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Evaluation of the Ortho-Clinical Diagnostics Vitros ECi Anti-HCV Test: Comparison With Three Other Methods

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After observing a high incidence of low positive hepatitis C virus (HCV) antibody screens by the Ortho-Clinical Vitros ECi test (Orthoclinical Diagnostics, Raritan, NJ), we compared results against those obtained using another chemiluminescent analyzer, as well as two U.S. Food and Drug Administration (FDA)-approved confirmatory methodologies. To ascertain the true anti-HCV status of samples deemed low-positive by the Ortho-Clinical Vitros ECi test, we tested samples using the ADVIA Centaur HCV screen test (Siemens Medical Solutions Diagnostics), the Chiron recombinant immunoblot assay (RIBA) test (Chiron Corp., Emeryville, CA), and the Roche COBAS Amplicor HCV qualitative test (Roche Diagnostics, Indianapolis, IN) in a series of studies. Of 94 specimens positive by Vitros ECi, 19% were observed to be negative by Centaur. A separate study of 91 samples with signal-to-cutoff (s/co) values less than 8.0 showed that all but one was negative for HCV ribonucleic acid (RNA). In comparison with RIBA, 100% (77) samples positive by the Vitros ECi test with s/co values less than 12.0 were negative or indeterminate by RIBA. A final study comparing all four methods side-by-side showed 63% disagreement by Centaur for Vitros ECi low-positive samples, 75% disagreement by RIBA, and 97% disagreement by polymerase chain reaction (PCR). In conclusion, the Ortho-Clinical Vitros ECi Anti-HCV test yields a high rate of false-positive results in the low s/co range in our patient population. J. Clin. Lab. Anal. 21:162–166, 2007.

Key words: hepatitis C virus; HCV; Vitros ECi; ADVIA Centaur; RIBA

INTRODUCTION

Hepatitis C virus (HCV) is a major public health concern and a leading cause of liver disease. According to the Centers for Disease Control and Prevention (CDC), HCV is the most common chronic blood-borne infection in the United States, with an estimated 2.7 million Americans chronically infected and 30,000 new infections occurring each year (1). The laboratory plays an important role in diagnosis of HCV infection. The CDC recommends screening followed by confirmatory testing of screen-positive samples (2,3). The confirmatory algorithm includes various permutations of antibody testing by recombinant immunoblot assay (RIBA) and molecular testing by nucleic acid test (NAT) to assess viremia (3). Fully automated HCV antibody screening has become available in recent years, offering both high throughput and state-of-the-art technology. Two such systems are the Ortho-Clinical Vitros ECi test and the Bayer ADVIA Centaur test.

Our laboratory performs over 45,000 HCV antibody tests per year. After implementing the Vitros ECi test in March 2004 for our HCV antibody screening, we observed an increase in the incidence of low-positive results. While we initially interpreted this to reflect increased sensitivity of the Vitros ECi reagents compared to the enzyme immunoassay (EIA) reagents we previously utilized, we undertook this study to assess the true positivity of results we obtained.

MATERIALS AND METHODS

Diagnostic specimens were received for HCV antibody testing through routine channels. Patient

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population consisted of U.S. military members, retirees, and/or dependents. Samples were initially tested using the Ortho-Clinical Vitros ECi Anti-HCV test, and for purposes of this study, a selection of Vitros ECi–positive samples were also tested using the Bayer ADVIA Centaur Anti-HCV test, the Chiron RIBA HCV 3.0 strip immunoblot assay (SIA) test, and/or the Roche COBAS Amplicor HCV 2.0 qualitative test. We were especially interested in comparing instrument results for samples yielding signal-to-cutoff (s/co) ratios between 1.0 and 8.0, and so we sampled more heavily from this group. Otherwise, samples were chosen randomly, with the only qualifying factor being sufficient sample volume to perform the requisite tests.

