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Accuracy of Versant HCV Genotype 2.0 (LIPA) to determine hepatitis C virus genotype and subtype

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Background: Hepatitis C virus (HCV) geno-subtyping is currently crucial for the antiviral treatment choice. The wider and more experienced used technique for years was VERSANT-HCV Genotype (LIPA), which 2.0 version was improved including the analysis of 5' 5'UTR and Core. Other commonly used techniques are real-time PCR (RTPCR) and Sanger/NGS NS5B sequencing (gold standard). The objectives of this study are: 1) Comparing LIPA 2.0 genotyping test versus NS5B sequencing in a prospective manner in order to evaluate the accuracy of this test to determine genotype/subtype. 2) Meta-analysis of HCV genotyping test evaluations previously published.

Material/methods: 376 serum samples were analysed by LIPA 2.0 test according to manufacturer's instructions during a 5-month period. Specific primers for NS5B partial amplification and sequencing (Sanger) were used. Phylogenies were performed with MEGA 7.0 software using neighbour Joining test at 1000 repetitions, including reference sequences.

Results: 1) 368 from 376 (97.8%) could be genotyped using LIPA 2.0 test: A total of 173 from 368 NS5B sequences were obtained corresponding to 1a (n=53); 1b (n=57); 2a (n=3); 2i (n=2); 2j (n=1); 3a (n=35); 4a (n=2); 4d (n=18) and 4f (n=2). Matching of HCV genotype was 100% in all cases among LIPA 2.0 and NS5B sequencing. Genotype 1 and 3 Subtypes (1a, 1b and 3a) were identified with high accuracy (100% of targets). Subtypes were detected correctly in 17% and 86% of genotypes 2 and 4 respectively. 8 from 376 (2.2%) were LIPA 2.0 indeterminate (not able to determine a genotype). Sequencing results were as follows: 4f, 5a, 1b+4a, 1a, 1b+4d and 1b. The remaining two had negative PCR amplification.

2) Meta-analysis of literature: Six studies[1-6] comparing genotyping methods to NS5B sequencing were selected. 0.58% of samples (2/345) were not genotyped by LIPA 2.0 while 3/380 (0.78%) were not genotyped by RTPCR. Each of the techniques showed one incorrect genotype assignment. The ability of determining subtype information for genotype 1 was of 94.55% for LIPA 2.0 and 91.89 for RTPCR. Three publications[1,5,6], including a total of 252 samples, reported wrong subtype assignation for LIPA 2.0 (10/252). By contrast, the most extensive analysis[2] (including 500 genotype 1 samples) did not report any mistake on subtype assignation.

Conclusions:

• LIPA 2.0 test was 100% accurate to identify genotype and subtypes 1a, 1b and 3 in 97.8% of analysed samples, giving indeterminate results in 2.2% most of them corresponding to coinfections of two genotypes or non-common subtypes.

• Subtypes for genotype 2 were poorly differenced with LIPA 2.0, while genotype 4a-4d and 4f were correctly assigned in 86% of cases.

• The observed accuracy in genotype 1 subtyping is consistent with the most extensive analysis previously published

References: [1]Bouchardeau 2007; [2]Chevaliez 2009; [3]Mallory 2013; [4]Quer 2014; [5]Chen-Hua 2015; [6]Chueca 2016