REBUTTAL:

Reviewer 1

Major comments:

1. **Comment from the reviewer 1:** ”Thermo Fisher TaqPath™ COVID-19 assay kit was a multiplex diagnostic solution that contains both the assays and controls needed for the RT-PCR detection of SARS-CoV-2 viral RNA. Thermo Fisher TaqPath™ COVID-19 assay kit targets S gene, N gene, ORF1ab gene of SARS-CoV-2 and MS2 gene (internal control). Approximately, 6.3µLof extracted RNA was added to 2µL of 1X Taqpath 1-step multiplex Master Mixture (mix) (Thermo Fischer Scientific, Pleasanton, CA, USA), 0.3µL of the probe and 2µL MS2... When the internal control was negative, the results were considered invalid due to the inefficiency of the RNA extraction method. Figure 1 illustrates a summary of three alternative RNA extraction methods compared to the Thermo Fisher PureLink™ kit

   Internal control MS2 of the Thermo Fisher TaqPath COVID-19 assay kit used in many steps of this study was not used correctly. The MS2 bacteriophage (not gene) control need to be added to the samples before extraction of the RNA. Here, the addition of the control during the RT-PCR step could allow PCR inhibitor detection but do not evaluate the inefficiency of the RNA extraction method leading to misinterpretation of some results.

   Response: We apologize for the confusion. The MS2 was added to patient samples **before** the extraction except for housekeeping control when the MS2 was only added **during** RT-PCR step. The changes are made in the methods section (page 4).

2. **Comment from the reviewer:** The number of samples is weak for this kind of study. It can be sufficient for the RNA extraction comparison but it is too weak to establish sensitivity and specificity of a test. In addition, PCR were only performed once, it is sufficient to establish diagnosis but not for a valuable Ct value comparison.

   Response: We acknowledge that the sample size was small and hence this was added as a limitation (page 16).

3. **Comment from the reviewer:** The Chi square statistic test used during the extraction methods comparison is not adapted for paired data.

   Response: Changes were made. We apologize for the word error (page 7).

4. **Comment from the reviewer:** I don’t found any information about both Lucigen lysis buffer and Bosphore lysis buffer on corresponding website. It is their manufacturer names? One problem for nasopharyngeal samples is the possible presence of inhibitors. What is the impact of these reagents in inhibitors removal?

Response: Noted. Please see the changes are made in the manuscript (page 4 and 14).

5. **Comment from the reviewer:** Start-up cost for SARS-CoV-2 extraction methods are confusing. This price includes extraction kit, reagents and consumables but how many samples can be processed with this start up cost? Sample price depend to the same variables but very different number can be observed by comparing both startup and sample price.

   Response: Noted. The section for start-up costs for SARS-CoV-2 extraction methods was removed from the manuscript because it does lead to confusion Start-up cost represents the overall cost of reagents, consumables and excludes equipment for the total number of samples which is the same as the cost per sample. The reason is that the cost per sample also includes reagents, consumables and excludes equipment in Table 6 (page 13).

Manor comments:

6. **Comment from the reviewer:** all the rpm have to be transformed in g

   Response: Noted. Changes were made (page 4 and page 5).

7. **Comment from the reviewer:** in §2.2.4 Correct both Epperndorf/Eppendorf

   Response: Noted. Changes were made (page 5).

8. **Comment from the reviewer:** in §2.4.2. PCLMD nCoV kit: What is the role of the PPM1, is it the positive control? Why three types of master mix?

   Response: Noted. This was addressed in the manuscript (page 6).

9. **Comment from the reviewer:** Figure 1 have no interest. She do not illustrates a summary of alternative RNA extraction nor highlights the comparison of alternative RT-PCR kits.

   Response: Noted. The figure was removed.

10. **Comment from the reviewer:** Table 1: PCLMD: the target gene for the detection of SARS is the N gene.

    Response: Noted. Changes were made on Table 4 (page 12)

11. **Comment from the reviewer:** Table 2: For MS2 gene / Lucigen lysis buffer: the difference between means is +0.8 instead of -0.8.

    Response: Noted. Changes were made on Table 1 (page 9).

12. **Comment from the reviewer:** Table 3: For MS2 gene / Lucigen lysis buffer: the difference between means is -0.04 instead of -0.4

    Response: Noted. Changes were made on Table 2 (page 9).

13. **Comment from the reviewer:** Correct SARS instead of Sars

    Response: Noted. Changes were made on Table 2 (page 9).

14. **Comment from the reviewer:** Table 5: Proteinase K instead of Protein K

    Response: Noted. Changes were made on Table 6 (page 13).

15. **Comment from the reviewer:** Figure 2: legend: “S” gene in italics and *Orf1ab* instead of *Orf*

    Response: Noted. Changes were made on Figure 2 and 3 (page 10).

16. **Comment from the reviewer:** Table 7: The running time indicated in the table seems overestimated when comparing to the time of cycles in the material and methods section and with time indicated by the manufacturer when available. Which part of the PCR were taken in account?

    Response: Noted. This was addressed in the manuscript on Table 7 (page 14).

17. **Comment from the reviewer:** “When comparing the cost of the Sonicator method (~0.18 USD per sample) to the Thermo Fisher PureLink™ kit (~2.96 USD per sample), there were more than a 60% price reduction with Sonicator methods, making the Sonicator method the cheapest method”. The price reduction between the two methods is more than 16 fold leading to a 94% price reduction.

    Response: Noted. Changes were made under the discussion section (page 14).