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High-Flow-Rate Impinger for the Study of Concentration, Viability, Metabolic Activity, and Ice-Nucleation Activity of Airborne Bacteria

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► **To cite this version:**

Tina Šantl-Temkiv, Pierre Amato, Ulrich Gosewinkel, Runar Thyrrhaug, Anaïs Charton, et al.. High-Flow-Rate Impinger for the Study of Concentration, Viability, Metabolic Activity, and Ice-Nucleation Activity of Airborne Bacteria. *Environmental Science and Technology*, 2017, 51 (19), pp.11224 - 11234. 10.1021/acs.est.7b01480 . hal-01654827

HAL Id: hal-01654827

<https://hal.science/hal-01654827v1>

Submitted on 6 Nov 2020

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

3
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20
21 ^Ω We dedicate this paper to our colleague Runar Thyrrhaug, who initiated the study, but sadly
22 passed away during its initial phase.

23 **Abstract**

24

25 The study of airborne bacteria relies on a sampling strategy that preserves their integrity and *in*
26 *situ* physiological state, e.g. viability, cultivability, metabolic activity, and ice-nucleation activity.

27 As ambient air harbours low concentrations of bacteria, an effective bioaerosol sampler should

28 have a high sampling efficiency and a high airflow. We characterize a high-flow-rate impinger

29 with respect to particle collection and retention efficiencies in the range 0.5- 3.0 μm , and we

30 investigated its ability to preserve the physiological state of selected bacterial species and

31 seawater bacterial community in comparison with 4 commercial bioaerosol samplers. The

32 collection efficiency increased with particle size and the cut-off diameter was between 0.5 and 1

33 μm . During sampling periods of 120-300 min, the impinger retained cultivability, metabolic

34 activity, viability, and ice-nucleation activity of investigated bacteria. Field studies in semi-urban,

35 high-altitude, and polar environments included periods of low bacterial air-concentrations thus

36 demonstrating the benefits of the impinger's high flow rate. In conclusion, the impinger

37 described here has many advantages compared with other bioaerosol samplers currently on the

38 market: a potential for long sampling time, a high flow rate, a high sampling and retention

39 efficiency, low costs, and applicability for diverse downstream microbiological and molecular

40 analyses.

41

42 **1. Introduction**

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

54
55 Atmospheric concentrations of bacteria are much lower than in many other environments,
56 including deep marine and terrestrial subsurfaces¹⁴. It has been observed that there are on average
57 between 5 and 350 cells found per L of air over terrestrial and marine surfaces¹⁵. These low
58 bacterial concentrations imply sampling at high air flow rates (on the order of 100 L/min or
59 higher), and/or for prolonged duration (on the order of hours or days) in order to obtain sufficient
60 biomass for downstream analyses. Nevertheless, excessively long sampling time may deteriorate
61 the physiological state of sampled bacterial cells¹⁶, and prevent the assessment of short-term
62 variability¹⁷. Consequently, bioaerosol sampling requires efficient particle collection at high flow
63 rate, while preserving biological properties of interest, such as cell integrity and metabolic
64 activity.

65

66 A number of samplers are available on the market for collection of bacteria on filters, on agar, or
67 into liquid¹⁸. Filter samplers typically capture most of the biological material with a high
68 efficiency and can be adapted for the use at high flows¹⁹. However, cell properties are largely
69 affected by desiccation and other stresses over prolonged sampling time by air filtration²⁰.
70 Alternatives include the collection of microorganisms directly on nutrient plates for cultivation-
71 dependent studies²¹⁻²³. However, aside from being biased towards the cultivable fraction of the
72 collected cells, this method impedes downstream community analysis using state-of-the-art
73 molecular approaches and single-cell analyses. Living biological material is therefore often
74 preferably sampled into a liquid (*i.e.* by impingement), which more efficiently preserves cell
75 viability and allows fixation of cellular biological properties in real-time^{20,24,25}.

76
77 Among the most common devices for sampling into liquid, both the all-glass impinger (AGI-30)
78 and the SKC BioSampler use airflows of 12.5 L/min²⁴. There are also devices for sampling at
79 higher flow rates, such as the XMX-CV that collects particles in the 1-10 μm range at a flow rate
80 of 530 L/min. However, due to evaporation of the sampling liquid, the total sampling duration
81 cannot exceed 10 min according to user's manual. Hence, the current impingers appear poorly
82 adapted to sampling airborne microorganisms in environments where their concentration is
83 lowest, like at high altitude and in remote areas.

84
85 The objective of this study was to evaluate the use of a commercially available vacuum cleaner,
86 which collects aerosols into a liquid, as a high-flow-rate impinger. We hypothesize that: (i) The
87 performance of this impinger in terms of sampling and retention efficiency is comparable to
88 currently available impingers for bio-aerosol sampling; (ii) The high-flow rate of the impinger
89 allows it to be used in diverse environments, including pristine environments with low bacterial

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

93

94 **2. Materials and Methods**

95

96 **2.1 Description of the impinger and tests performed**

97

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

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

117
118 The samplers were validated using: (i) sampling efficiency laboratory tests, (ii) retention
119 efficiency laboratory tests, and (iii) validation field tests. An overview of all experiments is
120 presented in Table 1. Field tests were performed in Norway, France, and Greenland. In two of the
121 tests commercially available bioaerosol samplers were used as references.

