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Performance of Third-Generation Confirmatory Tests for Detection of Antibody to Hepatitis C Virus

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We investigated three immunoblot assays (RIBA 3.0 from Chiron, Matrix from Abbott Laboratories, and LiaTek III from Organon Teknika) for the detection of antibody to hepatitis C virus. RIBA 3.0 and Matrix require reactivity to two antigens and LiaTek III requires reactivity to one for a sample to be positive. We tested 80 samples that were positive in repeat enzyme immunoassays by supplemental tests. The results showed that 55, 46, and 28% were reactive by LiaTek III, RIBA 3.0, and Matrix, respectively; 54, 33, and 13% of the samples were indeterminate by Matrix, RIBA 3.0, and LiaTek III, respectively. There were 32, 21, and 16% nonreactive samples by LiaTek III, RIBA 3.0, and Matrix, respectively. Of the samples positive by RIBA 3.0, only 50 and 76% were reactive by Matrix and LiaTek III, respectively. A large number of samples that were indeterminate by RIBA 3.0 were positive by LiaTek III (52%). The core antigen was the most reactive antigen in all three tests (48 to 57%). The NS_4 antigen in Matrix (20%) and LiaTek III (16%) was poorly reactive, although it performed better in RIBA 3.0 (45%). The NS_5 and E_2/NS_1 antigens made minor contributions to reactivity. The combinations of the core, NS_3, and NS_4 antigens produced 77% of the RIBA 3.0 and 100% of the Matrix reactive samples. The results showed a poor correlation among the three tests.

Hepatitis C virus (HCV) is the primary etiologic agent of parenterally transmitted non-A, non-B hepatitis (2). The laboratory diagnosis of HCV infection depends primarily on detecting antibody to HCV (anti-HCV) by enzyme immunoassays (EIAs). The first-generation EIAs were based on a recombinant HCV antigen, C100-3 (NS_4). However, it soon became obvious that the first-generation tests were lacking in sensitivity and specificity (10). This resulted in the development of second-generation EIAs and recombinant immunoblot assays (RIBA 2.0) (Chiron Corporation, Emeryville, Calif.). The second-generation EIA used three recombinant antigens, C200 (NS_4), C22-3 (core), and C100-3 (NS_4). RIBA 2.0 used four recombinant antigens, C100-3, 5-1-1, C33C (NS_3), and C22-3. The second-generation tests were more sensitive and specific (4, 5, 11, 16). However, the recombinant immunoblot assay (RIBA 2.0) was still identifying a significant number of samples as indeterminate (4, 10, 15). The biological and clinical significance of such a pattern is unknown (1).

Finally, a third-generation test (RIBA 3.0) was developed (Chiron Corporation) and was followed by other immunoblot assays, such as Matrix (Abbott Laboratories, North Chicago, Ill.) and LiaTek III (Organon Teknika, Amsterdam, The Netherlands). In the case of RIBA 3.0, the C100-3 and C22-3 bands are synthetic peptides, while C33C and the new band, NS_5, are recombinant proteins. The synthetic peptides have been designed to provide increased sensitivity.

The Matrix immunoblot assay (Abbott) has four recombinant antigen dots, C22-3, NS_3, NS_4 (*Escherichia coli*), and NS_4 (yeast). The LiaTek III has five synthetic bands, C1 (core), C2 (core), E_2/NS_1, NS_4, and NS_5, and one recombinant (NS_3) antigen band.

These three immunoblot assays are available commercially for supplemental testing in Canada. Preliminary results have shown that 85 to 90% of samples that were indeterminate by RIBA 2.0 were reactive by prototype RIBA 3.0 (7–9), whereas in another study (5), only 31% of samples that were indeterminate by RIBA 2.0 were reactive by prototype RIBA 3.0. However, a comparison of the performance of the three immunoblot tests is not available. Also, even though the antigens on the strips in different tests are from the same region of HCV, it is not known if they perform similarly.

In this report, we describe the comparative performance of the RIBA 3.0, Matrix, and LiaTek III tests for the detection of anti-HCV in a panel of human serum samples positive by EIA.

Eighty samples repeatedly positive for anti-HCV by EIA-2 were tested by RIBA 3.0, Matrix, and LiaTek III. These samples were submitted to our laboratory for supplemental testing. Risk factors for these patients were not available.

The tests were performed and interpreted according to the recommended protocols of the respective kits. RIBA 3.0 has two positive control bands and a superoxide dismutase control band. A specimen was considered positive when it reacted with two or more bands with an intensity equal to or greater than that of the weak immunoglobulin G (IgG) control ($\geq$1+) without reactivity to the superoxide dismutase band. A specimen that reacted with only one band ($\geq$1+) was considered an indeterminate result. The absence of reactivity to all of the HCV antigen bands was considered a negative result.

