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Performance Characteristics of a Transcription-Mediated Nucleic Acid Amplification Assay for Qualitative Detection of Hepatitis C Virus RNA

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The detection of hepatitis C virus (HCV) RNA by nucleic acid amplification techniques is the method of choice to differentiate between ongoing and past infection, and can be used to monitor the course of HCV infection. In this study, we evaluated the performance characteristics of a newly developed transcription-mediated amplification (TMA)-based assay, the VERSANT® HCV RNA qualitative assay, which was designed to qualitatively detect HCV RNA. Samples tested by the TMA assay included 100 HCV antibody negative sera; serial dilutions of an HCV genotype 1a panel; the WHO HCV RNA standard 76/790; an HCV genotyping panel; and 150 clinical specimens, including sera from patients who had received α interferon (IFN) treatment or liver transplants. TMA test results were compared with the Cobas Amplicor® HCV polymerase chain reaction (PCR) assay. The analytical specificity of the HCV TMA assay was > 98%. No carry-over contaminations were observed. The assay demonstrated an analytical sensitivity of 100% at 41 HCV RNA copies/mL (genotype 1a panel) and 5 IU/mL (WHO standard), respectively. HCV genotypes and subtypes did not affect the results. Qualitative RNA detection by diagnostic Amplicor® PCR and TMA was in agreement in > 97% of all 150 clinical samples tested. In our study, the TMA-based assay proved to be a specific and sensitive method for qualitative HCV RNA detection. The test may turn out to be an attractive alternative to already established techniques for HCV RNA amplification in routine clinical laboratories. J. Clin. Lab. Anal. 15:308–313, 2001.

Key words: hepatitis C virus; transcription-mediated amplification; genotypes; new technology

INTRODUCTION

Routine virological diagnosis of hepatitis C virus (HCV) infection is currently based on the detection of antibodies to HCV by serological tests. However, these techniques are not very sensitive in the early stages of HCV infection, and cannot differentiate between ongoing and resolved infection. Furthermore, in patients who are on hemodialysis or are immunocompromised, the development of antibodies to HCV may be delayed or even absent. Therefore, the direct molecular qualitative detection of HCV RNA is considered the gold standard for the diagnosis of HCV infection and for assessing the response to antiviral therapy (1–3). Since in the vast majority of laboratories HCV RNA is detected through reverse-transcription polymerase chain reaction (RT-PCR) by in-house assays or widely distributed automated systems (4–8), other nucleic acid amplification techniques have not yet gained a comparable importance in routine diagnosis of HCV infection. Among the methodologies that may have more widespread practical application is transcription-mediated amplification (TMA). As it is similar to some aspects of NASBA® technology, the amplification portion of this new assay has been shown to be useful in the field of HCV research (7,9–11). In the current study, we report on the performance characteristics of a newly developed TMA-based assay that has been validated prior to commercialization. This assay is designed for specific, sensitive, and rapid HCV RNA detection in a routine diagnostic laboratory.

PATIENTS AND METHODS

TMA Assay

TMA is an isothermal nucleic acid amplification technique that evolved from RNA transcription-based amplification, first described in 1989 (12). The VERSANT® HCV RNA qualitative assay (Bayer Diagnostics, Emeryville, CA) relies on reverse transcriptase and T 7 RNA polymerase to generate

