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Atmospheric Measurement Techniques
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Interactive comment on "Development of the drop Freezing Ice Nuclei Counter (FINC), intercomparison of droplet freezing techniques, and use of soluble lignin as an atmospheric ice nucleation standard" *by* Anna J. Miller et al.

Anonymous Referee #2

Received and published: 19 December 2020

General comments:

Miller et al. developed a droplet freezing assay to quantify ice nucleating particles (INPs) in immersion freezing mode. 288 microliter droplets can be observed simultaneously while cooling down to approx. -25 °C with a temperature uncertainty of 0.5 °C. The authors extensively discussed possible sources of contamination and performed an intercomparison study with two other droplet freezing assays to validate the new instrument. Additionally, they tested and discussed the water-soluble biopolymer lignin as a suitable ice nucleation standard material. The development of a new droplet

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freezing assay is not a substantial new concept for the ice nucleation community, but the authors extensively describe, test and discuss their new instrument and also many aspects beyond. This aspect along with the research for a good ice nucleation standard is highly valuable for the community. The manuscript is suitable for publication in AMT after the following comments have been addressed.

Specific comments:

Line 66: The high freezing temperature of -1 °C was not only reported for bacterial IN (e.g., Maki et al. 1974) but also for fungal IN (e.g., Richard et al. 1996). Please consider to include this aspect as well.

Line 69: Please include the following references: Felgitsch et al. 2018, Kunert et al. 2019, Pummer et al. 2015.

Line 98: Please add the following reference: Kunert et al. 2018.

Lines 98f: It would be nice if the authors could elaborate a bit more on advantages and disadvantages of the different methods.

Line 112: Snomax does not only consist of proteins from P. syringae. It is rather a preparation of freeze-dried, irradiated cells from P. syringae, which are non-viable and damaged after this procedure. It contains all parts of the bacterial cells including IN-active proteins. Please clarify this sentence.

Line 153: Also other droplet freezing assays can measure INP with high statistics using other types of PCR trays as for example the high-throughput droplet freezing assay TINA, which can be operated with two 384-well plates in one experiment. Please attenuate the term “unique feature”.

Lines 165ff: How do you ensure that the warm ethanol, which you add to the cooling bath during an experiment, does not affect your cooling rate of 1 °C per min?

Line 194: The link to Fig. S1 is not clear here. If you want to keep it in this sentence,

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you should include two arrows in the picture showing the direction top to bottom and left to right.

Lines 205ff, 310ff: You should consider to add an additional preparation step and spin down the prepared PCR trays before placing them into FINC to remove possible bubbles and ensure a comparable position of the droplet in each well.

Lines 206f: I would recommend to move the sentence “We found that these cleaning procedures…” to line 202 after “…for at least one hour.” and continue with “Sample solutions were then prepared…”.

Lines 239f, 258, 263f, 489: I would recommend to only use one decimal as your temperature uncertainty is 0.5 °C and you cannot be more precise than that.

Line 349: What is mg C L-1?

Lines 373ff, 402ff, 434ff: How did you manage that the three instruments all measured aliquots of the same suspension? Did you move all instruments to one lab or did you prepare the solution and transported aliquots to the different locations? Please elaborate.

Line 453: The parameterization of Wilson et al. 2015 is far off the results obtained in this study. What is the additional benefit of including it in Fig. 6?

Lines 461f: Have you also tried to test lignin from a different supplier? It would be nice for a universal standard not to be dependent on one company.

Line 467: Several research groups within the ice nucleation community work additionally with the initial freezing temperature. The requirements for a good ice nucleation standard should be as well to have reproducible initial freezing temperatures. If I see correctly in Figure 6 (the blue colors are difficult to distinguish), the initial freezing temperatures are in a range at least between -13 °C and – 15 °C. Please elaborate a bit more on this aspect also with regard to other substances such as fungal IN, which have a highly reproducible initial freezing temperature even after different treatments

(Fröhlich et al. 2015, Kunert et al. 2019).

Line 472: I agree that there is no trend over time. But I cannot agree that the T50 values are all within the temperature uncertainty of the instrument. If I compare the medians in Fig. 7, I estimate that the difference between day 1 and day 6 is about 1 °C. Please correct your statement. It would be nice to also discuss the applicability of the initial freezing temperature here. Is lignin as standard only useful with regard to the T50 value?

Line 496: Why -38 °C? Fig. 6 shows only data until -25 °C, which is the limit of detection for your instrument.

Fig. 4: The quality of the figure could be improved. The symbols seem to be very blurry, which makes it hard to see. Also the light grey color is very difficult to follow.

Fig. 6: The blue colors are impossible to distinguish. Please choose more colors or at least more different blue colors.

Section S1: Location of device for TINA is Max Planck Institute for Chemistry in Mainz. Please correct.

Section S2: Here, you state that the camera takes a picture every 2 °C. How can the script then record an image every 0.2 °C (see line 175 of the manuscript)?

Table S2: I would recommend to only use one decimal as your temperature uncertainty is 0.5 °C and you cannot be more precise than that.

Technical comments:

Line 205: “solution”

Line 206: a parenthesis is missing after 4.4.4.

Line 238: please add “droplets” after “5 uL”

Line 247: “shape”

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Line 251: “in FINC” not “on FINC”

Line 256: a parenthesis is missing after Wang (2013)

Line 268: “than” not “that”

Fig. S8 caption: Please remove the dot in “the. Milli-Q”

Sec. S8: Parentheses are missing after Eq. S2 and Eq. S4.

Figs. S9, S11, S12, captions: Missing dots at the end of the last sentences.

References:

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