Author's response to reviews

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

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Referee 1

Major Compulsory Revisions:

As shown in figure 5d, the detection spots were clearly discriminated from the negative control spots while their intensity was similar with that of the positive control spots. The result was determined by comparison between the detection spots and the negative control spots on the same chip. In figure 6a, the intensity of the HBsAg spots (line2) was similar with that of the positive control spots (line1) while clearly discriminated from the negative control spots (line6). While the intensity of the HBeAg spots (line3), the HBcAg spots (line4) and the HCVAg spots (line5) was similar with that of the negative control spots and clearly discriminated from the HBsAg spots and the positive control spots. The same applied to figure 6b-d.

Figure 5 has been re-submitted and it is clearer than before.

Referee 3

1 In Abstract ('Methods'):

The sentence 'To determine the detection limit of the protein chip assay, a set of model arrays in which human IgG was spotted were structured and the model arrays were incubated with different concentrations of anti-IgG. 'is added between the sentence 'Colloidal nano-gold labelled staphylococcal protein A (SPA) was used as an indicator and immunogold silver staining enhancement technique was applied to amplify the detection signals, producing black image on array spots, which were visible with the naked eye.' and 'A total of 305 sera previously characterized with commercial ELISA were divided into 4 groups and tested in this assay.'

2 In Abstract:

The Results was re-structured and revised into 'Results: We prepared mono-dispersed, spherical nano-gold particles with an average diameter of 15+/-.2 nm. Colloidal nano-gold-SPA particles observed by TEM were well-distributed, maintaining uniform and stable. The optimum silver enhancement time ranged from 8 to 12 minutes. In our assay, the protein chips could detect serum antibodies against HBsAg, HBeAg, HBCAg and HCVAg with the absence of the cross reaction. In the model arrays, the anti-IgG as low as 3 ng/ml could be detected. The data for comparing the protein chip assay with ELISA indicated that no distinct difference (P>0.05) existed between the results determined by our assay and ELISA respectively.'

3 We have optimized the concentration of proteins coating on the chip. In our assay, we immobilized a series of concentrations of human IgG solutions on the chip. The result showed the optimum concentration for protein coating ranged from 50µg/ml to 150µg/ml. When preparing the chip, we immobilized the proteins with the concentration of 100µg/ml. As what we mentioned in the paper, we developed a protein chip technology based on NIASS method. The objective of our present work is the non-quantitative visual detection of HBV and HCV antibodies. Work is ongoing and we are researching the relationships between the concentrations of human IgG and the hybridization signal intensity. In our present assay, we have found
that the hybridization signal intensity is linearly dependent on the logarithm of anti-IgG concentrations. The research about the quantitative detection of the protein chip assay will be carried out in our next work.

Some language corrections have been made about the manuscript. And we have gone through the manuscript formatting checklist.