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Evaluation of the Invader Assay for Genotyping Hepatitis C Virus

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The Invader 1.0 assay (Invader HCV Genotyping Assay, version 1.0; Third Wave Technologies, Inc., Madison, WI) has been developed for the rapid differentiation of hepatitis C virus (HCV) genotypes 1 to 6 based on sequence variation within the HCV 5′ noncoding (NC) region. In the present study, we evaluated the compatibility of Invader 1.0 with the COBAS MONITOR (COBAS AMPLICOR HCV MONITOR Test, version 2.0; Roche Molecular Systems, Inc., Branchburg, NJ), COBAS AMPLICOR (COBAS AMPLICOR Hepatitis C Virus Test, version 2.0; Roche Molecular Systems, Inc.), and COBAS TaqMan (COBAS TaqMan HCV Test; Roche Molecular Systems, Inc.) assays. The minimum HCV RNA titers required for successful HCV genotyping (≥90% success rate) were 1,000 IU/ml for COBAS MONITOR, 100 IU/ml for COBAS AMPLICOR, and 10 IU/ml for COBAS TaqMan. Invader 1.0 results obtained from unpurified COBAS TaqMan amplification products of 111 retrospectively selected clinical serum specimens (genotypes 1 to 6, with virus titers ranging from 15.1 to 2.1×10^7 IU/ml) showed 98% concordance with results obtained from the TRUGENE *HCV 5′ NC* Genotyping Kit (Bayer HealthCare LLC, Tarrytown, NY), used in conjunction with COBAS AMPLICOR. Although the assay is sensitive, accurate, and easy to perform, additional optimization of the Invader 1.0 interpretive software (Invader Data Analysis Worksheet) may be necessary to reduce potential misidentification of HCV genotypes in low-titer specimens. In summary, Invader 1.0 is compatible with a variety of commercially available PCR-based HCV 5′ NC region amplification assays and is suitable for routine HCV genotyping in clinical laboratories.

Several hepatitis C virus (HCV) genotyping assays are currently commercially available in the United States, including semiautomated DNA sequence analysis by TRUGENE (TRUGENE *HCV 5′ NC* Genotyping Kit; Bayer HealthCare LLC, Tarrytown NY), differential hybridization of HCV RNA amplification products with LiPA (VERSANT HCV Genotype Assay; Bayer HealthCare LLC), and homogeneous reverse transcription (RT)-PCR using the Abbott HCV Genotyping ASR assay (Abbott Molecular Diagnostics, Abbott Park, IL). TRUGENE and LiPA each require approximately 4 h to complete following RT-PCR amplification (8), while the Abbott HCV Genotyping ASR assay requires a separate RT-PCR amplification procedure that can take up to 5 h to complete, according to the manufacturer. Furthermore, both TRUGENE and LiPA are currently incompatible with both the recently introduced COBAS TaqMan (COBAS TaqMan HCV Test; Roche Molecular Systems, Inc., Branchburg, NJ) and TaqMan HCV Analyte Specific Reagent (Roche Molecular Systems, Inc.) assays.

The Invader HCV Genotyping Assay (Third Wave Technologies, Inc., Madison, WI) is an alternative test method recently made available for the rapid differentiation of HCV genotypes 1 to 6 based on sequence variation within the HCV 5′ noncoding (NC) region. This assay, currently available as Invader 1.0 (Invader HCV Genotyping Assay, version 1.0) for research use only (RUO) or as Invader HCV_G Analyte Specific Reagents (ASR), can be performed directly on HCV 5′ NC region amplification products from a variety of commercially available RT-PCR assays without purification. Invader 1.0 utilizes Cleavase technology (Third Wave Technologies, Inc.) and fluorescence resonance energy transfer (FRET) technology combined with automated, computerized data analysis to provide test results in about 1 h (5).

