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# Test and evaluation of a new bioaerosol collector in a laboratory setting

*A Canada—United Kingdom collaboration*

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## IMPORTANT INFORMATIVE STATEMENTS

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# Abstract

The aim of the project was to test a personal aerosol sampler based on electrostatic precipitation and electrowetting on dielectric (ESP-EWOD). A bioaerosol chamber trial was conducted to compare ESP-EWOD to two standard bioaerosol collectors (a SKC BioSampler and a 37 mm filter cassette hosting 0.8 µm polycarbonate filters). Three bacteria (*Bacillus atrophaeus* spores, *Pantoea agglomerans,* and *Escherichia coli*) were aerosolized, individually and in a mixture, into an aerosol chamber to introduce bacterial samples into the samplers simultaneously. The samples were collected and eluted for culture, endotoxin and quantitative polymerase chain reaction (qPCR) assays. The semi-quantitative comparison showed that ESP has comparable collection and elution efficiency as the BioSampler and filter cassette for *E. coli* and *P. agglomerans*, but reduced elution efficiency with *B. atrophaeus* spores. Sample concentration with the ESP was 1000x higher for *E. coli* and *P. agglomerans* and 33x higher than with the SKC BioSampler for *B. atrophaeus* spores. This first of a series of planned trials showed that ESP-EWOD is a promising technology for bioaerosol detector system integration. More work should be done to optimize ESP parameters to improve collection and elution efficiency and explore the effects of Tween detergent and different spore preparation methods on the ESP performance. Future test plans include field trials in easily accessible locations with complex environmental background, such as animal barns or wastewater treatment plants, and comparison to other low burden sampling systems would further demonstrate the use of the ESP-EWOD system.

# Significance to defence and security

Defence Research and Development Canada (DRDC) is developing an automated, fully integrated, low burden bioaerosol personal detector in response to a Canadian Armed Forces (CAF) requirement for a small footprint personal aerosol sampler. This Scientific Report describes the test results of a candidate aerosol sampler that could meet this requirement and is also amenable to integration with the multiplexed Toll-like receptor (TLR) electrochemical biosensor, which was developed through previous work. This chamber test trial showed that the prototype ESP-EWOD is a promising technology for this application.

# Résumé

Le but de ce projet est de tester un échantillonneur d'air personnel basé sur la précipitation électrostatique et l'élution sur diélectrique (ESP-EWOD). Un essai en chambre de bioaérosols a été mené pour comparer (ESP-EWOD) à deux échantillonneurs de référence (SKC BioSampler et cassette 37 mm avec un filtres d'une porosité de 0,8 µm). Trois microorganismes (spores de *Bacillus atrophaeus*, *Pantoea agglomerans* et *Escherichia coli*) ont été aérosolisés individuellement et en mélange, dans une chambre d'aérosol de sorte à exposer les trois échantillonneurs simultanément au même aérosol. Les échantillons prélevés ont été élués et analysés par culture, mesure d'endotoxines et de réaction en chaîne par polymérase quantitative (qPCR). La comparaison des concentrations relatives récoltées a montré que l'ESP a une efficacité de collecte et d'élution comparable à celle du SKC BioSampler et de la cassette avec filtre 0,8 µm pour *E. coli* et *P. agglomerans*. L'efficacité d'élution est cependant réduite avec les spores de B. atrophaeous. La concentration des échantillons obtenus avec l'ESP était 1000 fois plus élevée pour *E. coli* et *P. agglomerans* et 33 fois plus élevée pour les spores de *B. atrophaeus* qu'avec le SKC BioSampler. Ce premier essai d'une série d'essais planifiés a montré que l'ESP-EWOD est une technologie prometteuse pour l'intégration avec des systèmes de détection de bioaérosols. Des essais seront effectués pour améliorer la collecte avec l'ESP et l'élution avec EWOD, notammant pour vérifier l'effet de différentes préparations de spores et de détergeants comme le Tween. Des essaient comparatifs sur le terrain, avec d'autres appareils de détection à faible charge de travail, dans des environnements fortement chargés en microorganismes, comme des fermes et des usines de traitement des eaux usées, permettront de valider les performances de ESP-EWOD.

