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Letter to the Editor

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Comparison of AmpliSens® HCV genotype-FRT-g-1-6 PCR kit with Abbott® Real Time HCV genotype II assay for hepatitis C virus genotyping

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Sir,

The World Health Organization (WHO) estimates the global seroprevalence of hepatitis C virus (HCV) to be 3% [1]. Although most antiretrovirals are currently HCV pangenotypic, the clinical importance of genetic variability in HCV is due to the fact that certain HCV genotypes are associated with low response to treatment. The HCV genotypes 3b, 4r, and 1l have shown more difficult-to-cure populations [2]. Knowledge of the HCV genotype can be used to inform treatment options, even in the era of pangenotypic direct acting antivirals. According to European recommendations, it may be of clinical interest in special populations at risk of a particular subtype (*i.e* 1l, 4r, 3b, 3g, 6u, 6v, between others) shown to be less sensitive to treatment, or in patients for whom re-infection is suspected [3]. There are eight different HCV genotypes identified by sequencing methods. Messina *et. al* conducted a meta-analysis and estimated that HCV genotype 1 was the most prevalent genotype worldwide, with 83.4 million cases (46.2% of all cases), of which one-third are found in East Asia. The HCV genotype 3 was the second most prevalent globally (54.3 million, 30.1%); HCV genotypes 2, 4, and 6 accounted for 22.8% of all cases, and HCV genotype 5 only comprised the remaining <1% cases. While the HCV genotypes 1 and 3 are in the most countries, regardless of economic status, the highest proportions of HCV genotypes 4 and 5 are found in low-income countries [4]. In Spain, a retrospective study from 1997 to 2019 showed that 1.29% of hospital admissions included HCV as a diagnosis with the HCV genotypes detected 1b, 1a, and 3 [5,6].

In a retrospective cohort study, we compared the detection by real-time polymerase chain reaction (RT-PCR) of HCV genotypes with the AmpliSens® HCV genotype-FRT-g-1-6 PCR kit (Ecoli Dx, Buštěhrad, Czech Republic), an update of the AmpliSens® HCV-1/2/3-FRT kit including 1a, 1b, 2, 3a with 4, 5a, and 6 HCV genotypes. In our study we used as reference the Abbott® Real Time HCV genotype II assay (Abbott, Chicago, USA) including HCV target and 1, 1a, 1b, 2, 3, 4, 5, and 6 HCV genotypes. AmpliSens® assay including Core, 5´-untranslated region (5'-UTR), and NS5b HCV targets. However, the Abbott® assay only includes 5'-UTR and NS5b regions [1a (NS5b), 1b (NS5b), 1, 2, 3, 4, 5, 6 (5'-UTR)].

For this purpose, we used the AmpliSens® kit for screening 80 positive HCV samples of different patients from 2016 to 2022. Samples were kept properly frozen at -80°C during this period.

For both assays, RNA extraction was performed in 400 μL of serum with internal control using an EMAG™ extractor instrument (BioMérieux®, Marcy l´Etoile, France) following the manufacturer's instructions. The HCV genotypes detected with AmpliSens kit were amplified using CFX96™ Touch Real-Time PCR Detection System thermal cycler (Bio-Rad®, Hercules, California). However, the HCV genotypes using the Abbott® assay were amplified using an *m*2000*rt* Real Time System (Abbott, Chicago, USA).

We tested 80 HCV genotypes, including 26 type-1a, 20 type-1b, 16 type-4, 7 type-3a, 1 type-2, and 10 mixed genotypes classified by the Abbott® assay. The correlation for the non-mixed and mixed HCV genotypes detection was 97.2% and 22.2%, respectively between the two assays (Table 1). In our series, the Abbott® assay did not detect any HCV-genotype in two samples. These genotypes were classified as HCV genotype 3 as confirmed by Sanger sequencing analysis at the National Centre of Microbiology (Majadahonda, Madrid, Spain). However, the AmpliSens® assay detected both HCV genotypes 3a.