In this study, we have determined the HCV RNA titer thresholds, in IU/ml, necessary for successful HCV genotyping by Invader 1.0 using amplification products generated by three commercially available HCV RNA amplification assays: COBAS MONITOR (COBAS AMPLICOR HCV MONITOR Test, version 2.0; Roche Molecular Systems, Inc.), COBAS AMPLICOR (COBAS AMPLICOR Hepatitis C Virus Test, version 2.0; Roche Molecular Systems, Inc.), and COBAS TaqMan. We also examined the concordance of HCV genotype results generated by Invader 1.0 and TRUGENE among retrospectively collected clinical specimens submitted to our diagnostic laboratory for HCV genotype determination by TRUGENE.

MATERIALS AND METHODS

HCV analytical standards. A commercially available standard containing HCV genotype 1 at a concentration of 200,000 IU/ml (OptiQuant HCV RNA; AcroMetrix Corp., Benicia, CA) was used along with NAT Dilution Matrix (AcroMetrix Corp.) to prepare HCV dilutions for use in this evaluation. Dilutions of 5,000, 1,000, 500, 100, 50, and 10 IU/ml were prepared along with HCV RNA-negative (0 IU/ml) control replicates. All dilutions were prepared and stored at −70°C prior to RT-PCR amplification by COBAS MONITOR (5,000, 1,000, and 0 IU/ml), COBAS AMPLICOR (5,000, 1,000, 500, 100, and 0 IU/ml), and COBAS TaqMan (5,000, 1,000, 500, 100, 50, 10, and 0 IU/ml). The concentrations of HCV RNA selected for testing by these three assays were based on the lower limits of quantification for COBAS MONITOR (600 IU/ml) (6) and

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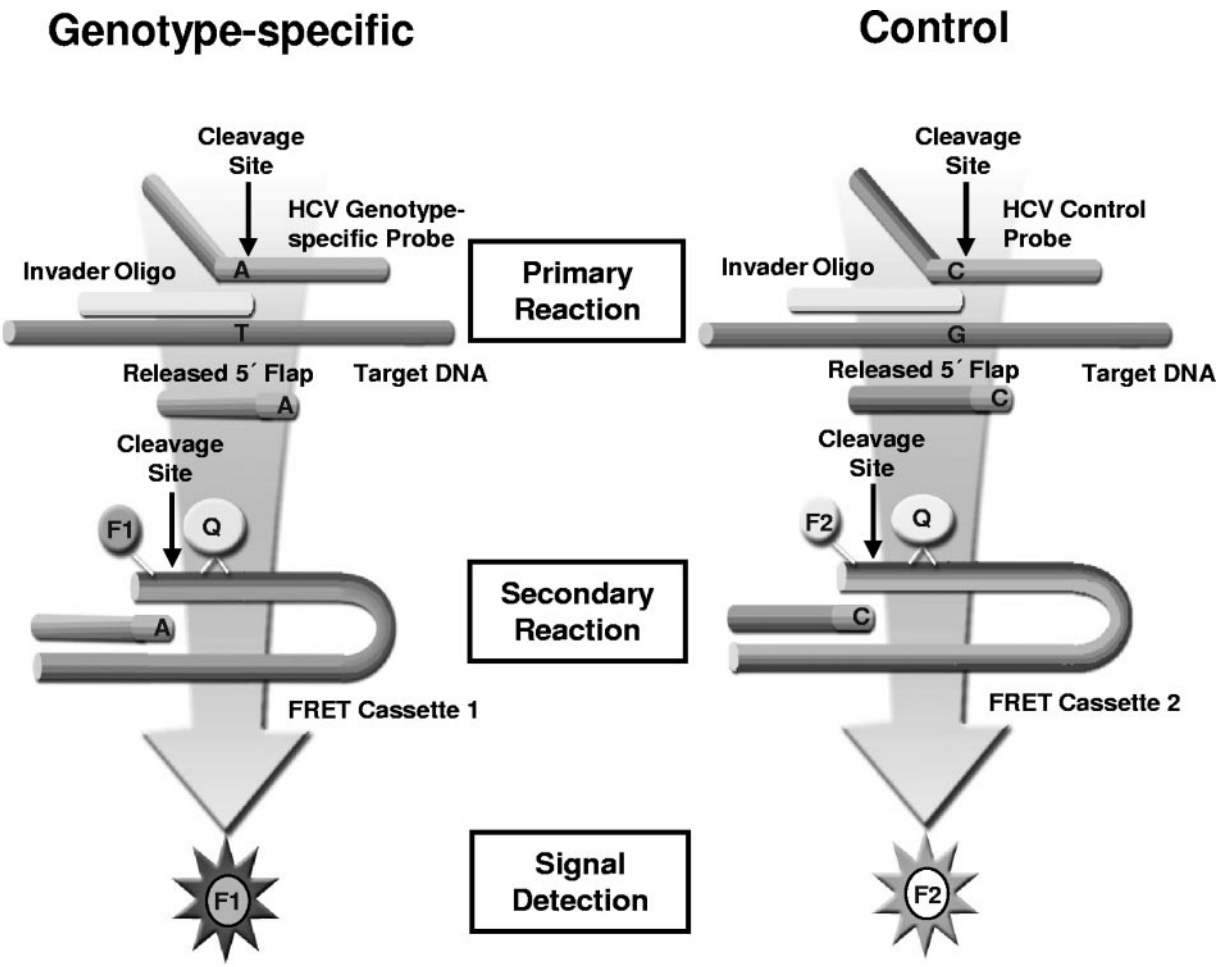

