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# Signal-to-cutoff ratios of current anti-HCV assays and a suggestion of new algorithm of supplementary testing

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ABSTRACT

*Background:* The detection of hepatitis C virus antibody (anti-HCV) is known to have high false-positive rates. Using signal-to-cutoff (S/Co) ratios in reflex supplemental testing, however, could reduce false-positive rates. Here, we analyzed the 2-year data of an anti-HCV assay to understand the significance of the S/Co ratio and make a new algorithm by confirming with a second anti-HCV assay.

*Methods:* We reviewed 32,573 samples of the Architect assay (Abbott Diagnostics) from a tertiary hospital. Retests with the Elecsys (Roche Diagnostics) and Vitros (Ortho Clinical Diagnostics) assays were performed in 346 anti-HCV-positive samples. HCV RNA PCR and recombinant immunoblot assay (RIBA) were performed in 147 and 11 anti-HCV-positive samples, respectively.

*Results:* Among 32,573 samples, 446 (1.37%) yielded positive results and 32,127 (98.6%) yielded negative results. Concordance rates in low S/Co samples (0.9–10.0) were 35.2%, 43.8%, and 81.9% for the Architect-Elecsys, Architect-Vitros, and Elecsys-Vitros comparisons, respectively. Correlation coefficients of S/Co ratios were as follows: Architect-Elecsys, 0.20; Architect-Vitros, 0.42; and Elecsys-Vitros, 0.46. In logistic regression, the S/Co value for predicting positivity with 95% probability was 3.13, while that for predicting 50% probability was 8.85. S/Co ratios of 1.70–3.34 showed one reactive antigen out of five antigens, and S/Co ratios of 13.54–17.72 showed three to five positive reactions out of five antigens used in the RIBA.

*Conclusions:* Supplementary testing of anti-HCV screening results is necessary to distinguish between true positivity and biological false positivity for anti-HCV. In this study, we presented an algorithm of supplementary testing by a retest with a second reagent, which could be useful in clinical laboratories.

## 1. Introduction

Hepatitis C virus (HCV) infection is a major public health issue, with 170 million people chronically infected and at risk of developing serious liver diseases, including cirrhosis and hepatocellular carcinoma [1]. Diagnosis of HCV infection is based on the detection of a specific antibody to HCV (anti-HCV) and the components of HCV viral particles, such as HCV RNA and core antigen. For primary screening for HCV infection, detection of anti-HCV is recommended. Anti-HCV can be detected by two main anti-HCV assays: enzyme immunoassay (EIA) and chemiluminescence immunoassay (CIA). Although the CIA screening method demonstrates improved specificity compared with the EIA, both methods have high false-positive rates, particularly in low-prevalence populations [2,3].

Several studies have demonstrated that the higher the signal-to-cutoff (S/Co) ratio, the higher the positive predictive values (PPVs) [3,4]. The US CDC recommends the use of S/Co ratios in reflex supplemental testing algorithms to reduce false-positive rates [3]. Understanding the clinical significance of the S/Co value of an anti-HCV assay is crucial to both patient management and setting of HCV testing algorithms. Clinicians could refer to S/Co ratios during clinical decision making, and the laboratory staff could set the algorithm for diagnosing HCV infection depending on S/Co ratios. However, although the Architect Anti-HCV assay (Abbott Diagnostics) is one of the commonly used CIAs in the clinical setting, limited evaluations for the significance of S/Co values by such assay have been reported [5–7]. This study aimed to understand the significance of S/Co values of the Architect anti-HCV assay and determine the optimal S/Co values for minimizing

*Abbreviations:* HCV, hepatitis C virus; anti-HCV, hepatitis C virus antibody; S/Co, signal-to-cutoff; RIBA, recombinant immunoblot assay; EIA, enzyme immunoassay; CIA, chemiluminescence immunoassay; PPV, positive predictive value

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the need for supplemental testing and improving the reliability of reported test results.

## 2. Materials and methods

### 2.1. Patients

A total of 32,573 samples that were requested for anti-HCV test from November 2014 to February 2017 at the cancer center of a tertiary hospital in Seoul, Korea, were included in this study.

