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Serological Determination of Hepatitis C Virus Genotype: Comparison with a Standardized Genotyping Assay

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In patients with chronic hepatitis C, determination of hepatitis C virus (HCV) genotype could be routinely run in the future to tailor treatment schedules. The suitabilities of two versions of a serological, so-called serotyping assay (Murex HCV Serotyping Assay version 1-3 [SA1-3] and Murex HCV Serotyping Assay version 1-6 [SA1-6]; Murex Diagnostics Ltd.), based on the detection of genotype-specific antibodies directed to epitopes encoded by the NS4 region of the genome, for the routine determination of HCV genotypes were studied. The results were compared with those of a molecular biology-based genotyping method (HCV Line Probe Assay [INNO-LiPA HCV]; Innogenetics S.A.), based on hybridization of PCR products onto genotype-specific probes designed in the 5′ noncoding region of the genome, obtained with pretreatment serum samples from 88 patients with chronic hepatitis C eligible for interferon therapy. Definitive genotyping was performed by sequence analysis of three regions of the viral genome in all samples with discrepant typing results found among at least two of the three assays studied. In all instances, sequence analysis confirmed the result of the INNO-LiPA HCV test. The sensitivity of SA1-3 was 75% relative to the results obtained by the genotyping assay. The results were concordant with those of genotyping for 92% of the samples typeable by SA1-3. The sensitivity of SA1-6 was 89% relative to the results obtained by the genotyping assay. The results were concordant with those of genotyping for 94% of the samples typeable by SA1-6. Overall, SA1-6 had increased sensitivity relative to SA1-3 but remained less sensitive than the genotyping assay on the basis of PCR amplification of HCV RNA. Cross-reactivities between different HCV genotypes could be responsible for the mistyping of 8 (SA1-3) and 6% (SA1-6) of the samples. Subtyping of 1a and 1b is still not possible with the existing peptides, but discriminating between subtypes may not be necessary for routine use.

Recently, sequences of different hepatitis C virus (HCV) variants were classified into different genotypes on the basis of overall sequence similarity (2, 20, 22, 24). HCV genotypes are likely the result of spontaneous mutations that occurred in the genome, that were selected in specific geographical areas, and that could, later, spread widely owing to population mixing and new routes of transmission. A consensus nomenclature for HCV genotypes has been proposed (19), in which the six main HCV types identified so far are numbered in the order of their discovery, i.e., 1 to 6. Within each type, subtypes have been identified by lowercase letters, which are also given in order of discovery. Most of the HCV strains present in industrialized countries are HCV genotype 1, 2, or 3 (6, 16).

HCV genotypes and subtypes seem to have important significance in the epidemiology and pathogenesis of HCV-related disease. We recently observed that HCV genotypes were significantly related to the source of infection (18). On the other hand, HCV genotype 1b could be associated with more severe liver disease than other types, as recently reported for HCV-positive liver graft recipients (5). Finally, HCV genotype 1 seems to be associated with a worse response to interferon alfa therapy in patients with chronic hepatitis C (7, 11, 16). Whether the routine determination of HCV genotype could be useful in the management of patients with chronic hepatitis C remains unclear. With the refinement of interferon schedules and the development of new antiviral molecules in the foreseeable future, tailoring of therapeutic schedules to various pretreatment parameters, including HCV genotype, will probably be required.

Molecular biology-based genotyping methods have been widely used for research purposes. However, they are time-consuming and expensive, require specific molecular biology equipment and experience, and are not adapted to large-scale HCV genotype determination. This emphasizes the need for more practicable typing technologies, and several investigators have proposed serological techniques of HCV genotype determination (so-called serotyping techniques) based on the detection of genotype-specific antibodies by immunoenzymatic methods (1, 4, 10, 21, 25, 26). In addition to their theoretically better suitability than molecular biology-based methods for routine use, serotyping methods could prove particularly useful in determining the HCV genotype in HCV RNA-negative patients and in identifying infections with multiple genotypes.

