JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2003, p. 4855–4857 Vol. 41, No. 10
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.10.4855–4857.2003

Evaluation of the TRUGENE *HCV 5′NC* Genotyping Kit with the New GeneLibrarian Module 3.1.2 for Genotyping of Hepatitis C Virus from Clinical Specimens

Jeffrey J. Germer,[1] David W. Majewski,[1] Michael Rosser,[2] Amber Thompson,[2] P. Shawn Mitchell,[1] Thomas F. Smith,[1] Slava Elagin,[2] and Joseph D.C. Yao[1]*

Division of Clinical Microbiology, Mayo Clinic, Rochester, Minnesota 55905,[1] and Bayer HealthCare LLC, Suwanee, Georgia 30024[2]

Received 13 May 2003/Returned for modification 14 July 2003/Accepted 21 July 2003

The TRUGENE *HCV 5′NC* genotyping kit (GeneLibrarian modules 3.1.1 and 3.1.2) and VERSANT HCV genotyping assay were compared by using 96 hepatitis C virus (HCV) RNA-positive patient specimens, including HCV genotypes 1, 2, 3, 4, 5, 6, and 10. The TRUGENE *HCV 5′NC* genotyping kit (GeneLibrarian module 3.1.2) yielded the most accurate genotyping results.

Hepatitis C virus (HCV) has been classified into at least six distinct genotypes that can be further differentiated into multiple subtypes (12, 13). Recent anti-HCV treatment algorithms suggest that tailoring antiviral therapy based on HCV genotype as well as HCV RNA titer can optimize therapeutic outcome (2, 7). While sequence variability is found throughout the HCV genome, the 5′ noncoding (5′NC) region remains highly conserved and is the target of choice for detection by reverse transcription-PCR (RT-PCR) (5, 14, 15). Despite the limited sequence diversity found within the HCV 5′NC region compared to that of a highly diversified genomic target such as the nonstructural (NS) 5B region, practical considerations have made the 5′NC region the preferred target for HCV genotyping in most diagnostic laboratories (3, 6, 8). Several HCV genotyping assays are currently commercially available, including the TRUGENE *HCV 5′NC* genotyping kit (TRUGENE 5′NC; Bayer HealthCare LLC, Berkeley, Calif.) and the VERSANT HCV genotype assay (LiPA; Bayer HealthCare LLC). Recently, there have been several published comparisons of these two assays (1, 8, 10), in addition to several other evaluations of the TRUGENE 5′NC (4, 11). To date, none of these comparisons has evaluated the TRUGENE 5′NC in conjunction with the new *HCV 5′NC* GeneLibrarian module 3.1.2 (GL 3.1.2; Bayer HealthCare LLC). The GL 3.1.2 is a new 5′NC sequence database validated by phylogenetic analysis of the 5′NC and corresponding NS5B sequences from over 250 HCV strains collected worldwide and is designed for use with the TRUGENE 5′NC (A. Grunwald-Petty, C. Sturchio, P. Berger, M. Rosser, P. Nordstrom, A. Beyou, A. Buckton, B. Clarke, S. Elagin, and A. De La Rosa, Abstr. 53rd Annu. Mtg. Am. Assoc. Study Liver Dis., abstr. 757, 2002).

This study was undertaken to evaluate and compare the HCV genotyping capability of the TRUGENE 5′NC using both GL 3.1.1 and 3.1.2 with that of LiPA. Concordance between HCV genotyping results obtained from TRUGENE 5′NC and LiPA with clinical specimens was also compared. An in-house TRUGENE *HCV NS5B* genotyping assay (TRUGENE NS5B; Bayer HealthCare LLC) was used to test specimens yielding discordant results between the TRUGENE 5′NC with GL 3.1.2 and LiPA.

A total of 96 unique HCV RNA-positive patient serum specimens submitted to our diagnostic laboratory (Mayo Medical Laboratories, Rochester, Minn.) for HCV genotyping by LiPA were selected for this study. Of the 53 specimens collected retrospectively between June 2000 and March 2002 (including genotypes 1, 2, 3, 4, 5, 6, and 10), 4 specimens were unsuccessfully genotyped by LiPA. The remaining 43 specimens were collected prospectively between March 2002 and April 2002. This study was approved by the Mayo Institutional Review Board.