122

123 **2.2. Cleaning and sterilizing the impinger**

124

125 Samplers and sampling liquids were decontaminated prior to each assay by applying sterilization
126 and rinsing procedures depending on the application (Table 1). *E.g.* a simple rinsing (Milli-Q
127 H₂O rinse) was required when using microspheres for determining sampling efficiency, a mild
128 sterilization (consecutive Ethanol and Milli-Q H₂O rinses) was required when examining
129 retention and preservation efficiency with model bacteria, and a stronger sterilization procedure
130 was required when collecting environmental samples (consecutive rinses with either HCl /
131 sterile Milli-Q H₂O / 96% Ethanol or 10% HNO₃ / deionized H₂O / bleach / deionized sterile
132 H₂O). The different sterilization liquids were either applied manually or by running the impinger
133 for 5 min having the vortex chamber filled with the respective liquid. The filter that protects the
134 motor (filter in Figure 1) was rinsed with the same sterilization liquids and replaced to its original
135 position. When transported into the field, the containers were kept enclosed in sterile plastic bags
136 and opened just before use. All sampling solutions were autoclaved (1-3 times) and then filtered
137 (0.22 μm or 0.1 μm porosity) to remove particles. Sterility and absence of particulate

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

145

146 **2.3 Experimental evaluation of the impinger**

147

148 2.3.1. Collection efficiency using an aerosol chamber

149

150 The collection efficiency of the samplers was evaluated with monodisperse fluorescent
151 polystyrene microspheres (Corpuscular Inc., Thermo Fisher Scientific Inc.) of different diameters
152 (500 nm, 790 nm, 1000 nm, 1400 nm, 2000 nm, and 3000nm) (Figure 1). The microspheres were
153 sprayed into an aerosol mixing chamber made of galvanized steel (diameter 0.5 m, volume 0.2
154 m³) and sampled with the impinger. Fluorescent microspheres were mixed by shaking and a brief
155 ultrasonication (~5 s) in an ultrasonic bath. Thereafter, the microspheres were diluted in Milli-Q
156 (MQ) water to a final concentration of 1.3×10^7 – 1.8×10^8 beads mL⁻¹. The microsphere suspension
157 was fed into a sparging liquid aerosol generator at 60 mL h⁻¹ (SLAG, CH Technologies, Inc),
158 which creates a relatively high particle number concentration at an airflow of 12 L/min of
159 aerosols that is stable over time²⁷. A high dilution, 3100 L/min, was needed in order to achieve
160 sufficient flow rates for the impinger. The collection efficiency was determined by measuring
161 particle concentration in the air and in the impinger liquid. The particle concentration in the

162 aerosol chamber was kept at the same order of magnitude as atmospheric concentrations of
163 bacteria (SI-Figure 2): $270 \pm 2 \text{ L}^{-1}$ for 500 nm microspheres (average \pm standard error of the
164 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
165 1400 nm microspheres, $84 \pm 2 \text{ L}^{-1}$ for 2000 nm microspheres, and $32 \pm 1 \text{ L}^{-1}$ for 3000 nm
166 microspheres. The concentration was measured at one-minute time resolution and typically
167 varied by $\pm 30\%$ over time with periods of low concentration when the microsphere solution was
168 refilled.

169
170 Aerosolized fluorescent microspheres were collected from the aerosol chamber using impinger
171 model DS5600 for either 1 h or 5 hrs. Three 1.5 mL MQ water aliquots were taken as negative
172 controls before pouring the MQ water into the vortex chamber, and from the vortex chamber after
173 collecting clean air within the aerosol chamber for 5 min. During the 1 h tests, triplicate 1.5 mL
174 sub-samples were removed from sampling liquid every 10 min, and during the 5 h tests 1.5 mL
175 sub-samples were withdrawn after 5, 30, 60, 90, 120, 180, 240, and 300 min. Both negative
176 controls and samples were kept in the dark at 4°C before they were analysed by flow cytometry
177 (FC).

178
179 A scanning mobility particle sizer SMPS (10-900 nm particles, design: Lund University) and an
180 aerodynamic particle sizer (0.5-20 μm particles, APS, model 3321, TSI Inc., US) were used to
181 obtain the aerosol number concentrations aloft in the chamber. Usually, microspheres appeared as
182 monodisperse particle number concentration peaks, which were integrated to determine airborne
183 concentrations. These were used to infer the theoretical numbers of microspheres sampled by the
184 impinger and to calculate size-resolved particle collection efficiencies by comparison with the

185 concentration derived from FC counts in the sampling liquid (c_{FCM}) as: $Eff [\%] = (c_{FC} / (c_{APS}$
186 $+c_{SMPS})) \cdot 100$.