### 2.2. Anti-HCV tests

Anti-HCV tests at the cancer center were performed using the Architect assay with an Architect i2000SR analyzer. If a positive result of the test was first time, we confirmed it by testing with other reagents, using the Elecsys Anti-HCV II (Roche Diagnostics) and Vitros Anti-HCV (Ortho Clinical Diagnostics), within 1 h. Criteria for true positivity were as follows: positive result by a previous anti-HCV test, positive result by HCV RNA PCR or recombinant immunoblot assay (RIBA), and positive anti-HCV result using another reagent. Criteria for true negativity were as follows: negative result by a previous anti-HCV test, negative result by HCV RNA PCR and RIBA, and negative anti-HCV result using another reagent. If a discrepancy existed between the anti-HCV results of three reagents, concordant results with two reagents were assumed to be true positive or true negative. We compared the results of the Architect, Elecsys, and Vitros assays in samples with low S/Co values (0.9–10.0). We calculated agreement using kappa values and carried out a correlation test of the S/Co values of the three reagents using Spearman's rank test. Moreover, we performed logistic regression to find an S/Co value that predicts positivity with 95% probability to determine the cutoff value for retest.

### 2.3. HCV RNA PCR and RIBA

We retrospectively compared anti-HCV S/Co values with HCV RNA PCR and RIBA results within 7 days of testing. The HCV RNA test was ordered in 147 samples within 7 days and was performed using the COBAS AmpliPrep/COBAS TaqMan HCV Test (Roche Molecular Systems), which consisted of 72 tests. Meanwhile, RIBA was performed using HCV BLOT 3.0 (MP Diagnostics). We performed logistic regression to find S/Co values that predict viremia and indicate positive results for HCV RNA with 5%, 50%, and 95% probability. RIBA was ordered in 11 samples within 7 days. Subsequently, we compared the anti-HCV S/Co values with the number of reactive antigens of the RIBA results.

### 2.4. Statistical analyses

All statistical analyses were performed using code developed in R version 3.0.1 (R Development Core Team, 2010; www.R-project.org) and run in RStudio (www.rstudio.com). Figures were generated using the R package, ggplot2 [8].

## 3. Results

Among the 32,573 samples, 446 (1.4%) yielded positive results (S/Co ≥ 1) and 32,127 (98.6%) yielded negative results (S/Co < 1) using the Architect assay (Fig. 1). Furthermore, 9.42% (42/446) of positive results were changed to negative, and 0.02% (8/32,127) of negative results were changed to positive by our criteria based on previous results, HCV RNA PCR and RIBA results, and retest results by other reagents.

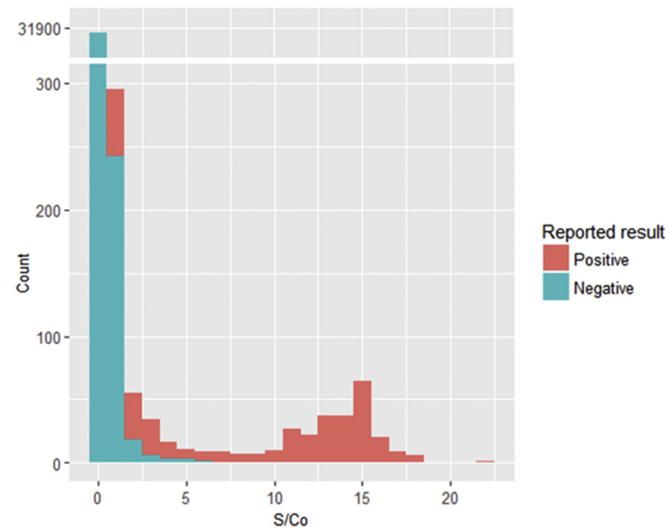


**Fig. 1.** Distribution of signal-to-cutoff (S/Co) ratios in 32,275 routine clinical samples assayed using the Architect hepatitis C virus (HCV) antibody assay. Reported results were defined by criteria based on previous results, HCV RNA PCR and recombinant immunoblot assay results, and retest results by other reagents.

### 3.1. Comparison of the Architect, Elecsys, and Vitros assays in low-S/Co specimens

With data from Elecsys and Vitros results to confirm Architect results, we used specimens with low S/Co values by the Architect assay (0.9–10.0) ($n$ = 104) and compared the values with Elecsys and Vitros results. Concordance rates were 35.2%, 43.8%, and 81.9% in the Architect-Elecsys, Architect-Vitros, and Elecsys-Vitros comparative tests, respectively (Table 1). Kappa coefficients were 0.05, 0.07, and 0.57 in the same Architect-Elecsys, Architect-Vitros, and Elecsys-Vitros comparisons, respectively. Correlation coefficients of S/Co values of the three reagents were as follows: Architect-Elecsys, 0.20 ($p$ = .042); Architect-Vitros, 0.42 ($p \leq .001$); and Elecsys-Vitros, 0.46 ($p \leq .0001$) (Fig. 2). The highest S/Co value by the Architect assay showed a discrepancy of 7.58 (0.156 by Elecsys, 1.28 by Vitros). The highest S/Co value by the Architect assay reported as negative was 5.55 (0.089 by Elecsys, 0.51 by Vitros).

