short report
Haematologica 1997; 82:690-691

A COMPARATIVE STUDY OF TWO THIRD-GENERATION ANTI-HEPATITIS C VIRUS ELISAs

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ABSTRACT

The sensitivity and specificity of two third-generation screening tests for the detection of antibody to hepatitis C virus (anti-HCV) was evaluated in a side-by-side study (Abbott HCV EIA 3.0/Ortho HCV ELISA 3.0). Specimens that were reactive in either ELISA were then retested by the other ELISA, and confirmed by RIBA-3. The screening of 15,540 serum samples from healthy blood donors showed a significantly lower number of reactive cases tested by Ortho (21 out of 8,805, 0.24%), than tested by Abbott (35 out of 6,735, 0.52%). In the side-by-side comparison, we found that significantly (p=0.005) more Ortho-positive samples were also reactive in Abbott (14/21, 66.6%), than in Abbott followed by Ortho (7/35, 20%). Moreover, the RIBA analysis revealed a significantly (p=0.014) higher number of RIBA-positive specimens among those reactive in Ortho 10/21 (47.6%), than among those reactive in Abbott 6/35 (17.2%), thus the former provides a greater positive predictive value. However, we did not observed differences in the sensitivity between Abbott and Ortho, because all RIBA-positive samples demonstrated reactivity in both ELISAs.

Key words: hepatitis C, diagnosis, ELISA, virus, third-generation ELISA, screening, hepatitis C virus

Since the introduction of routine screenings for antibodies to hepatitis C virus (anti-HCV), increased efforts have been made to achieve assays with higher sensitivity and specificity. Nowadays, several third-generation enzyme-linked immunosorbent assays (ELISA-3) are commercially available, but side-by-side comparisons between them have been scarce.[1,2] The purpose of our study was to compare the sensitivity and specificity of two third-generation anti-HCV ELISAs (Abbott HCV EIA 3.0/Ortho HCV ELISA 3.0). The relevance of HCV for hematologic disorders has been shown by recent papers in this Journal.[3-7]

Materials and Methods

A total of 15,540 serum samples from healthy blood donors were assayed for the presence of anti-HCV with either the Abbott 3.0 [Abbott Diagnostics, Irving, TX, USA] (6,735 specimens), or the Ortho 3.0 [Ortho Diagnostic Systems, Germany] (8,805 samples), both in their original versions. Initially reactive samples were re-assayed in duplicate by the same procedure, and were considered as positive only when they demonstrated reactivity in two out of the three analyses. In addition, all positive samples in either ELISA group were then retested by the other ELISA, and subjected to a confirmation assay with a third-generation recombinant immunoblot assay HCV RIBA 3.0 (Ortho Diagnostic). Results in RIBA-3 were reported as positive (reactive with at least two antigen bands), indeterminate (reactive with a single antigen band), or negative (no reactive bands). For statistical analysis, a chi-square test in 2×2 tables was used, and a *p* value of below 0.05 was considered significant.

Results and Discussion

Table 1 summarizes our findings in the screening of 15,540 sera. Using the Abbott EIA, 35 out of 6,735 screened samples (0.52%) were found positive. In comparison, a significantly lower number of positive cases (21 out of 8805, 0.24%) was detected within the group tested by Ortho. Interestingly, when cases reactive in either ELISA were retested by the other, we found that only 7/35 (20%) of the specimens positive in Abbott also provided reactivity in the Ortho EIA. By contrast, among the 21 Ortho-positive samples, 14 (66.6%) were also positive in Abbott. Thus, significantly more (p=0.005) Ortho-positive samples provide reactivity in the Abbott EIA, than *viceversa*.

The RIBA 3.0 analysis (Table 1) revealed a significantly (p=0.014) higher number of RIBA-positive specimens among those reactive in Ortho 10/21 (47.6%), than among those reactive in Abbott 6/35 (17.2%). No significant differences were detected in the number of RIBA-indeterminate (p=0.54) and RIBA-negative (p=0.094) results between these groups.

In Table 2 we present the reactivity provided in

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Received August 25, 1997; accepted October 1, 1997.

