Author's response to reviews

Title: Use of biokitHSV-2 Rapid Assay to Improve the Positive Predictive Value of Focus HerpeSelect HSV-2 ELISA

Authors:
Rhoda Ashley Morrow Ph.D. (rhoda.morrow@seattlechildrens.org)
David Friedrich BS (david.friedrich@seattlechildrens.org)
Amalia Meier Ph.D. (ameier@u.washington.edu)
Lawrence Corey MD (lcorey@u.washington.edu)

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We have revised our manuscript to respond to the comments of the reviewers as follows:

Comment 1 (Use of low positive reading control limits generalizability of the findings).
Response: We agree that the study will have more immediate applicability for readers if the reading of the biokitHSV-2 Rapid Assay conformed to the manufacturer's kit instructions. Strictly following these instructions, the results that we read as "low positive" (color less than our low positive control would be considered negative since the color was not clearly discernible. Therefore, we reanalyzed the data scoring these 26 sera as negative. While the conclusions do not change, the numbers in Tables 2 and 3 and in Figure 2 have been changed. For example, the positive predictive value of the Focus test increased from 80.5 to 94.7 in our original paper but from 80.5 to 95.6 in the revision.

Comment 2: (Duplicated findings in text, figures, and tables)
Response: We shortened the text of the results section by over 10% to reduce redundancy with Tables or Figures.

Comment 3: (Why does the combined testing work?)
Response: The gG-2 preparations used by Focus and Biokit differ (the former uses a recombinant gG-2 while the latter uses a lectin-purified native protein. The format of the tests; microwell plate ELISA versus laminar flow membrane also may affect the presentation of antigen. These resulting epitopes and their presentation almost certainly differ between the two tests. Different subsets of a typical polyclonal,
polyvalent human antibody response may bind under these differing conditions. This speculation has been added to the discussion. Simply repeating the Focus test may alter results slightly (as noted in the last paragraph of the discussion) but the most rigorous confirmatory test is likely to be one with a different antigen and test format.

Comment 4: (Recommended algorithm not clear).

Response: The data in Table 2 and 3 are presented with all positive samples being submitted to confirmatory testing. This algorithm (a "two-step testing algorithm") discussed extensively (see the 3 paragraphs on page 12). Laboratories and clinical practices may wish to use the raw data (Figure 1) or the mathematical model (Table 3) to create their own algorithms based on seroprevalence, cost, and time issues specific to their practices. To be clearer, we have added to the Conclusions section the term "of Focus positive results (index values >1.1)."

The revised manuscript now conforms to formatting guidelines of BMC.

We appreciate the reviews of our manuscript and feel we have met the concerns of the reviewers with our changes. We hope the paper is now ready for final approval and publication.

Rhoda Ashley Morrow PhD