### 3.2. Anti-HCV S/Co value for the retest cutoff

We used the anti-HCV test results we reported earlier and their S/Co values ($n$ = 32,577) to predict positivity with 95% probability (Fig. 3). In logistic regression, the S/Co value for predicting positivity with 95% probability was 3.13 (95% CI, 2.69–3.65). When applying this cutoff to actual data, it showed 97.7% (295/302) PPV and 99.6% (32,152/32,275) negative predictive value. Theoretically, when using 3.13 as the cutoff value for determining the need for an additional test, 63.6% [(S/Co > 3.13, $n$ = 302)/(S/Co > 0.9, $n$ = 475)] of retests can be reduced. When a sample of the Architect assay was positive (S/Co > 1.0) but the S/Co value was below the 3.13 cutoff, 28.5% of samples were classified as false positive because these were negative by both the

**Table 1**
Concordance rates and kappa coefficients of the Architect, Elecsys, and Vitros anti-HCV antibody assays in low-S/Co samples (0.9–10.0).

| Comparative test | Concordance rate (%) | Kappa coefficient | 95% CI |
|---|---|---|---|
| Architect vs Elecsys | 35.2 | 0.05 | −0.03 to 0.12 |
| Architect vs Vitros | 43.8 | 0.07 | −0.03 to 0.17 |
| Elecsys vs Vitros | 81.9 | 0.57 | 0.41 to 0.74 |

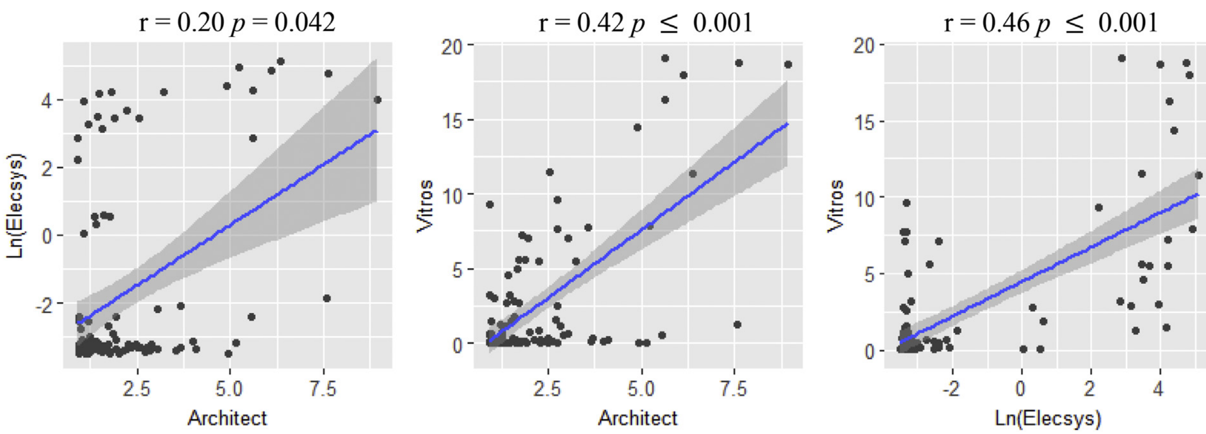


**Fig. 2.** Correlation between signal-to-cutoff (S/Co) values of the Architect, Elecsys, and Vitros hepatitis C virus antibody assays in low-S/Co samples (0.9–10.0).

