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A high-flow-rate impinger for the study of concentration, viability, metabolic activity, and ice nucleation activity of airborne bacteria

Tina Šantl-Temkiv^{1,2,3*}, Pierre Amato⁴, Ulrich Gosewinkel^{5*}, Runar Thyrhaug^{6Ω}, Anaïs Charton⁴, Benjamin Chicot⁴, Kai Finster^{2,3}, Gunnar Bratbak⁶, and Jakob Löndahl¹

¹ Lund University, Department of Design Sciences, Ergonomics and Aerosol Technology, P.O. box 118, SE-22100 Lund, Sweden

² Aarhus University, Stellar Astrophysics Centre, Department of Physics and Astronomy, Ny Munkegade 120, DK-8000 Aarhus, Denmark

³ Aarhus University, Department of Bioscience, Microbiology Section, Ny Munkegade 116, DK-8000 Aarhus, Denmark

⁴ Université Clermont Auvergne, CNRS, Institut de Chimie de Clermont-Ferrand, BP 10448, F-63000 Clermont-Ferrand, France.

⁵ Aarhus University, Department of Environmental Science, Frederiksborgvej 399, DK-4000 Roskilde, Denmark

⁶ University of Bergen, Dept. of Biology, Thormøhlensgt. 53 A/B, PO box 7803, N-5020 Bergen, Norway.

* Correspondence to: U Gosewinkel: Ulrich.Gosewinkel@envs.au.dk, tel: +45 871 58617; T Šantl-Temkiv: temkiv@phys.au.dk, tel: + 45 871 68464

^Ω We dedicate this paper to our colleague Runar Thyrhaug, who initiated the study, but sadly passed away during its initial phase.

Abstract

The study of airborne bacteria relies on a sampling strategy that preserves their integrity and *in situ* physiological state, e.g. viability, cultivability, metabolic activity, and ice-nucleation activity. As ambient air harbours low concentrations of bacteria, an effective bioaerosol sampler should have a high sampling efficiency and a high airflow. We characterize a high-flow-rate impinger with respect to particle collection and retention efficiencies in the range 0.5- 3.0 μm , and we investigated its ability to preserve the physiological state of selected bacterial species and seawater bacterial community in comparison with 4 commercial bioaerosol samplers. The collection efficiency increased with particle size and the cut-off diameter was between 0.5 and 1 μm . During sampling periods of 120-300 min, the impinger retained cultivability, metabolic activity, viability, and ice-nucleation activity of investigated bacteria. Field studies in semi-urban, high-altitude, and polar environments included periods of low bacterial air-concentrations thus demonstrating the benefits of the impinger's high flow rate. In conclusion, the impinger described here has many advantages compared with other bioaerosol samplers currently on the market: a potential for long sampling time, a high flow rate, a high sampling and retention efficiency, low costs, and applicability for diverse downstream microbiological and molecular analyses.

1. Introduction

Knowledge on the activity and abundance of primary biological aerosol particles (PBAP) is important for a variety of fields and applications, including atmospheric chemistry and physics, biogeography, meteorology, hospital hygiene, epidemiology as well as industry that is sensitive to contamination, such as pharmaceuticals or food processing. For the assessment of PBAP, a sampling strategy is needed that preserves the integrity and *in situ* state of these particles. Airborne bacteria are a particularly important group of PBAP. (i) They are potential human, animal and plant pathogens¹. (ii) Airborne bacteria that maintain their metabolic activity can affect atmospheric chemical processes, in particular radical chemistry², carbon³⁻⁵ and nitrogen⁶ cycles. (iii) Some bacterial species, such as *Pseudomonas syringae*, can be involved in cloud formation, thus influencing patterns of precipitation, meteorology and climate⁷⁻¹¹ due to physiological properties like ice-nucleation activity¹² and biosurfactant production¹³.

Atmospheric concentrations of bacteria are much lower than in many other environments, including deep marine and terrestrial subsurfaces¹⁴. It has been observed that there are on average between 5 and 350 cells found per L of air over terrestrial and marine surfaces¹⁵. These low bacterial concentrations imply sampling at high air flow rates (on the order of 100 L/min or higher), and/or for prolonged duration (on the order of hours or days) in order to obtain sufficient biomass for downstream analyses. Nevertheless, excessively long sampling time may deteriorate the physiological state of sampled bacterial cells¹⁶, and prevent the assessment of short-term variability¹⁷. Consequently, bioaerosol sampling requires efficient particle collection at high flow rate, while preserving biological properties of interest, such as cell integrity and metabolic activity.

A number of samplers are available on the market for collection of bacteria on filters, on agar, or into liquid¹⁸. Filter samplers typically capture most of the biological material with a high efficiency and can be adapted for the use at high flows¹⁹. However, cell properties are largely affected by desiccation and other stresses over prolonged sampling time by air filtration²⁰. Alternatives include the collection of microorganisms directly on nutrient plates for cultivation-dependent studies^{21–23}. However, aside from being biased towards the cultivable fraction of the collected cells, this method impedes downstream community analysis using state-of-the-art molecular approaches and single-cell analyses. Living biological material is therefore often preferably sampled into a liquid (*i.e.* by impingement), which more efficiently preserves cell viability and allows fixation of cellular biological properties in real-time^{20,24,25}.

Among the most common devices for sampling into liquid, both the all-glass impinger (AGI-30) and the SKC BioSampler use airflows of 12.5 L/min²⁴. There are also devices for sampling at higher flow rates, such as the XMX-CV that collects particles in the 1-10 μm range at a flow rate of 530 L/min. However, due to evaporation of the sampling liquid, the total sampling duration cannot exceed 10 min according to user's manual. Hence, the current impingers appear poorly adapted to sampling airborne microorganisms in environments where their concentration is lowest, like at high altitude and in remote areas.

The objective of this study was to evaluate the use of a commercially available vacuum cleaner, which collects aerosols into a liquid, as a high-flow-rate impinger. We hypothesize that: (i) The performance of this impinger in terms of sampling and retention efficiency is comparable to currently available impingers for bio-aerosol sampling; (ii) The high-flow rate of the impinger allows it to be used in diverse environments, including pristine environments with low bacterial

loads; (iii) The impinger preserves bacterial cultivability, viability, metabolic activity, and ice-nucleation activity; and (iv) The samples collected by the impinger are suitable for downstream analyses.

