JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2005, p. 3877–3883 Vol. 43, No. 8
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.8.3877–3883.2005

Simultaneous Detection of Hepatitis C Virus (HCV) Core Antigen and Anti-HCV Antibodies Improves the Early Detection of HCV Infection

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Received 28 December 2004/Returned for modification 9 March 2005/Accepted 9 May 2005

To evaluate whether a new enzyme immunoassay developed for the simultaneous detection of hepatitis C virus (HCV) core antigen (Ag) and anti-HCV antibodies (anti-HCV Ab) (Monolisa HCV Ag/Ab ULTRA; Bio-Rad) could improve the early detection of HCV infection, we compared its sensitivity to that of anti-HCV, HCV core Ag, and HCV RNA assays. The populations studied included 12 blood donor samples positive for HCV RNA and HCV core Ag but negative for anti-HCV antibodies and 23 hemodialysis patients who developed anti-HCV Ab (seroconversion) during the follow-up. From these 23 individuals, 83 samples sequentially collected prior to seroconversion and 108 samples collected after seroconversion were tested. Six of 12 blood donations were positive by the HCV Ag/Ab assay. In the hemodialysis cohort, the 24 HCV RNA-negative samples were negative by the HCV Ag/Ab assay and 23 of the 59 HCV RNA-positive samples (39%) were positive. The HCV Ag/Ab assay detected HCV infection on average 21.6 days before the most sensitive antibody assay. The HCV Ag/Ab assay did not detect HCV infection as early as the HCV RNA assay (mean delay, 30.3 days) or HCV Ag assay (mean delays, 27.9, and 16.3 days by the HCV core Ag quantification assay and the HCV Ag blood screening assay, respectively). This new assay provides a notable improvement for the early detection of HCV infection during the so-called window period compared with anti-HCV Ab assays and could be a useful alternative to HCV RNA detection or HCV core Ag assays for diagnosis or blood screening when nucleic acid technologies or HCV core Ag detection are not implemented.

Since the development of the first assay in 1989 (20), assays for detection of hepatitis C virus (HCV) antibodies (Ab) have allowed progress in the early detection of HCV infection (46). This increased sensitivity of the last-generation assays has dramatically reduced the risk of HCV transmission by blood components by reducing the window period from 82 days (5) to 66 days (3, 12). To further reduce the residual risk (2, 5, 16, 18, 36, 37, 41, 48), nucleic acid testing (NAT) for HCV RNA was introduced in several high-income countries (2, 14, 15, 21, 30, 39). In some countries, an assay for the detection of HCV core antigen (Ag) by use of the enzyme immunoassay (EIA) technology has been chosen as an alternative to NAT for the early diagnosis of infection (1, 8, 25, 38). In addition, some authors emphasized the clinical advantage of HCV core Ag quantification as a direct marker of viral replication in the chronic phase of infection (4) and as a relevant marker for predicting and monitoring the response to therapy (7, 29, 31). Indeed, the HCV core Ag assays have sensitivities close to that of NAT, with mean detection differences of 1 to 2 days in the window period with the specific assay developed for blood screening (11, 32, 35, 45) and 0.29 day with the immunoassay capable of detecting and quantifying HCV core Ag (23). A recent study reported that a prototype assay based on the simultaneous detection of HCV core Ag and anti-HCV Ab significantly closed the time gap between HCV RNA detection and the first appearance of detectable anti-HCV Ab (42). However, this assay is not yet available for routine use. More recently, a new combination assay has been developed and licensed in Europe (Monolisa HCV Ag/Ab ULTRA; Bio-Rad, Marnes la Coquette, France). To assess its sensitivity for the detection of HCV infection during the window period or at the early phase after seroconversion, we tested two panels and compared the results with those obtained using the two available assays for HCV Ag (HCV core Ag EIA blood screening assay and trak-C assay) and HCV RNA. The overall objective was to determine if this new test could constitute an alternative to NAT for the diagnosis of HCV infection during the window period and whether the sensitivity for antibody detection is preserved.

