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# Title: An interlaboratory comparison to quantify oxidative potential measurement in aerosol particles: challenges and recommendations for harmonisation

## **General comments**

The inter-laboratory comparison (ILC) involves 18 laboratories performing the DTTbased OP assay on four samples (SP1-SP4). Such an ILC is very much needed to achieve the aim of harmonizing this type of OP across different labs and ultimately to ensure comparability of monitoring OP as a proxy measure of PM toxicity over a wide spatial coverage and a longer temporal span. I have a few major comments, which are detailed below, regarding the analysis of measurement uncertainty sources.

## **Major comments**

(1) Reading the SOP described in S1-1, I deduce the equation to calculate the DTT consumption rate in nmol min<sup>-1</sup> for a sample is:

$$\Delta DTT_t \ (nmol \ min^{-1}) = \frac{V}{\Delta t} \left( DTT_o - \frac{A_t - A_{int}}{k} \right) - \Delta DTT_{a.o.r} \qquad \text{Eq (1)}$$

Where V is the volume of the DTT solution for which absorbance measurement is taken;  $\Delta t$  is the incubation time (e.g., 10, 20, 30 min),  $DTT_o$  is the initial DTTT concentration used in the assay; A<sub>t</sub> is final absorbance reading for T= t min incubation experiment (from step 16); A<sub>int</sub> is the intrinsic absorption of each sample (from step 7), k is the calibration slope of absorbance vs concentration of DTT, and  $\Delta DTT_{a.o.r}$  is the inherent DTT auto-oxidation rate (slope of Control<sub>ox</sub> sample).

(Note: It is unclear about  $DTT_{\circ}$  (the initial DTTT concentration used in the assay) is determined. Is it calculated from the known concentration (0.25mM) and the dilution factor (300/50 =6)? Or it is calculated from Absorbance measurement at incubation time 0 min?)

With the calculation equation established, a few questions ensue:

First, the calculation equation (1) needs to be provided in the SOP to avoid any second guessing on readers' or users' part. Second, if Eq (1) is the correct interpretation of the DTT oxidation rate calculation, then we can see  $A_t$ ,  $A_{int}$ , k, and  $\Delta DTT_{a.o.r}$  all contribute to the measurement uncertainty to  $\Delta DTT_t$ . Their measurement values and uncertainties (COV) from participating labs need to be presented in the paper so that we can understand which measurement step/variable contributed most to the overall uncertainty of  $\Delta DTT_t$ , especially for those labs that produced results of larger COV for the ILC samples (e.g., L1, L12, L13, L14, L15, and L21 shown in Figure 1).

(2) Lines284-286: "SP4: a sample extracted from a blank/clean quartz filter was sent to the participants, but it was not included in the evaluation since the measured values

were close to the instrument limits of detection for most participants." It is misleading when stating "close to the instrument limits of detection. If eq. (1) shown in the preceding comment is correct, then SP4's  $\Delta DTT$  being not detected is actually the first term in eq (1) being indistinguishable to  $\Delta DTT_{a.o.r}$ . Then, it is relevant to characterize the COV of  $\Delta DTT_{a.o.r}$ .  $\Delta DTT_{a.o.r}$ . is likely lab-specific, as ultrapure water and reagents (e.g., DTT, phosphate buffer, DTNB, etc) were prepared by individual participating labs. The lab cleanliness conditions and source and storage history of reagents could all affect  $\Delta DTTa.o.r.$  and its variability.

In any case, the ILC results for SP4, as well as  $\Delta DTT_{a.o.r.}$ , needs to be discussed. Related to this, the limits of detection as related to determination of  $\Delta DTT$  merits clarification.

(3): Selection of the four ILC samples: please elaborate reasons for their selection. Why a copper standard solution is not considered as an ILC sample, considering the strong response of OP DTT to Cu?

(4) Shown by Figure 3, all underestimations are lower than -2 z, however, 8 labs had one or more z-scores exceeding +2 z. This implies propensity for these labs to get overestimations. Do these labs tend to have higher  $\Delta DTT_{a.o.r}$ ?

## **Minor comments**

- Line 313: Here the authors state that a total of 20 research groups participated in the ILS, but Figures 1, 3, 7, and S7 show laboratory labels up to "L21". Please explain the discrepancy.
- S1-1: "weight" is mistakenly used throughout the description of Method 1. Please replace with "weigh".
- line 264 and S1-1, line 52: why the experiment temperature was 37.4oC, not 37oC?
- Please provide instruction on preparing particulate matter suspension solutions in the simplified DTT RI-URBANS SOP.
- Line 288: "SP3 were powders": unclear how could SP3 (originated from urban PM) could be in powder form?
- Line390: insert a comma between "solutions" and "liquid".
- Define COV at its first appearance (line 337-338) instead of later on (line 403)
- Figure 5: suggest using different legends for DTT-"home "and R1-URBANS protocols for easy differentiation. For example, hatched lines could be included for DTT-"home" data.
- Line 712: pay the way  $\rightarrow$  pave the way
- Line 713-716: Text here is largely redundant, as it repeats much of the preceding sentence.