2. Materials and Methods

2.1 Description of the impinger and tests performed

The characterized high-flow-rate impinger is a new application of a water based commercial vacuum cleaner by Kärcher (Alfred Kärcher GmbH & Co. KG, Germany, SI-Figure 1). Air samples were collected using the two models, DS5600, and its more recent version DS5800. The Kärcher impinger consists of an outer part that generates the suction, and an inner part, the “vortex chamber”²⁶ that contains the sampling liquid. The air flow within the vortex chamber of the Kärcher DS5600/DS5800 is illustrated in Figure 1. Both models operate by the same principle, but the DS5800 has been slightly modified by the manufacturer, reducing the size of the vortex chamber to facilitate higher airflow. The Kärcher impinger works as a high-flow-rate aerosol collector: the air enters the vortex chamber and swirls through the sampling liquid, which thereby captures the airborne particles and water-soluble gases. The sampling liquid is added by lifting the lid of the vortex chamber. The air flow through the vortex chamber was measured using a wind speed meter with a rotating vane sensor (Kimo LVB, Marne-la-Vallee, France) connected to the inlet pipe. For measuring the flow, the impinger was run in the same way as when collecting samples. The effect of different collecting liquids was evaluated, but did not have a large impact on the flow (data not presented). The flow was kept at $(0.8-0.9) \times 10^3$ L/min during sampling when powered by generators in the field and at 3.1×10^3 L/min when connected

to the electrical circuit, which was close to the values provided by the manufacturer (3.3×10^3 L/min). For the model DS5800, the airflow rate specified by the manufacturer (4.1×10^3 L/min) was adopted, as it was run connected to the electrical circuit.

The samplers were validated using: (i) sampling efficiency laboratory tests, (ii) retention efficiency laboratory tests, and (iii) validation field tests. An overview of all experiments is presented in Table 1. Field tests were performed in Norway, France, and Greenland. In two of the tests commercially available bioaerosol samplers were used as references.

2.2. Cleaning and sterilizing the impinger

Samplers and sampling liquids were decontaminated prior to each assay by applying sterilization and rinsing procedures depending on the application (Table 1). *E.g.* a simple rinsing (Milli-Q H₂O rinse) was required when using microspheres for determining sampling efficiency, a mild sterilization (consecutive Ethanol and Milli-Q H₂O rinses) was required when examining retention and preservation efficiency with model bacteria, and a stronger sterilization procedure was required when collecting environmental samples (consecutive rinses with either HCl / sterile Milli-Q H₂O / 96% Ethanol or 10% HNO₃ / deionized H₂O / bleach / deionized sterile H₂O). The different sterilization liquids were either applied manually or by running the impinger for 5 min having the vortex chamber filled with the respective liquid. The filter that protects the motor (filter in Figure 1) was rinsed with the same sterilization liquids and replaced to its original position. When transported into the field, the containers were kept enclosed in sterile plastic bags and opened just before use. All sampling solutions were autoclaved (1-3 times) and then filtered (0.22 µm or 0.1 µm porosity) to remove particles. Sterility and absence of particulate

contaminants were verified by collecting control samples at the start of each sampling event. For negative controls, the impinger was run for 5 min with the sampling solution, so that the liquid came into contact with the entire container. An aliquot of the sampling solution was then withdrawn from the chamber and analysed in parallel to the samples. In most cases the negative control was produced under sterile conditions, with the exception of samples collected in Greenland, where the negative control was taken in the field. For the latter negative controls, we present the values in SI-Figure 9A.

2.3 Experimental evaluation of the impinger

2.3.1. Collection efficiency using an aerosol chamber

The collection efficiency of the samplers was evaluated with monodisperse fluorescent polystyrene microspheres (Corpuscular Inc., Thermo Fisher Scientific Inc.) of different diameters (500 nm, 790 nm, 1000 nm, 1400 nm, 2000 nm, and 3000nm) (Figure 1). The microspheres were sprayed into an aerosol mixing chamber made of galvanized steel (diameter 0.5 m, volume 0.2 m³) and sampled with the impinger. Fluorescent microspheres were mixed by shaking and a brief ultrasonication (~5 s) in an ultrasonic bath. Thereafter, the microspheres were diluted in Milli-Q (MQ) water to a final concentration of 1.3×10^7 – 1.8×10^8 beads mL⁻¹. The microsphere suspension was fed into a sparging liquid aerosol generator at 60 mL h⁻¹ (SLAG, CH Technologies, Inc), which creates a relatively high particle number concentration at an airflow of 12 L/min of aerosols that is stable over time²⁷. A high dilution, 3100 L/min, was needed in order to achieve sufficient flow rates for the impinger. The collection efficiency was determined by measuring particle concentration in the air and in the impinger liquid. The particle concentration in the

aerosol chamber was kept at the same order of magnitude as atmospheric concentrations of bacteria (SI-Figure 2): $270 \pm 2 \text{ L}^{-1}$ for 500 nm microspheres (average \pm standard error of the mean), $41 \pm 2 \text{ L}^{-1}$ for 790 nm microspheres, $198 \pm 4 \text{ L}^{-1}$ for 1000 nm microspheres, $46 \pm 1 \text{ L}^{-1}$ for 1400 nm microspheres, $84 \pm 2 \text{ L}^{-1}$ for 2000 nm microspheres, and $32 \pm 1 \text{ L}^{-1}$ for 3000 nm microspheres. The concentration was measured at one-minute time resolution and typically varied by $\pm 30\%$ over time with periods of low concentration when the microsphere solution was refilled.

Aerosolized fluorescent microspheres were collected from the aerosol chamber using impinger model DS5600 for either 1 h or 5 hrs. Three 1.5 mL MQ water aliquots were taken as negative controls before pouring the MQ water into the vortex chamber, and from the vortex chamber after collecting clean air within the aerosol chamber for 5 min. During the 1 h tests, triplicate 1.5 mL sub-samples were removed from sampling liquid every 10 min, and during the 5 h tests 1.5 mL sub-samples were withdrawn after 5, 30, 60, 90, 120, 180, 240, and 300 min. Both negative controls and samples were kept in the dark at 4°C before they were analysed by flow cytometry (FC).

A scanning mobility particle sizer SMPS (10-900 nm particles, design: Lund University) and an aerodynamic particle sizer (0.5-20 μm particles, APS, model 3321, TSI Inc., US) were used to obtain the aerosol number concentrations aloft in the chamber. Usually, microspheres appeared as monodisperse particle number concentration peaks, which were integrated to determine airborne concentrations. These were used to infer the theoretical numbers of microspheres sampled by the impinger and to calculate size-resolved particle collection efficiencies by comparison with the

concentration derived from FC counts in the sampling liquid (c_{FCM}) as: $Eff [\%] = (c_{FC} / (c_{APS} + c_{SMPS})) \cdot 100$.

