positive and indeterminate samples

LIA		C1	C2	E2	NS3	NS4	NS5
Positive	(n)	790	741	418	764	590	212
	%	96.3	90.4	51	93.2	71.9	25.8
Indeterminate	(n)	22	8	2	44	4	0
	%	27.5	10	2.5	55	5	0

LIA = Line Immunoassay

Table 4. S/Co mean values relative to positive band counts

Positive Band Counts	n	%	Mean S/Co
2	83	10.1	50
3	129	15.7	61
4	213	26	68
5	260	31.7	56
6	135	16.5	45
Total	820	100	

S/co = signal to cut-off

determinate samples (Table 3).

When multiple-band positivity was examined, 5-band positivity (31.7%) was the most common, followed by four-band (26%), six-band (16.5%), three-band (15.7%) and two-band (10.1%) positivity. The S/Co mean values by the number of positive bands are given in Table 4.

DISCUSSION

With the help of new technologies, significant progress was made in identifying patients who are infected with HCV. It is important to screen general population, blood and organ donors to prevent the spread of HCV. More than %95 of individuals infected with HCV can full recovery with correct diagnosis and new treatment methods. The complications of HCV can be significantly reduced as a result of a successful diagnosis and treatment [13]. With the help of new technologies, significant progress was made in identifying patients who are infected with HCV. It is important to screen general population, blood and organ donors to prevent the spread of HCV. More than %95 of individuals infected with HCV can full recovery with correct diagnosis and new treatment methods. The complications of HCV can be significantly reduced as a result of a successful diagnosis and treatment [13].

The addition of more antigens to third-generation EIA used as screening tests increased sensitivity, however, this also increased the false positivity rates. To prevent false positive results, confirmatory tests must be used on samples with low S/Co ratios [21]. CDC recommends determining anti-HCV S/Co values that reflect true positivity in individuals, in order to reduce false positive results, particularly in populations with low prevalence of HCV [22]. The actual positive S/Co ratio may differ for each manufacturer's products. Regardless of the prevalence, laboratories could determine their optimal S/Co ratios and use confirmatory tests accordingly [21, 23].

Researchers tried to find an anti-HCV threshold value by using different confirmatory tests. Altuğlu *et al*. [12] used Architect Anti-HCV assay (Abbott Laboratories) and confirmed the reactive EIA results with LIA and evaluated by ROC curve analysis. They stated that, when the S/Co ratio was 3.27, they could detect true antibody positivity in 94.9% of their cases [12]. Karakoç *et al*. [24] evaluated the S/Co value as 8.1 when using PCR as confirmatory test for the samples screened with the Ortho test, and found the sensitivity as 100% and the specificity as 95%. When the RIBA test was used for confirmation, they found the S/Co value as 7.5 and determined the sensitivity and specificity as 95% and 81%, respectively [24]. Pan *et al*. [25] used Elecsys Anti-HCV II (Roche Diagnostics) screening test and confirmed the results with RIBA and found the S/Co ratio as 12.27 with 97.8% sensitivity and 86% specificity. Kao *et al*. [26] examined the S/Co ratio for AxSYM Microparticle Enzyme Immunoassay (MEIA) (Abbott Laboratories) using PCR for confirmation and found the S/Co ration optimal at 24 and 44. Using a cutoff S/Co ratio of 24, they could confirm HCV viremia with a sensitivity and positive predictive value (PPV) of 91.7%, specificity and negative predictive value (NPV) of 82.4%. The sensitivity, specificity, PPV, and NPV were found to be 86.1%, 94.1%, 96.9%, 76.2% respectively when S/Co ratio was set 44 as cutoff [26].

In this study, anti-HCV EIA results confirmed with LIA were evaluated with the ROC Analysis, the Area Under the Curve was 0.987 (SE:0.003), and the optimal S/Co ratio was found to be 15.85 (Fig. 2). According to these results, the accuracy was found to be 94.9% when the S/Co ratio was set 15.85 in anti-HCV EIA screening.

Recombinant immunoblot analysis is a complementary serological confirmation test preferred with its robust specificity [10]. However, indeterminate results can also be detected in this system. In our study, 80 (5.6%) of 1.419 reactive samples that were confirmed with LIA were found to be indeterminate. LIA indeterminate results were previously considered to be false anti-HCV positive. However, in a study Makuria *et al*. [27] showed that indeterminate results represent decreased antibody responses in individuals who have recovered from HCV infection. Mazzarella *et al*. [7] has associated indeterminate LIA results with declining antibodies in old age patients. After viral clearance, HCV antibodies persist for several years, and can be detected in laboratory tests during this period. HCV clearance is associated with the appearance of antibodies and reversal of HCV-specific T cell depletion. In the event of spontaneous or treatment-induced HCV clearance, antibodies gradually decrease and disappear in the absence of HCV RNA [7]. Researchers have discovered that individuals who spontaneously

