PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

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New combination test for hepatitis C virus genotype and viral load determination using Amplicor GT HCV MONITOR test v2.0

Motokazu Mukaide, Yasuhito Tanaka, Hirokazu Kakuda, Kei Fujiwara, Fuat Kurbanov, Eturo Orito, Kentaro Yoshioka, Kiyotaka Fujise, Shoji Harada, Takazumi Kozaki, Kazuo Takemura, Kazumasa Hikiji, Masashi Mizokami

Motokazu Mukaide, Hirokazu Kakuda, Shoji Harada, Takazumi Kozaki, Kazuo Takemura, Kazumasa Hikiji, SRL, Inc., 5-6-50, Shinmachi, Hino-shi, Tokyo 191-0002, Japan
Yasuhito Tanaka, Fuat Kurbanov, Masashi Mizokami, Department of Clinical Molecular Informative Medicine, Nagoya City University Graduate School of Medical Sciences, Kawasumi, Mizuho, Nagoya 467-8601, Japan
Kei Fujiwara, Eturo Orito, Department of Internal Medicine and Molecular Science, Nagoya City University Graduate School of Medical Sciences, Kawasumi, Mizuho, Nagoya 467-8601, Japan
Kentaro Yoshioka, Third Department of Internal Medicine, Nagoya University, School of Medicine, 65 Tsuruma, Showa, Nagoya 466-8550, Japan
Kiyotaka Fujise, Division of Gastroenterology and Hepatology, Department of Internal Medicine (Kashiwa), The Jikei University School of Medicine, 163-1, Kashiwashita, Kashiwa, Chiba 277-8567, Japan
Correspondence to: Masashi Mizokami, M.D., Ph.D., Department of Clinical Molecular Informative Medicine, Nagoya City University Graduate School of Medical Science, Kawasumi, Mizuho, Nagoya 467-8601, Japan. mizokami@med.nagoya-cu.ac.jp
Telephone: +81-52-853-8292 **Fax:** +81-52-842-0021
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Abstract

AIM: To develop a new sensitive and inexpensive hepatitis C virus (HCV) combination test (HCV Guideline test) that enables the determination of HCV genotypes 1, 2 and 3, and simultaneous determination of HCV viral load using commercial Amplicor GT HCV MONITOR test v2.0 (microwell version).

METHODS: The HCV Guideline test used the PCR product generated in commercial Amplicor GT HCV Monitor test v2.0 for viral load measurement using microwell plate version of Amplicor HCV Monitor and also captured on separate plates containing capture probes and competitive oligonucleotide probes specific for HCV genotypes 1, 2 and 3, The HCV genotype was subsequently determined using the biotin-labeled PCR product and five biotin-labeled HCV-specific probes.

RESULTS: The sensitivity of the HCV Guideline test was 0.5 KIU/mL. Specificity of the HCV Guideline test was confirmed by direct sequencing of HCV core region and molecular evolutionary analyses based on a panel of 31 samples. The comparison of the HCV Guideline test and an in-house HCV core genotyping assay using 252 samples from chronic hepatitis C patients indicated concordant results for 97.2% of samples (59.5% genotype 1, 33.7% genotype 2, 6.0% genotype 3, and 0.8% mixed genotypes). Similarly, the HCV Guideline test showed concordance with a serological test, and the serological test failed to assign any serotype in 12.7% of the samples, indicating a better sensitivity of the HCV Guideline test.

CONCLUSION: Clinically, both viral load and genotypes (1, 2 and 3) have been found to be major predictors of antiviral therapy outcome regarding chronic hepatitis C based on guidelines and they are, in normal circumstances, performed as separate stand-alone assays. The HCV Guideline test is a useful method for screening large cohorts in a routine clinical setting for determining the treatment regimen and for predicting the outcome of antiviral therapy of chronic hepatitis C.

