Reviewer's report

Title: In situ Molecular Identification of the Influenza A (H1N1) 2009 Neuraminidase in patients with severe and fatal infections during a pandemic in Mexico City

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Reviewer: Jeannette Guarner

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The concept of the study is interesting: use of biopsies which are usually obtained endoscopically and have little tissue to do a variety of tests that can help in the diagnosis of H1N1. However, there are multiple issues with the manuscript including: a) the very reduced number of cases, b) were the tissues studied really biopsies, and c) use of material that was on a slide rather than taking a sequential section for the PCR/sequence study.

Abstract:
1. (Minor essential revision) In the methods the word retrospectively is incorrectly placed.
2. (Minor essential revision) The first sentence of the results is actually a method.
3. (Minor essential revision) In which cells was the H1N1 genetic material found (bronchoepithelial, pneumocytes, alveolar macrophages)?
4. (Minor essential revision) Conclusions are redundant, do not repeat what is already in the results.

Background
5. (Major compulsory revision) The authors should concentrate on describing techniques that are used in tissue since that is what they studied. The Background could be focused by deleting diagnostic methods that are used in nasopharyngeal swabs and incorporating description of the methods used in tissues (immunohistochemistry of both seasonal and 2009 H1N1, in-situ hybridization).
6. (Minor essential revision) Can one reference the work that is being presented as a reference?

Methods
7. (Major compulsory revision) The authors state that these were biopsies which would make for an interesting manuscript since the pathology using autopsy lung tissue with extensive immunohistochemical localization of the virus and PCR has already been published. However, upon reading the manuscript it appears the tissue studied was obtained postmortem and in figure 3, P02 appears to be lung which is not usually obtained with biopsies (P01 and P03 could potentially be biopsies obtained endoscopically). The authors need to specify a) how the biopsies were obtained, b) when (pre or post mortem), c) what kind of biopsies
(central or peribronchial lung versus peripheral lung), and d) the size of the
tissues.

8. (Minor essential revision) Do the authors have autopsy material to compare
the results of their biopsies to that of the autopsy?

9. (Major compulsory revision) Negative controls should have included a
consecutive tissue section that was treated the same as the in-situ PCR but
without the primers. The authors do not mention if this was done. The authors
mention an interesting negative tissue control in which the detection antibody
was omitted but which is not useful to define non-specific “staining/ sticking” by
the primer.

10. (Minor essential revision) Were the slides counter-stained with hematoxylin?

11. (Major compulsory revision) The sections should have been reviewed by a
pathologists to define amount of non-specific staining, localization of signal (cell
type, area in the cell) and correlation with other histopathologic findings.

12. (Minor essential revision) The in-situ PCR procedure is not contained and
“completely clean”, why did the authors use the material from the slide? It would
have been a much cleaner PCR/ sequence if they had used sequential sections
cut using a “clean procedure”.

13. (Minor essential revision) Did the authors do all the assays presented in the 8
bronchial biopsies?

14. (Major compulsory revision) Figure 3 is of very poor quality and cytoplasmic
staining is not observed. The control present should have been a sequential
tissue section in which the primer was omitted, not a negative patient. The
authors should present higher magnification, better quality photographs.

Discussion

15. (Minor essential revision) The first paragraph of the discussion has too many
concepts with the authors talking about diagnostic techniques, localization of the
virus, epidemiology. There needs to be a focus.

16. (Major compulsory revision) The second paragraph should be deleted since it
mentions a correlation of viral load and patient outcomes that is not shown.

17. (Minor essential revision) The third paragraph talks about the cells infected;
however, the authors never describe them and figure 3 is so blurry that it is
impossible to define which cells are infected. In this paragraph, the authors again
discuss at length cytokines and chemokines which were never studied and have
little relevance to the study they performed.

18. (Minor essential revision) The authors’ summary paragraph talks about these
techniques being able to provide prompt and definitive diagnosis. At this point,
the majority of pathology laboratories do not perform in-situ PCR techniques
which makes the statement possible in only very few institutions.

**Level of interest:** An article of limited interest
Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I do not have any financial or other conflicts of interest.