All specimens were subjected to RNA extraction and RT-PCR amplification using the COBAS AMPLICOR Hepatitis C Virus test, version 2.0 (Roche Molecular Systems, Inc., Branchburg, N.J.), following the manufacturer's instructions. The amplification products were used directly in LiPA and purified with the QIAquick PCR purification kit (Qiagen, Inc., Valencia, Calif.) prior to use in the TRUGENE 5′NC. All specimens further analyzed by TRUGENE NS5B were processed with the Epicentre Masterpure RNA purification kit (Epicentre Technologies, Madison, Wis.). For TRUGENE NS5B, a portion of the HCV NS5B region was amplified from each specimen with the Titan One Tube RT-PCR kit (Roche Applied Science, Indianapolis, Ind.) and used directly in the assay.

LiPA was performed with the AutoBlot 2000 instrument (MedTec, Inc., Chapel Hill, N.C.) following the manufacturer's instructions. TRUGENE 5′NC genotyping consisted of CLIP sequencing, a proprietary single-tube DNA sequencing technique utilizing fluorescently labeled primers to generate bidirectional sequence data, followed by sequence analysis with the OpenGene DNA sequencing system, GeneObjects DNA analysis software, and GL 3.1.1 or 3.1.2. TRUGENE NS5B genotyping was performed with HCV NS5B RT-PCR

* Corresponding author. Mailing address: Hilton-460, Division of Clinical Microbiology, Mayo Clinic, 200 First St. SW, Rochester, MN 55905. Phone: (507) 284-3697. Fax: (507) 284-4272. E-mail: jdcyao@mayo.edu.

TABLE 1. Concordance between the TRUGENE 5′NC (GL 3.1.1) and LiPA

LiPA genotype	(*n*)	No. of specimens with TRUGENE 5′NC (GL 3.1.1) genotype concordance[a]													
		1 NS	1a	1b	1c	2 NS	2b	2c	3 NS	3a	4 NS	4a	5a	6a	NT[b]
1 NS	(2)		1	1											
1a	(24)		22		2										
1b	(10)	2		7											1
1a/1b	(3)		3												
2 NS	(3)					1	1	1							
2a/2c	(7)					7									
2b	(7)						6								1
3a	(7)								2	5					
4 NS	(8)										8				
4c/4d	(8)										8				
4e	(2)										1	1			
4f	(1)														1
4h	(2)										2				
5a	(2)		1										1		
6a	(4)													4	
10a	(1)								1						
NT	(5)						1				1	1			2

[a] Discordant results are underlined. NT, not typeable; NS, no subtype assigned.
[b] Includes three specimens yielding no sequence by the TRUGENE 5′NC assay (HCV RNA titer < 615 IU/ml).

amplification products directly in CLIP sequencing reactions. NS5B sequences were analyzed with the OpenGene DNA sequencing system, GeneObjects DNA analysis software, and a prototype *HCV NS5B* GeneLibrarian module 5.1 (Grunwald Petty et al., Abstr. 53rd Annu. Mtg. Am. Assoc. Study Liver Dis.).

Concordance between TRUGENE 5′NC (with GL 3.1.1 and 3.1.2) and LiPA is summarized in Tables 1 and 2. The typing efficiencies (genotype assignment with or without a subtype) of the TRUGENE 5′NC using GL 3.1.1 and 3.1.2 were 94.8% (91 of 96) and 92.7% (89 of 96), respectively, compared with 94.8% (91 of 96) when using LiPA. Subtyping efficiencies (specific subtype assignment) among the assays were 60.4% (58 to 96), 86.5% (83 to 96), and 62.5% (60 to 96), respectively. Discordant subtyping results (TRUGENE 5′NC versus LiPA) were found in 4.5% (4 of 88) and 14.9% (13 of 87) of the specimens with the GL 3.1.1 (Table 1) and the GL 3.1.2 (Table 2) modules, respectively.