Manufacturers' instructions were followed for all assays. The Ortho-Clinical Vitros ECi test and the Bayer ADVIA Centaur test both employ chemiluminescent technology, in which a light signal is created by a positive antibody-antigen reaction in direct proportion to the amount of antibody present. Both assays report results based on a cutoff of 1.00; the Centaur test reports "index value," whereas the Vitros ECi test reports "s/co." Both tests require duplicate repeat testing within a defined equivocal zone. Results greater than or equal to 1.00 are considered reactive for HCV antibodies by either assay.

RESULTS

In a comparison of the Ortho-Clinical Vitros ECi Anti-HCV assay with the Bayer ADVIA Centaur Anti-HCV assay, 92% agreement was observed, as shown in Table 1. In this initial study, 237 samples were tested without respect to s/co value; therefore, samples positive by the Vitros ECi test represented a wide range of s/co values above 1.00. Of the 94 specimens assessed as positive by the Vitros ECi test, 18 (19%) were deemed negative by the Centaur test. Only one sample tested positive by Centaur and negative by Vitros ECi.

Of 12,835 samples tested by the Ortho-Clinical Vitros ECi Anti-HCV test during the months of October 2004 through January 2005, 214 were positive for HCV antibodies by this screening method, and were tested by polymerase chain reaction (PCR) (Roche COBAS Amplicor) for confirmation. Figure 1 illustrates the distribution of s/co ratios and PCR results. Of all HCV screen-positive samples, 91 (43%) had a s/co ratio less than 8.0. Of these 91 samples, only one was positive for HCV ribonucleic acid (RNA).

In a separate analysis of 84 Vitros ECi screen-positive samples tested by RIBA, we found that all samples with s/co ratios under 12.0 were either negative or indeterminate by RIBA, as shown in Fig. 2. While the majority

TABLE 1. Correlation of ortho-clinical vitros ECi with Bayer ADVIA Centaur for anti-HCV

	Bayer ADVIA Centaur		
Ortho-Clinical Vitros ECi	Nonreactive	Equivocal	Reactive
Nonreactive	142	0	1
Equivocal	0	0	0
Reactive	18	0	76

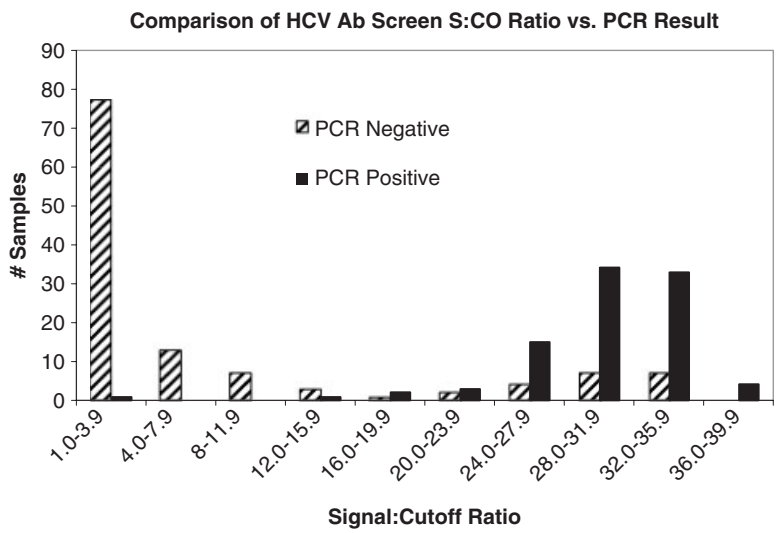

Fig. 1. Samples (n = 12,835) were tested by the Ortho-Clinical Vitros ECi Anti-HCV test as per manufacturer's instructions. Positive samples (n = 214) were then tested by PCR using the Roche COBAS Amplicor test, as per manufacturer's instructions. Results are plotted by PCR result (positive or negative), according to number of samples falling into each Vitros ECi s/co range.

