Author's response to reviews

Title: Persistence survey of Toxic Shock Syndrome toxin-1 producing Staphylococcus aureus and serum antibodies to this superantigen in five groups of menstruating women

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Version: 7 Date: 17 May 2010

Author's response to reviews: see over
Dear Drs. Alam and Cassidy-Cain

We appreciate the helpful comments from the reviewer regarding the manuscript “Persistence Survey of Toxic Shock Syndrome Toxin-1 producing Staphylococcus aureus and Serum Antibodies to this Superantigen in Five Groups of Menstruating Women” (1579702396300429). We have addressed his comments as follows with his concern presented first followed by our response. We hope that these revisions have strengthened the manuscript and will allow its acceptance for publication in *BMC Infectious Diseases*. On behalf of my co-authors, we look forward to receiving notification that it has been published.

Comment 1: In table 3, there are 2 group 4’s. In the text it is stated that only 17 patients from group 4 became SA-positive. Could it be that the second group 4 is actually group 5? In that case, why not 61 subjects instead of 58?

In the original 2005 *J. Clin. Micro.* study by Parsonnet et al, Group 4 consisted of non-carriers of *S. aureus*. In this manuscript, Table 3 divided Group 4 into two subgroups; toxigenic and non-toxigenic. Thus, Group 4 is not Group 5. The number of fifty-eight subjects is correct as it appears in the manuscript.

Comment 2: The legends of both figure 1 and figure 2 are lacking. On the Y-axis it is stated “number of visits”. What is this??

The following legend was added to the paper to address the reviewer’s inquiry:

Figure 1: Anti-TSST-1 antibody titers for Groups 1 & 2
A histogram of antibody titers expressed as a two-fold serial dilution starting at 1:4 to ≥ 1024 versus total number of subject visits.

Figure 2: Anti-TSST-1 antibody titers for Groups 3 & 4
A histogram of antibody titers expressed as a two-fold serial dilution starting at 1:4 to ≥ 1024 versus total number of subject visits

Comment 3: On page 10, it is stated that the threshold OD was determined previously by testing sera from patients with TSS caused by TSST-1. How many patients were used for determining this threshold?

The original, peer-reviewed and published *J Clinical Microbiology* 2005 paper outlines the method ELISA methodology used in this study. When the assay was developed in the early 1980’s by Dr. Parsonnet at the Channing Laboratory – Harvard Medical
School, several hundred patient samples from physician diagnosed menstrual toxic shock syndrome were utilized to determine the validity of the test (personal communication, Paul Modern DHMC). In fact, Dr. Parsonnet’s current laboratory at the Dartmouth Hitchcock Medical School is one of the reference laboratories that clinicians send patient sera for anti-TSST-1 antibody titer determination.

Comment 4: In the definition, it is stated that “0-24%” is classified as transient”. From table 3 it can be seen that 70.7% of non-carriers are now transient carriers. This is very confusing. . . . since actually all these subjects remained non-carriers.

The subjects were classified as non-colonized in the original 2005 JCM study by Parsonnet and subsequently characterized as transient in the follow up study as published in Table 3.

A: Comment 4: superantigens interact with T cells. The specific V beta repertoire expansion signature is not something new. . . . Therefore, I suggest that at least one sentence should be added in the discussion.

The following sentences were added to the conclusion section:

Colonization of *S. aureus* TSST-1 and the absence of antibodies to TSST-1 are important in the pathogenesis of mTSS. Other factors not studied in the report include V beta 2 TCR expansion and human leukocyte antigen (HLA) haplotype [42, 43].

B: Comment 11: I have no doubt that all procedures were performed correctly, but for publications, it is needed to describe what the intra- and inter-observer variation was.

C: Comment 12: it is especially for laboratory tests extremely important to assess inter- and intra-observer variations. Without this a laboratory test should not be published. . . .

The following is the response to both comment 11 & 12:

The first procedure, the isolation and identification of *S. aureus* by phenotypic methods is a widely accepted procedure used in clinical hospitals and research studies. It is of interest that this same *S. aureus* procedure was also used in a manuscript just published in BMC without any mention of intra- and inter- observer variation analyses. This paper was also referenced in the new version.
The second procedure is an ELISA procedure for estimating the anti TSST-1 antibody titer in humans - again a procedure which was originally published in *Journal of Clinical Microbiology* without any request for intra- and inter-observer variations. The authors clarified the manuscript noting that the methods are routine and published in peer-reviewed journals.

The same technicians completed the assays so it is not possible to complete inter-rater reliability.

I have discussed these comments for intra- and inter-observer variations with several immunologists and microbiologists in academia that use these methods on a routine basis for analysis of human specimens for diagnosis. They also could not provide a response to these comments.