Real-time quantitative assay of HCV RNA using the duplex scorpion primer

Brief Report

S.-X. Xu1, K. Lan2, Y.-L. Shan3, H. Wang1, J.-Y. Luo1, F. Cui4, Y.-S. Huang1, J.-P. Meng4, X.-M. Zhang1, and Y.-B. Yin1

1The Key Laboratory of Laboratory Medical Diagnostics, Ministry of Education, The Faculty of Laboratory Medicine, Chongqing University of Medical Sciences (CQUMS), Chongqing, P.R. China
2The Laboratory Department of the Guangdong Provincial Hospital of Traditional Chinese Medicine, Guangzhou, P.R. China
3The Second Affiliated Hospital of CQUMS, Chongqing, P.R. China
4The First Affiliated Hospital of CQUMS, Chongqing, P.R. China

Received December 1, 2005; accepted July 31, 2006
Published online September 25, 2006 © Springer-Verlag 2006

Summary. A novel real-time quantitative method for detecting HCV in serum was established in which the duplex scorpion primer was used to provide a unimolecular probing mechanism for hybridizing the highly conserved 5′ noncoding region (5′ NCR) of the HCV genome specifically. Through methodological evaluation, we found this new method had a wide linearity, high sensitivity, repeatability and specificity. Compared to the commercial TaqMan method, this method was found to be more sensitive and less costly, and the final results were obtained more quickly. Therefore, it could be applied to diagnose and monitor HCV infection in clinical practice.

Hepatitis C virus (HCV) infection has reached epidemic proportions and become a major global health issue. The WHO estimates that up to 3% of the world population has been infected by the virus, equating to more than 170 million carriers worldwide [19, 23]. Persistent HCV infection may be associated with a wide spectrum of outcomes, from mild nonprogressive liver damage to severe chronic hepatitis that could develop to cirrhosis, end-stage liver disease, or hepatocellular carcinomas. Several studies have tried to elucidate the viral characteristics involved in the progression of the disease. The infecting genotype, the degree of
viral diversity, and the viral load have been suggested to correlate with disease activity, degree of liver damage, and response to alpha interferon treatment, but the findings of some research groups remain controversial [2, 6, 10, 15]. It has been proven that the viral load is relatively different in the chronic phase of infection, associated with the response to interferon therapy [22], and that the quantification of HCV load seems to be highly significant for tailoring the treatment schedules as well as for monitoring the level of HCV replication during therapy. In cases where HCV circulates in the blood at a low load and its genome is extremely heterogeneous, the detection and quantification of HCV RNA in serum must be highly sensitive and specific [12]. The 5′ noncoding region (5′ NCR) is the most conserved region among genotypes [7] and can therefore be used to quantify HCV RNA. Various techniques have been used, such as branched-DNA (bDNA) or reverse transcription-PCR (RT-PCR), to attempt to precisely quantify the viral load in the sera of HCV-infected patients [1, 9]. However, these methods are marred by tedious protocols that are affected by many factors, yielding results that appear to be of low precision.

When compared to competitive or noncompetitive quantitative PCR methods, the real-time PCR invented by Higuchi et al. in 1993 [8], saved time and showed no plateau effect, no post-PCR manipulation, and no carryover contamination [5, 16]. Thereafter, real-time PCR proved itself to be invaluable in laboratories around the globe for building on the enormous amount of data generated by conventional PCR assays [11]. In 1999, a new real-time PCR system based on a primer with a tail attached to its 5′ end by a linker that inhibited the extension of the 5′ end of the primer was established. This probe, named “stem-loop” scorpion primer, is designed to hybridize to its target only when the target site has been incorporated into the same molecule by extension of the tailed primer [21]. Two years later, a duplex scorpion primer was invented that displayed many advantages over the precursor on which it was built [17]. The mode of action of the duplex scorpion primer is illustrated in Fig. 1. The intramolecular probe-target interaction is the most noteworthy feature of the scorpion system and allows for a faster and more reliable detection system than that of the TaqMan, molecular beacon, and hybridization probes. Furthermore, the fluorophore and quencher are presented on two different strands in the scorpion primers, which can decrease the cost of synthesizing, labeling and purifying probes compared to the TaqMan probe possessing the fluorophore and quencher on the ends of the same strand [17, 18, 21]. In this study, we explored a very fast and reliable detection system for HCV quantitation using the duplex scorpion primers targeting the 5′ NCR of the HCV genome.

An external standard was required for the quantification of the targeted sequence. Recently, standard RNA synthesized in vitro [13] and armored RNA [14, 20] have been put into application. Although the former can control for variability of reverse transcription, it can not solve the problem of the RNA extraction variability, and must be synthesized freshly for clinical diagnosis because of its instability over time. The armored RNA is stable, but its high cost prevents its further manipulation for clinical practice. Therefore, we incorporated