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Editorial Board

Journal of Visualized Experiments

Dear Editorial Board,

I want to thank you for the opportunity to revise our manuscript entitled *"Atomic absorbance spectroscopy as a tool to measure intracellular Zn pools in mammalian cells"* (JoVE59519) for consideration at the Journal of Visualized Experiments (JoVE), by Shellaina J.V. Gordon, Yao Xiao, Amanda L. Paskavitz, Napoleón Navarro-Tito, Juan G. Navea, and myself.

We have addressed all the editorial comments and concerns of the reviewers. A detailed response to all the comments accompanies this letter.

As I commented in my previous letter, trace elements are critical for mammalian tissue development but are potentially toxic at high levels, meaning their homeostasis must be tightly regulated. Therefore, the development of sensitive, accessible, and accurate techniques is fundamental for understanding imbalances and dysregulation of metal transport and also homeostasis. In this manuscript, we describe the detailed application of atomic absorbance spectroscopy for measuring zinc in whole cell and subcellular fractions of different primary and established mammalian cell lines. Our groups have worked on optimizing these techniques over the past couple of years in order to develop a reliable method for metal quantification in biological samples. It is noteworthy that in this protocol, we focus on zinc measurements, but our procedures can be easily adapted to measure other transition and heavy metals.

We hope you find our revised manuscript suitable for publication at JoVE. Thank you for your consideration.

Sincerely yours,

Teresita Padilla-Benavides, PhD.

Response to editorial and reviewer comments:

Editorial comments:

Changes to be made by the author(s) regarding the manuscript:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.
Response: Spelling and grammar issues were corrected by all the authors in the revised version of our manuscript.

2. Please revise lines 212-214, 216-218 to avoid previously published text.
Response: Thank you for identifying these mistakes. Both sentences were changed in the revised version.

3. Keywords: Please provide at least 6 keywords or phrases.
Response: the original version of the manuscript provided 5 keywords (lines 29-30). We have added one more.

4. Please abbreviate liters to L to avoid confusion.
Response: This issue has been corrected.

5. Protocol: Please refrain from using bullets, dashes, or indentations.
Response: This issue has been corrected. However, dashes in the authors' last names must be maintained.

6. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents. You may use the generic term followed by "(see Table of Materials)" to draw the readers' attention to specific commercial names. Examples of commercial sounding language in your manuscript are: OptiMEM, Bioruptor, Diagenode, Nonidet, etc.
Response: This issue has been corrected.

7. Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).
Response: The protocol text has been corrected for this issue.

8. Please revise the protocol (lines 174-182, etc.) to contain only action items that direct the reader to do something (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note." Please include all safety procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible. Please move the discussion about the protocol to the Discussion.
Response: The protocol text has been corrected for this issue.

9. Please move the introductory paragraphs of the protocol to the Introduction, Results, or Discussion (as appropriate) or break into steps.
Response: The protocol text has been corrected for this issue.

10. In the JoVE Protocol format, “Notes” should be concise and used sparingly. They should only be used to provide extraneous details, optional steps, or recommendations that are not critical to a step. Any text that provides details about how to perform a particular step should either be included in the step itself or added as a sub-step. Please consider moving some of the notes about the protocol to the discussion section.
Response: The protocol text has been corrected for this issue.

11. 1.1.1.4: Please specify the concentration of trypsin.
Response: The concentration has been added.

12. 2.9: Please provide more details regarding how to measure the samples using atomic absorbance spectroscopy, specifying the parameters used such as pyrolysis and atomization temperature.
We thank the editorial office for this suggestion. We have added details in the protocol in the text and a new Figure 3

13. 2.10: Please describe how to prepare the standard samples and how to measure them.
Response: We thank the editorial office for this suggestion. We have added details in the protocol in the text accompanying which included the measurements of standards and samples.

14. Please note that lines 455-468 describe important considerations that are not reflected in the protocol, please elaborate how to perform these critical steps in the protocol if appropriate.
Response: Thank you, the text has been added on how to perform the calculations.

15. Please combine some of the shorter Protocol steps so that individual steps contain 2-3 actions and maximum of 4 sentences per step.
Response: This issue has been corrected.

16. Please apply single line spacing throughout the manuscript, and include single-line spaces between all paragraphs, headings, steps, etc.
Response: This issue has been corrected.