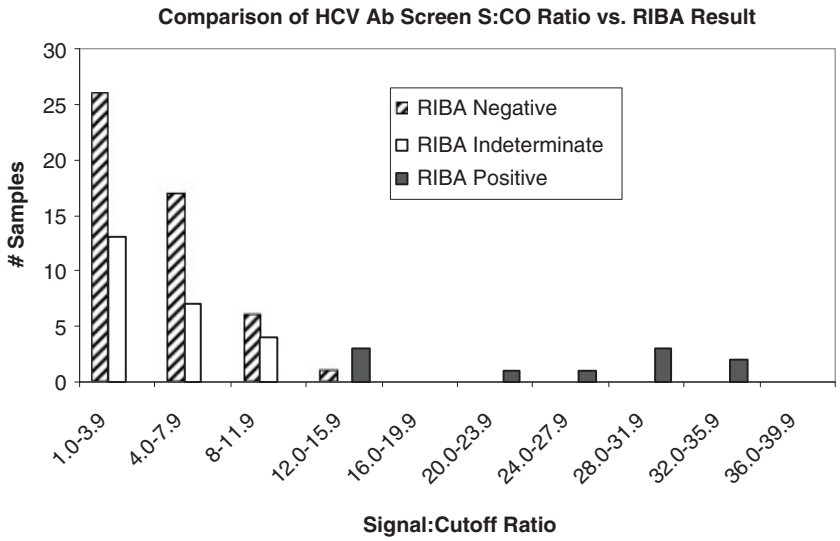

Fig. 2. A total of 84 samples positive by the Ortho-Clinical Vitros ECi test were tested by Chiron's RIBA test, as per manufacturer's instructions. Results are plotted by RIBA result (positive, negative, or indeterminate), according to number of samples falling into each Vitros ECi s/co range.

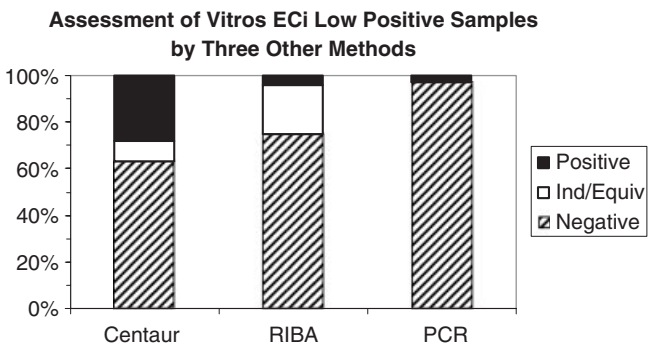

Fig. 3. A total of 32 samples were selected that had positive Ortho-Clinical Vitros ECi test results with s/co ratios under 10.0. All 32 samples were further tested by the Bayer ADVIA Centaur test and by PCR, and 28 of these samples were also tested by Chiron's RIBA test. All tests were performed as per manufacturers' instructions. Results (positive, negative, or indeterminate/equivocal) are plotted in percentages for each of the three comparison methods.

were negative, a full 33% of these samples (with s/co ratios under 12.0) were indeterminate. Of the few (seven) samples with s/co ratios over 20, all were positive by RIBA.

In a comparison of all four methods side-by-side, we tested samples positive by the Vitros ECi test with s/co ratios under 10.0. Figure 3 illustrates our findings. A total of 32 samples were tested by Vitros ECi, by Centaur, and by PCR, and 28 of these samples were also tested by RIBA. The Centaur result disagreed with the Vitros ECi result 63% of the time, calling 20 of the 32 samples negative. The Centaur called only nine (28%) of the Vitros ECi–positive samples positive and three (9%) equivocal. The confirmatory methods showed even less agreement with the original Vitros ECi results, with positive or indeterminate RIBA results for only 25% of Vitros ECi–positive samples, and positive PCR results for only 3% of samples. All but one sample was deemed negative by PCR. RIBA yielded 21 (75%) negative and six (21%) indeterminate results for 28 samples. Only one sample (4%) showed as positive by RIBA. This sample also showed as positive by the Centaur test.

DISCUSSION

Our findings show that the Ortho-Clinical Vitros ECi test yields a considerable number of low positive results with s/co values less than 8.0 (43% of all positive results), most of which cannot be confirmed as true positives. Our low-positive rate appears to be somewhat higher than that reported by other laboratories. One study reported 10–15% of positive samples exhibited s/co ratios less than 8.0 (4); another reported a rate of approximately 21% (5). Nonetheless, both laboratories reported a number of positive results by Ortho-Clinical Vitros ECi, which could not be confirmed by RIBA, NAT, or a combination of the two.

