Clin Chem Lab Med 2010;48(4):469–474 © 2010 by Walter de Gruyter • Berlin • New York. DOI 10.1515/CCLM.2010.093

Performance evaluation of the Abbott RealTi*me* HCV Genotype II for hepatitis C virus genotyping

Yong-Hak Sohn[1], Sun-Young Ko[2], Myeong Hee Kim[3] and Heung-Bum Oh[2,*]

[1] Department of Laboratory Medicine, Eulji University Hospital, Daejeon, South Korea
[2] Department of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center, Seoul, South Korea
[3] Department of Laboratory Medicine, East-West Neo-Medical Center, Kyunghee University, College of Medicine, Seoul, South Korea

Abstract

Background: The Abbott RealTi*me* hepatitis C virus (HCV) Genotype II (Abbott Molecular Inc.) for HCV genotyping, which uses real-time PCR technology, has recently been developed.
Methods: Accuracy and sensitivity of detection were assessed using the HCV RNA PHW202 performance panel (SeraCare Life Sciences). Consistency with restriction fragment mass polymorphism (RFMP) data, cross-reactivity with other viruses, and the ability to detect minor strains in mixtures of genotypes 1 and 2 were evaluated using clinical samples.
Results: All performance panel viruses were correctly genotyped at levels of >500 IU/mL. Results were 100% concordant with RFMP genotypic data (66/66). However, 5% (3/66) of the samples examined displayed probable genotypic cross reactivity. No cross reactivity with other viruses was evident. Minor strains in the mixtures were not effectively distinguished, even at quantities higher than the detection limit.
Conclusions: The Abbott RealTi*me* HCV Genotype II assay was very accurate and yielded results consistent with RFMP data. Although the assay has the advantages of automation and short turnaround time, we suggest that further improvements are necessary before it is used routinely in clinical practice. Efforts are needed to decrease cross reactivity among genotypes and to improve the ability to detect minor genotypes in mixed infections.
Clin Chem Lab Med 2010;48:469–74.

*Corresponding author: Heung-Bum Oh, MD, Department of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center, 388-1 Pungnap2-dong, Songpa-gu, Seoul 138-736, Korea
Phone: +82-2-3010-4505, Fax: +82-2-478-0884,
E-mail: hboh@amc.seoul.kr
Received October 6, 2009; accepted November 24, 2009; previously published online February 4, 2010

Keywords: genotype; genotyping; HCV; method evaluation; real-time PCR.

Introduction

Chronic hepatitis C virus (HCV) infection is a major cause of chronic liver disease worldwide, as are chronic hepatitis B infection and alcoholic liver problems (1). Although hepatitis B virus (HBV) infection is the most common of these conditions in East Asia, including Korea, the relative incidence of chronic HCV infection has increased after the introduction of HBV vaccination programs. Chronic liver disease develops in about 80% of patients with acute HCV infection, and 20% of patients who develop chronic liver disease progress to liver cirrhosis. In addition, patients with liver cirrhosis are at high risk of developing hepatic failure and hepatocellular carcinoma (HCC), with an annual incidence of 1%–3%. Thus, prevention of disease progression is an important goal of treatment in patients with chronic HCV infections (2). The most widely accepted treatment for such infections is combination therapy with pegylated interferon and ribavirin (3–5). It is generally accepted that treatment response differs with repect to HCV genotype. For instance, patients with HCV genotype 1 or 4 show poorer response and require longer duration of treatment than those infected with HCV genotype 2 or 3 (6). Accordingly, the American Association for the Study of Liver Disease (AASLD) guidelines (4, 5) and the NIH Consensus Conference (3) recommend implementation of HCV genotyping prior to treatment and individual planning of therapy with respect to the infecting genotype.

