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Pragmatic protocols for working cleanly when measuring ice nucleating particles

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ABSTRACT

Measuring ice nucleating particles (INPs) is critical for understanding, and modeling, cloud formation, reflectivity, and precipitation patterns. However, because INPs are very rare in the atmosphere, but abundant—sometimes alarmingly so—in the dust that covers all work surfaces, contamination during processing of collected aerosol samples can be a significant hindrance to obtaining accurate measures of INPs. In preparing this technical note, we questioned the cleanliness of every collection and processing step involved in making immersion freezing measurements of INPs re-suspended from filter samples. The aim was to identify, and then minimize, all potential sources of significant contamination, including containers and tools used to store filters and prepare liquid suspensions, gloves, work surfaces, and the polymerase chain reaction (PCR) trays commonly used for sample analysis. While plasticware released few INPs, most gloves readily shed them, but this can be mitigated by using washed cleanroom vinyl or polyethylene gloves. Work surfaces, even those that had been cleaned, were prodigious reservoirs of contaminating INPs, but simply covering them with aluminum foil will provide an INP-free surface. By applying these practices, we developed a method to reduce the background level of INPs on Nuclepore™ polycarbonate filters in our tests to 0 at −25 °C and < 20 at −27 °C, making them suitable for use in all sampling environments.

1. Introduction: Importance of working cleanly

Ice nucleating particles (INPs) are necessary for initiation of ice formation in clouds above the level of homogeneous freezing (−38 °C); mixed-phase and glaciated clouds serve as the primary source of precipitation outside of the tropical oceans (Mülmenstädt et al., 2015). Despite their importance in influencing precipitation, as well as cloud reflectivity and lifetime, INPs are rare in the atmosphere (DeMott et al., 2010; Kanji et al., 2017) and have a diverse range of sources (Šantl-Temkiv et al., 2019).

Filters and impingers are now commonly used to sample air for offline immersion freezing measurements of INPs, and their validity is supported by recent intercomparisons with other methods for sampling ambient INPs (DeMott et al., 2017; DeMott et al., 2018). Aerial samples and samples from remote marine regions can be especially challenging to analyze because few INPs are captured. For example, a filter sample of 20 m^3 of air from above the Southern Ocean will typically collect only around 20 INPs active at −20 °C (McCluskey et al., 2018). By contrast, the dust that covers every laboratory surface harbors millions of warm-temperature INPs that can easily taint such clean samples during preparation. Contamination will also limit the temperature to which a test can be taken, by raising the background INP level in the dilutions and negative controls.

Surprisingly, there are very few systematic assessments of the sources of INP contamination from substrates and water (e.g., Creamean et al., 2018; Polen et al., 2018). Since contamination may entail multiple inputs from numerous sources, the entire processing train needs to be scrutinized to quantify all identifiable potential sources so that preventative protocols can be designed. In this work, we measured the number of INPs present on tools, containers, and surfaces used for offline

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analysis of INPs. Since consumables designed primarily for molecular biology are increasingly used as receptacles for holding aliquots of suspensions in a range of immersion freezing instruments (Garcia et al., 2012; Conen et al., 2014; Moffett, 2016; Zaragotas et al., 2016; Beall et al., 2017; Schiebel, 2017; Chen et al., 2018; Harrison et al., 2018; Kunert et al., 2018; David et al., 2019; Gute and Abbatt, 2020; Miller et al., 2019), we also evaluated several brands of 96-well polymerase chain reaction (PCR) trays for intrinsic contamination. Using this comprehensive survey, we provide a pragmatic general handling and sampling protocol for measurement of INPs via immersion freezing. Since Nuclepore™ polycarbonate filters are a commonly used medium for aerosol filtering, we applied the protocol to develop an improved method for their cleaning.

2. Methods

All tests were performed using the CSU Ice Spectrometer (IS), which analyzes liquid suspensions for INPs active via immersion freezing. The IS is constructed using two aluminum blocks, designed for incubating PCR plates, encased by cold plates that contain copper coils through which coolant is circulated. The IS produces spectra spanning a wide dynamic range of temperatures and INP concentrations and is supported with well-established experimental protocols applied in diverse scenarios (DeMott et al., 2017; Beall et al., 2017; Hill et al., 2016; Hiranuma et al., 2015).

Prior to measurement of INPs in the IS, filters are placed into sterile 50 mL polypropylene centrifuge tubes, with 7–10 mL of 0.1 μm-filtered deionized (DI) water added and particles re-suspended by tumbling end-over-end. Next, 50 μL aliquots of suspensions are dispensed into sterile, 96-well PCR trays in a laminar flow hood and covered with clean lids and wrapped in foil before transport to the IS. The trays are then placed into the blocks, the device covered, and the headspace purged with high efficiency particulate air (HEPA)-filtered N_2 (750 mL min^{-1}). The IS is cooled at 0.33 °C min^{-1} using a recirculating low temperature bath, and the freezing of wells is recorded through an interface with a charge-coupled device camera system. The limit of measurement is between −27 and − 30 °C, depending on the number of INPs in the DI water background. Immersion freezing temperature spectra are obtained by converting the number of frozen wells at each temperature to the number of INPs mL^{-1} suspension using eq. 13 in Vali (1971). Ninety-five percent confidence intervals for binomial sampling are obtained from eq. 2 in Agresti and Coull (1998). All tests presented here were corrected for INPs in the 0.1 μm-filtered DI sample blank, unless otherwise noted.

2.1. Water

DI water is used for cleaning, for collection of INPs in impingers, and to re-suspend and dilute INPs caught on filters. Its INPs content will limit the temperature to which immersion freezing tests can be made. For all tests, we used deionized water from a centralized supply (Evoqua), "polished" using 0.1 μm-pore-diameter syringe filters (Whatman® Puradisc 25 TF) or 0.02 μm-pore-diameter Anotop® syringe filters (Whatman®).

2.2. Minimizing INP contamination during sample handling for analysis

As a preamble to this section, we suggest using a laminar flow hood, if available, for all preparatory steps to avoid contamination of filters and tools with ambient aerosol particles. We use a standard Table Top Work Station (Envirco), which comes fitted with a HEPA filter, and produces an ISO Class 5 environment. The particle number concentration inside the laminar flow hood, measured using an ultrafine condensation particle counter with a detection limit of 2.5 nm (TSI, model 3776), was 0 cm^{-3} (i.e., undetectable). By contrast, the laboratory air contained >1000 particles cm^{-3}.