The Matrix system is an immunodot assay in which all the washing, incubation, and reading are done automatically. In this test, the intensity of reflected light is measured at 655 nm and given a numerical value. The result is reported as a sample-to-cutoff ratio, and a value equal to or greater than 1.0 is considered a positive result. A sample is positive when it reacts to two or more antigens. A sample is negative when it does not react to any of the HCV proteins. If a sample reacts to only one HCV dot, then it is called single-antigen reactive, and we have considered this an indeterminate result.

LiaTek III is an immunoblot assay with six HCV antigen bands. A sample is reactive by LiaTek III when the reaction to only one antigen has a 2+ or higher rating according to the positive controls or that to two or more antigens has a 1+ or higher rating. A sample is indeterminate when it is reactive

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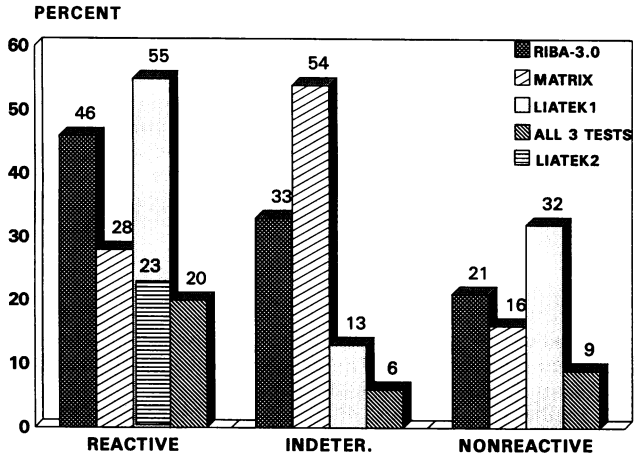

FIG. 1. Reactive, indeterminate, and nonreactive samples by RIBA 3.0, Matrix, and LiaTek III. LiaTek 1 shows all the samples which were reactive by their protocol, that is, ≥2+ for one antigen or ≥1+ for two or more antigens. LiaTek 2 represents only those samples reactive for two or more antigens (≥1+). "All 3 tests" means the number of samples reactive, indeterminate, and nonreactive by all three tests.

with only one antigen with a 1+ rating or with one or more antigens with a ± rating. A sample is negative when it does not react with any of the HCV antigens.

The results (Fig. 1) showed that 46, 28, and 55% of the samples were reactive by the RIBA 3.0, Matrix, and LiaTek III tests, respectively. When reactivity to two LiaTek III antigens was considered a positive result (LiaTek 2), then only 23% of the samples were positive. Twenty percent of the samples were positive by all three tests. A higher percentage of samples were positive by LiaTek III (55%) only because reactivity to one antigen (≥2+) was considered a positive result. When we applied the criterion of two-antigen reactivity to LiaTek III, the results showed that both RIBA 3.0 and Matrix performed better than LiaTek III.

The higher sensitivity of the RIBA 3.0 test has been reported previously (3, 7–9), and in all cases, the number of positive samples was higher by this test than by RIBA 2.0. However, similar data are not available for Matrix and LiaTek III.

The number of samples reactive by all three tests was low (20%), indicating that the three tests differ significantly in sensitivity or that they are often detecting different positive samples. The other possibility is that the synthetic peptides did not perform as well as the recombinant antigens, as reported previously (12).

Thirty-three, 54, and 13% of the samples were indeterminate by RIBA 3.0, Matrix, and LiaTek III, respectively, and 6% were indeterminate by all three tests. Nonreactive samples were 21, 16, and 32% by RIBA 3.0, Matrix, and LiaTek III, respectively, and the correlation among the three tests was 9%.

TABLE 2. Reactivity patterns of individual antigens from the RIBA 3.0, Matrix, and LiaTek III tests

Test	No. (%) of reactive samples				
	Core (C1, C2, C22)	NS_3 (C33C)	NS_4 (C100-3)	NS_5	E_2/NS_1
RIBA 3.0	43 (52)	36 (44)	37 (45)	9 (11)	NA[a]
Matrix	47 (57)	32 (39)	16[b] (20)	NA	NA
LiaTek III	39[c] (48)	13 (16)	13 (16)	5 (6)	5 (6)

[a] NA, not applicable.
[b] Two NS_4 antigen bands.
[c] Two core antigen bands.

The correlation among the three tests for indeterminate samples (6%) was even poorer than for the reactive samples. Matrix had the highest number of indeterminate samples, and LiaTek III had the lowest. The reason for the lowest number of indeterminates being found by LiaTek III is that samples positive for one antigen (≥2+) are considered reactive by this test, whereas they are considered indeterminate by RIBA 3.0 and Matrix.