FIG. 1. Principles of Invader 1.0.

COBAS TaqMan (10 IU/ml) (3) and the limit of detection for COBAS AMPLICOR (50 IU/ml) (6). Appropriate dilutions were tested in duplicate by all three assays daily for a total of five days.

Clinical specimens. A total of 111 clinical serum specimens submitted to our diagnostic laboratory for routine HCV genotyping between June 2001 and September 2004 were retrospectively selected for inclusion in this study. The specimens consisted of a genetically diverse group of HCV strains (including genotypes 1 to 6 with multiple subtypes) based on the genotyping results previously generated by TRUGENE. No additional patient information was available for specimen selection.

All specimens had been previously subjected to RT-PCR amplification by COBAS AMPLICOR according to the manufacturer's instructions. The amplification products were then purified using the QIAquick PCR Purification Kit (QIAGEN Inc., Valencia, CA) prior to analysis by TRUGENE, performed according to the manufacturer's instructions. All retrospectively selected specimens were stored at −70°C prior to RT-PCR amplification by COBAS TaqMan and subsequent analysis by Invader 1.0.

This study was reviewed and approved by the Mayo Foundation Institutional Review Board.

HCV RNA amplification. COBAS MONITOR and COBAS AMPLICOR were performed according to the manufacturer's instructions. Denatured reaction products (A rings) were stored at 4°C for up to five days or at −20°C for up to 16 weeks prior to analysis by Invader 1.0. The unpurified, denatured reactions were diluted 1:20 and 1:100 in nuclease-free water prior to analysis by Invader 1.0 according to the manufacturer's recommendations.

COBAS TaqMan sample preparation and testing were performed with the MagNA Pure LC and COBAS TaqMan 48 Analyzer (Roche Molecular Systems, Inc.) as previously described (3). Amplification reaction products were removed from the COBAS TaqMan 48 Analyzer immediately following completion of the amplification/detection process and were frozen at −20°C and held for up to 16 weeks prior to analysis by Invader 1.0. Unpurified amplification reaction products were diluted 1:20 and 1:500 in nuclease-free water prior to analysis by Invader 1.0 according to the manufacturer's recommendations.