2.3.2 Retention efficiency of the Kärcher impinger and of four bioaerosol impingers

The retention efficiency for the Kärcher impingers DS5600, DS5800 and four commercially available bioaerosol impingers were assessed in either laboratory or field tests (Supporting Information, SI, Section SI-1.1). The sampling liquid was spiked with an ice nucleation active (INA) *Pseudomonas syringae* 32b-74 (GenBank A.N. HQ256872)²⁸, *Pantoea agglomerans* (ATCC 33243), *Bacillus atrophaeus* (ATCC 9372), or seawater bacterial community that was collected in a sterile container just before the experiments. The retention efficiency was assessed by running the impingers for 2-5 hrs. The ability of the Kärcher DS5600 impinger to maintain cell integrity was compared to four commercially available impingers (XMX-CV, SKC BioSampler, SASS 2000PLUS, and SpinCon) in field tests, and the retention efficiency of Kärcher DS5800 was tested in a laminar flow hood. The samples were assessed by FC for total (Sybr Green I staining) and viable cell (Live/Dead staining) concentrations, colony forming units (CFU) on R2A nutrient plates, protein synthesis activity measurements using tritium-labelled leucine²⁹, and ice nucleation activity (droplet-freezing assays) (Table 1, Supporting Information, SI, Section SI-1.3).

2.4 Bioaerosol collection in ambient air

Bioaerosol sampling in ambient outdoor air was performed using Kärcher DS5800 impinger at 2 locations: from the roof of a building at the Clermont-Auvergne University campus “Les Cézeaux” (45.76111 N, 3.116667 E), at ~4 m above the ground, and from the roof platform of the atmospheric observatory of the Puy de Dôme mountain, nearby the city of Clermont-Ferrand (45.77222 N, 2.96583 E, 1465 m asl) (Table 1). Sampling was performed for 3 to 12 consecutive hours, with subsamples of the sampling liquid (sterile NaCl 0.9% solution) taken every hour in a laminar flow hood. Before subsampling, evaporation losses were determined by weighing and corrected by addition of sterile fresh sampling liquid or deionized water. For all calculations, we assumed 100% sampling efficiency and no significant cell loss from the sampling liquid (based on the laboratory test: see Figures 2A and 4A).

Three air samples were collected in duplicates in southwest Greenland from the M500 peak (500 m above sea level, 64.12264 N, 51.36619 W) at the Nuuk Basic research station (Table 1). The Kärcher impinger was powered by generators, thus the air flow was kept at $(0.8-0.9) \times 10^3$ L/min during the sampling period of 5 hrs, which resulted in the collection of aerosols from $(1.30-1.45) \times 10^5$ L of air. The samples were collected into a high-salt solution, which is designed for real-time preservation of sensitive RNA molecules³⁰. Duplicate samples were collected using two Kärcher impingers DS5600 in order to evaluate sampling reproducibility. The samples and the negative controls were concentrated onto SterivexTM filter units, and stored in presence of 1 mL of RNA later (Thermo Fisher Scientific Inc, Massachusetts) as described by Lever *et al*³⁰. The sampling solution was autoclaved twice and filter sterilized (0.1 μ m, VacuCap 90 Devices, Pall Corporation, New York). During sampling, evaporated water was replaced with sterile MQ water.

3. Results and discussion

3.1 Sampling efficiency at different particle sizes

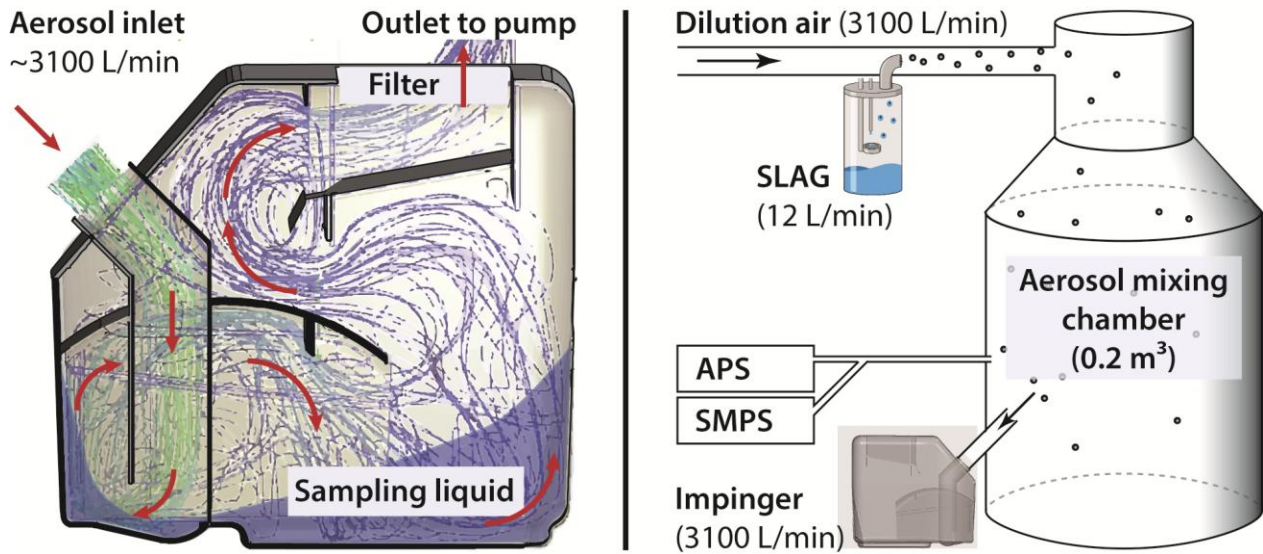


Figure 1: (Left) Model of the airflow in the Kärcher DS5800 impinger. Aerosol enters by the inlet (on the left), impacts and rotates through the liquid at the bottom, and leaves through an outlet on the top. Filter has the function of sealing the vortex chamber and protecting the motor of the vacuum. (Right) Schematic of the experimental setup deployed for sampling efficiency tests. SLAG stands for Sparging Liquid Aerosol Generator, APS for Aerodynamic Particle Sizer, and SMPS for Scanning Mobility Particle Sizer.

The collection efficiency and cut-off diameters of the sampler were assessed by sampling microspheres of defined size and known concentration from an aerosol chamber. The collection efficiency (Figure 2A) increased with increasing microsphere diameter, with $25\pm5\%$ for 500 nm microspheres and $69\pm17\%$ – $199\pm28\%$ for larger particles. Based on these experiments we estimated a cut-off diameter of between 0.5 and 1 μm . Efficiencies higher than 100 % are likely due to microsphere doublets causing the APS to underestimate their actual number concentration in air. Thus, there may be an overall overestimate of sampling efficiency. There is an additional

uncertainty from particle losses at the sampling inlets and heterogenous particle mixing, as laminar homogenous isokinetic sampling was not possible to achieve at these high flow rates.

There was no saturation with microspheres in the sampling liquid over a sampling period of 5 hours at microsphere air concentrations comparable to high bacterial concentrations in ambient air ($\sim 100 \text{ L}^{-1}$). Figure 2B confirms that the concentrations of microspheres with a diameter of 500 nm and 1400 nm in the sampling liquid increased linearly with sampling time (1400 nm: $R^2=0.97$, $p < 0.001$, 500 nm: $R^2= 0.97$, $p < 0.001$).

