Journal of Virological Methods 167 (2010) 205–207

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

Short communication

A multicenter evaluation of the Abbott RealTime HCV Genotype II assay

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Article history:
Received 31 December 2009
Received in revised form 16 March 2010
Accepted 22 March 2010
Available online 31 March 2010

Keywords:
HCV genotype
INNO-LiPA
Real-time PCR

A B S T R A C T

Genotype determination is recommended before starting anti-HCV therapy to determine the duration of treatment (PEG-Interferon + ribavirin). The Versant HCV Genotype 2.0 assay, based on the reverse hybridization of the 5′UTR segment and core region of hepatitis C virus (HCV), has been one of the assays used most widely for HCV genotyping. A multicenter evaluation of the more automated Abbott RealTime HCV Genotype II assay was carried out on 124 HCV positive sera tested previously with the Versant HCV Genotype 2.0 assay. There was good agreement between the two assays. Type concordance was 95.9% (117/122) while concordance at the subtype level for genotype 1 was 95.6% (43/45). The Abbott RealTime HCV Genotype II assay is automated, allowing a substantial reduction of time-to results and hands-on time. The combined features of full automation, objective interpretation and digital archiving make this assay useful in a diagnostic setting.

1. Introduction

Hepatitis C virus (HCV) is an important cause of chronic liver disease with the risk of progression to cirrhosis and hepatocellular carcinoma (HCC) (Hnatyszyn, 2005). Antiviral treatment is provided to avoid these complications, but the rate of success is influenced by several biological factors including the viral genotype. It has been shown that HCV genotypes 1 and 4 require longer treatment than genotypes 2 and 3 when treated with pegylated alpha interferon and ribavirin (Hnatyszyn, 2005). Therefore, HCV genotyping, measurement of viral load and liver fibrosis at the time of diagnosis are used to determine the duration of antiviral therapy and to predict the response to treatment (NIH Consensus Statement, 2002). HCV is classified into seven genotypes and each genotype is composed of multiple subtypes (Simmonds et al., 2005; Kuiken and Simmonds, 2009). While determination of the HCV genotype is sufficient to reach a clinical decision, HCV subtyping is important for epidemiological studies and to trace the source of infection. Differences among genotypes/subtypes in relation to pathogenicity are not clear and have not been proved fully. The only subtype difference of potential clinical relevance concerns genotype 1. A meta-analysis of 57 relevant publications (Raimondi et al., 2009) suggests that HCV genotype 1b plays a major role in the development of HCC and the risk is higher during the early stages of liver disease. In an observational study carried out on a large group of patients, sustained virological response was achieved in a larger proportion of patients with genotype 1b compared to genotype 1a, while there was no difference between genotype 4a and genotype 4d (Legrand-Abravanel et al., 2009).

Currently, one of the assays used most widely for HCV genotyping has been the Versant HCV Genotype 2.0 assay (INNO-LiPA HCV v.2.0, Siemens Healthcare Diagnostics, Eragny, France, afterwards known as LiPA v. 2.0) which is based on the reverse hybridization of the 5′UTR segment and core region. The core region capability of LiPA v. 2.0 was added to improve the accuracy of the assay when used to classify the subtypes of genotypes 1 and 6 (Verbeeck et al., 2008). A new real-time PCR based HCV genotyping assay has been developed recently by Abbott Molecular (Abbott Laboratories, RealTime HCV Genotype II assay, Des Plaines, IL, USA). This latter assay targets the 5′UTR region, which is highly conserved between HCV genotypes, and the NS5B gene for efficient discrimination between HCV genotypes 1a and 1b. The assay uses a minor groove binder (MGB) technology. Probes with MGB groups form extremely stable duplexes with single-stranded DNA targets thus allowing increased mismatch discrimination. The assay uses three reaction mixes with HCV oligonucleotide probes bound to three different reporter dyes (FAM, VIC, and NED) and a fourth dye (Quasar 670) assigned to a heterologous internal control (pumpkin gene). This design allows an accurate discrimination of HCV types 1 to 6 and subtypes 1a and 1b.