Direct sequencing (7, 8) and the line probe assays (9) are currently the most popular methods for genotyping. A novel HCV genotyping assay using the restriction fragment mass polymorphism (RFMP) method has also been used in some laboratories (10, 11). RFMP can be used to detect minor strains in mixed genotype infections because the technique distinguishes mass differences among nucleic acid restriction fragments with high analytical sensitivity. However, all of these approaches require nucleic acid sequencing or hybridization after PCR, and consequently have relatively long turnaround times. The current methods are based on amplification of the 5′-untranslated region (UTR) of HCV RNA only, which shows relatively little variation compared with the NS5B region. Whereas sequencing of the NS5B region is generally accepted as the gold standard for HCV genotyping, the 5′-UTR is considered the region of choice for routine laboratory work owing to the high level of conserved

sequences, which makes it easy to design reliable PCR primers, along with sufficient variability to provide clinically useful genotypic information (12).

Recently, a novel genotyping kit, the Abbott RealTi*me* HCV Genotype II (Abbott Molecular Inc., Des Plaines, IL, USA) has been developed. The kit uses real-time PCR technology. A considerable advantage of this procedure is that, in contrast to the current methods, only a single reaction is required to identify the HCV genotype. Moreover, the ability to discriminate between HCV subtypes 1a and 1b is improved through additional amplification of the NS5B region (13, 14). In the present study, we evaluated the performance of the Abbott RealTi*me* HCV Genotype II assay using samples from Korean patients with chronic HCV.

Materials and methods

Evaluation of kit accuracy and analytical sensitivity

The accuracy of the Abbott RealTi*me* Genotype II assay was evaluated using the PHW202 HCV RNA genotype performance panel (SeraCare Life Sciences, West Bridgewater, MA, USA) that includes seven (sub) genotypes (1a, 1b, 2, 3, 4, 5, and 6). We validated the analytical sensitivity by testing a series of three dilutions of all members of the PHW202 panel, down to a level of 500 IU/mL, which is claimed as the sensitivity limit by the manufacturer (Abbott Molecular Inc.).

The Abbott RealTi*me* Genotype II was used following the manufacturer's instructions using the Abbott m2000sp and Abbott m2000rt modules. HCV RNA was extracted from 500 μL of serum using the Abbott m2000sp process. Prior to extraction, internal controls were added to confirm the stability of RNA during extraction and PCR. Extracted RNA samples were aliquoted into three reaction wells within the test plate, and mixed with master mixes A, B, and C, respectively. RNA was transcribed into cDNA via reverse transcription and amplified using the Abbott m2000rt procedure. Four primer types were used for amplification of the 5′-UTR and NS5B regions (subtypes 1a and 1b) and the internal control. Amplified products were detected using the minor groove binder (MGB) probe in real time. Six genotypes, and subtypes 1a and 1b, were identified by combining the results from each tube. HCV genotype 3, subtype 1a, and all HCV isolates were generally detected in Tube A. Genotypes 1 and 2 and subtype 1b were detected in Tube B, and genotypes 4, 5, and 6 in Tube C. The Abbott Genotype II assay used three cut-off values to facilitate accurate designation of the HCV genotype. These included [1] the threshold cycle (Ct); [2] the Ct number difference (<6) for each genotype-specific probe, compared to the HCV all-genotype probe cycle threshold number; and [3] the maximum ratio (MR). In the case of genotype 1, subtypes 1a and 1b were identified only in PCR products obtained from the NS5B region.

Evaluation of kit capability to detect mixed HCV infections

The capability to detect minor strains in mixed HCV infections was assessed using clinical samples. HCV genotype 1b (3.0×10^6 IU/mL) and 2 (1.6×10^6 IU/mL) were mixed using 1:1 and 1:100 ratios (Mix-test 1). Similarly, genotypes of type 1 other than 1a and 1b (3.0×10^5 IU/mL) and genotype 2 strains (4.8×10^5 IU/mL) were mixed and tested (Mix-test 2). If the minor strain was not detected in a 1:100 mixture, a 1:10 mixture was prepared using the same samples, and tested.