Collecting subsamples of the sampling liquid at dedicated times allowed us to follow the particle number concentration in air at various time resolutions, which were higher than achievable with most bioaerosol samplers (Figure 2D). The concentrations measured in the collection liquid of the impinger reflected the airborne particle number concentrations measured with the APS, within an order of magnitude (Figure 2C). At bacterial number concentrations of $\sim 100 \text{ L}^{-1}$, which is at the higher end of bacterial cell concentrations in outdoor air¹⁵, sampling for analyses at a time resolution of 30 min was feasible.

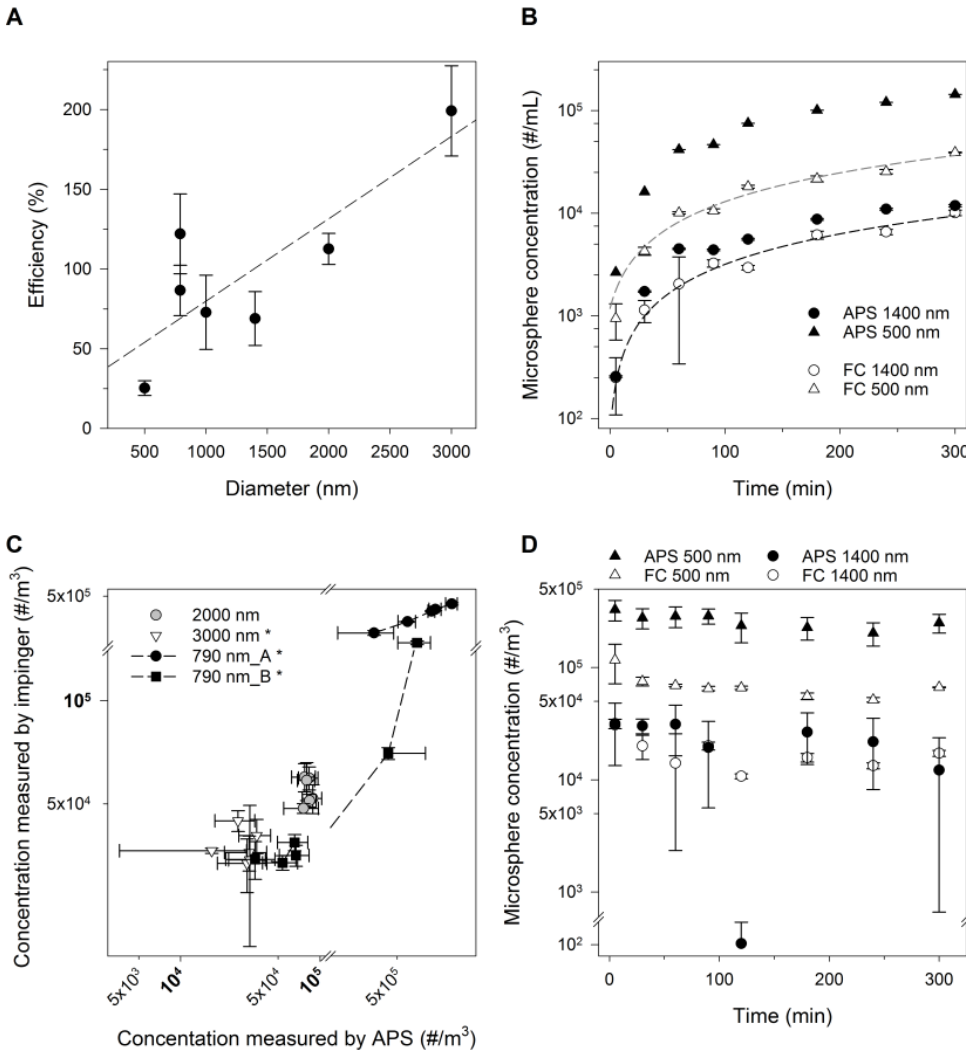


Figure 2: Five-hour sampling tests of fluorescent microspheres in an aerosol chamber at relevant particle number concentrations ($32\text{--}270\text{ L}^{-1}$ for 2A, $\sim 100\text{ L}^{-1}$ for 2B-D). 2A: Particle size resolved sampling efficiency. Experiment was performed twice with 790 nm microspheres and both values are presented. 2B: Comparison of the concentrations of microspheres in the sampling liquid measured by FC and the predicted concentration based on the aerosol concentration measured by APS. The linear model (dashed lines) is shown for the microspheres concentration measured by FC. 2C: Correlation between particle number concentration measured with the impinger and with the optical particle counter (APS) for different sphere sizes and for experiments performed for 1 h. An asterisk next to the legend indicates a significant linear regression model ($p < 0.001$). 2D: The average number concentration of microspheres in the aerosol chamber as assessed with the optical particle counter (APS) and the impinger (FC) for experiments performed for 5 hours.

Dybwad et al²⁰ showed that, aside from filter samplers, all bioaerosol samplers had low collection efficiency for 1 μm fluorescent microspheres and efficiently collected microspheres in the 4 μm size range. The high-flow-rate impinger XMX-CV had the highest efficiency reduction for 1 μm compared to 4 μm microspheres (26-fold), collecting between ~4% and ~20% of airborne 1 μm microspheres²⁰. In comparison, the Kärcher impinger performed much better with approximately 75% collection efficiency at ~1 μm . The AGI-30 impinger and the SKC BioSampler have collection efficiencies above 90% at 1 μm , but operate at flowrates that are three orders of magnitude lower, which allows for longer deposition times and more efficient impaction due to a larger pressure drop in collection zone. It has been shown that bacterial cell shape influences its capture efficiency, so that the rod-shaped cells are captured with higher efficiency than spherical cells³¹. Many bacterial cells, such as cells of the INA species *Pseudomonas fluorescens*, are rod shaped with diameters 300–800 nm, and lengths 1000–3000 nm, resulting in an aerodynamic diameter of approximately 800 nm³². However, the majority of airborne bacteria at inland sites were found associated with particles and thus had an aerodynamic diameter larger than 3000 nm³³. Thus, airborne biological particles, including bacteria, are in the diameter range that is covered by the high efficiency range of the Kärcher impingers. This demonstrates that the majority of airborne particles in size ranges similar to those observed for bacteria are collected by the impinger.

3.2 Retention efficiency and maintenance of viability

Preservation of cell viability is an important criterion for the applicability of bio-aerosol samplers, as survival is essential for further physiological studies. Three tests were performed on different types of model bacteria (Proteobacteria: *P. agglomerans* and *P. syringae*; Firmicutes: *B.*

atrophaeus) as well as on a complex bacterial community from a sea water sample. The Kärcher impinger performed reproducibly with respect to liquid loss rate, loss rate of bacterial cells, cell viability, cell activity, and cell ice nucleation activity over the total sampling period (Table 2, Figure 3). It also showed similar trends independent of the type of bacteria spiked in the samplers.

