Reviewer's report

Title: Vaccination with Hemagglutinin or Neuraminidase DNA Protects BALB/c Mice against Influenza Virus Infection in Presence of Maternal Antibody

Version: 3 Date: 8 June 2007

Reviewer: Ian Brett

Reviewer's report:

General
In general this is a very good revision that corrects grammatical errors and phrase misuse. The paper has a more logical flow and is much more readable. While this revision is a major improvement, some data still rests on a flawed technique, and thus the conclusions may not be sound.

Sincerely,
Ian C. Brett, BS
Countersigned
Bert E. Johansson, MD PhD

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1) Fluorescent NI-The authors have taken out the Potier and Deroo references for the fluorescent NI assay and replaced them instead with a paper by Sylte et al that uses the assay and references these two papers. This misses the point of my argument. Traditionally, the NI assay has been based on thiobarbituric acid/fetuin (REF). Any alternative method of measuring NI titers must compare the new method to this gold standard. Our lab and others have done this and found that the results are not necessarily correlative. Merely acknowledging that the titers would be falsely low and saying that another assay is being developed is unacceptable because these data are being used to compare vaccine groups and draw conclusions that may not necessarily be true.

There are three ways to make this acceptable.

A) Redo the serological experiments using the traditional thiobarbituric acid/fetuin based NI.
B) Publish the results of both assays and show that they correlate, either in this paper or in a separate paper.
C) Publish this paper with a few sentences acknowledging that the Fluorescent NI results may not be correlative and that this test has been shown to be unreliable.

The fact of the matter is that I believe that the trend that they are seeing is real—it makes perfect scientific sense. However, because some of the data that they are basing these conclusions on are drawn from an unreliable, unproven assay, the conclusions may not be valid. Furthermore, the fact that there is at least one published paper that indicates that the fluorescent-based NI assay is not useful, and that we have seen the same unreliability in switching the NI from fetuin-based to fluorescent-based; coupled with the fact that nobody has published a study comparing the two assays showing that they are interchangeable is grounds for questioning this data. I do not fault the authors for previously publishing in this manner—these facts were previously unknown. However, now that they are revealed, it would be irresponsible to continue to publish in this manner without at least an acknowledgment that these data, and therefore these conclusions may not be valid because of the lack of standardization of this assay.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Though the “2n” from the tables was explained in the ‘response to reviewers,’ it should be explained in the paper—perhaps in the table legend.

Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major
compulsory revisions

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

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

**Declaration of competing interests:**

I declare that I have no competing research interests, neither does Dr. Johansson. However, one or both of us have received monies for consulting or advising from companies who support influenza vaccine development in the past 5 years. None of these companies have a direct interest in this work, competing or otherwise.