Evaluation of Versant Hepatitis C Virus Genotype Assay (LiPA) 2.0[▽]

Jannick Verbeeck,[1] Mark J. Stanley,[2] Jen Shieh,[2] Linda Celis,[3] Els Huyck,[3] Elke Wollants,[1]
Judy Morimoto,[2] Alice Farrior,[2] Erwin Sablon,[3] Margaret Jankowski-Hennig,[4] Carl Schaper,[4]
Pamela Johnson,[4] Marc Van Ranst,[1]* and Marianne Van Brussel[3]

*Laboratory of Clinical Virology, Rega Institute for Medical Research, Leuven, Belgium[1]; Department of Microbiology,
Kaiser Permanente, TPMG Regional Laboratory, Berkeley, California[2]; Innogenetics NV, Gent, Belgium[3]; and
Siemens Healthcare Diagnostics, Berkeley, California[4]*

Hepatitis C virus (HCV) genotyping is a tool used to optimize antiviral treatment regimens. The newly developed Versant HCV genotype assay (LiPA) 2.0 uses sequence information from both the 5′ untranslated region and the core region, allowing distinction between HCV genotype 1 and subtypes c to l of genotype 6 and between subtypes a and b of genotype 1. HCV-positive samples were genotyped manually using the Versant HCV genotype assay (LiPA) 2.0 system according to the manufacturer's instructions. For the comparison study, Versant HCV genotype assay (LiPA) 1.0 was used. In this study, 99.7% of the samples could be amplified, the genotype of 96.0% of samples could be determined, and the agreement with the reference method was 99.4% when a genotype was determined. The reproducibility study showed no significant differences in performance across sites ($P = 0.43$) or across lots ($P = 0.88$). In the comparison study, 13 samples that were uninterpretable or incorrectly genotyped with Versant HCV genotype assay (LiPA) 1.0 were correctly genotyped by Versant HCV genotype assay (LiPA) 2.0. Versant HCV genotype assay (LiPA) 2.0 is a sensitive, accurate, and reliable assay for HCV genotyping. The inclusion of the core region probes in Versant HCV genotype assay (LiPA) 2.0 results in a genotyping success rate higher than that of the current Versant HCV genotype assay (LiPA) 1.0.

Hepatitis C virus (HCV) is a leading cause of chronic liver disease and has already infected at least 170 million people worldwide. Each year, 3 to 4 million people are newly infected. HCV creates an extensive disease burden, since it accounts for 20 to 30% of cases of acute hepatitis, 70 to 80% of cases of chronic hepatitis, 40% of cases of end-stage cirrhosis, 50 to 76% of cases of hepatocellular carcinoma, and 30 to 40% of liver transplants (15, 33, 34).

HCV belongs to the family of the *Flaviviridae* and can be divided into different genotypes based on phylogenetic analysis of full-length or partial sequences of HCV strains. The most current consensus proposal distinguishes six genotypes based on phylogenetic cluster analysis of complete genomes. The genotype formerly designated as 10a has been reassigned as genotype 3, subtype k. Genotypes 7, 8, 9, and 11, belonging to clade 6, have been reassigned to genotype 6, subtypes c to l (25, 26, 27). These six HCV genotypes have different geographical distributions (21, 30, 32).

Treatment options for chronic HCV infections are poor. At the moment, the only accepted antiviral therapy with proven effectiveness is a combination therapy of (peg)interferon alpha and ribavirin. The overall success rate of this antiviral treatment ranges from 50% to 90% (11). According to a National Institutes of Health (NIH) 2002 panel, several factors are associated with successful treatment response, including lower baseline HCV RNA levels, lower fibrosis and inflammation scores upon liver biopsy, lower body weight, and lower body surface area, but the most important predicting factor is HCV genotype (24). Patients infected with HCV genotype 1 respond least to therapy, while patients infected with genotypes 2 and 3 show the best responses (14, 17, 22). For HCV genotypes 4, 5, and 6, treatment data are scarce, but it is recommended to treat these individuals using the same regimen as for patients infected with genotype 1 (7, 13, 18, 31). Nearly all patients experience side effects with the antiviral therapy. These side effects can be severe and contribute to discontinuation rates of 10 to 14% and dose reductions for 7 to 42% of patients, depending on the type and length of treatment (16). Therefore, it is important that clinicians have the appropriate information to make individual treatment choices in order to maximize the chance of successful treatment outcome for each individual patient, rendering HCV genotyping assays important and useful tools to optimize treatment type, duration, and dose.

In this paper, we evaluate Versant HCV genotype assay (LiPA) 2.0 (CE marked in Europe; for research use only; not for use in diagnostic procedures in the United States) (manufactured by Innogenetics, distributed by Siemens Healthcare Diagnostics), which uses sequence information from the core region in addition to sequence information from the 5′ untranslated region (5′UTR), allowing an improved and more accurate distinction between HCV genotype 1 and subtypes c to l of genotype 6 and between subtypes a and b of genotype 1.