MATERIALS AND METHODS

Panels. (i) Panel 1: individual samples from volunteer blood donors. Panel 1 (Table 1) consisted of 12 blood donor samples which were negative for anti-HCV Ab (Ortho HCV 3.0 EIA test system Enhanced SAVe; Ortho Clinical Diagnostics, Raritan, NJ) but positive for HCV RNA. The plasma from each of these blood donations was immediately aliquoted and stored at −30°C until it was

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TABLE 1. Results of HCV Ag/Ab assay for the 12 HCV RNA-positive, anti-HCV Ab-negative blood donor samples included in panel 1

Case no.	Clinical context[a]	Genotype	HCV RNA level (IU/ml)[b]	HCV core Ag result by blood screening assay (S/CO)[c]	trak-C assay result (Ag level [pg/ml])	Monolisa HCV Ag/Ab ULTRA result (S/CO)[c]
1	WP	1a	1.6×10^7	+ (20.6)	+ (183.5)	+ (5.95)
2	IS	1b	8×10^5	+ (2.14)	+ (48.8)	− (0.65)
3	WP	2a/2c	8×10^6	+ (20.1)	+ (>217)	Gray zone (0.90)
4	WP	1b	6.5×10^5	+ (3.05)	+ (51.7)	− (0.40)
5	WP	3	3.6×10^4	− (0.27)	+ (2.8)	− (0.10)
6	WP	1	8×10^7	+ (>53.8)	+ (>217)	+ (>6.64)
7	WP	1a	4.7×10^7	+ (49)	+ (>217)	Gray zone (0.93)
8	IS	1a	1.7×10^7	NA[d]	+ (>217)	+ (1.20)
9	WP	3a	1.2×10^5	− (0.45)	+ (15.2)	− (0.10)
10	IS	4a	$>5 \times 10^5$	+ (9.1)	+ (>278)	+ (2.58)
11	?	3a	7×10^6	+ (1.3)	+ (105)	− (0.13)
12	WP	1b	1.8×10^7	+ (38)	+ (>240)	− (0.50)

[a] WP, window period; IS, immunosilent chronic carrier.
[b] Amplicor HCV Monitor Roche v 2.0.
[c] S/CO, OD for the sample/cutoff OD; S/CO ≥ 1 = positive; 0.9 ≤ S/CO < 1 = gray zone.
[d] NA, not available.

used. All these samples were subsequently found to be positive for HCV core Ag by the trak-C assay (23), and 9 of the 11 samples tested were also positive by the HCV core Ag blood screening assay. Eight samples had been collected before the seroconversion (among these, five had been identified by the French Fractionation and Biotechnology Laboratory by detection of HCV RNA in plasma pools, and three had been identified by NAT in a pool of 8 or 24 donations after its implementation in blood donation centers), and three had been collected from three chronic immunosilent HCV carriers. Among the donors who provided the latter three samples, the first one developed antibodies 2 years after donation (13), and the second and the third ones were still negative for anti-HCV Ab 1 year and 6 months after donation, respectively (unpublished data). The last one was not explored. Viral loads ranged from 3.6×10^4 to 8×10^7 IU/ml (Amplicor HCV Monitor test v 2.0; Roche Diagnostics, Branchburg, NJ). Genotypes were determined by the InnoLipa test (Innogenetics, Zwijndrecht, Belgium).

