

Point-by-point response to Reviewers

Reviewer #1:

General comment: The availability and performance of rapid and accurate tests for HBV and HCV levels in serum a sample is of high clinical and economic importance. The authors compared the relatively new, rapid, and affordable test NeuMoDx96 with the well-established, but slow and expensive COBAS test from Roche. They tested 186 patient samples with HBV or HCV infection.

1. Unfortunately, the preselected patients with known results from the Roche test are insufficiently described. Thus, it is not clear whether only easy to diagnose cases or difficult cases were also included. An independent determination of the limit of detection is missing.

Reply: As suggested by the reviewer, a clear definition of true positives and true negatives taken for both HBV and HCV assay has been mentioned in the manuscript, Briefly, out of 99 samples for HBV assay, 49 confirmed HBV infected (HBsAg, anti-HBc and HBV DNA positive) were taken as **HBV positive samples** and confirmed HBV non-infected (HBsAg, anti-HBc and HBV DNA negative) were taken as **HBV negative samples**. Similarly, out of 87 samples for HCV assay, 39 confirmed HCV infected (anti-HCV and HCV RNA positive) cases were taken as **HCV positive samples** and confirmed HCV non-infected (anti-HCV and HCV RNA negative) were taken as **HCV negative samples**. (Page no.2, Lines 36-39; Page no.5, Lines 105-113)

We agree with the reviewer that an independent determination of limit of detection is missing, but we have included a set of clinical samples with a broad range of known viral load (Range: 10^1 to $> 10^6 \log_{10}$ IU/ml) which would help in determining the overall analytical sensitivity for both HBV and HCV assays.

2. The formal statistics of the data looks at the first glance correct, but a closer look reveals inconsistencies.

Reply: As per suggestions, statistics has been rechecked in the revised manuscript. (Page No. 9 Lines 224-228, Page No.7, Lines 166-174 and Table 3).

3. The text contains several inaccuracies which need to be corrected.

Reply: We thank the reviewer for their kind observation. Suggestive changes have been incorporated in the main text (**Page No.2, Lines 36-41, Page No. 5, Lines 107-113, Page No. 7, Lines 165-174, Page No. 8, Lines 185-193, Page No. 8, 9 and Table 3**).

4. Abstract. “For HBV, out of 99 samples, 49 (49.49%) were DNA positive. For HCV out of 87 samples, 39 (44.82%) were RNA positive.” The authors should state how the diagnosis of HBV and HCV infection was established. Easiest would be: “Out of 99 HBsAg positive samples 49 (49.9%) were DNA positive, out of 87 anti-HCV positive samples were 39 (44.8%) were RNA positive.” However, the role of anti-HBc should not have been neglected.

Reply: As per suggestions, diagnosis of HBV and HCV infection has been mentioned in the revised manuscript (**Page No. 2 Lines 36-39; Page No.5 Lines 105-113**). We agree with the reviewer on the role of anti-HBc. We have included the same in the abstract and the revised manuscript (**Page No. 2 Lines 36-39; Page No.5 Lines 105-113**).

5. Introduction. “Moreover, viral hepatitis accounts for around 96% of mortality in low and middle income countries.” But there is one word missing: hepatitis mortality. According to reference 1, WHO writes: The report focuses on hepatitis B and C, which are responsible for 96% of all hepatitis mortality?

Reply: We thank the reviewer for pointing this out. We have edited the text in the revised manuscript (**Page No. 3, Lines 74-75**).

6. Introduction. The statement on the sensitivity and specificity of the new test are unsatisfactory. Does, e.g., 95.6% specificity mean that 4.3% of the positives are false positive? Or was the comparator assay false negative?

Reply: We have modified the manuscript accordingly with the correct sensitivity and specificity (**Page no. 7, Lines 161-162, 179-180**).

7. Materials and Methods. The authors forgot to report the selection criteria for HBV and HCV see also point 1. Figure 1 is not helpful in this respect.

Reply: As per the reviewer's query, text in the manuscript has been modified accordingly (Page No.2, Lines 36-39, Page No 5, Lines 105-117, Page No. 6, Lines 149-154). We have removed the Figure 1 as the same has been described in the main text now (Page No. 5, Lines 105-117, Page No. 6, Lines 149-154).