Anti-HCV antibody reactivities have been shown to be influenced by HCV genotypes (8, 17). In particular, epitopes encoded by the NS4 region of the HCV genome emerged as candidates for discriminating serologically among patients infected with different HCV genotypes (8, 17). A serotyping assay based on the use of branched synthetic peptides and

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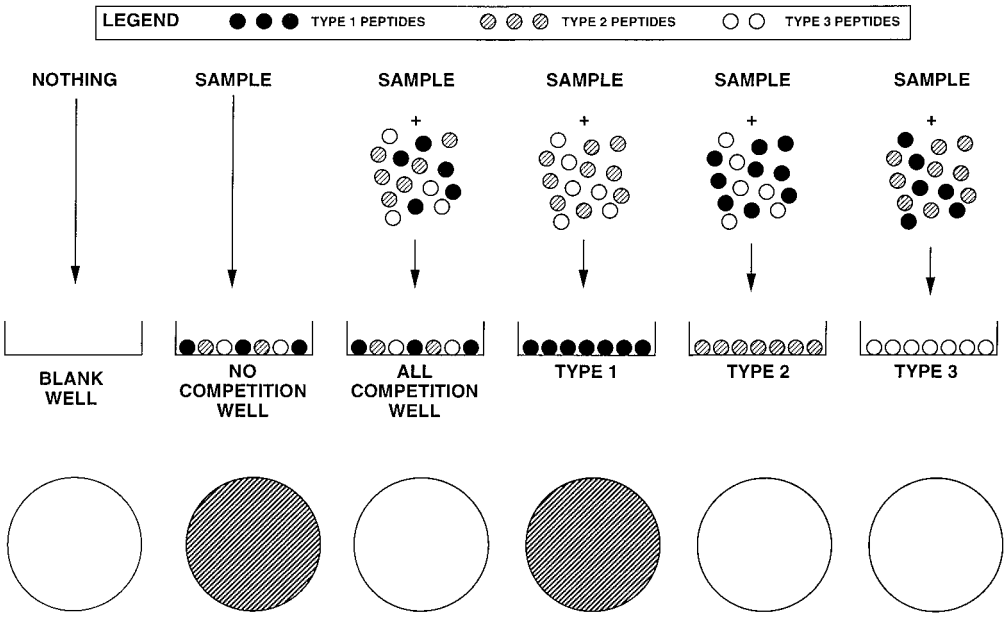

FIG. 1. Principles of SA1-3 and example of the results given by the assay for a patient infected with HCV genotype 1. Eight NS4-encoded genotype-specific branched peptides are used: three are specific for type 1, three are specific for type 2, and two are specific for type 3. Each determination needs six different microwells. For example, detection of genotype-specific antibody to type 1 is achieved by the addition of a 100-fold molar excess of peptides from genotypes 2 and 3 to the test serum or plasma samples. Thus, color appears in the no-competition well and in the specific type 1 well, while no color is visible in the other wells.

competition enzyme-linked immunosorbent assay (ELISA) has been developed to detect genotype-specific anti-NS4 antibodies directed to HCV genotypes 1, 2, and 3 (21). This assay has later been improved and commercialized in a standardized format (Murex Anti-HCV Serotyping Assay version 1-3 [SA1-3]; Murex Diagnostics Ltd., Dartford, United Kingdom). Recently, a new version of this serotyping assay (Murex Anti-HCV Serotyping Assay version 1-6 [SA16]) has been developed; the new version included a larger number of branched peptides to identify HCV genotypes 1 through 6 and several technical modifications (1). Both versions of the assay have been successfully used to determine HCV genotypes in selected populations of HCV-positive patients or blood donors (1, 21).

The aim of this study was to determine the suitability of the two versions of this competitive ELISA-based serotyping assay for routine determination of HCV genotype. For this, the results of the two serotyping assays were compared with those of a standardized molecular biology-based genotyping method based on hybridization of PCR products onto genotype-specific probes designed in the 5′ noncoding region of the genome. The study materials comprised pretreatment serum samples from patients with chronic hepatitis C eligible for interferon therapy, the main target group for the large-scale, routine use of HCV genotype determination in the future.