MATERIALS AND METHODS

Extraction and amplification. HCV RNA was extracted by using the QIAamp DSP virus kit (for in vitro diagnostic use in Europe) in combination with the QIAvac 24 Plus vacuum system (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. In each extraction run, positive and negative

---

* Corresponding author. Mailing address: Laboratory of Clinical & Epidemiological Virology, Rega Institute for Medical Research, University of Leuven, Minderbroedersstraat 10, BE-3000 Leuven, Belgium. Phone: 32-16-347908. Fax: 32-16-332131. E-mail: marc.vanranst@uz.kuleuven.ac.be.
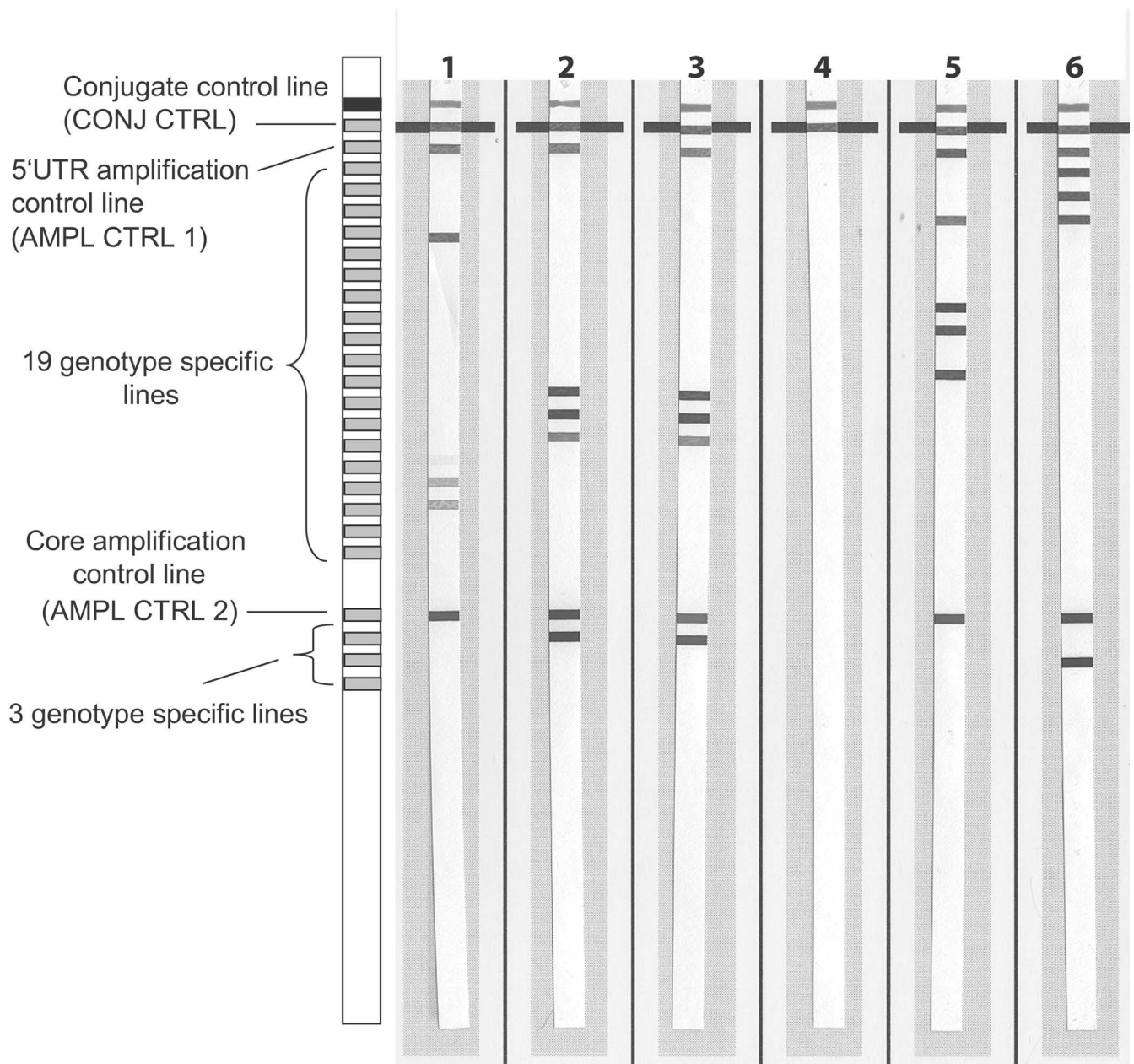
FIG. 1. Schematic representation of the Versant HCV genotype assay (LiPA) 2.0 strip design and six strips that were processed according to the manufacturer's instructions (strip 1, HCV genotype 4; strips 2 and 3, HCV genotype 3a; strip 4, negative control; strip 5, HCV genotype 2b; strip 6, HCV genotype 1a). CONJ CTRL, conjugate control.

controls (Versant HCV control 2.0 kit) were included and placed between the samples. The Versant HCV amplification 2.0 kit was used to amplify the appropriate fragments in the 5′UTR and the core region of the HCV genome.