187

188

189 2.3.2 Retention efficiency of the Kärcher impinger and of four bioaerosol impingers

190

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

205

206 2.4 Bioaerosol collection in ambient air

207

208 Bioaerosol sampling in ambient outdoor air was performed using Kärcher DS5800 impinger at 2
209 locations: from the roof of a building at the Clermont-Auvergne University campus “Les
210 Cézeaux” (45.76111 N, 3.116667 E), at ~4 m above the ground, and from the roof platform of the
211 atmospheric observatory of the Puy de Dôme mountain, nearby the city of Clermont-Ferrand
212 (45.77222 N, 2.96583 E, 1465 m asl) (Table 1). Sampling was performed for 3 to 12 consecutive
213 hours, with subsamples of the sampling liquid (sterile NaCl 0.9% solution) taken every hour in a
214 laminar flow hood. Before subsampling, evaporation losses were determined by weighing and
215 corrected by addition of sterile fresh sampling liquid or deionized water. For all calculations, we
216 assumed 100% sampling efficiency and no significant cell loss from the sampling liquid (based
217 on the laboratory test: see Figures 2A and 4A).

218
219 Three air samples were collected in duplicates in southwest Greenland from the M500 peak (500
220 m above sea level, 64.12264 N, 51.36619 W) at the Nuuk Basic research station (Table 1). The
221 Kärcher impinger was powered by generators, thus the air flow was kept at $(0.8-0.9) \times 10^3$ L/min
222 during the sampling period of 5 hrs, which resulted in the collection of aerosols from $(1.30-$
223 $1.45) \times 10^5$ L of air. The samples were collected into a high-salt solution, which is designed for
224 real-time preservation of sensitive RNA molecules³⁰. Duplicate samples were collected using two
225 Kärcher impingers DS5600 in order to evaluate sampling reproducibility. The samples and the
226 negative controls were concentrated onto Sterivex™ filter units, and stored in presence of 1
227 mL of RNA later (Thermo Fisher Scientific Inc, Massachusetts) as described by Lever *et al*³⁰.
228 The sampling solution was autoclaved twice and filter sterilized (0.1 μm, VacuCap 90 Devices,
229 Pall Corporation, New York). During sampling, evaporated water was replaced with sterile MQ
230 water.

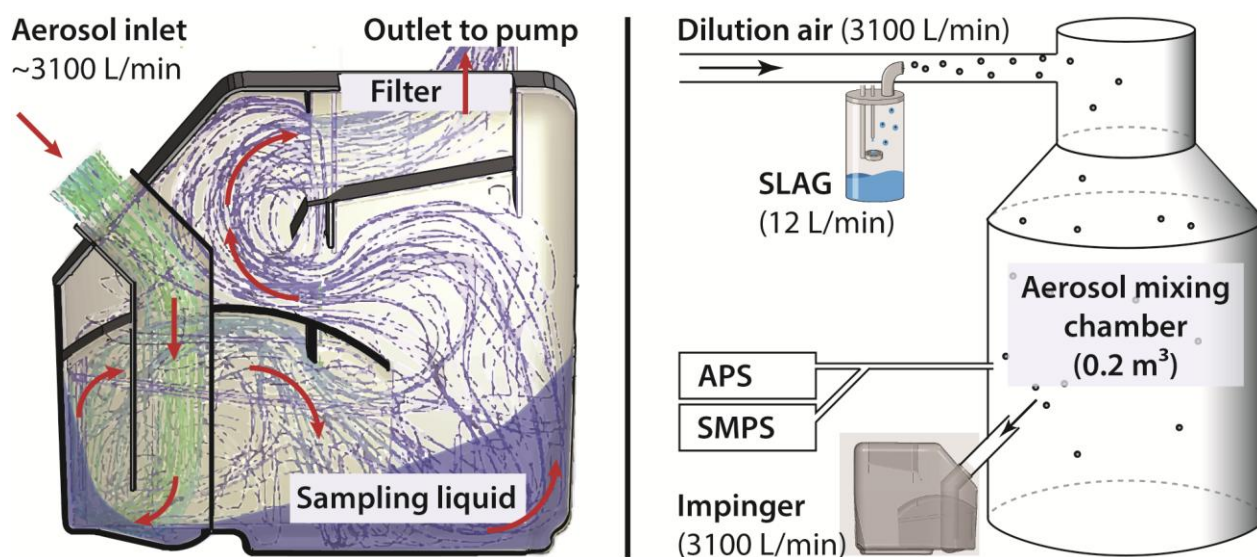
231

232 3. Results and discussion

233

234 3.1 Sampling efficiency at different particle sizes

235



236

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

242

243 The collection efficiency and cut-off diameters of the sampler were assessed by sampling
244 microspheres of defined size and known concentration from an aerosol chamber. The collection
245 efficiency (Figure 2A) increased with increasing microsphere diameter, with $25\pm 5\%$ for 500 nm
246 microspheres and $69\pm 17\%$ – $199\pm 28\%$ for larger particles. Based on these experiments we
247 estimated a cut-off diameter of between 0.5 and 1 μm . Efficiencies higher than 100 % are likely
248 due to microsphere doublets causing the APS to underestimate their actual number concentration
249 in air. Thus, there may be an overall overestimate of sampling efficiency. There is an additional