A model INA strain of *P. syringae* was spiked into the sampling liquid to evaluate retention of the total- and viable-cell concentrations as well as concentrations of ice nucleating particles (INP) at temperatures between -2°C and -7°C. The total cell concentration of *P. syringae* did not change over a 5 hour sampling period (Kruskal-Wallis test; $p > 0.05$) (Table 2), indicating that losses of bacteria due to reaerosolization from the vortex chamber during sampling are negligible. However, the proportion of viable cells, relative to total cells, dropped within the first hour of sampling, from 18%±10% to 2.2%±0.2% (i.e. ~12% of the initial viability was retained). Later, viability remained stable over time (Kruskal-Wallis test; $p > 0.05$). The concentrations of ice nucleating particles behaved similarly, with the concentration of highly active INP decreasing by two orders of magnitude within the first two hours of sampling, and no further change over time until the end of the sampling period (Figure 3). Sampling operations caused the ice nucleation profiles to be shifted down in temperature by less than 2°C, indicating that the impinger did not cause significant damage to the sample and is suitable for detecting INP in bioaerosol populations.

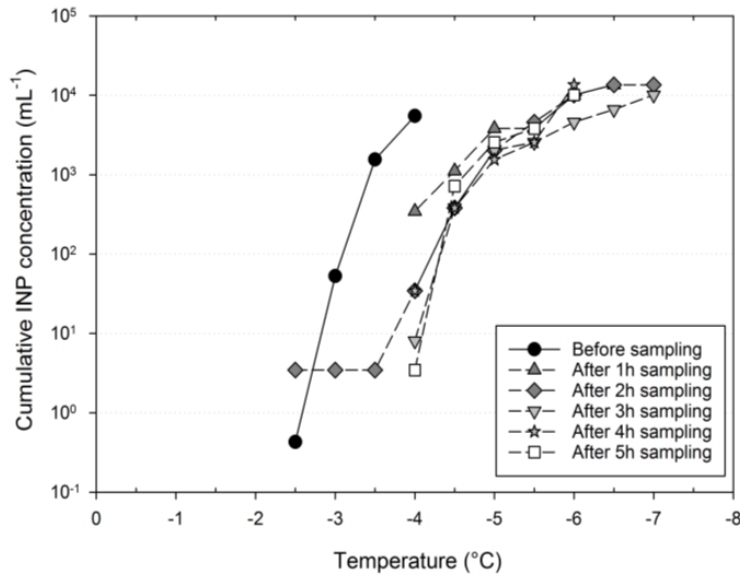


Figure 3: Retention efficiency of bacterial INP in the Kärcher DS5800 impinger spiked with INA *P. syringae* over 5 hours of sampling.

Two model bacterial strains and a complex bacterial seawater community were spiked into the vortex chamber in order to test the extent to which bacterial concentration, viability, and metabolic activity were affected by a sampling period of 120 min. The abundance of seawater bacteria remained unchanged while the abundances of *P. agglomerans* and *B. atrophaeus* cells were reduced to $70\pm3\%$ and $79\pm3\%$ of their initial values after 120 min (SI-Figure 3, Table 2). The concentration of cultivable cells decreased by 50-65% (SI-Figure 4, Table 2). Relative leucine incorporation in the seawater bacteria decreased by $61\pm7\%$ compared to the initial values (SI-Figure 4, Table 2). Also, the fraction of cultivable among total cells decreased (SI-Figure 4, Table 2). However, the fraction of cultivable and metabolically active cells among the total cell population decreased rapidly within the first 5 min of sampling for *P. agglomerans* and seawater bacteria, but was more stable during the later sampling period.

The retention efficiency of the Kärcher impinger was compared to four other commercially available impingers (Table 2, SI-Figure 3-4), including the SKC BioSampler, which was previously used as a reference sampler due to its well characterized properties²⁰. The Kärcher impinger has the highest flow rate (1-2 orders of magnitude higher) and the lowest liquid loss rate of all samplers (Table 2). One limitation could be that the liquid volume in the Kärcher impinger is hundredfold higher than in other samplers. We show, however, that the bioaerosols collected by the Kärcher impinger can be concentrated 1000-times, for example by using SterivexTM filter units for performing nucleic acid extractions (see section 2.4). Liquid can be replenished through the lid of the vortex chamber by pausing the sampling. In this way we can compensate for evaporation, thus extending the sampling period. Refilling the sampling liquid is not an option in all other models (e.g. XMX-CV), which limits the sampling time.

Using flow cytometry we show that the Kärcher impinger had lower cell losses than all other samplers except for the XMX-CV. About $\frac{1}{3}$ of the cultivable and $\frac{1}{2}$ of the active cells retained cultivability/activity at the end of the sampling period with the Kärcher impinger DS5600 (Table 2). The XMX-CV and the SKC BioSampler had a 0.9-2.6 times higher efficiency in retaining cell cultivability/activity than Kärcher impinger DS5600. Thus, the performance of the Kärcher impinger in terms of preserving bacterial cultivability/activity was comparable with the SKC BioSampler and worse than the performance of the XMX-CV in case of the sea-water community and *P. agglomerans*. We suggest that the short sampling time (10 min) of the XMX-CV is responsible for the better performance of the XMX-CV sampler in terms of both cell losses and bacterial activity losses. The SKC BioSampler is slightly better than the Kärcher DS5600 impinger at preserving the cultivability/activity even after one hour of operation. The flow rate of this sampler, however, is more than 100-times lower than the flow rate of the Kärcher impinger,

364 which means that highly time-resolved sampling is impossible. Overall, the Kärcher impinger has
365 many advantages for investigating environmental atmospheric bacteria compared to other tested
366 impingers, due to its high flow rate, together with a comparatively high retention efficiency and
367 the possibility of long sampling time.

Table 1. Overview of tests and samplers used and the weather conditions for outdoor tests.