8. Table 1 should show the clinical data for HBV and HCV patients separately and somewhere in the legend or a footnote it should be described what was the basis for the diagnosis HBV and HCV. The description in the results should also be extended. a. Were the cases exclusively chronic HBV infections (CHB)? b. How many had active hepatitis, how an inactive HBV infection? "The data of table 1 are not helpful. "Non-cirrhotic" may still be an active CHB. c. Were any occult HBV infections included? d. How many cases had received an antiviral HCV therapy?

Reply: Since this was lab-based retrospective study on previously tested archived once thawed samples, the clinical data available were: For HBV- HBsAg, anti-HBc and HBV DNA, For HCV: anti-HCV antibody and HCV RNA. Therefore, based on these, the samples were classified as "true positives" and "true negatives". As per the reviewer's suggestion, the table has been edited accordingly (**Table 2**).

9. Methods. Comparison of assays 1 and 2. The technical data of the two tests are well compiled in the text, but a well-designed table would allow for better comparison.

Reply: We thank the reviewer for this suggestion. A table has been added in the revised manuscript (**Table 1**).

10. Results. The data of table 2a and fig. 2a on HBV DNA do not agree.

- a. The table shows three cases with $>10^6$ IU/ml HBV DNA for assay 1 and two cases for assay 2. But the figure shows 6 dots for $\log \text{ IU/ml} > 6$ for assay 1, and 5 dots for assay 2.
- b. The numbers seem to agree for the mid-range of $\log \text{ IU/ml}$ 3-6 for both assays.
- c. I could not exactly count the dots for the low range 10- 10^3 , but there are less than 31 or 29 dots in the figure than the 31 cases of the table 2a.

Reply: We thank the reviewer for improving the manuscript with the above mentioned queries. (**Page No. 9, Lines 224-229, Table 3**)

11. Results. The dot at zero in fig. 2a cannot be meaningful, because the LOD of assays is 0.9 or 1.5 and not 0 log IU/ml (i.e., 1 IU/ml).

Reply: Since we analyzed both the HBV positive and negative samples for linear regression analysis, the dot at zero in the figure 2a indicates HBV negative samples tested on both NeuMoDx and Cobas platforms (No viral load, Log value is 0) (**Figure 1a and 2a**).

12. The main text must be revised or must explain the inconsistency.

Reply: We appreciate the reviewer for improving our overall manuscript. Necessary edits have been done in the revised manuscript. The overall inconsistencies have been revised

13. I could not find table 2b in my file.

Reply: We have included the Table 2 in the revised “Table” word document (86345-Table). There is no “2b” now with respect to table 2.

14. Correlation between the two assays in detection of HBV viral load. “Assay 1 and Assay 2 quantified 99 samples ...” a. This statement is wrong because only 49 samples were positive. A negative result cannot be quantified.

Reply: We understand the reviewer’s concern here. We actually meant that the for HBV assay, all 99 samples were analysed for HBV DNA levels to confirm true HBV positives and true HBV negatives for the assay. This statement has been edited in the revised manuscript (**Page No. 7 Line 166**).

15. The correlation between the two assays is indeed very good, but fig. 2a and b suggest that assay 2 slightly overestimates low values <4 log IU/ml and underestimates higher values in comparison to assay 1, as suggested also by the medians.

Reply: We agree with the reviewer’s observation. Indeed, the correlation was good, but a slight overestimation and underestimation of samples between both the real-time PCR platforms is possible. We have discussed the same in the “discussion” section of the revised manuscript (**Page No.9, lines 224-229**).

16. Correlation between the two assays in detection of HCV viral load. The authors write: “... a very good correlation ($R^2 = 0.957$) was observed between the two assays (Fig. 3a).” However, the correlation was not as good as with HBV. Fig. 3a and 3b identify 3 outliers

with far too low values in the Roche assay. Thus, the correlation is at best good, not very good.

Reply: We agree with the reviewer's comment. The manuscript has been edited accordingly (Page No. 8, Lines 186-187).