Various means can be used to clean laboratory utensils, such as soaking in dilute hydrochloric acid (HCl), followed by methanol/ethanol and DI rinses, or 10% hydrogen peroxide (H_2O_2) followed by DI rinses. Immersion in an ultrasonic bath (in a plastic bag) efficiently dislodges attached particles. For glassware, all organics can be removed by baking to 550 °C for 2–3 h. To clean most laboratory items, we use Windex®, followed by DI rinses, because it is designed to remove surface particles and organics without leaving a residue.

2.2.1. Minimizing INP contamination during filter manipulation

Forceps should be used to handle filters. We use acetyl plastic forceps (Fine Science Tools®, Cat. 11,700–00), because they are precisely manufactured and inert. To test them for contamination, the tips of 10 pairs of cleaned forceps were swirled in a reservoir of 5 mL of DI water. This was compared with a replicate 5 mL of DI water.

Since filters may need to be cut into pieces, we tested scalpel blades (Swann-Morton, No. 10) for cleanliness. The tips of 32 scalpel blades, taken from individual foil packs, were dipped, one per well, into 50 μL aliquots of deionized water in a PCR tray. This array was compared with an adjacent control array of 32 × 50 μL aliquots of DI water.

2.2.2. Reducing potential INP contamination from plasticware

Many types of plasticware are used during the collection, storage and analysis of filters, and in the preparation of DI water. Specifically, these include:

- Plastic slider bags, which are used for cleaning and storage of laboratory items. We tested them by adding 20 mL of 0.1 μm-filtered DI water and shaking vigorously for approximately 1 min. Hefty® Quart Freezer Storage Slider Bags were used for analysis. We also assessed the usefulness of washing bags by initially spraying the interior with Windex® followed by several DI rinses, before testing.
- Petri dishes are used for rinsing filters in DI water during cleaning, and for storage of filters after sampling. They were tested by adding 4 mL of 0.1 μm-filtered DI water to the upturned lid, swirling, and then transferring the liquid to the base and swirling again. These results were not corrected for INPs in the negative control since the values were comparable. The Petri dishes analyzed were Pall® Laboratory 50 × 9 mm sterile (Cat. 7232), and Life Science Products 60 × 15 mm sterile (Cat. LS-6706).
- Pipette tips dispense aliquots of filter suspensions and make the dilutions. They were examined by opening a new box and aspirating 1 mL of water from 10 mL of 0.1 μm-filtered DI water in a pre-rinsed centrifuge tube, and then dispensing it back into the reservoir. This was repeated with 15 tips using the same reservoir. Eppendorf™ ep Dualfilter T.I.P.S.® (50–1250 μL, Cat. 0030078594) were tested.
- Corning® 50 mL polypropylene centrifuge tubes (Cat. 89,093–190) serve as the receptacle for the filter suspensions and dilutions, as well as for the reservoir of the 0.1 μm-filtered DI water. To test whether they are a potential source of INP contamination, an unrinsed tube was compared with a pre-rinsed tube. For the unrinsed tube, 10 mL of 0.1 μm-filtered DI water was added and hand-shaken to re-suspend residual INPs in the tube. For the pre-rinsed tube, 5 mL of 0.1 μm-filtered DI water was initially added, hand-shaken for approximately 20 s, and discarded. This step was repeated before 10 mL of 0.1 μm-filtered DI water was added and shaken as for the unrinsed tube. These results were not corrected for INPs in the negative control since these tubes, pre-rinsed, serve as the reservoir for such.

2.3. INP mitigation with exterior surfaces

We use compressed air dusters to blow off dust that accumulates on surfaces, such as on pipettes, the plexiglass lids of the IS, and the aluminum blocks into which the PCR trays are placed. To check for potential INPs in their propellant, 20 mL of 0.1 μm-filtered DI water was added to a plastic slider bag, and the duster was sprayed into the bag in three 2 s pulses. The bag was then closed and shaken to re-suspend the

particles in the liquid. We tested GUST® Dusters, Stoner® - 94,203, 340 g, Difluoroethane (Cat. 89,065–918) which contain 0.2 μm-filtered propellant.

2.3.1. Personal coverings

Clothing, and especially sleeves, or bare skin, may be large sources of readily dislodged ice nucleation-active particles. Given the inherent variability of clothing we did not test it, but advise always using a dedicated and regularly washed lab coat (stored in a bag), disposable cleanroom sleeves (e.g. Kimberly-Clark Professional™ KIMTECH™, Cat. 49,815), and cleaned gloves to minimize shedding of INPs from the operator.

Gloves must be used at all points in making INP measurements, from the field to the laboratory analyses, to avoid contamination from numerous INPs on hands. We screened a variety of different materials (latex, nitrile, copolymer, vinyl, and polyethylene) and brands to find those with the lowest number of INPs adhering to their surface. Glove tests were performed by first adding 20 mL of 0.1 μm-filtered DI water to a fresh plastic slider bag. A new pack of gloves was opened, and a glove from the middle of the pack was carefully fitted onto the hand without touching the exterior. Next, the gloved hand was immersed and enclosed tightly in the plastic slider bag and vigorously shaken back and forth for approximately 1 min. Using a pipette, the liquid was dispensed into a pre-rinsed centrifuge tube for analysis. Washed gloves were first sprayed with Windex® and rinsed thoroughly under DI water before immersion in the plastic slider bag. For comparison, we also tested an unwashed and washed (with Windex®) hand following the same method.

The gloves surveyed included:

- Ansell™ FoodMates™ Disposable Polyethylene Gloves
- Ansell™ Microflex® Derma Free® Vinyl Gloves
- Ansell™ Microflex® Latex Gloves
- Ansell™ Microflex® MidKnight™ Powder-Free Nitrile Examination Gloves
- Fisher Scientific™ Safety Choice™ Economical Vinyl Exam Gloves
- Great Value™ Disposable Vinyl Gloves
- Great Value™ Disposable Poly Gloves
- Kimberly-Clark Professional™ KIMTECH™ Pure® G5 *Co*-Polymer Gloves
- PIP™ CleanTeam™ Vinyl Cleanroom Gloves (Cat No. 191201452B for medium)
- VWR® Soft Nitrile Examination Gloves

2.3.2. Working on surfaces

Clean surfaces, both in the laboratory and field, are essential for making accurate INP measures. To quantify differences between lab surfaces as sources of contaminating INPs, 0.05 or 0.1 m^2 of laboratory floor, uncleaned bench top (2 weeks since last clean), cleaned bench top (wiped with Windex® followed by a DI rinse), and the inner (intrinsically cleaner) surface of aluminum foil (Reynolds Wrap® Heavy Duty) were tested. Between 7.5 and 30 mL of DI water was swirled on each surface, and a proportion retrieved with a pipette and dispensed into a pre-rinsed centrifuge tubes for analysis.

