The **JOVE** Editorial Board

**Re: Revised manuscript**

                   Oct 9th, 2018

Dear Prof. Steindel,  
Thank you for all the efforts concerning our manuscript entitled “**Human serum anti-aquaporin-4 immunoglobulin G detection by cell-based assay**”. The comments from the reviewers have been most helpful in the revision of our manuscript. We have dealt fully with the comments from the reviewers and revised our manuscript accordingly, which is now re-submitted. We have specified the changes made in the manuscript (underlined), as well made point-to-pointresponses to the comments from the reviewers as follows:

**Response to editorial comments**  
“*Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues*.”

Thank you for the comment. We have proofread the manuscript carefully to avoid typos and grammar issues.

“*Please remove commercial language from the manuscript: Euroimmun, Tween, CellSens, etc. Please replace them with generic terms.*”

All the commercial language in the manuscript has been removed. However, as Tween is a chemical name instead of commercial name, it is not excluded.

*“Please revise the protocol to contain only action items that direct the reader to do something (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” Please include all safety procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible.”*

The manuscript has been revised accordingly.   
“*1.1: Please write the text in the imperative tense.”*

The statement has been revised accordingly (line 77).  
*“ 2.1.2, 2.4.1: Please list an approximate volume of buffer to be prepared.”*

The volume of buffer has been added to the protocol accordingly (line 111 and 138).   
*“Please provide the concentration of antibodies used in the protocol.”*

The manuscript has been revised accordingly (line 113 and 132).

*“Please include single-line spaces between all paragraphs, headings, steps, etc.”*

We have made sure to use single-line space.

“*After you have made all the recommended changes to your protocol (listed above), please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol*. *Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense*.”

The part of protocol for shooting has been highlighted.   
“*Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted*.”

The protocol has been highlighted accordingly.

*“Figures 3-7, Table 1: Please use a capital X to denote magnification. There should be no space between the number and the X (i.e., 4X, 10X, 20X).”*

Thank you for the suggestion. The table and figures have been revised accordingly.

*“References: Please do not abbreviate journal titles.”*

All the references have been revised.  
*“ Please revise the table of the essential supplies, reagents, and equipment to include the name, company, and catalog number of all relevant materials including software.”*

The table has been revised accordingly.   
*“Table of Equipment and Materials: Please provide lot numbers and RRIDs of antibodies, if available.”*  
The antibodies are included in the kit and the lot number is not available.   
**Response to comments from Reviewer #1**  
*“The protocol, line 101, the authors stated a wide range of the temperature for storing sera to maintain the stability of the samples. It would be great to specify the different acceptable periods of sera to store under -20 and -80 °C, for example, storage at ambient is acceptable for 72 hours, storage at -20 °C is acceptable to keep for months, and -80 °C is acceptable to keep for years.”*

Thank you for the suggestion. The protocol has been revised accordingly (line 100).  
*“The protocol, line 118, it was good that the authors noted "Do not touch the BIOCHIPs". Handling the slides before removing from the cover, for instance, holding the slide side by side, is also important and should be addressed.”*

This important tip has been added (line 118).   
*“The protocol, line 133, please review and confirm that it is necessary to cover the slides by dark box or foil. The advantage of the EUROIMMUN kit is it is unnecessary to cover the slides after adding secondary antibody.”*

The referred protocol has been modified (line 136).   
*“The protocol, line 138, it is recommended and practical to drop the mounting medium onto a cover glass rather than directly adding onto the BIOCHIPs. Then put the BIOCHIPs facing downwards onto the prepared cover glass.”*

Thank you for the comment. As the BIOCHIPs should face upwards for microscopy, it is more convenient to add mounting medium directly onto the BIOCHIPs. It works well.  
*“Discussion, line 234, multiple sclerosis so far is not proven to be exactly an autoimmune disease. Either immune-mediated or inflammatory disease would be appropriated in this context.”*

The description has been modified accordingly (line 236).   
*“Discussion, line 257-259, the statement authors mentioned is true for the "probable positive" result. Such a problematic result is difficult to judge. One possible solution would be repeating the CBA again.”*  
Thanks for the suggestion. The solution has been mentioned in the discussion section (line 259).  
**Response to comments from Reviewer #2**  
**Reviewer #2:**  
*“As the authors use the Euroimmun chip (from a commercial entity) it is unclear if the authors simply follow the manufacturer's instructions or have developed the implementation on their own. Thus, it is unclear if there are any IP/copyright issues.”*

Thank you for the comment. We have confirmed with editor that it shouldn't be a problem more or less following manufacturer's instructions. Firstly, although their BIOCHIP is special, the protocol, which is a widely used immunocytochemistry protocol, is not special. Such as ELISA, Western blot and cell culture protocols, everybody has a basic idea on how to perform the experiment. It is hard to avoid that some details need to be adapted to the instruction given by the distributor. Secondly, we do not simply follow the instruction, we have optimized and re-arranged the protocol.  
*“The paper needs to be seen by a native speaker.”*  
Typos and grammar errors have been carefully corrected.   
*“In how many patients and controls was the protocol validated? Was the assay compared with another detection method? How many false positives/false negatives have the authors detected?”*  
The detection has been performed on approximately 1,500 subjects (line 74). The assay was not compared with other detection methods. However, the conclusions from relevant comparison studies were summarized in introduction and discussion section.   
*“Several pertinent references are neglected or inappropriate. First sentence of the introduction: PMID: 26185772 applies here; "Anti-AQP4 is involved...." PMID: 28018943 applies here; Discussion/MRI findings: PMID: 28451627 and PMID: 25695963 apply here; Discussion: mention that testing for AQP4 abs in the CSF is not informative, see PMID: 27144221, PMID: 20825655; Discussion/MOG: different clinical phenotype and some recent work deserves mention, see PMID: 29724224, PMID: 30048919, PMID: 28105459; Discussion: mention titers may change with rituximab PMID: 28054001; Discussion: titers not predictive of disease course and response to immunotherapy, see and add PMID: 29055457, PMID: 28857723”*

Thank you for the comments. The referred parts of manuscript have been revised and the references have been updated (line 37, 42, 269, 261, 270 and 272).

*“"Sven et al. reported...." It must be "Jarius et al....”*

Thank you for the comment. The error has been corrected (line 64).   
*“1.1.1. optic neuritis: testing in any case is not recommended as the pre-test probability in an unselected cohort is low, see PMID: 20850793”*

Thank you for the comment. The description has been modified (line 80).  
*“2.5.1 any technical requirements for the microscope?”*

No special requirement is for the microscope.  
*“"blindly evaluated by at least 2 clinicians" any comment on the required experience of the grader?”*

The required experience of clinicians has been added (line 176).   
*“table 2: how many sera from nmo and other conditions/hc have the authors tested with this method?”*

As our detection is for diagnosis instead of for scientific researches, we do not perform titer analysis of anti-AQP4 IgG. We realized that the method was not verified by our diagnostic center. Table 2 and relevant description have been deleted.