MATERIALS AND METHODS

Patients. Eighty-eight consecutive patients with chronic hepatitis C (53 men and 35 women; mean age, 46 years) eligible for interferon alfa therapy were studied. The diagnosis of chronic hepatitis C was based on persistently elevated serum alanine aminotransferase activity, chronic hepatitis on liver biopsy, and the presence in serum of anti-HCV antibodies detected by third-generation detection and confirmatory assays (Ortho Diagnostic Systems, Raritan, N.J.). The patients were all negative for hepatitis B surface antigen and antibodies to human immunodeficiency virus. Informed consent was obtained from each patient, and the study protocol conformed to ethical guidelines, as reflected by approval by the institutions' (Hôpital Henri Mondor, Créteil, and Hôpital de Bicêtre, Le Kremlin-Bicêtre) human research review committee. Serum samples were aliquoted before any treatment, and aliquots were frozen at −80°C. The HCV genotype was determined by means of the genotyping assay and the two versions of the serotyping assay. When the results of the genotyping and the serotyping assays were discrepant, the 5′ noncoding, core, and NS4 regions of the samples were amplified by PCR, and the PCR products were sequenced directly and from clones (NS4) for definitive genotype determination.

Genotyping assay. The HCV Line Probe Assay (INNO-LiPA HCV; Innogenetics S.A., Ghent, Belgium) was used to determine HCV genotypes. For this procedure, the highly conserved 5′ noncoding region of the HCV genome was amplified by nested reverse transcription PCR with two sets of universal biotinylated HCV primers. The amplified products were then hybridized to oligonucleotide probes designed to be specific for the different HCV types and subtypes and were immobilized as parallel bands on nitrocellulose strips (23). The LiPA procedure was slightly adapted in our laboratory, as described recently (18). The version of the INNO-LiPA HCV assay used in this work allowed us to identify HCV genotypes 1a, 1b, 2, 3, and 4 or 5, with the last two not being distinguished by the assay.

SA1-3. The commercially available assay SA1-3 (Murex Diagnostics) was used for serotyping. In this assay, eight NS4-encoded genotype-specific branched peptides are used: three are specific for type 1, three are specific for type 2, and two are specific for type 3. Each determination needs six different microwells, including a blank well (no peptide is coated), a no-competition well (all peptides are coated and no competing peptides are added), an all-competition well (all peptides are coated and the eight free peptides are added for competition), and wells that are specific for type 1, type 2, and type 3 (all peptides are coated and free peptides specific for the types not sought for in the well are added) (Fig. 1). For example, detection of genotype-specific antibody to type 1 is achieved by the addition of a 100-fold molar excess of peptides from genotypes 2 and 3 to the test serum or plasma samples.

The assay was carried out according to the manufacturer's instructions. Briefly, 10 μl of diluted samples was incubated, in the presence of the serotype-specific competing peptides, with the five microwells coated with type-specific HCV antigens. The wells were incubated for 1 h at 37°C to allow the serotype-specific anti-HCV antibodies to bind to the immobilized antigens. Following washing to remove unbound material, the captured antibodies were incubated with peroxidase-conjugated monoclonal anti-human immunoglobulin G for 1 h at 37°C. After removal of excess conjugate, bound enzyme was detected by the addition of a solution containing 3,3′,5,5′-tetramethylbenzidine and hydrogen peroxide. A purple color developed in the wells which contained anti-HCV-reactive samples. The enzyme reaction was stopped with sulfuric acid to give an orange color which was read photometrically.

SA1-6. In the SA1-6 assay, 21 NS4-encoded branched peptides specific for HCV types 1, 2, 3, 4, 5, and 6 were used in eight wells (no blank well). The procedure was similar to that described above for SA1-3, except that the conjugate was incubated for 30 min at 37°C.

TABLE 1. Results of serotyping by SA1-3 and SA1-6 compared with genotyping by INNO-LiPA HCV for sera from 88 patients with chronic hepatitis C eligible for interferon therapy

Assay and serotype	No. of patients infected with the following HCV genotype, as determined by INNO-LiPA HCV:				
	1a (n = 19)	1b (n = 34)	2 (n = 8)	3 (n = 18)	4-5 (n = 9)
SA1-3					
1	16	24			5
2			7		
3				9	
1 and 3				5	
NR[a]	3	10	1	4	4
SA1-6					
1	19	30		2	1
2			7		
3				11	
4		2			3
5					1
1 and 3				2	
NR		1		2	3
NTS[b]		1	1	1	1

[a] NR, nonreactive.
[b] NTS, non-type specific.