2.4. Screening for contamination in PCR trays

Sterile, 96-well polypropylene PCR trays hold the aliquots of suspension for testing with the IS. They are typically certified to be free of DNA and RNA. However, this does not equate to them being particle free. PCR tray tests were performed by pipetting 50 μL aliquots of 0.1 μm-filtered DI water into three 32-well sectors per tray (left, center and right), and analyzing with the IS. The tray tests were not corrected for INPs in the DI water.

Brands used were:

- BIO-RAD Multiplate™, 96-well PCR Plates (Cat no.: MLP9601)
- Fisherbrand™, 96-well PCR Plates (Cat. No. 14–230-232)
- Lifeline™ PCR Plates Life Science Products (Cat. No. PCR-9620-01, from Life Science Products)
- OPTIMUM® ULTRA Brand, PCR Plates (Cat. No. LS-9796, from Life Science Products, Frederick, CO)
- Thermo Scientific™ ABgene™, 96-well PCR Plates (Cat. No.: AB0600)

2.5. Filter preparation protocol

An improved technique for cleaning Nuclepore™ polycarbonate membrane filters was developed to remove INPs introduced during manufacture and packaging. This method is especially ideal for sampling in low INP environments.

1) Fill a clean 500 mL polypropylene bottle completely with methanol (or ethanol). We used Fisher Scientific™ (Certified ACS) 99.9% methanol
2) Immerse filters (up to about 30) completely in the methanol
3) Cap the bottle, hold upright in an ultrasonic bath, and ultrasonicate for two 10 s pulses
4) Discard half of the methanol, fill with DI water, and gently mix
5) Discard half of the solution, fill with DI water, and repeat gentle mixing
6) Discard most solution, fill with DI water, and repeat gentle mixing
7) Discard most water and then tip filters in the residual water into a large Petri dish (Life Science Products, 150 × 15 mm, sterile, Cat. LS-6725). Drain out the DI water
8) Fill Petri dish with 100 mL of 0.1 μm-filtered DI water
9) Separate filters individually with clean plastic forceps to ensure efficient rinsing
10) Drain and repeat the filtered DI rinse
11) Drain water and lay filters to dry on new foil in a laminar flow hood
12) Wrap filters in individual aluminum foil pockets to be stored and transported for use in the field

3. Results and discussion

3.1. Water

Standard laboratory water deionizers should produce water usable to −20 °C or colder, depending on droplet size. The temperature limit to which measures can be taken can be extended by "polishing" the DI water by using, for example, 0.1 μm-pore-diameter syringe filters (Whatman® Puradisc 25 TF) as used in this study. Our polished deionized water contained ~1 INP mL^{-1} at −25 °C. Harrison et al. (2018) noted that filtering of Milli-Q water through Sartorius Minisart filters (0.2 μm, product code 17597-K) lowered the temperature at which pure water droplets froze by 2–3 °C, while O'Sullivan et al. (2015) found up to a 5 °C reduction by using a 100 kDa filter (Millipore, Amicon Ultra, UFC910008). By contrast, Polen et al. (2018), who performed a comprehensive water analysis, found erratic results when repeatedly testing Milli-Q water, even after 0.02 μm-filtration; they suggested bottled HPLC grade water instead, for consistency. Additionally, regularly changing syringe filters is recommended, as Polen et al. (2018) found an increase in INPs active colder than about −25 °C when using the same 0.02 μm filter for several weeks. If deionizers are not well maintained, they will release INPs, irrespective of whether the system outlet filter (generally 0.2 μm) is working, since those INPs active at cold temperatures tend to be small (O'Sullivan et al., 2015). In a system overdue for servicing, we found that 0.02 μm-filtering performed better than 0.1 μm-filtering (Fig. S1).

3.2. Minimizing INP contamination during sample handling

3.2.1. Minimizing INP contamination during filter manipulation

The cleaned acetyl plastic forceps did not shed any INPs (the spectrum was not significantly higher than the control), but the steel scalpel blades did release INPs active below −22 °C (Fig. S2). This may be caused by microscopic metal shards or INP contamination introduced during packaging; there are many seemingly innocuous sources of INP contamination. We therefore recommend using a small ceramic blade knife, since the blade is smooth, unreactive, and easily cleaned with Windex® or H_2O_2.

3.2.2. Minimizing INP contamination from plasticware

- Freshly opened (unwashed) plastic slider bags were found to contain less than 100 INPs per quart-sized bag active at −27 °C (Fig. 1a). Washing the plastic bags before use may slightly reduce the number, but in practice, this benefit may be offset by the additional handling introducing contamination. Cleaning items, such as plastic forceps, in plastic bags is advisable since most of the pre-existing INPs in the bags will also be removed during the wash step. Change bags used for cleaning or storage often in the field.
- Rinsing centrifuge tubes (Fig. 1b) with 0.1 μm-filtered DI water reduced the number of INPs introduced during manufacture by approximately 4-fold at −25 °C, although there was no difference by −28 °C. There was also a 2 degree colder onset temperature with the rinsed centrifuge tube.
- Both brands of newly-opened packs of Petri dishes possessed few if any INPs, with none at all detectable until around −25 °C.
- The pipette tips released no detectable INPs (no difference with the 0.1-μm DI water blank). Pipette tips with filters should always be used, since the interior of the pipette can be a major source of contamination. We also advise wiping down their exteriors with DI water before each use. Disassembling the lower part, rinsing the components in DI water, and using a compressed air duster to blow them dry should be done once a month.

3.3. Limiting INPs on exterior surfaces

Compressed air dusters emitted no detectable INPs in their gas stream once correction for the water blank and plastic bag had been made. Therefore, they can be safely used to blow dust off external surfaces. We recommend using dusters that have filtered propellant.