Invader 1.0. The Invader 1.0 assay utilizes a series of eight individual HCV genotype-specific assays (A to H) and is performed in microtiter plate format. Each individual assay contains a genotype-specific and a control component, each with a corresponding primary and secondary reaction, as shown in Fig. 1. This biplex assay format generates unique nucleic acid structures that are recognized by a proprietary Cleavase enzyme during the primary reaction, resulting in the formation of two target-specific cleavage products (5′ flaps). This design enables the detection of two targets (HCV genotype-specific and control targets) in a single well by using two different discriminatory HCV probes (each generating a unique 5′ flap) and two different FRET cassettes (each with a spectrally distinct fluorophore). Use of the microtiter format permits testing of up to 11 samples and a negative control per 96-well plate. Following incubation of diluted amplification products at 98°C for 10 min, addition of reaction mixtures, and incubation at 63°C for 30 to 60 min, fluorescent signals can be detected with a standard laboratory fluorescence plate reader.

Automated data analysis and HCV genotype determination are performed using a Microsoft Excel-based HCV Genotyping Invader Data Analysis Worksheet (IDAW) developed and provided by Third Wave Technologies, Inc., for research use only. Initially, each individual assay (A to H) control signal is evaluated to determine if the associated genotype-specific assay is valid (i.e., proper setup, adequate signal strength). Each valid assay is categorized further as either positive or negative by assay-specific algorithms established by the assay manufacturer and based on the fluorescence signal ratios generated in the individual assays. A genotype code is then generated from the genotype-specific reactivity pattern among the eight individual assays (A to H). An "unable-to-call"

TABLE 1. Analytical sensitivity of Invader 1.0 with amplification products obtained from various commercially available HCV RNA detection/quantification assays

HCV RNA concn (IU/ml)	No. of replicates tested (each assay)	% Replicates successfully genotyped (95% CI)[a]		
		COBAS MONITOR[b]	COBAS AMPLICOR[c]	COBAS TaqMan[d]
5,000	10	100 (69–100)	100 (69–100)	100 (69–100)
1,000	10	100 (69–100)	100 (69–100)	100 (69–100)
500	10	ND	100 (69–100)	100 (69–100)
100	10	ND	100 (69–100)	100 (69–100)
50	10	ND	ND	100 (69–100)
10	10	ND	ND	100 (69–100)
0	10	0 (0–31)	0 (0–31)	0 (0–31)

[a] CI, confidence interval; ND, not done.
[b] Amplification products diluted 1:20.
[c] Amplification products diluted 1:100.
[d] Amplification products diluted 1:500.

TABLE 2. HCV genotype concordance between Invader 1.0 and TRUGENE

Invader 1.0 genotype	*n*	TRUGENE genotype and subtype[a]																		
		1NS	1a	1b	2a	2b	3NS	3a	3b	3d	3h	4NS	4a	4b	4c	4i	5a	6NS	6a
1	24	3	14	7															1[d]
2	20				3	17[b]													
3	25						1	18	1	1	3								
4	21								1[c]			3	7	1	4	6			
5	4																4		
6	17																	1	15

[a] NS, no subtype assigned.
[b] 1:500 and 1:20 dilutions of a single specimen yielded coinfection by genotypes 1 and 2, according to Invader 1.0.
[c] A 1:20 dilution yielded genotype 4 by Invader 1.0 following a 30-min incubation but genotype 3 following an additional 30-min incubation.
[d] A 1:500 dilution yielded genotype 1 by Invader 1.0, but a 1:20 dilution yielded genotype 6.

result is generated if the genotype code is unrecognizable, while "invalid" or "low-template" results are generated if from one to three or more than three individual assays yield low signal strength, respectively. In the event of an invalid or low-template result following the initial 30-min incubation at 63°C, the Invader reaction plate can be incubated for an additional 30 min (60 min total) and fluorescent signals can be read again. If the invalid or low-template result persists following a 60-min incubation at 63°C, testing of a second concentration (i.e., 1:20) of amplification products may be required to generate a valid genotype result.

