Effects of Storage and Viral Load on Hepatitis C Viral Genotyping

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Hepatitis C virus (HCV) genotyping is important for determining the treatment protocol for hepatitis C patients. Since amplified material from the Roche HCV Monitor kit is compatible with the Innogenetics INNO-LiPA HCV II kit (line probe assay), amplicons from the Monitor assay can be used to identify the HCV genotype. The Monitor package insert recommends using amplicons within a 7-day period (at 4°C) following amplification. It was hypothesized that storage of amplicons for 4 weeks and longer (at –20°C) would not affect the sensitivity of the genotyping assay. After denaturation, amplicons from two genotypes were stored for 7–386 days prior to perform-

ing the genotyping assay. Storage of amplicons did not hamper the ability to identify the genotype. Additionally, the sensitivity of the assay was evaluated by analyzing five genotypes with low viral loads. HCV genotypes were detected most consistently at viral levels of 10,000 copies/mL. In conclusion, the Innogenetics genotyping assay can use stored amplicons, thus reducing the cost of the assay by avoiding additional PCR reactions. Determining the sensitivity of this assay facilitates the efficient use of this test by incorporating a sensitivity cutoff of $\geq 10,000$ copies/mL. *J. Clin. Lab. Anal.* 15:331–333, 2001. © 2001 Wiley-Liss, Inc.

Key words: monitor; line probe assay; PCR; HCV; genotyping

INTRODUCTION

Hepatitis C virus is a single-stranded RNA virus (1). This virus has substantial nucleic acid sequence heterogeneity (2,3). Characterizing the HCV genotype is important for determining risk of hepatocellular carcinoma and treatment protocols for patients with chronic hepatitis C infections (4–9). HCV genotyping also provides cost savings to both the patient and medical community by guiding the duration of combination therapy (10).

Since amplified material from the viral load analysis using the Roche HCV Monitor kit (Roche, Indianapolis, IN) is compatible with the INNO-LiPA HCV II kit (Innogenetics Inc., Alpharetta, GA), amplicons from this assay are often used to identify the HCV genotype (11,12). Briefly, the denatured biotinylated polymerase chain reaction (PCR) product is hybridized to a membrane containing immobilized genotype-specific oligomers. Following washing steps, an alkaline phosphatase conjugate is incubated with the hybridization product. The bound alkaline phosphatase catalyzes a chromogen that produces a purple precipitate, which is then used to identify the genotype. Currently, the Roche HCV Monitor package insert recommends the use of amplicons within a 7-day period following amplification. Limiting the use of the amplicons to this time frame increases the cost by decreasing the throughput of the assay and/or adding the additional expense of a separate

PCR for the genotyping assay. A utilization issue that also adversely affects the cost of this assay is the lack of information regarding the sensitivity of the assay using Roche-derived amplicons. To improve the workflow of the Innogenetics genotyping assay, storage and HCV viral load were evaluated for their affect on the INNO-LiPA HCV II assay.

MATERIALS AND METHODS

For analysis of prolonged storage, serum specimens containing HCV genotypes 1a (1,462,600 copies/mL) and 1b (3,223,900 copies/mL) were extracted according to the Roche Monitor version 1.0 (manual) protocol, and then each specimen was amplified as five different samples. One amplicon of each specimen was denatured at the end of amplification and genotyped using the INNO-LiPA HCV II kit according to the manufacturer's directions. All other amplicons were denatured and frozen at –20°C. Genotyping of one amplicon from each specimen was performed at days 0, 7, 21, and 28, and finally at 13 months.

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Received 29 September 2000; Accepted 11 April 2001

For analysis of assay sensitivity, seven patient samples that had been quantified using the manual HCV monitor version 1.0 with viral loads of 144,200–5,356 copies/mL were genotyped. Two different specimens contain 3a. Subsequently, aliquots of patient samples with viral loads > 30,000 copies/mL and an additional patient with type 4 were diluted using serum determined to be HCV negative by qualitative PCR (HCV Amplicor) to a concentration of 5,000–10,000 copies/mL. These viral loads were confirmed using the HCV monitor version 1. Genotyping was performed to determine the sensitivity cutoff for this assay.

RESULTS

All samples for HCV genotype 1a and 1b resulted in the correct typing following prolonged storage. The intensity of the bands did not vary between the initial testing and final testing, which covered a span of more than 13 months. Prolonged storage of denatured amplicons appears not to have a negative affect on identifying HCV genotype (Table 1).

In addition, the sensitivity of the Innogenetics INNO-LiPA HCV II kit was evaluated by analyzing specimens with viral loads of 144,200–5,000 copies/mL. Five HCV genotypes were detected at viral levels of approximately 10,000 copies/mL. Type 1 could not be genotyped at 11,742 copies/mL. Specimens that could not be genotyped with viral loads of 5,000 copies/mL were types 1a, 2b, 3a, and 4. In contrast, genotypes 1a/1b and another specimen containing 3a could be detected at 5,000 and 5,356 copies/mL, respectively (Table 2).

DISCUSSION

No difference in intensity of the banding pattern was observed for the day 0, 7, 21, 28, or 386 amplicons for either subtype. Thus, the INNO-LiPA HCV II genotyping assay can utilize stored, denatured Roche Monitor amplicons without adversely affecting the reportable results. Storage of the denatured amplicons at –20°C may increase their longevity by decreasing the activity of contaminating nucleases. Additionally, the sensitivity of the assay is capable of detecting amplicons of

TABLE 2. Effect of viral load on genotyping assay

Genotype	Viral load (copies/mL)	Results ^a
1a	104,442	+
1a	12,360	+
1a	10,000	w+
1a	5,000	–
1a/1b	30,282	+
1a/1b	5,000	w+
1	11,742	–
2b	35,020	+
2b	10,000	w+
2b	5,000	–
3a	144,200	+
3a	10,000	w+
3a	5,356	w+
3a	5,000	–
4	10,000	w+
4	5,000	–

^aPositive result (+); weak positive (w+); negative (–).

one specimen containing genotype 3a with a viral load of 5,356 copies/mL, and a specimen containing genotypes 1a and 1b at 5,000 copies/mL. However, another genotype 3a specimen containing 5,000 copies/mL did not yield detectable bands. With the exception of a genotype 1 specimen with a viral load of 11,742 copies/mL, the remaining genotypes gave reproducible results at 10,000 copies/mL or greater.

This study provides data that can be used to streamline the workflow of a molecular lab with respect to HCV genotyping and viral load analysis. Specifically, storing the amplicons until full batches are obtained will decrease laboratory expense due to increased efficiency of technician time. Using stored amplicons from the HCV viral load assay will reduce reagent costs by circumventing the need to perform additional PCR reactions. Implementing utilization parameters for HCV genotyping assays for patients with viral loads of greater than 10,000 copies/mL will also prevent waste of reagents and technician time due to failed assays.

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TABLE 1. Effect of storage on genotyping assay

Genotype	Days stored	Results ^a
1a	0	+
1b	0	+
1a	7	+
1b	7	+
1a	14	+
1b	14	+
1a	21	+
1b	21	+
1a	28	+
1b	28	+
1a	386	+
1b	386	+

^aPositive result (+); negative (–).

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