17. Performance of the NeuMoDx 96 in different genotypes of HBV& HCV. Since the author have the sequence of the HBV and HCV samples, the sub genotypes could also be identified. E.g., HBV sub genotypes A1 and A2 are very different and may occur in India.

Reply: We agree with the reviewer's comment. The revised manuscript now contains the sub genotype information also (Page No. 6, Lines 149-154).

Reviewer #2: Major Comments:

1. Are there controversies in this field? What are the most recent and important achievements in the field? In my opinion, answers to these questions should be emphasized. Perhaps, in some cases, novelty of the recent achievements should be highlighted by indicating the year of publication in the text of the manuscript.

Reply: As per the reviewer's suggestions, we have edited the manuscript accordingly (Page No. 9, Lines 219-223 Page No. 9-10, Lines 230-243).

2. The results and discussion section is very weak and no emphasis is given on the discussion of the results like why certain effects are coming in to existence and what could be the possible reason behind them?

Reply: As per the suggestions we have modified the results and discussion sections completely. (Page No. 9, Lines 224-229).

3. Conclusion: not properly written.

Reply: We have updated the conclusion section in the revised manuscript (Page No.10, Lines 249-254).

4. Results and conclusion: The section devoted to the explanation of the results suffers from the same problems revealed so far. Your storyline in the results section (and conclusion) is hard to follow. Moreover, the conclusions reached are really far from what one can infer from the empirical results.

Reply: As per suggestion, the results and conclusion section is revised (Page No. 7, Lines 159-164, 177-181 Page No. 10 Lines 249-254).

5. The discussion should be rather organized around arguments avoiding simply describing details without providing much meaning. A real discussion should also link the findings of the study to theory and/or literature.

Reply: We have revised our discussion section accordingly (Page No. 9, Lines 224-229).

6. Spacing, punctuation marks, grammar, and spelling errors should be reviewed thoroughly. I found so many typos throughout the manuscript.

Reply: We are grateful to the reviewer for overall improving the manuscript. We have carefully gone through the manuscript and edited it accordingly to avoid any inconsistencies.

7. English is modest. Therefore, the authors need to improve their writing style. In addition, the whole manuscript needs to be checked by native English speakers.

Reply: As per suggestion, we have improved the overall writing and flow of the manuscript.

Answers to Editorial comments

Comment 1: We are very pleased to receive your revised manuscript (No. 86345). However, there are some questions that need to be addressed. -----1. Please provide specific point-to-point replies to each reviewer's comments (according to reviewers' comments)

Answer: The authors have already provided "point-to-point response" file along with the revised manuscript submitted on 19th July 2023. We have attached again the same for your reference.

Comment 2: Any article describing a study (basic research and clinical research) involving human and/or animal subjects is required to have the institutional review board (IRB) name, whether institutional (part of the author(s)' academic/medical institution, such as the Oak Grove Children's Hospital Institutional Review Board) or commercial/independent/private (contracted for-profit organizations, such as the ClinicCare Coalition for Human Rights Institutional Review Board), stated explicitly on the title page. Please provide the approval file of Institutional review board, and state it on the page.

Answer: The authors have provided the institutional review board statement and the approval file as suggested by the editor.

Comment 3: In order to attract readers to read your full-text article, we request that the first author make an audio file describing your final core tip. This audio file will be published online, along with your article. Please submit audio files according to the following specifications: Acceptable file formats: .mp3, .wav, or .aiff Maximum file size: 10 MB. -----Please reply within seven days, thank you! Only one file is available in F6publishing system, please upload all files with zip format, or send to me by email (y.l.chen@wjgnet.com).

Answer: The authors have provided the audio file (mp3 format) regarding the core tip. We have uploaded all files in zip format too.

Comment 4: For manuscripts submitted by non-native speakers of English, the authors are required to send the manuscript to a professional English language editing company or a native English-speaking expert to polish the language further. When you submit the subsequent polished manuscript to us, you must provide a language certificate along with it. Please visit the following website for the professional English language editing companies we recommend: <https://www.wjgnet.com/bpg/gerinfo/240>.

Answer: We thank the editor for this suggestion. The authors have edited the manuscript extensively, which has improved the overall writing of the text. We have used freely available online grammar-checking tools to further improve the manuscript language