recover from HCV infection had much stronger Cell-Mediated Immune (CMI) responses compared to controls that had never been infected with HCV before [27]. Hitzinger *et al*. [28] retested the individuals tested five and two years ago, found a decrease in positivity, similar to the study of Makuria *et al*. [7]. Also, the researchers showed a gradual decrease in antibody level by using the quantitative Luciferase Immuno-Precipitation System (LIPS), as a patient moves from being chronic carrier (highest antibody level) to spontaneously recover (mid-level) and RIBA indeterminate (low level) [27]. Seeff *et al*. [29], who supported this concept, showed that there was complete antibody loss in some patients who were followed up as anti-HCV positive for 20 years. In another study, anti-HCV prevalence rates tended to decrease in a 12-year follow-up, and positivity declined from 43.6% to 29.2% during the study period [30].

Another approach is that perhaps the initial viral inoculum or exposure may be very low in individuals losing their serological response to HCV [27]. These studies show that anti-HCV positive, LIA-indeterminate results are not false positive, and there might be a declining antibody response. In addition, Pereira *et al*. [31] stated that false positive results may occur in populations with low HCV prevalence or in cases with cross-reactivity due to other viral factors in immunocompromised individuals.

In our research, 44 (55%) of the 80 LIA tests that resulted indeterminate were found to be positive for NS3 band, 22 (27.5%) for C1 band and 8 (10%) for C2 band. Consistent with our research, Pereira *et al*. [31], found that NS3 was the most detected band with 86% (12/14) in indeterminate samples. When interpreting the LIA test, if one HCV antigen band has a reactivity rating of 1+ or higher or if the NS3 band reacts with a reactivity of ± or higher and all other antigen bands are negative, the sample is considered indeterminate. For this reason, higher positivity rate of NS3 band can be considered as an expected result. Most patients who are infected with HCV give a humoral and cellular response to core antigens. Persistent HCV infection is induced with the suppression of early host immune response by core antigens released during the acute phase in HCV infection. High levels of core antigens disrupt the function of T-lymphocytes by interacting with the complement receptor gC1qR, and lead to a decrease in immune response against HCV. It is considered that HCV persistence occurs with immune destruction mechanism [32]. Rafik *et al*. [10] found reactivity of 91.2% in C1 band, 76.5% in C2 band, 97.3% in NS3 band. Pereira *et al*. [31], on the other hand, found 96% reactivity in C1 band and 100% in NS3 band. In our research, LIA test yielded %93,2 reactivity in NS3 band, 96.3% and 90.4% reactivity in core proteins C1 and C2, respectively for EIA reactive samples. Strong antibody response against NS3 antigen have been associated with viral persistence. Beld *et al*. [33] reported that individuals with viral persistence had higher antibody response to NS3 than individuals with significant viral clearance. Viral clearance was associated with significant decrease in antibodies against NS3, independent of HCV genotype, compared to individuals with persistent viremia [33]. In individuals with spontaneous recovery of infection, anti-HCV may persist and remain detectable for a lifetime while it may decrease slightly or gradually disappear after a few years. Anti-HCV continues indefinitely in patients who develop chronic infection, but antibodies may become undetectable in immunocompromised or hemodialysis patients [20].

It has been suggested that high anti-HCV EIA index values may reflect the actual state of infection or viremia. Since anti-HCV is produced by antigen stimulation secondary to viral replication, anti-HCV antibody levels appear to increase with strong viral stimulation. For this reason, the anti-HCV-S/Co ratio is likely to be higher in patients with HCV viremia, who have strong and continuous viral stimulation compared to patients with spontaneous recovery [19, 34]. The conclusion of a study conducted by Seo *et al*. [19] is that the anti-HCV S/Co ratio is significantly dependent on the presence of HCV viremia and contributes significantly to predicting the presence of HCV viremia. Pereira *et al*. [31] established a statistically significant relationship between high anti-HCV index values and the presence of viremia in 92% of RIBA positive samples with an HCV index value > 5.0. Studies have shown that in cases where the anti-HCV antibody titer is higher in the patient's sera, the chances of the test result being real positive are higher than the false positivity [20]. In our study, PPV was found to be 96.6% when S/Co ratio was set 15.85.
In a study conducted by Rafik *et al*. [10] 3-band-and-above positivity was found as 90.9% in individuals with chronic HCV infection, and 86.2% in HCV in-

Table 5. LIA positive band groups, number of level 3 + bands within the group and S/Co mean values

Positive Band Counts	Level 3+ Bands	n	%	Mean S/Co
2 bands	1	27	32.5	52.7
	2	22	26.5	73.8
3 bands	1	28	21.7	60
	2	34	26.4	73
	3	21	16.3	82
4 bands	1	24	11.3	85
	2	39	18.3	84
	3	70	32.9	74
	4	60	28.1	46
5 bands	1	10	3.8	81
	2	18	6.9	80
	3	69	26.5	62
	4	98	37.8	50
	5	62	23.9	46.7
6 bands	1	2	1.5	90
	2	5	3.7	74
	3	9	6.7	67
	4	42	31.1	51
	5	50	37	35
	6	26	19.3	38

LIA = Line Immunoassay, quartile range, S/co = signal to cut-off

fected healthcare workers via LIA confirmatory test. In another study using RIBA, authors found 4-band positivity as 60%, 3-band positivity as 28%, and total of 3-band-and-above positivity as 88% [31]. In this study, positivity rate of 3-band-and-above was found as 89.9% in all cases (Table 4).

