Dear Dr. Kostek,

Your manuscript JoVE54130R1 "Inter-tester reliability of a cost-efficient method to measure sensation and pain in humans" has been peer-reviewed and the following comments need to be addressed. Please keep JoVE's formatting requirements and the editorial comments from previous revisions in mind as you revise the manuscript to address peer review comments. Please maintain these overall manuscript changes, e.g., if formatting or other changes were made, commercial language was removed, etc.

Please track the changes in your word processor (e.g., Microsoft Word) or change the text color to identify all of the manuscript edits. When you have revised your submission, please also upload a separate document listing all of changes that address each of the editorial and peer review comments individually with the revised manuscript. Please provide either (1) a description of how the comment was addressed within the manuscript or (2) a rebuttal describing why the comment was not addressed if you feel it was incorrect or out of the scope of this work for publication in JoVE.

Your revision is due by **Apr 28, 2016.** Please note that due to the high volume of JoVE submissions, failure to meet this deadline will result in publication delays. To submit a revision, go to the [JoVE Submission Site](http://www.editorialmanager.com/jove" \t "_blank) and log in as an author. You will find your submission under the heading 'Submission Needing Revision'.

Jaydev Upponi, Ph.D.  
Science Editor

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**Editorial comments:**

•**NOTE: Please download this version of the Microsoft word document (File name: 54130\_R1\_021016) for any subsequent changes.**

 •Please keep the editorial comments from your previous revisions in mind as you revise your manuscript to address peer review comments. For instance, if formatting or other changes were made, commercial language was removed, etc., please maintain these overall manuscript changes.

•Formatting:  
-Please rephrase the short abstract to describe the method presented, not its cost-effectiveness.

We have made this change.  
-Please include a space between numbers and units of measurement.

We have made this change.  
-Please correct the numbering in the protocol so that all steps/substeps are in numerical order.

We have made this change.  
-Please remove references to the video.

We have made this change.  
-Please use the less than or equal to symbol in word rather than underlining the less than symbol.

We have made this change.-Please include a space between the note and step 4.1.

We have made this change.  
-Please remove all underlining from the manuscript. For example, protocol subheading should not be underlined.

We have made this change.  
-Please provide email addresses for all authors.

We have made this change  
-References – Please abbreviate all journal titles.

•Grammar:  
-Please copyedit the manuscript for numerous typographical errors.

We have made this change.  
-Long abstract – Please correct “that described here and demonstrate require”

We have made this change.  
-Introduction – “Puntuated” typo

We have made this change.  
-Line 444 – “In the representative results shown above, are reported strong inter-experimenter reliability”

We have edited this sentence.  
•Visualization: Please provide a diagram of the VAS. This can be included as a supplemental file.

This will be included as part of the video.  
 •Additional detail is required: 3.3 – How is the intensity of the pressure stimulus to be used determined? This should appear at the beginning (3.3.1) rather than step 3.3.5.

We have made this change.  
 •Branding and commercial language should be removed:  
-The focus on cost is excessive. The mentions of equipment cost should be reduced to only one per section of the manuscript.

We have made this change and reemphasized the focus of the manuscript to be on the video presentation of the technique rather than it’s cost.  
-Please remove “cost-efficient” from the title of the manuscript.

We have modified the title to more accurately reflect our manuscript.  
-Introduction – TSA II

We have made this change.  
-4.4 – SPSS

We have made this change.

•Discussion: Please discuss the significance with respect to alternative methods, including mention of what those methods are and appropriate citations.

This is now discussed in the introduction including citations.

 •If your figures and tables are original and not published previously, please ignore this comment. For figures and tables that have been published before, please include phrases such as “Re-print with permission from (reference#)” or “Modified from..” etc. And please send a copy of the re-print permission for JoVE’s record keeping purposes.

Comment ignored, they are original.

•JoVE reference format requires that DOIs are included, when available, for all references listed in the article. This is helpful for readers to locate the included references and obtain more information. Please note that often DOIs are not listed with PubMed abstracts and as such, may not be properly included when citing directly from PubMed. In these cases, please manually include DOIs in reference information.

We have now included DOI’s for every publication where they were available to us.

 •NOTE: Please copyedit the entire manuscript for any grammatical errors you may find. This editing should be performed by a native English speaker (or professional copyediting services) and is essential for clarity of the protocol. Please thoroughly review the language and grammar of your article text prior to resubmission. Your JoVE editor will not copy-edit your manuscript and any errors in your submitted revision may be present in the published version.