RESULTS

Distribution of HCV genotypes. All 88 serum samples tested in this study were HCV RNA positive by PCR and could thus be genotyped by the INNO-LiPA HCV. The distribution of HCV genotypes, as determined by this assay, was as follows: 19 patients (22%) were infected with HCV type 1a, 34 (39%) were infected with type 1b, 8 (9%) were infected with type 2, 18 (20%) were infected with type 3, and 9 (10%) were infected with type 4-5.

Serotyping by SA1-3. The results of SA1-3 are presented in Table 1 according to the genotype determined by the INNO-LiPA HCV. Samples from 22 patients (25%) were nonreactive in the assay; i.e., no antibodies to the NS4-encoded region where the HCV peptides were designed could be detected. The sensitivity of the assay was thus 75%. As indicated in Table 1, the lack of reactivity in the assay was not associated with a particular genotype. Among the 66 patients who could be serotyped by SA1-3, SA1-3 and the genotyping method gave concordant results for 61 (92%). However, for sera from five patients that were reactive only with the type 3 probes in the INNO-LiPA HCV, SA1-3 was reactive both in the type 1 and in the type 3 wells, suggesting either mixed type 1 and 3 infection not detected by the genotyping assay or type 1 and 3 cross-reactivities. Discrepant results were observed for five patients (8%). The corresponding five samples reacted with the genotype 4-5 probes in the INNO-LiPA HCV, but with type 1 peptides in SA1-3.

Serotyping by SA1-6. The results of SA1-6 are presented in Table 1 according to the genotype determined by the INNO-LiPA HCV. Sera from six patients (7%) were nonreactive in this assay. In four additional serum samples (4%), including two that had been serotyped by SA1-3, antibodies were detected but reactivities were non-type specific. This means that the sample was positive in the no-competition well, but had no reactivity in any other well (Fig. 1). As indicated in Table 1, neither the lack of reactivity nor the lack of specificity of the reactivities was associated with a particular genotype, but no type 1a sample was found to be nonreactive or non-type specific in the assay. The overall sensitivity of SA1-6 was 89% for the study population. Among the 78 patients who could be serotyped by SA1-6, SA1-6 and the genotyping assay gave concordant results for 73 (94%). However, two samples that hybridized only with type 3 probes reacted with both type 1 and type 3 peptides in SA1-6 (including one sample in which types 1 and 3 were found and one sample in which type 3 alone was found by SA1-3). Discrepant results between SA1-6 and INNO-LiPA HCV were observed for samples from five patients (6%). They included two patients infected with genotype 3 and one patient infected with genotype 4-5 found to be serotype 1 by SA1-6 and two patients infected with genotype 1b found to be serotype 4 by SA1-6.

Concordance between the two serotyping assays. Table 2 gives the correspondence between the results of the two successive versions of the serotyping assay for the same sera. Eighty-four percent of the samples found to be positive by both serotyping assays gave concordant results. Sera from 8 of the 22 patients nonreactive by SA1-3 were nonreactive (six patients) or non-type specific (two patients) by SA1-6. SA1-6 resolved the results for four of five patients found by SA1-3 to be infected with types 1 and 3, indicating that they were infected with type 3 alone, whereas the result for one patient remained type 1 and 3. One additional sample that reacted only with type 3 probes in the INNO-LiPA HCV and with type 3 peptides in SA1-3 reacted with both type 1 and type 3 peptides in SA1-6, suggesting either mixed infection or type 1 and 3 cross-reactivity. SA1-6 discriminated between type 1 and type 4-5 better than SA1-3 did, because among the five samples that hybridized with the type 4-5 probes and that reacted with the type 1 peptides in SA1-3, three reacted with the type 4 peptides and one reacted with the type 5 peptides in SA1-6. One sample still reacted with the type 1 peptides, and isolates from the four patients infected with genotype 4-5 that were nonreactive by SA1-3 were still not typeable by SA1-6. Twelve samples found to be nonreactive by SA1-3 were type 1 by SA1-6, suggesting better sensitivity of SA1-6 for type 1, whereas one type 1

TABLE 2. Concordance of the results of the two serotyping assays (SA1-3 and SA1-6) for sera from 88 patients with chronic hepatitis C eligible for interferon therapy

Serotype by SA1-3	No. of patients infected with the following HCV genotype as determined by SA1-6:							
	1 (n = 52)	2 (n = 7)	3 (n = 11)	4 (n = 5)	5 (n = 1)	1 and 3 (n = 2)	NR[a] (n = 6)	NTS[b] (n = 4)
1 (n = 45)	40			3	1			1
2 (n = 7)		7						
3 (n = 9)			7			1		1
1 and 3 (n = 5)			4			1		
NR (n = 22)	12			2			6	2

[a] NR, nonreactive.
[b] NTS, non-type specific.