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

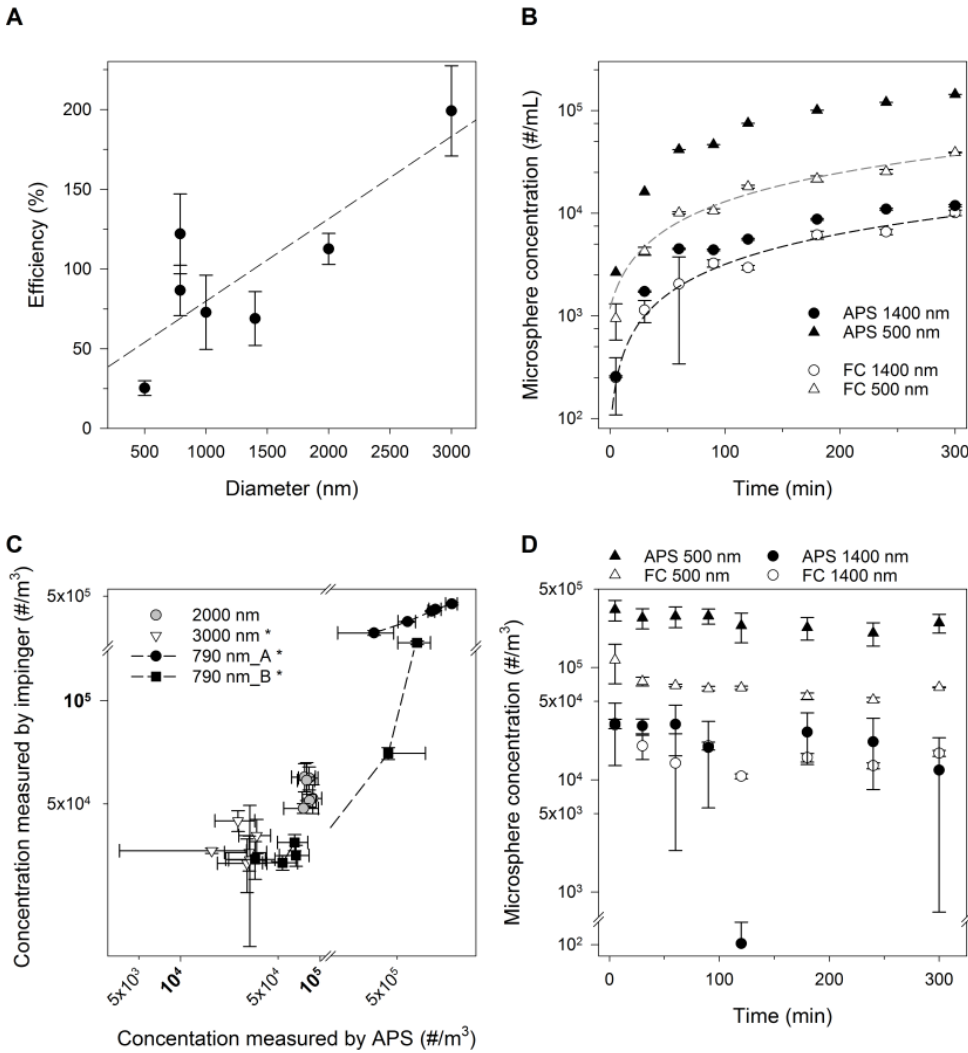
252

253 There was no saturation with microspheres in the sampling liquid over a sampling period of 5
254 hours at microsphere air concentrations comparable to high bacterial concentrations in ambient
255 air ($\sim 100 \text{ L}^{-1}$). Figure 2B confirms that the concentrations of microspheres with a diameter of 500
256 nm and 1400 nm in the sampling liquid increased linearly with sampling time (1400 nm:
257 $R^2=0.97, p < 0.001$, 500 nm: $R^2= 0.97, p < 0.001$).

258

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

266



267

268 **Figure 2:** Five-hour sampling tests of fluorescent microspheres in an aerosol chamber at relevant particle number

269 concentrations (32-270 L⁻¹ for 2A, ~100 L⁻¹ for 2B-D). 2A: Particle size resolved sampling efficiency. Experiment

270 was performed twice with 790 nm microspheres and both values are presented. 2B: Comparison of the

271 concentrations of microspheres in the sampling liquid measured by FC and the predicted concentration based on the

272 aerosol concentration measured by APS. The linear model (dashed lines) is shown for the microspheres

273 concentration measured by FC. 2C: Correlation between particle number concentration measured with the impinger

274 and with the optical particle counter (APS) for different sphere sizes and for experiments performed for 1 h. An

275 asterisk next to the legend indicates a significant linear regression model ($p < 0.001$). 2D: The average number

276 concentration of microspheres in the aerosol chamber as assessed with the optical particle counter (APS) and the

277 impinger (FC) for experiments performed for 5 hours.