In our study, as many as two out of three results tested positive by Ortho-Clinical Vitros ECi but then negative by Bayer ADVIA Centaur, an instrument employing the same methodology. Moreover, the vast majority of Vitros ECi–positive samples were not confirmed as positive by either RIBA or NAT. A number of laboratories have observed similar discordance while

attempting to correlate the various HCV tests on the market today (4–7). Oethinger et al. (5) reported findings very similar to ours when comparing Ortho-Clinical Vitros ECi with PCR and RIBA for a population of U.S. Department of Veterans Administration (VA) patients. Their study showed that 99% of patient samples with very low s/co ratios (≤ 5) were false-positive, with no evidence of HCV infection.

False-positive results are common in low prevalence populations, and the CDC has estimated that false-positive results by enzyme-linked immunosorbent assay (ELISA) methodologies average about 35% in the general immunocompetent population (3). Therefore, it is perhaps not unusual to observe such a high incidence of false-positive results in our relatively low-prevalence population of active duty military members and beneficiaries. In fact, Pierson et al. (8) reported a false-positive rate as high as 82% in a population of 1,600 soldiers returning from Iraq, as determined by RIBA confirmatory testing of EIA-positive samples. On the other hand, in populations where the prevalence of HCV infection is higher, false-positive anti-HCV results should be relatively rare. The VA patient population of Oethinger et al.'s (5) study could be considered such a group, with an estimated HCV seroprevalence of 8–10%, and yet, even in this higher prevalence group, a high rate of false-positive anti-HCV results was observed with the Ortho-Clinical Vitros ECi test. These findings suggest a lower specificity of the Ortho-Clinical Vitros ECi Anti-HCV test compared with other methods currently available, including the newest FDA-approved automated test on the market, the Bayer ADVIA Centaur Anti-HCV assay.

Minimizing the occurrence of false-positive results is of benefit to both patient and clinician, for a number of reasons, not the least of which is the cost and time involved in downstream confirmatory testing. The CDC currently recommends reflex or automatic supplemental testing for HCV to confirm initial HCV antibody screen results and further guide the clinician's treatment plan. In brief, the laboratory should either reflex test all anti-HCV screen-positive samples, or reflex test those with results below a specified s/co ratio (2,3). The bulk of the false positives we discuss herein would presumably fall into this category. By our estimation, a significant number of samples (>200 per year in our laboratory) would be tested for confirmation unnecessarily. Confirmatory testing by RIBA and/or NAT can be relatively expensive. The RIBA is offered by only one vendor in the United States, and HCV NAT tests are offered by only a few vendors in the United States. Therefore, significant cost savings may be realized by maximizing the specificity of the HCV screen employed, and thereby decreasing the need for costly reflexive testing. On the other hand, increasing specificity generally comes at the expense of sensitivity, and it is possible that Ortho-Clinical Diagnostics has chosen to maximize sensitivity in their anti-HCV test design; it is up to the users to determine the optimal test and characteristics for their patient population.

A final note of interest is that of reagent design. Reagent design certainly plays a part in the sensitivity and specificity of any laboratory assay. Abbott Diagnostics has recently introduced an FDA-approved Anti-HCV test for the AxSYM, and in this new formulation, the recombinant HCV protein NS5 has been omitted. It has been proposed that this new formulation will increase specificity of the anti-HCV test. Indeed, one research laboratory minimized false-reactivity with HCV-negative samples by designing new recombinant forms of the HCV protein NS5A (9). Ortho-Clinical Diagnostics and other vendors (including Bayer) use the NS5 protein in their assay preparations. Nonetheless, one laboratory has shown the Bayer ADVIA Centaur Anti-HCV assay to have higher specificity than the international Abbott AxSYM Anti-HCV assay preparation (6). While it is possible that elimination of the NS5 protein in the test formulation may improve specificity and minimize false positivity, more research will be required to fully address this issue.

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