# Importance pour la défense et la sécurité

RDDC met au point un détecteur personnel de bioaérosols automatisé, entièrement intégré et requérant une faible charge de travail en réponse a une exigence des forces armées canadiennes (FAC). Ce rapport décrit les résultats de tests d'un petit échantillonneur personnel qui remplit ces exigences et qui pourrait être intégré avec le biocapteur électrochimique multiplex basé sur le récepteur de type Toll développé lors de travaux précédents. Cet essai en chambre a montré que le prototype ESP-EWOD est une technologie prometteuse pour cette application.

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# Acknowledgements

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# 1 Introduction

Defence Research and Development Canada (DRDC) is developing an integrated bioaerosol detection system. DRDC developed a handheld electrochemical (EC) biosensor based on Toll-like receptor (TLR) technology that has the potential to classify pathogens, including those causing emerging diseases, in real time with a low false alarm rate [1]. The project has since progressed to a 5-plex multiplex system at technology readiness level (TRL) 6 by Innovation for Defence Excellence and Security (IDEaS) innovators [2]. The ongoing development efforts involve the integration of aerosol sampling and concentration and sample processing with the TLR EC biosensor. As such, an international collaboration was formed among DRDC, the Centre de recherche de l'institut universitaire de cardiologie et de pneumologie de Québec (CRIUCPQ), the National Research Council Nantotechnology Research Centre (NRC NANO), the University of Hertfordshire (UH), and the Defence Science and Technology Laboratory (Dstl) Porton Down, UK with expertise in bioaerosol collection and measurements, digital microfluidic (DMF), system integration, and chemical and biological (CB) sensing.

The electrostatic precipitator (ESP) air sampler is a bioaerosol sampling prototype developed at the UH, UK, in contract with Dstl. The ESP has a corona discharge at the device entry that charges incoming aerosol particles, which are then collected onto the surface of a indium tin oxide (ITO) and Cytop® coated glass slide. ITO is conductive and allows the creation of an electric field to attract the charged particles. Cytop® is a hydrophobic chemical that facilitates the recovery of the sample after air sampling. The slide is then placed in a digital microfluidic liquid handling system developed at UH (also in contract with Dstl), which uses the electrowetting-on-dielectric (EWOD) principle to recover particles from the collection surface. The EWOD system uses electrowetting to actuate a 3 µL water droplet on the slide to elute the sample [3].

There are three key advantages of the ESP-EWOD sampling approach. The first is the low pressure drop inherent in electrostatic precipitation compared to other aerosol collection methods (e.g., filtration or inertial impaction), as there is no physical restriction to flow. The second is the high concentration rate, i.e., the ratio of airborne aerosol concentration to sample concentration. The concentration rate for the ESP-EWOD sampler is orders of magnitude higher than that of other commercial, small size aerosol samplers. Third, being a DMF device, the ESP-EWOD sampler presents the collected sample in a format that should allow for easy integration with a lab-on-a-chip sensor. These features make it amenable to integration with the DMF-TLR EC biosensor.

The purpose of this series of experiments was to evaluate the ESP-EWOD system and to compare it with two commonly used air samplers, namely the SKC BioSampler and 37 mm filter cassettes hosting 0.8 µm polycarbonate filters, in an aerosol chamber that allows exposure of numerous air samplers to similar aerosol content for comparison purposes. The eluted samples were analyzed using culture, endotoxin assay, and quantitative polymerase chain reaction (qPCR).

# 2 Materials and methods

## 2.1 Materials and preparations

Three microorganisms were aerosolized in the system: Bacillus atrophaeus spores (American Type Culture Collection [ATCC] 9372 obtained from Dugway Proving Ground, USA), *Pantoea agglomerans* (formerly called *Erwinia herbicola*), and *Escherichia coli* ATCC 15597 (Felix d'Hérelle Reference Centre for Bacterial Viruses). Unless otherwise stated, all chemical reagents were purchased from Millipore Sigma (Oakville, ON).