The comparative analysis of the data is given in Table 1. Among samples reactive by RIBA 3.0, 50 and 76% were reactive by Matrix and LiaTek III, respectively. This further supports the differences in the performance of the three tests. On the other hand, 47 and 13% of samples reactive by RIBA 3.0 were indeterminate by Matrix and LiaTek III, respectively. Some of the RIBA 3.0 reactive samples were nonreactive by Matrix (3%) and LiaTek III (11%). Most of the RIBA 3.0 indeterminate samples remained the same by Matrix (64%), with the rest split between reactive (16%) and nonreactive (20%). However, by LiaTek III, 52% of the RIBA 3.0 indeterminate samples were reactive, only 22% stayed indeterminate, and the rest were nonreactive (26%). The results showed a better correlation between RIBA 3.0 and Matrix for the indeterminate samples. The majority of RIBA 3.0 nonreactive samples were also nonreactive by LiaTek III (88%), whereas only 41% were nonreactive by Matrix and 59% were indeterminate, while none were indeterminate by LiaTek III. However, none of the RIBA 3.0 nonreactive samples were reactive by Matrix, but 12% were reactive by LiaTek III. We cannot explain why a high percentage of RIBA 3.0 nonreactive samples were indeterminate by Matrix.

The pattern of reactivity for individual antigens is given in Table 2. The results showed that in the case of RIBA 3.0, the percentages of samples reacting with the core (52%), NS_3 (44%), and NS_4 (45%) antigens were similar. However, reactivity to NS_5 antigen was poor (11%). In the case of Matrix, reactivity to the core antigen was higher (57%) than that to the NS_3 (39%) or NS_4 (20%) antigens. The core antigen was the most reactive antigen (48%) in LiaTek III, while the NS_3 and NS_4 antigens each showed 16% reactivity and NS_5 and E_2/NS_1 each reacted with only 6% of the samples. The importance of

TABLE 1. Comparative evaluation of RIBA 3.0, Matrix, and LiaTek III results

RIBA 3.0 result (no. of samples)	Matrix result [no. (%) of samples]			LiaTek III result [no. (%) of samples]		
	Reactive	Indeterminate	Nonreactive	Reactive	Indeterminate	Nonreactive
Reactive (38)	19 (50)	18 (47)	1 (3)	29 (76)	5 (13)	4 (11)
Indeterminate (25)	4 (16)	16 (64)	5 (20)	14 (52)	6 (22)	7 (26)
Nonreactive (17)	0	10 (59)	7 (41)	2 (12)	0	15 (88)
Total (80)	23	44	13	43	13	26

TABLE 3. Comparison of positivity patterns for RIBA 3.0, Matrix, and LiaTek III

Test used	No. (%) of reactive samples								
	Core + NS_3	Core + NS_3 + NS_4	Core + NS_3 + NS_4 + NS_5	Core + NS_4	Core + NS_5	NS_3 + NS_4	NS_3 + NS_5	Combination[a]	Total
RIBA 3.0	4 (10.5)	9 (24)	7 (18.4)	8 (21)	1 (3)	8 (21)	1 (3)	NA[b]	38 (100)
Matrix	9 (39)	9 (39)	NA	2 (9)	NA	3 (13)	NA	NA	23 (100)
LiaTek III	1 (5)	0	1 (5)	3 (16)	1 (5)	1 (5)	0	12 (63)	19 (100)

[a] Different combinations of E_2/NS_1, core, NS_3, NS_4, and NS_5 antigens.
[b] NA, not applicable.

the core and NS_3 antigens was also demonstrated in studies (9, 13, 14) with the RIBA 2.0 and 3.0 tests.

The combination of different antigens which produced a positive result by RIBA 3.0, Matrix, and LiaTek III is given in Table 3. RIBA 3.0 positive samples in most cases (77%) reacted with the core, NS_3, and NS_4 antigens in different combinations. Samples positive by Matrix also reacted with these three antigens in different combinations. However, in LiaTek III, the core was the only major antigen, and 53% of samples were positive because of reactivity with this antigen (data not shown).

It is surprising to observe that the three tests are performing differently for the detection of anti-HCV in spite of the fact that they use antigens from the same regions of the genome, although a previous report (6) has shown that kits using the same antigens produced similar results. One possible explanation for these differences is that the concentration or reactivity of these antigens is different in each test or that the antigens are from a slightly different part of the same region of the HCV genome. Further analysis of the results showed that the NS_5 and E_2/NS_1 antigens were not making any significant contribution to the tests, as only a very small number of samples were positive because of reactivity with these two antigens.

The data indicated that the performance of these three supplemental tests for the detection of anti-HCV varies significantly and that RIBA 3.0 seems to be comparatively more sensitive than the other two tests if the same interpretation criteria are applied to LiaTek III.

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