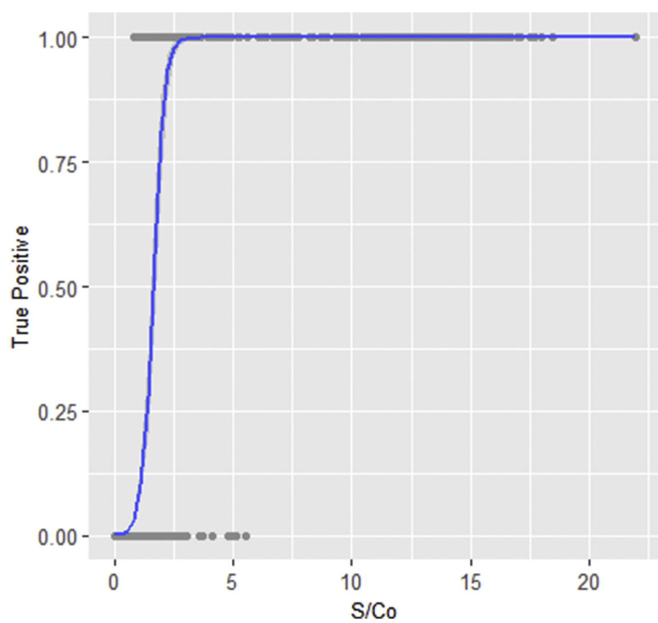


**Fig. 3.** Logistic regression of signal-to-cutoff (S/Co) ratios and true positivity defined by criteria based on previous results, hepatitis C virus RNA PCR and recombinant immunoblot assay results, and retest results by other reagents.

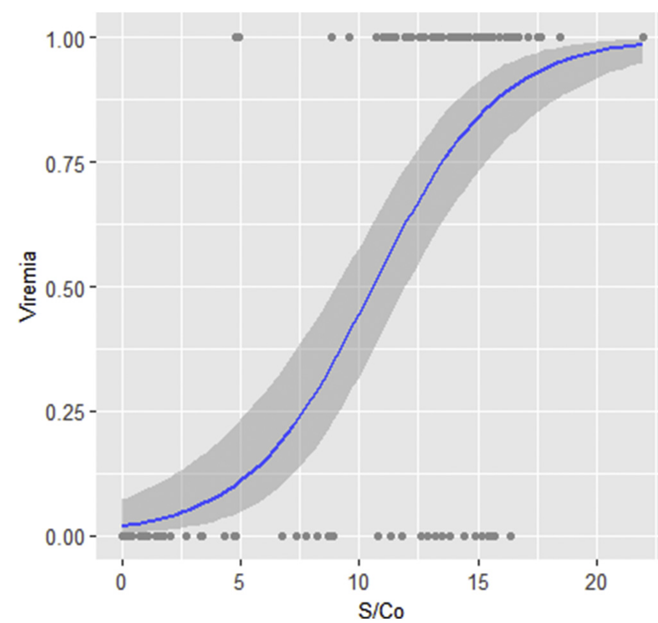


**Fig. 4.** Logistic regression of signal-to-cutoff (S/Co) ratios and viremia defined by hepatitis C virus RNA positivity.

Elecsys and Vitros assays.

### 3.3. Anti-HCV S/Co values that predicts viremia

We used S/Co values of the anti-HCV tests with HCV RNA PCR results to determine S/Co values that predict viremia (positive by HCV RNA PCR) (Fig. 4). The anti-HCV S/Co value for predicting 5% probability of viremia was 2.94 (95% CI, 1.40–6.18); for predicting 50% probability, 8.85 (95% CI, 6.94–11.28); and for predicting 95% probability, 26.61 (95% CI, 16.79–42.19). Using an S/Co value of 8.85 as the cutoff, the PPV for viremia was 77.1%, and the negative predictive value, 96.1%.

### 3.4. Anti-HCV S/Co values and RIBA results

We retrospectively compared the anti-HCV S/Co values and RIBA results. Among the 11 samples in which RIBA was ordered, 5 samples with S/Co values within 1.70–3.34 showed one positive reaction out of five antigens, while 6 samples with S/Co values within 13.54–17.72 showed three to five positive reactions out of five antigens.

## 4. Discussion

The 2003 “Guidelines for Laboratory Testing and Result Reporting of Antibody to Hepatitis C Virus” from the CDC recommend that a low S/Co ratio by an anti-HCV screening test needs confirmation with a more-specific supplemental test such as RIBA or HCV RNA PCR [3]. However, high cost, requirement of specialized equipment, extended turnaround time, short supply, and indeterminate results of RIBA brought modification of the strategy. A 2013 guideline reported that RIBA was excluded from supplemental testing and a second anti-HCV assay different from the initial antibody testing could be used to distinguish between true positivity and biological false positivity for anti-HCV [9]. In this study, the results of the Architect assay were retested by those of the Elecsys and Vitros assays.