Versant HCV genotype assay (LiPA) 2.0. Versant HCV genotype assay (LiPA) 2.0 is a reverse hybridization line probe assay in which biotinylated DNA PCR products are hybridized to immobilized oligonucleotide probes that are specific for the 5′UTRs and core regions of the six HCV genotypes. The probes are bound to a nitrocellulose strip by a poly(T) tail. After hybridization of the biotinylated targets to the probes, unhybridized PCR products are washed from the strips, and alkaline phosphatase-labeled streptavidin (conjugate) is bound to the biotinylated hybrid. After washing the strips, BCIP (5-bromo-4-chloro-3-indolylphosphate)–nitroblue tetrazolium chromogen (substrate) reacts with the conjugate forming a purple/brown precipitate, which results in a visible line pattern on the strip that is specific for each genotype. Each strip has 3 control

lines and 22 parallel DNA probe lines containing sequences specific for HCV genotypes 1 to 6 (Fig. 1). The conjugate control at line 1 monitors the color development reaction and gives a positive result if the strip is correctly processed. The amplification control at line 2 (AMPL CTRL 1) contains universal probes that hybridize to PCR products from the 5′UTR. AMPL CTRL 2 is located at line 23 and contains universal probes that hybridize to PCR products from the core region. HCV genotypes are determined by aligning the strips with a reading card and comparing the line patterns from the strip with the patterns on the interpretation chart. In this study, version 25885 v1 of the interpretation chart was used (version 26020 v0 was used for the CE-marked kit). The limits of detection described in the user manual are 2,106 IU/ml to >7.7 × 10⁶ IU/ml.

Clinical accuracy study. A total of 326 HCV-positive clinical specimens (serum, EDTA plasma, and citrate-phosphate-dextrose anticoagulant plasma) with viral loads above 2,000 IU/ml were collected. Genotypes and subtypes were

TABLE 1. Overview of the original test results and the results after one repeat test in clinical accuracy study

Test result	No. of specimens (%) with agreement between reference method test result and:	
	Original test result	Test result upon 1 repeat test
Correct genotype	302 (92.6)	311 (95.4)
Incorrect genotype	2 (0.6)	2[a] (0.6)
Uninterpretable	15 (4.6)	12 (3.5)
No amplification	7 (2.1)	1 (0.3)
Total	326 (100)	326 (100)

[a] If a genotype result was obtained from the original test, repeat test results are not included.

assigned by sequencing part of the NS5b region (from nucleotide 8256 to nucleotide 8636, approximately 340 bp) or the core region (from nucleotide 341 to nucleotide 686, approximately 350 bp), followed by phylogenetic analysis. Phylogenetic analysis was performed using a set of reference sequences derived from HCV complete genome sequences according to the work of Simmonds et al. (26). Alignments were created using the Clustal X program. Distance matrices were produced by DNADIST using the Kimura two-parameter settings and further analyzed in NEIGHBOR using the neighbor-joining settings. Throughout the text, this method is referred to as the "reference method." All samples were additionally genotyped using Versant HCV genotype assay (LiPA) 2.0. Invalid runs and samples with invalid results were repeated/retested. Study results were compared with the specimen genotype reference sequence. Runs producing results which were discordant with the specimen genotype or which were uninterpretable were repeated.

Reproducibility study. To evaluate the reproducibility of Versant HCV genotype assay (LiPA) 2.0, a nine-member HCV genotype panel was tested in duplicate in three separate runs per kit lot of the assay system, using three lots, at three different sites by one operator per site. By use of this study design, each panel member was tested 54 times, resulting in a total number of 486 (9 times 54) reactions. Operators were blinded to the HCV genotype of the specimen. The panel members were serum and EDTA plasma samples of each genotype, with HCV viral loads ranging from 16,024 IU/ml to 1,379,458 IU/ml, and they were prepared by dilution. There was one panel member for each of the HCV genotypes 1a, 1b, 2, 3, 4, and 5. HCV genotype 6 was represented by subtypes 6a and b and two representatives of subtypes c to l, the last formerly known as genotypes 7, 8, 9, and 11. Eight of nine panel members were sequenced in the NS5b region to determine the true genotype of the samples. One panel member was sequenced in the core region. Invalid runs and samples with invalid results were repeated/retested.

