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Brief Original Article

Reflex threshold of signal-to-cut-off ratios of the Elecsys anti-HCV II assay for hepatitis C virus infection

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Abstract

Introduction: Fast screening tests for hepatitis C virus (HCV) antibody often give false-positive results. Signal-to-cut-off (S/Co) ratios were suggested to be used as reflex confirmation of anti-HCV. The Elecsys Anti-HCV II assay is an effective test for the detection of hepatitis C, but no S/Co cutoff has been reported. The aim of this study was to determine the S/Co ratio threshold of anti-HCV test using Elecsys Anti-HCV II screening and supplemental recombinant immunoblot assay (RIBA) test results as the gold standard.

Methodology: A total of 36,341 serum samples were tested for HCV antibody using the Elecsys Anti-HCV II assay and 276 positive samples were then tested with supplemental RIBA (Mikrogen recomLine HCV IgG strip immunoassay). Receiver operation curve (ROC) analysis was used to determine the cutoff, sensitivity, and specificity of the optimal S/Co ratio.

Results: The Elecsys Anti-HCV II assay was positive (S/Co ratio ≥ 1) in 288 of the 36,341 samples (0.79%). RIBA testing on 276 of these 288 positive samples showed that all but one of 44 samples with an S/Co ratio of ≥ 1 and < 10 were negative, whereas the vast majority of samples (223/232, 96.1%) with an S/Co ratio ≥ 10 were positive. ROC analysis revealed that an optimal S/Co ratio cut-off value was 12.27.

Conclusions: An S/Co ratio of 12.27 obtained with the Elecsys Anti-HCV II assay could be used as reflex confirmation of anti-HCV tests.

Key words: hepatitis C; anti-HCV RIBA; Elecsys hepatitis C virus antibody assay; S/Co ratio.

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Introduction

Fast anti-hepatitis C virus (HCV) tests are usually used as primary screening for HCV infection, but these tests have a high false-positive rate [1]. Recombinant immunoblot assays (RIBAs) are widely used as supplemental tests due to their high specificity. However, the production of RIBAs was stopped in 2010 in the United States and it is therefore no longer recommended as a supplemental test in the 2013 guidelines from the Centers for Disease Control and Prevention (CDC). Current CDC guidelines suggest the use of assay-specific signal-to-cut-off (S/Co) ratios as anti-HCV confirmation to limit the number of samples needing supplemental testing [1,2]. Nevertheless, RIBA is still commonly used in other countries, including China [3-5].

Thresholds of S/Co ratios are inconsistent between studies, even for the same assay [6,7]. The Roche Elecsys Anti-HCV II immunoassay is a new anti-HCV test system that has been reported to have superior sensitivity and similar or superior specificity for detecting early HCV infection compared to available assays [8].

However, the S/Co ratios for anti-HCV confirmation of this system have not been determined. Therefore, the aim of the present study was to determine the threshold S/Co ratio for the Elecsys assay that would predict a true positive ≥ 95% of the time, using RIBA as the gold standard.

Methodology

A database of 36,341 serum samples (28,269 from hospitalized patients and 8,072 from outpatients) from three centers in China tested between January 2013 and December 2013 was retrospectively screened. Anti-HCV tests were performed using the Elecsys Anti-HCV II assay (Roche Diagnostics International Ltd., Rotkreuz, Switzerland). Positive sera (S/Co ratio > 1) were further evaluated with the RecomLine HCV IgG strip immunoassay (Mikrogen GmbH, Neuried, Germany). Ethical approval was obtained in September 2012 from the ethics committee of each participating center. As this was a study of leftover serum samples

that had been retained in storage, individual informed consent was waived by all three committees.

All the reaction steps are performed automatically by the Elecsys 2010 system. Samples with an S/Co ≥ 1 are considered positive. The RecomLine HCV IgG assay was performed according to the manufacturer's protocol.

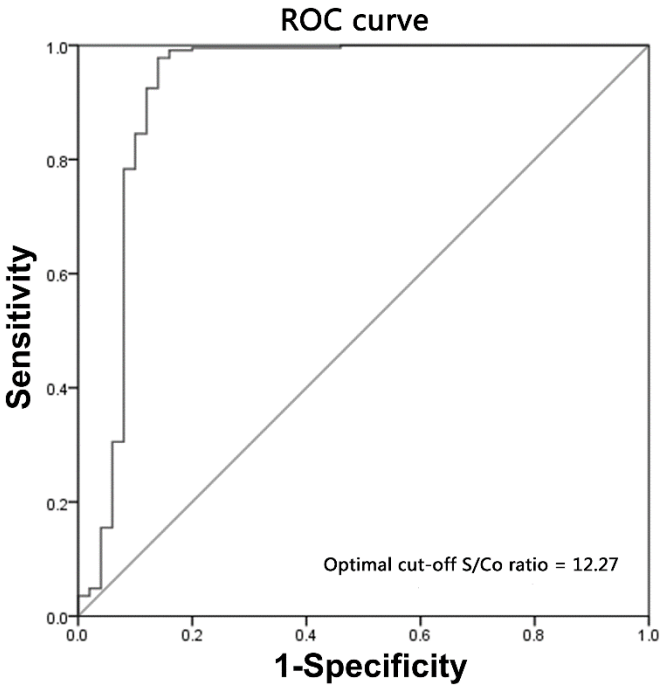
Statistical analysis was performed using SPSS 21.0 (IBM, Armonk, NY, USA). The receiver operating characteristic (ROC) analysis and the Youden index (calculated as sensitivity + specificity - 1) were used to identify the optimal threshold of S/Co ratio for the confirmation of anti-HCV, with RIBA test results as the gold standard.