278

279 Dybwad et al²⁰ showed that, aside from filter samplers, all bioaerosol samplers had low collection
280 efficiency for 1 μm fluorescent microspheres and efficiently collected microspheres in the 4 μm
281 size range. The high-flow-rate impinger XMX-CV had the highest efficiency reduction for 1 μm
282 compared to 4 μm microspheres (26-fold), collecting between $\sim 4\%$ and $\sim 20\%$ of airborne 1 μm
283 microspheres²⁰. In comparison, the Kärcher impinger performed much better with approximately
284 75% collection efficiency at $\sim 1 \mu\text{m}$. The AGI-30 impinger and the SKC BioSampler have
285 collection efficiencies above 90% at 1 μm , but operate at flowrates that are three orders of
286 magnitude lower, which allows for longer deposition times and more efficient impaction due to a
287 larger pressure drop in collection zone. It has been shown that bacterial cell shape influences its
288 capture efficiency, so that the rod-shaped cells are captured with higher efficiency than spherical
289 cells³¹. Many bacterial cells, such as cells of the INA species *Pseudomonas fluorescens*, are rod
290 shaped with diameters 300–800 nm, and lengths 1000–3000 nm, resulting in an aerodynamic
291 diameter of approximately 800 nm³². However, the majority of airborne bacteria at inland sites
292 were found associated with particles and thus had an aerodynamic diameter larger than 3000
293 nm³³. Thus, airborne biological particles, including bacteria, are in the diameter range that its
294 covered by the high efficiency range of the Kärcher impingers. This demonstrates that the
295 majority of airborne particles in size ranges similar to those observed for bacteria are collected by
296 the impinger.

297

298 3.2 Retention efficiency and maintenance of viability

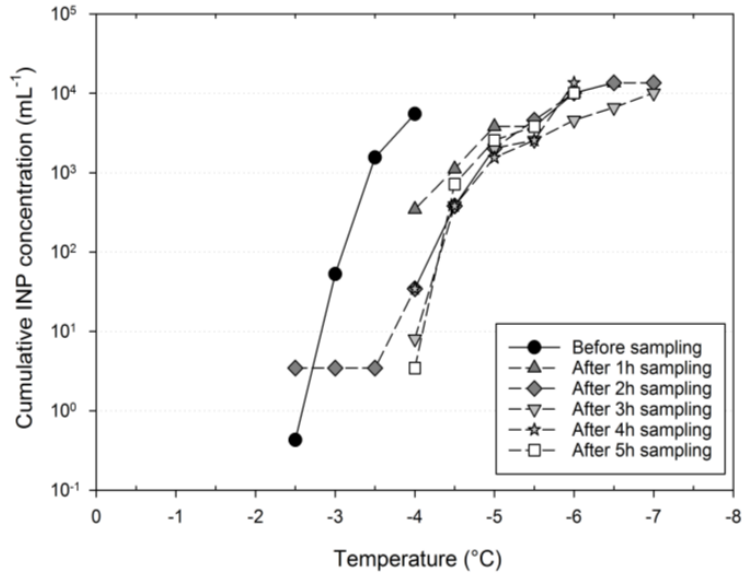
299

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

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

308
309 A model INA strain of *P. syringae* was spiked into the sampling liquid to evaluate retention of
310 the total- and viable-cell concentrations as well as concentrations of ice nucleating particles (INP)
311 at temperatures between -2°C and -7°C . The total cell concentration of *P. syringae* did not
312 change over a 5 hour sampling period (Kruskal-Wallis test; $p > 0.05$) (Table 2), indicating that
313 losses of bacteria due to reaerosolization from the vortex chamber during sampling are negligible.
314 However, the proportion of viable cells, relative to total cells, dropped within the first hour of
315 sampling, from $18\% \pm 10\%$ to $2.2\% \pm 0.2\%$ (i.e. $\sim 12\%$ of the initial viability was retained). Later,
316 viability remained stable over time (Kruskal-Wallis test; $p > 0.05$). The concentrations of ice
317 nucleating particles behaved similarly, with the concentration of highly active INP decreasing by
318 two orders of magnitude within the first two hours of sampling, and no further change over time
319 until the end of the sampling period (Figure 3). Sampling operations caused the ice nucleation
320 profiles to be shifted down in temperature by less than 2°C , indicating that the impinger did not
321 cause significant damage to the sample and is suitable for detecting INP in bioaerosol
322 populations.

323



324
 325 Figure 3: Retention efficiency of bacterial INP in the Kärcher DS5800 impinger spiked with INA *P. syringae* over 5
 326 hours of sampling.

327
 328 Two model bacterial strains and a complex bacterial seawater community were spiked into the
 329 vortex chamber in order to test the extent to which bacterial concentration, viability, and
 330 metabolic activity were affected by a sampling period of 120 min. The abundance of seawater
 331 bacteria remained unchanged while the abundances of *P. agglomerans* and *B. atrophaeus* cells
 332 were reduced to $70\pm 3\%$ and $79\pm 3\%$ of their initial values after 120 min (SI-Figure 3, Table 2).
 333 The concentration of cultivable cells decreased by 50-65% (SI-Figure 4, Table 2). Relative
 334 leucine incorporation in the seawater bacteria decreased by $61\pm 7\%$ compared to the initial values
 335 (SI-Figure 4, Table 2). Also, the fraction of cultivable among total cells decreased (SI-Figure 4,
 336 Table 2). However, the fraction of cultivable and metabolically active cells among the total cell
 337 population decreased rapidly within the first 5 min of sampling for *P. agglomerans* and seawater
 338 bacteria, but was more stable during the later sampling period.