Comparison study. In order to demonstrate that the performance of Versant HCV genotype assay (LiPA) 2.0 is equivalent to or better than that of the currently established Versant HCV genotype assay (LiPA) 1.0, a total of 100 HCV-positive samples (serum, EDTA plasma, and citrate-phosphate-dextrose anticoagulant plasma) with viral loads above 10,000 IU/ml were tested at two independent sites with the two assays according to the manufacturer's instructions. Operators were blinded to the HCV genotype of the specimens. The extracted RNA was divided into two aliquots. One aliquot was used with the current kit, and the other aliquot was used for parallel testing with Versant HCV genotype assay (LiPA) 1.0. The amplicon for use with LiPA 1.0 was reverse transcribed and the 5′UTR was amplified using site-specific reverse transcriptase PCR methodology. At one site, the amplification was performed using a modified method starting from transcriptionally mediated amplification (HCV TMA; Siemens Healthcare Diagnostics, CA). At the other site, amplification of the 5′UTR was performed with specific biotinylated primers (more detailed information on the methodology used at both sites is available on request). Invalid runs and samples with invalid results were repeated/retested. Specimens with indeterminate results were retested by both methods. Specimens with discordant results were sequenced in the NS5b region of the HCV genome.

Terms and statistical analysis. The analysis includes results only from valid runs, i.e., from runs in which the positive and negative control strips gave the correct results. Results were classified as either interpretable or indeterminate. An indeterminate result occurs when a specimen yields either an amplification failure or an uninterpretable result. An uninterpretable result occurs when the strip has a positive AMPL CTRL 1 but has a line pattern that does not match any of the patterns shown on the interpretation chart.

The genotype success rate is defined as percentage of valid results that give an interpretable result. In the comparison study, specimens were considered to have discordant results if the two versions of the assay produced results with different genotypes or produced the same genotype result with different subtypes. An improvement is defined as obtaining the correct result (based on NS5b sequencing) with Versant HCV genotype assay (LiPA) 2.0 when Versant HCV genotype assay (LiPA) 1.0 does not produce a result or produces an incorrect result.

Lower 95% confidence limits (LCL) for percentages were all calculated using the Clopper-Pearson method. In the reproducibility study, a permutation distribution was used to estimate the P values for comparing sites and for comparing lots.

RESULTS

Clinical accuracy study. Table 1 summarizes the results for the 326 specimens that were used for the reference method comparison. Upon initial testing, 93.3% (304/326) of the specimens gave interpretable genotype results, 2.1% (7/326) failed to amplify, and 4.6% (15/326) amplified but gave uninterpretable results. Of the 304 specimens that yielded a genotype result, 99.3% (302/304) gave results that agreed with the reference method. After specimens that yielded no genotype result were retested, 96.0% (313/326) of the specimens gave interpretable genotype results, 3.5% (12/326) amplified but remained uninterpretable, and 0.3% (1/326) failed to amplify. Of the 313 specimens that yielded a genotype result after repeat testing, 99.4% (311/313) gave results that agreed with the reference method. Table 2 shows that the specimens that did not amplify or give interpretable results were distributed

TABLE 2. Summary of the results of the clinical accuracy study for each genotype

Reference HCV genotype	Total sample no.	No. of specimens exhibiting:			
		Amplification failure	Amplification failure repeat	Uninterpretable result	Uninterpretable repeat
Genotype 1	2	0	0	0	0
Genotype 1a	94	2	0	1	0
Genotype 1b	41	0	0	2	2
Genotype 2	53	2	1	3	2
Genotype 3	55	2	0	0	0
Genotype 4	30	0	0	4	4
Genotype 5a	15	1	0	1	0
Genotype 6	17	0	0	2	2
Genotype 6 (c to l)	19	0	0	2	2
Total	326	7	1	15	12

TABLE 3. Summary of valid, indeterminate, correct, and incorrect genotype results for each panel member of reproducibility study

Panel member	HCV genotype	No. of valid results	No. of specimens with indicated result			
			Indeterminate		Interpretable	
			Did not amplify	Uninterpretable	Incorrect genotype	Correct genotype
1	1a	54	1	2	0	51
2	1b	54	0	0	0	54
3	2	54	0	3	0	51
4	3	54	0	0	0	54
5	4	54	1	0	0	53
6	5	54	2	2	0	50
7	6	54	1	2	0	51
8	6[a]	54	0	2	0	52
9	6[a]	54	0	0	0	54
Total	All	486	5	11	0	470

[a] Required both 5′UTR and core region for genotyping.

TABLE 4. Overview of the original test results and the results after one repeat test for comparison study

Result	No. of specimens giving:	
	Original test result	Test result upon 1 repeat test
Identical result by both methods	63	67
Matched genotypes but one assay having no subtype	16	16
Matched genotype but different subtypes	8	8
Uninterpretable by both assays	3	3
Uninterpretable by LiPA 1.0 only	8	5
Uninterpretable by LiPA 2.0 only	2	1
Total	100	100

across genotypes. The two specimens that initially gave results that disagreed with those obtained by the reference method were retested, and both gave retest results that agreed with those obtained by the reference method.