(ii) Panel 2: sequential samples from hemodialysis patients. Panel 2 consisted of 191 samples collected from 23 hemodialysis patients who developed anti-HCV antibodies (seroconversion) during the follow-up. Each sample had been imme-

TABLE 2. Number of positive samples among anti-HCV Ab-negative samples included in panel 2, according to the assay

Patient no.	Genotype[b]	No. of samples tested (n = 83)	No. of samples[c]: RNA HCV positive	HCV core Ag blood screening assay positive	trak-C assay positive	Monolisa HCV Ag/Ab ULTRA positive[a]
S9	1b	3	2	2	2	0
S10	1b	2	1	1	1	1
S11	nt	7	6	3	6	0
S12	1b	2	1	1	1	1
S13	3a	1	0	0	0	0
S14	1a	3	2	2	2	2
S16	1b	1	0	0	0	0
S17	1b	11	10	6/6	9/9	6
S18	1b	3	2	1	2	0
S19	1	5	5	3	4	0
S20	1b	1	0	0	0	0
S21	2a/2c	3	2	2	2	1
S23	1b	2	1	0	1	0
S24	3a	5	4	4	4	3
S29	1b	4	2	2	2	2
S31	4c/4d	2	1	1	1	0
R1	2a/2c	7	5	4	4	3
R2	2a/2c	6	5	0	3/3	0
R5	2a/2c	3	1	0	1	0
R6	2a/2c	4	4	3	3/3	2
R7	2a/2c	3	2	1/1	2	2
R8	2a/2c	1	0	0	0	0
R9	2a/2c	4	3	3	2/2	0
No. positive/no. of HCV RNA-positive samples tested (%)			59/59 (100)	39/54 (72.2)	52/54 (96.3)	23/59 (39)

[a] Including four samples in the gray zone.
[b] nt, nontypeable.
[c] Where not all samples were tested, values are numbers of positive samples/numbers tested.

TABLE 3. Delays in the detection of early HCV infection between assays for patients included in panel 2

Patient	Delay (days) between the two mentioned tests (no. of samples/total no. of samples tested)[d]				
	HCV RNA and Monolisa HCV Ag/Ab	HCV core Ag blood screening assay and Monolisa HCV Ag/Ab	trak-C assay and Monolisa HCV Ag/Ab	Monolisa HCV Ag/Ab and the most sensitive anti-HCV assay[a]	Monolisa HCV Ag/Ab and Monolisa HCV
S9	46 (2)	46 (2)	46 (2)	0	0
S10	0	0	0	29 (1)	44 (2)
S11	61 (6)	Not assessable	61 (6)	−7 (1)[c]	0
S12	0	0	0	28 (1)	28 (1)
S13	0	−28 (1)[b]	0	0	0
S14	0	0	0	47 (2)	47 (2)
S16	0	0	0	0	25 (4)
S17	354 (4)	Not assessable	Not assessable	287 (6)	287 (6)
S18	28 (2)	19 (1)	28 (2)	0	0
S19	≥103 (5)	48 (3)	76 (4)	0	16 (1)
S20	0	0	0	0	0
S21	25 (1)	25 (1)	25 (1)	30 (1)	30 (1)
S23	15 (1)	0	15 (1)	0	31 (1)
S24	28 (1)	28 (1)	28 (1)	28 (3)	35 (2)
S29	0	0	0	69 (2)	90 (3)
S31	28 (1)	28 (1)	28 (1)	0	0
R1	5 (2)	0	0	51 (4)	51 (4)
R2	133 (5)	Not assessable	133 (3)	0	12 (2)
R5	91 (1)	0	91 (1)	0	0
R6	28 (2)	19 (1)	19 (1)	93 (2)	93 (2)
R7	0	0	0	77 (2)	77 (2)
R8	0	0	0	0	0
R9	44 (3)	44 (3)	44 (2)	0	40 (2)
Mean delay	30.3 (36/59)[e]	16.3 (11/39)[f]	27.9 (29/52)[g]	21.6[h]	30.1[i]

