Versant HCV Genotype 2.0 Assay (LiPA) in Hepatitis C Virus Genotype Determination

Testul Versant HCV Genotype 2.0 Assay (LiPA) în determinarea genotipurilor virusului hepatitic de tip C

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Abstract

Hepatitis C virus (HCV) is a major cause of chronic liver disease, the prevalence of the infection being currently estimated at approximately 170 million persons worldwide. HCV isolates have been classified into six main genotypes. Quick and accurate genotyping of HCV is becoming increasingly important for clinical management of chronic infection and as an epidemiological marker. Genotyping methods are frequently based on the analysis of the 5’ untranslated region (5’UTR) of HCV genome, which is not appropriate for the accurate discrimination of HCV strains at a subtype level. The newly developed Versant HCV Genotype 2.0 Assay (LiPA) uses sequence information from both the 5’UTR and the core region allowing the distinction between subtypes 1a and 1b inside genotype 1 and between genotype 1 and subtypes c to l of genotype 6. Hereby, we make a review of the literature evaluating this new genotyping assay.

Keywords: hepatitis C virus, genotyping, reverse hybridization, sequencing.

Rezumat

Virusul hepatitic de tip C (VHC) este o cauză majoră a bolilor hepatice cronoce, prevenește infecției în lume fiind estimată în prezent la aproximativ 170 milioane de persoane. Tulpinile de VHC au fost clasificate în 6 genotipuri. Genotipurerea rapidă și precisă a VHC este din ce în ce mai importantă pentru tratamentul infecției cronice și ca marker epidemiologic. Metodele de genotipare sunt frecvent bazate pe analiza regiunii 5’ netraduse a genomului VHC, care nu este potrivită pentru discriminarea cu acuratețe a tulpinilor de VHC la nivel de subtip. Noul test Versant HCV Genotype 2.0 Assay (LiPA) utilizează informații prezentate atât în regiunea 5’ netradusă, cât și în regiunea core, permițând distincția dintre subtipurile 1a și 1b ale genotipului 1 și dintre genotipul 1 și subtipurile c-l ale genotipului 6. Acest articol trece în revistă datele actuale privind evaluarea acestui nou test de genotipare.

Cuvinte-cheie: virusul hepatitic de tip C, genotipare, revers-hibridizare, secvențiere.

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Hepatitis C virus (HCV) is a major cause of chronic liver disease worldwide. After its first description in 1989, it was soon identified as the main causative agent of the previously so-called posttransfusion non-A, non-B hepatitis. HCV has been the subject of intense research and clinical investigations as its major role in human disease has emerged.

The prevalence of HCV infection is difficult to evaluate because many of the infected persons have a clinically and biochemically silent infection. It was estimated that around 170 million patients are chronically infected with HCV. Each year, 3 to 4 million people are newly infected. In industrialized countries, HCV accounts for 20% of cases of chronic hepatitis, 40% of cases of end-stage cirrhosis, 60% of cases of hepatocellular carcinoma and 30% of liver transplants.

HCV is a single-stranded positive RNA virus belonging to the family Flaviviridae, genus Hepacivirus. HCV genome (Figure 1) is about 9.6 kb in length and codes for a polyprotein which is posttranslationally cleaved in 10 major polypeptides: the core protein (C), the envelope glycoproteins (E1 and E2) and the nonstructural proteins: p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B. At the 5’ and 3’ ends of the genome, HCV has two untranslated regions (UTRs).

Based on genetic differences between HCV isolates, the hepatitis C virus species are classified into six genotypes (1-6) with several subtypes (represented by letters) within each genotype. The genotypes differ by as much as 31% to 34% in their nucleotide sequences. In a former classification, the existence of more than six HCV genotypes was acknowledged, but with the development of new sequencing and clustering algorithms, the newer classification was adopted. Thus, the former genotypes 7, 8, 9, 11 have been reclassified as subtypes 6c-6l inside genotype 6 and genotype 10 was classified as subtype 3k of genotype 3. Table 1 presents the comparison between the present and the past classification of HCV genotypes.

The preponderence and distribution of HCV genotypes varies globally. In Europe, genotype 1b is predominant, followed by 2a, 2b, 2c and 3a.

In addition to viral load and liver histology, the genotype of the infecting HCV strain appears to be an important determinant of the severity and aggressiveness of liver infection. The determination of HCV genotype also provides clinically important information that can be used to direct the duration and type of antiviral therapy and to predict the likelihood of sustained HCV clearance after therapy. Many studies unequivocally demonstrated the association between the HCV genotype and the response to therapy.

Table 1. Comparison of old and new HCV genotype nomenclature.

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<th>Current classification</th>
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Figure 1. Schematic representation of HCV genome.
siveness to antiviral combination therapy with pegylated interferon alpha and ribavirin. Genotypes 1 and 4 are less responsive to interferon-based treatment than are the other genotypes (2, 3, 5 and 6). Indeed, only 45% of genotype 1 infected patients but 80% of those infected with genotype 2 or 3 reach a sustained viral response with the current therapy combining ribavirin and peginterferon.