TABLE 3. Results of INNO-LiPA HCV, the two serotyping assays, and sequence analysis of the 5′ noncoding, core, and NS4 regions of the HCV genome for 12 samples with discrepant results among at least two of the three assays evaluated

Sample	Typing results			Sequencing results[a]		
	INNO-LiPA HCV	SA1-3	SA1-6	5′ NCR	Core	NS4
BO	1b	NR[b]	4	1b	PCR (−)	1b
42	4-5	1	4	4	4	4
44	3a	1+3	1+3	3a	3	3a
41	4-5	1	4	4a	4a	PCR (−)
12	4-5	1	5	5	5	5
61	3a	1+3	3	3a	3a	3a
24	4-5	1	1	5	5	5
AE	3a	1+3	3	3a	PCR (−)	3a
AJ	3a	1+3	3	PCR (−)	PCR (−)	PCR (−)
BF	3a	NR	1	3a	3a	3a
34	3a	1+3	3	3a	3a	3a
60	3a	NR	1	3a	3a	3a

[a] 5′ NCR, 5′ noncoding region; PCR (−), PCR negative.
[b] NR, nonreactive.

sample and one type 3 sample by SA1-3 were antibody positive but non-type specific by SA1-6.

Resolution of discrepant results. Table 3 gives the results of the three assays and the results of genotyping by sequence analysis of the 5′ noncoding, core, and NS4 regions for 12 of the 14 samples with discrepant results between at least two of the three assays. As indicated in Table 3, sequence analysis confirmed the results of INNO-LiPA HCV for all samples. In addition, sequencing allowed for the discrimination of types 4 and 5 in those samples identified as genotype 4-5 in INNO-LiPA HCV. In the patients found to be infected with genotype 3a by INNO-LiPA HCV and types 1 and 3 by one or both of the serotyping assays, sequence analysis confirmed the results of INNO-LiPA HCV. However, like INNO-LiPA HCV, sequence analysis is based on PCR amplification and can overlook mixed infections in the event that one genotype replicates at a higher level than the other one.

Sensitivities of the two serotyping assays and concordance of results with those of the genotyping assay. Since the result of INNO-LiPA HCV was confirmed by sequence analysis of three genome regions for all samples with discrepant results, INNO-LiPA HCV was used as the reference test to determine the sensitivities and specificities of SA1-3 and SA1-6. The results are summarized in Table 4. According to the results of the INNO-LiPA HCV, the sensitivity of SA1-3 was 75, 87, and 78% for the determination of HCV types 1, 2, and 3, respectively. There was a 100% concordance between the results of SA1-3 and INNO-LiPA HCV in the identification of HCV types 2 and 3, but the serotyping assay was concordant with INNO-LiPA HCV for only 80% of the patients identified as being infected with genotype 1. This was due to mistyping by SA1-3 of samples hybridizing with type 4-5 probes and reacting with type 1 peptides, later resolved in SA1-6. Compared to SA1-3, SA1-6 had an increased sensitivity for the detection of type 1-specific antibodies (92%), whereas its sensitivity for type 2 and 3 peptides remained essentially unchanged (87 and 72%, respectively). SA1-6 detected type 4 or 5 antibodies in only 44% of the samples identified by INNO-LiPA HCV as being infected with type 4 or 5. The assay had better concordance with INNO-LiPA HCV for patients identified as being infected with HCV genotype 1 (91%), whereas there was still a 100% concordance for the identification of HCV types 2 and 3 between SA1-6 and the genotyping assay. Finally, the results of SA1-6 and INNO-LiPA HCV were concordant for only 66% of the samples hybridizing with type 4-5 probes in the genotyping assay.