Evaluation of cross-reactivity

Cross reactivity of primers or probes with HBV, Epstein-Barr virus (EBV), cytomegalovirus (CMV), and human immunodeficiency virus (HIV) was examined using two samples of each virus. HBV samples were acquired from patients with chronic hepatitis B, and the DNA concentrations were estimated as $>1.0\times10^9$ IU/mL and 5.0×10^8 IU/mL using the Abbott Real-Time HBV quantification kit. EBV- and CMV-positive samples were obtained from immunocompromised patients following transplantation. DNA concentrations of 2.3×10^3 and 5.0×10^4 copies/mL of EBV and 5.4×10^3 and 3.2×10^3 copies/mL of CMV were measured using an in-house real-time PCR methodology. For HIV, the Abbott Real-Time HIV-1 Calibrator B was used at a concentration of 6.2 log IU/mL. After viral suspensions were mixed with the same amounts of sera containing HCV of the known genotype, we assessed whether the genotype results were similar to samples where HCV was not admixed with other viruses.

Comparison of the Abbott RealTi*me* Genotype II assay and the restriction fragment mass polymorphism method

HCV genotype results were compared with those obtained using the RFMP method (10, 11). A total of 53 samples were randomly selected from those with HCV RNA concentrations over 10^3 IU/mL, as determined using the Abbott RealTi*me* HCV quantification kit (Abbott Molecular Inc., Des Plaines, IL, USA). These were also simultaneously tested using both Abbott RealTi*me* Genotype II and RFMP methods. Additionally, to compare results for genotypes other than 1b and 2, 13 stored samples that had been genotyped as other than these (sub) genotypes using the RFMP method were tested using the Abbott RealTi*me* Genotype II assay. When discrepancies between results tested with the different methods were observed, the 5′-UTR regions were sequenced as described previously (10, 15). This study was approved by the Institutional Review Board of the Asan Medical Center, Seoul, Korea.

Results

Accuracy and analytical sensitivity

The Abbott RealTi*me* Genotype II kit gave correct results for all members of the virus reference panel at concentrations of >500 IU/mL, which is the detection limit of the assay (Table 1). Correct genotyping of subtype 1a was obtained from PCR reactions using NS5B-specific primers. With the exception of the genotype 1b and 2 panels, samples diluted to <500 IU/mL were correctly genotyped using this method, as shown in Table 1.

Capability of identifying minor genotypes from mixed HCV infections

The Abbott RealTi*me* Genotype II kit correctly identified both genotypes in 1:1 mixtures. However, in 1:100 mixtures, only the minor strain of subtype 1b was correctly identified in Mix-test 1 samples by amplification of the NS5B region

Table 1 Verification of detection sensitivity using the PHW202 HCV RNA genotype performance panel (SeraCare Life Sciences, West Bridgewater, MA, USA).

Genotype (sample)	HCV RNA quantitation, IU/mL[a]	Genotype result
Panel GT 1a	1100	1a
	550	1a
	275	1a
Panel GT 1b	2600	1, 1b
	520	1b
	260	Unidentified
Panel GT 2	1300	2
	325	2
	163	Unidentified
Panel GT 3	3000	3
	750	3
	375	3
Panel GT 4	5700	4
	950	4
	317	4
Panel GT 5	1200	5
	300	5
	150	5
Panel GT 6	1600	6
	800	6
	400	6

[a]Diluted concentrations based on RNA quantification results using the Roche COBAS Amplicor HCV Monitor, Version 1.5 (Roche Diagnostics Corp., Indianapolis, IN, USA).

using specific primers (Table 2). All remaining minor strains were not detected in 1:100 mixtures. When the 1:10 mixtures were tested to determine the ability to detect and genotype minor strains, the following results were obtained. First, the minor strain of genotype 2 was not identified in the mix-test 1. Although the amplified signal for genotype 2 was detected as a Ct value of 24.3, this was not accepted for reporting because the Ct number difference with respect to the HCV all-genotype probe was >6. Second, HCV strains of subtype 1 other than 1a and 1b were not detected in the 10-fold dilution of Mix-test two samples, whereas genotype 2 strains were identified at a 10-fold dilution (Table 2).

Cross reactivity with other viruses

No cross reactivity was observed in samples containing mixtures of HCV genotype 1b or 2 viruses and HBV-, EBV-, CMV- or HIV-positive sera.