In our confirmation with LIA, we grouped the antigen positivity as 2, 3, 4, 5 and 6-band positivity. While mean S/Co values increased from two band positivity and reached the highest level as 68 in four band positivity, it was noteworthy that individuals with five and six band positivity showed a decrease in mean S/Co values (Table 4). When the positive band groups were examined, it was observed that the mean S/Co values increased in individuals with level 3 positivity in two and three bands within each group, and a decrease was observed if the number of level 3 positive bands was four and above (Table 5). The reason for the decrease in the mean value when level 3 positive band number increased to four and above was investigated. Among the samples newly confirmed with the LIA test, those with a level 3 positive band number

over 3 or 4 were retested in serial dilutions. The index values of these samples were between 26-50 with EIA, and when retested with serial dilution, it was observed that the index values increased, reaching up to 3000s. However, there was no change in index values for the samples with an index value of 119, three level 3 positive bands, and indeterminate samples with an index value of 20 when tested with serial dilution. These results show that when the number of level 3 positive bands is four and above, the actual index value can be reached with dilution study, since the absorbance value of the sample may be above linearity due to excess antibody, which suggests that there may be a correlation between strong band positivity and high index value. We believe that further researches in concordance with the HCV clinical course and treatment response are needed on this issue.

The NAAT or Western Blot modifications are confirmatory tests for HCV infection. Elaborate procedures, the need for equipment and qualified personnel limit the widespread use of molecular techniques [14]. RIBA is an alternative to NAAT which needs hardware and infrastructure for countries with limited resources [13, 35]. A previous study recommends the RIBA test to confirm HCV exposure after anti-HCV reactive results, and if the RIBA test is positive, additional PCR test is recommended to evaluate the status of HCV viremia [36]. In another study, it is stated that RIBA is needed for patients with EIA reactive, NAAT negative results, thus a better evaluation could be made by following these patients who recovered from HCV infection. Nevertheless, it has been stated that neglecting RIBA has a minimal effect on HCV diagnosis, provided that the anti-HCV S / Co ratio is included in the diagnostic algorithm [37]. Despite the decision of CDC to remove RIBA from the diagnostic algorithm for HCV, some authors reported that their results indicate the RIBA should continue to be used [10]. Similarly, there are some studies arguing that EIA reactive results are not indicative of HCV infection without a complementary RIBA test [8]. The CDC stated that future studies are needed for good practices [9].

Limitations

One of the limitations of our study is that the population we screened for anti-HCV includes not only possible hepatitis cases, but also individuals with routine medical examinations. Besides, just as there were patients who had been treated in different hospitals, there were individuals who were followed up in different hospitals. Therefore, we were not able to include HCV-RNA and alanine aminotransferase (ALT) results. The strengths of our study are that it contains a high number samples screened for anti-HCV and the antibody bands, the level of positivity and S/Co mean values are determined and analyzed for all the samples tested positive or indeterminate by LIA confirmatory test.

CONCLUSION

EIA tests are indispensable tools for laboratories as anti-HCV screening tests due to its low cost, easy and time-saving procedure, ease of working with automated devices. However, the number of false-positive results is high in anti-HCV EIA screening test, but the use of the S/Co value will make a significant contribution to reducing both false-positive results and the LIA confirmatory test consumption. In our study, the S/Co value was found to be 15,85 with an accuracy of 94.9%. In addition, it has been observed that there is a correlation between the increase in index value and the number of level 3 positive bands in the LIA confirmatory test. We are of the opinion that this relationship, which does not have much data in the literature, should be examined together with the clinic course of HCV infection, and further researches are needed on this subject.

Authors' Contribution

Study Conception: MEB, MO; Study Design: MEB, MO; Supervision: MEB; Funding: N/A; Materials: MEB; Data Collection and/or Processing: MO, MEB; Statistical Analysis and/or Data Interpretation: MO, MEB; Literature Review: MEB; Manuscript Preparation: MO, MEB and Critical Review: MO, MEB.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Ethical approval

This study has been approved by the University of Health Sciences, Şişli Hamidiye Etfal Training and Research Hospital Ethics Committee, Resolution 2832.

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