TABLE 4. Sensitivity and concordance with molecular biology-based genotyping of two serotyping assays (SA1-3 and SA1-6) for identification of HCV genotypes 1 through 5 in 88 patients with chronic hepatitis C eligible for interferon therapy

Assay and genotype	Sensitivity (%)	% Concordance
SA1-3		
1	75	80
2	87	100
3	78	100
SA1-6		
1	92	91
2	87	100
3	72	100
4-5	44	67

DISCUSSION

The reference method for HCV genotype determination is full-length genomic sequence analysis followed by phylogenetic analysis for definitive classification. Such a procedure is not always necessary, and sequence determination of more limited regions of the genome is used in most cases. However, this method is time-consuming and not adapted to clinical studies or large-scale routine use. Several fast typing methods have been developed, including subtype-specific amplification (13), restriction fragment length polymorphism analysis of PCR products (3, 8), and reverse hybridization (23). One of the main advantages of INNO-LiPA HCV is standardization of the commercially available assay. The version of INNO-LiPA HCV used in the present study was shown to be both sensitive and specific for the identification of HCV genotypes 1 to 5 (23). However, it is conceivable that spontaneous mutations in the 5′ noncoding region of the genome could be responsible for mistyping in some cases. This was shown to be the case for 8% of the patients infected with HCV genotype 1 (22). In these samples, subtypes 1a and 1b cross-reacted in the assay, because their specific probes differed by only one nucleotide, located at position −99 of the genome (22). However, possible mistyping of subtypes of type 1 strains did not influence the results of our study, because the serotyping assays evaluated did not discrim-

inate between subtypes 1a and 1b. Overall, INNO-LiPA HCV could be considered a suitable test for comparison in this study due to its sensitivity and specificity in the recognition of HCV types 1, 2, 3, and 4-5. Its accuracy was indeed confirmed in this work by sequence analysis of three viral genomic regions in samples with discrepant serotyping assay results, the results of which always confirmed those of INNO-LiPA HCV.

The formats of the two versions of the Murex Anti-HCV Serotyping Assay investigated in this study (SA1-3 and SA1-6) differed considerably from those of previously described serotyping assays, which were based on the coating with different antigens corresponding to different genotypes of different wells or of nitrocellulose strips (4, 10, 25, 26). Indeed, all wells were coated with peptides from all studied genotypes. Type-specific antibody was detected by competition between the solid-phase antigens and a 100-fold molar excess of peptides added in solution with the plasma or serum sample (21). This method guarantees that only those antibodies that can bind to the solid phase are those that do not cross-react with genotypes heterologous to HCV. In addition, the practicability of the assays and their easy use by people routinely performing enzyme immunoassays make them good candidates for use for routine genotype (serotype) determination.

Our results showed that the results of the first-generation serotyping assay (SA1-3) were always concordant with those of the genotyping assay in identifying HCV genotypes 2 and 3. Its major disadvantages were (i) an overall lack of sensitivity (75%) that ranged from 75 to 87% according to HCV genotype; (ii) a lack of specificity in the detection of type 1-specific antibodies, due to type 1 reactivities in serum samples that otherwise hybridized with type 4-5 probes in INNO-LiPA HCV; and (iii) frequent results indicating mixed type 1 and type 3 infections. The latter finding was, however, difficult to interpret because it could be due either to type 1 and type 3 cross-reactivities or to mixed infections with HCV types 1 and 3 not recognized by molecular biology-based genotyping methods. Molecular biology-based methods often fail to identify mixed infections because one strain usually becomes predominant and is the only one detected by PCR, an exponential amplification technique (12). It must be stressed, however, that mixed infections with distinct HCV genotypes are probably rare because HCV genotype is strongly related to the source of infection (18), so that patients are likely most often reinfected through the same route (e.g., multiple transfusions and intravenous drug use) with heterologous strains belonging to the same genotype. In the absence of a molecular biology-based test that could be used as a "gold standard," stating whether type 1 and 3 infections by SA1-3 were really mixed infections or related to type 1 and type 3 cross-reactivities was thus difficult. However, the fact that results for four of these patients were resolved as type 3 and the results for only one remained type 1 and type 3 in the more specific SA1-6 strongly suggested unexpected type 1 and type 3 cross-reactivities in SA1-3 that were avoided in SA1-6. The lack of discrimination between subtypes 1a and 1b could also be considered a disadvantage of the SA1-3 because type 1a infects 25 to 60% of the patients infected with HCV type 1 in the United States and Western Europe (6, 7, 18). However, recent findings suggested that HCV types 1a and 1b, although often transmitted through different routes, were not different with regard to their role in the pathogenesis of the disease and the sensitivity to interferon alfa therapy (15). This suggests that subtyping might be useless if genotype determination is routinely used to tailor treatment schedules.

