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Statistical analysis of instrumental reproducibility as internal quality control in high-resolution respirometry

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Reviewer 2

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*Only major points from review and responses included.

Reviewer 2

The paper proposes a SOP as internal quality control for experimental reproducibility. However, although they show the values of the calibration signal, a comparison of respiration in biological samples is missing. For instance, it would be interesting to compare the respiration from a cell preparation in an instrument daily calibrated with a large interval-calibrated instrument to show the impact of inaccuracy on a biological sample measurement. For this comparison, the authors could utilize the values of experiments shown on Figure 2.

Authors

We added the following sentence in the introduction: *A follow-up report on these experiments is in preparation to analyze the reproducibility achieved with cultured cells as biological sample.*

Reviewer 2

In the material and methods section, it is unclear whether the instruments utilized in the comparison (1-3 days after and 27+ days after) were randomly chosen. In other words, it is not clear if all instruments were previously submitted to the same SOP-POS/SOP-BG routine before R1#1.

Authors

To clarify, we changed the order of figures 3a, b and c to emphasize that *SOP-BG was performed first for all sensors and chambers, followed by SOP-POS for the same sensors.* We

added the following sentence in section 3.1.: *In contrast to R0, R1 calibrations were performed on these sensors not only in monthly time intervals but on each day before performing experiments with biological sample.*

Reviewer 2

In the section 3.1 the authors begin the second paragraph describing the comparison of calibrations without a prior explanation of the rationale of the experiment. Some concepts, such as the meaning of "zero-corrected air calibration" and how these values can be utilized as an O2 accuracy detection, are not clear in the text. I suggest beginning the second paragraph with a sentence explaining this concept and why the calibration values over time are a parameter to measure POS sensitivity. Also, including a table before figure 3 with a brief and simple definition of terms such as "zero-corrected air calibration", "air calibration" and "background test" for new users or users that are not familiar with HRR.

Authors

We added the definition of "zero-corrected air calibration signal" in the table of abbreviations. We modified the definition of POS sensitivity in the first paragraph of section 3.1.: *Sensitivity is the change of the raw signal [µA] divided by the change of O_2 concentration [µM] and averaged 0.011 µA/µM.* In the same paragraph we added the definition of stability: *Stability is evaluated either as a function of the deviation between the raw signals [µA] at two calibration points (from which the time-averaged drift can be calculated), or as a function of continuously measured drift [pmol·s^{-1}·mL^{-1}].*

Reviewer 2

In Figure 3a and figure 3b, the authors utilize two different SOP to compare values from calibration from two different intervals of time. To better show the loss of signal stability of sensors over time, I suggest comparing the correlation of SOP-POS 1-3 after and 27 days after and include in figure 3.

Authors

This is a valuable suggestion. We prepared a corresponding analysis and added Figure S1 c and d to the supplement.

Reviewer 2

The data in figure 6 show a stable O2 background detection in the instrument 35 days after SOP-BG. However, it is not clear for how long the instrument can sustain stability. It would be interesting to show this measure for a longer time to suggest to users a suitable interval of SOP-BG to be performed.

Authors

We added the following sentence in last paragraph of section 3.2: *The optimal time interval of performing SOP-BG may be modified based on individual experience and adjusted to the specific requirements for quality control in a particular project.*