The genotyping accuracy of TRUGENE 5′NC with GL 3.1.2 was confirmed by TRUGENE NS5B among specimens showing discordant results between TRUGENE 5′NC with GL 3.1.2 and LiPA (Table 3). A single genotype-discordant result (10a versus 3) between the TRUGENE 5′NC and LiPA can be attributed to differences in the classification schemes used by each assay. The genotype 3 designation is in concordance with currently accepted HCV taxonomy (9). Among the specimens included in Table 3, the subtyping results of TRUGENE 5′NC with GL 3.1.2 were largely supported by those obtained with TRUGENE NS5B. The two assays were in complete agreement in 10 of 13 subtype-discordant specimens and 2 of 9

TABLE 2. Concordance between the TRUGENE 5′NC (GL 3.1.2) and LiPA

LiPA genotype	(*n*)	No. of specimens with TRUGENE 5′NC (GL 3.1.2) genotype concordance[a]													
		1 NS	1a	1b	2a	2b	2c	3 NS	3a	4 NS	4a	4i	5a	6a	NT[b]
1 NS	(2)		1	1											
1a	(24)	2	22												
1b	(10)	1	1	7											1
1a/1b	(3)		3												
2 NS	(3)				1	1	1								
2a/2c	(7)				7										
2b	(7)					6									1
3a	(7)								7						
4 NS	(8)									2	4	2			
4c/4d	(8)										8				
4e	(2)										2				
4f	(1)														1
4h	(2)										2				
5a	(2)												1		1
6a	(4)													4	
10a	(1)							1							
NT	(5)					1					1				3

[a] Discordant results are underlined. NT, not typeable; NS, no subtype assigned.
[b] Includes three specimens yielding no sequence by the TRUGENE 5′NC assay (HCV RNA titers < 615 IU/ml).

TABLE 3. Assay comparison among discordant LiPA and TRUGENE 5′NC (GL 3.1.2) specimens

Discordance (*n*)	Genetype determined by[a]:			
	LiPA	TRUGENE 5′NC		TRUGENE NS5B (GL 5.1)
		GL 3.1.1	GL 3.1.2	
Type (1)	10a	3 NS[b]	3 NS[b]	10a
Subtype (13)	4e	4a	4a	4e
	4h	4a	4a	4a
	4h	4 NS	4a	4a
	4e	4 NS	4a	4a
	4c/4d	4 NS	4a	4a
	4c/4d	4 NS	4a	4a
	4c/4d	4 NS	4a	4a
	4c/4d	4 NS	4a	4a
	4c/4d	4 NS	4a	4a
	4c/4d	4 NS	4a	4a
	4c/4d	4 NS	4a	4a
	4c/4d	4 NS	4a	FS
	1b	1 NS	1a	FS
Undetermined (9)	NT	4 NS	4a	4a
	NT	2b	2b	2b
	NT	NT	NT	4e
	NT	4a	NT	1a
	5a	1b	NT	1a
	4f	NT	NT	FS
	NT	FS[c]	ND	ND
	2b	FS[c]	ND	ND
	1b	FS[c]	ND	ND

[a] FS, failed sequence; ND, not done; NS, no subtype assigned; NT, not typeable.
[b] HCV genotype 10a is reported as a subtype of genotype 3 in concordance with NS5B phylogenetic analysis.
[c] HCV RNA titer < 615 IU/ml.

specimens of "undetermined" discordance, whereas TRUGENE NS5B supported the LiPA result in only 1 of 13 subtype-discordant specimens (Table 3). TRUGENE 5′NC with GL 3.1.1 assigned a subtype in only 2 of 13 subtype-discordant specimens and 3 of 9 specimens of "undetermined" discordance. Furthermore, LiPA and TRUGENE 5′NC with GL 3.1.1 each yielded an additional type discrepancy compared to TRUGENE NS5B (5a versus 1a and 4a versus 1a, respectively) among the "undetermined" discordant results (Table 3).

The clinical significance of HCV genotyping has been well documented, and many clinical laboratories are routinely performing genotyping by a variety of methods. However, methods utilizing nucleic acid amplification products generated directly from the HCV 5′NC region are typically the most efficient, since amplification products can be consistently generated from diverse HCV strains and may be readily available from qualitative or quantitative HCV RNA testing. Both LiPA and TRUGENE 5′NC are commercially available and are compatible with HCV RNA target amplification assays targeting the HCV 5′NC region.