In relation to mixed HCV genotypes, two out of nine patients were detected with the same mixed HCV genotypes by both assays: one patient was positive for HCV genotypes 1a + 2, and another patient was positive for HCV genotypes 3a + 4. In the remaining seven patients, the AmpliSens® assay detected only one of the two HCV genotypes detected by the

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Table 1 **Correlation of HCV-genotypes detected between Abbott® HCV-genotype-FRT-g-1-6 and AmpliSens® Real Time HCV genotype II assay and percent of correlation (%) (n=80)**

Non-mixed HCV-genotype (n=71)	Abbott® RealTime HCV Genotype II assay	AmpliSens® HCV Genotype-FRT-g-1-6
1a	27	26
1b	20	21
2	1	1
3/3a	5	7
4	16	16
Total non-mixed HCV-genotypes	69 (97.2%)[a]	71 (100%)
Mixed HCV-genotype (n=9)	1a + 2	1a + 2
	3 + 4	3a + 4
	1a + 4	4
	1a + 1b	1b
	1a + 4	1a
	1b + 4	1b
	1a + 1b	1b
	1a + 1b	1b
	1b + 4	1b
Total mixed HCV-genotypes	9 (100%)	2 (22.2%)[b]

[a]Two 3 HCV-genotypes not detected by Abbott® assay: 27 Abbott® vs. 26 AmpliSens® (1a), 20 Abbott® vs. 21 AmpliSens® (1b), 5 Abbott® vs. 7 AmpliSens® (3/3a).

[b]Seven mixed HCV-genotypes not detected by AmpliSens® assay.

Abbott® assay. The AmpliSens® kit did not detect four HCV genotypes 1a, and two HCV genotypes 4 in the mixed samples studied (Table 1). Sohn *et. al* reported that Abbott® assay could not detect all strains in mixed HCV genotype 1 and 2 samples [7]. However, in our study, the Abbott® and AmpliSens® assays detected one mixed HCV genotypes 1 + 2.

Mutlu *et. al* reported for Abbott® Real Time HCV genotype II assay some misclassified of HCV genotypes 1a for 1b [8]. However, we found only one discrepancy for HCV genotypes 1a/1b between the Abbott® and AmpliSens® assays. One HCV genotype was classified as HCV genotype 1a by Abbott® assay and as HCV genotype 1b by the AmpliSens® assay. Sequencing analysis was not performed on these samples.

Yang *et al.* demonstrated that the Abbott® assay had limitations for detecting HCV genotype 6 in Southeast Asia [9]. The different HCV genotype targets represent an important limitation of the HCV Abbott® assay for HCV genotype 6 detection. Mohamed *et. al* reported that AmpliSens® kit did not able to discriminate the HCV genotypes 6c and 1, which can be mistyped as HCV genotype 1/1b because of sequence homology [10]. Although in our study we did not detect any HCV genotype 5 or 6 for any assay due to the HCV genotype epidemiology in Spain, neither of the HCV genotypes screened were misclassified as HCV genotype 6 using the AmpliSens® assay.

From an economic perspective, both tests have a similar price per determination. In terms of technical difficulty and time requirements, the Abbott® assay contains an HCV target that allows the verification of RNA amplification. Moreover, Abbott® assay imply a single-step RT-PCR with autointerpretation, while the AmpliSens® assay requires a first PCR to obtain cDNA, and a second RT-PCR for HCV genotyping, implying a higher risk of contamination, equipment to be used, and turnaround time.

Our limitations were the retrospective analysis, the lack of availability of Sanger sequencing analysis to confirm the discordant results, differences in multiplexing capability, sensitivity of each assay for different genotypes, and degradation of HCV RNA over time.

In conclusion, both RT-PCR assays showed good correlation for detecting non-mixed hepatitis C virus genotypes. Based on our experience, we consider that the most suitable assay for routine clinical microbiology laboratory use would be the Abbott® assay due to the one-step RT-PCR, auto-interpretation of results, and better detection of HCV mixed genotypes. The AmpliSens® assay allowed the detection of the non-detected HCV genotypes 3 by Abbott® assay but showed worse results for the detection of mixed HCV genotypes. In the case of use the Abbott® assay and not obtaining amplification of any HCV genotype, Sanger sequencing is necessary to rule out the detection of genotype 3. Further prospective studies using WGS are necessary to corroborate these results, especially for mixed HCV genotype analysis.

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CONFLICT OF INTEREST

Authors declare no have conflict of interest.

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