3.3.1. Gloves

Gloves should always be used, since hands are covered in INPs: A bare hand, even after washing, can release over 1000 INPs active at −16 °C (Fig. 2a). Gloves, however, can also be a source of INPs. Washing gloves by spraying them with Windex® followed by rinsing under DI water will remove INPs from dust acquired during production or which has settled upon an open pack. By contrast, wiping gloves with alcohol will have little benefit other than denaturing proteinaceous INPs, which are likely a minor contributor. Avoid powdered gloves. For a consistent comparison, only washed glove results are presented in Fig. 2a. Gloves that shed the fewest INPs are the cleanroom vinyl and polyethylene gloves. Both released around 50 INPs per glove at −25 °C. Polyethylene gloves (typical food preparation gloves) are inexpensive but hard to use as they lack a textured surface for grip. They are also harder to clean and have lower durability. Copolymer (cleanroom produced) and nitrile gloves have similar INP-temperature spectra, releasing approximately 700 INPs per glove at −25 °C, over 10-fold more than a washed cleanroom vinyl glove.

Since nitrile gloves are popular, we show a direct comparison between washed and unwashed cleanroom vinyl and nitrile gloves in Fig. 2b. An unwashed cleanroom vinyl glove is better than a washed nitrile glove. Comparing unwashed and washed cleanroom vinyl gloves, the number of INPs at −25 °C were 360 for the unwashed compared with only 50 for the washed. As they were taken from a fresh, cleanroom-produced pack, it underscores the importance of always washing gloves and never assuming anything is INP-free. Washing nitrile gloves usefully removed warmer temperature INPs, lowering the onset from −16.5 °C to −21 °C. However, there was essentially no difference by −24 °C, suggesting there is something intrinsic to the nitrile material itself that ice nucleates at colder temperatures. This contrasts with the washed cleanroom vinyl that showed a reduction in INPs across the entire temperature spectrum. This superior performance is consistent with the findings of Garçon et al. (2017), who showed that vinyl gloves released lower amounts of most trace elements than other gloves.

3.3.2. Cleanliness of work surfaces

Surfaces are major direct as well as indirect (by transferring INPs to items) sources of INP contamination. Not surprisingly, Fig. 3 confirms that laboratory floors are an especially high source, with over 10,000 m^{-2} by −7.5 °C. Benches that are not regularly cleaned will also support many INPs, with over 10,000 m^{-2} at −12 °C. Cleaning them definitely helps, as this number is reduced to only 100 m^{-2} at the same temperature. However, with the number of INPs active at colder temperatures remaining large (i.e., >10,000 m^{-2} at −20 °C), this is still not sufficient since even terrestrial ground-based daily filter samples, which are quite

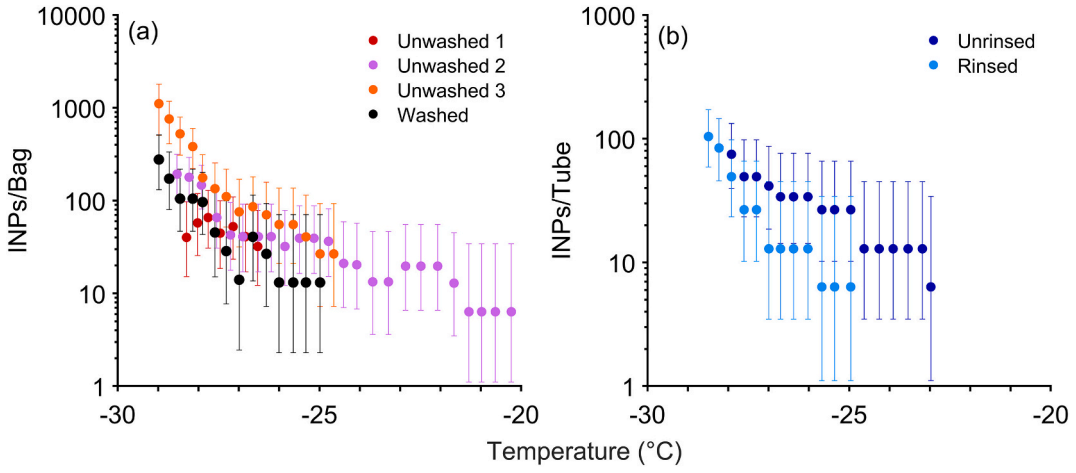

Fig. 1. Cumulative INP spectra from (a) unwashed and washed quart plastic slider bags (with background DI water INPs removed), and (b) an unrinsed and rinsed Corning 50 mL polypropylene centrifuge tube.

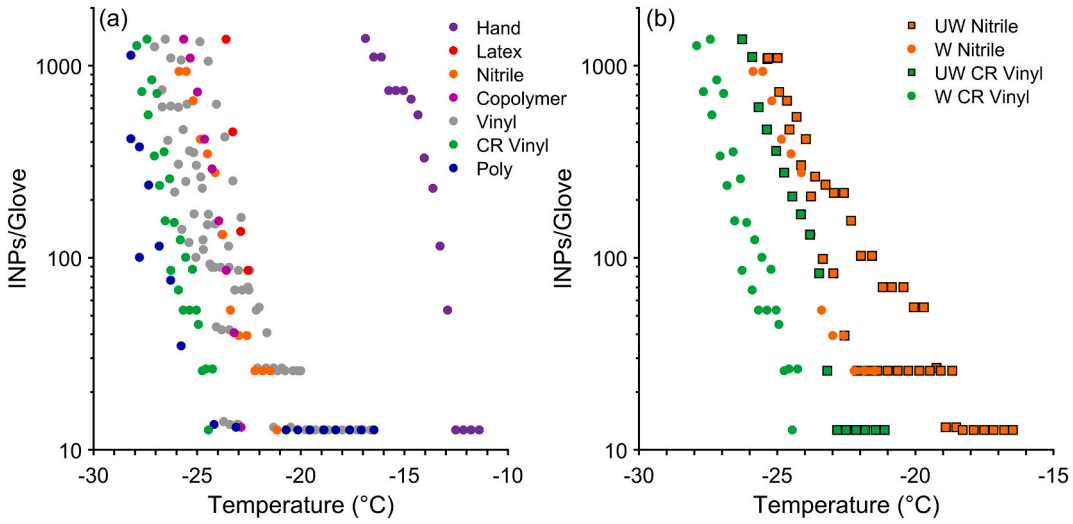

Fig. 2. Cumulative spectra of INPs on (a) the surfaces of washed gloves compared with a washed hand, and (b) on washed (W) and unwashed (UW) nitrile and cleanroom (CR) vinyl gloves. All glove tests were corrected for background INPs in the DI water blank.