[a] The most sensitive assay is defined as the first assay which detected the appearance of anti-HCV Ab in each patient included in the panel.
[b] Monolisa HCV Ag/Ab ULTRA positive before HCV core Ag positive by HCV core Ag blood screening assay.
[c] Signal in RIBA test before Monolisa HCV Ag/Ab ULTRA positive result.
[d] The data for some patients were excluded from calculation of the mean due to the absence of available results of suitable delays between sampling. These patients are indicated in footnotes *e* to *i*.
[e] S13, S17, S20, R5, R8. For patient S19 with a follow-up beginning with an HCV RNA positive sample with a low viral load (400 copies/ml), we considered that the delay between the first HCV RNA-positive sample and the first Monolisa HCV Ag/Ab ULTRA was 104 days.
[f] S11, S17, S20, S29, R2, R5, and R8.
[g] S13, S17, S20, R5, and R8.
[h] S13, S17, S20, and R8.
[i] S13, S17, S20, R7, and R8.

diately aliquoted after collection and stored at −30°C until it was used. Samples were previously tested for (i) anti-HCV Ab by using third-generation assays (Ortho HCV 3.0 EIA test system Enhanced SAVe and RIBA 3.0; Ortho Clinical Diagnostics); (ii) HCV RNA (Amplicor HCV and Amplicor HCV Monitor test v 1.2 [Roche Diagnostics], which have detection limits of 50 IU/ml and 400 copies/ml, respectively); (iii) HCV core Ag, determined with the two available assays (HCV core Ag blood screening assay and trak-C assay; Ortho Clinical Diagnostics); and (iv) HCV genotype (InnoLipa test; Innogenetics) (11, 23). For each patient except the one who was positive only for HCV RNA in the first available sample, a negative sample for all above-mentioned HCV markers was obtained. This negative sample preceded the first available sample positive for at least one of these markers. Among the 191 samples included in panel 2, 83 (1 to 11 samples for each patient) were collected during the HCV window period (59 were positive and 24 were negative for HCV RNA) and 108 were collected after seroconversion, which was defined as a positive HCV Ab EIA result or a signal, even weak, for NS3 or core protein by a recombinant immunoblot assay (RIBA test).

Methods. Each sample was tested simultaneously by Monolisa anti-HCV Plus version 2 for anti-HCV Ab detection and by Monolisa HCV Ag/Ab ULTRA for the combined detection of HCV antigen and HCV antibodies, according to the manufacturer's instructions.

Monolisa HCV Ag/Ab ULTRA is based on the combination of an indirect test for the antibodies and a sandwich test for Ag detection. The main steps of the procedure were as follows: 50 μl of each sample and controls was incubated at 37°C for 90 min with 100 μl of conjugate 1 (containing biotinylated anti-HCV core monoclonal antibody) in microwells coated with a monoclonal antibodies specific for HCV core protein, purified NS3 and NS4 recombinant HCV proteins (from genotypes 1 and 3), and a core Ag-specific peptide. After a cycle of washings, 100 μl of conjugate 2 (containing peroxidase-labeled antibodies to human immunoglobulin G and streptavidin-peroxidase) was added to each well and the plate was incubated for 30 min at 37°C. After a second cycle of washing, the antigen-antibody complex was revealed by the addition of substrate, and the reaction was stopped after a 30-min incubation at room temperature by the addition of 100 μl of a 1 N sulfuric acid solution to each well. The absorbance was measured at 450 nm with a 620-nm reference wavelength. A sample was considered positive when its optical density (OD) was greater than or equal to the cutoff value (determined by the mean of the positive control OD divided by 4). Moreover, a 10% gray zone was recommended by the manufacturer, which advises consideration of such a sample as possibly positive.