17. After you have made all the recommended changes to your protocol (listed above), please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.
Response: Essential steps have been highlighted in green.

18. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense. Notes cannot usually be filmed and should be excluded from the highlighting.
Response: Essential steps have been highlighted in green.

19. Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted.
Response: Essential steps have been highlighted in green.

20. Table of Materials: Please sort the items in alphabetical order according to the name of material/equipment.
Response: The table of materials has been formatted accordingly.

21. For in-text references, the corresponding reference numbers should appear as superscripts after the appropriate statement(s) in the text (before punctuation but after closed parenthesis). The references should be numbered in order of appearance.
Response: References have been properly formatted.

22. Please ensure that the references appear as the following: [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source. Volume (Issue), FirstPage – LastPage (YEAR).] For more than 6 authors, list only the first author then et al. Please do not abbreviate journal titles. See the example below:
Bedford, C.D., Harris, R.N., Howd, R.A., Goff, D.A., Koolpe, G.A. Quaternary salts of 2-[(hydroxyimino)methyl]imidazole. Journal of Medicinal Chemistry. 32 (2), 493-503 (1998).
Response: References have been properly formatted.

Reviewers' comments:

Reviewer #1:
The authors have contributed a nice article in regards to using GF-AAS to quantify zinc levels in various mammalian cell types. A few points should be considered prior to moving forward with this submission.
Response: We thank the reviewer for the positive comments on our manuscript.

1. A previous JoVE article showed that cation levels could be quantified using AAS (Duerr et al. 72, e50201, doi: 10.3791/50201 2013). Adding this manuscript and a description of it in the background section would be a nice addition for this manuscript.
Response: We thank the reviewer for the suggestion. The reference has been added to the introduction and discussion sections.

2. To better understand the method, a flow chart of the procedure would be highly beneficial.
Response: We thank the reviewer for this suggestion. A flow chart has been added as Figure 1.

3. A number of times in the protocol, an "appropriate" amount is suggested. At these points, it would be beneficial to provide more quantitative details. Otherwise, users will have to look to other papers to find the correct methodology, which seems odd for a methods paper.
Response: We thank the reviewer for pointing this out. We have removed the word appropriate from several points. A few were maintained as they are the prelude to examples in following sections.

4. In the cell fractionation step, how do the authors account from variations between nuclei preparations that result in differing metal concentrations?

Response: We thank the reviewer for this inquiry. We have added explanantory text in the protocol and result section, also a new representative Western blot of the subcellular fractions typically obtained from this protocol (Figure 2) to clarify this point was included.

5. In the representative results section, the authors talk about differences in metal levels within the sole figure. Were these differences significantly different? No indication of significance testing is apparent in the text or figure legend.
Response: We thank the reviewer for pointing out this important omission, and apologize for the mistake. Statistical significance has been added to the figure.

Reviewer #2:
Manuscript Summary:
The manuscript by Gordon et al describes the measurement of zinc concentrations in cell lysates and subcellular fractions of different cell lines, using graphite furnace atomic absorption spectroscopy (GF-AAS). Protocols are well described, the manuscript is well written and it displays how useful this technique can be to determine accurately zinc concentrations. Of course, there are more modern and fancy technologies for this, such as ICP-MS, but not as accessible to the scientific community. This manuscript will enable researchers at small universities or research institutions to take advantage of their GF-AAS equipment to make this kind of metal concentration determinations. Almost any chemistry department has a GF-AAS equipment.
Response: We thank the reviewer for the positive comments on our manuscript.

Major Concerns:
None.

Minor Concerns:
I find the first sentence of the abstract a bit confusing: How do essential transition metal ions compete with non-physiological ones?
Response: We thank the reviewer for noticing this redaction mistake. It has been corrected.

Statistical analysis for the data shown in Figure 1 must be included.
Response: We thank the reviewer for pointing out this important omission. Statistical significance has been added to the figure.

How do the findings of authors regarding changes of zinc levels compare to previously reported data? A discussion on this would enrich the manuscript, especially if previously reported data come from other techniques.
Response: We thank the reviewer for this suggestion. Text has been added in the discussion to address this point.

A comment regarding how AAS compares to ICP-MS in terms of amount of sample needed and detection limit would also be useful.
Response: We thank the reviewer for this suggestion. Text has been added to the discussion to address this issue.