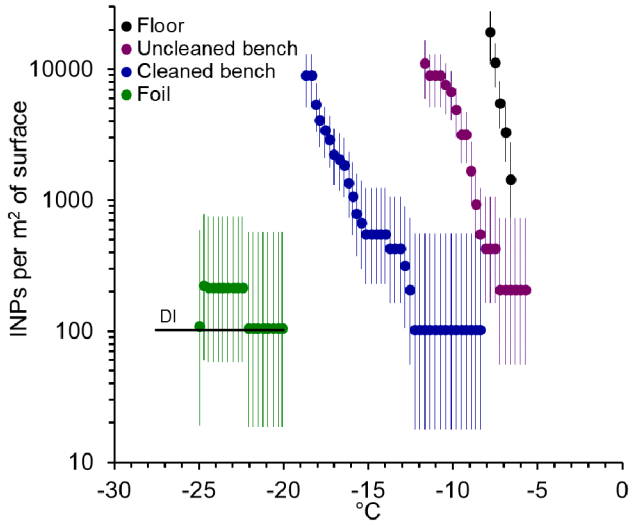

Fig. 3. Cumulative spectra from laboratory testing of background INPs on the uncleaned floor, 2-week uncleaned benchtop, cleaned benchtop, and aluminum foil (the inner side of the roll). The "DI" line indicates INPs in the 0.1 μm-filtered DI water sample blank, which was subtracted from all surface tests.

heavily loaded with particles, may only collect a few thousand INPs at −20 °C. By stark contrast, aluminum foil had essentially zero detectable INPs down to the limit of testing at −28 °C. Hence, simply doing all work, both in the laboratory and field, on fresh foil will eliminate this major source of contamination. Foil should be changed every day before commencing work, and the underlying surface pre-cleaned to minimize lofting of dust when the foil is laid down. Additionally, regularly wiping down all bench tops and storing items on foil will reduce the overall number of INPs in the general workspace.

3.4. PCR trays as a source of INPs

Polypropylene trays are mass produced for performing PCR reaction tests. They are, thus, a convenient platform for measuring INPs, and are used in a growing number of ice spectrometer designs (mentioned in Section 1). We compared 96-well PCR trays from five suppliers, each loaded with 0.1 μm-filtered DI water, for their low temperature performance. Unexpectedly, one brand, OPTIMUM® ULTRA, consistently performed better, by a margin of 2–3 °C, over the other well-known brands (Fig. 4). A consistent feature of the other brands was a habit of freezing in the center first (Fig. 5), which was not, or minimally, displayed by the OPTIMUM® plates. This appears to be a signature of some form of contamination introduced during manufacture, and suggests that the lower temperature limit of immersion freezing tests using PCR plates is typically controlled by contamination in the plate itself. Low temperature limitation caused by the PCR plate brand was alluded to by Kunert et al. (2018) when comparing 96-well plates from one company with 384-well plates from another. In the former, 50% of 3 μL droplets of DI water froze by around −25.5 °C, while in the latter, this level wasn't reached until −29 °C. The lower temperature limit of the OPTIMUM® plates may also be due to the plate or to impurities in the DI water. While PCR trays are DNA/RNA-free they are clearly not particle-free, and this could partially explain, in addition to droplet volume differences, why cold plates can reach colder temperatures (e.g. Tobo, 2016; Creamean et al., 2018).

3.5. Filter cleaning for sample collection

Contamination of filters with INPs during manufacture and packaging can limit the lower temperature to which they can be used. Our new method for cleaning Nuclepore™ polycarbonate filters reduces the intrinsic INPs on each filter to less than 20 at −27 °C (Fig. 6). This translates to an approximately three-degree improvement from the previously used method of soaking in 10% H_2O_2, and over a five-degree improvement from using unwashed filters. In the process of sampling, the filters may collect more INPs in their handling, but this method works to eliminate a major source of contamination. Although the difference in background INPs released might not be essential for terrestrial ground-based measures, this will certainly improve detection limits for aerial and marine studies. Most importantly, the two short pulses of ultrasonication did not affect the integrity of the filters; three filters were checked before and after cleaning with a mass flow meter, and the flows and pressure drops across them were unchanged. Ultrasonication for longer periods of time (i.e., minutes) can result in tears at the edges of filters.

4. Conclusions

This work aimed to quantify, and subsequently minimize, all potential sources of INP contamination when making immersion freezing measures with filters and using PCR trays as the measurement platform.