339

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

351
352 Using flow cytometry we show that the Kärcher impinger had lower cell losses than all other
353 samplers except for the XMX-CV. About $\frac{1}{3}$ of the cultivable and $\frac{1}{2}$ of the active cells retained
354 cultivability/activity at the end of the sampling period with the Kärcher impinger DS5600 (Table
355 2). The XMX-CV and the SKC BioSampler had a 0.9-2.6 times higher efficiency in retaining cell
356 cultivability/activity than Kärcher impinger DS5600. Thus, the performance of the Kärcher
357 impinger in terms of preserving bacterial cultivability/activity was comparable with the SKC
358 BioSampler and worse than the performance of the XMX-CV in case of the sea-water community
359 and *P. agglomerans*. We suggest that the short sampling time (10 min) of the XMX-CV is
360 responsible for the better performance of the XMX-CV sampler in terms of both cell losses and
361 bacterial activity losses. The SKC BioSampler is slightly better than the Kärcher DS5600
362 impinger at preserving the cultivability/activity even after one hour of operation. The flow rate of
363 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			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	Ethanol/MQ rinse	Sterile PBS		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 INP₋₄ 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.

7 3.3 Application to ambient bioaerosol sampling at semi-urban, mountain, and arctic sites

8

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

18

19 3.3.1. Quantification of total cells

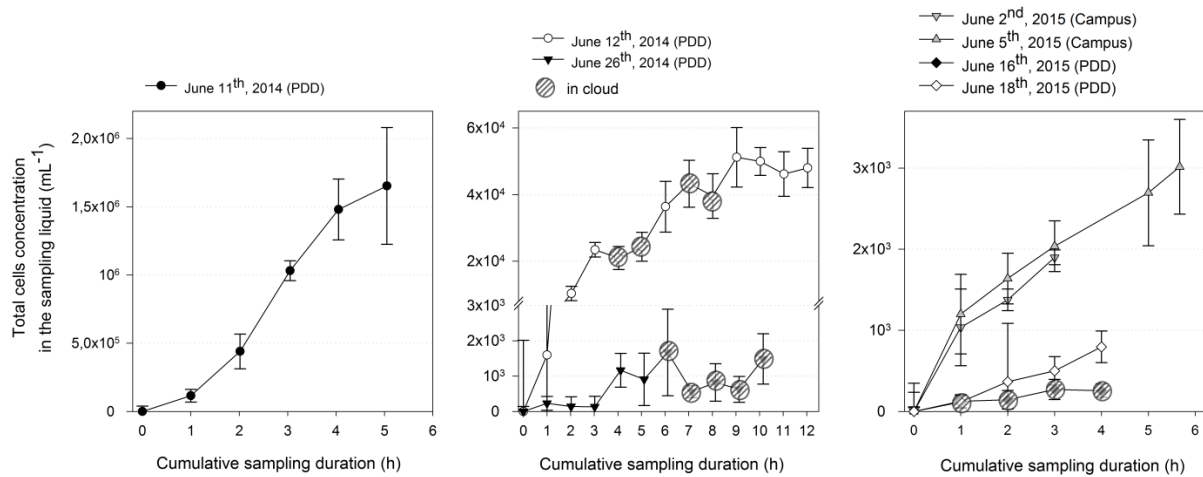
20

21 Cell concentration in the sampling liquid increased over time, reaching concentrations of $\sim 10^2$
22 to $\sim 10^5$ cells mL⁻¹ over the entire sampling period (SI-Figure 6). Two samples were collected
23 at a semi-urban site on the Clermont-Auvergne University campus (Clermont-Ferrand,
24 France). The inferred airborne cell concentrations ranged between 3.7 ± 0.6 and 12.5 ± 3.4 cells
25 L⁻¹. Five samples were collected at the Puy de Dôme mountain site (1465 m asl) at different
26 periods (day and night) and under different weather conditions, including the presence of a
27 cloud at the sampling site (Table 1). Overall, the inferred airborne bacterial concentrations
28 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
29 sampling date and time (Table 1, Figure 4). Diurnal variations in bioaerosol concentrations
30 have been previously described, but only based on culturable fraction of the airborne
31 communities^{35,36}. Here, similarly but focusing on total cells, we recorded higher cell number
32 concentrations around midday (June 11th 2015) than during the night and in the morning (June

33 12th, 16th, 18th, and 26th 2015). This illustrates that the Kärcher impinger can potentially be
 34 used for describing diurnal short-term variations of total bacterial concentrations.