RESULTS

Panel 1: individual blood donor samples. Table 1 summarizes the results of HCV RNA testing, HCV core Ag blood screening assay, trak-C assay, and Monolisa HCV Ag/Ab ULTRA for the 12 HCV RNA-positive and anti-HCV Ab-negative blood donor samples of panel 1. Four samples (from cases 1, 6, 8, and 10) were positive by the HCV Ag/Ab assay, and two samples (from cases 3 and 7) were in the gray zone. Except for the sample from case 12 (HCV RNA level, 1.8×10^7 IU/ml), which was repeatedly negative by the HCV Ag/Ab assay (data not shown in Table 1), the six HCV Ag/Ab-positive samples

TABLE 4. Characteristics of the 22 samples collected after HCV seroconversion from 10 patients who became negative for anti-HCV antibody (Monolisa HCV Ab assay) ($n = 8$) and/or HCV core Ag (trak-C assay) ($n = 20$) during the follow-up[d]

Patient	Delay after seroconversion (mo)	Anti-HCV antibodies		HCV RNA	HCV core Ag trak-C assay result (level [pg/ml])	Monolisa HCV Ag/Ab ULTRA result (S/CO)
		RIBA test	Monolisa HCV Ab (S/CO)			
S13[a]	2	Neg	Neg (0.6)	Pos	Pos (>224)	Pos (1.64)
S19	1	NS4, NS3, core	Pos (6.4)	NT	Neg (0.0)	Pos (3.9)
S21	3	NS3, core	Pos (>7.2)	NT	Neg (0.0)	Pos (5.6)
S23[b]	90	NT	Neg (0.5)	Neg	NT	Pos (1.8)
	114	NS3+/−, core+/−	Neg (0.5)	NT	Neg (0.1)	Pos (1.4)
S31	15	NS4, NS3, core	Pos (>8.6)	NT	Neg (0.5)	Pos (5.9)
R1	1.5	NS3, core	Pos (8.1)	Neg	Neg (0.7)	Pos (5.2)
	9	NS3, core	Pos (6.7)	Neg	Neg (0.7)	Pos (5.1)
R2[c]	24	NS4, NS3, core	Pos (8.1)	Neg	Neg (0.6)	Pos (5.3)
R5[c]	0.8	NT	Pos (6.3)	Neg	Neg (0.6)	Pos (5.7)
	6.5	NS3, core	Pos (1.9)	Neg	Neg (0.6)	Pos (3.3)
	18	NS3 +/−, core +/−	Neg (0.2)	Neg	Neg (0.6)	Neg (0.3)
	41	Neg	Neg (0.8)	Neg	Neg (0.6)	Pos (1.0)
R7[b]	6	NS3, core	Pos (1.3)	Neg	Neg (0.6)	Pos (3.3)
	25	NT	Neg (0.2)	Neg	Neg (0.7)	Neg (0.3)
	33	NT	Neg (0.2)	Neg	Neg (0.6)	Neg (0.2)
R9[c]	1.5	NS4, NS3, core	Pos (7.4)	Neg	Neg (0.7)	Pos (5.7)
	2	NT	Pos (6.6)	Neg	Neg (0.7)	Pos (5.7)
	3	NS4, NS3, core	Pos (5.5)	Neg	Neg (0.9)	Pos (5.6)
	4	NT	Pos (4.3)	Neg	Neg (0.9)	Pos (5.0)
	7	NS3+/−, core	Pos (1.3)	Neg	Neg (0.8)	Pos (2.1)
	15	Core	Neg (0.5)	Neg	Neg (0.8)	Pos (1.6)

[a] Intermediate antibody-negative result.
[b] Spontaneous resolved infection.
[c] Interferon treatment (R2, 15 months after seroconversion; R5, 1 month after seroconversion; R9, 1 month after seroconversion).
[d] Abbreviations: NT, not tested; Neg, negative; Pos, positive; S/CO, sample OD/cutoff OD.

corresponded to the highest viral loads (HCV RNA levels, from 8×10^6 IU/ml to 8×10^7 IU/ml).