Key words: Hepatitis C Virus; HCV Guideline test; Viral Load; Genotype

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INTRODUCTION

Affecting nearly 300 million people worldwide, hepatitis C virus (HCV) infection is considered to be the most frequent cause of post-transfusion non-A, non-B hepatitis worldwide[1,2]. Epidemiological observations predict that the burden of hepato cellular carcinoma (HCC) caused by HCV in USA will increase in the next two to three decades, possibly matching Japan[3]. HCV, a member of the Flaviviridae family, is a single-stranded RNA virus, containing a genome of 9400 nucleotides. The HCV genome demonstrates considerable diversity of nucleotide sequences and is of a different genotype[4]. HCV genotype 1, which is the most prevalent worldwide, is associated with more severe clinical manifestations, higher levels of HCV viremia and less amenable to treatments such as interferon-alfa or PEG-interferon-alfa/ribavirin therapy than HCV genotypes 2 or 3[5-9]. Therefore, clinical trials of antiviral therapies require genotype information for appropriate stratification of subjects because HCV genotypes (genotype 1,2 and 3) and RNA levels are clinically important factors that determine the response to antiviral therapy, cost benefit[10,11] and algorithm for treatmentive patients[12], and should thus be used to determine the duration of treatment of chronic C hepatitis as in guideline recommendations (Management of hepatitis C: 2002 by National Institutes of Health consensus development conference statement (http://consensus.nih.gov/cons/116/091202116cdc_statement.htm), clinical guidelines on the management of hepatitis C by British Society of Gastroenterology (http://www.bsg.org.uk/pdf_word_docs/clinguidehepc.pdf) and the guideline for IFN treatment of chronic hepatitis C patients by the Japan Ministry of Health, Labour and Welfare). Moreover, the interaction between HCV genotypes 1 and 2 was reportedly considered for the prediction of carcinogenesis using a multiplicative proportional hazard model[13].

Assays for HCV genotyping are generally based on sequencing technology[14-17], but these assays are labor intensive, expensive and require significant expertise and additional devices such as an autosequencer. Although convenient HCV core genotyping assay using genotype-specific primers[18,19] or a commercial serotyping test[20] is also used, if there is a need to obtain information on HCV load, a separate method should be carried out.

In this study, we developed a new sensitive and inexpensive HCV combination test (HCV Guideline test) that enables the determination of HCV genotypes 1, 2 and 3 using genotype-specific probes, and simultaneous HCV viral load determination using the Amplicor GT HCV Monitor test v2.0. The HCV Guideline test was compared with direct sequencing of HCV core region and molecular evolutionary analysis, an in-house HCV core genotyping assay and a convenient serotyping test to evaluate its accuracy.

MATERIALS AND METHODS

Serum samples

Serum samples from a total of 264 subjects with chronic HCV infection in Japan were collected for the evaluation of the HCV Guideline test. Fifteen subjects with chronic HCV infection in Uzbekistan were used for reference of HCV genotype 3. All samples were sero-positive for anti-HCV by the third-generation enzyme immunoassay (Dinabot Laboratories, Tokyo, Japan). Serum samples ($n = 36$) from control Japanese individuals who were positive for anti-HCV were used to determine the cutoff point of the assay in parallel with anti-HCV negative samples ($n = 36$). Additional 30 samples from healthy blood donors were used as negative controls. An informed consent was obtained from each subject.

Extraction of HCV-RNA and quantification of HCV-RNA for HCV Guideline test

HCV RNA was isolated from serum using the Amplicor GT HCV Monitor test v2.0 (microwell plate version, Roche Diagnostics, Tokyo, Japan), the robotic processor GT12 and an automated RNA extraction system (Precision System Science Co. Ltd. Tokyo, Japan). The extracted HCV-RNA was quantified according to the manufacturer's recommendations by using the Amplicor GT HCV Monitor test v2.0 and an automatic system (Figure 1), which consisted of the processor BEPIII (washing, dispensing, incubating, and reading instruments for microwell plates (incl. Supply Unit, Apple Macintosh), Date Behring, Inc., Illinois, USA) and the robotic dispenser NSP-7000 (Nitiryo Co., LTD., Tokyo, Japan).

Genotype plates for HCV Guideline test

Genotype capture probes introduced of an aliphatic primary amine at the 5' end of oligonucleotides by Aminolink 2 (Applied biosystems, CA, USA, Table 1) were diluted in phosphate buffer (50 mmol/L Na_2PO_4, pH8.5, 1 mmol/L EDTA) at a concentration of 250 pmol/mL. Then, 0.1 mL of each probe solution was added to wells of a microwell plate (DNA-BIND: COSTAR 2505, Corning, NY, USA), in which the "pre-activated surfaces" were covalently bound to abstractable hydrogens of oligonucleotide probes, incubated at 37 ℃ for 1 h, and washed three times with the buffer of the Amplicor GT HCV Monitor test v2.0. The plate was then treated with 0.1% gelatin (Maruha, Tokyo, Japan) in phosphate buffer for 30 min. Thereafter, the plate was washed three times with the same buffer.