In this study, diluted amplification products (1:100 and 1:20) of HCV standards generated by COBAS MONITOR and COBAS AMPLICOR were tested subsequently by Invader 1.0, and fluorescence signals were read after 30- and 60-min incubations. Diluted amplification products (1:500 and 1:20) generated by COBAS TaqMan were tested and read also after 30- and 60-min incubations. Invader 1.0 reaction mixtures were incubated on a GeneAmp PCR System 9700 Thermal Cycler (Applied Biosystems, Foster City, CA), while fluorescence signals were detected with a fluorescence plate reader (GENios FL; Tecan Systems, Inc., San Jose, CA). Data analysis and interpretation were performed with HCV Genotyping IDAW, version 101804 (Third Wave Technologies, Inc.), which contains a total of 46 reportable HCV genotype codes, including 27 mixed-genotype codes.

Other HCV genotyping assays. LiPA and the TRUGENE *HCV NS5B* Genotyping Assay (Bayer HealthCare LLC) were performed according to protocols at the Bayer Reference Testing Laboratory, Berkeley, CA.

HCV 5′ NC region cloning and sequencing. COBAS TaqMan or COBAS AMPLICOR amplification products from clinical specimens yielding mixed HCV genotypes by Invader 1.0 or discordant results unresolved by LiPA testing were subjected to nested PCR amplification for cloning purposes by a previously established method (4). This approach was undertaken to generate non-dUTP-containing sequences necessary for cloning. Cloning was performed with the TOPO TA Cloning Kit (Invitrogen Corp., Carlsbad, CA) according to the manufacturer's instructions. Individual clones were screened by PCR amplification of HCV 5′ NC region sequences using the nested PCR primers described above followed by Invader 1.0 testing and direct sequencing (Mayo Sequencing Core Facility, Rochester, Minnesota) when appropriate.

RESULTS

The findings of our analytical study confirm the compatibility of Invader 1.0 with amplification products generated by COBAS MONITOR, COBAS AMPLICOR, and COBAS TaqMan. The minimum analytical threshold titers required for reliable performance (≥90% success rate) of Invader 1.0 using amplification products generated from HCV standards by COBAS MONITOR, COBAS AMPLICOR, and COBAS TaqMan were determined to be 1,000, 100, and 10 IU/ml, respectively (Table 1). These thresholds were based on the results obtained by incubating the 1:20 (COBAS MONITOR), 1:100 (COBAS AMPLICOR), and 1:500 (COBAS TaqMan) dilutions of unpurified amplification products for 60 min.

All HCV standards subjected to amplification by COBAS AMPLICOR and COBAS TaqMan were successfully genotyped by Invader 1.0 following a 30-min incubation period using 1:100 and 1:500 dilutions of amplification products, respectively. Identical results were observed with the same dilutions of amplification products after incubation for an additional 30 min and at a dilution of 1:20 following 30- and 60-min incubations. COBAS MONITOR amplification products diluted 1:100 yielded valid Invader 1.0 genotype results from just 20% (2 of 10) of the 1,000-IU/ml dilutions after 30 min and from 90% (9 of 10) after a 60-min incubation. COBAS MONITOR amplification products diluted 1:20 yielded valid Invader 1.0 genotype results in 90% and 100% of the 1,000-IU/ml dilutions after 30- and 60-min incubations, respectively. All HCV RNA-negative (0 IU/ml) replicates tested by the three RT-PCR assays yielded low-template results at both high and low dilutions of amplification products and at both 30- and 60-min incubation time points, indicating the absence of nonspecific Invader 1.0 reactivity.

