Author's response to reviews

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

Authors:

Rodolfo Ocadiz-Delgado (wilox@cinvestav.mx)
Martha E Albíno-Sánchez (malbino@cinvestav.mx)
Enrique García-Villa (engavi@cinvestav.mx)
María G Aguilar-González (maguilar@cinvestav.mx)
Carlos Cabello (taiincq@yahoo.com.mx)
Dora P Rosete (Dorosete67@yahoo.com.mx)
Fidencio Mejía (biolfimene@yahoo.com.mx)
María E Manjarrez (e_manjarrez@yahoo.com)
Carmen Ondara-Aguilera (coa62@yahoo.com.mx)
Rosa M Rivera-Rosales (rosa.rivera48@yahoo.com.mx)
Patricio Gariglio (vidal@cinvestav.mx)

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Author's response to reviews: see over
Dear Miss Pangilinan,

Thank you for your letter dated March 2nd, 2012, concerning the manuscript: “In situ Molecular Identification of the Influenza A (H1N1) 2009 Neuraminidase in patients with severe and fatal infections during a pandemic in Mexico City” by Ocadiz-Delgado, et al. (MS: 5856165636464167). Considering the content of your letter and the enclosed suggestions of the handling Editor, we now submit a revised version in which we have made several changes. In addition, we include specific arguments for each reviewer’s comment, where we discuss and try to clarify, as much as possible, each point raised by the Editor. Our new version of the manuscript incorporates the changes bolded in the text. In addition, the quality of Figures was enhanced according to handling Editor’s suggestions.

We really appreciate all suggestions since they greatly improved our manuscript. We hope that now you would find our paper suitable for publication in your important Journal.

Thank you in advance,

Sincerely yours,

Ma. Eugenia Manjarrez Zavala, PhD
Departamento de Investigación en Virología,
Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas.
Calz. De Tlalpan 4502, Colonia Sección XVI, CP 14080
Mexico DF, Mexico
Tel. 54 81 17 00-5123
E-mail: e_manjarrez@yahoo.com
Alternative E-mail: wilox@cinvestav.mx
Handling Editor:
There a number of topics that need to be addressed before the manuscript can be sent for peer review:

1) Title: The described method detects neuraminidase-gene derived nucleic acid of influenza A virus, not the NA enzyme. The title should be changed accordingly.

Answer to Point 1:
According to Editor’s comment, we have changed the title of our manuscript to: “Retrospectively in situ identification of the Neuroaminidase-gene derived nucleic acid of Influenza A (H1N1) 2009 virus in patients with severe and fatal respiratory infections during the pandemic outbreak in Mexico City”

Handling Editor:
2) Page 6: There is hardly any information on the identity / institution of the ethic committee that approved the study. Please insert more details into the manuscript.

Answer to point 2
In page 6 we inserted more details on the Institution of the ethic committee.

This project was approved after being checked by the “Institute Science and Bioethics Committee” (INER-Mexico). The Committee is responsible for evaluating the research projects to be performed, in order to monitor that every study meets the principles for research involving human subjects, established in the Declaration of Helsinki and its different revisions.

Handling Editor:
3) Page 13: If the authors wish to make any conclusions about the relationship of their patient viruses to other strains, it is necessary to perform phylogenetic analyses in addition to give only degrees of sequence identity. Also, it is difficult to envision that a sequence from a patient has the strongest identity with the lab strain A/PR/8/34 ! This needs to be clarified as it suggests a contamination with the positive control.

Answer to Point 3:
We are agree with Editor so we only made a suggestion about the possibility of relationship of Mexican patients with different variants. According to this, we have included the following paragraph (Page 16, line 4):

“Although the primary goal of this study was to validate the in situ RT-PCR detection of NA influenza viral gene, we thought that these data may suggest that Mexico City could have played an important role for the dissemination of some variants throughout the world as indicated by the match between the Mexican samples included in this study and sequences described in other Countries such as.....”

On the other hand, since some years ago we have been performing molecular diagnostic of Influenza in hundreds of patients (manuscript in preparation), including those mentioned in
this manuscript. Fortunately to date, we do not have contaminations with different positive controls (we have used both clinical samples and viral nucleic acid obtained from *in vitro* cell cultures). In order to clarify, as much as possible, this point raised by the Editor we have included the following paragraph (Page 14, line 5):

“In all experiments, negative Influenza-like illness patient [Neg ILI (-)] and positive (H1N1 Puerto Rico/Puerto Rico/IvPR8/Puerto Rico; 98% homology) controls were included. In order to avoid contamination and subsequent false results, we have performed sequence determinations at least in triplicate including a negative control without template.”

**Handling Editor:**
4) Page 15: There are no Figs 3A and 3B in the accompanying figures although the text says so.

**Answer to Point 4:**
We agree with this observation, the manuscript was already revised entirely and corrected this mistake (Fig. 3 instead Fig. 3A or Fig. 3B).

**Handling Editor:**
5) Page 15: Please insert the evidence that viral RNA is mainly detected in type I and II pneumocytes as stated in the text. No double-staining with suitable marker antigens has been performed and also no quantification has been done.

**Answer to Point 5:**
According with reviewer’s observation, we have included the following paragraphs:

(Page 16, line 19)

“As previously described by other groups, the AH1N1 influenza virus infects mainly Type I and Type II alveolar cells (ATI and ATII) [25-31].”

(Page 17, line 20):

“In summary, the identification of A (H1N1) viral sequences into type II pneumocytes in the respiratory tract [35-44], provides insights into the viral tropism of the main cell types found within the lung and may be relevant to understanding the pathogenesis of severe human influenza disease. Interestingly, in this study morphological analyses suggest that in situ signal was detected in pneumocyte-like cells; however, we have not used any cellular marker to identify these cell types, therefore, additional strategies will be necessary to confirm this issue.”

**Handling Editor:**
6) The quality of Figure 3 needs to be enhanced. In particular, I have some concern on the lower background stainings in the fields of the negative control (“Neg ILI(-)”), which appear to have been selectively reduced. Can you provide more convincing negative controls?
Answer to Point 6:
As reviewer suggested, we have improved the quality of images showed in Figure 3. In addition, we have included less contrasted photos of negative controls (please see new Figure 3).

Handling Editor:
7) Requesting copyediting before review: After reading through your manuscript, we feel that the quality of written English needs to be improved before the manuscript can be considered further. We advise you to seek the assistance of a fluent English speaking colleague, or to have a professional editing service correct your language. Please ensure that particular attention is paid to the abstract.

Answer to Point 7:
According with reviewer’s observation, Manuscript was improved after carefully editing of a fluent English speaking colleague. Our new version of the manuscript incorporates the changes bolded in the text.