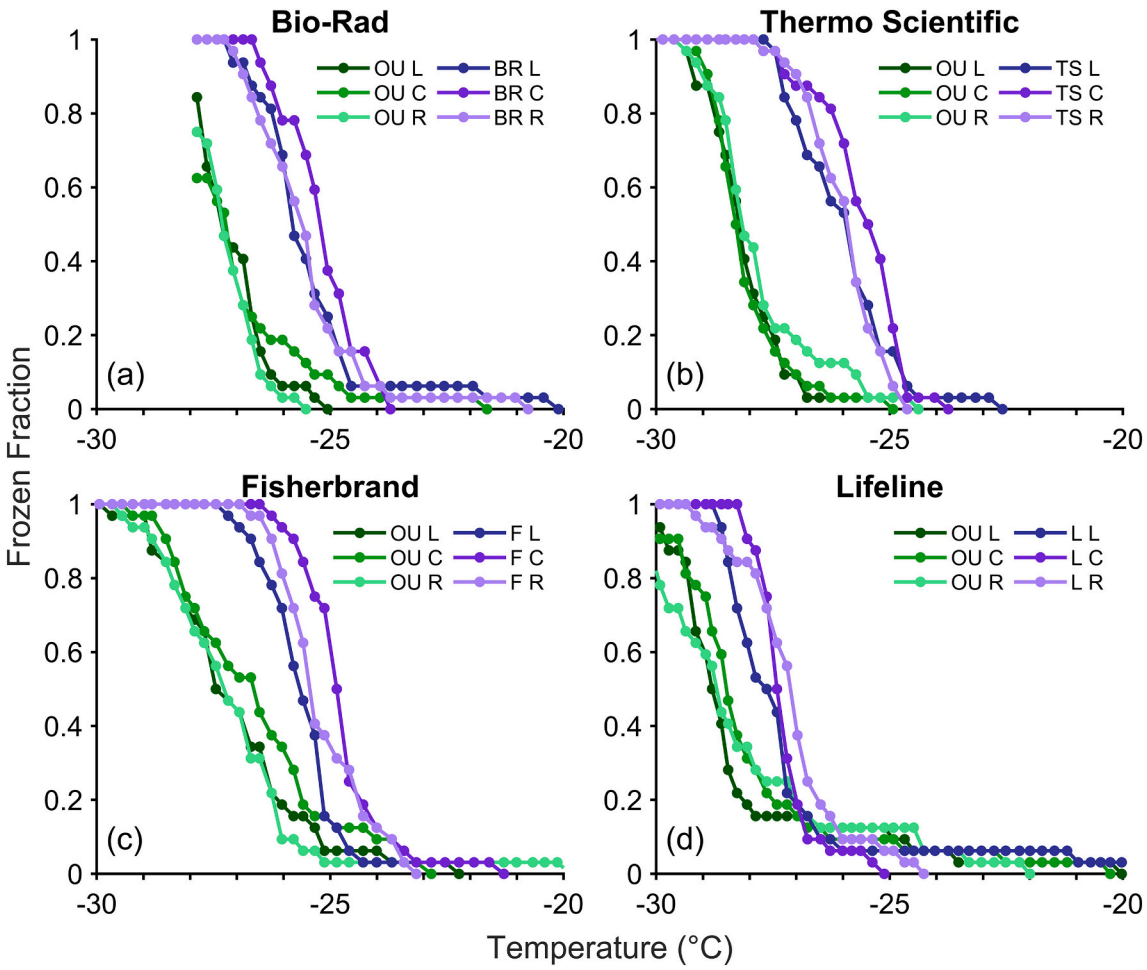

Fig. 4. Frozen fraction comparison of (a) Bio-Rad (BR), (b) Thermo Scientific™ (TS), (c) Fisherbrand™ (F), and (d) Lifeline™ (L) with OPTIMUM® ULTRA (OU) PCR trays. Each 96-well tray was divided into three 32-well sectors designated left (L) center (C) and right (R). All wells contained 50 µL of 0.1 µm-filtered DI water.

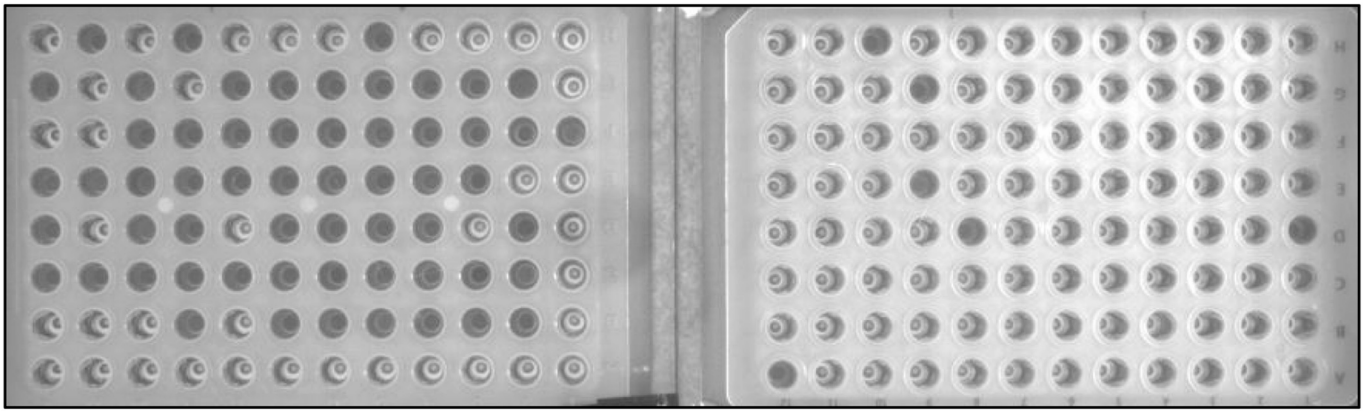

Fig. 5. Frozen wells of 0.1 µm-filtered DI water (dark gray) in adjacent PCR trays in the Ice Spectrometer at −26 °C. The left tray was made by Thermo Scientific™ and the right-side tray by OPTIMUM® ULTRA. Note that in the left tray most frozen wells were clustered in the center.

Out of an abundance of caution, we assumed that every tool, surface, and container was a significant extraneous INP source. The resulting general handling protocol delivers improvements to the limit of detection, as well as ensuring consistently accurate and representative measures over a wide temperature range. Although this continues to be a work in progress, we can recommend many straightforward and easily-adopted practices to limit INP contamination and so improve performance:

1) Work in a laminar flow hood, if available.
2) Fresh, unwashed plastic slider bags are recommended for storage and cleaning of laboratory tools as they contain few INPs.
3) The tested pipette tips, filtered compressed air dusters, Petri dishes, and pre-rinsed polypropylene centrifuge tubes are minor sources of INPs.
4) Washed cleanroom vinyl and polyethylene gloves retain very low numbers of INPs on their surfaces. Vinyl gloves are easier to work in

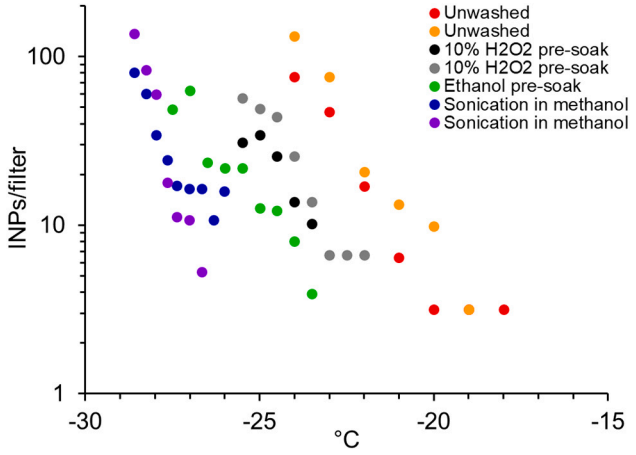

Fig. 6. Cumulative spectra of INPs released from Nuclepore™ polycarbonate filters after different cleaning protocols. Apart from unwashed Nuclepore™ filters, which were taken directly from a new pack, all filters were rinsed three times in DI water post treatment, the last two rinses using 0.1 μm-filtered DI. All filter tests were corrected for INPs in the DI water blank.

than polyethylene gloves, and are comparable in cost to nitrile gloves, the current standard.

