Answering Reviewers

Reviewer 1

Dear Author You have done good job. But I have few concern regarding submitted article, which is as follows: 1. Reference range of IgA tTG and IgG DGP is different for different serum samples. It appears that different kits are used for ELISA. Why it is not done by the kit of single manufacturer? 2. References needs to be critically revised as title of ref. 13 is missing, etc.

Conclusion: Minor revision

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Q.1 Reference range of IgA tTG and IgG DGP is different for different serum samples. It appears that different kits are used for ELISA. Why it is not done by the kit of single manufacturer?

Ans: Thanks for reviewing our paper very much.

Ans 1. The sample cohort analysed in this study has been collected and stored over the period of 5 years. During this time different available serological tests were used for testing, therefore, the different reference ranges.

Q.2 References needs to be critically revised as title of ref. 13 is missing

Ans 2. The title of Reference 13 has been added.

Reviewer 2

This article is about a new test for celiac disease using functionalized peptides with gold nanoparticles and uses alpha gliadin as a specific peptide for the diagnosis. In this paper, the specificity of this sensor has been investigated, but the precision of this sensor has not been compared with existing ones such as ELISA. Specificity with control samples and differences in color intensity were investigated (difference in color intensity in AuNP-peptide-AGA and AuNP-peptide-IgG, which did not change the color intensity of AuNP-peptide-IgG in comparison to the antibody

fraction, but in AuNP- peptide-AGA change) The detection of binding by the technique of the difference in color intensity indicates that when the antigliadin antibody binds to the antigen, the color intensity decreases as compared to the control sample and when there is no antigen. The antibody binding method is to the level of gold nanoparticles peptides using the avidin biotin method, which these two substances have the ability to interconnect. Gold nanoparticles peptides are first coated with NeutrAvidin, followed by Biotin- (PEG) 11-Maleimide, which adds biotin and avidin to each other, and then the anti-gliadin antibody is added to the resulting peptide. Samples of patients with celiac disease are found on the peptide and, by changing the color intensity of the binding of the antibody and the antigen. For clarity and simplicity, the nanoparticles can be coated with avidin for greater accuracy and clarity. Using the functional groups, the antibody was placed on the surface. To diagnose the sample, the patient labelled with a biotin-tagged antigen, labelled with a biotin binding to avidin and color changes, noticed the binding of the antibody to the antigen and the presence or absence of the disease. Also, this article claims to be the best method for diagnosis after mucosal biopsy for celiac disease, but has not provided sufficient reasons such as accuracy and cheapness and ... etc.

Conclusion: Major revision

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Question: This article claims to be the best method for diagnosis after mucosal biopsy for celiac disease, but has not provided sufficient reasons?

Ans: Thanks for reviewing our paper very much.

Line 13 in the conclusion section the following line has been changed as 'The developed assay has high accuracy levels and is relatively cheaper to develop, the assay format has potential to be adapted as point-of-care test that would be useful in an exclusion diagnostic strategy as positive result would strengthen the possibility of CD that can be confirmed using intestinal biopsy'.

Reviewer 3

Congratulations to the authors for beautiful work! The authors presenting an interesting study by determining spectrophotometric properties of gold nanoparticles coated with the peptide sequence reactive activities in celiac disease (CD). This novel work has a high potential in improving the diagnostic accuracy and

adding additional biomarker to CD' workup. Unfortunately this paper has been written in a unconventional fashion. The results is extremely long and the paper lack a classical structure like introduction, patients/methods/ results and discussion with proportional information. I suggest balancing the paper with limited material and methods/results. The results section should be shortened and contain the very essential information suitable for WJG readers. The rest can be submitted as additional supplemental files. The information related to patients is inadequate. I suggest adding a section lile patient and Methods were the patient can clearly discuss how the have selected their patients and controls (volunteers) and at which stage of diagnosis the test were accomplished. Other comments Again, why do we have to read about "villous atrophy" when no such process is involved! Why do authors persist in repeating these meaningless phrases and words [see Marsh et al, Gastroenterology 151: 784, 2016]. Likewise Oberhuber's changes have for many years been shown to be unworkable, and pretty useless in terms of diagnosis, treatment, or follow-up (Rostami K, et al. Gut 2017;66:2080-2086). In light of recent studies the Oberhubers classification lack scientific support and shouldn't be used.

Conclusion: Major revision

Scientific Quality: Grade A (Excellent)

Language Quality: Grade B (Minor language polishing)

Q. Methods were the patient can clearly discuss how the have selected their patients and controls (volunteers) and at which stage of diagnosis the test were accomplished?

Ans: Thanks for reviewing our paper very much.

