Reviewer’s report

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

Version: 0 Date: 25 Oct 2018

Reviewer: Mark Hopkins

Reviewer's report:

The authors present an interesting study which looks at the compatibility of LiPA amplicons for downstream sequencing protocols to resolve indeterminate HCV genotyping results. I agree with the authors that these protocols would be of interest to diagnostic laboratories using the LiPA system. However, the manuscript would benefit from clearer presentation of the data and a focused discussion of the outcome, especially how the data support implementation of these protocols in routine practice. It is not clear what the author is recommending. I think the manuscript requires revision but hope this does not deter the authors from pursuing publication.

General points:

Methods

Please define how you are determining "true" genotype of the LiPA indeterminate samples. If discrepant analysis is not required, please justify.

There is no reference to optimisation of the sequencing protocols. Please state if there is supporting data for these protocols elsewhere.

Analysis of third party reference material, including some reproducibility testing would strengthen the dataset.

Results

The data is presented as a single table containing all results. This is interesting but could be supplementary data if there is lack of space. The manuscript needs a summary of this so the reader can quickly compare how the sequencing assays performed. Possibly using a table by genotype and LiPA result with of the number of samples successfully genotyped by each of the three sequencing regions.

Either within this table or as a separate table there could be a sub-analysis of the LiPA indeterminate results which were successfully resolved by each region, especially since this is the primary aim.
Explain discrepancies between the methods and highlight which individual or combination of protocols is better at resolving the genotype.

Are the viral loads known for the samples? Are you able to state anything about the sensitivity of each assay?

Discussion and conclusions

Within the text it is not clear if you think these methods are comparable or one is better than another. Or do you recommend both are run simultaneously on indeterminate samples? You could state your proposed algorithm for dealing with indeterminate samples, especially if this has been implemented in the Belgian reference laboratory, and how many results you expect this to resolve. It may be clearer to structure the results to fit with the algorithm you are proposing.

Specifically

Abstract line 24 - Is misleading stating all the samples were characterised by the LiPA assay but it seems only 65% gave a genotype? Please clarify.

Background line 29 - the statement conflicts with line 4.

Results line 5 - see comment for abstract line 24.

Results line 15 - Only 64 samples were sequenced by UTR and core. Why was this so low? Was viral load a factor or is it likely interference of the two amplicons in the sequencing reaction? Potential reasons should be raised in the discussion.

Results line 16 - why were 77 samples analysed for NS5B? Did 23 not amplify or were these not attempted.

Table 1 - Please cite the source of these oligonucleotides.

Table 2 - states that sequencing enabled discrimination of all 27/100 samples not identified by LiPA yet table 2 contains 2 samples which have a LiPa result only. Therefore the comparison is of 98 samples? Also the footnotes "not realized" and "undetermined" require clarification.
Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Unable to assess

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

Quality of written English
Please indicate the quality of language in the manuscript:

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