35

36



37

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

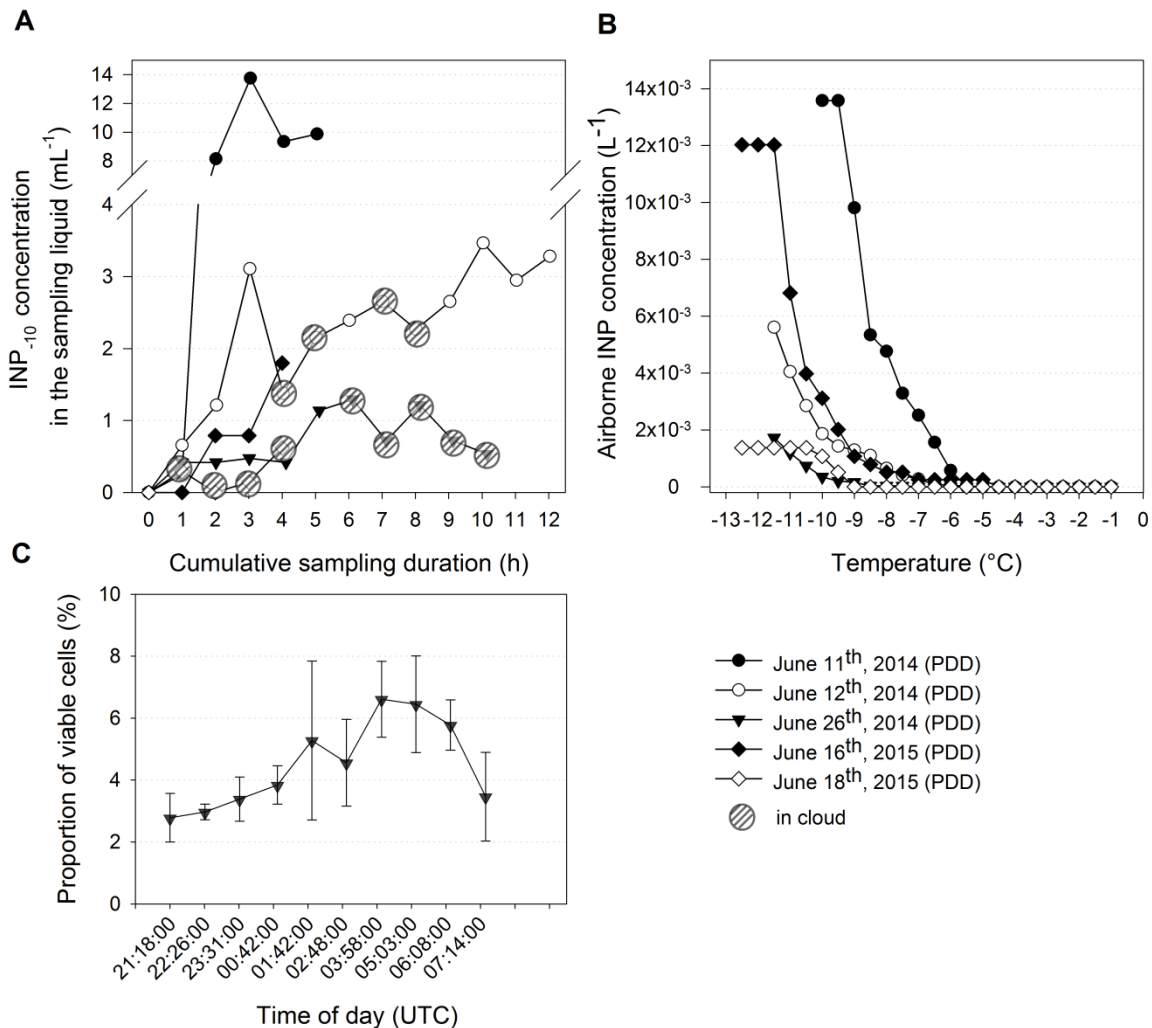
44

45 3.3.2. Quantification of culturable and viable cells

46

47 Three air samples were investigated for culturable bacteria. Their concentration in the
 48 sampling liquid increased with sampling time and reached around ~200 CFU mL⁻¹ over the
 49 entire sampling period, corresponding to an average airborne concentration of 3.9x10⁻² to 1.1
 50 cultivable cells L⁻¹ (SI-Figure 7), or 0.02% to 11.2% of the total bacteria counted by flow
 51 cytometry. At realistic airborne concentration of 1-10 CFU L⁻¹, the detection limit for CFU
 52 counts (*i.e.* 20 colony per plate) is reached in 8-80 minutes with the Kärcher impinger, due to
 53 its high airflow rate. However, large uncertainties were often associated with CFU counts

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



62
 63 **Figure 5:** 5A. Evolution of INP₋₁₀ concentration in the sampling liquid during sampling at the mountain site
 64 (PDD). 5B. Corresponding cumulative freezing profiles at the end of the sampling period expressed as airborne
 65 concentrations averaged over the duration of sampling. 5C. Evolution of the proportion of viable cells
 66 determined by LIVE/DEAD staining, during day/night sampling. Error bars are standard deviations from the

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

69

70 3.3.3. Quantification of ice nucleating particles (INP)