Experiment ^A	Sample/Locaton	Date	Samplers	Sterilization/ Cleaning ^B	Sampling liquid	Sample analysis	Temperature (°C) ^{C,D}	Humidity (%) ^{C,D}	Wind Speed (m s ⁻¹) ^{C,D}	Rain (mm) ^C	Cloud (LWC, g m ⁻³) ^D
RET	Sea water, Norway	2007-04-20	DS5600, XMX-CV, SKC	Ethanol/MQ rinse	Sterile PBS	Enumeration of bacterial cells and cultivable bacteria, activity measurements	-1.1-8.1	27-41	5-12	00.03	/
RET	<i>P. agglomerans</i> , Norway	2007-04-24	BioSampler, SASS				4.5-15.7	96-99	2-4	0	/
RET	<i>B. atrophaeus</i> , Norway	2007-04-25	2000PLUS, SpinCon				8.3-11.8	97-98	1-3	02.03	/
RET	<i>P. syringae</i> , France	/	DS5800	Ethanol/MQ rinse	sterile 0.9% NaCl	Enumeration of total and viable bacterial cells and IN	/	/	/	/	/
SET	LU	/	DS5600	MQ rinse	MQ water	Enumeration of fluorescent microspheres	/	/	/	/	/
FT	Nuuk Basic RS	2013-07-30	DS5600	HCl/MQ/Ethanol rinse	Sterile high-salt solution	DNA/RNA co-extraction and quantification	13.4-17.5	31.0-48.5	/	0	/
FT		2013-08-01					8.4-9.4	64.0-79.2	/	0	/
FT		2013-08-01					9.1-10.2	77.2-83.7	/	0	/
FT (HS)	Puy de Dôme	2014-06-11	DS5800	HNO ₃ /dH ₂ O/bleach rinse	sterile 0.9% NaCl	Enumeration of bacterial cells, viable and cultivable bacteria, quantification of IN particles	/	/	/	0	0
FT (HS)		2014-12/13-6					13.7-17.7	77.8-100.0	2.1-6.8	0	0.00-0.07
FT (HS)		2015-06-18					10.2-13.8	79.7-94.6	1.8-2.2	0	0
FT (HS)		2015-06-16					8.1-8.8	100.00.00	2.4-3.0	0	
FT (HS)		2014-26/27-6					8.3-10.3	96.5-100.0	5.5-12.0	0	0.00-0.37
FT (HS)	Clermont Auvergne University Campus	2015-06-02	DS5800	HNO ₃ /dH ₂ O/bleach rinse	sterile 0.9% NaCl	Enumeration of bacterial cells and viable bacteria	21.3-24.5	21.2-41.1	2.8-4.1	0	0
FT (HS)		2015-06-05					25.6-32.4	9.6-37.3	2.9-4.7	0	0

^A RET – retention efficiency test ; SET – sampling efficiency test; FT – field test. HS – hourly subsampling

^B a – *Ethanol/MQ rinse*: the impinger was cleaned with ethanol and rinsed with sterile MQ. b – *HCl/MQ/Ethanol rinse*: the impinger was cleaned with 1 M HCl and then rinsed first with autoclaved, filter sterilized (0.1 µm, VacuCap 90 Devices, Pall Corporation, New York) MQ water followed by 96% ethanol. The remainders of ethanol were left to evaporate under sterile conditions. c – *MQ rinse*: the vortex chamber was rinsed with MQ. d – *HNO₃/dH₂O/bleach rinse*: the impinger was sterilized by an overnight application of the container with 10% nitric acid. The container was rinsed several times with deionized H₂O (dH₂O), then with diluted bleach (~0.1%) before being finally thoroughly rinsed with sterile dH₂O.

^c Norwegian Weather data loaded from <http://www.eklima.no> (The Norwegian Meteorological Institute). Data from Gardermoen weather station show min-max during the experimental period, from 0600 to 1800. For France (Clermont-Ferrand University Campus and Puy de Dôme), weather data were obtained from the Observatory of the Globe Physics of Clermont-Ferrand (OPGC).

^d Monitoring data for this paper are unpublished data, provided by L.H. Christensen, GeoBasis program run by University of Copenhagen (Dept. Geography) and Aarhus University (Dept. Bioscience). The program is part of the Greenland Environmental Monitoring (GEM) Program financed by the Danish Environmental Protection Agency, Danish Ministry of the Environment.

1 **Table 2.** Technical characteristics and performance of the impingers tested in this study.

Sampler	Flow rate	Liquid volume	Liquid loss rate	Sampling time	Sample Volume	Total cells		Cultivable cells	Leucine uptake	Fraction of viable cells	INA cell proportion at -4°C
	[L/min]	[mL]	[% min ⁻¹]	[min]	m ³	Test bacteria	[% T ₀]	[% T ₀]	[% T ₀]	[% T ₀]	[% T ₀]
XMX-CV	530	5	0.6±0.0	10	5.3	SW	97.2±2.4		103.3±10.8		
						<i>P. aggl</i>	83.9±1.4	73.2±75.7			
						<i>B. atr</i>	75.7±6.2	63.2±23.0			
SASS 2000PLUS	325	5	3.9±0.3	60	19.5	SW	26.0±4.8		0.5±0.2		
						<i>P. aggl</i>	22.9±17.1	78.7±819.4			
SpinCon	450	10	3.3±0.5	60	27	SW	55.3±2.1		1.6±0.8		
						<i>P. aggl</i>	20.6±5.8	78.7±819.4			
SKC BioSampler	12.5	20	0.6±0.0	60	0.75	SW	64.1±2.3		68.1±4.9		
						<i>P. aggl</i>	99.7±0.2	43.7±3.1			
						<i>B. atr</i>	26.3±9.1	35.5±25.7			
Kärcher DS5600	3300	1500	0.1±0.0	120	396	SW	97.0±3.0		48.7±8.3		
						<i>P. aggl</i>	70.0±3.4	27.7±5.1			
						<i>B. atr</i>	79.0±2.6	38.5±6.6			
Kärcher DS5800	4080	1700	0.51±0.04	60	245	<i>P. syr.</i>	118.2±18.6			12.1±3.00	5.31%
				300	1224	<i>P. syr.</i>	112.7±16.8			14.2±10.7	0.06%

2

3 The flow rate and the sampling liquid volume provided by the manufacturer are listed, as well as the length of the sampling period as chosen by the authors (Kärcher

4 impinger) or recommended by the manufacturers (all others). We present the fraction of remaining total, viable, and cultivable cells as well as the INP₄ proportion (at -4°C)

5 out of initial values. Also, the fraction of final leucine uptake out of initial leucine uptake is given for the seawater bacterial community (SW). Values are listed as means ±

6 standard deviations; % T₀ denotes the percent of the initial value.

3.3 Application to ambient bioaerosol sampling at semi-urban, mountain, and arctic sites

Deploying the sampler at a semi-urban, a remote mountain, and a pristine arctic site, we demonstrate that due to its high flow rate the Kärcher impinger can be used at high-temporal resolution (1- to 5-hours resolution) and a wide range of environmental conditions, in particular for sampling air with low bacterial loads. The airborne bacterial communities are highly diverse in terms of cell sizes, genetic diversity, and their phenotypic characteristics. We show here that the Kärcher impinger can be used for, but is not limited to, quantifying these communities in terms of (i) the total bacterial cells, (ii) cultivable bacterial cells, (iii) ice nucleating particles, and (iv) nucleic acids. Using this sampler for collecting airborne viruses in remote air was also attempted³⁴, but this would require further development.