In order to determine the core amplification efficiency, 156 genotype 1 and genotype 6 (c to l) samples were analyzed. Two samples showed negative AMPL CTRL 1 lines and were excluded from further analysis. Of the remaining 154 genotype 1 and genotype 6 (c to l) samples, 1 sample had a negative AMPL CTRL 2, resulting in the amplification of 99.4% (153/154) samples.

The clinical subtype efficiency for HCV genotypes 1a and 1b was determined using 129 samples that were genotype 1a or 1b based on reference sequencing and genotype 1, 1a, or 1b based on LiPA genotyping; this determination was based on initial testing only, excluding repeat testing of initial amplification failures and uninterpretable results. Three out of 129 samples were indeterminate at the subtype level, resulting in a clinical HCV genotype 1 subtype efficiency of 97.7% after initial testing. Upon repeat testing, all samples gave a correct consensus subtype result. All of the 126 samples that were genotype 1a or 1b by LiPA were concordant with sequencing.

In order to check whether Versant HCV genotype assay (LiPA) 2.0 was able to determine the correct genotype for samples with viral loads at the upper limit of detection, 22 samples with viral loads ranging from 4.0×10^6 IU/ml to 8.7×10^6 IU/ml were selected, and the genotype success rate and the percentage of agreement with the reference method for these high-concentration specimens were estimated. For all these samples, Versant HCV genotype assay (LiPA) 2.0 produced the same genotype results as the genotype result determined by NS5b sequencing and phylogenetic analysis, resulting in both a genotype success rate and an agreement with the reference method of 100%.

Reproducibility study. Table 3 summarizes the valid, indeterminate, correct, and incorrect genotype results for each reproducibility panel member. In total, 3.3% (16/486) of reactions gave indeterminate results (defined as specimens with either an amplification failure or an uninterpretable result) and 96.7% (LCL, 95.0%) yielded an interpretable genotype

result. Of the 470 specimens with interpretable results, 100% (LCL, 99.4%) gave the correct genotype. The indeterminate results occurred at all sites, with all three reagent lots, and in multiple assay runs. There were no significant performance differences seen for the Versant HCV genotype assay (LiPA) 2.0 system across sites/operators ($P = 0.43$) or across reagent lots ($P = 0.88$). The genotype success rates at the individual sites were 98.1% for site 1, 97.6% for site 2, and 94.4% for site 3. The genotype success rates for the individual lots were 96.9% using lot 1, 97.5% using lot 2, and 95.7% using lot 3.

Comparison study. Table 4 gives an overview of the results of the comparison study after original testing and after repeat testing. Of the 100 specimens tested, 13 specimens initially produced uninterpretable results by either Versant HCV genotype assay (LiPA) 1.0 or Versant HCV genotype assay (LiPA) 2.0 or both assays. The HCV RNA concentrations of these 13 samples ranged from 14,615 to 2,500,000 IU/ml. These specimens were retested using both assays. After repeat testing, three specimens remained uninterpretable by both versions of the assay, five remained uninterpretable by Versant HCV genotype assay (LiPA) 1.0, and one remained uninterpretable by Versant HCV genotype assay (LiPA) 2.0. For all six specimens that gave a genotype result by only one version of the assay, the observed genotype result agreed with that obtained by sequencing the NS5b region of the HCV genome. After repeat testing, 83 specimens were concordant by both assays at the genotype level. Of these, 16 specimens had concordant genotypes by both assays, but one of the assays failed to give a subtype, resulting in a total of 67 concordant specimens when the subtype level is taken into account. Results from eight specimens were discordant between the two assays, and results from nine specimens were uninterpretable by at least one of the assays. The total number of interpretable specimens by both assays was 91, of which 83 had concordant results at the genotype level only (91.2%; LCL, 84.7%). Table 5 shows the number of genotype and subtype results produced by both assays for the 100 specimens tested after repeat testing.

The eight samples that showed discordant results were sequenced in the NS5b region of the HCV genome (two samples were HCV genotype 6 subtypes c to l, and six samples were HCV genotype 1a). Results indicated that Versant HCV genotype assay (LiPA) 2.0 gave the correct HCV genotype and subtype, as determined by NS5b sequencing. In contrast, Versant HCV ge-

TABLE 5. Genotype and subtype results for 100 clinical specimens tested by both Versant HCV genotype assay (LiPA) 1.0 and Versant HCV genotype assay (LiPA) 2.0

Versant HCV LiPA 1.0 result	No. of specimens exhibiting indicated result by Versant HCV LiPA 2.0[a]															Total
	1	1a	1b	2	2a/2c	2b	3	3a	3b	4	4a/4c/4d	5a	6a/6b	6 (c to l)	Unint[b]	
1		**12**	2													14
1a		**12**														12
1b		6	**12**											2		20
2				**2**												2
2a/2c				2	**8**										1	11
2b						**7**										7
3							**2**									2
3a								**11**								11
3b									**1**							1
4										**5**						5
4a																0
4c/4d											**4**					4
5a												**2**				2
6a/6b													**1**			1
6 (c to l)																0
Unint[b]							1			2		1	1		3	8
Total	0	30	14	4	8	7	3	11	1	7	4	3	2	2	4	100