Comparison of kit results with restriction fragment mass polymorphism data

Among the 66 samples examined, 39 were identified as genotype 1, 22 as genotype 2, and five as genotype 3 using the RFMP method. Although concordant results were usually obtained with the Abbott RealTi*me* Genotype II kit, genotypes 1b+3 were detected in two samples and genotypes 2+4 in another sample. The three samples with mixed genotypes detected using the Abbott RealTi*me* Genotype II assay were re-genotyped using RFMP as genotypes 1, 1, and 2 only. Subtypes 1a and 1b were further distinguished using the Abbott Genotype II assay kit. Among the 39 samples identified as genotype 1 by RFMP (30 of subtype 1b, 1 of subtype 1a, 2 of subtypes 1a+1b, and 6 of subtype 1 other than 1a or 1b), results from the Abbott RealTi*me* Genotype II assay kit were consistent for 21 of the samples (Table 3). The most common discrepancies included nine instances of subtype 1b as shown by RFMP, but of subtype 1 other than 1a or 1b with the Abbott RealTi*me* Genotype II assay, and three examples where the opposite was true (Table 3). When the 5′-UTR region was sequenced for 11 of the 18 samples

Table 2 Test results for mixed samples containing genotypes 1 and 2.

Test no. (mixed genotypes)	Mixture ratio (RNA concentration, IU/mL)		Ct (ΔCt based on HCV)				Abbott results
	Genotype 1	Genotype 2	HCV all	1	1b	2	
Mix-test 1	0.01	1	18.5	ND	21.5 (3.0)	17.6 (–0.9)	1b and 2
(1b and 2)	1 (3.0×10^6)	1 (1.0×10^6)	18.6	18.5 (–0.1)	17.7 (–0.9)	19.3 (0.7)	1b and 2
	1	0.1	18.0	17.4 (–0.6)	17.2 (–0.8)	24.3 (6.3)	1b[a]
	1	0.01	17.3	16.4 (–0.9)	16.3 (–1.0)	ND	1b
Mix-test 2	0.01	1	21.4	ND	ND	19.7 (–1.7)	2
(1[c] and 2)	0.1	1	19.2	ND	ND	18.5 (–0.7)	2[b]
	1 (3.0×10^5)	1 (4.8×10^5)	19.3	19.7 (0.4)	ND	19.2 (–0.1)	1 and 2
	1	0.1	20.8	19.3 (–1.5)	ND	22.2 (1.4)	1 and 2
	1	0.01	21.4	19.7 (–1.7)	ND	ND	1

[a]The amplified signal for genotype 2 was detected as a Ct value of 24.3, but was not accepted for reporting, as the Ct number difference with respect to the HCV all-genotype probe was higher than six. [b]The test with a 10-fold dilution of genotype 1 was performed after a negative result was obtained in a test with a 100-fold dilution of the sample. [c]Genotype 1 subtypes other than 1a and 1b. Ct, threshold cycle; ND, not detected.

Table 3 Comparison of genotype results for 66 clinical samples using restriction fragment mass polymorphism (RFMP) and the Abbott RealTi*me* HCV Genotype II method.

		Abbott RealTi*me* HCV Genotype II						
		1a	1b	1[a]	2	3	1b+3	2+4
RFMP	1a		1					
	1a+1b		2					
	1b		21	9				
	1[a]	1	3				2	
	2a/c				13			
	2b				2			
	2[b]				6			1
	3a					5		

[a]Genotype 1 subtypes other than 1a and 1b. [b]Genotype 2 subtypes other than 2a/c and 2b, by the RFMP method.

that were available and that showed discrepancies, all results were concordant with those of the RFMP assay.

Discussion

The clinical course and treatment response to chronic hepatitis C varies according to the HCV genotype. For example, HCV genotype 1b causes more severe hepatic damage, rapid progression to cirrhosis, and is associated with higher serum viral concentrations than are genotypes 2 and 3 (16–18). Additionally, genotypes 1 and 4 respond poorly to current medications for HCV, and patients with these genotypes require prolonged treatment compared with those with genotypes 2 or 3 (6, 16). Thus, HCV genotyping is important for planning treatment schedules and predicting prognosis of patients with chronic HCV infections. HCV genotype frequencies vary geographically. HCV genotype 1 is the most common worldwide, particularly in North and South America, Western and Eastern Europe, and Northeast Asia, where this genotype accounts for more than 50% of infections. HCV genotype 2 is also common in Western Europe, North America, and Northeast Asia (19). In Korean individuals, genotypes 1b (50%) and 2a (35%) are relatively common (20).

