**Brasília, July 27, 2018.**

**From: Antonio Teixeira**

**To:** Vineeta Bajaj, Editor **JoVE**

**Ref.:** manuscript JoVE57985R2

Dear Editor:

Thank you for your extensive and excellent editing job. Please, find my answers and comments on the specific questions and comments of the reviewers:

**Editorial and production comments**

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

Answer: the manuscript was reviewed by an English first Language.  
  
2. Please complete the first Page of the Author License Agreement (ALA). Please then scan and upload the signed ALA with the manuscript files to your Editorial Manager account.

Answer: The filled-in and signed ALA uploaded.

3. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account.

Answer: Copyright permissions obtained and sent to the Editorial Manager account\*.

4. Figure 1: Please add a scale bar to provide context to the magnification used. Scale bar is defined in the figure legend but missing from the figure. Please also explain the black arrows in the figure legend.

Answer: The scale bar introduced in the figure legend and the black arrows explained. Note that the high-resolution figures 1 to 13 are 300 pixels.

5. Figure 2: There are no panels G, H and I. Please revise the figure legend.

Answer: Certainly, panels G, H and I replaced by D, E and F in the figure legend.

6. Figure 9: Please define error bars in the figure legend.

Answer: Error bars defined in the figure legend.

7. Please define all abbreviations before use.

Answer: Abbreviations defined in the text.

8. Please use SI abbreviations for all units: L, mL, µL, h, min, s, etc.

Answer: Abbreviations now appear accordingly with SI

9. Please include a space between all numbers and their corresponding units: 15 mL, 37 °C, 60 s; etc.

Answer: Yes. The space included between numbers and their units.

10. Please change centigrade to °C.

Answer: The change made in the text.

11. Please adjust the numbering of the Protocol to follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary. Please refrain from using bullets, dashes, or indentations.

Answer: The formatting is now in agreement with the JoVE Instructions for Authors.

12. For culture media and buffer such as DMEM, etc., please spell out at first use and provide composition. If they purchased, please cite the materials table.

Answer: The purchased culture ingredients (such as DMEM) defined in the text and cited in the table Materials and Equipment.  
13. Please add more details to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. Some examples:  
Line 98: What are the symptoms of acute Chagas disease?

Answer: symptoms of acute Chagas disease defined at lines 95 and 96 of the Protocol

Line 100: Please describe how to obtain serum and blood mononuclear cells.

Answer: please find description at lines 98 to 100 of the Protocol.

Line 101: Please describe how to isolate the wild type T. cruzi from the blood.

Answer: please find description at lines 103 to 126 of the Protocol.

Lines 103-105: What container is used in this step? What volume of DMEM is used? What do haploid cells refer to? Please specify which supernatant.

Answer: those definitions made at lines 114 to 120 of the Protocol.

Lines 117-118: Please specify growth conditions.

Answer: the growth conditions defined at lines 114 to 120 of the Protocol.

Lines 119-121, 139-142: Please describe how this is actually done.

Answer: Actually, this made as described at lines 103 to 126 of the Protocol.

14. As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:  
a) Critical steps within the protocol  
b) Any modifications and troubleshooting of the technique  
c) Any limitations of the technique  
d) The significance with respect to existing methods  
e) Any future applications of the technique

Answers: the steps a) to e) explicitly covered in a full paragraph at lines 550 to 575 of the Discussion.

15. References: Please do not abbreviate journal titles. Please include volume and issue numbers for all references.

Answer: journal titles are no longer abbreviate, and the volume, issue numbers are included for all references.

16. Table of Equipment and Materials: Please provide lot numbers and RRIDs of antibodies, if available.

Answer: sorry, but lot numbers and RRIDs of antibodies are no long available.

Changes to be made by the Author(s) regarding the video:

1. Please increase the homogeneity between the written protocol and the narration in the video. It would be best if the narration is a word for word from the written protocol text.

Answer: the homogeneity between the written protocol and the narration in the video achieved to considerable extent, according with the Editor’s instruction. For example, see description at lines 92 to 100 of the Protocol, which now appear in the video section on the ‘Study population and isolating the wild type *Trypanosoma cruzi’.* These changes appear at red lines in the current article text.

2. The details in the video are not the same as the details in the written manuscript. For example:  
01:28: The video mentions the physicians delivered health care for a period of five years while the written manuscript does not mention this.

Answer: gap is filled in at line 97 of the protocol, which appears in the video: ‘the research team delivered health care for the study population’.

01:41-02:23: The video does not contain any actions but only shows figures. Steps in lines 99-121 of the written manuscript not covered in the video.

Answer: actually, the video shows actions described at lines 92 to 122 of the Protocol.

02:26-03:52: Steps 3 and 4 of the video are difficult to follow because they do not match the written manuscript.

Answer: those steps of the video now match the manuscript.

02:48: The section title card shows now we are at step 3 (DNA extraction), while the details stated in the video are provided in step 5 of the written manuscript.  
02:59: The video says 1.5 while the written manuscript states 15 μM MgCl2. Please revise to be consistent.

Answer: The video now says 15 μM MgCl2 in agreement with the sentence at lines 152 to 154 of the Protocol.

03:07: The video mentions and shows centrifugation while the written manuscript does not state centrifugation.

Answer: the narration in the video is in agreement with lines 129 and 130 of the Protocol.