The HCV RNA titers among the 111 clinical serum specimens, as determined by COBAS TaqMan, ranged from 15.1 to 2.1×10^7 IU/ml, with a median titer of 1.1×10^6 IU/ml. Invader 1.0 results obtained from these unpurified COBAS TaqMan amplification products were directly compared to the results obtained by TRUGENE. While HCV subtype determinations by TRUGENE could not be directly evaluated with Invader 1.0, the analysis of a diverse group of HCV strains by Invader 1.0 yielded good agreement (genotype without subtype) with TRUGENE among all genotypes and subtypes included in this study. There was 98% (109 of 111) concordance of genotype results (without subtype) among these specimens, with two discordant results (Table 2). The discordant results were associated with specimens containing HCV genotypes 3b and 6a, as determined by TRUGENE. Of note is a mixed-genotype infection (1 and 2) detected by Invader 1.0 in one of the 109 specimens with concordant results. This specimen was determined previously to contain only genotype 2b by TRUGENE.

For the discordant specimen containing genotype 3b, initial Invader 1.0 testing of the 1:500 dilution yielded invalid and unable-to-call results following 30- and 60-min incubations, respectively. Additional Invader 1.0 testing, performed in duplicate from the original amplification products from this specimen diluted 1:20, yielded genotype 3 and 4 assignments following a 30-min incubation and genotype 3 assignments (both replicates) after a 60-min incubation. Examination of the Invader 1.0 fluorescence data for assay F, which is critical for differentiation of genotypes 3 and 4, revealed that the genotype 4 assignment at 30 min was the direct result of a low signal ratio (0.290) in this particular assay, compared to the signal ratio (0.325) of the other replicate with a genotype 3 assignment and to the ratio threshold (0.300) required for a positive assay F result. Following a 60-min incubation, the assay F signal ratio for the replicate previously identified as genotype 4 (30-min incubation) was 0.516 (i.e., above the threshold of 0.300), thus resulting in a genotype 3 assignment. Repeat TRUGENE testing was not performed with this specimen due to the extremely low HCV RNA titer (15.1 IU/ml) and the low likelihood of obtaining a second interpretable result from this assay. Due to the limited quantity and low viral titer of this specimen, supplemental LiPA testing was also not performed. However, HCV NS5B sequencing by the TRUGENE *HCV NS5B* Genotyping Assay was attempted with this specimen but failed to generate a result. COBAS TaqMan amplification products from this specimen were also subjected to nested PCR, and testing of these nested PCR amplification products by Invader 1.0 yielded a genotype 3 assignment following a 30-min incubation. Invader 1.0 testing of 22 individual clones derived from this nested PCR also yielded genotype 3 assignments in all but one clone, which consistently yielded a genotype 4 assignment. While the HCV 5′ NC region sequence of this clone was consistent with that of genotype 3b, further examination of the sequence indicated that two unexpected single-nucleotide polymorphisms in the assay F target region (with respect to the Invader 1.0 probe sequences) may have led to the erroneous genotype 4 assignment (Scott Law, personal communication). The presence of this novel sequence in the COBAS TaqMan amplification products of this clinical specimen may have contributed to the inconsistent results observed initially with Invader 1.0.

For the discordant specimen containing genotype 6a, initial Invader 1.0 testing of a 1:500 dilution of amplification products yielded a genotype 1 assignment following 30- and 60-min incubations. Additional Invader 1.0 testing was performed in duplicate with both 1:500 and 1:20 dilutions of the original COBAS TaqMan amplification products. The 1:20 dilution was tested despite the fact that COBAS TaqMan yielded a relatively high HCV RNA titer of 3,690,000 IU/ml in this specimen. Both replicates of the 1:500 dilution yielded genotype 1 assignments following 30- and 60-min incubations, while the 1:20 dilution replicates consistently yielded genotype 6 assignments. Examination of the Invader 1.0 fluorescence data from assay H following both 30- and 60-min incubations of the 1:500 dilution replicates revealed signal ratios (0.114 to 0.491) that were well above background (0.003 to 0.013) but still below the assay H threshold ratio (1.00) necessary for a genotype 6 assignment (instead of genotype 1). Repeat testing of this specimen by TRUGENE consistently yielded a genotype 6a assignment. While LiPA was unable to provide a genotype assignment with this discordant specimen, the TRUGENE result was confirmed by the TRUGENE *HCV NS5B* Genotyping Assay (data not shown).