Despite their inability to accurately subtype some HCV strains, both TRUGENE 5′NC and LiPA are currently acceptable for routine clinical HCV genotyping. This is particularly true because the clinical significance of HCV subtyping remains unknown. In contrast to LiPA, TRUGENE 5′NC (with GL 3.1.1 or 3.1.2) provided more detailed sequence information, and, unlike LiPA, the OpenGene platform proved to be flexible and easily updated with new sequence data (GL 3.1.2). In summary, the introduction of the GL 3.1.2 represents a significant improvement in the TRUGENE 5′NC, yielding improved genotyping accuracy compared to that of TRUGENE 5′NC with GL 3.1.1 or LiPA. However, the TRUGENE 5′NC with GL 3.1.2 remains limited in its ability to accurately subtype all HCV strains due to the high degree of sequence conservation found within the HCV 5′NC region.

REFERENCES

1. **Ansaldi, F., F. Torre, B. M. Bruzzone, A. Picciotto, P. Crovari, 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.
2. **European Association for the Study of the Liver.** 1999. Consensus statement. J. Hepatol. **30:**956–961.
3. **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.
4. **Halfon, P., P. Trimoulet, M. Bourliere, H. Khiri, V. de Lédinghen, P. Couzigou, J. M. Feryn, P. Alcaraz, C. Renou, H. J. A. Fleury, and D. Ouzan.** 2001. Hepatitis C virus genotyping based on 5′ noncoding sequence analysis (Trugene). J. Clin. Microbiol. **39:**1771–1773.
5. **Han, J. H., V. Shyamala, K. H. Richman, M. J. Brauer, B. Irvine, M. S. Urdea, P. Tekamp-Olson, G. Kuo, Q. L. Choo, and M. Houghton.** 1991. Characterization of the terminal regions of hepatitis C viral RNA: identification of conserved sequences in the 5′ untranslated region and poly(A) tails at the 3′ end. Proc. Natl. Acad. Sci. USA **88:**1711–1715.
6. **Holland, J., I. Bastian, R. M. Ratcliff, M. Y. Beers, P. Hahesy, H. Harley, D. R. Shaw, and G. D. Higgins.** 1998. Hepatitis C genotyping by direct sequencing of the product from the Roche AMPLICOR test: methodology and application to a South Australian population. Pathology **30:**192–195.
7. **National Institutes of Health.** 2002. Consensus development conference: management of hepatitis C: 2002. Hepatology **36:**S3–S20.
8. **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.
9. **Robertson, B., G. Myers, C. Howard, T. Brettin, J. Bukh, B. Gaschen, T. Gojobori, G. Maertens, M. Mizokami, O. Nainan, S. Netesov, K. Nishioka, T. Shin i, P. Simmonds, D. Smith, L. Stuyver, A. Weiner, et al.** 1998. Classification, nomenclature, and database development for hepatitis C virus (HCV) and related viruses: proposals for standardization. Arch. Virol. **143:**2493–2503.
10. **Roque-Afonso, A. M., M. P. Ferey, J. D. Poveda, E. Marchadier, and E. Dussaix.** 2002. Performance of TRUGENE hepatitis C virus 5′ noncoding genotyping kit, a new CLIP sequencing-based assay for hepatitis C virus genotype determination. J. Viral Hepat. **9:**385–389.
11. **Ross, R. S., S. O. Viazov, C. D. Holtzer, A. Beyou, A. Monnet, C. Mazure, and M. Roggendorf.** 2000. Genotyping of hepatitis C virus isolates using CLIP sequencing. J. Clin. Microbiol. **38:**3581–3584.
12. **Simmonds, P., A. Alberti, H. J. Alter, F. Bonino, D. W. Bradley, C. Brechot, J. T. Brouwer, S. W. Chan, K. Chayama, D. S. Chen, Q. L. Choo, M. Colombo, T. Cuypers, T. Date, G. Dusheiko, J. I. Esteban, O. Fay, S. 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. Schalm, T. Shikata, K. Shimotohno, L. Stuyver, C. Trepo, 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.
13. **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 NS-5 region. J. Gen. Virol. **74:**2391–2399.
14. **Smith, D. B., J. Mellor, L. M. Jarvis, F. Davidson, J. Kolberg, M. Urdea, P. L. Yap, P. Simmonds, et al.** 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.
15. **Young, K. K., R. M. Resnick, and T. W. Myers.** 1993. Detection of hepatitis C virus RNA by a combined reverse transcription-polymerase chain reaction assay. J. Clin. Microbiol. **31:**882–886.