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Evaluation of Third-Generation Assays for Detection of Anti-Hepatitis C Virus (HCV) Antibodies and Comparison with Presence of HCV RNA in Blood Donors Reactive to c100-3 Antigen

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We tested serum samples from blood donors by using first-, second-, and third-generation enzyme immunoassays or recombinant immunoblot assays. Second- and third-generation assays gave comparable results. Circulating hepatitis C virus RNA was found in a high proportion of reactive samples. A lack of reactivity or low-level reactivity predicted the absence of hepatitis C virus RNA in 100% of the cases.

The purpose of our research was to investigate the specificity of the new anti-hepatitis C virus (anti-HCV) serological assays. A commercially available first-generation anti-HCV enzyme immunoassay (EIA) (anti-HCV EIA; Ortho Diagnostics) was used for the prospective (between 1 August 1990 and 1 July 1991) screening of serum samples from 23,500 volunteer blood donors. Samples reactive twice in this assay were further investigated by a supplementary test (anti-HCV neutralization; Ortho Diagnostics). Subsequently, the samples reactive three times were further investigated with a second supplementary assay provided by another manufacturer (anti-HCV supplementary EIA; Abbott Diagnostics) and with three second-generation EIAs (anti-HCV EIA [Abbott Diagnostics], anti-HCV [Pasteur], and anti-Non-A-Non-B [Wellcome]), two third-generation EIAs (Ortho HCV 3.0 and Murex anti-HCV), one second-generation recombinant immunoblot assay (RIBA-2; Ortho Diagnostics), one third-generation recombinant immunoblot assay (RIBA prototype 3.0; Ortho Diagnostics), and one third-generation Western blot (immunoblot) assay (Wellcozyme HCV Western blot; Wellcome). Samples with absorbencies of less than 10% below or above the cutoff value were considered indeterminate. In addition 20 serum samples from donors nonreactive in the anti-HCV EIA antibody screening assay but showing elevated serum alanine aminotransferase (ALT) levels were also included.

Sixty-eight samples were reactive in the first-generation and first-generation supplementary assays. The seroprevalence of 0.29% is comparable to that found by another Swiss blood transfusion center (4). No donor had anamnestic or serological evidence of acute or chronic hepatitis B. Sixty-three samples (92.6%) were confirmed by the second supplementary assay (Table 1). All samples found positive when the second- and third-generation assays were used were included in these 63 samples. Twelve samples with discrepancies were found (Table 2). All of the remaining 56 samples were reactive in all assays. A comparison of the serological results with histories, risk factors, and clinical characteristics has been published elsewhere (24).

All 68 samples were also tested for the presence of HCV RNA by a nested PCR, using established procedures (6, 15).

TABLE 1. Comparison of anti-HCV first-generation supplementary assay, second- and third-generation EIAs, and second- and third-generation recombinant immunoblot/Western blot assays for blood donor samples repeatedly reactive to c100-3

Assay	No. (%) of Ortho c100-3-reactive samples (n = 68) with anti-HCV assay result			No. (%) of Ortho c100-3-nonreactive samples (n = 20) with anti-HCV assay result	
	Reactive	Nonreactive	Indeterminate	Reactive	Indeterminate
Abbott supplementary	63 (92.6)	5 (7.4)	0 (0.0)	0	0
Abbott second generation	57 (83.8)	10 (14.7)	1 (1.5)	0	0
Pasteur	54 (79.4)	12 (17.6)	2 (2.9)	0	0
Wellcome anti-Non-A-Non-B	53 (77.9)	15 (22.1)	0 (0.0)	0	0
Ortho HCV 3.0 EIA	56 (82.4)	12 (17.6)	0 (0.0)	1 (5.0)	0
Murex anti-HCV	56 (82.4)	12 (17.6)	0 (0.0)	0	0
RIBA-2	55 (80.9)	11 (16.2)	2 (2.9)	0	0
RIBA-3	55 (80.9)	8 (11.8)	5 (7.4)	0	1 (5.0)
Wellcozyme Western blot	56 (82.4)	8 (11.8)	4 (5.9)	0	2 (10.0)
HCV RNA	50 (73.5)	18 (26.5)	0 (0.0)	0	0

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TABLE 2. Serum samples with divergent results for the various assays performed

