

You will find Editorial comments and Peer-Review comments listed below. Please read this entire email before making edits to your manuscript.

NOTE: Please include a line-by-line response to each of the editorial and reviewer comments in the form of a letter along with the resubmission.

Editorial Comments:

- Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.

Response: *The manuscript has been thoroughly proofread.*

- Please avoid abbreviations in the title (both video and text).

Response: *The title has been revised as requested.*

• Protocol Detail:

- 1) Please ensure that all details and actions mentioned in the video are in the protocol text.

Response: *All details and actions in the video are provided in the text.*

- 2) Please include an ethics statement before your numbered protocol steps indicating that the protocol follows the guidelines of your institutions human research ethics committee.

Response: *An ethics statement has been added as requested.*

- 3) Ficoll-Paque is a commercial name and must be replaced with a generic alternative in the video and text.

Response: *Reference to Ficoll-Paque has been changed as requested.*

- **Discussion:** JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs): 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

Response: *Discussion covers the requested information.*

• Figure/Tables:

- 1) Please remove the embedded figures from the manuscript. Figure legends, however, should remain within the manuscript text, directly below the Representative Results text.

Response: *Figures have been removed as requested.*

- 2) Define error bars in fig 2BC, 3AB

Response: *Error bars have been defined as requested.*

- 3) Please remove the figure legends from the figure files and place them directly below the Representative Results text.

Response: *Figure legends have been removed from figure files.*

• References:

- 1) Please number your references in the order they appear and use superscripted in-text citations numbering.

Response: *References have been adjusted as requested.*

2) Please make sure that your references comply with JoVE instructions for authors. Citation formatting should appear as follows: (For 6 authors or less list all authors. For more than 6 authors, list only the first author then *et al.*): [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. *Source*. **Volume** (Issue), FirstPage – LastPage, doi:DOI (YEAR).]

Response: *References have been adjusted as requested.*

3) Please spell out journal names.

Response: *Journal names are now spelled out as requested.*

• If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."

Response: *All figures and tables used are original.*

Video Comments:

• 0:01 - The university logo should be removed here. It can be included at the end of the video.

Response: *The requested video edits have been made.*

• 0:50-1:31, 5:28-5:55, 8:01-8:20 - The run time of this video 14:23, which is getting close to our 15 minute cap and much longer than the average Jove video. We recommend removing these materials lists from the video. They do not provide any important visual information beyond the text of the list, and that list will be included in the text protocol on the same webpage as the video. On screen text can be added that refers the video to those materials lists.

Response: *The requested video edits have been made.*

• 2:06 - There are one or two black frames in the middle of this edit. This edit should be a crossfade to avoid a jump cut.
• 3:16, 3:24, 3:32, 9:05 - Jump cuts, like the one here, where the angle is not changed between shots, should be avoided.

Response: *The requested video edits have been made.*

Jump cuts tend to have a jarring effect on the audience, and don't fit with our visual style. Please use cross dissolves (aka fades) to cover those edits.

Response: *The requested video edits have been made.*

• 8:51-9:01 - This shot is shown over silence. The narration describing it ("...and resuspend the pelleted bacteria at 1 mm DPBS.") is played over the previous shot. This part of the narration should be moved over this shot so that it is clear what we are seeing.

Response: *The requested video edits have been made.*

• Please upload your new video here : <https://www.dropbox.com/request/oGbE9Z8Fx6flgFLT3RKO?oref=e>

Comments from Peer-Reviewers:

Reviewer #1:

Manuscript Summary:

Overall, the manuscript is very well written, informative and concise. The steps were easy to understand and they were presented in a clear and detailed manner. However, there were several major concerns regarding the manuscript & video that the authors could look into.

Major Concerns:

Manuscript

1. *S. aureus* USA300 strain used in this study is a highly virulent pathogen. However, there was no mention of the use of a biosafety cabinet or appropriate PEP in the handling of this strain.

Response: *Handling of S. aureus and the use of appropriate PEP shown in the video section conformed to all Montana State University safety requirements for this pathogen. We did not specify these requirements as they will vary between research institutions. The use of a biosafety cabinet or appropriate PEP are not specified in numerous previously published JoVE manuscripts that also used S. aureus.*

2. The authors have successfully shown the rapid testing of *S. aureus* cytotoxicity on PMN using flow-cytometry. However, there was no comparison done to other cytotoxicity assays such as LDH or MTT that utilize the colorimetric method. As flow cytometry might not be available in some laboratories (lower-income regions), colorimetric methods are usually preferred as they are relatively simple to handle and provide good detection. Perhaps the authors could discuss more on why flow-cytometry is chosen instead of the other methods.

Response: *One of the most important components of this procedure is the isolation of pure PMNs that exhibit minimal activation. We cannot condone protocols that do not carefully evaluate isolated PMNs for other contaminating cells or PMN activation that can confound experimental results. Although other methods can measure the disruption of PMN membrane integrity, only flow cytometry allows the rapid evaluation of PMN purity and activation that is required for every preparation prior to using these cells.*

Video

1. As in the manuscript, the handling of both *S. aureus* strains was not done within an appropriate BSC.

Response: *Please see comment above.*

2. The list of materials/items for each step can be excluded from the video as listeners can always refer to the manuscript.

Response: *The requested video edits have been made.*

3. The video is too lengthy and wordy. It will be easier for the listeners if the authors only include pertinent steps in the video.

Response: *The video length has been shortened.*

4. The clarity of voice can be improved.

Response: *We feel the clarity of voice in the video is adequate and cannot be dramatically improved*

Minor Concerns:

1. Line 105 - To revise the subtitle to - "Isolation of Human Serum and Purification of Human Polymorphonuclear Leukocytes"

Response: *We prefer to emphasize the most important component of this part of the procedure, the isolation of PMNs. As such we have left this subtitle as is.*

2. The authors can include a simple flow-chart to accompany each section in the video for better clarity.

Response: *We have not included a flow-chart because we feel the manuscript and video provide adequate clarity for the procedure described, as supported by Reviewer 2 comments.*

Reviewer #2:

Manuscript Summary:

This manuscript provides a series of protocols designed to test the cytotoxicity of *S. aureus* and its secreted factors against purified human PMNs. The assays use propidium iodide and flow cytometry to measure damage to the PMN membrane as a measure of cytotoxicity. The protocols are clearly described with sufficient detail and the representative results provide a nice example of expected outcomes. The video was nicely done and easy to follow. This will be a useful protocol.

Response: *We appreciate the comments and support provided by Reviewer 2.*

Major Concerns:

None

Minor Concerns:

None