Reviewer's report

Title: Rapid and Simultaneous Detection of Human HBV and HCV Antibodies Based on a Protein Chip Assay Using Nano-gold Immunological Amplification and Silver Staining Method

Version: 1 Date: 16 February 2005

Reviewer: Maria Martell

Reviewer's report:

General
The manuscript by Duan et al. describes the application of a protein chip assay that combines nano-gold immunological amplification and silver staining, for the simultaneous detection of human HBV and HCV antibodies. The data presented here follow on from previous studies by this group, having as the main objective the rapid, cheap and less time consuming diagnostic of viral hepatitis due to hepatitis B virus and hepatitis C virus. A visual and simple detection assay, like they propose, would be most useful for those places where the equipment needed to perform more sophisticated methods are not available. However, the data presented here seems incomplete due to an apparent lack of replicate experiments, proper quantification of signal intensities and statistical analysis.

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

Detection limit.
1. Figure 5 is too small and the quality of the picture is too poor to draw many conclusions from this version of the manuscript. What's the difference between "detection spot" and "positive spot"?
2. Anyhow, dot blots are notoriously difficult to reproduce. In Figure 5 it seems to be a visually apparent variability between hybridization signals from the same concentration replicates. How many times was this experiment repeated? Were the results reproducible? Coefficients of variation (CV), intra and inter assay, for all concentrations are needed to convince the reader that these results are reproducible and also that the anti-IgG as low as 3 ng/ml could be detected in their assay.
3. Also, does the proposed detection method have a linear dynamic range? A plot showing the hybridization signal intensities versus anti-IgG concentrations should be included.

Clinical validation.
4. A representative picture of the scanned array for at least one serum sample from each group needs to be shown, hopefully of better quality than the one shown for the detection limit. How many spots were printed on each array?
5. A table describing the different groups of serum samples would be also helpful for the reader. Being a simultaneous detection method for HCV and HBV, it would be interesting to include also some co-infected serum samples.
6. Tables 1a, 1b and 1c are confusing. It is difficult to understand what exactly are the authors comparing and how are they doing it. The simplest way one can imagine is a x2 test between the Protein chip assay (the observed results) and the ELISA test (the expected results). In this case does not a value of p<0.05 indicates a significant difference, in contrast with the authors' interpretation? The authors should make the tables more comprehensive and explain the statistical method used to compare the results.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
1. Page 6, line 14. Manually printed arrays (0.1 µl spots) can not be considered microarrays but
macroarrays or just arrays.
2. 2. Page 8, line 4. Transmission Electron Microscopy should be fully written before the first abbreviation.
3. 3. Page 12, line 11. The authors should either explain how the results of this study could have potential in proteomics research, or moderate their statement.
4. 4. Page 14, line 6. There is a mistake in reference 10: this paper corresponds to a Journal of Medical Virology.

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 whose findings are important to those with closely related research interests

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

Statistical review: No

Declaration of competing interests:

I declare that I have no competing interests