The *B. atrophaeus* spores were obtained from the Dugway Proving Ground. Briefly, the spores were prepared with a production media that used enzymatically digested casein. The spent media was concentrated to about 20% solids (but not washed) and then spray dried. The dry powder from all fermentations were blended to give a uniform product. 5% Aerosol 812 R (Evonik Industries, formally Degussa AG), a hydrophobic fumed silica fluidizer, was added to the *B. atrophaeus* spores at the time of mixing. The quality and concentration of the spores were assessed in Suffield Research Centre prior to use in these experiments. Spores were suspended in deionized water for aerosolization, and suspensions from $10^6$ CFU/mL to $10^9$ CFU/mL were tested. *E. coli* was grown overnight at 37°C in 30 mL of Trypticase soy broth (TSB) (Difco Laboratories, Becton, Dickinson and Company, Sparks, MD), centrifuged and washed three times with deionized water, and resuspended in 50 mL of deionized water. *P. agglomerans* was grown overnight at 37°C in 30 mL of nutrient broth (NB) (Difco Laboratories), centrifuged and washed three times with deionized water, and resuspended in 50 mL of deionized water. A fresh culture of *E. coli* and *P. agglomerans* was prepared for each day of aerosolization. A mixture of *E. coli* and *B. atrophaeus* spores was also prepared in deionized water and aerosolized.

## 2.2 Aerosol experiments

Aerosol experiments were conducted in a GenaMini aerosol chamber (SCL MedTech, Montréal, QC), specifically designed to compare air samplers [4]–[10]. The experimental setup was contained in a Class II biosafety cabinet Type A2, Figure 1. The chamber has a capacity of up to 30 L/min, creating a laminar airflow where up to 8 air samplers can be connected. The airflow in the chamber can be adjusted with regards to the air samplers' flow rates. The chamber can adjust the exhaust flowrate according to aerosol flowrate and air sampling flowrate to allow air sampling with no differential pressure; however, the adjustment performed with electronic valves is not instantaneous. Aerosols were produced using a single jet atomizer (Model 9302, TSI Inc. Shoreview, MN), connected to a diffusion dryer (Model 3062, TSI Inc.), and a neutralizer (Model 3012A, TSI Inc.) before entering the GenaMini chamber. Atomizer airflow and dilution air flow were adjusted to obtain between 2 and 200 particles/mL in the chamber. The atomizer was set at 10 L/min, with dilution at 22 L/min for nebulization of *B. atrophaeus* spores; 2.5 L/min with 29 L/min dilution for *E. coli*; and 3.5 L/min with 28 L/min dilutions for *P. agglomerans* and for the mixture of *B. atrophaeus* spores and *E. coli*. At the start of each day, blanks were collected using deionized water in the nebulizer. Nine replicates were done per aerosolized solution resulting in a total of 36 sets of samples for subsequent analyses.

The ESP was modified in order to connect it to an outlet port of the aerosol chamber. When operating as intended, the air is drawn into the ESP with a fan located at the device's exhaust. However, this fan was influenced by pressure variation in the chamber resulting in an unknown flow rate through the sampler. Also, the ESP housing is not airtight, thus replacing the outlet fan with a pump (less affected by pressure changes in the chamber) caused a pressure drop inside the instrument and resulted in the air coming in from unsealed joints. The consequence of such perturbation on airflow and hence on sampling efficiency was unknown. Therefore, the fan was removed and the flow from the GenaMini chamber entered the ESP unassisted during the experiments. The chamber was set with a slight overflow to allow more air to enter the ESP. In these conditions, the pressure drop across the ESP was minimal. Therefore, we expected no leakage in ESP in these conditions.