For the specimen initially yielding mixed genotypes 1 and 2 by Invader 1.0, additional Invader 1.0 testing of duplicate 1:500 and 1:20 dilutions of the COBAS TaqMan amplification products confirmed the original mixed-genotype result, while LiPA detected only genotype 2 in this specimen. Invader 1.0 testing of the COBAS AMPLICOR amplification products from this specimen performed in duplicate at a dilution of 1:20 yielded genotype 2 assignments following 30-min incubations but yielded uninterpretable results with increased signal strength in assays A and B (characteristic of genotype 1) detected after a 60-min incubation. Thus, the Invader 1.0 results obtained from COBAS TaqMan and COBAS AMPLICOR amplification products both yielded results suggestive of mixed genotypes 1 and 2 in this specimen. However, the presence of HCV genotype 1 in this specimen could not be demonstrated conclusively despite the screening of 62 individual clones derived from nested PCR amplification products (COBAS AMPLICOR) with Invader 1.0.

Performance of Invader 1.0 was further evaluated by reviewing our clinical study data to determine our test failure rate. The failure rate, defined as the frequency of invalid and unable-to-call Invader 1.0 results, was $<2\%$ over the 11 runs required to complete testing of the 111 clinical specimens. One failure involved an invalid test result presumably due to inadequate mixing of Invader 1.0 reaction components. Setting up Invader 1.0 involves the pipetting of small volumes of reaction mixture (10 μl) and diluted amplification products (10 μl) using a multichannel pipette. Based on our initial experience with Invader 1.0, failure to deliver and adequately mix these reaction components can result in an invalid result due to a lack of HCV internal control and/or genotype-specific reactivity in one or more of the individual reactions (A to H). In this case, repeat testing of a 1:500 dilution of amplification product resulted in a valid Invader 1.0 result. A second failure involved an unable-to-call result associated with a very low titer specimen (15.1 IU/ml). In this case, testing at a 1:20 dilution was required to generate a valid Invader 1.0 result.

The total duration of the entire Invader 1.0 test performed with a full 96-well plate (11 samples plus a negative control) and a 30-min incubation was approximately 71 min, including 35 min of actual hands-on time. The time to completion for two staggered runs (22 samples plus negative controls in two plates), each with a 30-min incubation, was approximately 121 min.

DISCUSSION

Clinical laboratories will likely continue to face increasing HCV test volumes, according to projected increases in the diagnosis of chronic HCV infection in the United States over the next several decades (1). As a result, clinical laboratories must continue to rapidly adopt new technologies capable of improving HCV test performance and efficiency. In addition to sensitive detection and accurate quantification of HCV RNA, HCV genotype determination will also likely continue to play an important role in anti-HCV treatment algorithms. The In-

vader assay can be performed with pre-existing HCV 5′ NC region amplification products from qualitative or quantitative RT-PCR testing, allowing clinical laboratories to efficiently perform the assay as a reflex test or upon physician request following the initial amplification of HCV RNA.

The assay is performed most efficiently in a full 96-well plate containing 11 specimens and a negative control. In our experience, a single plate could be processed from start to finish in just over 1 h. Alternatively, two plates (each containing 11 samples and a negative control) can be efficiently processed in staggered fashion using a single thermal cycler and fluorescence plate reader. It may also be advantageous to use multiple thermal cyclers in clinical laboratories with high specimen throughput demands. In the event that additional incubation time and/or testing of additional dilutions is required, the test turnaround time may be increased. However, our analysis of 111 clinical specimens required an additional 30-min incubation (60 min total) in only two instances, with just one of these reactions requiring the testing of an additional dilution.