Panel 2: hemodialysis patient samples. Of the 83 anti-HCV Ab-negative samples collected before the seroconversion (Table 2), the 24 HCV RNA- and HCV core Ag assay-negative samples were also negative by the Monolisa HCV Ag/Ab ULTRA assay. Among the 59 anti-HCV Ab-negative, HCV RNA-positive samples (39 of 54 were HCV core Ag blood screening assay positive and 52 of 54 were positive by the trak-C assay), 23 (39%) were positive by the HCV Ag/Ab assay. No difference was observed according to genotype. All 23 samples were also positive by both HCV core Ag assays. Table 3 shows the mean delays for the early detection of HCV infection between assays. The mean delays between positive results by HCV RNA testing and positive results by the Monolisa HCV Ag/Ab, between positive results by the trak-C assay and Monolisa HCV Ag/Ab ULTRA assay, and between positive results by the HCV core Ag blood screening assay and the Monolisa HCV Ag/Ab ULTRA assay were 30.3 days (36 of 59 = 61% of discrepant results), 27.9 days (29 of 52 = 55.8% of discrepant results), and 16.3 days (11 of 39 = 28% of discrepant results), respectively. In addition, the Monolisa HCV Ag/Ab ULTRA could detect HCV infection before the most sensitive HCV Ab assay (defined as the first HCV Ab assay which detected the appearance of anti-HCV Ab for each patient) and Monolisa anti-HCV Plus version 2 could, with mean delays of 21.6 days and 30.1 days, respectively.

Among the 52 trak-C assay-positive samples that were collected during the window phase, 22 of 26 samples with an HCV core Ag concentration greater than 260 pg/ml were Monolisa HCV Ag/Ab ULTRA positive, whereas only 1 of 26 samples with a core Ag level below 260 pg/ml was Monolisa HCV Ag/Ab ULTRA positive ($P < 10^{-3}$).

Of the 108 samples collected during or after seroconversion, 4 were Monolisa HCV Ag/Ab ULTRA negative. One sample (patient S11, Table 3) corresponded to an early seroconversion (RIBA test c33+/−) and was positive for HCV RNA but negative by the Monolisa HCV Ab (the trak-C assay was not performed). The three other Monolisa HCV Ag/Ab ULTRA-negative samples (which were also negative for all other HCV markers) (Table 4) were collected from two patients who recovered from infection (patient R5, one sample collected 17 months after interferon treatment; patient R7, two samples collected 25 and 33 months after seroconversion and corre-

TABLE 5. Sensitivity of Monolisa HCV Ag/Ab ULTRA for HCV RNA-positive but anti-HCV Ab-negative samples

Sample	Total no. of HCV RNA-positive samples	No. of samples positive by individual assay/total no. tested (%):		
		HCV core Ag blood screening assay	trak-C assay	Monolisa HCV Ag/Ab ULTRA assay
Blood donors	12	9/11 (81.8)	12 (100)	6[a] (50)
Hemodialyzed patients	59	39/54 (72.2)	52/54 (96.3)	23/59[b] (39)
Total	71	48/65 (73.8)	64/66 (96.9)	29/71 (40.8)

[a] Two of six samples were in the gray zone.
[b] Four of 23 samples were in the gray zone.

sponding to a spontaneous resolved infection). Of the 104 Monolisa HCV Ag/Ab ULTRA-positive samples, 17 were trak-C assay negative (14 were anti-HCV Ab positive and 3 were anti-HCV Ab negative by the Monolisa HCV Ab assay) and 8 were Monolisa HCV Ab assay negative (3 were trak-C assay negative). All these 17 samples (Table 4) (14 of which were HCV RNA negative and 3 of which were not tested) were collected from patients who recovered from infection spontaneously or after antiviral treatment.

