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

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# Reviewer 1

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*Only major points from review and responses included.

### Reviewer 1

It is not readily evident in the materials and methods and in the figure legends what the sample size is, as well as what is considered to be a sample in each instance (chamber? measurements in a single chamber?).

### Authors

Assuming that sample size refers to the statistical meaning (number of technical repeats), we clarified the distinction between O2k instruments and chambers in the last sentence of Section 2.4.

### Reviewer 1

Section 2.3: please explain for the audience unfamiliar with the equipment that Tip2k is a micropump injection module and that DatLab is a software also provided together with the Oroboros; also, explain how one can inject even small volumes of fluids in the chambers without pressure build-up if in principle there is no gas phase inside the chamber (that would be compressible) and the volume of the chamber is constant at 2 mL. Is there a drain in the chamber?; lastly, it would be instrumental just to mention there that the dithionite protocol described refers to the SOP-BG.

### Authors

We moved Section 2.3 after section "High-resolution respirometry" and added information about TIP2k (new Section 2.4) and DatLab (new Section 2.3). In Section 2.4 we clarified the constant volume- constant pressure operation.

**Reviewer 1**

Page 8, first paragraph "In 30 different POS, zero-corrected air calibration signals, R1-R0, ranged from 1.8 to 2.3 uA": Could this variability be due to differences in atmospheric water vapor pressure?

**Authors**

We added the following sentence in Section 3.1: *The variability is due to intrinsic differences between sensors and variation of membrane thickness when mounting and stretching the membrane on the sensor.*

**Reviewer 1**

Page 8, second paragraph "to a drift per day of 0.16 %, 0.21 %, and 0.10 % or 0.003, 0.004, and 0.002 pmol·s−1·mL−1 for 2019, 2020 and 2021, respectively (Figures 3d to f).": What exactly is plotted then in figs 3d-f? Did the authors take the absolute drift between the two measurements and divided it by 38 in 2019, 27 in 2020 and 35 in 2021 and plotted that ratio? If so, there is an assumption of oriented (linear) drift between measurements that does not fit with the apparent directional randomness that happens in 2019 and 2020 (but not in 2021 - do the authors have an explanation for that?). Or do they present the absolute drift between measurements if figs 3.d-f? This should be clarified either in the methods, or in the legend.

**Authors**

We addressed these points by modifying the following sentence: "The deviations between two calibration points are shown in Figures 3d to f. The maximal deviations, divided by the number of days between measurements, corresponded to a time-averaged drift per day of 0.16 %, 0.21 %, and 0.10 % or 0.003, 0.004, and 0.002 pmol·s−1·mL−1 for 2019, 2020 and 2021, respectively."

We agree that "an assumption of oriented (linear) drift between measurements" does not apply, which we stated explicitly in Section 3.1: *Comparing these values with the maximal slope of 0.004 pmol·s−1·mL−1 calculated between calibrations separated by 27 to 38 days (Figure 3b), indicates that the maximal drift does not persist over the month.*

We do not have explanation for the different patterns from 2019 to 2021.

**Reviewer 1**

Legend to Figure 3 "Chambers P6A and P7B were not used in the MiR05-Kit study in 2020.": If not, what do the two pairs of gray data in P7 and P8 correspond to? (but not in 2021 - do the authors have an explanation for that?). Or do they present the absolute drift between measurements if figs 3.d-f? This should be clarified either in the methods, or in the legend.

**Authors**

Chambers P6A and P7B were not used for experiments with sample in the MiR05-Kit study in the context of which we analyzed instrumental performance, but background tests were anyway performed. These background data are represented by gray data points.

## Reviewer 1

Discussion, page 14 "At this level of agreement, it may be asked why and how frequently a user of the O2k should perform the chamber test. Regular testing provides IQC, ensuring that (*1*) the instrument is in an optimally functional state, (*2*) the operators are shown to be skilled to follow properly the SOP, and therefore (*3*) instrumental artifacts are excluded. In summary, instrumental QC is a cornerstone for experimental reproducibility.": Is it a question of days between calibrations, or absolute number of experiments between calibrations? I.e., were the chambers used for how many experiments/samples between the chamber calibrations, for instance?

## Authors

This is a very valid point, therefore we added in Section 3.2: *Use of the instruments besides the MiR05-Kit study varied for different O2k, but a correlation between usage and background stability does not add information due to the small variation of J°O2 between chambers in different instruments (Figure 8).*