TITLE

Hepatitis C virus antibody detection: the role of signal-to-cut-off ratios.

RUNNING TITLE

Efficiency of anti-HCV S/Co ratios.

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ABSTRACT

We reviewed results from 12,800 samples tested for hepatitis C virus (HCV) antibody detection in our laboratory by screening (ORTHO® CIA) and supplemental tests (CHIRON® RIBA). We found that a signal-to-cut-off ratio (S/Co) of 10.3 was, in our setting, the most efficient cut off point to improve the diagnostic algorithm of HCV infection.

Hepatitis C virus (HCV) infection is a major public health concern (2, 12), about 3% of the world’s population has been infected with more than 170 million chronic carriers (17).
HCV infection can lead to end-stage liver disease, cirrhosis and hepatocellular carcinoma and mortality will continue to increase over the next two decades (1, 10).

The diagnosis of HCV infection is based on serological assays that detect specific antibodies to HCV (anti-HCV) and molecular assays that detect viral nucleic acid (HCV RNA) (14). Testing for the presence of anti-HCV antibodies is recommended for initially identifying persons with HCV infection (3,7, 16). Anti-HCV detection by immunoassay screening tests is generally the first step in clinical diagnosis and screening of asymptomatic subjects. Screening tests have high false-positive rates particularly among populations with a low (<10%) prevalence of HCV infection (4). For this reason more specific supplemental tests such as recombinant immunoblot assay (RIBA) or a nucleic acid test (NAT) using reverse transcriptase polymerase chain reaction (RT-PCR) for HCV RNA detection are used to confirm positive anti-HCV screening tests (15).

As many as nine testing strategies for detection of HCV infection have been analyzed (6). The Centers for Disease Control and Prevention (CDC) published guidelines in order to provide a systematic approach for the laboratory diagnosis of HCV infection suggesting algorithms for accurate, efficient and cost-effective strategies using screening and supplemental tests (4).

Screening for anti-HCV antibodies is carried out in our laboratory using the ORTHO® VITROS anti-HCV 3.0 chemiluminescence assay (Ortho-Clinical Diagnostics Johnson & Johnson, U.K.) on VITROS® ECiQ automated analyzer (ORTHO® CIA) (8, 11, 13). This is a two-step sandwich enhanced chemiluminescence immunoassay for the detection of human antibodies to several HCV recombinant antigens (c22-3, c200 and NS-5).

Results are calculated as a normalized signal-to-cut-off ratios (S/Co) obtained by measuring the signal strength of sample and the signal strength of an internal cutoff. Samples with a S/Co ≥1.0 are defined by the manufacturer as positives.
Each positive sample by ORTHO® CIA screen is followed by CHIRON® RIBA® HCV 3.0 Strip Immuno Assay (Chiron Corporation, Emeryville, CA, USA) (CHIRON® RIBA) a more specific supplemental anti-HCV assay to confirm screening test results.

CHIRON RIBA is a qualitative enzyme immunoblot assay for the detection of antibodies against recombinant antigens (c33c and NS5) and HCV-encoded synthetic peptides (c22, c100 and 5-1-1).

The anti-HCV reactivity of specimens is determined by visually comparing each HCV band to the intensity of the low and high human IgG internal control bands blotted onto each strip. A negative, indeterminate or positive interpretation is based on the reaction pattern present on the strip.

The CDC guidelines for laboratory testing reported that screening test positive results are classified as having high S/Co ratios if their ratios are at or above a pre-determined value that predicts a supplemental test positive result ≥95% of the time, regardless of the anti-HCV prevalence or characteristics of the population being tested.

The CDC, on its website (5) gives S/Co ratios predictive of a true positive ≥95% of the time for each screening test available. For ORTHO® CIA, high S/Co ratios are defined as S/Co ≥8.0 ratios.

Several studies have been published about the ability of this screening test to predict the supplemental test result (9, 11, 14, 15). Lai et al. (14) concluded that for ORTHO® CIA it is not necessary to confirm negative or positive values if the S/Co is <3.0 or ≥20.0 because of the high rate of true negative and true positive results respectively; other authors suggested that confirmatory tests are not necessary for patients with S/Co ratios <5.0 and <4.5, (15,9).

The objective of the present study was to evaluate in our setting the relationship between ORTHO® CIA positive S/Co samples and CHIRON® RIBA results to assess if our diagnostic algorithm might be modified in order to reduce unnecessary supplementary tests.