HCV genotyping assay by HCV Guideline test

The methodology of the HCV Guideline test is summarized in Figure 2. The wash buffer, hybridization buffer, enzyme conjugate and substrate were all supplied with the Amplicor kit. It consisted of four major steps. In step 1, the polymerase chain reaction (PCR) product was hybridized to the HCV genotype specific capture probe on the microwell plate, and in step 2, unbound PCR product was washed out from the plate. In brief, 0.1 mL of the hybridization buffer, containing 10 pmol/mL of each competitive oligonucleotide probe (Table 1), was dispensed into the microwell plate and a small portion (25 μL) of denaturated amplified PCR products obtained from the Amplicor GT HCV Monitor test v2.0 was added. The plate was incubated at 37 ℃ for 1 h, followed by washing twice with the buffer (supplied with the Amplicor kit). One hundred μL of the hybridization buffer and the biotin labeled-probes (10 pmol/mL each, Table 2) were added to each well. Step 3 consisted of signal amplification with biotin labeled probes and the HCV genotype was determined by a colorimetric method. After incubation at 37 ℃ for 30 min, the wells were washed twice with the wash buffer and 0.1 mL of avidin-horse radish peroxidase conjugates was added. The plate was incubated at 37 ℃ for 15 min, and washed five times. One hundred μL of substrates (3,3',5,5'-tetramethylbentidine and hydrogen peroxide) was added to each well, and the plate was incubated again at 37 ℃ for 15 min. Subsequently, 0.1 mL stop solution was added and the absorbance (A) was measured at 450 nm. HCV genotype was determined using an automatic system, which consisted of the processor BEPIII (Date Behring, Inc., Illinois, USA) and the robotic dispenser NSP-7000 (Nitiryo Co., LTD., Tokyo, Japan). An A greater than 0.2 was considered a positive result.

Direct sequencing and molecular evolutionary analysis

Nucleotide sequencing in the core and NS5B regions of HCV genome was performed as described[3,19]. For the PCR in HCV core, the first round utilized primers Sc2 (5'-GGGAGGTCTCGTAGACCGTGCACCATG-3'-24-3) and Ac2 (5'-GAG(AC)GG(GT)AT(AG)TACCCCATGAG(AG)TCGGC-3' 417-391). Briefly, the second-round PCR was performed with primers S7 (5'-AGACCGTGCACCATGAGCAC-3'-12-8) and A5 (5'-TACGCCGGGGGTCA(TG)T(GA)GGGCCCCA-3' 343-319). For PCR in NS5B region, NS5B8278S (8258-8278): TGATACCCGCTGYTTTGACTC and NS5B8618AS (8618-8639): GTACCTGGTCATAGCCTCCGTG were used. The PCR products of a single target molecule were directly sequenced using an automated DNA sequencer (ABI PRISM DNA Sequencer 3100, Applied biosystems, CA, USA). To clarify the relationship among different HCV isolates, a phylogenetic tree was constructed as described elsewhere[21]. Briefly, using computer program HCV database (http://172.17.49.11/), the number of nucleotide substitutions per site and the genetic distances between the isolates were estimated by the 6-parameter method[22]. Based on these values, a phylogenetic tree was constructed by the neighbor-joining (N-J) method[23].

RESULTS

Optimization of the assay

To increase the signal-to-background ratio of the test, the addition of competitive oligonucleotide probes (Table 1) during the hybridization step (Figures 1, 2) of PCR products was evaluated. Three samples with genotypes 1b, 2a and 3a and with high HCV RNA levels (> 850 000 IU/mL) were used. Without the addition of competitive oligonucleotides, the samples harboring genotype 1 produced a very strong signal ($A_{450} = 3.25 \pm 0.14$, $n = 5$) on genotype plate 1. However, a signal ($A_{450} = 0.34 \pm 0.09$, $n = 5$) was also detectable in samples containing genotypes 2 and 3, indicating nonspecific binding between the capture probe for genotype 1 and the PCR-amplified products of genotypes 2 and 3. On the other hand, the addition of competitive probes resulted in a reduction in the extent of unspecific binding and an improvement in signal ($A_{450} = 0.054 \pm 0.015$, $n = 5$)-to-background ratio.