[a] Specimens with concordant genotype results are shown in bold.
[b] Unint, uninterpretable result.

notype assay (LiPA) 1.0 had misclassified all eight samples as HCV genotype 1b. Of the 96 specimens that were interpretable with Versant HCV genotype assay (LiPA) 2.0, 83 showed concordant results with Versant HCV genotype assay (LiPA) 1.0, while 13 showed improved results over Versant HCV genotype assay (LiPA) 1.0, which leads to 100% concordant or improved results (LCL, 96.9%).

DISCUSSION

Phylogenetic analysis of a coding region, or even more, the complete genome, is considered the gold standard for identifying different HCV genotypes (6). However, since this method is expensive and time-consuming, it is impractical for large-scale genotyping projects (8). For this reason, commercial genotyping kits were developed for routine determination of HCV genotypes. Most commercially available HCV genotyping assays, including Versant HCV genotype assay (LiPA) 1.0, use the 5′UTR, since this region is highly conserved and therefore well suited for the development of detection methods. The reliability of genotyping methods highly depends on the amount of information (i.e., the number of informative sites) that is utilized for the discrimination of genetic variants. The 5′UTR is sufficiently variable for discrimination of HCV genotypes 1 to 5 and most subtypes of HCV genotype 6 (12, 28, 29, 32). However, it does not allow discrimination of HCV genotype 6 subtypes c to l from HCV genotype 1 and has only a limited subtyping accuracy (5, 29). To overcome the limitations of the 5′UTR, a new assay which uses additional sequence information from the core region of the HCV genome, Versant HCV genotype assay (LiPA) 2.0, has recently been developed (20). In this study, we evaluated the new assay and compared it with the previous version of the assay.

Our results indicate that Versant HCV genotype assay (LiPA) 2.0 yielded an interpretable genotype result for 96.0% of the samples and that 99.4% of the interpretable results agreed with the reference method, rendering it an accurate and reliable assay suitable for large-scale genotyping. This new assay outperforms the previous version of the line probe assay, since Versant HCV genotype assay (LiPA) 1.0 has an overall accuracy of 74%, taking subtype information into account (8, 23).

In the comparison study, eight specimens showed discordant results when tested with both assays. The NS5b sequencing results for these samples showed that Versant HCV genotype assay (LiPA) 2.0 gave the correct HCV genotype and subtype and thereby showed an improvement in identifying HCV-positive samples which are subtypes c to l of genotype 6 and in identifying the correct subtype of genotype 1. This improvement can be attributed to the additional information available from the core region of the HCV genome, which can better distinguish between genotype 1 and subtypes c to l of genotype 6 and between subtype a and b of genotype 1. This core information is not available in Versant HCV genotype assay (LiPA) 1.0, and this can lead to misinterpretation. For example, in a study by Chinchai et al., this assay could not discriminate HCV genotype 6a variants from HCV genotype 1b, and two samples found to be genotype 1 by the assay contained genotype 3 core sequences (5). Chen and Weck showed that Versant HCV genotype assay (LiPA) 1.0 cannot accurately distinguish HCV genotypes 1a and 1b, since in most cases, the 5′UTR is not heterogeneous enough for use in determining the HCV subtype (4). Several other studies report on moderate distinction at the subtype level (1, 2, 9, 12, 19). This is not surprising, since the 5′UTR is the most highly conserved region of the HCV genome, and only one or two nucleotide changes distinguish unique subtypes. Assigning correct genotypes and subtypes to HCV specimens is important for several research purposes, including epidemiological, phylogenetic, and natural history studies. Some studies even report that there is a slight difference in treatment outcomes between HCV genotype 1a- and

HCV genotype 1b-infected patients, showing that correct subtype assignment is indispensable (3, 10, 30).

In conclusion, Versant HCV genotype assay (LiPA) 2.0 provides a rapid, sensitive, and accurate means of HCV genotyping and can be used as a routine tool to distinguish between the different HCV genotypes and subtypes. Considering the importance of genotype determination in understanding the epidemiology of the virus and in the management of hepatitis C treatment strategies, efficient genotyping tools are indispensable in clinical diagnostic settings.