71

72 The temporal evolution of the INP₋₁₀ (INP that initiate freezing at temperatures $\geq -10^{\circ}\text{C}$)
73 concentrations in the sampling liquid during sampling, as well as the endpoint freezing
74 profiles of air samples collected at the mountain site, are shown in Figure 5. SI-Figure 8
75 shows the hourly evolution of the freezing profiles measured between 0°C and -12.5°C during
76 sampling. The average airborne concentrations of INP₋₁₀ over the duration of sampling,
77 inferred from measurements in the sampling liquid, ranged between $< 10^{-3}$ INP L^{-1} and
78 $\sim 14 \times 10^{-3}$ INP L^{-1} . These are consistent with observations made at comparable sites using
79 other sampling methods^{38,39}. At such low concentrations of airborne IN, the detection limit of
80 our method (0.05 IN mL^{-1} of liquid) was reached in less than 20 minutes with the Kärcher
81 impinger, while about 80 minutes would be needed to reach the detection limit with a low-
82 flow-rate impinger, such as the BioSampler.

83

84

85

86 3.3.4 Quantification of nucleic acids in Arctic ambient air

87
88 Three samples were collected in duplicates and used for the quantification of 16S-rRNA-gene
89 and 16S-rRNA-molecules in air (SI-Figure 9). Three duplicated (duplicates denoted _A and _B)
90 samples showed consistent results for bacterial cell concentrations (sample A11: $(5.5\pm 0.2)\times 10^{-1}$
91 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
92 demonstrates that the Kärcher impinger can be used in pristine air to quantify bacterial cells
93 based on molecular microbiological methods. The background concentrations of 16S rRNA
94 genes reached between 0.4% and 26% of the concentrations measured in air (SI Figure 9),
95 pointing at a low signal to noise ratio. This indicates that we were in some cases very close to the
96 detection limit, which was due to the extremely low bacterial loads ($\sim 10^3$ per m³ of air). Thus, the
97 high flow rate of the Kärcher impinger allows us to quantify bacterial cell at such conditions. The
98 16S rRNA copy number concentration, which roughly corresponds to the ribosome concentration
99 in air, was between 8.3×10^1 and 1.1×10^4 copies L⁻¹ and was highly variable between duplicates,
100 indicating the heterogeneity of the atmospheric bacterial community in terms of its activity. The
101 background contamination on the RNA level was assessed on two occasions and was 0.1% and
102 1.6% of the 16S rRNA copy numbers (SI-Figure 9).

103
104 Other samplers have previously been employed for molecular analysis of genomic DNA and
105 RNA. Most studies used filter samplers to collect samples for DNA analysis^{19,40,41}. SKC
106 BioSampler impingers have been used to investigate the diversity of airborne bacterial
107 populations as well as to quantify airborne *ina* genes with qPCR targeting genomic DNA^{17,42}. *Ina*
108 gene concentrations were below the detection limit in most cases⁴², which was likely due to a low
109 sampling capacity of the SKC BioSampler. It has been shown that the SKC BioSamplers can be

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

115
116 In conclusion, we characterized a high-volume impinger and confirm that it is highly effective for
117 quantitative and qualitative research on airborne microorganisms. The impinger has a high
118 collection- and retention efficiency, facilitates sampling under diverse environmental conditions,
119 and gives reliable results in environments with low biomass. In comparison to four other
120 commercially available impingers, the Kärcher impinger had lower cell losses during sampling
121 and was suited for preserving viability, activity, and ice nucleation activity of bacterial cells. Its
122 main advantages over alternative impingers are its higher flow rate, low liquid loss, and ability to
123 sample over long periods. Finally, the Kärcher impinger is an accessible device that most
124 research groups could employ, which is in support of standardization within aero-microbiology.

125

126 **Acknowledgements**

127 This work was supported by grants from the EU PASR 2006 programme (project Aerobactics,
128 contract SEC6-PR-214400), The Research Council of Norway (project MicrobAir, contract no.
129 177802), the Swedish Research Council FORMAS (project 2014-1460) and the Danish Council
130 for Independent Research (project NUMEN, DFF-FTP-4005-00485B). TST is grateful for
131 funding from the Stellar Astrophysics Centre, which is provided by The Danish National
132 Research Foundation (Grant agreement DNRF106). KF, UG and TST acknowledge support by
133 the AUFF Nova programme (AUFF-E-2015-FLS-9-10). We gratefully acknowledge the

134 contributions of Arctic Research Centre (ARC), Aarhus University and the Canada Excellence
135 Research Chair (CERC). This work is a contribution to the Arctic Science Partnership (ASP). PA
136 is grateful to LaMP-OPGC, in particular J.M. Pichon, for providing meteorological data, and to
137 Fédération de Recherche en Environnement (CNRS FR3467) for partial financial support.
138 Special thanks go to F. Conen, L. Zimmerman and E. Stopelli, University of Basel, for kindly
139 providing LINDA, and to S. Souzani and H. Licina, Lund University, for providing the model of
140 the airflow.

141

142 **Supporting Information.**

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

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

149

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