Results

The results from the Elecsys Anti-HCV II assay were positive for 288 of the 36,341 samples (0.79%). The RecomLine HCV strip immunoassay was done on 276 of the 288 positive samples (this was a study performed using leftover samples in a clinical setting; therefore, 12 samples could not be tested because of an insufficient amount of remaining samples). Of the samples with an S/Co between 1 and 10, 43/44 (97.7%) tested negative by RIBA (Table 1). In addition, samples with negative RIBA test results were also negative for HCV RNA with the COBAS AmpliPrep/COBAS TaqMan assay (data not shown; detection limit of 15 HCV RNA IU/mL). Most samples (223/232, 96.1%) with an S/Co ≥ 10 were positive for HCV by RIBA (Table 1).

The area under the ROC curve (Figure 1) was 0.920 (95% confidence interval, 0.854–0.986). The maximal Youden index was 0.838 (sensitivity = 0.978, specificity = 0.860) at an optimal cut-off S/Co ratio of 12.27.

Figure 1. ROC analysis of data from 276 samples that tested positive (S/Co ratio ≥ 1) using the Elecsys Anti-HCV II assays, and subsequently analyzed using the RecomLine HCV strip immunoassay. The cut-off, sensitivity and specificity of S/Co ratio for anti-HCV were calculated using RIBA as reference. The area under the curve was estimated to be 0.920 (95% confidence interval 0.854-0.986). The maximal Youden index was determined to be 0.838, at an optimal cut-off value for the S/Co ratio of 12.27 (sensitivity = 0.978, specificity = 0.860).



Discussion

RIBA has been used as a supplemental test for anti-HCV confirmation due to its high specificity. RIBA is still widely used in countries other than the United States due to its cost effectiveness [3-5]. In the present study, samples with an S/Co between 1 and 10 tested with Elecsys Anti-HCV II assay were mostly false-positive by RIBA. These results suggest that supplemental testing is necessary for these samples. Reverse transcriptase polymerase chain reaction (RT-PCR) is available for the qualitative detection of HCV RNA, but only in samples from patients with an active infection. According to the Chinese guidelines, an antibody test is the first choice. Therefore, RIBA might still be a valuable tool for anti-HCV confirmation in samples with an S/Co value between 1 and 10.

Table 1. Results of the RecomLine HCV strip immunoassay performed in 276 samples with an S/Co ratio ≥ 1.

S/Co ratio	Samples (n)	Immunoassay results	
		Positive (n)	Negative (n)
1 ≤ S/Co ratio < 10	44	1	43
10 ≤ S/Co ratio < 20	34	32	2
20 ≤ S/Co ratio < 30	49	47	2
30 ≤ S/Co ratio < 40	56	54	2
40 ≤ S/Co ratio <5 0	53	53	0
S/Co ratio ≥ 50	40	37	3
Total	**276**	**224**	**52**

S/Co: signal/cut-off.

A number of previous studies have reported the threshold S/Co ratios for a variety of anti-HCV IgG assays [6,7,9-11], and the CDC has published a list of S/Co ratios predictive of a true positive result ≥ 95% of the time for six different immunoassays [1,2]. The Elecsys Anti-HCV II immunoassay was reported to have superior performance compared to several other commercially available assays [8,12]. A previous study has shown that the Elecsys Anti-HCV II immunoassay was sensible, specific, and suitable for the detection of anti-HCV antibodies [8]. The high sensitivity of the assay makes it suitable for the screening of blood donors [13]. Previous studies have shown that the high sensitivity of the assay shortens the seroconversion window and that it is suitable for screening, even in immunocompromised patients [12,14]. Therefore, the Elecsys Anti-HCV II assay has great potential for routine clinical practice.

However, lower and/or upper cut-off values for the S/Co ratio vary between studies, even for the same assay [6,7,9-11]. This study provides the first results for the Elecsys Anti-HCV II assay, but further studies are necessary to confirm these findings.

Considering the possibility that negative RIBA testing results may be due to the early stage of infection, PCR testing of HCV was used to confirm the results for some subjects. In fifteen subjects with negative RIBA test results, all PCR results were negative. In two subjects with positive RIBA test results, PCR results were positive for one subject and negative for the other. Liver functional tests were all negative for these seventeen subjects. These results confirmed the false positivity. However, the small number of subjects in this subgroup precludes any conclusions. Further study of a larger sample size is necessary.

The present study is not without limitations. First, the use of RIBA as the reference test for anti-HCV could be debated. Nevertheless, due to the reported specificity of the assay and limited availability of other assays, we feel that it is appropriate, at least for routine clinical practice, to use RIBA as an anti-HCV confirmation for the indication of further medical attention. Since the production of RIBA has been stopped in the United States, early estimation of an S/Co threshold for RIBA positivity may help avoid clinical limitations in the future. Confirmation of serological reactive tests may be done using a nucleic acid amplification test for HCV RNA instead. On the other hand, RIBA is still used as the gold standard in many developing countries, and it is still available in most hospitals in China (guidelines for laboratory tests for HCV from the Chinese Center for Disease Control, in which RIBA is still recommended as the primary test for anti-HCV, were last renewed in 2011) [12,14-16]. This study included 276 samples, which is a relatively small sample size, but it was obtained from 36,341 samples, suggesting the rarity of anti-HCV positivity. Finally, the natural history of the disease was not taken into account, and the seropositivity indicates either a past resolved infection or a false HCV antibody positivity [1]. Further studies with a larger sample size are needed to confirm our findings.

Conclusions

Fast screening using the Elecsys Anti-HCV II assay with cutoff setting of S/Co ratio ≥ 1 may generate few false-positive results. Increasing the cutoff to S/Co ratio ≥ 10 effectively avoid the false-positive results. ROC analysis revealed that S/Co ratio ≥ 12.27 could be used as optimal settings for the confirmation of anti-HCV tests.

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