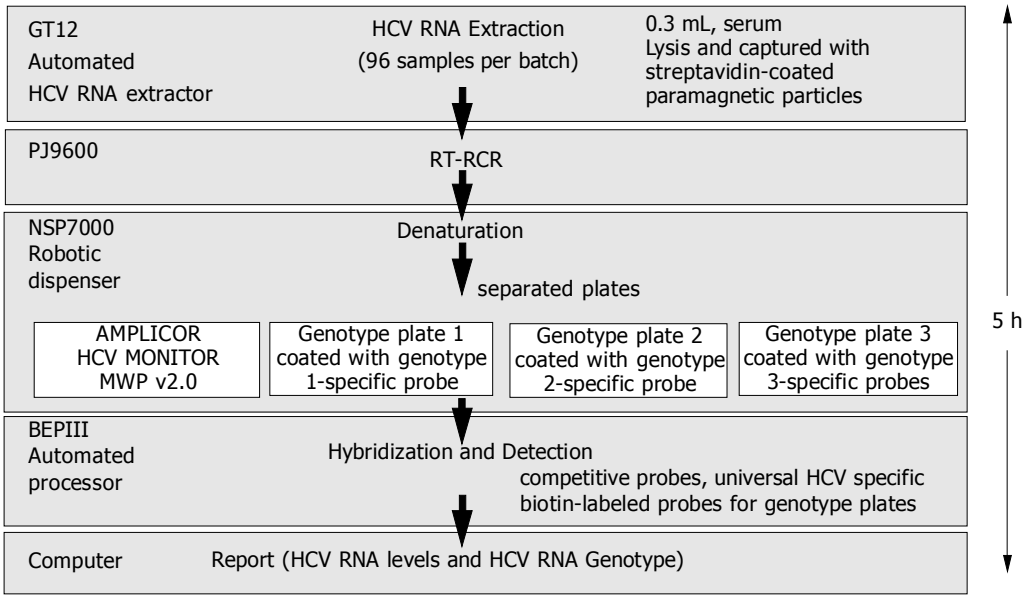

Figure 1 HCV Guideline test flow.