DISCUSSION

The diagnosis of HCV infection is currently based on the detection of anti-HCV Ab by EIAs, and it is confirmed by a positive result obtained by an immunoblot assay or by the presence of HCV RNA (27, 34). However, when an early diagnosis of HCV infection is required, such as in emergency situations (health care worker exposures) (47), in blood screening to reduce the residual risk of transmission through blood (5) or grafts (9, 24), or as a pretreatment screening for at-risk populations, two diagnostic approaches based on the detection of the HCV RNA or HCV core Ag are currently available. The prevention of viral transmission by blood, thanks to the implementation of these methods for routine blood screening, has been reported (10, 14, 25, 39, 40, 44). However, since nucleic acid testing could not totally prevent HCV transmission, anti-HCV serologic screening must be continued (6, 22, 33) and viral screening approaches can be implemented only as supplemental tests. Thus, a new assay that combines the detection of HCV antigen and antibodies could preclude the need for additional assays in routine screening and thereby minimize the cost of the improvement of blood safety, especially with regard to NAT procedures (17, 28, 43).

Concerning the early diagnosis of HCV infection in seronegative individuals, our study indicates that the Monolisa HCV Ag/Ab ULTRA assay was able to detect the infection in 40.8% of HCV-RNA positive samples. The performance in terms of sensitivity was not as high as that observed by HCV core Ag assays (73.8% and 96.9% of positive samples for HCV core Ag blood screening assay and trak-C assay, respectively) (Table 5). These differences were due to the lower sensitivity of the Monolisa HCV Ag/Ab ULTRA assay for the detection of HCV core proteins than those of specific HCV core Ag assays. However, this HCV Ag/Ab combined assay significantly reduces the window period to 21.6 days and 30.1 days compared with that of the most sensitive assay and Monolisa HCV Ab assay, respectively. Moreover, 50% of the blood donation samples in panel 1 that were positive only by NAT were also detected by the Monolisa HCV Ag/Ab ULTRA assay, indicating a better sensitivity compared to those of the antibody-only assays. When the present seronegative window period of 66 days is considered (2), the yield of this HCV Ag/Ab assay would correspond to a 33 to 46% reduction in the HCV transfusion-transmitted residual risk. Thus, we can assume that the implementation of this assay in countries where only the anti-HCV antibody screening is performed would be an efficient measure that could be used to improve blood safety.

After seroconversion, HCV RNA, as well as HCV antigen, become undetectable (especially if the blood screening HCV Ag core assay is used). This is the consequence of a progressive decline in viremia after seroconversion (5), and it is also probably due to the fact that HCV antigens are in the form of immune complexes. In such cases, NAT and HCV Ag-specific assays could be inefficient for the detection of HCV infection (19). Monolisa HCV Ag/Ab ULTRA also gave negative results, but only after spontaneous or treatment-induced resolution of infections, when, evidently, all markers of HCV infection are undetectable. Contrary to what is observed in rare circumstances of early infection with human immunodeficiency virus (HIV) with some HIV Ag/Ab combined assays (26), we did not evidence a gap between Ag and immunoglobulin G detection, despite the fact that the HCV combined assay uses an indirect EIA for anti-HCV antibody detection. Thus, the improved sensitivity of the Ag detection test did not impair antibody detection.

Although this new assay is not as sensitive as HCV Ag-specific assays, it presents undeniable performance in terms of sensitivity for the detection of HCV infection, especially in the early phase, when antibodies are undetectable. Its represents a notable improvement for the detection of HCV infection when it is compared with the anti-HCV Ab detection assays. The simultaneous detection of HCV antigen and antibodies provides a valuable alternative to methods that directly detect viremia, such as NAT or an HCV Ag-specific assay, especially with regards to cost, organization, emergency, and logistic difficulties. It could be implemented for blood screening in developing countries where NAT or HCV Ag assays are not affordable or not feasible. In addition, the requirement for a fast screening of organ transplant donors makes the HCV Ag/Ab combined assay an attractive option, since a high HCV prevalence has been observed in this population (9). Finally, it could contribute to decision making when preventive antiviral therapy has to be implemented in the case of occupational exposure. As observed for HIV Ag/Ab combined assays (26), the improvement of the HCV antigen detection of HCV

Ag/Ab assays would allow the benefit provided by these new tools to be increased.

ACKNOWLEDGMENT

We thank Camille Sureau for helpful advice and comments.

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