REFERENCES

1. **Andonov, A., and R. K. Chaudhary.** 1995. Subtyping of hepatitis C virus isolates by a line probe assay using hybridization. J. Clin. Microbiol. **33:**254–256.
2. **Ansaldi, F., F. Torre, B. M. Bruzzone, A. Picciotto, P. Crovri, and G. Icardi.** 2001. Evaluation of a new hepatitis C virus sequencing assay as a routine method for genotyping. J. Med. Virol. **63:**17–21.
3. **Bell, H., K. Hellum, S. Harthug, A. Maeland, S. Ritland, B. Myrvang, B. von der Lippe, N. Raknerud, K. Skaug, B. G. Gutigard, R. Skjaerven, L. E. Prescott, P. Simmonds, et al.** 1997. Genotype, viral load and age as independent predictors of treatment outcome of interferon-alpha 2a treatment in patients with chronic hepatitis C. Scand. J. Infect. Dis. **29:**17–22.
4. **Chen, Z., and K. E. Weck.** 2002. Hepatitis C virus genotyping: interrogation of the 5′ untranslated region cannot accurately distinguish genotypes 1a and 1b. J. Clin. Microbiol. **40:**3127–3134.
5. **Chinchai, T., J. Labout, S. Noppornpanth, A. Theamboonlers, B. L. Haagmans, A. Osterhaus, and Y. Poovorawan.** 2003. Comparative study of different methods to genotype hepatitis C virus type 6 variants. J. Virol. Methods **109:**195–201.
6. **Germer, J. J., P. N. Rys, J. N. Thorvilson, and D. H. Persing.** 1999. Determination of hepatitis C virus genotype by direct sequence analysis of products generated with the Amplicor HCV test. J. Clin. Microbiol. **37:**2625–2630.
7. **Halfon, P., A. U. Neumann, M. Bourliere, A. Rieu, S. Chadapaud, H. Khiri, Ouzan, D., and P. Cacoub.** 2003. Slow viral dynamics of hepatitis C virus genotype 4. J. Viral Hepat. **10:**351–353.
8. **Halfon, P., P. Trimoulet, M. Bourliere, H. Khiri, V. De Ledinghen, P. Couzigou, J. M. Feryn, P. Alcaraz, C. Renou, H. J. Fleury, and D. Ouzan.** 2001. Hepatitis C virus genotyping based on 5′ noncoding sequence analysis (Trugene). J. Clin. Microbiol. **39:**1771–1773.
9. **Haushofer, A. C., J. Berg, R. Hauer, D. Trubert-Exinger, H. G. Stekel, and H. H. Kessler.** 2003. Genotyping of hepatitis C virus—comparison of three assays. J. Clin. Virol. **27:**276–285.
10. **Jiao, J., and J. B. Wang.** 2005. Hepatitis C virus genotypes, HLA-DRB alleles and their response to interferon-alpha and ribavirin in patients with chronic hepatitis C. Hepatobiliary Pancreat. Dis. Int. **4:**80–83.
11. **Koike, K.** 2006. Antiviral treatment of hepatitis C: present status and future prospects. J. Infect. Chemother. **12:**227–232.
12. **Lee, J. H., W. K. Roth, and S. Zeuzem.** 1997. Evaluation and comparison of different hepatitis C virus genotyping and serotyping assay. J. Hepatol. **26:**1001–1009.
13. **Legrand-Abravanel, F., K. Sandres-Saune, L. Barange, L. Alric, J. Moreau, P. Desmorat, J. P. Vinel, and J. Isopet.** 2004. Hepatitis C virus genotype 5: epidemiological characteristics and sensitivity to combination therapy with interferon-alpha plus ribavirine. J. Infect. Dis. **189:**1397–1400.
14. **Medeiros-Filho, J. E., I. M. de Carvalho Mello, J. R. Pinho, A. U. Neumann,** F. de Mello Malta, L. C. da Silva, and F. J. Carrilho. 2006. Differences in viral kinetics between genotypes 1 and 3 of hepatitis C virus and between cirrhotic and non-cirrhotic patients during antiviral therapy. World J. Gastroenterol. **12:**7271–7277.
15. **Moradpour, D., A. Cerny, and M. H. Hein.** 2001. Hepatitis C: an update. Swiss Med. Wkly. **131:**291–298.
16. **National Institutes of Health.** 2002. National Institutes of Health consensus development conference statement: management of hepatitis C. 2002. –June 10–12, 2002. Hepatology **36:**S3–S15.
17. **Neumann, A. U., N. P. Lam, H. Dahari, M. Davidian, T. E. Wiley, B. P. Mika, A. S. Perelson, and T. J. Layden.** 2000. Differences in viral dynamics between genotypes 1 and 2 of hepatitis C virus. J. Infect. Dis. **182:**28–35.
18. **Nguyen, M. H., and E. B. Keeffe.** 2005. Prevalence and treatment of hepatitis C virus genotypes 4, 5 and 6. Clin. Gastroenterol. Hepatol. **3:**S97–S101.