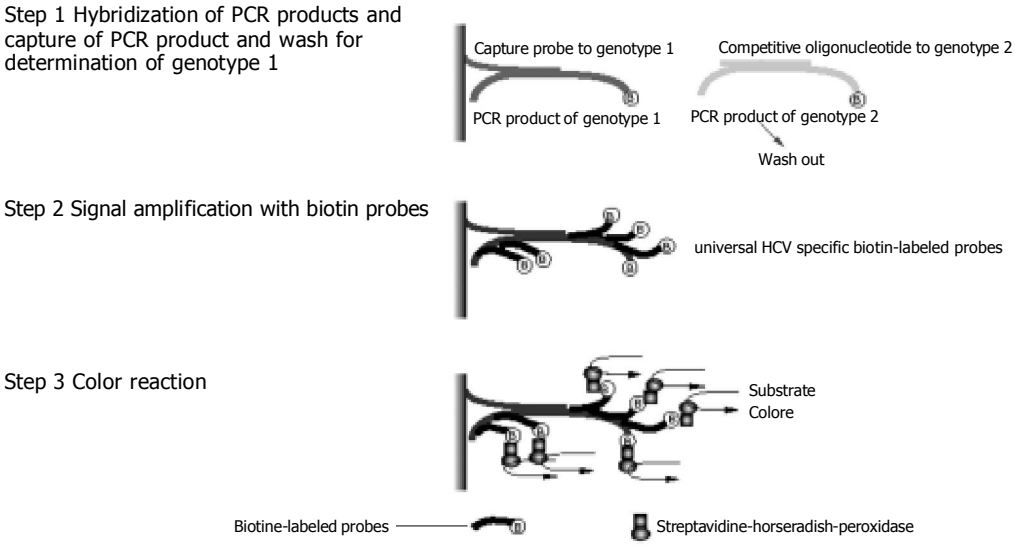

Figure 2 Strategy of HCV Guideline test. Step 1: The PCR product (here genotype 1) was hybridized to the specific capture probe, immobilized on a micro-plate. Other PCR products (e.g. of genotype 2) did not hybridize to the immobilized probe and were captured by the competitive oligonucleotide probes. The genotype specific-hybridized PCR product is retained on the micro-plate, while other PCR products were washed out from the plate. Step 2: Additional universal biotin-labeled-probes hybridized to the specific PCR product resulting in signal amplification. Step 3: Biotin reacted with streptavidine which was conjugated to the horseradish-peroxidase (HRP) enzyme. The color reaction was measured after the addition of enzyme specific substrates.

Table 1 Genotype specific capture probes (Aminolink[1]) and competitive oligonucleotide probes

Probe name	Sequences	Use
MH1Ami	5′-Aminolink-tttttgctcaatgcctggagatttg-3′	Genotype 1 specific capture probe
MHCG1C	5′-cactctatgcccggcc-3′	Competitor oligonucleotide to genotype 2
MHCG1C3	5′-tcaatrcccraaatttg-3′	Competitor oligonucleotide to genotype 3
MH2Ami	5′- Aminolink-tttttcttggataaacccactctatgyc-3′	Genotype 2 specific capture probe
MHCG2C	5′-ggatcaacccgctcaatg-3′	Competitive oligonucleotide to genotype 1
MHCG2C3	5′-arcaacccgctcaatg-3′	Competitive oligonucleotide to genotype 3
MH3Ami	5′- Aminolink-tttttgaatcgctggggtgaccg-3′	Genotype 3 specific capture probe
MH3′Ami	5′- Aminolink-tttttcgcgagatcactagccgagtag-3′	Genotype 3 specific capture probe
MHCG3C	5′-aattgccrggamgac-3′	Competitive oligonucleotide to genotype 1, 2
MHCG3′C	5′-cgcragactgctagccga-3′	Competitive oligonucleotide to genotype 1, 2

[1]Aminolink is a registered trademark of applied biosystems.

To improve the efficiency of signal amplification, the effect of biotin-labeled universal probes during the hybridization step was evaluated using five samples with a low HCV RNA level, ranging from 500 to 700 IU/mL. The universal biotin-labeled probes were specific for the HCV 5'-UTR region (Table 2). If the HCV Guideline test was performed with only a single biotin primer, as in the Amplicor GT HCV Monitor test v2.0, the absorbance in terms of A_{450} (0.15±0.076, $n = 5$) remained below 0.2 in three of five samples. In contrast, the addition of five biotin-labeled universal probes enhanced signal amplification. All the five samples showed ODs at 450 nm (A_{450}) well above the cutoff of 0.2, ranging from 0.33 to 0.53 (0.45±0.10, $n = 5$).

Table 2 Universal biotin-labeled HCV specific probes for the amplification of the detection signal

Probe name	Sequences
MH211B+	5′-Biotin-ctagccgagtagcgttgggttgcgaaagg-3′
MH111B+	5′-Biotin-ctagccgagtagtgttgggtcgcgaaagg-3′
MHBT1	5′-Biotin-tagtatgagtgtcgtgcagcctccaggc-3′
MHBT2	5′-Biotin-gagagccatagtggtctgcggaaccggt-3′
MHBT3	5′-Biotin-gagtacaccggaattgccaggacgacc-3′
MHBT4	5′-Biotin ccttgtggtactgcctgatagggtgcttg-3′

Optimal cutoff of absorbance for HCV Guideline test

Serum samples ($n = 36$) from control Japanese individuals who were positive for anti-HCV were tested in parallel with anti-HCV negative samples ($n = 36$). The mean A_{450} value for anti-HCV-positive samples (mean = 3.15; range, 0.75 to 3.6; 95% confidence = 2.97 to 3.32) was 52 times greater than that observed for anti-HCV negative samples (mean = 0.061; range 0.03 to 0.15; 95% confidence = 0.054 to 0.068). The cut-off value was defined as more than 6 standard deviation of the mean value for anti-HCV-negative samples. For a valid run, samples with an A_{450} of 0.20 were considered positive for HCV. ODs less than 0.20 were considered invalid negative results (Figure 3).