HCV genotyping is now an indispensable tool for the tailoring of antiviral treatment of the patients. It is also an essential tool for epidemiological studies and for tracing a source of contamination. For clinical concerns, the determination of the genetic group is sufficient, whereas the subtype designation is crucial for epidemiological and transmission investigations. It has been suggested that genotypes 3a and 1a are closely associated with intravenous drug use and that genotype 1b is seen more often in patients who acquired HCV through blood transfusion. Suspected nonconventional routes of HCV transmission could also be investigated by molecular analysis of HCV strains from different persons. These include the vertical and sexual routes.

In order to determine HCV genotypes and subtypes, the choice of the genome region to be analyzed is crucial. This region must present genotype-specific and subtype-specific motifs. Additionally, it must be highly conserved to be detected by most of the assays based on nucleic acid amplification. Several assays were developed to identify HCV genotypes and subtypes from the 5'UTR because this region is readily amplified by PCR. On the other hand, this region does not contain sufficient information for the recognition of all different types and subtypes. The NS5B region appears to be much more accurate for identifying variations in the nucleotide sequences, but is too variable to be suitable for PCR.

In clinical laboratories, HCV genotypes are most frequently determined by sequencing or by the line-probe assay. Although the recognized gold standard for genotype determination is direct DNA sequencing, this methodology has been criticized as too expensive and labor intensive for routine clinical use. High-throughput sequencing has been developed for HCV genotyping, but the methodology and equipment are best suited to large laboratories with high test volumes.

The line-probe assay (LiPA) requires reverse transcription - PCR (RT-PCR) of part of the viral genome with biotinylated primers. The resulting biotin-labeled amplicons are hybridized to an array of genotype/subtype-specific probes that have been immobilized to strips of nitrocellulose membrane. The immobilized amplicons are detected by use of an enzymatic colorimetric detection system.

Versant HCV Genotype 2.0 Assay (LiPA), the most recently introduced new generation of line probe assay, utilizes 20 immobilized oligonucleotide probes specific for the 5'UTRs and 4 that are specific for core regions of the six HCV genotypes. The probes are bound to a nitrocellulose strip by a poly(T) tail. After hybridization of the biotinylated amplification products to the probes under highly stringent conditions, unhybridized PCR products are washed from the strips, and streptavidin coupled to alkaline phosphatase (conjugate) is bound to the biotinylated hybrid. After washing the strips, BCIP/NBT (5-bromo-4-chloro-3-indolyolphosphate/ nitroblue tetrazolium) chromogen (substrate) reacts with the conjugate forming a purple/brown precipitate, which results in specific banding patterns on the nitrocellulose strips.

Each strip has 22 parallel DNA probe lines containing sequences specific for HCV genotypes 1 to 6 and 3 control lines: 1 conjugate control line and 2 amplification controls (Figure 2). The conjugate control at line 1 monitors the color development reaction and gives a positive result if the strip is correctly processed. The amplification control at line 2 (AMPL CTRL 1) contains universal probes that hy-
bridize to PCR products from the 5'UTR. AMPL CTRL 2 is located at line 23 and contains universal probes that hybridize to PCR products from the core region. HCV genotypes are determined by aligning the strips with a reading card and comparing the line patterns from the strip with the patterns on the interpretation chart.

Since its introduction on the market, Versant HCV Genotype 2.0 Assay (LiPA) has been compared in several studies with other HCV genotyping assays. The most tempting comparison to make is against the previous version of the assay.

Versant HCV genotype assay (LiPA) 1.0, like most commercially available HCV genotyping assays, uses the 5'UTR, since this region is highly conserved and therefore well suited for the development of detection methods. The 5'UTR contains multiple genotype-specific sequences distributed over small variable regions. They provide accurate genotyping information for genotypes 1 to 5 and for genotype 6 subtypes a and b; however, owing to high sequence similarity, subtypes c to l of genotype 6 cannot be distinguished from genotype 1 by analysis of the 5'UTR alone. Also, the accuracy of differentiating subtypes 1a and 1b is limited by 5'UTR analysis.

In a side-by-side comparison of versions 1 and 2 of the assay, samples that showed discordant results were analyzed by direct sequencing of the NS5B region. The results for these samples showed that Versant HCV Genotype 2.0 Assay (LiPA) gave the correct HCV genotype and subtype and thereby showed an improvement in identifying HCV-positive samples which were subtypes c to l of genotype 6 and in identifying the correct subtype of genotype 1. This improvement was attributed to the additional information available in the newer test from the core region of the HCV genome.