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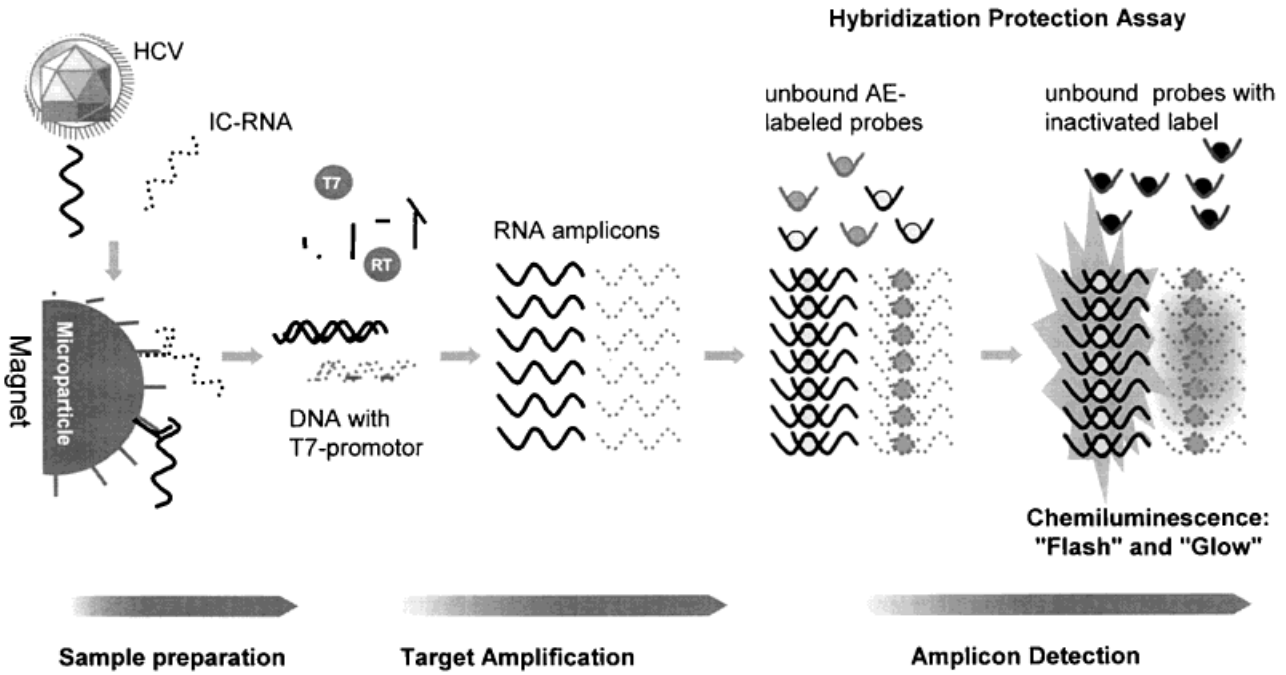

Fig. 1. Qualitative detection of HCV RNA by transcription-mediated amplification (VERSANT® HCV qualitative assay, reproduction with permission of Bayer Diagnostics). IC RNA = internal HCV RNA control; RT = reverse transcriptase; T 7 = T 7 RNA polymerase; AE = acridinium ester; "flash" signal = rapid emission of light produced by the chemiluminescent tag of the internal control RNA; "glow" signal = relatively slower light emission by the probes that are specific to the target amplicon.

multiple RNA copies of an HCV RNA template. As depicted in Fig. 1, after lysing the specimens with detergent, the free HCV RNA is hybridized in solution with capture oligonucleotides complementary to the highly conserved 5′ noncoding region (5′NCR) of the HCV genome. The hybridized targets are then captured on magnetic microparticles and are separated from the remaining sample components by a magnet. Primers, nucleotides, reverse transcriptase, and T 7 polymerase are then added to amplify the 5′NCR of the captured HCV RNA. Finally, the resulting amplicons are detected by a hybridization protection assay using single-stranded, chemiluminescent probes that are complementary to the amplicons. All steps, including sample lysis and extraction, are carried out in a single tube. Assay performance from target RNA isolation to amplification and amplicon detection is monitored by adding an internal control RNA at the beginning of the assay (13).

Assessment of Analytical Specificity

Sera from 100 healthy individuals with no history of previous hepatitis infections were tested by the VERSANT® HCV RNA qualitative assay four times in independent runs. The sera were negative for antibodies to HCV in a third-generation enzyme-linked immunosorbent assay (ELISA) (Sanofi Pasteur, Freiburg, Germany). To check for possible carry-over contaminations, 20 samples with very high HCV RNA titers (1.1×10^7 to 1.1×10^8 HCV RNA copies/mL) in Quantiplex® 2.0 bDNA assay (Bayer Diagnostics) were tested two times in parallel with HCV RNA-negative specimens.

Assessment of Analytical Sensitivity

The analytical sensitivity of the VERSANT® HCV qualitative assay was assessed by serial dilutions of an HCV genotype 1a panel consisting of six specimens. The number of HCV RNA copies (8–164/mL) in these samples was determined by Quantiplex® bDNA test that had been calibrated against RNA transcripts, as described previously (14–16). The specimens of the dilution panel were assayed by HCV TMA six times each in four independent runs. Additionally, six serial dilutions (1–100 IU/mL) of the WHO HCV RNA standard 96/790 (17) were tested in duplicate in two separate runs. The possible influence of different HCV genotypes and subtypes on the analytical sensitivity of the VERSANT® HCV qualitative assay was evaluated by means of a well-characterized HCV genotyping panel (18). The samples of this panel were quantified by the HCV bDNA 2.0 assay [which is not affected by the HCV genotype (15,16,19)], diluted to 200, 100, 50, and 25 HCV RNA copies/mL in HCV RNA-negative serum, respectively, and tested in duplicate in two separate runs by HCV TMA.