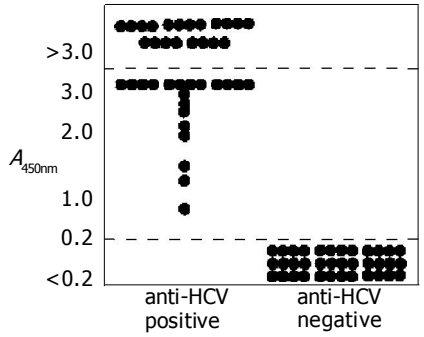

Figure 3 Specificity of HCV Guideline test. 36 anti-HCV-positive samples and 36 anti-HCV-negative samples were used to determine the specificity of the HCV Guideline test. All the anti-HCV-positive samples showed an absorbance greater than 0.2, whereas all anti-HCV-negative samples showed an absorbance below 0.2.

Detection limit of HCV Guideline test

The detection limit of the HCV Guideline test was determined using 264 subjects with chronic HCV infection in Japan (Table 3). The proportions of samples were 0% for samples with a HCV viral load <500 IU/mL and 100% for samples with a HCV viral load 500 IU/mL. Furthermore, they were confirmed by the limited dilutions using samples with genotypes 1b, 2a, and 2b.

Table 3 Detection limit of the HCV Guideline test in 264 samples infected with HCV

Amplicor monitor (IU/mL)	Number	HCV-Guideline positivity, *n* (%)
<500	12	0 (0)
500-1 000	18	18 (100.0)
1 000-10 000	73	73 (100.0)
10 000-100 000	71	71 (100.0)
100 000-850 000	62	62 (100.0)
850 000<	28	28 (100.0)

Reproducibility of HCV Guideline test

To determine the variance of repeated runs, isolates of all the three genotypes were tested 10 times on different days. Each sample was typed clearly, and no differences were noted between the results for the respective days. Thirty samples from healthy blood donors served as negative controls. None of these showed a positive result.

Comparison of HCV Guideline test and direct sequencing with molecular evolutionary analysis

A phylogenetic tree was constructed on the basis of alignment of the HCV core genes (from nucleotide positions, 12 to 319) determined by direct sequencing of 31 HCV samples (JA1 to 31) and 11 reference sequences recruited from the DDBJ/EMBL/GenBank DNA database [HCVH/1a (M67463), HCVJ/1a (D00831), HCVJ/1b (D00574), HCVN/1b (D90208), HCJ6/2a (D00944), HC8J/2b (D10988), K3a/3a (D28917), TrKj/3b (D49374), ED43/4a (Y11604), SA13/5 (AF064490), Th580/6 (AF064490)]. Comparison of HCV Guideline test and molecular evolutionary analysis using the 31 HCV samples indicated 100% concordance between the two assays. Namely, genotype 1 corresponded to JA1, JA2, JA9, JA10, JA11, JA14, JA15, JA22, JA23, JA28; genotype2 corresponded to JA5, JA7, JA8, JA12, JA13, JA16, JA17, JA18, JA19, JA20, JA21, JA24, JA25, JA26, JA27, JA29, JA30, JA31; and genotype 3 corresponded to JA3, JA4, JA6.

Comparison of HCV Guideline test and HCV core genotyping assay

After optimization of the assay, the HCV Guideline test was compared with a HCV core genotyping assay[19] using 252 samples positive for HCV RNA (Table 4). HCV genotype 1 was detected in 150 samples (59.5%) by the HCV Guideline test. Of these samples, 147 were of showed subtype 1b, and 1 sample was of subtype 1a and 2 samples were of the mixed type, 1b plus 2a. Genotype 2 was found in 85 samples (33.7%) using the HCV Guideline test. Overall, an excellent concordance of 97.2% (245/252) between the two assays was observed. Discordant samples were mainly the mixed genotype or the samples not classified by the core genotyping assay (Table 4).

