Comments to “A Semi-automated Instrument for Cellular Oxidative Potential Evaluation (SCOPE) of Water-soluble Extracts of Ambient Particulate Matter” by Sudheer Salana et al. (MS No.: amt-2021-188).

This study introduced a semi-automated instrument for measuring cellular ROS formation potential (OP) of ambient PM and associated components in murine alveolar cells. This system was calibrated using dichlorofluorescein diacetate (DCFH-DA) as ROS probe and tert-Butyl hydroperoxide (t-BOOH) as standard compound for positive control. The authors found that metals, quinones, PAHs and inorganic salts exhibit different macrophage OP, claiming for the feasibility of using this system for assessing the cytotoxicity of different type of air pollutants. Overall the study is interesting and the topic fits the journal of AMT. However, the written of the manuscript needs some improvement before consideration of publishable potency. Detailed comments are as follows:

1. The authors need to justify and demonstrate why t-BOOH is chosen as standard compound for calibration.
2. Why choose rat alveolar macrophages? In previous studies, canine, human, and other different types of macrophages have been used as metrics (e.g. Beck-Speier et al., Oxidative stress and lipid mediators induced in alveolar macrophages by ultrafine particles. Free Radic. Biol. Med. 38, 1080-1092, 2005.). The calibrations in these studies were based on different standards. It is almost certain that OP of same PM samples from different macrophage assays will be different, including the current method. How do illustrate the baseline and OP differences across different methods?
3. The selectivity of the DCFH method toward different types of ROS should be discussed. If the ROS yields of certain concentrations of ambient PM and t-BOOH are the same, but the types of ROS (e.g. radicals and H_2O_2) formed by them are different, how to justify the health impact of ambient PM? The sensitivity/reactivity of the DCFH with different PM components (e.g. metal ions vs quinones) rather than with ROS should be considered and discussed.
4. Line 21 of page 1: Show the full name of PAH please. Whether oxygenated PAH is more accurate here? It looks like parent PAH generally do not exhibit prominent OP.
5. Line 41-43 of page 2: it is worthy to introduce the electron paramagnetic resonance (EPR) assay/method here.
6. Line 95-96: the ‘one week’ storing time is necessary? You may want to say use it up in one week or make fresh stocks each week.

7. Line 166 of page 6: Why ‘2 h incubation’ is the best for measurement? In addition, for incubation of human macrophages, the mechanism and time period (much slower) for the metabolic process are quite different. More discussions are needed to clarify the gap between murine alveolar cells and human alveolar cells.

8. Line 181-192 of page 6-7: Clarify whether the filters have been prebaked (condition) or not?

9. Line 194 on page 7: The impact of sonication on ROS formation should be mentioned.

10. Line 197: What is the impact of fluorescent particle smaller than 0.45 μm in ambient particles to the measurement?

11. Line 235: the ‘1”’ is confusing.

12. Sections 2.2 and 2.4 can be merged to form one section. Section 3.3 and 3.4 can be merged to form one section. The current Section 2.6 can be the last subsection in Section 2.