Direct sequencing (7, 8) and the line probe assay (9) are the most commonly used methods for genotyping. These procedures require additional reactions, such as hybridization and sequencing after PCR, and thus are labor-intensive and time-consuming. Recently, these problems have been reduced by the development of new genotyping methods using real-time PCR technology (21–23). Furthermore, as this technology, in conjunction with a semi-automated platform, is widely used to determine viral load in patients with hepatitis, it is expected that genotyping will be much easier to perform when using an approach similar to that of the Abbott RealTi*me* Genotype II assay.

The Abbott Genotype II assay was accurate when evaluated using a viral reference panel, and appeared consistent with RFMP data at the genotype level following analysis of 66 clinical samples, suggesting that it may be of use in the clinic. Interestingly, the Abbott RealTi*me* Genotype II assay detected previously unsuspected genotype 3 and 4 viruses in three clinical samples (Table 3). Although it is not clear which method produces the correct data most consistently, one explanation is that cross reaction occurs between supposedly genotype-specific primers or probes, since genotypes 3 and 4 are very rare in Korean individuals, and the kit specifications state that cross reactions can take place, albeit rarely. Specifically, 0.32% of viruses with genotype 1 sequences and 0.4% of viruses with genotype 2 sequences also react with genotype 3 and 4 probes, respectively. Finally, the 5′-UTR sequencing of one sample (genotype 1+3) among these identified genotype 1.

Differentiation of the HCV subtype is not considered to be important in currently available treatment protocols. Nevertheless, the Abbott RealTi*me* Genotype II assay is designed to distinguish subtypes of genotype 1, reflecting earlier reports that patients with subtype 1b have a worse prognosis than those with subtype 1a HCV (24, 25). However, sequence analyses based only on 5′-UTR regions cannot fully discriminate between genotypes 1a and 1b (26, 27), as subtype differentiation in the 5′-UTR is based solely on A or G differences at the –99 nucleotide position (28). The challenge in distinguishing subtypes of group 1 using methods such as RFMP based only on 5′-UTR sequences may be the major reason for the discrepancies between the two procedures. As the Abbott RealTi*me* Genotype II assay involves the amplification of NS5B in addition to 5′-UTR, it is likely that this assay is more accurate in subgenotyping genotype 1 samples. The correct typing results obtained for reference panel samples containing genotypes 1a and 1b support this theory.

Unfortunately, the Abbott RealTi*me* Genotype II assay could not detect all strains in mixed samples of genotypes 1 and 2, although the two genotype strains were present at levels higher than the detection limit. Although both genotypes were clearly detected when mixed at similar concentrations, minor strains were not always evident in 1:100, or even 1:10, mixtures (Table 2). However, the genotype 1b strain was detected with the kit, even in 1:100 mixtures. Thus, the Abbott RealTi*me* Genotype II assay has the advan-