While our initial experience with Invader 1.0 suggested that improper assay setup could result in a significant increase in the failure rate, we experienced no significant problems performing Invader 1.0 during the course of this study, and our failure rate among clinical specimens was quite low (<2%). Although our analytical studies did not evaluate all HCV genotypes, our data from analytical standards containing HCV genotype 1 suggest that Invader 1.0 is compatible with COBAS MONITOR, COBAS AMPLICOR, and COBAS TaqMan amplification products and is capable of successfully producing HCV genotype determinations from most, if not all, specimens yielding HCV RNA amplification products with these assays. These data suggest that Invader 1.0 has the potential to significantly reduce the failure rates associated with HCV genotyping of low-titer specimens by other commercially available genotyping methods (8; N. Wylie, S. Burrows, A. Perlina, S. Magdalani, B. Boyadzhyan, and T. Yashina, Abstr. 20th Annu. Clin. Virol. Symp., abstr. S29, 2004).

Compatibility of Invader 1.0 with COBAS TaqMan is of particular importance to clinical laboratories, since COBAS TaqMan is incompatible with both LiPA (due to the lack of biotinylated primers with COBAS TaqMan) and the current version of TRUGENE (due to a COBAS TaqMan primer shift relative to TRUGENE primers). As a result of these incompatibilities, there is currently a lack of commercially available HCV genotyping assays, other than the Invader assay, that are compatible with COBAS TaqMan. Our findings, generated with COBAS TaqMan (an RUO test kit) suggest that Invader 1.0 would also be suitable for use with amplification products generated with the TaqMan HCV Analyte Specific Reagent.

While inconsistent Invader 1.0 results were not evident during testing of low-titer analytical standards (all genotype 1), testing of COBAS TaqMan amplification products derived from clinical specimens did reveal such discordances. While two clinical specimens yielded inconsistent Invader 1.0 results (genotype 3 versus 4 and genotype 1 versus 6) with repeat testing, it is important to note that the discordance with TRUGENE results was resolved when Invader 1.0 testing was performed with either a longer incubation time (60 min) or a higher concentration of amplification products (1:20 dilution). Detailed review of the Invader 1.0 raw data from these clinical specimens suggests that the data analysis criteria for assays F and H (IDAW, version 101804) were likely suboptimal. Our findings suggest that optimization of the Invader 1.0 interpretive software may require adjustment of assay-specific thresholds or the implementation of "equivocal ranges" for the signal ratios of assays F and H in order to reduce the potential for incorrectly genotyping HCV present in low-titer clinical specimens. Despite these deficiencies in the current Invader 1.0 interpretive software (IDAW, version 101804), our data indicate that Invader 1.0 performed well overall with HCV genotypes 1 to 6 and is suitable for routine clinical HCV genotype determination despite an inability to subtype HCV strains (2, 7, 9).

The potential for Invader 1.0 to efficiently detect mixed HCV genotype infections was not addressed specifically in our study. However, Invader 1.0 did detect a mixture of genotypes 1 and 2 in a single clinical specimen found to contain only genotype 2 by both TRUGENE and LiPA. Both TRUGENE and LiPA have been reported to be capable of detecting mixed HCV genotypes in clinical specimens containing minor genotype populations of ≥10% and ≥5%, respectively (8). While experimental data available from the assay manufacturer indicate that Invader 1.0 can detect mixed HCV genotypes in simulated specimens containing major and minor genotype populations at a ≥20:1 ratio (Slava Elagin, personal communication), the mixed genotype result observed with Invader 1.0 in our study could not be confirmed by subsequent cloning and analysis of >50 individual clones. The potential of Invader 1.0 to detect mixed genotypes will require further investigation with clinical specimens known to contain mixed HCV genotypes.

In summary, Invader 1.0 can be performed in conjunction with a variety of commercially available RT-PCR assays targeting the HCV 5′ NC region, and it is sensitive, accurate, and easy to perform. Although not designed for discriminating HCV subtypes, the assay is suitable for routine HCV genotyping in clinical diagnostic laboratories based on our current understanding of the influence of HCV genotype on clinical outcome with current anti-HCV therapies.

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