

Dear editor Despina Kalogianni,

Please find enclosed the revised manuscript entitled **“Development and application of an SPR nanobiosensor based on AuNPs for the detection of SARS-CoV-2 in food surfaces”**.

We thank you for managing our article. The manuscript has been severely reviewed in light of the referee's comments. We also thank the reviewers for their extremely useful assessment of this article. Our detailed responses are provided below and demonstrate that all comments significantly improved this work. Responses to the referee's comments and changes are highlighted in red for clarity. We hope these changes prove satisfactory, although any additional suggestions are welcome.

Reviewer 2

SPR nano biosensor was developed for the detection of SARS-CoV-2 using AuNPs functionalized with oligonucleotides in food surfaces. The manuscript is well organized but exists some shortcomings. I could not advice to publish the study on the journal with the current version. The manuscript needs a major revision. Here are some questions and advice in the following that may be useful for the manuscript.

Response: We would like to thank you for doing constructive remarks on the manuscript. All of the comments and suggestions together with the observations from the other reviewers helped to improve the scientific level of our article.

1. The text in figure is not readable. This should be change with best image. Prefer to TIFF file.

Response: Thank you. The text of the figures has been enlarged to improve its visualization. In addition, all figures were saved in TIFF format, with a resolution of 600 dpi.

2. Please mention the company name, address etc. of each equipment like you mentioned RNeasy 133 Mini Kit information.

Response: Thank you for the suggestion. We mention in detail all equipment and reagents, and we highlight in red the changes.

3. I will recommend the author to discuss in detail about the stability and shelf life of the mentioned SPR biosensor.

Response: Thank you for the comment. The shelf life of the SPR biosensor was discussed in lines 218- 221.

4. Why BST.2.0 is selected? Why not 3.0 and others? Justify it

Response: Thank you. We reproduced the exact protocol by Zhang et al., 2020 and Aoki et al., 2021. We were afraid that changes in these protocols could interfere with the normal amplification yield. So, we followed the sequence of the primers, and the BST polymerase and master mix were purchased from the same manufacturer.

5. How about the density of the AuNPs? How much dense they are? As the numbers of nanoparticles affect on the binding and detection.

Response: The density of the solution found in the vial was 1 g/mL. The number of nanoparticles present in the solution influences the intensity of the color generated after aggregation and consequently detection.

6. I appreciate if you convert the LOD in terms of copies/reaction.

Response: As suggested, the LOD was converted in terms of copies, lines 37, 275 and 366.

7. Explain the LOD in terms of better way, e.g., provide statistical analysis

Response: It is therefore evident that statistical methods for assessing and comparing limits of detection are of importance. In general terms, the limit of detection of an analyte may be described as that concentration which gives an instrument signal significantly different from the 'blank' or 'background' signal. This description gives the analyst a good deal of freedom to decide the exact definition of the limit of detection, based on a suitable interpretation of the phrase 'significantly different'. There is still no full agreement between researchers, publishers, and professional and statutory bodies on this point. Taking into mind, we estimated the LOD using Figure 6 in which the concentration of 1.0 ng.mL^{-1} was significantly different from the 'blank'. We added some pieces of these sentences in the manuscript, Page 7.

8. Explain the LOD in terms of FDA guidelines. Is this biosensor being good for commercialization and scalability?

Response: We understand that the FDA guidelines determine the LOD estimative assuming values set to 3-time standard deviations above the blank response by linear instrument response. However, as said above, we estimated the LOD according to the spectrums of Figure 6, with a concentration of 1.0 ng. mL^{-1} that was significantly different from the 'blank'. Currently, this short communication focused on announcing the applicability of food samples, and in the next study, we will verify its suitability for commercialization and scalability.

9. Figure 7 (c) scale bar is not readable

Response: Figure 7 (C) has been reformulated with an increase in the scale bar to improve visualization.

10. All figures should be readable and clear for the readers.

Response: Thank you for the observation. All figures were saved in TIFF format, with a resolution of 600 dpi.

11. How about the detection time? Also explain this parameter of a sensor.

Response: The system time for detection is 100 min (line 372). The detection time is a very important parameter, the goal is to design sensors that require less time than standard techniques. The standard PCR and ELISA techniques are considered time-consuming because they can reach from 3h to 9 days, and sensors that overcome this limitation are appreciable.