07:45: The video says that “instill … into the peritoneal cavity or into the vagina”, while the written manuscript states that “into the peritoneal and an equal amount into the vagina of group-B mice”.

Answer: The description in the protocol (lines 139 and 140) now coincides with the audio – ‘instill semen aliquots into the peritoneal cavity and into the vagina”.

3. Please show and describe representative results after the protocol, not during the protocol.

Answer: The modification made in the text and in the video, such as for the growth of the wild type parasites (lines 103 to 126 of the Protocol) that proceeds in the next step of the investigation.

4. Please upload a revised high-resolution video. Answer: The high-resolution video uploaded here: <http://www.jove.com/files_upload.php?src=17685473>

Video Production Notes 5/1/18:

Formatting issues  
• 0:00-0:10 - The opening 10 seconds of the title card can be cut out. We recommend starting the music at the beginning of the video. Answer: The recommendation is ok.

Audio issues  
• The audio quality clearly jumps around throughout the video. It sounds like different pieces were recorded in different sessions. All of the audio is sufficient for our minimum quality standards, but it does sound like an audio patchwork. If it is possible to re-record in one session, this would be a large improvement.

Answer: Sure, the audio patchwork recorded at different laboratories in different sessions because the laboratory no long exists after my retirement.

• 1:03 - There is some stray audio here that should be cut out.

Answer: Some stray audio was cut out.

• 1:20 - The beginning of this sentence fades in while "The" is being spoken. The fade in should be removed in the audio.

Answer: The fade is resolved.

• 7:19-7:20 - The punch-in audio recording here sounds too different for it to work as a mid-sentence patch. This entire sentence should be re-recorded.

Answer: The punch-in is resolved in the new sentence recorded.

Animal use issues  
• 1:01 - Please give the name of the institution in the disclaimer card.

The name of the institution (Faculty of Medicine of the University of Brasilia) now appear in the Study population disclaimer card.

Editing issues  
• 4:03, 6:45, 6:48, 6:50, 6:53, 6:55, 7:03 - The edits here are jump cuts, which tend to have a jarring effect on the viewer. They should be smoothed out with crossfades instead.

Answer: jump cuts and jarring effect smoothed out.

• 5:06-5:11 - The combination of the shaky camera and the quick edits makes this part seem chaotic and difficult to follow. Either the shots should be redone, or they should at least be allowed to play out for a bit longer so that the viewer doesn't feel rushed.

Answer: the video plays a bit longer in order the viewer doesn´t feel rushed.

• 3:20-3:26, 7:42-7:50 - The edits during this time are too rapid. The viewer's eye never gets to settle on any shot, which makes it very difficult to follow what is happening on screen. The pacing of the edits should be slowed down.

Answer: The pacing of the edits slowed down.

• 8:27 - There needs to be more space between the audio statements here.

Answer: more space added.

Video quality issues  
• 7:56-8:06 - Rather than 3 video clips that shake a bit with one that is 90° different from the other two, we recommend taking still photos of the placards and using those in the video. It will be easier for the viewer to read and will look more uniform.

Answer: this query solved with the following demand.

• 7:56-8:31 - This section talks about performing specific steps but none are shown in the video. The only visual is slowed down B-roll of mice in cages. We recommend cutting this section out and adding a reference to the text in the previous section of the video.

Answer: this full section cut and replaced by a card reference to the text Protocol section.

In agreement with the JoVE’s Video Technical team, the Introduction and the Discussion video and audio recorded again in order to obtain homogeneity. In this new look, the Discussion is now six paragraphs in length, because it includes the suggestion of the Reviewer to cover explicitly the details: a) Critical steps within the protocol; b) Modifications ant troubleshooting; c) Limitations of the technique and significance; d) future applications of the technique. In this regard, the video is over 16 min now.

Dear Editor, I look forward to hearing from you.

Sincerely,

Antonio Teixeira,

Corresponding Author.

\*Annexes subjected to the JoVE Manager account:

* Letters of permissions from the Author and the publishers:
* A) Memórias do Instituto Oswaldo Cruz, Rio de Janeiro, Brazil (ref. 1).
* B) Acta Venezolana de Malariología y Salud Publica (ref. 24).
* C) International Journal of STD Research & Reviews (ref. 19)
* D) PLoS Neglected Tropical Diseases (ref. 26).

Title: Sexual transmission of the American trypanosomes from males and females to naive mates during intercourse

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| --- | --- | --- | --- | --- | --- |
| **#** | **Time in the video** | **comment** | **Change in video required**  **Yes/No** | **Change in text is sufficient**  **Yes/No** | **Suggested Changes** |
| 1 | Laboratory mice | No corrections in the video | NO | Yes | The research Ethical Committee approved the protocol 054/09: the mice received humane care; the animals were sacrificed under anesthesia |
| 2 |  | Mice aren’t housed in hoods | N | Y. line 237 | Remove “kept in hoods” |
|  |  | The diet should be whatever is offered at the facility. | N | y. line 238 | Remove “Purina Chow” |
|  |  | Mice should be under anesthesia before performing a cardiac puncture. | N | Y. Lines 260-262 | Draw 0.5 mL of blood by heart puncture from the parental (FO) and progeny (F1) mice under anesthesia; then submit the body tissue sections for pathology analysis. |

URL: <https://www.jove.com/video/57985/title?status=a59991k>

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