Table 1. Anti-HCV screening of 15,540 serum samples from volunteer blood donors, by two third-generation ELISAs and patterns of anti-HCV reactivity observed with RIBA-3 in ELISA-positive specimens.

	Abbott 3.0	*Ortho 3.0*	*Total*
Samples	6,735	8,805	15,540
Anti-HCV positive	35 (0.52%)	21 (0.24%)	56 (0.36%)
RIBA-3			
Positive	6/35 (17.2%)	10/21 (47.6%)	16/56 (28.6%)
Negative	18/35 (51.4%)	6/21 (28.6%)	24/56 (42.8)
Indeterminate	11/35 (31.4%)	5/21 (23.8%)	16/56 (28.6%)

Table 2. ELISA reactivity in samples analyzed by RIBA 3.0.

	RIBA + (n=16) *EIA*		*RIBA indeter(n=16)* *EIA*		*RIBA – (n=24)* *EIA*	
	+	–	+	–	+	–
Abbott	16	0	13	3	20	4
Ortho	16	0	5	11	7	17

both ELISAs of all samples analyzed by RIBA. As shown, all RIBA-positive samples demonstrated reactivity in both Abbott and Ortho EIA. Among RIBA-negative samples (false positives from EIA), 3 provided reactivity in both ELISAs, 17 were reactive only in Abbott EIA, and 4 were reactive only in Ortho. Finally, within the RIBA-indeterminate group, 2 samples were detected as positive in both ELISAs, 11 were positive only by Abbott, and 3 were positive exclusively in the Ortho EIA. As concerns the antigen specificity of RIBA-indeterminate samples, we observed that the 3 RIBA-indeterminate specimens detected only by Ortho and the 2 RIBA-indeterminate samples detected in both ELISAs were reactive on c33c. By contrast, the 11 RIBA-indeterminate samples in the group positive only in Abbott demonstrated reactivity to either NS5 (8/11, 73%), c22p (2/11, 18%), or c100p (1/11, 9%) antigens.

The results of our study suggest that 3rd generation anti-HCV ELISAs of Abbott and Ortho have a similar sensitivity, because all RIBA-positive specimens demonstrated reactivity in both ELISAs. However, the positive predictive value (PPV) of the Ortho EIA was significantly higher than (p=0.01) the correspondent value of Abbott. Thus, 47.6% of the reactive specimens detected by Ortho were confirmed in RIBA-3, whereas only 17.2% of the reactive samples in the Abbott EIA were RIBA-positive. The reasons for the lower PPV of the Abbott assay are unclear. Since most Abbott positive-RIBA indeterminate specimens reacted against the NS5 antigen, unspecific reactivity induced by the presence of this antigen in the Abbott EIA could be speculated. Indeed, the inclusion of the NS5 antigen in third-generation screening assays has been controversial because of its limited contribution in improving sensitivity in the detection of anti-HCV.[8] In addition, its impact on the specificity of the HCV screening in low-risk donors is uncertain, since there are sera that have been identified as positive in RIBA 3.0 against the NS5 recombinant protein, but that are PCR negative.[9] Nevertheless, the elimination of the NS5 antigen in an attempt to exclude false-positive reactions may result in an increased risk of false-negative interpretations. One case of early seroconversion to NS5 in post-transfusion hepatitis has been reported.[10]

In our study, we detected 8 Abbott-positive sera which only reacted against the NS5 antigen in RIBA. These samples were found negative in Ortho EIA 3.0, and may correspond to the Ortho false-negatives. This argument is also valid for the 2 Abbott-positive samples that were reactive only against the c-22p antigen that did not demonstrate reactivity in Ortho. On the other hand, of the 5 Ortho positive-RIBA indeterminate samples reactive only against the c33c, 3 were not detected by Abbott. These results are in agreement with the findings of an earlier trial by Couroucé *et al*.[1] comparing the sensitivity of several anti-HCV screening tests including Abbott 3.0 and Ortho 3.0. These authors reported two false-negatives in Abbott corresponding to c33c reactive specimens, and one Ortho false-negative with anti-c22p reactivity.

In conclusion, our results show that the 3rd-generation anti-HCV ELISA of Ortho provides a greater PPV than the Abbott EIA 3.0, although the sensitivity of both techniques is similar.

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