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doi:10.1016/j.jviromet.2010.03.017

Table 1
Comparison of the Abbott RealTime HCV Genotype II and Versant HCV Genotype 2.0 assays at type level (subtype for genotype 1).

	LiPA									
	1	1a	1b	2	3	4	1a + 3a	5	IND*	TOT**
Abbott										
1			2							2
1a	2	16	1				1			20
1b		1	27						1	29
2				33					1	34
3					17					17
4			1			17				18
5								1		1
1b + 3			1							1
1a + 4		2								2
TOT	2	19	32	33	17	17	1	1	2	124

* Indeterminate.
** Total.

2. Material and methods

2.1. Abbott RealTime HCV Genotype II assay

In this study, a multicenter evaluation of the Abbott assay was carried out in three Italian laboratories on 124 serum samples from hepatitis C infected patients tested previously with the LiPA v. 2.0 assay. The samples were run on the *m*2000 system, a platform capable of automated RNA extraction and PCR set-up, followed by amplification/detection.

The samples showing discrepant results between LiPA v. 2.0 and RealTime were re-amplified with primers encompassing the NS5B region (Laperche et al., 2005) and sequenced using the Beckman Coulter CEQ 8000 Genetic Analysis System (Fullerton, CA, USA).

3. Results

Reportable type results with both assays were determined for 122 out of 124 (98.4%) samples with two samples indeterminate by the LiPA v. 2.0 assay. Type concordance was 95.9% (117/122) (95% confidence limit: 92.4–99.4%).

Due to the different subtyping capabilities of the two assays, concordance by subtype is given only for genotype 1. A subtype was determined by both assays in 45 out of 49 (91.8%) genotype 1 samples (four samples, two with Abbott and two with LiPA provided only a type result).

Excluding the four unsubtyped samples from this assessment, concordant subtype results were found for 43 out of 45 samples. A subtype concordance of 95.6% was then reached (95% confidence limit: 89.5–100.0%). The kappa statistic for 1a/1b agreement is 0.905 (CI 95%: 0.777–1.00).

Only one sample had a discordant type result (Abbott 4, LiPA 1b). This sample was resolved as type 4 subtype 4a after sequencing and phylogenetic analysis. The type 4 subtype 4a result was also compatible with the Kurdish origin of the patient. There were four mixed results, three with Abbott (two 1a + 4 and one 1b + 3) and one with LiPA v. 2.0 (1a + 3a). In all cases the comparison test showed a single genotype 1 infection. The results are summarized in Table 1.

4. Discussion

Genotype determination is recommended before starting therapy in order to apply the most appropriate therapeutic regimen, since HCV genotype is predictive of the response to therapy. There has not been clear evidence, as yet, that HCV subtype has an impact on the choice of and response to treatment (Zein, 2000; Legrand-Abravanel et al., 2009).

Currently patients carrying HCV genotypes 2 and 3 require 24 weeks of therapy, while those with genotypes 1 and 4 require 48 weeks of treatment. Improved treatment response rates have been observed with genotypes 2 and 3, as compared to genotypes 1 and 4.

LiPA v. 2.0 has been the assay used most widely for HCV genotype determination. In this study, the performance of the LiPA v. 2.0 assay was compared with that of the more automated Abbott assay. Overall, the Abbott assay showed a good agreement with LiPA v. 2.0 at both the type (95.2%) and subtype (95.6%) level for genotype 1.

When excluding the two undetermined samples with the LiPA assay and the four genotype samples unsubtyped by both methods (two with LiPA and two with Abbott) the overall concordance (including subtype concordance for genotype 1) is 111/118 (94.6%; 95% confidence limit: 89.8–98.3%).