The second-generation serotyping assay (SA1-6) was aimed (i) at improving the sensitivity and the specificity of the serotyping assay and (ii) at providing identification of HCV genotypes 4, 5, and 6. The latter was justified by the fact that HCV genotype 4 is present in 5 to 10% of the patients with chronic hepatitis C in Western Europe and that type 5 can also be encountered in industrialized countries, although it is mostly present in the South African Republic (3, 18). In contrast, type 6 appears to be limited to Hong Kong, Macao, and Vietnam (9). Preliminary results obtained with a precommercial version of SA1-6 did not suggest significant improvement in sensitivity compared to that of SA1-3 with the 21 genotype-specific HCV peptides initially included in the assay (14). In the last year, the sequences used for the peptides have been modified to address some of the problems associated with reduced sensitivity and specificity. In addition, sophisticated and effective methods have been used to coat the ELISA plates with peptides, and the composition of competing solutions has been changed in the commercially available version of the ELISA. Overall, our results suggest improved sensitivity and specificity in the definitive commercially available version of SA1-6 compared to those of SA1-3. Identification of HCV types 4 and 5 could be achieved (no sera from a patient infected with type 6 was included in the study), although both the sensitivities and the specificities of type 4 and 5 peptides appeared to need further improvement. The main improvement in SA1-6 was increased sensitivity and specificity of type 1 antibody detection. Improved specificity for type 1 determination mainly resulted from the lack of cross-reactivities with samples identified as type 4-5 by genotyping, and this could mostly be due to the introduction in the assay of peptides competing with type 4. The fact that five patients were identified as being infected with types 1 and 3 by SA1-3, but four were found to be infected only with type 3 by SA1-6, also suggested better specificity for the type 1 peptides by SA1-6. In contrast, there was no improvement in the sensitivity of the assay for HCV genotypes 2 and 3, whereas the concordance of the results with those of the genotyping assay in identifying these types remained excellent. Possible cross-reactivities could still be observed, although at a low rate. They were mainly found with type 1 and type 4 peptides. However, two patients were found to be infected with types 1 and 3 by SA1-6, and in these two patients, neither cross-reactivities nor mixed infections could be ruled out. The discrepancies between the two serotyping assays in the diagnosis of mixed infections questions, however, their real abilities to identify such cases in the population studied. Finally, in SA1-6, determination of subtypes 1a and 1b was no more possible than in SA1-3, but because these two subtypes do not appear to be pathogenetically different, subtyping might not be absolutely necessary. Overall, due to the concordance between SA1-6 and INNO-LiPA HCV for sera from patients such as those studied here, i.e., patients with chronic hepatitis C eligible for interferon therapy, SA1-6 could routinely be used as an alternative to molecular biology-based methods for determining HCV genotype so that this information can be used as a tool to tailor treatment schedules. However, in about 10% of the patients, a molecular biology-based typing method will be required because of the lack of sensitivity of the assay in its present form.

In conclusion, the recently commercialized SA1-6 had increased overall sensitivity compared to that of SA1-3 for sera from unselected patients eligible for interferon therapy, mainly because of the increased sensitivity of HCV genotype 1-specific antibody detection. It remained, however, less sensitive than a standardized genotyping assay based on PCR amplification of HCV RNA. Both versions of the assay had good concordance with the genotyping assay, but cross-reactivities between different HCV genotypes could be responsible for mistyping for 8 (SA1-3) and 6% (SA1-6) of the patients. Finally, subtyping of

types 1a and 1b is still not possible with the existing peptides. For that reason, serotyping cannot reliably be used for epidemiological studies. However, it could routinely be used in the future to tailor treatment schedules according to the infecting HCV genotype, because discriminating between subtypes does not appear to be necessary in this setting.

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