Author's response to reviews

Title: A new method for determination of varicella-zoster virus immunoglobulin G avidity in serum and cerebrospinal fluid

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Author's response to reviews: see over
Submission of revised manuscript:
“A new method for determination of varicella-zoster virus immunoglobulin G avidity in serum and cerebrospinal fluid“

Dear Editorial Team,

The manuscript has been revised according to the comments of the reviewers. A detailed response is given below.

Sincerely,

Benedikt Weissbrich, MD
Response to reviewers and changes in the revised manuscript

Reviewer: Ilkka Seppala

Major revisions

- We appreciate that Dr. Seppala considers the use of urea hydrogen peroxide as denaturant in avidity assays a highly interesting claim. We have been using urea hydrogen peroxide successfully as denaturing agent in other avidity assays in our lab (unpublished). Therefore, we also used this reagent in preliminary experiments when we tried to establish a VZV avidity assay. Because urea hydrogen peroxide was superior to the urea washing procedure in these experiments, we used it further on and this aspect became a “by-product” of the paper. The main point we attempted to make with our paper is the introduction of a new calculation method.

The optimisation of the denaturing washing procedure is the most crucial step when establishing an avidity assay. However, because of the multitude of parameters which are involved (nature of denaturing agent, concentration of denaturing agent, number of incubations, incubation time), only a selection of conditions and a selection of samples can be optimised in a reasonable way. The final prove of the quality of the denaturing washing step is usually the distinction between acute and past infections by means of the avidity index. As shown in Figure 2, this aim has been achieved by using urea hydrogen peroxide in the VZV avidity assay. Therefore, our point is that urea hydrogen peroxide may be used as denaturing agent. However, we do neither claim urea would not have worked, nor that urea hydrogen peroxide is superior to urea in general. To make this clearer, we have modified the first sentence in the second paragraph of the discussion. Because of these limitations of our claims regarding urea hydrogen peroxide, we feel that the additional experiments proposed by the reviewer may not be necessary to support our findings.

As suggested, we have included the primary data of the preliminary optimisation experiment as Table 1. From the absorbance values obtained with denaturation, it is apparent that a 1 x 3 min 4 M urea hydrogen peroxide washing step is more
dissociating than washing 3 x 5 min with a 5 M urea solution.

- A graphic presentation of the new method has been added in Figure 4a for two examples.

The main difference between the new calculation method (virtual absorbance ratio) and the log-log line fitting described in Korhonen et al. (ref. 15) is that the new method is based on line fitting for an absorbance-absorbance curve, while the log-log line fitting is based on a dilution-absorbance relationship. As a result, the new method involves only one curve, while the log-log line fitting requires curve fitting for two titration curves.

Because the logistic model used by “Avidity 1.2” was superior to the log-log curve fitting in the paper by Korhonen et al., we didn’t evaluate the log-log curve fitting in our study.

We agree that the educated guesses for the variables a through d may influence the CV obtained with “Avidity 1.2”. The linear range of the CSF avidity assay extends to higher absorbance values than the linear range of the enzyme immunoassays used to establish “Avidity 1.2”. However, the use of the avidity values based on the least diluted samples does not uniformly increase the CVs for “Avidity 1.2”. For example, omission of the results from the dilution pair “1:1 & 1:8” will not minimize but increase the CVs of “Avidity 1.2” for all examples (Table 3 in revised manuscript). The issue that adaptations of “Avidity 1.2” may improve its CVs for the application in our study has been addressed in the last sentence of the second last paragraph in the discussion.

The CVs in Table 2 (Table 1 in original manuscript) are based on 5 - 6 dilutions per sample. This information has been added in the legend.

**Minor and discretionary revisions**

- We are not aware of previous publications on the use of urea hydrogen peroxide for the disruption of protein-protein interactions.
- Page 7, line 9: “from dilution series” has been added.
- “urea in high molarity” refers to solutions such as 8 M urea, which are also commonly used.
- “hydrogen peroxide” was not included in the title because it is not meant to be the major point of the paper.
- We have performed calculations with the assumption that factor c is zero. However, the results were inferior to the results based on the use of the experimentally determined factor c.
Reviewer: Oliver Liesenfeld:

1. For the routine determination of VZV-specific IgG in CSF, we have been using a VZV enzyme immunoassay with modified assay conditions similar to the ones described in the paper since several years. So far, we have tested more than 150 CSF samples of VZV-seronegative patients. All were negative for VZV IgG in the CSF assay. Therefore, there is no relevant impact on the specificity for VZV IgG detection by the sensitivity increase of the enzyme immunoassay.

2. Predictive values for our assay cannot be calculated from our data in a meaningful way, because we have selected samples of patients with acute and past infections. Therefore, the prevalence of patients with acute VZV infections in our data does not reflect the true prevalence of acute VZV infections.

3. A linear equation for a sample with the slope m and the intercept c can be calculated from the absorbance values of at least two dilutions. This can be performed by standard office software such as Excel (Microsoft) or by statistical programs using linear regression analysis. For evaluation purposes, we have used four or more dilutions per sample, but for routine use, two dilutions may be sufficient. These issues have been described and discussed in the method section (page 7), the result section (page 10) and in the last paragraph of the discussion (page 14).

4. The non-linearity of the assay at low absorbance values (without denaturation) is an important point. The lower limit of the linear range of our assay is approximately 0.200 OD. Dilutions with absorbance values lower than 0.200 in the reference assay (assay without denaturation), were not used for the calculation of the linear equations. This information has been added in the last paragraph of the method section (page 7).