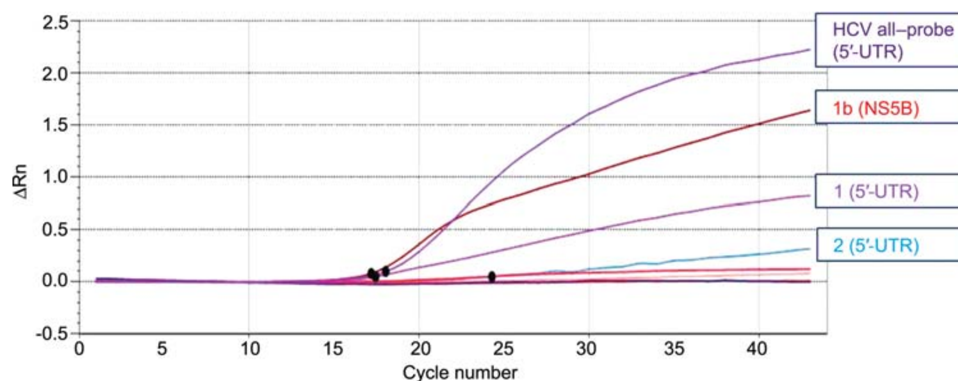

Figure 1 Amplification plot of genotype 1b and 2 strains in the 10:1 mixture in Mix-test 1. An amplified signal for genotype 2 (light blue) was detected, but not accepted, as the Ct number difference with respect to the HCV all-genotype probe (dark purple) was higher than six.

tages of detecting minor HCV strains of genotype 1b HCV because amplification of the NS5B primer is not disrupted by genotype two samples.

The Abbott RealTi*me* Genotype II assay differs from the previous version in that all reactions can be performed in the Abbott m2000 automated preparation instrument in the new version, thus taking advantage of all the validation checks provided by the new software. In addition, with the Abbott RealTi*me* Genotype II assay, a Ct number difference between a genotype-specific probe and an HCV all-genotype probe is accepted only when the value is lower than six. This new algorithm was developed to improve the detection of mixed genotypes, compared with that offered by the previous version of the assay. The earlier version, the Abbott HCV genotyping analyte-specific reagent (ASR), had different assay criteria and samples with genotype Ct differences of <3 were accepted as mixed infections (14). In Mix-test 1, the Ct value of genotype 2 was 24.3 at a 10-fold dilution (Figure 1). However, the Ct value was not accepted by the assay criteria because the Ct value of the HCV all-genotype probe was 18.0, leading to a difference of more than six. This finding indicates that further modification of the algorithm is required to improve the ability of the assay to detect mixed genotypes.

In conclusion, the Abbott Real-Ti*me* HCV Genotype II assay is highly accurate with a verifiable detection limit of about 500 IU/mL. The data obtained with this method are consistent with results obtained with the RFMP method at the genotype level. Although the Abbott Genotype II assay has the additional advantages of automation and shorter turnaround time, we suggest that further improvements are required before the assay can be used safely in routine clinical laboratories. It is necessary to decrease the cross reactivity between genotypes and to improve the ability of the kit for the detection of minor genotypes in mixed infections.

Acknowledgements

This research was supported by the Pioneer R&D program for converging technology through the Korea Science and Engineering Foundation funded by the Ministry of Education, Science and Technology (Grant number: M10711270001-08M1127-00110).

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.

References

1. Poynard T, Yuen MF, Ratziu V, Lai CL. Viral hepatitis C. Lancet 2003;362:2095–100.
2. Lauer GM, Walker BD. Hepatitis C virus infection. N Engl J Med 2001;345:41–52.
3. NIH Consensus Statement on Management of Hepatitis C: 2002. NIH Consens State Sci Statements 2002;19:1–46.
4. Strader DB, Wright T, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C. Hepatology 2004;39: 1147–71.
5. Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. Hepatology 2009;49:1335–74.
6. Hnatyszyn H. Chronic hepatitis C and genotyping: the clinical significance of determining HCV genotypes. Antivir Ther 2005;10:1–11.
7. Arens M. Clinically relevant sequence-based genotyping of HBV, HCV, CMV, and HIV. J Clin Virol 2001;22:11–29.
8. Ansaldi F, Torre F, Bruzzone B, Picciotto A, Crovari P, Icardi G. Evaluation of a new hepatitis C virus sequencing assay as a routine method for genotyping. J Med Virol 2001;63:17–21.
9. Verbeeck J, Stanley M, Shieh J, Celis L, Huyck E, Wollants E, et al. Evaluation of Versant hepatitis C virus genotype assay (LiPA) 2.0. J Clin Microbiol 2008;46:1901–6.
10. Kim Y, Kim S, Chung H, Jee M, Kim B, Kim K, et al. Population genotyping of hepatitis C virus by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analysis of short DNA fragments. Clin Chem 2005;51:1123–31.