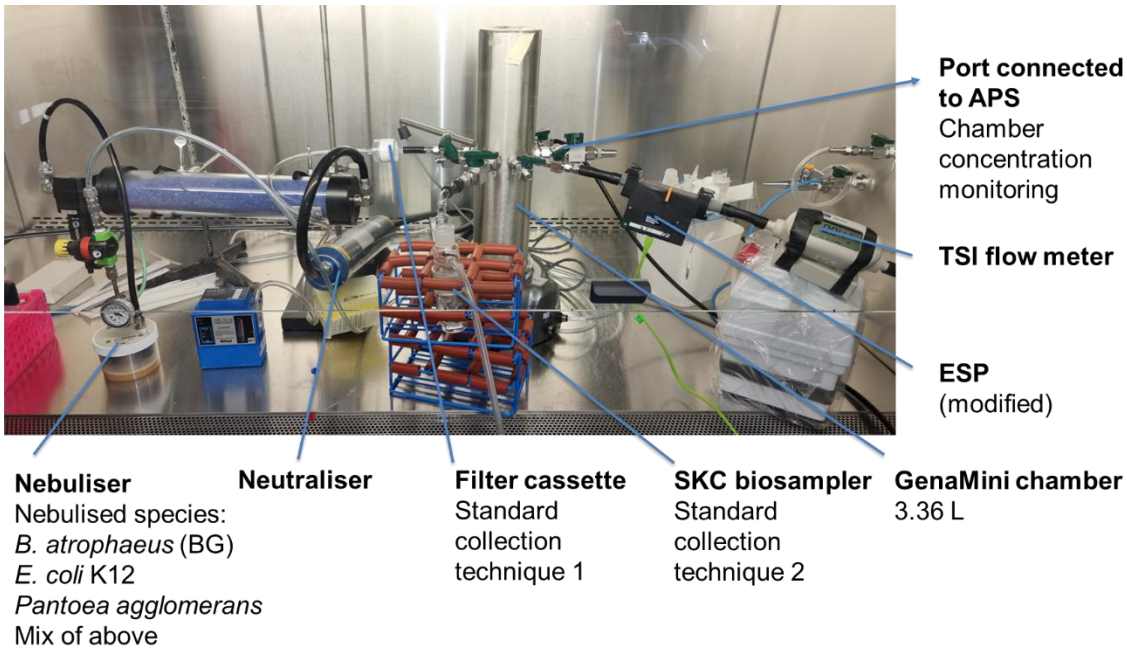


***Figure 1:*** *The experimental setup of the aerosol test trials. The setup included a nebulizer, the GenaMini aerosol chamber with the output to the three samplers (SKC BioSampler, 37 mm filter cassette, and the modified ESP). Sampling ports on the GenaMini are placed at 90° to the laminar air flow. An outlet port is also connected to the aerodynamic particle sizer (APS) to monitor bioaerosol concentration within the chamber.*

Aerosols were monitored using the APS 3321 (TSI Inc.). Only the capillary of the APS (and not the sheath airflow) was connected to the chamber; therefore, it was taking in 1 L/min from the GenaMini. The ESP bioaerosol collector (and EWOD elution) was compared with SKC BioSamplers (SKC Inc, PA, USA), which were filled with 20 mL of phosphate-buffered saline (PBS) and 37 mm closed face cassettes with 0.8 µm polycarbonate filters (SKC Inc). The critical orifice of the instrument determines airflow (12.5 L/min) in the SKC BioSampler. The pump for the filter cassette was calibrated at 2 L/min. An inline flowmeter (TSI, 4000 series) was placed on the outlet of the ESP to monitor the airflow within the device during sampling. The airflow was recorded every minute, allowing for measurements of the air volume passing through the ESP, with an average of 5 L/min over the duration of the test. To note, the resulting sampling flow rate was half of the 10 L/min at which the ESP was designed to operate, possibly causing lower than expected collection performance. It is safe to assume that the flow rate measured downstream of the ESP is the same as, or very close to, the flow rate entering the ESP. The pressure difference between the inside of the ESP and the atmosphere is likely small as the precipitation channel is so open. We can safely assume there is minimal to no loss around the ESP housing. For each experiment, the air sampling lasted 20 min. Nine replicates were performed for each microorganism and for the mixture. The particle size distribution in the aerosol chamber for each microorganism is presented in Figure 2.

