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Comparative Evaluation of Three Commercially Available Methodologies for Hepatitis C Virus Genotyping

Ted E. Schutzbank,[1]* Susan E. Sefers,[2] Nicole Kahmann,[1] Haijing Li,[3] and Yi-Wei Tang[2,3]

CompuNet Clinical Laboratories, Dayton, Ohio,[1] and Departments of Pathology[2] and Medicine,[3] Vanderbilt University School of Medicine, Nashville, Tennessee

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We compared the performances of three hepatitis C virus genotyping methodologies supplied by Bayer, Abbott, and Third Wave Technologies. Genotypes were determined for 136 of 137 specimens by the Bayer method, 121 of 137 specimens by the Invader assay, and only 77 of 137 specimens by the Abbott assay. All reported genotypes were concordant by all three methods.

Infections caused by specific hepatitis C virus (HCV) genotypes, such as 1a and 1b, are more refractory to antiviral therapy than those caused by other genotypes commonly seen in the United States (3–4, 10). For this reason, HCV genotyping has become a critical component of the standard of care of HCV-infected patients (6). Several commercial HCV genotyping assays are currently available to clinical laboratories. Two Food and Drug Administration-approved methods in wide use are the Trugene 5′ NC genotyping kit (2, 7) and the Versant HCV genotyping kit (both from Bayer Diagnostics, Tarrytown, NY) (1, 7). The former method is based on the sequencing of a segment of the 5′ noncoding (5′-NC) region of the HCV genome. The latter method is based on differential hybridization of the same region of the HCV genome to oligonucleotides immobilized on a nylon strip.

More recently, two alternative methods have become available as analyte-specific reagents. The Abbott HCV genotyping kit is based on real-time reverse transcription-PCR technology. Unlike the two Bayer assays, the Abbott method is performed directly on RNA extracted from a patient specimen (plasma) and not from previously amplified viral nucleic acid. As with the Trugene and Versant genotyping methods, the Abbott assay also targets sequences from the 5′-NC region of the HCV genome. The other method is the Invader HCV genotyping assay, developed by Third Wave Technologies, Inc. (TWT) (1). This assay also targets sequences in the 5′-NC region of the HCV genome to determine the genotype. The assay utilizes cleavase and fluorescence resonance energy transfer technologies, combined with automated, computerized data analysis, to provide test results in about 1 h (1). The RNA extraction step is not necessary to perform the TWT Invader assay.

In this study, we compared the performance of the Trugene, Abbott, and Invader methods using a panel of 137 plasma samples submitted to the Molecular Infectious Diseases Laboratory at Vanderbilt University Medical Center. HCV viral loads and genotyping were determined previously using the Cobas Amplicor HCV Monitor test (Roche Molecular Systems, Inc.) and the Trugene HCV 5′ genotyping kit, respectively. The viral loads of the samples ranged from less than 300 to 1.4×10^7 HCV RNA IU/ml. The starting samples for the Trugene and the TWT genotyping methods were amplicons generated using a COBAS MONITOR HCV test (Roche Diagnostic Corporation, Indianapolis, IN) and a user-developed, real-time TaqMan assay, respectively, as described previously (8, 9). These samples were extracted with the QIAGEN Viral RNA Mini kit before amplification. RNA samples to be analyzed by the Abbott method were extracted from 400 μl of plasma using the QIAGEN M48 Biorobot and MagAttract Viral RNA Mini kit reagents using the virus IC1.1.1 protocol. The final elution volume of extracted RNA was 100 μl in RNase-free water. Genotyping with the Invader assay was performed as previously described (1).

Genotyping using the Abbott assay was performed as follows: three parallel real-time PCR master mixes were assembled, each containing Abbott's proprietary HCV genotyping reagents, Z05 DNA polymerase, manganese, and one of three different primer/probe cocktails. Eight microliters of the extracted RNA was added to 22 μl of each master mix. Amplification and detection were performed using an ABI 7000 real-time PCR system provided by Abbott. Data analysis was performed using software provided by the manufacturer.