3.3.1. Quantification of total cells

Cell concentration in the sampling liquid increased over time, reaching concentrations of $\sim 10^2$ to $\sim 10^5$ cells mL⁻¹ over the entire sampling period (SI-Figure 6). Two samples were collected at a semi-urban site on the Clermont-Auvergne University campus (Clermont-Ferrand, France). The inferred airborne cell concentrations ranged between 3.7 ± 0.6 and 12.5 ± 3.4 cells L⁻¹. Five samples were collected at the Puy de Dôme mountain site (1465 m asl) at different periods (day and night) and under different weather conditions, including the presence of a cloud at the sampling site (Table 1). Overall, the inferred airborne bacterial concentrations ranged between $(0.6 \pm 0.1) \times 10^0$ and $(2.8 \pm 0.2) \times 10^3$ cells L⁻¹, with variations depending on sampling date and time (Table 1, Figure 4). Diurnal variations in bioaerosol concentrations have been previously described, but only based on culturable fraction of the airborne communities^{35,36}. Here, similarly but focusing on total cells, we recorded higher cell number concentrations around midday (June 11th 2015) than during the night and in the morning (June

12th, 16th, 18th, and 26th 2015). This illustrates that the Kärcher impinger can potentially be used for describing diurnal short-term variations of total bacterial concentrations.

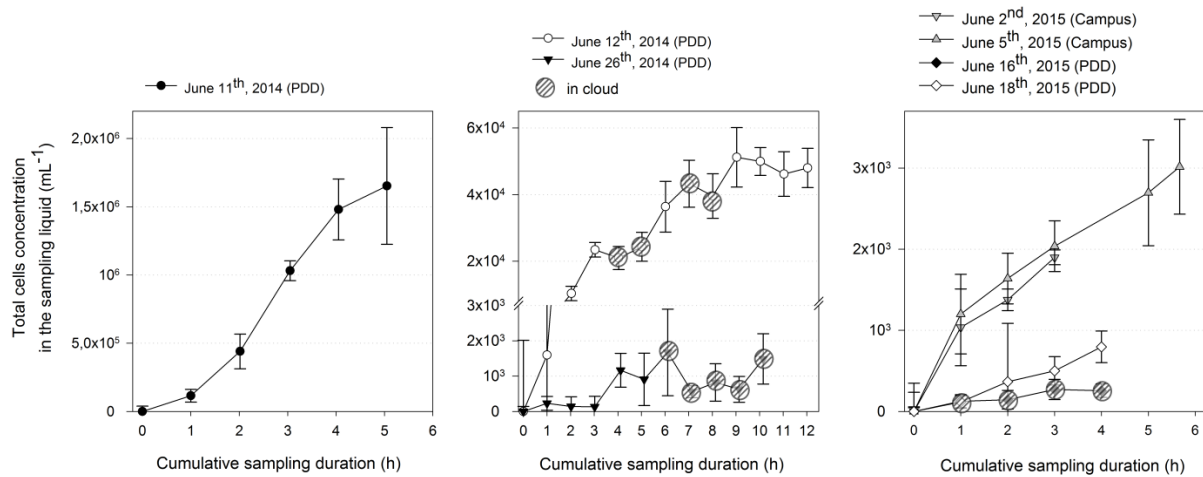


Figure 4: Evolution of total airborne bacteria concentrations inferred from measurements in the sampling liquid of the Kärcher DS5800 impinger, averaged over the cumulated duration of sampling at the semi-urban site (University campus) and at the mountain site puy de Dôme (PDD) in France. Error bars are standard errors of measurements on at least triplicate subsamples of the sampling liquid. The concentration measured just before sampling (time “zero”) at each occasion was deducted from the data. A dashed circle on a symbol indicates the presence of a cloud during the preceding sampling hour.

3.3.2. Quantification of culturable and viable cells

Three air samples were investigated for cultivable bacteria. Their concentration in the sampling liquid increased with sampling time and reached around ~200 CFU mL⁻¹ over the entire sampling period, corresponding to an average airborne concentration of 3.9×10^{-2} to 1.1 cultivable cells L⁻¹ (SI-Figure 7), or 0.02% to 11.2% of the total bacteria counted by flow cytometry. At realistic airborne concentration of 1-10 CFU L⁻¹, the detection limit for CFU counts (*i.e.* 20 colony per plate) is reached in 8-80 minutes with the Kärcher impinger, due to its high airflow rate. However, large uncertainties were often associated with CFU counts

(coefficient of variation of the mean count of triplicate plating: $13\% < CV < 96\%$, $\sim 50\%$ in average). On the other hand, when viability was assessed by flow cytometry using LIVE/DEAD staining (Figure 5), results were more consistent, with CV ranging between 4% and 34% of the mean. Viable cells accounted for 2.8% to 6.6% of the total cell number, and viability was not linked with cultivability. Viability did not decrease over sampling duration, contrary to observations made on the model bacterial strain (SI-Figure 4), but seemed rather linked with environmental conditions: we observed a higher cell viability during the night (Figure 5), consistently with previous reports³⁷.