We retrospectively reviewed results from a database of 12,800 serum samples that were tested from 1st July 2008 to 31st December 2010. Of these, 7,000 samples (54.7%) were from hospitalized patients and 5,800 (45.3%) were from outpatients.
All samples were analyzed for anti-HCV antibodies screening detection using the ORTHO® CIA and all positive sera were evaluated with CHIRON® RIBA assay as supplemental test. Statistical analysis was carried out with STATA® RELEASE statistical software version 11.0 (STATA Corp. LP, College Station, Texas, USA) and Visual Basic (VBA) for Windows®; P-value <0.05 was considered as significantly different. Among 12,800 patients tested 313 (2.4%) resulted positive (S/Co ≥1.0) by ORTHO® CIA. The S/Co ratio of positive samples ranged from 1.0 (min) to 30.1 (max). The mean value was 19.1 (SD 9.4) and 5th and 95th percentiles were 1.28 and 28.50, respectively. Of the 313 ORTHO® CIA positive patients, 222 (71.0%), 46 (14.7%) and 45 patients (14.3%) were positive, negative and indeterminate, respectively, by CHIRON® RIBA. We categorized positive samples on the basis of S/Co and calculated ratio of negative, indeterminate and positive results by CHIRON® RIBA (Table 1). The diagnostic sensitivity and specificity, the positive predictive value (PPV) and negative predictive value (NPV) were calculated at values of 3.0, 8.0 and 20.0 S/Co ratios, respectively (Table 2). Ordinal Regression analysis was performed using S/Co screening test as continuous predictive variables and confirmatory test as ordinal dependent variable (0=negative, 1=indeterminate, 2=positive). The analysis with Ordinal Regression shows that the screening test value is strongly associated to the ordinal result of confirmatory test (χ²=226.1, p<0.0001) suggesting a strong relationship between screening and supplemental tests. Despite the relationship there are a statistically significant amount of samples with an indeterminate result (Figure 1). The values of S/Co ratios associated with 95% PPV and 95% NPV were 10.3 and 3.0, respectively. On the basis of our current study and literature data we modified our algorithm for HCV testing. If the S/Co ratio is <10.3 ORTHO® CIA results should be confirmed by supplemental CHIRON® RIBA.
We decided to report specimens with S/Co ≥10.3 without a supplemental test but with an explanatory comment. We declare in the comment that supplemental serological testing was not performed for a sample with S/Co ≥10.3 since in these cases screening test predicts a true antibody positive result ≥95% of the time. We inform the test-ordering physician also that a more specific testing can be requested if necessary, especially for people being tested for HCV infection for the first time or on the basis of other clinical or laboratory information. For this eventuality we store the specimens for supplemental test. Therefore, we suggest to perform RT-PCR to detect HCV viremia in positive samples at screening test and indeterminate or positive RIBA results.

We believe that implementation of this algorithm will improve the accuracy, efficiency and utility of anti-HCV testing with more reliable results for physicians and their patients and can reduce unnecessary supplementary testing. We also suggest that this type of validation will need to be done by each laboratory since the population characteristics and the assay used will both have an effect on the cutoff selection.

**ACKNOWLEDGMENTS**

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REFERENCES


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Table 1. RIBA results in relation to CIA S/Co ratios subset.

<table>
<thead>
<tr>
<th>CIA</th>
<th>S/Co ratio</th>
<th>Negative</th>
<th>Indeterminate</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0</td>
<td>≤3.0 (43)</td>
<td>31 (72.1%)</td>
<td>11 (25.6%)</td>
<td>1 (2.3%)</td>
</tr>
<tr>
<td>3.01-8.0</td>
<td>3.01-8.0 (26)</td>
<td>8 (30.8%)</td>
<td>16 (61.5%)</td>
<td>2 (7.7%)</td>
</tr>
<tr>
<td>8.01-20.0</td>
<td>8.01-20.0 (36)</td>
<td>4 (11.1%)</td>
<td>8 (22.2%)</td>
<td>24 (66.7%)</td>
</tr>
<tr>
<td>&gt;20.01</td>
<td>&gt;20.01 (208)</td>
<td>2 (1.0%)</td>
<td>11 (5.3%)</td>
<td>195 (93.7%)</td>
</tr>
<tr>
<td>Total</td>
<td>313</td>
<td>45</td>
<td>46</td>
<td>222</td>
</tr>
</tbody>
</table>
Table 2. Diagnostic performance of CIA screening test in the prediction of RIBA results.  

<table>
<thead>
<tr>
<th>CIA S/Co ratio</th>
<th>3.0</th>
<th>8.0</th>
<th>20.0</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity (95%CI)</strong></td>
<td>99.5% (97.5-100)</td>
<td>98.6% (96.1-99.7)</td>
<td>87.8% (82.8-91.8)</td>
</tr>
<tr>
<td><strong>Specificity (95%CI)</strong></td>
<td>46.2% (35.6-56.9)</td>
<td>72.5% (62.2-81.4)</td>
<td>85.7% (76.8-92.2)</td>
</tr>
<tr>
<td><strong>PPV(^2) (95%CI)</strong></td>
<td>81.9% (76.7-86.3)</td>
<td>89.8% (85.2-93.3)</td>
<td>93.8% (89.5-96.6)</td>
</tr>
<tr>
<td><strong>NPV(^3) (95% CI)</strong></td>
<td>97.7% (87.7-99.9)</td>
<td>95.7% (87.8-99.1)</td>
<td>74.3% (64.8-82.3)</td>
</tr>
</tbody>
</table>

1 = 95% confidence interval
2 = Positive Predictive Value
3 = Negative Predictive Value
\(^{a}\) (values in parentheses are the limits of 95% CI)