We have made this change.

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

The current document represents a line-by-line response.

**Reviewers' comments:**

**Reviewer #1:**  
*Manuscript Summary:*  
This is a well summarized practical guide that will help those without extensive resources. I have a few comments/corrections detailed below.

We appreciate the reviewer’s thoughtful comments.  
  
*Major Concerns:*  
Cutaneous mech sensitivity: I found the presentation to be confusing because of the way the numbering was done. Might I suggest that 1.3 and 1.2 should be flipped, so that the instructions to have the subject look away are given before beginning the testing.

We have made this change.

Also, renumber so that 1.4 becomes 1.3.1 and 1.4.1 become 1.3.2. That way, it becomes clearer to the reader that the 4 trials of 1.3.1 and the 1 trial of 1.3.2 are part of the 5 trials mentioned in the new 1.3 (old 1.2).

We have made these changes.

Finally, having 1.6 start with "for a single filament" made me question what was being done in 1.5. Suggestion: Make a new heading like "Score the trials" and put 1.5 and 1.6 as subheadings below that.

We have made these changes.  
  
Radiant heat: The clarity of this would be much improved with a picture of the glass plate apparatus, since it is custom built according to the table of materials. I assume that it will be pictured in the video; make sure that the video pauses long enough to allow viewers to understand the design, or include a picture with the figures for the paper.

We will note this during video production.

The note under 2.3 is confusing, move and combine with the note under 2.5. Use consistent naming: innocuous detection, temperature detection, and temperature sensitivity are all used in 2.4 through the 2.5 note. Please pick one (innocuous temperature detection is my suggestion).

We have made this change and used the suggested wording.

2.6.1 is under 2.5 and should probably be 2.5.1?

We have made this change.

Finally, the section that begins with 2.6 is confusing. What "same equipment": the heat lamp, the testing platform, both? I think that, since P2L70 mentions radiant and contact heat, the contact heat should be in a separate section. By the way, the numbering for 2.7.1-2.7.3 is probably incorrect; should it be 2.6?

We have adjusted this section including edits for clarity and numbering.  
  
Pressure sensitivity: 3.2.4 should be 3.2.3?

Numbering corrections have been made throughout.  
  
As eight separate intraclass correlation values are calculated, it is important to correct for multiple comparisons. This could have an impact on the interpretation of the results. For example, if a Bonferroni correction was used, the critical p-value would be .05/8 = .006. With this critical p-value, Hargreaves pain threshold would not be significant, and perhaps some of the other ICCs would not be significant as well. If the authors feel that it is appropriate to not correct for multiple comparisons, some justification for this should be provided.

Justification is now included in the results and discussion sections. The primary focus of this manuscript is meant to be the video/visual representation of the testing. In retrospect, we placed too much emphasis on the cost and reliability. We have revised the manuscript throughout to emphasize the testing technique and we strongly recommend that each clinic or lab conduct their own reliability analysis using our trial of reproducibility as an example.  
  
One of the main selling points of this study is that this protocol is generalizable to other clinics and research labs, where other raters can conduct the procedure and get consistent results. As such, the ICCs should be calculated in a random effects model, rather than a mixed model. A mixed model treats rater as a fixed factor and as a result, inferences are limited to the particular raters used in the study.

We again thank the reviewer for their comments. It allowed us to focus on the primary purpose of the manuscript. The true need for, and novelty of our manuscript, is not the reliability data but the visual representation of the QST protocol. As noted by reviewers our sample size is somewhat small and we tested healthy subjects. Yet our detailed procedures can be replicated in any patient population. Our intention was to demonstrate all tests and a *procedure for how* reliability can be examined for each laboratory. We have now clarified this point and suggest that each laboratory should conduct their own analysis for internal validity. Our analysis is meant as an example in terms of timing, number of subjects, and general procedures, and calculations  
  
One important limitation that should be noted is the use of healthy volunteers. The protocol was "designed with chronic pain conditions in mind", yet the reliability data presented only include healthy volunteers.

This is a critical point that we have incorporated into the discussion section. Because there are many types and sub-types and classifications for pain syndromes we suggest that rather than limit our protocol to one type of patient, healthy controls provide a baseline from which each clinic can start.  
  
*Minor Concerns:*  
Line 72: "the techniques and equipment that described here and demonstrate" word missing

Correction made  
Line 191: "Till date" should this be "to date"?

Correction made  
Line 329: specify whether the description of the VAS scale should be given prior to the stimulus or after?