Comparison of Analytical Procedures

The VERSANT® HCV qualitative assay was compared with the Cobas Amplicor® HCV 2.0 assay (Roche Diagnostics,

Mannheim, Germany), an integrated PCR system widely used for qualitative HCV RNA detection in diagnostic laboratories (4,5). A total of 150 patient specimens were tested with both systems. These samples consisted of 75 randomly chosen frozen sera drawn from 38 men and 37 women (mean age: 48 years, range: 18–79 years). Antibodies to HCV could be detected in 45 of these sera. Two of the patients were suffering from chronic HBV infection, and six showed serologic signs of past HBV infection. None were positive for antibodies to HIV. Additionally, 58 serial samples from 10 chronically HCV-infected patients treated with α interferon (IFN) were tested by both the VERSANT® HCV qualitative assay and the Cobas Amplicor®. The specimens were derived before, during, and after therapy from six men and four women (mean age: 44 years, range: 11–67 years) infected with HCV subtypes 1b (six patients), 3a (three patients), and 2b (one patient), respectively. Finally, 17 serial sera from five male patients (mean age: 53 years, range: 42–59 years) before and after orthotopic liver transplantation due to end-stage chronic HCV infection were included in the analysis. All specimens that showed discrepant results by the VERSANT® HCV qualitative assay and the Cobas Amplicor® 2.0 assay were retested with nested in-house RT-PCR, as described previously (20).

RESULTS

Analytical Specificity

Testing sera from 100 healthy individuals in four independent runs revealed an analytical specificity for the HCV TMA assay of 98%, 99%, 99%, and 99%, respectively. One sample was positive in all four runs. HCV RNA could also be detected in this serum by both the Cobas Amplicor® 2.0 assay and nested in-house RT-PCR. Genotyping identified an HCV 1a isolate. Further evaluation of the donor showed that he was suffering from acute HCV infection and had developed antibodies to HCV a few weeks after the sample was taken from him in the window phase. Accepting this sample as truly positive for HCV RNA, the analytical specificity of the four runs would be 99%, 100%, 100%, and 100%, respectively. No carry-over contaminations were observed when assaying the 20 high-titer samples together with HCV RNA-negative sera in the same run.

Analytical Sensitivity

As shown in Table 1, all six replicates of the HCV genotype 1a dilution panel containing 41 RNA copies/mL were invariably positive by the VERSANT® HCV qualitative assay. Sometimes, ever lower HCV RNA copy numbers could be detected. Using serial dilutions of the first international HCV RNA standard, a detection limit of 5 IU/mL was established. Differences in HCV genotypes did not influence the analytical sensitivity of the VERSANT® HCV qualitative assay, since approximately 50 HCV RNA copies/mL were always detectable regardless of the genotype or subtype (Table 2).

Comparison of Analytical Procedures

The overall concordance between the test results obtained by the VERSANT® HCV qualitative assay and Cobas Amplicor® 2.0 assay was >97% (Fig. 2). Four samples that were taken from chronically HCV-infected patients after initiation of α-IFN treatment tested negative by Amplicor®, but were positive for HCV RNA in the VERSANT® HCV quali-

TABLE 1. Analytical sensitivity of the VERSANT® HCV qualitative assay

	HCV genotype 1a dilution panel							
	Run 1		Run 2		Run 3		Run 4	
Copies/mL	Pos. (*n*)	Neg. (*n*)	Pos. (*n*)	Neg. (*n*)	Pos. (*n*)	Neg. (*n*)	Pos. (*n*)	Neg. (*n*)
164	6	0	6	0	6	0	6	0
82	6	0	6	0	6	0	6	0
61	6	0	6	0	6	0	6	0
41	**6**	**0**	**6**	**0**	**6**	**0**	**6**	**0**
20	4	2	6	0	5	1	6	0
8	4	2	4	2	1	5	1	5
	WHO HCV RNA standard 96/790							
	Run 1		Run 2		Run 3		Run 4	
IU/mL	Pos. (*n*)	Neg. (*n*)	Pos. (*n*)	Neg. (*n*)	Pos. (*n*)	Neg. (*n*)	Pos. (*n*)	Neg. (*n*)
100	2	0	2	0	2	0	2	0
10	2	0	2	0	2	0	2	0
7.5	2	0	2	0	2	0	2	0
5	**2**	**0**	**2**	**0**	**2**	**0**	**2**	**0**
2.5	0	2	0	2	0	2	0	2
1	0	2	0	2	0	2	0	2

Serial dilutions of an HCV genotype 1a panel and the WHO HCV RNA Standard 96/790 (17) were repeatedly tested in four separate runs. Lines in bold indicate the minimal number of HCV RNA copies/mL or IU/mL, respectively, that could be detected with a sensitivity of 100%.