5) Always work on fresh aluminum foil (inner surface) in the field, laboratory, and laminar flow hood.
6) PCR trays typically contain contaminating INPs. Of the five brands tested, the OPTIMUM® ULTRA brand was clearly superior.

Enhanced by these clean-working methods, we developed a simple but effective protocol for background INP removal from Nuclepore™ polycarbonate filters. This will be particularly beneficial for INP measures in clean environments, such as over oceans and in the free troposphere. If stringent handling protocols are followed at every step of preparation, sampling, and analysis, the number of INPs on field blanks will approach the cleanliness of the ultra-clean filters themselves.

Data availability

The data that support the findings of this study are available at: DOI: 10.17632/v3c3wc9gtg.1

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atmosres.2020.105419.

References

Agresti, A., Coull, B.A., 1998. Approximate is better than "exact" for interval estimation of binomial proportions. Am. Stat. 52, 119–126. https://doi.org/10.2307/2685469.

Beall, C.M., Stokes, M.D., Hill, T.C., DeMott, P.J., DeWald, J.T., DeWald, J.T., Prather, K. A., 2017. Automation and heat transfer characterization of immersion mode spectroscopy for analysis of ice nucleating particles. Atmos. Meas. Tech. 10, 2613–2626. https://doi.org/10.5194/amt-10-2613-2017.

Chen, J., Wu, Z.J., Augustin-Bauditz, S., Grawe, S., Hartmann, M., Pei, X.Y., Liu, Z.R., Ji, D.S., Wex, H.K., 2018. Ice-nucleating particle concentrations unaffected by urban air pollution in Beijing, China. Atmos. Chem. Phys. 18, 3523–3539. https://doi.org/10.5194/acp-18-3523-2018.

Creamean, J., Primm, K., Tolbert, M.A., Hall, E.G., Wendell, J., Jordan, A., Sheridan, P.J., Smith, J., Schnell, R., 2018. HOVERCAT: a novel aerial system for evaluation of aerosol-cloud interactions. Atmos. Meas. Tech. 11, 3969–3985. https://doi.org/10.5194/amt-11-3969-2018.

David, R.O., Cascajo-Castresana, M., Brennan, K.P., Rosch, M., Els, N., Werz, J., Weichlinger, V., Boynton, L.S., Bogler, S., Borduas-Dedekind, N., Marcolli, C., Kanji, Z.A., 2019. Development of the DRoplet Ice Nuclei Counter Zurich (DRINCZ): validation and application to field-collected snow samples. Atmos. Meas. Tech. 12, 6865–6888. https://doi.org/10.5194/amt-12-6865-2019.

DeMott, P.J., Prenni, A.J., Liu, X., Kreidenweis, S.M., Petters, M.D., Twohy, C.H., Richardson, M.S., Eidhammer, T., Rogers, D.C., 2010. Predicting global atmospheric ice nuclei distributions and their impacts on climate. Proc. Natl. Acad. Sci. U. S. A. 107, 11217–11222. https://doi.org/10.1073/pnas.0910818107.

DeMott, P.J., Hill, T.C.J., Petters, M.D., Bertram, A.K., Tobo, Y., Mason, R.H., Suski, K.J., McCluskey, C.S., Levin, E.J.T., Schill, G.P., Boose, Y., Rauker, A.M., Miller, A.J., Zaragoza, J., Rocci, K., Rothfuss, N.E., Taylor, H.P., Hader, J.D., Chou, C., Huffman, J.A., Poschl, U., Prenni, A.J., Kreidenweis, S.M., 2017. Comparative measurements of ambient atmospheric concentrations of ice nucleating particles using multiple immersion freezing methods and a continuous flow diffusion chamber. Atmos. Chem. Phys. 17, 11227–11245. https://doi.org/10.5194/acp-17-11227-2017.

DeMott, P.J., Mohler, O., Cziczo, D.J., Hiranuma, N., Petters, M.D., Petters, S.S., Belosi, F., Bingemer, H.G., Brooks, S.D., Budke, C., Burkert-Kohn, M., Collier, K.N., Danielczok, A., Eppers, O., Felgitsch, L., Garimella, S., Grothe, H., Herenz, P., Hill, T. C.J., Hohler, K., Kanji, Z.A., Kiselev, A., Koop, T., Kristensen, T.B., Kruger, K., Kulkarni, G., Levin, E.J.T., Murray, B.J., Nicosia, A., O'Sullivan, D., Peckhaus, A., Polen, M.J., Price, H.C., Reicher, N., Rothenberg, D.A., Rudich, Y., Santachiara, G., Schiebel, T., Schrod, J., Seifried, T.M., Stratmann, F., Sullivan, R.C., Suski, K.J., Szakall, M., Taylor, H.P., Ullrich, R., Vergara-Temprado, J., Wagner, R., Whale, T.F., Weber, D., Welti, A., Wilson, T.W., Wolf, M.J., Zenker, J., 2018. The Fifth International Workshop on Ice Nucleation phase 2 (FIN-02): laboratory intercomparison of ice nucleation measurements. Atmos. Meas. Tech. 11, 6231–6257. https://doi.org/10.5194/amt-11-6231-2018.

Garcia, E., Hill, T.C.J., Prenni, A.J., DeMott, P.J., Franc, G.D., Kreidenweis, S.M., 2012. Bacterial and organic ice nuclei in air over two U.S. High Plains agricultural regions. J. Geophys. Res. 117 https://doi.org/10.1029/2012JD018343. D18209.

Garçon, M., Sauzéat, L., Carlson, R.W., Shirey, S.B., Simon, M., Balter, V., Boyet, M., 2017. Nitrile, latex, neoprene and vinyl gloves: a primary source of contamination for trace element and Zn isotopic analyses in geological and biological samples. Geostand. Geoanal. Res. 41, 367–380. https://doi.org/10.1111/ggr.12161.

Gute, E., Abbatt, J.P., 2020. Ice nucleating behavior of different tree pollen in the immersion mode. Atmos. Environ. 231, 117488. https://doi.org/10.1016/j.atmosenv.2020.117488.