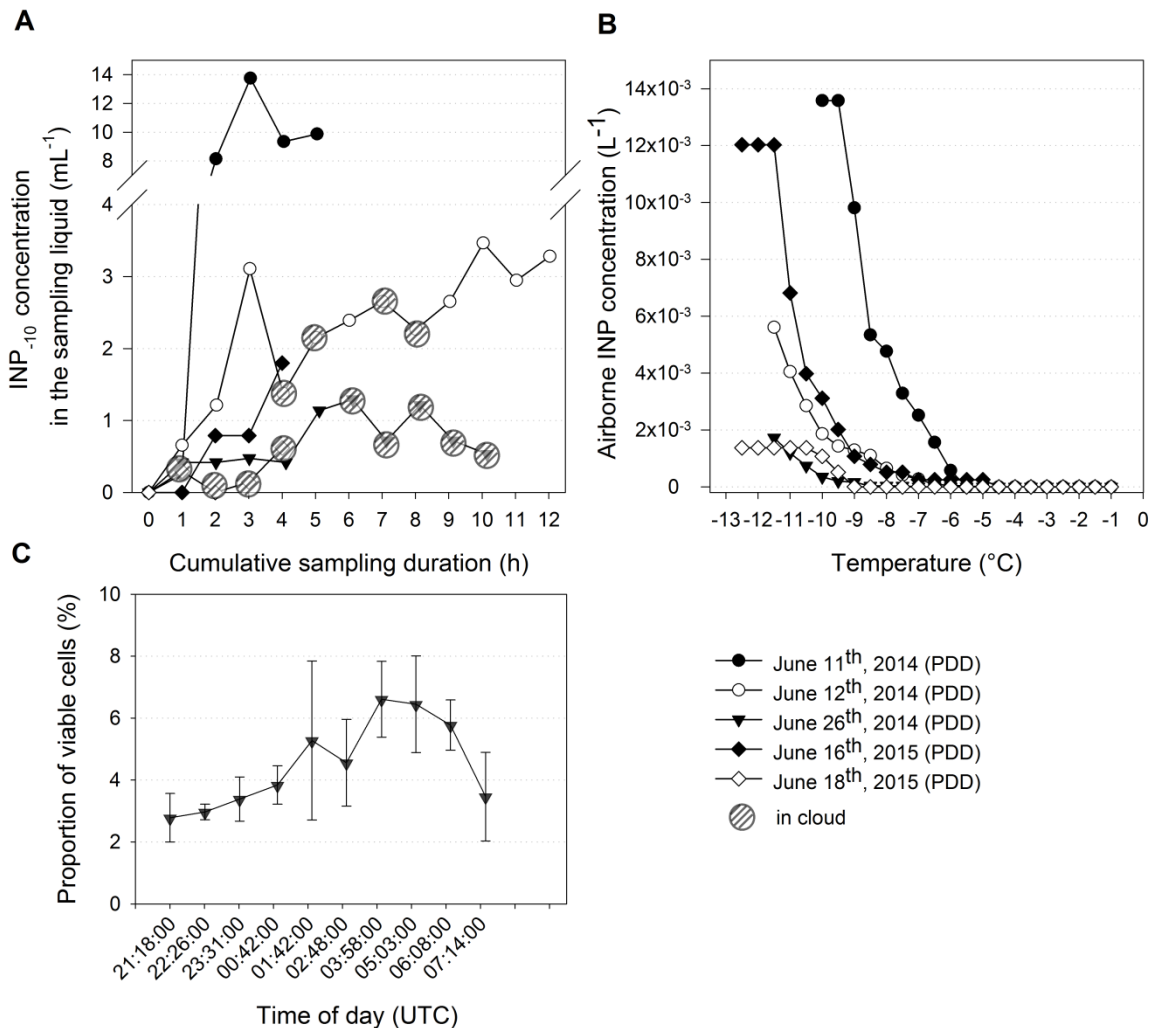


Figure 5: 5A. Evolution of INP₁₀ concentration in the sampling liquid during sampling at the mountain site (PDD). 5B. Corresponding cumulative freezing profiles at the end of the sampling period expressed as airborne concentrations averaged over the duration of sampling. 5C. Evolution of the proportion of viable cells determined by LIVE/DEAD staining, during day/night sampling. Error bars are standard deviations from the

mean of measurements on triplicate subsamples of the sampling liquid. A dashed circle on a symbol indicates the presence of a cloud during the preceding sampling hour.

3.3.3. Quantification of ice nucleating particles (INP)

The temporal evolution of the INP₋₁₀ (INP that initiate freezing at temperatures $\geq -10^{\circ}\text{C}$) concentrations in the sampling liquid during sampling, as well as the endpoint freezing profiles of air samples collected at the mountain site, are shown in Figure 5. SI-Figure 8 shows the hourly evolution of the freezing profiles measured between 0°C and -12.5°C during sampling. The average airborne concentrations of INP₋₁₀ over the duration of sampling, inferred from measurements in the sampling liquid, ranged between $< 10^{-3}$ INP L^{-1} and $\sim 14 \times 10^{-3}$ INP L^{-1} . These are consistent with observations made at comparable sites using other sampling methods^{38,39}. At such low concentrations of airborne IN, the detection limit of our method (0.05 IN mL^{-1} of liquid) was reached in less than 20 minutes with the Kärcher impinger, while about 80 minutes would be needed to reach the detection limit with a low-flow-rate impinger, such as the BioSampler.

3.3.4 Quantification of nucleic acids in Arctic ambient air

Three samples were collected in duplicates and used for the quantification of 16S-rRNA-gene and 16S-rRNA-molecules in air (SI-Figure 9). Three duplicated (duplicates denoted _A and _B) samples showed consistent results for bacterial cell concentrations (sample A11: $(5.5 \pm 0.2) \times 10^{-1}$ cell L⁻¹, sample A16: 6.8 ± 3.1 cell L⁻¹, and sample A17: $(2.9 \pm 0.7) \times 10^{-1}$ cell L⁻¹), which demonstrates that the Kärcher impinger can be used in pristine air to quantify bacterial cells based on molecular microbiological methods. The background concentrations of 16S rRNA genes reached between 0.4% and 26% of the concentrations measured in air (SI Figure 9), pointing at a low signal to noise ratio. This indicates that we were in some cases very close to the detection limit, which was due to the extremely low bacterial loads ($\sim 10^3$ per m³ of air). Thus, the high flow rate of the Kärcher impinger allows us to quantify bacterial cell at such conditions. The 16S rRNA copy number concentration, which roughly corresponds to the ribosome concentration in air, was between 8.3×10^1 and 1.1×10^4 copies L⁻¹ and was highly variable between duplicates, indicating the heterogeneity of the atmospheric bacterial community in terms of its activity. The background contamination on the RNA level was assessed on two occasions and was 0.1% and 1.6% of the 16S rRNA copy numbers (SI-Figure 9).

Other samplers have previously been employed for molecular analysis of genomic DNA and RNA. Most studies used filter samplers to collect samples for DNA analysis^{19,40,41}. SKC BioSampler impingers have been used to investigate the diversity of airborne bacterial populations as well as to quantify airborne *ina* genes with qPCR targeting genomic DNA^{17,42}. *Ina* gene concentrations were below the detection limit in most cases⁴², which was likely due to a low sampling capacity of the SKC BioSampler. It has been shown that the SKC BioSamplers can be

used for molecular studies on the RNA level either in environments heavily loaded with RNA viruses or by using 24 SKC BioSamplers in parallel to be able to perform RNA analysis of bacteria⁴³. We show that the Kärcher impinger allows both obtaining a sufficient amount of cells in pristine environments with low bacterial loads, and also preserving bioaerosols for sensitive RNA analysis, making it suitable for carrying out state-of-the-art molecular analysis.

In conclusion, we characterized a high-volume impinger and confirm that it is highly effective for quantitative and qualitative research on airborne microorganisms. The impinger has a high collection- and retention efficiency, facilitates sampling under diverse environmental conditions, and gives reliable results in environments with low biomass. In comparison to four other commercially available impingers, the Kärcher impinger had lower cell losses during sampling and was suited for preserving viability, activity, and ice nucleation activity of bacterial cells. Its main advantages over alternative impingers are its higher flow rate, low liquid loss, and ability to sample over long periods. Finally, the Kärcher impinger is an accessible device that most research groups could employ, which is in support of standardization within aero-microbiology.

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Supporting Information.

The Supporting Information is available free of charge on the ACS Publications website.

The content of the Supporting Information includes: Supporting Methods, Supporting Figures 1-9 & Tables, and Supporting References. The methods used for assessing sampling and retention efficiencies, as well as for bioaerosol analysis (*i.e.* total, viable and cultivable bacterial cells, fluorescent microspheres, ice nuclei and nucleic acids quantifications and metabolic activity measurements) are presented in Supporting Information (SI, Section SI-1.2 and SI-1.3).

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