To explain the discrepancy in results, samples of genotypes 1b and 2a were prepared. The analysis of these samples revealed that mixed virus populations of genotypes 1b and 2a could be accurately detected, if the proportion of the minority strains exceeds at least 10%. Three samples that were not classified by the core genotyping assay were determined to be of genotype 2 by the HCV Guideline test, which were also confirmed by direct sequencing of the HCV NS5B region (data not shown). Additionally, 14 of the 15 samples identified to be of genotype 3 by the HCV Guideline test were confirmed to be of genotype 3a or 3b which represented a positive predictive value of 93.3%. One discordant sample was determined to be of genotype 3 by the HCV Guideline test, but of genotype 1b plus 3a by the core genotyping assay. Direct sequencing of the NS5B region indicated that genotype 3a was a major clone (data not shown).

Table 4 HCV genotypes of 252 serum samples from patients with chronic HCV infection as determined by HCV-Guideline test and a core genotyping method reported by Ohno and colleagues[19]

Core genotyping	HCV-Guideline test					
	1	2	3	1+2	1+3	Total
1a	1	0	0	0	0	1
1b	147	0	0	0	0	147
2a	0	64	0	0	0	64
2b	0	15	0	0	0	15
3a	0	0	13	0	0	13
3b	0	0	1	0	0	1
2a+2b	0	2	0	0	0	2
1b+2a	2	1	0	0	0	3
1b+2b	0	0	0	1	0	1
1b+3a	0	0	1	0	1	2
Not classified	0	3	0	0	0	3
Total	150	85	15	1	1	252

Comparison of HCV-Guideline test and serotyping assay

A total of 252 samples were used to compare the HCV-Guideline test (Table 5) with a serotyping assay (Immunocheck HCV GR assay, Kokusai, Kobe, Japan). In the HCV-Guideline test, 150 samples (59.5%) were determined to be of genotype 1, 85 (33.7%) of genotype 2 and 15 (6%) of genotype 3. Consistent with these results, 143 (56.7%) samples were found to be of serotype 1 (G1) and 77 (30.6%) samples of serotype 2 (G2) using the serotyping assay. One discordant sample was detected, which was shown to be of serotype 2, but of genotype 1 in the HCV-Guideline test. The result obtained by direct sequencing supported that by the HCV-Guideline test. Additionally, the serotyping failed in the assignment of serotype in 32 (12.7%) including 15 samples of genotype 3, indicating a better sensitivity of the HCV-Guideline test than that of the serotyping assay.

Table 5 HCV genotypes and serotypes of 252 serum samples from patients with chronic HCV infection as determined by HCV-Guideline test and serological Immucheck HCV GR test

Serotyping	HCV-Guideline test					
	1	2	3	1+2	1+3	Total
G1	142	0	0	0	1	143
G2	1[1]	76	0	0	0	77
Not determined	7	9	15	1	0	32
Total	150	85	15	1	1	252

[1]HCV core sequence by direct sequencing indicated genotype 1b, and confirmed by a core genotyping method[19].

DISCUSSION

We developed the HCV-Guideline test for the determination of HCV genotypes 1, 2 and 3; The test enabled the simultaneous HCV viral load determination using the Amplicor GT HCV Monitor test v2.0. The Amplicor GT HCV Monitor test v2.0 amplified the 5'-UTR region of HCV RNA, which could be used for the subsequent the HCV-genotyping test (Figure 1). The HCV-Guideline test using the PCR products produced by the Amplicor GT HCV Monitor test v2.0 allowed the rapid determination of HCV genotype simultaneous with HCV viral load determination within 5 h. This also gave a good cost-benefit during the follow-up of HCV-infected patients.

Several strategies were adopted in order to optimize the HCV-Guideline test. To improve the signal-to-background ratio, the blocking solution of the microwell plate was carefully selected, which largely reduced the background signal. Moreover, a second biotin-labeled probe was added to enhance the signal intensity. The sensitivity of the HCV-Guideline test was approximately 500 IU/mL with the quantitative Ampicor GT HCV MONITOR test v2.0, which was much higher than that obtained using a commercial direct-sequencing genotyping kit (TRUGENE HCV 5'NC) or a commercial reverse hybridization line probe assay (INNO-LiPA HCV II) for genotyping of HCV, which was approximately 20 000 IU/mL[17]. Our assay revealed a wide dynamic range that extended over a 4-log range of HCV input. Additionally, as the HCV-Guideline test was subsequently determined using a colorimetric method with biotin-labeled HCV specific probes, the test might be adapted for PCR products from in-house RT-PCR of HCV, such as real-time PCR, using products with the region of the specific probes and amplification probes, and may be used to detect mutations of some other genes. The specificity of genotyping by this HCV-Guideline test was also confirmed by direct sequencing.