The description of the scale should occur first, we have made this correction.  
Line 361: "subject" should this be subjective?

It should be subjects. Correction made.  
Line 393: were subjects tested 30 minutes apart, or for 30 minutes?

30 minutes apart, correction made.  
Line 395: "intraclass correlation coefficients (model 3,2) [ICC(3,2)] were calculated using a two-way mixed analysis of variance (ANOVA) with absolute agreement" Presumably separate ANOVAs were run for each dependent variable (i.e., 8 ANOVAs corresponding to the 8 outcomes as presented in table 1). This should be stated more clearly, as the current text implies that a single ANOVA was conducted.

We have now clarified the analysis.  
Line 400: "Here, " weird phrasing, maybe no comma?

Edit made.  
Lines 415, 416: "inter-reliability" is this meant to be inter-experimenter reliability?

Edit made.  
Line 415: "which is considered a good agreement between raters" according to whom?

Phrase removed.  
Line 443: experimenter training "(one trial observation, one trial implement)" should be described in more detail. Does this mean that the only training the experimenter received was observing each of the various assays one time? Were verbal instructions given to the experimenter as well? This is important information for the implementation of this protocol.

One trial observation, one trial implement, is correctly stated and we now emphasize this point because we agree with the reviewer that this is an important point to emphasize. The verbal instructions were those heard during the observation trial.  
Table 1: Constant pressure unpleasantness not included in table 1, while it is included in figure 1. Perhaps this is an oversight?

It is now included.  
Figure 2 caption: what test do the p values come from? Presumably intraclass correlation coefficients, but this should be stated.

It was stated in the second to last sentence of the legend.  
  
*Additional Comments to Authors:*  
N/A  
  
**Reviewer #2:**  
*Manuscript Summary:*  
N/A  
  
*Major Concerns:*  
The authors would do well to include comments regarding the following:  
1. Previously published multidimensional QST protocols.

We now make note of this in the introduction.  
2. Previously published normative data for thresholds using von Frey filaments.

We now make reference to this in the introduction.  
3. Information regarding the cost of the radiant heat device recommended.

The unit cost is ~$3,000 and will be included as part of the video.  
4. Discussion of the two cases of very poor correlation in determining the mechanical sensitivity threshold.  
Insofar as the authors present a dataset on implementation of the protocol, a larger dataset with more evaluators and subjects would be more compelling.

We agree that more data is almost always better. However, the primary intent of the current submission was to provide a visual representation of this protocol as there are currently no such video reports in the literature. A description of how to collect the ICC data, how it could be analyzed, and some representative results are included for the purpose of helping each lab conduct an internal validity examination for their respective clinic or laboratory. We have now stressed our original intent more clearly throughout the document including the title by removing mention of cost and reliability data. And although an inter-rater correlation of 0.70 is considered good by most literature reports for these tests, we did reexamine the collection of these data to see if any irregularities may have occurred that would explain the lower correlations for two of the subjects. None were found. We have added this as a point in the discussion.  
  
*Minor Concerns:*  
N/A  
  
*Additional Comments to Authors:*  
N/A  
  
**Reviewer #3:**  
*Manuscript Summary:*  
Chronic pain is a serious worldwide problem and more clinics will need reliable, low cost equipment and techniques to determine pain sensitivities in patients. The authors demonstrate the use of several thermal and pressure techniques that address this need. The authors also show high inter-rater results with these techniques. The manuscript is well written and should provide many clinicians the ability to evaluate pain in their patient populations.

We appreciate the reviewer’s thoughtful comments and we believe the edits made based on the reviewer’s comments clarify and strengthen our manuscript.  
This manuscript is acceptable, but there are a few grammatical issues and one technical comment that should be corrected.

We have made this correction.  
  
*Major Concerns:*  
none  
  
*Minor Concerns:*  
Page 4, lines 138-139: "To examine touch sensitivity, mechanical stimulation of skin with von Frey filaments are used." This should be changed to "is used." Stimulation is the singular subject.

We have made this correction.  
Page 4, lines 151-152: "Therefore, one measure of thermal sensation and two measures of thermal pain is included." I believe that this can be grammatically correct, but "are included" is correct, also.

We have made this edit.Page 4, lines 159-160: "Heating gives a preferential activation of C-fibers (thought to be most important for peripheral opioid receptors) and the best evaluation of second pain." This seems to be an awkward sentence. It is unclear what the section "thought to be most important for peripheral opioid receptors" adds to the sentence. Consider removing it.