TABLE 2. Analytical sensitivity of the VERSANT® HCV qualitative assay with regard to different HCV genotypes and subtypes

	HCV genotyping panel		
Genotype	Copies/mL	Run 1	Run 2
1a	250	Pos.	Pos.
	100	Pos.	Pos.
	50	Pos.	Pos.
	25	**Pos.**	**Pos.**
1b	250	Pos.	Pos.
	100	Pos.	Pos.
	50	Pos.	Pos.
	25	**Pos.**	**Pos.**
2a	250	Pos.	Pos.
	100	Pos.	Pos.
	50	**Pos.**	**Pos.**
	25	Neg.	Neg.
2b	250	Pos.	Pos.
	100	Pos.	Pos.
	50	**Pos.**	**Pos.**
	25	Neg.	Neg.
2c	250	Pos.	Pos.
	100	Pos.	Pos.
	50	**Pos.**	**Pos.**
	25	Pos.	Neg.
3a	250	Pos.	Pos.
	100	Pos.	Pos.
	50	Pos.	Pos.
	25	**Pos.**	**Pos.**
4	250	Pos.	Pos.
	100	Pos.	Pos.
	50	Pos.	Pos.
	25	**Pos.**	**Pos.**
5	250	Pos.	Pos.
	100	Pos.	Pos.
	50	Pos.	Pos.
	25	**Pos.**	**Pos.**

Serial dilutions of an HCV genotyping panel (18) were tested in two separate runs. Lines in bold indicate the minimal number of HCV RNA copies/mL that could be detected with a sensitivity of 100%.

tative assay (Fig. 3). These results were confirmed by retesting the sera with the sensitive nested in-house RT-PCR. In the 17 samples obtained from patients before and after orthotopic liver transplantation due to end-stage chronic HCV infection, the VERSANT® HCV qualitative assay and Amplicor® results were in complete concordance.

DISCUSSION

To qualify for routine use in a diagnostic laboratory, a newly developed test for qualitative detection of HCV RNA needs to be specific, sensitive, convenient to handle, and concordant with the results of other assays that have already been validated in clinical settings. Therefore, our unicenter study of the VERSANT® HCV qualitative assay was designed to demonstrate these necessary performance characteristics prior to commercialization by testing more than 300 samples in a total of 850 determinations.

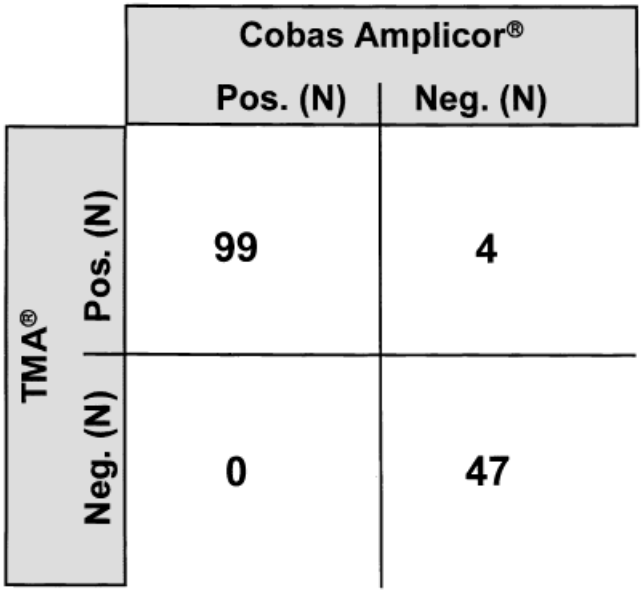

Fig. 2. Comparison of HCV TMA and Cobas Amplicor® assay test results.

The assay proved to be specific. Only one truly false-positive result was observed, which is in line with the rates of unspecificity reported previously for other commercially available HCV RNA amplification approaches, such as the widely used Amplicor® system (4–7) and NASBA® (7). The fact that no contaminations occurred even when testing specimens with HCV RNA titers of more than 10^8 copies/mL definitely contributed to the high specificity of the TMA assay. Unlike PCR, TMA generates multiple unstable RNA copies from an RNA template, so, in general, contamination from previous amplifications is less probable in TMA than in PCR. However, the robustness of the system against contamination is also ensured by two conceptual precautions of the assay configuration: 1) The TMA amplicons consist primarily of antisense HCV RNA, but only positive viral RNA is captured. 2) The primer sequences used in the assay are located downstream from the binding sites of the oligonucleotide capture probes. As a result, RNA amplicons still present from a preceding amplification cannot bind to the magnetic microparticles, and hence are not able to contaminate the next run (13).

