



SDI Review Form 1.6

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

General guideline for Peer Review process:

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.

To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

(<http://www.sciencedomain.org/page.php?id=sdi-general-editorial-policy#Peer-Review-Guideline>)



SDI Review Form 1.6

PART 1: Review Comments

	Reviewer's comment	Author's comment (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)
Compulsory REVISION comments	The manuscript [2014_IJBcRR_8940] entitled 'Tissue Standards for Aging Research' is a Methods Paper that has evaluated the linearity and general reproducibility of common protein products of house-keeping genes such as GAPDH, tubulin, and actin as loading standards, as compared to alternative assessments of total protein loading in gels using dyes such as Direct-Blue or Stain-Free. In general, reproducible and comparable protein loading is essential for using immunoblotting as a robust and reliable assay to determine differences in the concentration of distinct proteins in gel electrophoretically separated fractions. The lack of reproducibility of commonly used comparative methods is a general and serious problem in analytical biochemistry, including aging research. Major point: In addition, to the data shown in Figure 1 that compare the suitability of total protein staining and marker protein blotting, ideally titration curves should be provided. This should include the linearity of the antibody staining intensity with respect to increasing amounts of loaded protein and changes in antibody concentration. Such optimization experiments are crucial for setting up any blotting experiment and should therefore be included in a Methods Paper on loading control issues. The same is true for total protein staining approaches. To support the conclusions made in this paper with respect to the suitability of housekeeping proteins versus total protein labeling, a titration curve of staining of increasing amounts of young and aged tissue specimens should be shown. This would make an important contribution and show at what protein concentration range dyes are reliable indicators of equal loading.	Major point: We agree with the reviewer, and we apologize for not making it clear that the linearity analysis is just such a titration curve. We evaluated the linearity of all the methods under increasing concentrations of proteins loaded. We did not vary antibody concentrations, as we used the minimum concentrations to achieve detection with our apparatus and other investigators have found decreasing incubation time rather than antibody concentration to improve linearity, again a point we failed to include. We have revised the manuscript to correct these errors.
Minor REVISION comments	(i) Introduction: The Introduction section is brief and lacks referencing, which is highly unusual for a Methods Paper. The authors are encouraged to firstly expand the introductory statements to properly outline the critical issues of loading controls in comparative biochemistry and in verification experiments of protein hits identified by mass spectrometry-based proteomics and add a list of suitable references to support the underlying objective of their study. The statements of loading controls found in the scientific literature should be properly referenced in the Introduction section.	(i): We have expanded the introductory statements and fully referenced our statements. (ii): The reviewer is correct; we have now used the term housekeeping proteins throughout



SDI Review Form 1.6

	(ii) The authors refer to the proteins tested as 'housekeeping genes', whereby the correct term should probbaly be 'protein products of housekeeping genes' or 'housekeeping proteins', since they measured protein content; and not the genes themselves.	the paper.
<u>Optional/General</u> comments		