Sample no.[a]	Result[b] (antigen[s]) with assay									
	Ortho screening	Abbott supplementary	Abbott second generation	Pasteur	Wellcome anti-Non-A-Non-B	Ortho HCV 3.0 EIA	Murex anti-HCV	RIBA-2	RIBA-3	Wellcozyme Western blot
1	+	+	+	−	−	−	−	± (c100-3)	−	−
2	+	+	−	±	−	+	−	−	−	−
3	+	+	±	±	−	+	+	−	+ (c22-3, c-100-3)	+ (core, NS4)
4	+	+	+	−	−	−	−	+ (c100-3, 5-1-1)	± (c100-3)	−
5	+	+	+	−	−	−	−	−	−	−
6	+	+	+	+	−	+	+	+ (all bands)	+ (all bands except NS5)	+ (all bands)
7	+	+	−	−	−	−	+	−	± (c100-3)	± (core)
8	+	+	+	+	−	+	+	+ (all bands)	+ (all bands except NS5)	+ (all bands)
9	+	+	−	−	−	−	−	−	± (c100-3)	+ (NS3, NS5)
10	+	+	−	−	−	−	−	± (c100-3)	± (NS5)	± (NS5)
11[c]	−	−	−	−	−	+	−	−	± (c22-3)	± (core)
12[c]	−	−	−	−	−	−	−	−	−	± (core)

[a] All samples were negative for HCV RNA.
[b] +, reactive; ±, indeterminate; −, nonreactive.
[c] Donor with elevated ALT level.

TABLE 3. Comparison of EIA and recombinant immunoblot/Western blot assay reactivities with the presence of HCV RNA

Assay	Result	No. (%) of samples (n = 68) anti-c100-3 reactive and with RNA:	
		Positive[a]	Negative
Ortho c100-3	Reactive	50 (73.5)	18 (26.5)
Abbott supplementary	Reactive	50 (79.4)	13 (20.6)
	Nonreactive	0 (0.0)	5 (100.0)
Abbott second generation	Reactive	50 (87.7)	7 (12.3)
	OD > 2.0	50 (98.0)	1 (2.0)
	cutoff < OD < 2.0	0 (0.0)	6 (100.0)
	Indeterminate	0 (0.0)	1 (100.0)
	Nonreactive	0 (0.0)	10 (100.0)
Pasteur	Reactive	50 (92.6)	4 (7.4)
	OD > 3.0	26 (100.0)	0 (0.0)
	OD > 2.0	50 (94.3)	3 (5.7)
	cutoff < OD < 2.0	0 (0.0)	1 (100.0)
	Indeterminate	0 (0.0)	2 (100.0)
	Nonreactive	0 (0.0)	12 (100.0)
Wellcome	Reactive	50 (94.3)	3 (5.7)
	OD > 2.0	50 (94.3)	3 (5.7)
	cutoff < OD < 2.0	0 (0.0)	0 (0.0)
	Nonreactive	0 (0.0)	15 (100.0)
Ortho HCV 3.0 EIA	Reactive	50 (89.3)	6 (10.7)
	OD > 2.0	50 (92.6)	4 (7.4)
	cutoff < OD < 2.0	0 (0.0)	2 (100.0)
	Nonreactive	0 (0.0)	12 (100.0)
Murex anti-HCV	Reactive	50 (89.3)	6 (10.7)
	OD > 2.0	50 (92.6)	4 (7.4)
	cutoff < OD < 2.0	0 (0.0)	2 (100.0)
	Nonreactive	0 (0.0)	12 (100.0)
RIBA-2	Reactive	50 (90.9)	5 (9.1)
	Indeterminate	0 (0.0)	2 (100.0)
	Nonreactive	0 (0.0)	11 (100.0)
RIBA-3	Reactive	50 (90.9)	5 (9.1)
	Indeterminate	0 (0.0)	5 (100.0)
	Nonreactive	0 (0.0)	8 (100.0)
Wellcozyme Western blot	Reactive	50 (89.3)	6 (10.7)
	Indeterminate	0 (0.0)	4 (100.0)
	Nonreactive	0 (0.0)	8 (100.0)

[a] Twenty-nine donors had normal ALT levels; 21 donors had elevated ALT levels.

TABLE 4. Comparison of reactivity patterns of second- and third-generation recombinant immunoblot assays (RIBA-2 and RIBA-3) and third-generation Western blot (Wellcozyme) assay with the presence of HCV RNA

