Name of Journal: *World Journal of Nephrology*
ESPS Manuscript NO: 24315
Manuscript Type: Diagnostic Advances

Title: Immunofluorescence on paraffin embedded renal biopsies: Experience of a tertiary care center with review of literature

Authors: Geetika Singh, Lavleen Singh, Ranajoy Ghosh, Devajit Nath, Amit Kumar Dinda

Response letter

Thank you for your valuable comments and we will try to address all the concerns raised.

Reviewer 1(00503282):
In this study, authors share their experience of immunofluorescence on formalin-fixed, paraffin-embedded (IF-P) renal biopsy tissue. They attempted IF-P on 246 cases and among these, 32 cases were excluded. In the end, 214 cases were analyzed. These were the case of GN. The authors conclude that IF-P can act as a salvage technique for the demonstration of immunoreactants in paraffin-embedded renal biopsies. However, there are potential caveats. The results are interesting and useful for renal pathologists in developing world. However, the paper can not be accepted in the present form. There are several major and minor points in the paper, which need correction as under:

1. The authors should explain the rationale for doing this study in the Introduction. Was it done as a pilot project, in parallel with IF-F or what??? Please add a para on this aspect of the study.
2. English language and punctuation needs careful correction throughout the paper.
3. The use of abbreviations is not standardized. Some abbreviations are not fully spelled out, eg. FITC.
4. Give percentage figures in brackets in Table 2 and 3.

Response:

1. As mentioned in the 3rd paragraph of the introduction, this study describes 'our experience' with utilizing the technique of immunofluorescence on enzyme digested formalin fixed paraffin embedded renal biopsy material, since its introduction into the laboratory. Comparison with routine immunofluorescence on fresh frozen material was possible in a small cohort of cases when both techniques were utilized, usually to confirm the IF findings in a case. The description of our experience, includes technical and interpretation issues faced by us and these pointers may be helpful to any laboratory planning to introduce this technique. (Line added to introduction)

2. English language and punctuation has been corrected.

3. Abbreviations have been spelled out.

4. Table 2 and 3 have been modified as suggested.

Reviewer 2 (00352969):
The authors present an interesting article on immunofluorescence on paraffin embedded renal biopsies. It is hard to see how it is really novel since so much of this has been done already; however, I suppose that seeing this work in their practice setting is a nice. They also provide a decent meta-analysis.

I have the following observations:
- I felt that the introduction was too brief and really didn't provide enough background. For example, there is only 1 reference in the introduction. I feel that more could be included here. They go on to have a pretty nice discussion, so maybe they could include some of the discussion in the introduction and also expand the introduction? Maybe I'm being too picky.
- It seems that some spaces are missing in places in the article. For example, there seem to be words that run together a great deal in the references and also some of the tables. Perhaps this is some sort of issue related to conversion from 1 file type to another.
- I realize that they at least partially provide it elsewhere in the paper, but I think that it would be nice if they provided more information regarding their methods in Table 1 (e.g., manufacturer, manufacturer location, titration, etc). I think that this table is nice and may be used as a reference by laboratories in the future; therefore, if Table 1 stands on its own as "recipe", then it could become a tremendous reference for other laboratories.
- I don't fully understand Table 3. It might be nice if they also provide a % of cases that had the given differences (i.e., intensity where the 2 methods were equal, difference of 1+, and difference of 2+). Do these differences pertain to a specific antibody (IgG, IgA, IgM, C3, C1q, etc.)? Alternatively, do these differences pertain to some overall average?
- A tally at the bottom of Table 4 might have been nice. For example, how many cases of each diagnosis have been tried? How many studies use each of the different reagents (e.g., pronase, etc.)? This is just a suggestion.
- It seems to me that they need to list the definition of some acronyms @ the bottom of Table 4 [as well as other tables]. I personally like tables to stand on their own, but maybe this is just me.
- I wish we knew more about how these reagents (enzymes, etc.) work. How do they expose the antigens? How gentle vs. how harsh? It would be nice if we were provided with a guide to this, but I realize that it would be difficult to complete such a comprehensive description. I realize that it would be difficult to do a head-to-head comparison since the tissue would be exhausted. The cost might be prohibitive also. Therefore, maybe the article stands as a nice description as it does now.

Response:

1. A line has been added to the introduction, however the authors feel that adding more to the introduction may interfere with the flow of the paper.

2. The spacing of letters has been corrected.

3. More details have been provided in Table 1.

4. The differences in intensity of staining between IF-F and IF-P pertain to the 'diagnostic antibody or complement'. For example IgA in IgA nephropathy or IgG in Membranous nephropathy. The table has been appropriately modified and percentages have been added.

5. Table 4 was envisaged by the authors as a chronological review of the literature on the use of immunofluorescence on enzyme digested tissue. A variety of enzymes have been used on a variety of glomerular and tubulointersitial diseases. Definitions of the acronyms used have been added as a footnote to the table.

6. As mentioned in the 2nd paragraph of the discussion the enzyme breaks the cross linkages formed by formalin, to expose the antigens on the extracellular immune complex deposits, which are recognized by the FITC labeled antibodies. No uniform protocol for enzyme digestion exists, as is evident from Table 4 and different laboratories use different concentrations and duration of enzyme for digestion.

Reviewer 3:
The paper of Singh et al shows the possibility to have a good option for IF with formalin fixed paraffin embedded tissue, that could be considered a useful 'salvage' technique in case of non-availability of representative fresh frozen tissue.
They shows also the limit to use this technique but as well it can be extremely useful where frozen tissue is not available.
The paper needs some language editing.

Response:
Appropriate language editing has been done.

To the Editor in Chief

1st August 2016

Name of Journal: *World Journal of Nephrology*
ESPS Manuscript NO: 24315
Manuscript Type: Diagnostic Advances

Title: Immunofluorescence on paraffin embedded renal biopsies: Experience of a tertiary care center with review of literature

Authors: Geetika Singh, Lavleen Singh, Ranajoy Ghosh, Devajit Nath, Amit Kumar Dinda

Dear sir,

Thankyou for your comments. Please find below the clarifications required.

1. Of the 246 cases on which IF on paraffin was performed **32 cases could not be interpreted due to technical issues** i.e. underdigested tissue or tissue floated off the slide.

2. OF the remainder 214 cases , in **9 cases with technically sound IF on paraffin** the **findings did not support or contribute to the light microscopic diagnosis** and were therefore **considered non diagnostic**, for example lack of IgG in case of Membranous nephropathy.

3. Table 2 descibes the findings in the 214 technically sound cases, including 150 cases listed with different diagnoses. In the row of IgA nephropathy, 64 cases are mentioned where the role of IF P was to exclude an IgA nephropathy. Thus the total matches the complete number of cases which were interpretable i.e. 214.

Thanking you

Best regards
Dr Geetika Singh