The misclassification of genotype 1a as 1b or the contrary has been described on the basis of the sequence polymorphism at position -99 of the genome, frequently used to differentiate genotypes 17–10. Cantaloube et al. reported that, in a study performed on a set of 357...
HCV strains isolated from blood donors in France in 2002 and 2003 (a total of 120 strains were initially identified as subtype 1b based on 5' UTR analysis) but sequence analysis of the NS5B region was concordant only for 93 strains (77.5%). Sequencing of the 5' UTR showed that all discordant isolates had a G in position –99.

Moreover, information based solely on 5'UTR analysis is not reliable for correctly identifying the diversity within genetic groups as seen in genotypes 2 and 4. In the same study reported by Cantaloube et al., 61% of strains initially identified as 2a/2c by the analysis of 5'UTR were reclassified after sequencing of NS5B region as non-2a and non-2c subtypes, and 45% of strains initially identified as 4c/4d subtypes were reclassified as non-4c and non-4d subtypes.

Another benefit of Versant HCV Genotype 2.0 Assay (LiPA) is its ability to correctly classify genotype 6. In a work conducted on sera obtained from South-East Asia, the new assay correctly genotyped samples in 96% of the cases compared to only 71% for the old assay that only targeted 5'UTR. 33 samples from a total of 73 were genotype 6. The sample set not only contained subtype 6a, but also 6d, 6c, 6f, 6i, 6l and 6n. All of these were correctly identified by the assay of the newer generation.

As stated before, phylogenetic analysis of a coding region, or even more, the complete genome, is considered the gold standard for identifying different HCV genotypes. When compared to direct sequencing methods, Versant HCV Genotype 2.0 Assay (LiPA) performs remarkably well in identifying HCV genotypes and subtypes. Verbeeck et al. reported having obtained interpretable results in 96% of the tested samples and that 99.4% of the interpretable results agreed with the reference method used, which was sequencing part of the NS5B region. Other studies reported success rates of over 95% in identifying the correct genotype and subtype as compared to sequencing. Boucherdeau et al. reported a discordant result in a sample that was classified as subtype 1a by Versant HCV Genotype 2.0 Assay (LiPA) and 1b by NS5B sequencing method. A 5'UTR-based sequencing assay found the presence of a nucleotide A at position –99 and confirmed the subtype 1a. These findings could be explained by a mixed infection of subtypes 1a and 1b, by a possible A/G polymorphism existing at nucleotide -99 in some HCV isolates, or by an infection with a recombinant form 1a/1b. The same phenomenon had also been observed by others.

Mixed-genotype infections may be more common than previously reported given the typical routes of HCV infection and the inadequate sensitivity of most genotyping assays to detect them. Among HCV-infected Canadians, mixed genotypes have been found in 8% of HCV-positive blood donors, 14% of patients with chronic hepatitis C, and 17% of thalassemia patients who had received multiple transfusions. The capacity for the first generation of hybridization-based HCV genotyping to correctly identify mixed genotypes is poor. The need for HCV genotyping assays able to accurately detect mixed infections is warranted by the appreciable occurrence of such infections and their potential impact on the patient response to antiviral treatment.

Although not frequent, but probably increasing due to the hybrid generation in multiply exposed individuals, the recombination forms limit the accuracy of genotyping assays when only a segment of the genome is analyzed. The spread of the naturally occurring HCV recombinant forms might eventually invalidate the entire current concept of HCV genotyping which is essentially founded on the intrinsic assumption that the genotype and subtype inferred from one region represents the genome as a whole.

For the purpose of current treatment
management. 5’UTR based genotyping assays are acceptably accurate since they have been shown to present more than 95% concordance with genotypes identified by nucleotide sequencing. Assigning correct genotypes and subtypes to HCV specimens is however important for several research purposes, including epidemiological, phylogenetic, and natural history studies. The rather poor subtype recognition of 5’ UTR-based techniques for HCV typing was shown to impair the outcome of studies on HCV epidemiology. Furthermore, results obtained by these assay formats will not enable the proper choice of new antiviral compounds like inhibitors of the NS3-serine protease of HCV, which are likely to show distinct activities against isolates belonging to different subtypes of HCV genotype 1.

Typing of HCV isolates became increasingly important during recent years and is today routinely performed in many laboratories throughout the world. Genotyping methods based on hybridization represent an attractive option compared to sequencing methods. Incontestably, Versant HCV Genotype 2.0 Assay (LiPA) demonstrates better performance than its predecessor, especially for the subtyping of genotype 1 samples and the characterization of genotype 6, due to the addition of core motifs, which provide a useful complement of information. Versant HCV Genotype 2.0 Assay (LiPA) provides a rapid, sensitive, and accurate means of HCV genotyping and can be used as a routine tool to distinguish between the different HCV genotypes and subtypes. Clinical laboratories must continue to rapidly adopt new technologies capable of improving HCV test performance and efficiency, as HCV genotype determination will likely continue to play an important role in anti-HCV treatment algorithms.

References


