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Journal Name:	International Journal of Biochemistry Research & Review
Manuscript Number:	2014_IJBcRR_8940
Title of the Manuscript:	Tissue Standards for Aging Research
Type of the Article	Method Article

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This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

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PART 1: Review Comments

	Reviewer's comment	Author's comment <i>(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)</i>
<u>Compulsory</u> REVISION comments	As a method article, the manuscript titled "Tissue standards for Aging Research" did not meet the quality of IJBcRR for publication. There are some major flaws in the manuscript that need to be addressed. 1. There is a lack of new methods or new development on existing methods in this manuscript. It was merely observation based. The authors also comment on line 178 that similar conclusions were drawn previously. A recent publication (J Vis Exp. 2013 Dec 30;(82):50948.) had demonstrated similar results using Stain free system as western blot loading control. The author should state clearly whether there's any new development or improvement on existing methods, or the work is repeating previous studies. If this is the first time the author apply this methodology studying tissue aging, it should also be discussed in the text. 2. The title is somewhat misleading, the author should consider something more directly align with the content, such as "comparison of reliability between western blot loading standards", or something similar. 3. There is concern on the data quality and consistency in this manuscript. For eg., line 83, 18 well gel with samples triplicated, however, in Fig.1, data was shown in duplicate	1. This is the first application to aging research which we have now discussed. Additionally this is the first comparison over multiple tissues and the first direct comparison of all these methods in a single study. 2. We disagree with the comment on the title, we sought to highlight the utility of this study in aging research. We appreciate the suggestion of the alternative title. 3. We apologize for not making this cleared in the original paper. All of the gels used in this study used a single tissue. To visually highlight the remarkable variability in housekeeping protein staining in multiple tissues that we and others described and numerically showed in the tables, we ran a demonstration blot. This demonstration blot was to visually reveal the poor performance of tubulin and actin as housekeeping



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	on a 12-well gel. The blot image presented in Fig1 has a high background, especially in the Tubulin blot, which will impact the accuracy of imageJ analysis. In the Actin blot of the old liver sample, there might be transfer issue during blotting since one of the band seemed partially blocked by a bubble. The two gels on the bottom, lower molecular weight proteins were already run out of the gel, introducing the question that whether the intensity analysis is accurate and sensitive enough. In both tables, no P value was shown to suggest whether the differences are statistically significant. 4. The author missed a very critical point that can actually argue for their results – in aging research, not only intracellular protein, but very often extracellular proteins are studied. The traditional loading standards (GAPDH, ACTIN, etc) are not good controls for loading extracellular proteins secreted. In this case, overall protein loading is clearly superior to these housekeeping controls.	proteins when multiple tissues were compared. This was a demonstration blot , so only a 12 well blot was used with duplicate samples was used, and no Image J analysis or statistical studies were done . 4. We agree and we are thankful for this suggestion and have included this discussion in our revised manuscript.
<u>Minor</u> REVISION comments	1. The language and the flow of the text need work. For eg., Line8, “surprised” should be changed to something neutral, such as “unexpected”. A clear conclusive statement should be added to the abstract and at the end of the manuscript. 2. As a method paper, the materials and methods section lack of details in general.	1. We have removed surprised from line 8 as requested. The conclusion “The best overall loading control in all tissues, at both ages, was the Stain Free method.” has been added to both the abstract and discussion as suggested by the reviewer. 2. More details have been added to the methods section.
<u>Optional/General</u> comments		