A number of HCV typing methods have been developed in recent years. A serotyping assay Immunochech HCV GR assay[20] is only available for HCV typing test which was approved by the Ministry of Health, Labour and Welfare in Japan. Therefore, for routine diagnostic procedures the HCV serotype is usually employed in Japan. The serologic typing of HCV, lacked a sufficient sensitivity compared with molecular-based techniques[24]. Alternatively, genotype-specific amplification of the core region could determine the HCV genotype[18,19,25]. HCV genotyping by genotype-specific amplification, direct sequencing and line probe assay have been reported to give concordant results of HCV genotypes in all the cases studied[26-28]. However, these techniques are too expensive or too time-consuming to be useful outside a research setting.

Accordingly, to evaluate the new HCV-Guideline test we compared it with the HCV core genotyping assay and serotyping assay. Using the optimized method, an excellent concordance value of 97.2% was found between the HCV-Guideline test and the HCV core genotyping assay. Discordant samples between the HCV-Guideline test and the core genotyping assay were found mainly in mixed genotypes or unclassified samples (Table 4). Six mixed genotypes were detected by the core genotyping assay, however only 2 were correctly detected by the HCV-Guideline test, suggesting that the core genotyping assay could detect mixed infections more efficiently. On the other hand, 3 samples were not classified by the core genotyping assay. As the HCV RNA levels of these samples were relatively low (500-800 KIU/mL), the HCV-Guideline test seemed to be more suitable for detecting such low-viral load samples.

The genotypes 4, 5 and 6 were not found in our population. Regarding these genotypes, clinical data are not sufficient for the determination of the response to treatment and they are very rare in the general population particularly in the USA[29], Europe[25,30,31], and Japan[19,32]. The most common HCV genotype in these countries is genotype 1. From a clinical viewpoint, the knowledge of the precise HCV genotypes (e.g., 1a and 1b) seems to be unnecessary for the therapeutic decisions regarding chronic hepatitis C using IFN based on guidelines. In this context, it is most important to determine HCV genotypes 1, 2 and 3. Patients infected with HCV genotype 1 would need a different therapy regimen different from that for patients with HCV genotype 2 or 3 {(Management of hepatitis C: 2002 by National Institutes of Health consensus development conference statement (http://consensus.nih.gov/cons/116/

091202116cdc_statement.htm), clinical guidelines on the management of hepatitis C by British Society of Gastroenterology (http://www.bsg.org.uk/pdf_word_docs/clinguidehepc.pdf) and the guideline for IFN treatment of chronic hepatitis C patients by the Japan Ministry of Health, Labor and Welfare)}, because the sustained response to the current standard peg-interferon alpha-2b plus ribavirin therapy regimens for chronic hepatitis C is strongly associated with HCV viral road, genotypes 1, 2 and 3[33,34]. Thus, the HCV-Guideline test is useful for regions where HCV genotypes 1, 2 and 3 are predominant, such as in the USA, Europe, and Japan.

The directional medical cost of HCV in the USA is projected to increase $10 billion yearly between 2010 and 2019[11]. The incremental cost-effectiveness of combination therapy with peg-IFN for men ranged from 26 000 to 64 000 dollars per quality-adjusted life-year (QALY) for genotype 1 and from 10 000 to 28 000 dollars per QALY for other genotypes; and for women, ranged from 32 000 to 90 000 dollars for genotype 1 and from 12 000 to 42 000 dollars for other genotypes[10]. Therefore, medical cost can be reduced if the patients are determined to be of genotype 2 or 3 using the sensitive and inexpensive HCV-Guideline test.

In conclusion, the HCV-Guideline test is specific, sensitive and easier to perform automatically, and less expensive than a serotype assay, HCV core genotyping assay and direct sequencing. In addition, this test can be used in the same laboratory setting and reagents as those of the Amplicor GT HCV Monitor test v2.0 without requiring special or an additional expensive devices such as an auto-sequencer or a real-time PCR system. The HCV-Guideline test is therefore more suitable for screening large cohorts in a clinical routine practice for the prediction of clinical course, and the determination of treatment regimens and the outcome of antiviral therapy of hepatitis C.

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