Reviewer’s report

Title: Label-free blood serum detection by using surface-enhanced Raman spectroscopy and support vector machine for the preoperative diagnosis of parotid gland tumors

Version: 3 Date: 16 June 2015

Reviewer: Alois Bonifacio

Reviewer’s report:

MAJOR COMPUSORY REVISIONS

In the revised manuscript, the authors did not adequately comply to most of the major compulsory revisions suggested in my review. Thus, the major compulsory revisions are almost the same present in the first original review. Some additional comments to the authors responses are outlined below:

1. EXPERIMENTAL METHODS

“Significantly” is too vague as a term. The authors must straightly state if the patients were treated or not. The fact that they were not treated “significantly” does imply that they were treated, but that the authors judge the treatment as not significant, which is a subjective assumption.

Moreover, they should also specify how they corrected the spectra for autofluorescence and noise (e.g. by subtraction of a 4th polynomial function and by a Savitzky-Golay smoothing).

2. DATA ANALYSIS

The authors claim they normalized all spectra before data analysis, and specified it in the revised version of the manuscript. They corrected the y-axis values in figure 3. However, the values in the y-axis spectra in figure 2 are still characteristic of un-normalized spectra. Why? In fact, the average spectra should be calculated out of normalized spectra, but apparently they aren’t.

Moreover, the authors decided not to comply with my indication about using a leave-one-patient-out cross validation method instead of a leave-one-sample-out. I do not agree with the authors on the fact that the spectra from the same patient can be considered as independent spectra. Treating such spectra as independent can lead to huge errors on the specificity, sensitivity and accuracy calculated. The number of patients involved in the study is enough to go for a leave-one-patient-out cross validation, and the authors should apply it to get consistent and realistic values for their diagnostic performance parameters (sens, spec, acc). I insist on this point as a compulsory revision for this paper.

3. COMPARISON OF THE SPECTRA REPORTED WITH THOSE AVAILABLE IN LITERATURE

Even when considering SERS of plasma (as for instance the work by Li et al.}
cited by the authors in their response) instead of serum, the differences between the spectra available in the literature and the ones presented in this manuscript cannot be explained by simply invoking the use of different baseline-subtraction method. The figure presented by the authors in their response, which compares the two spectra, clearly show big differences in both Raman shift and relative intensity of major bands (see my supplementary figure: red dashed lines are evidencing major discrepancies). Thus, background subtraction cannot be invoked as an explanation. The authors should explicitly mention such differences (stressing the differences, not the similarities) in the manuscript, trying to find an explanation, or if they can’t, explicitly stating that they do not have an explanation yet for such differences.

Moreover, as the author acknowledge that the band assignments are controversial (even more so considering that their spectra differ from those in literature), they should explicitly write so in the manuscript, when discussing band assignments.

4. LIMITS OF THE WORK

The authors adequately addressed the issue of discussing SVM limits, as well as pointing out the issue of sample size, stressing that their results are “preliminary”. However, they should clearly state the problem with the age matching directly in the manuscript, as expressed in their response. This might be a serious limitation of this study, as the changes observed might be due to age and not to the disease, and thus it must be put in evidence in the text.

MINOR ESSENTIAL REVISIONS.

None. The authors adequately addressed all the issues raised in the first review.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

'I declare that I have no competing interests