The S/Co ratios of commercially available anti-HCV assays are well studied in many literatures. Using the Vitros assay, low-S/Co (lower than 2–5) samples showed negative HCV RNA results and/or no clinical evidence of HCV infection, resulting in the elimination of supplemental testing, and could be reported as “borderline” [4,10]. Otherwise, high-S/Co (> 6–8) samples showed PPVs high enough to eliminate supplemental testing [3,11]. The optimal S/Co range of the Elecsys assay was determined by the ROC curve and regression analysis as 12.27–19.0

**Table 2**
Hepatitis C virus (HCV) RNA PCR results and recombinant immunoblot assay (RIBA) results at different signal-to-cutoff (S/Co) ratios by the Architect anti-HCV assay.

| Architect S/Co | RIBA | | | | | | HCV RNA PCR |
|---|---|---|---|---|---|---|---|
| | Interpretation | core | NS3-1 | NS3-2 | NS4 | NS5 | |
| 1.70 | Indeterminate | – | 1+ | – | – | – | ND |
| 2.12 | Indeterminate | 1+ | – | – | – | – | ND |
| 2.31 | Positive | 2+ | – | – | – | – | ND |
| 2.98 | Positive | 2+ | – | – | – | – | ND |
| 3.34 | Positive | 2+ | – | – | – | – | NT |
| 13.54 | Positive | 4+ | 4+ | 4+ | 3+ | 4+ | $6.40 \times 10^6$ |
| 14.53 | Positive | 3+ | 3+ | – | – | 3+ | NT |
| 15.01 | Positive | 3+ | 2+ | – | 2+ | 1+ | $2.98 \times 10^5$ |
| 15.28 | Positive | 3+ | 3+ | – | 2+ | 2+ | $5.68 \times 10^5$ |
| 15.42 | Positive | 3+ | 3+ | 2+ | 2+ | 2+ | $2.94 \times 10^6$ |
| 17.72 | Positive | 3+ | 3+ | 3+ | 3+ | 2+ | NT |

ND, not detected; NT, not tested.

compared with RIBA results or clinical diagnosis [12,13]. Using the Architect assay, the S/Co ratio was defined as 5.0 based on the ROC analysis compared with HCV RNA results and 95% PPV for RIBA [5,6]. Most of the algorithms suggested above included RIBA, which was excluded in the 2013 CDC guideline [9]. We analyzed the 2-year data of our anti-HCV assay to understand the clinical significance of the S/Co ratio and make a new algorithm by confirming the data with a second anti-HCV assay.

We presented the comparison results of the three assays with low S/Co (0.9–10.0) values. The Architect, Elecsys, and Vitros anti-HCV assays are reported to be highly comparable in several studies [14,15]. However, in our comparison in samples with low S/Co values (0.9–10), it showed quite low concordance rates (35.2–81.9%) and correlation coefficients (0.20–0.46). Since the number of reactive antigens in the immunoblot correlates with the S/CO values, antibodies in low-S/Co specimens mostly react with a few antigens [14]. In our study, samples with low S/Co ratios (1.70–3.34) showed one reactive antigen out of five antigens in the RIBA (Table 2). Consequently, the diverse composition of recombinant antigens or epitopes in commercially available anti-HCV immunoassays could result in discrepancy between different anti-HCV reagents.

We analyzed the anti-HCV results to define the cutoff for retest using logistic regression. An S/Co value of 3.13 was predicted to have 95% probability of true positivity. Other studies on the Architect assay determined the S/Co ratio to be 5.0 based on the ROC analysis compared with the HCV RNA results and 95% PPV for RIBA [5,6] and 7.5 based on the ROC analysis compared with RIBA results [7]. Applying the cutoffs of 3.13, 5.0, and 7.5 to our data showed 97.35%, 98.89%, and 99.60% PPV, respectively. When reporting positive results in S/Co ratios above the cutoffs of 3.13, 5.0, and 7.5 without retesting, 67.7%, 60.3%, and 56.5% of retests could be reduced, respectively. Thus, the S/Co ratios of 3.13, 5.0, and 7.5 for the Architect anti-HCV assay were suitable for the clinical setting and could reduce a large number of unnecessary retests. The reason for the lower S/Co cutoff in our study could be associated with the algorithm we used to define true positivity that was based mainly on retest results by other anti-HCV reagents. Because commercially available anti-HCV immunoassays have a similar composition of recombinant antigens, the threshold for true positivity of anti-HCV could be lower than when using RIBA.

In our study, the S/Co ratio that predicts viremia with 50% probability was 8.85. Meanwhile, Seo et al. reported that an S/Co ratio of 10.9 could accurately predict the presence of viremia [16]. In our study, there was tendency that a high anti-HCV titer indicated the presence of viremia; however, the S/Co ratios were not able to predict for viremia precisely, which could be explained by the fact that detection of anti-HCV does not necessarily mean viremia. Anti-HCV with low S/Co ratios could represent a subject who recovered from a self-limiting HCV infection or who is on a seroconversion status [17].

