Lot-to-Lot Variation in Anti-Hepatitis C Signal-to-Cutoff Ratio, D. Robert Dufour (Pathology and Laboratory Medicine Service, Veterans Affairs Medical Center, and Department of Pathology, The George Washington University Medical Center, Washington, DC; address for correspondence: Pathology and Laboratory Medicine Service-113, Veterans Affairs Medical Center, 50 Irving St. NW, Washington DC 20422; fax 202-745-8284, e-mail d.robert.dufour@med.va.gov)

Hepatitis C virus (HCV), the most common chronic viral infection in North America, affects an estimated 2.7 million individuals in the US (1). Hepatitis C is more common in certain populations, including injection drug users, prison inmates, the homeless, dialysis patients, and those seeking care in Veterans Affairs medical centers (2-5). Hepatitis C is the leading cause for end-stage liver complications, including hepatocellular carcinoma and need for liver transplantation; the frequency of these is expected to increase two- to threefold by 2030 (6).

Antibodies to HCV (anti-HCV) can indicate one of three possible conditions: current active infection with HCV, past infection with HCV, or a false-positive reaction (7). Although tests are usually interpreted as positive or negative, samples with low-positive anti-HCV results are usually falsely positive, whereas >90% of samples that are high-positive are from patients who test positive for HCV RNA (8, 9). Recently, the CDC revised guidelines for laboratories, recommending confirmatory testing for samples with low signal-to-cutoff (S/C) ratios (10). When enzyme immunoassays (EIAs) are used, they suggested a S/C ratio <3.8 to identify low-positive samples; when chemiluminescence assays (CAS) are used, a S/C ratio <8.0 was recommended (10).

No data exist on the reproducibility of S/C ratios between lots. Because calibration of both EIA and CA methods involves a single point (the cutoff value), S/C ratios may vary significantly between lots. I retrospectively reviewed data for S/C ratios and confirmatory test results by lot of reagent for both EIAs and CAS.

The Pathology and Laboratory Medicine Service maintains a blinded database of all patients with positive anti-HCV results; details of the screening program and the assays used have been reported previously (8, 9). A total of 13,714 individuals were tested over a 13-month period by a third-generation EIA (Ortho Clinical Diagnostics), with 2140 (15.6%) positive, whereas 19,518 were tested over a 21-month period by CA (Vitros Eci; Ortho Clinical Diagnostics), with 2257 (11.6%) positive (Data are available in supplemental files EIA and CA in the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol50/issue5/).

Before August 2002, confirmatory testing was largely based on physician decisions (except in samples with low-positive anti-HCV). Since then, routine reflex testing has been performed on all anti-HCV-positive samples if adequate sample remained (reflex testing was completed on 98% of positive samples). Recombinant immunoblot assays (RIBAs; Chiron Diagnostics) were performed on all samples with low-positive anti-HCV, and HCV RNA was assayed by quantitative bDNA (Quantiplex 3.0, Bayer Diagnostics) for all samples with high-positive anti-HCV or with indeterminate or positive RIBA results. Data on anti-HCV were segregated by lots; seven different EIA lots and eight different CA lots were used. Data on quality-control material S/C ratios were available by lot for CA testing. The study design was approved by the facility Institutional Review Board.

Differences between mean results among the lots were evaluated by ANOVA. Differences in proportions of results were evaluated by the \( \chi^2 \) test. Differences in mean control S/C values between two different lots of reagent were assessed by t-test. Results were considered significant if \( P < 0.05 \).

There was a significant difference in mean EIA-positive S/C values among the lots tested (ANOVA; F value, 7.49; \( P < 0.0001 \)). Results were grouped into lots with mean positive S/C ratios <4.5 and those with ratios \( \geq 4.5 \) (see Table 1 in the online Data Supplement). Positive results were significantly more common in lots with mean positive S/C ratios <4.5 (16.5%) than in lots with higher mean positive S/C ratios (14.8%; \( P = 0.007 \)). Most of the difference was attributable to a higher percentage of low-positive results (20.5%) in lots with low mean positive S/C ratios than in lots with higher mean positive S/C ratios (15.2%; \( P = 0.0016 \)). The percentage of total results that were high-positive was not significantly different (13.6% vs 12.8%; \( P = 0.22 \)). There was no difference in the proportion of low-positive samples that were indeterminate or positive by RIBA between lots with low and high S/C ratios (\( P = 0.44 \)). Even after elimination of the low-positive results, the lot-to-lot differences in mean samples with high-positive results were highly significant (ANOVA; F value, 37.78; \( P < 0.0001 \)).