Harrison, A.D., Whale, T.F., Rutledge, R., Lamb, S., Tarn, M.D., Porter, G.C.E., Adams, M., McQuaid, J.B., Morris, G.J., Murray, B.J., 2018. An instrument for quantifying heterogeneous ice nucleation in multiwell plates using infrared emissions to detect freezing. Atmos. Meas. Tech. 11, 5629–5641. https://doi.org/10.5194/amt-2018-177.

Hill, T.C.J., DeMott, P.J., Tobo, Y., Froehlich-Nowoisky, J., Moffett, B.F., Franc, G.D., Kreidenweis, S.M., 2016. Sources of organic ice nucleating particles in soils. Atmos. Chem. Phys. 16, 7195–7211. https://doi.org/10.5194/acp-16-7195-2016.

Hiranuma, N., Augustin-Bauditz, S., Bingemer, H., Budke, C., Curtius, J., Danielczok, A., Diehl, K., Dreischmeier, K., Ebert, M., Frank, F., Hoffmann, N., Kandler, K., Kiselev, A., Koop, T., Leisner, T., Mohler, O., Nillius, B., Peckhaus, A., Rose, D., Weinbruch, S., Wex, H., Boose, Y., DeMott, P.J., Hader, J.D., Hill, T.C.J., Kanji, Z.A., Kulkarni, G., Levin, E.J.T., McCluskey, C.S., Murakami, M., Murray, B.J., Niedermeier, D., Petters, M.D., O'Sullivan, D., Saito, A., Schill, G.P., Tajiri, T., Tolbert, M.A., Welti, A., Whale, T.F., Wright, T.P., Yamashita, K., 2015. A comprehensive laboratory study on the immersion freezing behavior of illite NX particles: a comparison of 17 ice nucleation measurement techniques. Atmos. Chem. Phys. 15, 2489–2518. https://doi.org/10.5194/acp-15-2489-2015.

Kanji, Z.A., Ladino, L.A., Wex, H., Boose, Y., Burkert-Kohn, M., Cziczo, D.J., Kramer, M., 2017. Overview of ice nucleating particles. Meteorol. Monogr. 58, 1.1–1.33. https://doi.org/10.1175/Amsmonographs-D-16-0006.1.

Kunert, A.T., Lamneck, M., Helleis, F., Poschl, U., Pohlker, M.L., Frohlich-Nowoisky, J., 2018. Twin-plate Ice Nucleation Assay (TINA) with infrared detection for high-throughput droplet freezing experiments with biological ice nuclei in laboratory and field samples. Atmos. Meas. Tech. 11, 6327–6337. https://doi.org/10.5194/amt-11-6327-2018.

McCluskey, C.S., Hill, T.C.J., Humphries, R.S., Rauker, A.M., Moreau, S., Strutton, P.G., Chambers, S.D., Williams, A.G., McRobert, I., Ward, J., Keywood, M.D., Harnwell, J., Ponsonby, W., Loh, Z.M., Krummel, P.B., Protat, A., Kreidenweis, S.M., DeMott, P.J., 2018. Observations of ice nucleating particles over Southern Ocean waters. Geophys. Res. Lett. 45, 11989–11997. https://doi.org/10.1029/2018gl079981.

Miller, A., Brennan, K., Wieder, J., Mignani, C., Zipori, A., Borduas-Dedekind, N., 2019. Development of drop freezing ice nucleation chamber (FINC), validation using lignin, and application to organic matter samples. In: EGU General Assembly 2020, pp. EGU2020–630. https://doi.org/10.5194/egusphere-egu2020-630.

Moffett, B.F., 2016. Fresh water ice nuclei. In: Fundam. Appl. Limnol./Archiv für Hydrobiologie, 188, pp. 19–23. https://doi.org/10.1127/fal/2016/0851.

Mülmenstädt, J., Sourdeval, O., Delanoë, J., Quaas, J., 2015. Frequency of occurrence of rain from liquid-, mixed-, and ice-phase clouds derived from A-Train satellite retrievals: rain from liquid- and ice-phase clouds. Geophys. Res. Lett. 42, 6502–6509. https://doi.org/10.1002/2015GL064604.

O'Sullivan, D., Murray, B.J., Ross, J.F., Whale, T.F., Price, H.C., Atkinson, J.D., Umo, N. S., Webb, M.E., 2015. The relevance of nanoscale biological fragments for ice nucleation in clouds. Sci. Rep. 5, 8082. https://doi.org/10.1038/srep08082.

Polen, M., Brubaker, T., Somers, J., Sullivan, R.C., 2018. Cleaning up our water: reducing interferences from nonhomogeneous freezing of "pure" water in droplet freezing assays of ice-nucleating particles. Atmos. Meas. Tech. 11, 5315–5334. https://doi.org/10.5194/amt-11-5315-2018.

Šantl-Temkiv, T., Sikoparija, B., Maki, T., Carotenuto, F., Amato, P., Yao, M., Morris, C. E., Schnell, R., Jaenicke, R., Pöhlker, C., DeMott, P.J., Hill, T.C.J., Huffman, J.A., 2019. Bioaerosol field measurements: challenges and perspectives in outdoor studies. Aerosol Sci. Technol. 54, 520–546. https://doi.org/10.1080/02786826.2019.1676395.

Schiebel, T., 2017. Ice Nucleation Activity of Soil Dust Aerosols. Karlsruhe Institute of Technology, Karlsruhe, Germany.

Stopelli, E., Conen, F., Zimmermann, L., Alewell, C., Morris, C.E., 2014. Freezing nucleation apparatus puts new slant on study of biological ice nucleators in precipitation. Atmos. Meas. Tech. 7, 129–134. https://doi.org/10.5194/amt-7-129-2014.

Tobo, Y., 2016. An improved approach for measuring immersion freezing in large droplets over a wide temperature range. Sci. Rep. 6, 32930. https://doi.org/10.1038/srep32930.

Vali, G., 1971. Quantitative evaluation of experimental results on heterogeneous freezing nucleation of supercooled liquids. J. Atmos. Sci. 28, 402–409. https://doi.org/10.1175/1520-0469(1971)028<0402:QEOERA>2.0.CO;2.

Zaragotas, D., Liolios, N.T., Anastassopoulos, E., 2016. Supercooling, ice nucleation and crystal growth: a systematic study in plant samples. Cryobiology 72, 239–243. https://doi.org/10.1016/j.cryobiol.2016.03.012.