The earlier LiPA v. 1.0 assay, based only on the amplification of the 5′-untranslated region, failed to discriminate between subtypes 1a and 1b in many circumstances. The recent addition of the Core region for genotype 1 improved its capacity to discriminate between subtypes 1a and 1b and between genotypes 1 and 6 (Bouchardeau et al., 2007).

It has been reported in a previous study that the Abbott assay failed to identify correctly the HCV genotype 1 subtype in approximately 10% of cases (Chevaliez et al., 2009). In that study most of the incorrect Abbott RealTime results were not due to mistyping between 1a and 1b subtypes, but rather to a genotype 1 call without further subtype assignment. Indeed, out of 493 reportable results (excluding samples with indeterminate results, PCR failure or with insufficient volume) there were 30 genotype 1 cases with no subtype reported results (6.01%). Although a far lower number of genotype 1 samples were analyzed by us, the agreement with LiPA v. 2.0 (95.6%) for genotype 1 subtype determination suggests a somewhat better discrimination. In this analysis, both the LiPA v. 2.0 and the Abbott test gave the same number of type 1 unsubtyped results (2/59 = 3.4%). The seeming difference between the two studies may be explained by the fact that the final subtype in the Chevaliez study was assigned based on the result of the NS5B sequence. However, it should be noted that the main object of this study was limited to comparing the performance of two commercially available assays for HCV genotyping rather than to use the NS5B sequence as a reference sequence to assign the HCV genotype.

Mixed infections were observed in four samples, three with Abbott and one with LiPA. The clinical and pathogenic significance of mixed infection is still unclear. Some investigators suggested that infection by different HCV genotypes may be an important factor in the acute exacerbation of HCV-related chronic hepatitis (Kao et al., 1994). In this study it was not possible to confirm the pres-

ence of all mixed infections, by cloning and sequencing, because of the lack of additional serum from these patients. However, when serum volume was available, the sequence analysis confirmed the results obtained with the Abbott assay as in the case of the two samples with an indeterminate result with LiPA. The viral load in these two samples was 193×10^3 and 68×10^3 IU/ml, respectively. So, the indeterminate result obtained by LiPA was not due to a low viral load but rather to the appearance of non-specific bands on the strips.

In order to gain an insight on the practical aspects of workflow, a time-motion assessment based on a run of 24 samples, including run controls, was undertaken. The time-to-result was 9 h, 39 min and 6 h, 8 min for LiPA v. 2.0 (QIAcube from QIAgen was used for extraction) and Abbott, respectively. Hands-on times requirement of 3 h, 1 min and 38 min, were found for the LiPA v. 2.0 and Abbott assays, respectively.

The Abbott *m*2000 system, besides providing an increased level of automation for HCV genotyping, runs other viral assays such as HBV-DNA, HCV-RNA and HIV-1 RNA viral loads. Consolidation is a crucial aspect in today's instrument-crowded virology laboratories, allowing streamlined workflow and easier staff turnover. Additional *m*2000 benefits, compared to the LiPA v. 2.0 technique, include an objective interpretation vs a subjective strip read-out and the availability of results recorded digitally. As a further safeguard against interpretation issues, the accuracy of the real-time signal is double-checked against an embedded proprietary algorithm based on mathematical principles (maxRatio) (Shain and Clemens, 2008).

This study bears some limitations due to sample size and the lack of resolution of all discrepant results, especially in regard to potential mixed infections. While the degree of concordance with the LiPA v. 2.0 assay observed here suggests that the Abbott RealTime Genotype II is suitable for current clinical practice, further evaluations are needed to ascertain–as it would appear from this preliminary study – if it can play a role in future clinical practice, when accurate 1a vs 1b discrimination will be demanded by new antiviral agents. Taken together, the data showed that the Abbott RealTime HCV Genotype II assay is in good agreement with the Versant HCV Genotype 2.0 assay and is suitable for current clinical practice. The automation on the Abbott *m*2000 system makes the Abbott RealTime HCV Genotype II assay particularly useful for diagnostic laboratories.

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