We removed it.  
Page 6, lines 248-249: "During each trial, ask the subject to look away from their forearm/calf. Apply the filament to the subject's forearm or calf and ask if they feel the filament." Nothing is noted about the bending of the filament during the application. This should be addressed.

We have made this correction and it will be emphasized during the video.  
  
*Additional Comments to Authors:*  
N/A  
  
  
**Reviewer #4:**  
*Manuscript Summary:*  
The authors describe an inexpensive and portable method of quantitative sensory testing, which is in itself a good aim.  
  
-At present, the description does not seem clear enough to me to be able to reproduce it. For details see below. I have no access to the video, which may explain a lot.

The journal does not shoot the video until after the manuscript is accepted. We agree that it will help tremendously because the video is the overall manuscript intent.  
-The introduction is redundant. For example, the authors write several times that they use von Frey hairs. Once would be enough.

We searched the use of the term “von Frey” to make the edit. However, it is only mentioned once when the tests are listed, then again when we explain what it is, and is used a final time as a transition to the next set of tests in a following paragraph. We eliminated one use of the word in an attempt to reduce the redundant use of the term.  
-"New technologies have made the measurement of pain and neural sensation precise and reliable.." I don't think there is any technology yet that can measure pain. What is "neural sensation"? Please reword this sentence.

The reviewer raises an intriguing point as to whether pain can be measured. We do not know if this was meant to be a question of semantics or a philosophical viewpoint. We agree that from some perspectives, “pain” cannot really be quantitatively assessed or measured; it is a phenomenological experience. However we believe that, scientists that study pain, clinicians that treat it, and people who experience it are able to quantify it through the use of controlled methods and technologies. If these technologies could not assess “pain” there would be no reason for the current submission or the numerous other published scientific manuscripts that claim to have measured “pain”. In an effort to appease all philosophical schools of thought (and/or our word choice) we changed the word “measure” to “assess”. To clarify the concept of neural sensation we have further edited the sentence.  
-The introduction goes back and forth between the description of available systems, the global burden of pain, nociceptor function, and description of the new system. Please bring into order and shorten.

We thank the reviewer for their thoughtful perspective and have made specific eliminations and edits to the introduction to address this issue. Unfortunately we also received requests from reviewers and the editors to add more description and background. Additionally JoVE has requirements of what must be addressed in the introduction which is unique to their journal. For most journals the authors would address issues only as they saw fit to justify the need for their research. This created a slightly longer introduction than would be typical. Additionally, the final three paragraphs of the introduction should be noted as only addressing safety issues, and while very important, they appear to make the introduction longer than it really is. Overall, the introduction has been revised in order to address all four reviewers and the JoVE editorial review. We believe the introduction is now more orderly and again thank the reviewer for this observation.  
-Protocol:  
I do not entirely understand the choice of forearm and lower leg for the testing areas. In distally accentuated conditions, like peripheral neuropathy, these may give normal results, although the patients sensory functions are not normal. Please explain the advantages and disadvantages of this choice.

A patient having a distally accentuated condition is an important issue that we did not consider. We thank the reviewer for identifying this oversight. We have now addressed this in the eighth paragraph of the introduction. As to the general reasons for using the arms and legs we refer the reviewer to the same paragraph (eighth) of the introduction where we addressed anatomical location. It was noted that site specific testing may be needed for some conditions, but that the arms and legs are the optimal choice for most chronic pain conditions. A description and reasoning of the issue, and citations, are in the paragraph.  
-Fig. 2 A:  
The mechanical sensitivity threshold seems to vary immensely between the male and the female experimenter. Please discuss and explain.

We have now added a paragraph to the discussion to interpret these results.  
-The text in the equipment table is cut off in the boxes, so there are some parts I cannot read. For example, I would be interested in knowing how the glass is warmed up and to which temperature. Is the proband's skin temperature measured and taken into account?

The glass plate is equipped with heat strips (similar to that of a windshield defroster), that are connected to a power source that supplies ~4 volts of power to the setup. The glass plate is heated to be set to a temp of ~28C before the subject applies their forearm to the plate. Upon application of their forearm against the glass and the thermode, the temperature of that area heats up to 32C. It is at this approximate temperature that we commence the trial. The skin temperature is not directly measured, but the temperature is measured at the interface of glass and skin.

*Major Concerns:*  
N/A  
  
*Minor Concerns:*  
N/A  
  
*Additional Comments to Authors:*  
N/A