Assay	Reactivity pattern for HCV antigens					No. (%) of samples		
						Total (n = 68)	HCV RNA positive (n = 50)	HCV RNA negative (n = 18)
RIBA-2	5-1-1	c100-3	c33c	c22-3	Interpretation			
	+	+	+	+	Reactive	33 (48.5)	32	1
	−	+	+	+	Reactive	7 (10.3)	7	0
	+	−	+	+	Reactive	4 (5.9)	4	0
	+	+	+	−	Reactive	2 (2.9)	0	2
	−	−	+	+	Reactive	8 (11.8)	7	1
	+	+	−	−	Reactive	1 (1.5)	0	1
	−	+	−	−	Indeterminate	2 (2.9)	0	2
	−	−	−	−	Nonreactive	11 (16.2)	0	11
RIBA-3	NS5	c100-3	c33c	c22-3	Interpretation			
	+	+	+	+	Reactive	46 (67.6)	45	1
	−	+	+	+	Reactive	8 (11.8)	5	3
	−	+	+	−	Reactive	1 (1.5)	0	1
	+	−	−	−	Indeterminate	1 (1.5)	0	1
	−	−	−	+	Indeterminate	1 (1.5)	0	1
	−	+	−	−	Indeterminate	3 (4.4)	0	3
	−	−	−	−	Nonreactive	8 (11.8)	0	8
Wellcozyme Western blot	NS5	NS4	NS3	Core	Interpretation			
	+	+	+	+	Reactive	39 (57.4)	37	2
	−	+	+	+	Reactive	12 (17.6)	10	2
	+	−	+	−	Reactive	1 (1.5)	0	1
	−	+	−	+	Reactive	3 (4.4)	3	0
	−	−	+	+	Reactive	1 (1.5)	0	1
	+	−	−	−	Indeterminate	1 (1.5)	0	1
	−	−	−	+	Indeterminate	3 (4.4)	0	3
	−	−	−	−	Nonreactive	8 (11.8)	0	8

Fifty of the 68 samples were positive for the presence of HCV RNA (Table 1); this represented 73.5 to 94.3% of the reactive samples, depending on the assay used (Table 3). When only high antibody levels (optical densities [ODs] exceeding the densitometer range) were considered, a better correlation with the presence of HCV RNA (92.6 to 100%) was obtained. Conversely, all samples found to be nonreactive or possessing low or intermediate antibody levels (OD < 2.0) were negative for circulating HCV RNA. The Pasteur EIA showed the highest specificity (100%) for circulating HCV RNA when the detection limit of the densitometer (overflow) was used, but only 26 of 50 samples gave results above this limit. With an arbitrary limit of an OD of >2.0, which was more comparable to the overflow limits of the other EIAs, the Pasteur EIA showed comparable results (Table 3). Table 4 shows the results for detection of HCV RNA in comparison to the anti-HCV antibody patterns found when the RIBA-2, RIBA-3, and Wellcozyme assays were used. The reactivity pattern of the RIBA-2 assay was interesting because reaction to the c22-3 antigen predicted the presence of circulating virus in 96.2% of samples, whereas its absence predicted absence of circulating virus in 100% of samples, a finding also noted by others (3, 7, 13, 18, 19, 23). Antibodies against 5-1-1 and c100-3 did not represent a valuable correlate for HCV RNA. With the two third-generation immunoblot assays, a positive result was associated with the presence of HCV RNA in 89.3 to 90.9% of the samples. All negative or indeterminate samples were negative for HCV RNA.

The use of second-generation assays reduced the number of HCV-positive samples by 8.8 to 22.1%. The third-generation assays were comparable, since they reduced the rate of positive results by the same order of magnitude as did the second-generation assays. Results diverging between first-, second-, and third-generation assays were found only for HCV RNA-negative samples and for donors who showed no biochemical or clinical evidence of liver disease and had no history of exposure to HCV. This indicates probable false-positive results of the first-generation EIA, of the first supplementary assay, and, to a lesser extent, of the second supplementary assay. From these data, one cannot conclude that some of the second- or third-generation assays evaluated yielded false-positive results or that some showed greater sensitivity than others. Thus, all second- and third-generation assays evaluated showed a marked and comparable improvement in specificity compared with the first-generation assays. Such an observation has already been made for second-generation assays (5, 8, 10–12, 18, 21, 22).

High anti-HCV antibody levels, as estimated by high densitometric absorbencies, predicted the presence of circulating HCV RNA in 92.6 to 100% of the samples. On the other hand, low absorbencies predicted the absence of circulating HCV RNA in 100% of the samples. Thus, densitometric absorbencies in the second- and third-generation EIAs can contribute to predicting the presence of HCV viremia in blood donors with acceptable accuracy and thereby can help reduce the need for PCR technology, which is currently not suitable for routine laboratory screening. A positive result in the third-generation immunoblot assays also offered a good estimation of the presence of circulating HCV RNA. This was also found for the RIBA-2 assay, except that the overall interpretation was less clear-cut.

All 20 seronegative blood donors with elevated ALT levels

were negative for circulating HCV RNA. This indicates that ALT elevation in seronegative blood donors is generally due to causes other than HCV infection, at least in our blood donor population.

Of the 50 HCV RNA-positive donors, 21 had elevated ALT levels and 29 had normal ALT levels at the time of blood sampling. Thus, serum ALT screening, used as a surrogate marker of HCV infection, would have reduced the risk of HCV transmission for only 42% of our subjects (1, 2, 14, 16, 20).

The exclusion from blood donation of all donors with sera reactive in a second- or third-generation assay should be recommended, since because of the known fluctuation of HCV RNA levels (3), the few second- or third-generation assay-positive subjects without demonstrable HCV RNA might indeed still be infectious (9, 17).

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