1. Materials and method section for AGA assay in clinical human serum has been updated (line 13) as 'The histological interpretation and the serology levels for each of the clinical sample tested using biopsy and the existing commercially available serology tests is presented in Tables 1 and 2'.

Reviewer Comment: The results is extremely long and the paper lack a classical structure like introduction, patients/methods/ results and discussion with proportional information.

Ans. The results and discussion section have been separated into two sections along with the supplementary information.

Reviewer Comment: In light of recent studies the Oberhubers classification lack scientific support and shouldn't be used.

Ans: Introduction (line 18) the Oberhuber classification has been removed from the introduction and the reference 15 has been replaced with the one suggested by the reviewer (Rostami K, et al. Gut 2017; 66:20180-2086) that describes detailed role and importance of the intraepithelial lymphocytes in celiac disease diagnosis.

Reviewer 4

A novel screening test for celiac disease using peptide functionalised gold nanoparticles. The investigators have developed a novel and innovative process to screen for celiac disease. The process works by determining spectrophotometric properties of gold nanoparticles coated with the peptide sequence reactive in celiac disease. The investigators report a change to redder spectrum in reacting substrate. Although the authors mention the sensitivity and specificity of the method, I didn't notice any resulting values. The authors state - We next assessed the sensitivity and specificity levels of the test using serum samples spiked with AGA. What are the values? All cases of CD were medically diagnosed and based on typical small intestinal histology usually in conjunction with positive CD serology. Need better explanation. What was the serology and Marsh score of each patient versus diagnosis of CD? Results and Discussion - should be separate. A formal Discussion section is needed to compare and contrast the study with published works in the literature, with citations. Separate Results and Discussion sections are needed. Cites needed in the Discussion, with compare and contrast. Out of these thirty samples, fourteen samples that were diagnosed with active CD with high antibody titres as shown by serology and intestinal damage as per biopsy were identified as CD positive using AuNP-Peptide-AGA assay as well. What about the other samples? while 26 samples showed comparable results with existing serology and histology, What does comparable results mean? 2 false positive results and 2 false negative results were obtained using the AuNP-Peptide-AGA assay giving the AuNP-Peptide-AGA assay an overall accuracy of 86.6%. What were the existing serology and histology values/scores for these?

Conclusion: Major revision

Scientific Quality: Grade D (Fair)

Language Quality: Grade A (Priority publishing)

Q: What are the values of sensitivity and specificity in spiked serum?

Ans: Thanks for reviewing our paper very much.

1. Lines 6 and 9 in the sub-section Testing AGA in spiked serum in the results have been added.

Q: What was the serology and Marsh score of each patient versus diagnosis of CD?

2. Materials and method section for AGA assay in clinical human serum has been updated (Line 13) as 'The histological interpretation and the serology levels for each of the clinical sample tested using biopsy and the existing commercially available serology tests is presented in Tables 1 and 2'.
   The serology levels and histology interpretation of sample cohort is described in the Tables 1 and 2 in the manuscript.

Q: Separate Results and Discussion sections are needed.

3. The results and discussion sections have been separated as suggested. The discussion section contains cited literature.

Q: What about the other samples? while 26 samples showed comparable results with existing serology and histology, What does comparable results mean?

4. Line 59 under the discussion section has been revised as 'Out of the thirty samples analysed, fourteen samples that were diagnosed with active CD with high antibody titres as shown by serology and intestinal damage as per biopsy were identified as CD positive using AuNP-Peptide-AGA assay as well. These samples showed the formation of a precipitate and had a clear shift as well as drop in UV-Vis absorbance values as well as a high colorimetric response value (refer Table 1). The remaining samples were then

classified into various sub-classes based on the analysis using the AuNP-Peptide-AGA assay as described below (refer Tables 1 and 2, Figure 5)'.

Line 109 under the discussion section changed as 'The cohort included four samples identified as negative for CD based on biopsy and existing serology tests (volunteer numbers n.2, n.13, n.20 and n.21, Table 1). Out of the four samples, two samples were correctly identified as CD negative by AuNP-Peptide-AGA assay while the other two samples showed the formation of aggregates and were identified as positive (volunteer numbers n.2 and n.20). As intestinal biopsy has been used as the gold standard for CD confirmation, these two samples have been referred to as a false positive'.

Line 116 under the discussion section changed as 'Overall, upon comparing the results for the 30 clinical samples, while 26 samples were interpreted similar to the analysis using the existing serology and histology, 2 false positive results and 2 false negative results were obtained using the AuNP-Peptide-AGA assay giving the AuNP-Peptide-AGA assay an overall accuracy of 86.6%.