Author’s response to reviews

Title: Comparison of Sanger sequencing for Hepatitis C Virus Genotyping with a Commercial Line Probe Assay in a tertiary hospital

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Author’s response to reviews:

Sir,

Would you please consider the submission of our revised manuscript, “Comparison of Sanger sequencing for Hepatitis C Virus Genotyping with a Commercial Line Probe Assay in a tertiary hospital” (INFD-D-18-01576). We responded to the requests of the reviewer as specified in the attached document.

We feel this paper should now be acceptable for publication in BMC Infectious Diseases.

Yours sincerely

Sylvie Goletti
Reviewers comments to the author:

Reviewer’s comment: C

Author’s response: R

Reviewer 1:

C1: The authors have performed phylogenetic analysis using NJ and UPGMA. As far as NJ is concerned, the authors do not describe which model has been employed to calculate distances (Kimura 2P, Tamura Nei?). Moreover, the UPGMA method is obsolete and not applicable in phylogenetic tree construction of HCV sequences. Authors should use an alternative method either maximum likelihood or Bayesian inference of phylogeny.

R1: We agree with the reviewer’s comment, we had information about the model used to calculate distances (method section page 6 lines 34-35). We also added a maximum likelihood approach in place of the UPGMA method (method section page 6 lines 29 to 33 and Figures 1 to 3).

C2: There is no statistical validation for the groups in the phylogenetic tree. Authors should perform bootstrapping (at least 1000 replicates) and present the significant results (i.e. results more than 75%).

R2: A statistical validation was used, Branch support was obtained by approximate likelihood-ratio test (aLRT, SH-like) (a likelihood based alternative to the computationally intensive bootstrapping (method section page 6 lines 32-33 and Figures 1 to 3).

C3: Authors should present distance weighted phylogenetic trees and not UPGMA trees. Bootstrap values should appear on trees so as to assess the significance of various groupings.

R3: We fully agree with the reviewer's comment made the modification on Figures 1, 2 and 3.

C4: Based on Table 4, there are many samples with discordant genotyping between various HCV regions; for example sample HCV004 classifies as genotype 1b in 5' UTR region and genotype 6
in core region. How the authors explain those discordant results? Recombination? Mixed genotype infection?

R4: The discordance was explained by the fact that 5’UTR sequencing is not able to make a good distinction between genotype 1 and genotype 6 unlike the core sequencing.

C5: Sample HCV075 has been assigned to three different genotypes ie 1/3/6 whereas on 5'UTR tree seems to be classified as genotype 1b. Authors should check again the table and correct errors if any.

R5: The classification of sample HCV075 change according to the tree. On the 5’UTR tree, we can’t make a differentiation between genotype 1 and 6.

Reviewer 2:

C1: The authors report to have constructed phylogenetic trees using neighbour-joining and UPGMA methods. However, the latter implies that the tree that can be explained by minimum evolution, is the so-called 'best' tree, a statement that we know is incorrect. I would rather advise the authors to include a maximum-likelihood phylogenetic analysis, but certainly not UPGMA.

R1: We agree with the reviewer’s comment and added a maximum likelihood approach in place of the UPGMA method (method section page 6 lines 29 to 33 and Figures 1 to 3).

C2: Related to the phylogenetic analysis, is the representation of the trees. The authors state that the 5’UTR region does not contain sufficient phylogenetic signal to distinguish the different HCV genotypes/subtypes, but that core and NS5B region do. I do not agree with this conclusion, as you clearly see in Figure 2 that, although the different HCV subtypes are separated, the inter-genotypic relations are not correctly represented. For instance, HCV subtypes 1a and 1b belong to the same HCV genotype, however in the tree inferred from the core region, they are not clustering together. The latter is however correctly represented in Figure 3, using nucleotide information from the NS5B region. For samples for which more than one genetic region is sequenced, the authors could consider to concatenate fragments, to increase the phylogenetic signal in the tree.

R2: We change our conclusion: As shown in the analysis of phylogenetic trees, the sequencing of the NS5B region allows for better discrimination of HCV genotyping and subtyping, the sequencing of the core region gives results close to those of NS5B sequencing, the 5’ UTR
region is the least recommended of the 3 regions due to low discrimination. Discussion section page 12 lines 21 to 25.

C3: To end, I highly recommend the authors to improve their visualisation of the phylogenetic trees. The choice of colours is not the best, but more importantly I would certainly remove the genetic distances that are presented on the branches. In general, it is more appropriate to visualise the bootstrap support values, to show the correct diversification of the tree in HCV genotypes. Also, instead of having a separate legend, I would label the respective clades on the tree itself.

R3: We agree with the reviewer’s comment and modify the presentation of the trees (Figures 1, 2 and 3).