19. **Nolte, F. S., A. M. Green, K. R. Fiebelkorn, A. M. Caliendo, C. Sturchio, A. Grunwald, and M. Healy.** 2003. Clinical evaluation of two methods for genotyping hepatitis C virus based on analysis of the 5′ noncoding region. J. Clin. Microbiol. **41:**1558–1564.
20. **Noppornpanth, S., E. Sablon, K. De Nys, X. L. Truong, J. Brouwer, M. Van Brussel, S. L. Smits, Y. Poovorawan, A. D. Osterhaus, and B. L. Haagmans.** 2006. Genotyping of hepatitis C viruses from Southeast Asia by a novel line probe assay that simultaneously detects core and 5′ untranslated regions. J. Clin. Microbiol. **44:**3969–3974.
21. **Pawlotsky, J. M.** 2003. Hepatitis C virus genetic variability: pathogenic and clinical implications. Clin. Liver Dis. **7:**45–66.
22. **Pawlotsky, J. M.** 2003. Mechanisms of antiviral treatment efficacy and failure in chronic hepatitis C. Antiviral Res. **59:**1–11.
23. **Richter, S. S.** 2002. Laboratory assays for diagnosis and management of hepatitis C virus infection. J. Clin. Microbiol. **40:**4407–4412.
24. **Seeff, L. B., and J. H. Hoofnagle.** 2002. National Institutes of Health consensus development conference: management of hepatitis C: 2002. Hepatology **36:**S1–S2.
25. **Simmonds, P., A. Alberti, H. J. Alter, F. Bonino, D. W. Bradley, C. Bechot, J. T. Brouwer, S. W. Chan, K. Chayama, D. S. Chen, Q. L. Choo, M. Colombo, H. T. M. Cuypers, T. Date, G. M. Dusheiko, J. I. Esteban, O. Fay, S. J. Hadziyannis, J. Han, A. Hatzakis, E. C. Holmes, H. Hotta, M. Houghton, B. Irvine, M. Kohara, J. A. Kolberg, G. Kuo, J. Y. N. Lau, P. N. Lelie, G. Maertens, F. McOmish, T. Miyamura, M. Mizokami, A. Nomoto, A. M. Prince, H. W. Reesink, C. Rice, M. Roggendorf, S. W. Schalm, T. Shikata, K. Shimotohno, L. Stuyver, C. Trépo, A. Weiner, P. L. Yap, and M. S. Urdea.** 1994. A proposed system for the nomenclature of hepatitis C viral genotypes. Hepatology **19:**1321–1324.
26. **Simmonds, P., J. Bukh, C. Combet, G. Deleage, N. Enomoto, S. Feinstone, P. Halfon, G. Inchauspé, C. Kuiken, G. Maertens, M. Mizokami, D. G. Murphy, H. Okamoto, J. M. Pawlotsky, F. Penin, E. Sablon, I. T. Shin, L. J. Stuyver, H. J. Thiel, S. Viazov, A. J. Weiner, and A. Windell.** 2005. Consensus proposal for a unified system of nomenclature of hepatitis C virus genotypes. Hepatology **42:**962–973.
27. **Simmonds, P., E. C. Holmes, T. A. Cha, S. W. Chan, F. McOmish, B. Irvine, E. Beall, P. L. Yap, J. Kolberg, and M. S. Urdea.** 1993. Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS5 region. J. Gen. Virol. **74:**2391–2399.
28. **Smith, D. B., J. Mellor, L. M. Jarvis, F. Davidson, J. Kolberg, M. Urdea, P. L. Yap, and P. Simmonds.** 1995. Variation of the hepatitis C virus 5′ non-coding region: implications for secondary structure, virus detection and typing. J. Gen. Virol. **76:**1749–1761.
29. **Stuyver, L., A. Wyseur, W. Van Arnhem, F. Hernandez, and G. Maertens.** 1996. Second-generation line probe assay for hepatitis C virus genotyping. J. Clin. Microbiol. **34:**2259–2266.
30. **Tang, S., I. K. Cheng, V. K. Leung, U. I. Kuok, A. W. Tang, Y. Wing Ho, K. Neng Lai, and T. Mao Chan.** 2003. Successful treatment of hepatitis C after kidney transplantation with combined interferon alpha-2b and ribavirin. J. Hepatol. **39:**875–878.
31. **Verbeeck, J., P. Maes, P. Lemey, O. G. Pybus, E. Wollants, E. Song, F. Nevens, J. Févery, W. Delport, S. Van der Merwe, and M. Van Ranst.** 2006. Investigating the origin and spread of hepatitis C virus genotype 5a. J. Virol. **80:**4220–4226.
32. **Weck, K.** 2005. Molecular methods of hepatitis C genotyping. Expert Rev. Mol. Diagn. **5:**507–520.
33. **World Health Organization.** 1999. Global surveillance and control of hepatitis C. Report of a WHO consultation organized in collaboration with the Viral Hepatitis Prevention Board, Antwerp, Belgium. J. Viral Hepatol. **6:**35–47.
34. **World Health Organization.** 2007. Initiative for vaccine research. http://www.who.int/vaccine_research/diseases/viral_cancers/en/index2.html.