There was also a significant difference in mean positive CA S/C values (Table 2 in the online Data Supplement) among the lots tested (ANOVA; F value, 33.0; \( P < 0.0001 \)). There was a borderline significant difference in positive rate between lots with mean positive S/C ratios >32 (12.4%) and those with lower mean positive S/C ratios (11.2%; \( P = 0.02 \)), but no significant difference in the percentage of positive samples with low S/C ratios (11.9% vs 11.6%; \( P = 0.81 \)). There was no significant difference between lots in the percentage of samples with a S/C ratio between 1 and 3.9 that were RIBA negative (75% vs 78%; \( P = 0.65 \)). In samples with a S/C ratio between 4.0 and 7.9, there was a borderline significant trend toward a lower percentage of negative RIBA in lots with lower mean positive S/C ratios (57% vs 33%; \( P = 0.07 \)). There were
also significant differences in control values (where comparisons using the same lot of control were possible with two different lots of reagent).

With the EIA, there was a sharp cutoff in likelihood of HCV RNA positivity at a S/C ratio of 3.8 (Fig. 1A); this did not differ between lots with low and high mean positive S/C ratios. With the CA, there was a gradual increase in likelihood of positive HCV RNA in samples with S/C ratios >8. This relationship differed markedly between the three lots with mean positive S/C ratios >32 and those with lower mean positive S/C ratios (Fig. 1B).

To minimize lot-to-lot variation, I evaluated interpreting the S/C ratio by dividing it by the median value for the lot (median ratio). Use of the median ratio reduced the differences in HCV RNA positivity rate between lots with low and high mean positive S/C ratios (Fig. 1C). Use of the median ratio also eliminated the difference in RIBA positivity between lots with high and low positive S/C ratios. In samples with a median ratio ≤0.1, 78% were RIBA negative with no difference between lots ($P = 0.83$). In samples with a median ratio of 0.1–0.4, 32.1% were RIBA negative with no difference between lots ($P = 0.44$).

I also evaluated how many observations would be needed to be reasonably certain of the true median. In six of eight lots, the median calculated on the first 10 positive samples was within 10% of the median for the entire lot.

![Fig. 1. Rate of HCV RNA positivity classified by S/C and median ratio.](image)

(A), for the EIA, there is only a slight increase in positive rate as S/C ratio increases up to the low cutoff point of 3.8. At S/C ratios ≥3.8, there is little difference in the rate of HCV RNA positivity as S/C ratio increases further. (B), in contrast to the pattern seen with the EIA, the mean positive S/C ratio for the CA affects the likelihood of positive HCV RNA. In lots with high mean positive S/C ratios (solid line), no samples with a S/C ratio between 8 and 20 had positive HCV RNA. Above a S/C ratio of 20, the likelihood of positive HCV RNA increases and plateaus at S/C ratios >32. In lots with low mean positive S/C ratios (dotted line), there is a gradual increase in rate of HCV RNA positivity at all S/C ratios up to 28, with a plateau above that point. The likelihood of HCV RNA positivity differs markedly between lots with high and low mean S/C ratios. (C), use of the median ratio minimizes the difference between lots with high and low mean positive S/C ratios.
To be certain within 5% of the true median value required 50 samples in six lots and 60 samples in the remaining two lots. Although the median ratio improved diagnostic comparability among lots, differences in positive control median ratios among lots were still significant (data not shown).

The data confirm significant lot-to-lot variation in positive S/C ratios in both EIA and CA tests. With the EIA, this was attributable to a significant increase in low (false)-positive results, but with no difference in significance of cutoff values, and thus of no clinical importance. In the CA, there were significant lot-to-lot variations in the frequency of negative RIBA results for samples with low S/C ratios. Among samples with high S/C ratios, the likelihood of HCV RNA positivity was directly related to S/C ratio; however, the correlation differed based on the mean positive S/C ratio of the lot. Because these differences may have important clinical consequences, use of a single S/C cutoff ratio to decide on additional testing does not appear justified when using CAs.

Anti-HCV assays are calibrated based on a single point (the cutoff value for distinguishing positive and negative results). With single-point calibration, it is difficult to determine the slope of the calibration curve and thus the accuracy of quantitative results. To achieve a reasonably reproducible S/C ratio, it would be necessary to use additional known positive calibrators. Because anti-HCV is not available in purified form, it could be difficult to achieve standardization of results between assay lots. My data suggest that use of the median ratio may minimize lot-to-lot variation in S/C ratio. There was no significant difference among lots of CA tests in the frequency of negative results on RIBA or positive results by HCV RNA when the median ratio was used, in contrast to the variability seen when the S/C ratio was used. In most cases, the median ratio can be accurately estimated from the first 10 positive samples, and in all lots tested, the median estimate from the first 60 positive samples was within 5% of the true value. Manufacturers could also collect panels of positive samples, evaluate the median for these, and provide that value in their package inserts; laboratories could then validate the median in their own samples. We have not yet adopted the median ratio pending confirmatory studies by other investigators. Should additional studies confirm its usefulness, the median ratio could reduce the significance of lot-to-lot variation in interpretation of low-positive anti-HCV results.

I have received travel support from Ortho Clinical Diagnostics to speak at a meeting.

References

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