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

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Reviewer: Peggy Lymberi

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The manuscript by Duan et al. reports the development of an assay based on the use of nano-gold particles carrying Protein A (and immunogold silver staining), for the rapid and simultaneous detection of human HBV and HCV antibodies. The immunological reaction takes place on chemically-modified glass slides and the result is visible by naked eye. It is described as a sensitive and specific assay, convenient, less expensive and time-consuming than ELISA. The proposed methodology seems interesting and promising for both, research and diagnostic purposes. In the latter case, it needs further validation.

There are some corrections which should be addressed (Major Compulsory revisions):
1) In Abstract, line 16, the sentence “...the anti-IgG as low as...” is unclear, since it is not previously supported in the text.
2) The use of human IgG to detect anti-IgG in order to evaluate the detection limit of the assay, is not mentioned in Methods (origin, species, and full protocol must be added), but only in Results (on p. 9, line 13, anti-IgG) and in such a way that is not clear to the reader.
3) In Methods, p.8, the authors should give details about the commercial ELISAs they used (origin, principle, etc), since they perform a comparative study with their assay leading to important conclusions. Furthermore, it is not clear whether the authors chose only highly positive sera or borderline ones as well?
4) The authors do not explain why only one concentration (100 μg/ml) for the viral antigen coating on the chemically derivated slides was used (optimization data?). The same applies to the control IgG.
5) The Discussion should be modified. Clinical performance of the assay and comparison with classical ELISA are not being commented upon. The authors discuss almost exclusively on the preparation of optimum nano-gold particles and suitable antigen immobilization support.

Minor points
1) On p.7, the title “Detection of the serum samples...” should be replaced by “Detection of antibodies in patients’ sera...” or “Screening of patients’ sera...”.
2) Pages 7 and 9 (lines 18 and 19, respectively): “ELISA test protocols...” should be changed to “ELISA...”.
3) P. 11, line 23: the nine references in parenthesis could be replaced by “(25-33)...”.
4) In Results, the title “Comparison of test protocols between the protein chip assay and ELISA...” should be replaced by “Comparison of protein chip assay and ELISA...”, or “Comparison of serological findings obtained with protein chip assay and ELISA...”.
5) The authors should avoid the use of words such as “probes” or “hybridisation”, common in gene detection methodology, when referring to immunoassays.

The manuscript appears to have been written hastily and needs to be edited by an English-speaking individual prior to re-submission.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions.
Quality of written English: Needs some language corrections before being published