Although a small number of samples had RIBA results, S/Co ratios of 1.70–3.34 showed one reactive antigen out of five antigens and S/Co ratios of 13.54–17.72 showed three to five positive reactions out of five antigens used in the RIBA (Table 2), consistent with the report of Wu et al. that positive RIBA samples had S/Co ratios of 11.40–14.29 and that negative or indeterminate RIBA results had S/Co ratios of 1.652–5.729 by the Architect assay [5]. Several studies suggested the elimination of supplemental testing of samples with very low S/Co ratios (< 4.5–5.0 by Vitros) in blood banks based on negative or indeterminate RIBA results and negative HCV RNA results [4,18]. While RIBA-indeterminate results have generally been considered to represent false-positive reactions, RIBA-indeterminate reactions are known to represent decreased anti-HCV responses such as in persons who recovered from a remote HCV infection [19]. Therefore, in clinical laboratories, samples with low S/Co ratios should not be ignored as false positive; thus, follow-up is necessary.

Collectively, we made an algorithm of the anti-HCV assay using supplementary testing by a retest with a second reagent (Fig. 5). S/Co ratios below the 3.13 cutoff would react with few recombinant antigens, resulting in a high rate of discrepancy with other reagents. Therefore, a retest with a second reagent is needed to reduce false positivity for anti-HCV. In positive samples with S/Co ratios below the 3.13 cutoff, when a second assay is negative, it can be reported as

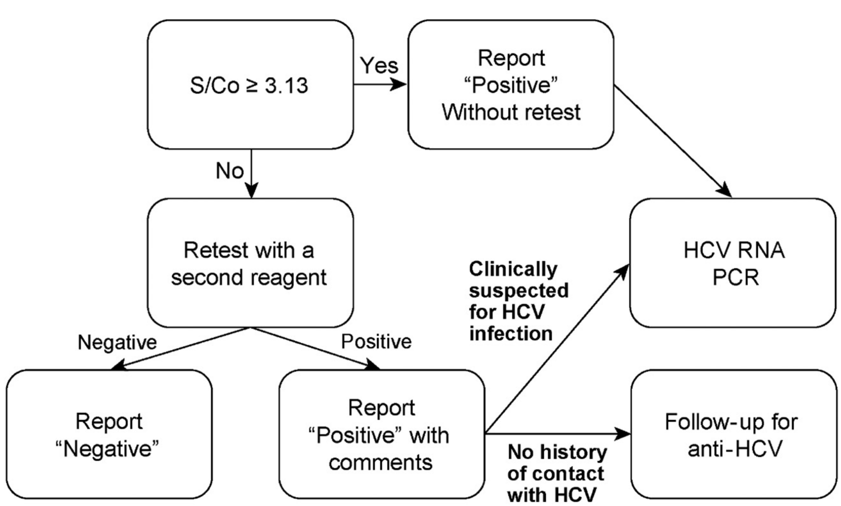


**Fig. 5.** A new algorithm of a hepatitis C virus antibody (anti-HCV) assay using supplementary testing by a retest with a second reagent.

"negative." When a second assay is positive in a low-S/Co sample, it can be reported as "positive" written with the S/Co ratio. It would be mostly negative for HCV RNA because a 5% probability of viremia at an S/Co ratio of 2.94 was predicted, and could represent a subject who recovered from a self-limiting HCV infection or who is on a seroconversion status. Thus, careful interpretation of the results and close follow-up of the patient are required. When the S/Co ratio is above 3.13 at the screening anti-HCV assay, it can be reported as "positive" without a retest by a second reagent. The patient should be further tested by HCV RNA PCR to detect viremia.

This research, however, mainly evaluated S/Co ratios of Architect anti-HCV assay. This algorithm could be applied to other chemiluminescence immunoassay (CIA) like Vitros anti-HCV assay. However Elecsys anti HCV assay that uses electrochemiluminescence (ECIA) showed quite different values from CIA (Fig. 2). Further study on assays using other methods is required to set optimal S/Co ratios for reflex supplemental testing.

In conclusion, supplementary testing of anti-HCV screening results is necessary to distinguish between true positivity and biological false positivity for anti-HCV. In this study, we presented an algorithm of supplementary testing by a retest with a second reagent, which could be useful in clinical laboratories.

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