Regarding the analytical sensitivity of the VERSANT® HCV qualitative assay, 41 HCV RNA copies/mL and 5 IU/mL of the first international HCV RNA standard were detectable with a sensitivity of 100%, respectively. The detection limit was not influenced by the HCV genotype or subtype. Thus, in our study the VERSANT® HCV qualitative assay proved to be slightly more sensitive than the current version of the Cobas Amplicor® HCV assay, which is believed to be the most sensitive commercially available kit (11) and was shown in an evaluation to reliably identify approximately 100 HCV RNA copies/mL (5).

The overall concordance between the results obtained by

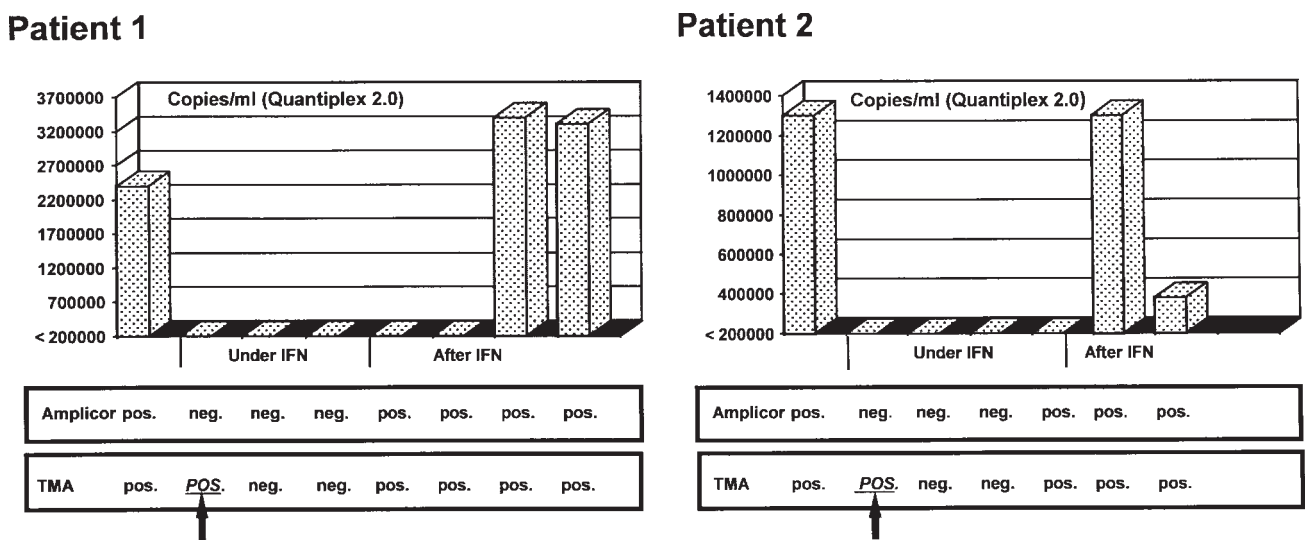

Fig. 3. VERSANT® HCV qualitative assay, Cobas Amplicor®, and Quantiplex® bDNA test results in two chronically HCV-infected patients treated with α-IFN. Discrepant results recorded by VERSANT® HCV qualitative assay and Cobas Amplicor® are indicated by arrows.

the VERSANT® HCV qualitative assay and Cobas Amplicor® 2.0 HCV assay was excellent. Due to the slightly higher analytical sensitivity, HCV RNA was still detectable in four Amplicor®-negative samples taken from chronically HCV-infected patients after the onset of α-IFN treatment, which is usually accompanied by a rapid and sharp decline of HCV RNA copy numbers to low-titer levels (21).

The internal control was undetectable in only three of 850 samples processed, indicating that inhibition of the isothermal amplification by serum components did not play a significant role in the VERSANT® HCV qualitative assay. Disadvantages of the system are the rather high sample volume of 500 µl and the fact that the equipment requires a lot of space. This may be problematic in some laboratories. The performance characteristics of the VERSANT® HCV qualitative assay proved to be excellent. Our results show that the newly developed test is a specific, sensitive, and reliable diagnostic tool for qualitative HCV RNA detection, providing results consistent with those of the already well-established Cobas Amplicor® system. These analytical data, in combination with a favorable turnaround time (processing of 92 samples in about 5 hr), a user-friendly design, and technical robustness, make the VERSANT® HCV qualitative assay suitable for use in routine clinical laboratories.

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