In terms of complexity of use, the Bayer Trugene method was the most labor-intensive, requiring several manual pipetting steps, sequencing gel preparation, and setup of the sequencing gel towers. Both the Abbott and TWT Invader methods required significantly fewer pipetting steps and less preparation time (Table 1). In this study, the time to result for the Bayer assay was approximately 6 h (exclusive of RNA extraction), significantly longer than the 4 h previously described (5). Not including RNA extraction, the times to result of the Abbott and TWT genotyping methods were approximately 2.5 h and 1.5 h, respectively (Table 1). Throughput was highest with the Abbott TaqMan method (32 samples per run), followed by Bayer and TWT (12 and 11 patient samples, respectively) (Table 1).

The Bayer Trugene method was able to successfully genotype 136 of the 137 patient plasma specimens utilized in this study, giving a typeable rate of 99.3%. Genotypes were determined for 121 (88.3%) and 77 (56.2%) specimens using the TWT Invader and Abbott TaqMan PCR assays, respectively.

* Corresponding author. Mailing address: CompuNet Clinical Laboratories, 2308 Sandridge Rd., Dayton, OH 45439. Phone: (937) 290-7326. Fax: (937) 297-8229. E-mail: ted.e.schutzbank@questdiagnostics.com.

TABLE 1. Comparison of Abbott, Third Wave, and Bayer HCV genotyping assays

Method	Starting sample	Extraction required	Total no. of samples/run[a]	Time required for:			
				Extraction	Assay setup	Incubation	Data analysis
Abbott	Plasma	Yes	32	3.5 h[c]	1 h	1 h 20 min	15 min
Third Wave	Amplified DNA[b]	No	11	2.5 h[d]	0.5 h	0.5 h	15 min
Bayer Trugene	Amplified DNA[b]	No	12	2.5 h[d]	1.5 h	3 h 30 min[e]	1 h

[a] Including controls.
[b] Amplification products from the Roche Cobas Amplicor HCV Monitor viral load assay.
[c] For a batch of 48 samples using the QIAGEN M48 Biorobot.
[d] Time used for RNA extraction from original specimens was included.
[e] This includes the sequencing reaction plus the time to run three gel electrophoresis experiments (four samples per gel using two electrophoresis towers).

When generated, genotyping results were concordant across all three assays, with one exception: both the TWT and Abbott methods were able to detect a mixed infection with genotypes 1 and 2 (1b/2b with the Abbot assay), which was detected as type 1b by the Bayer Trugene method (Table 2). The TWT Invader method gave a "low-template" result for 15 specimens, 14 of which had HCV viral loads below 300 IU/ml, with one specimen having a viral load of 12,100 IU/ml. There is no explanation as to why there was a "low-template" result for the latter specimen. The Abbott method was unable to generate genotypes for all 17 specimens with viral loads less than 300 IU/ml: 15 results were not typeable, and 2 were reported as indeterminate. An additional 42 specimens, all with viral loads well over 1,000 IU/ml were reported to be not typeable (20 specimens) or indeterminate (22 specimens) (Table 2).

Both the Abbott and Trugene methods were able to subtype genotypes 1 and 2. Additionally, the latter method could subtype genotypes 3, 4, and 6. No subtyping results were generated using the TWT Invader methodology.

TABLE 2. Results of the Bayer Trugene, TWT Invader, and Abbott TaqMan HCV genotyping assays

Genotype result	No. of specimens		
	Bayer Trugene	Third Wave Invader	Abbot Diagnostics
1	9	94	0
1a	63	0	40
1b	30	0	16
2	0	12	0
2a	3	0	0
2b	13	0	0
3	1	7	6
3a	8	0	0
4	0	5	2
4a	4	0	0
4c	1	0	0
4i	1	0	0
6	1	2	2
6b	1	0	0
Mixed (1 and 2)	0	1	1
Low template	0	15	0
Not typeable	1	0	35
Indeterminate	0	0	24
Total[a]	136	121	77

[a] Total number of samples with a successful genotype determination.

In conclusion, both the Bayer Trugene and TWT Invader HCV genotyping methods successfully genotyped nearly all of the plasma specimens with viral loads above 300 IU/ml; genotyping was successful with the former assay for all but one specimen used in this study. Complete concordance was obtained across all three platforms when genotypes were successfully reported. Both the Bayer Trugene and TWT Invader assays resulted in the highest number of successful genotypes. Similar to the TWT Invader method, the Abbott TaqMan assay was unsuccessful at genotyping all plasma samples containing less than 300 IU/ml and failed to type an additional 42 samples, even though viral loads were in excess of 1,000 IU/ml.

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