11. Chung HJ, Yang JH, Hwang SH, Hong SP, Oh HB. The comparison of restriction fragment mass polymorphism with sequencing method for the hepatitis C virus genotyping. Korean J Lab Med 2005;25:352–60.
12. Simmonds P, Bukh J, Combet C, Deleage G, Enomoto N, Feinstone S, et al. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. Hepatology 2005; 42:962–73.
13. Cook L, Sullivan K, Krantz E, Bagabag A, Jerome K. Multiplex real-time reverse transcription-PCR assay for determination of hepatitis C virus genotypes. J Clin Microbiol 2006;44:4149–56.
14. Martro E, Gonzalez V, Buckton A, Saludes V, Fernandez G, Matas L, et al. Evaluation of a new assay in comparison with reverse hybridization and sequencing methods for hepatitis C virus genotyping targeting both 5′ noncoding and nonstructural 5b genomic regions. J Clin Microbiol 2008;46:192–7.
15. Mizokami M, Gojobori T, Lau JY. Molecular evolutionary virology: its application to hepatitis C virus. Gastroenterology 1994;107:1181–2.
16. Fried M, Shiffman M, Reddy K, Smith C, Marinos G, Goncales FJ, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347:975–82.
17. Kobayashi M, Tanaka E, Sodeyama T, Urushihara A, Matsumoto A, Kiyosawa K. The natural course of chronic hepatitis C: a comparison between patients with genotypes 1 and 2 hepatitis C viruses. Hepatology 1996;23:695–9.
18. Poynard T, McHutchison J, Goodman Z, Ling M, Albrecht J. Is an ‘a la carte’ combination interferon alfa-2b plus ribavirin regimen possible for the first line treatment in patients with chronic hepatitis C? The ALGOVIRC Project Group. Hepatology 2000;31:211–8.
19. Zein N. Clinical significance of hepatitis C virus genotypes. Clin Microbiol Rev 2000;13:223–35.
20. Suh DJ, Jeong SH. Current status of hepatitis C virus infection in Korea. Intervirology 2006;49:70–5.
21. Lindh M, Hannoun C. Genotyping of hepatitis C virus by Taqman real-time PCR. J Clin Virol 2005;34:108–14.
22. Rolfe K, Alexander G, Wreghitt T, Parmar S, Jalal H, Curran M. A real-time Taqman method for hepatitis C virus genotyping. J Clin Virol 2005;34:115–21.
23. Schroter M, Zollner B, Schafer P, Landt O, Lass U, Laufs R, et al. Genotyping of hepatitis C virus types 1, 2, 3, and 4 by a one-step LightCycler method using three different pairs of hybridization probes. J Clin Microbiol 2002;40:2046–50.
24. Zein NN, Rakela J, Krawitt EL, Reddy KR, Tominaga T, Persing DH. Hepatitis C virus genotypes in the United States: epidemiology, pathogenicity, and response to interferon therapy. Collaborative Study Group. Ann Intern Med 1996;125:634–9.
25. Bell H, Hellum K, Harthug S, Maeland A, Ritland S, Myrvang B, et al. Genotype, viral load and age as independent predictors of treatment outcome of interferon-alpha 2a treatment in patients with chronic hepatitis C. Construct group. Scand J Infect Dis 1997;29:17–22.
26. Chen Z, Weck K. Hepatitis C virus genotyping: interrogation of the 5′ untranslated region cannot accurately distinguish genotypes 1a and 1b. J Clin Microbiol 2002;40:3127–34.
27. Laperche S, Lunel F, Izopet J, Alain S, Deny P, Duverlie G, et al. Comparison of hepatitis C virus NS5b and 5′ noncoding gene sequencing methods in a multicenter study. J Clin Microbiol 2005;43:733–9.
28. Stuyver L, Wyseur A, van Arnhem W, Lunel F, Laurent-Puig P, Pawlotsky JM, et al. Hepatitis C virus genotyping by means of 5′-UR/core line probe assays and molecular analysis of untypeable samples. Virus Res 1995;38:137–57.

Copyright of Clinical Chemistry & Laboratory Medicine is the property of De Gruyter and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.