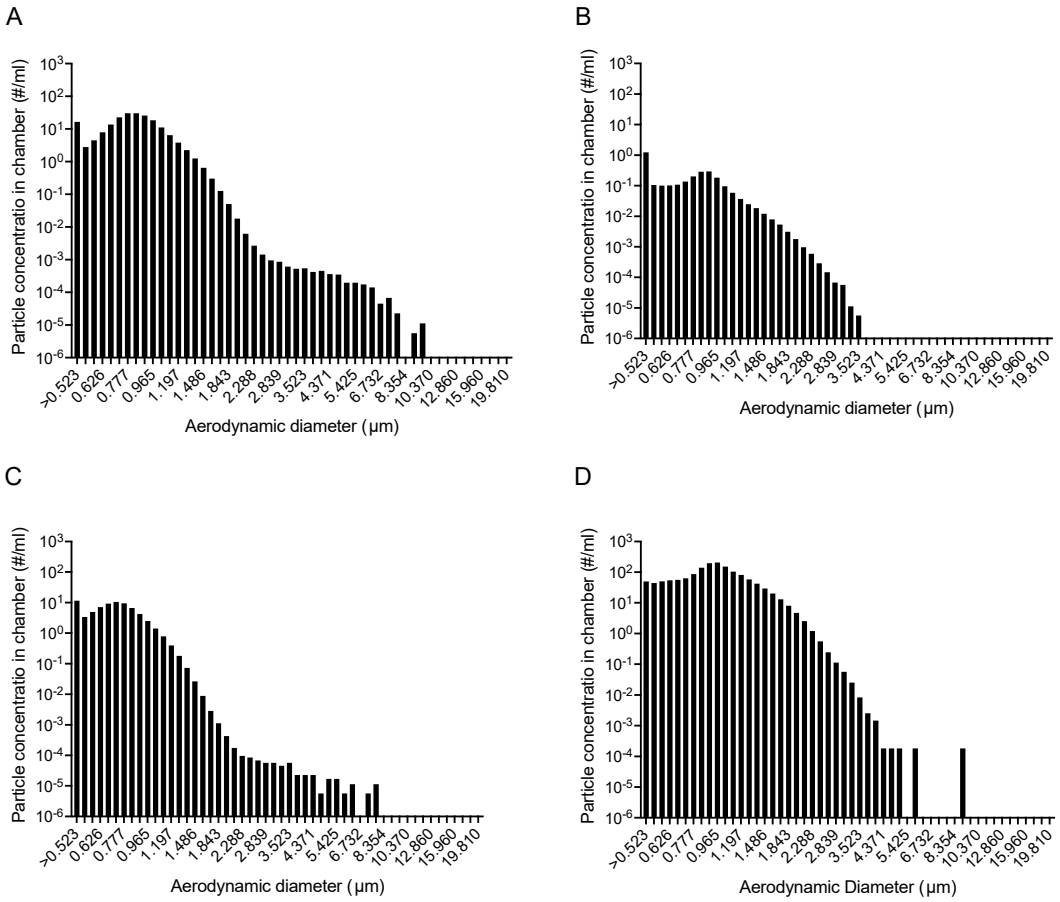


***Figure 2:*** *Particles size distribution of the nebulized aerosols as measured by the APS 3321 of a E. coli solution at a concentration of 3.5 × $10^9$ genomes/mL (A), B. atrophaeus spores solution at a concentration of 1 × $10^5$ genomes/mL (B), P. agglomerans solution at a concentration of 1.1 × $10^9$ genomes/mL (C), and a solution mixture of E. coli and B. atrophaeus at concentrations of 1.2 × $10^9$ genomes/mL and at 8 × $10^7$ genomes/mL respectively (D).*

## 2.3 Sample elution and analyses

Filter cassettes were processed by adding 5 mL of PBS with 0.025% Tween 20 followed by 15 min shaking on a 3-D Rotator Waver (VWR International LLC) at 30 rpm speed and 15º tilt angle. The liquid and filters were collected from the cassettes and vortexed together for an additional 2 min to elute the sample. The remaining liquid (some of the liquid is lost during sampling due to evaporation and/or reaerosolization) in the SKC BioSamplers was measured following collection and topped up to 20 mL with PBS and 0.1% Tween 20. Samples from the ESP collection slide were eluted into an automatically delivered volume of water (target volume = 2 µL) using the automated EWOD sample recovery system. The droplet was then added to 1 mL of water, weighed for estimation of the elution volume, and diluted with 2 mL of PBS and 0.1% Tween 20 for further analysis. During the trial, we observed a sticky effect of the droplet during elution in the *B. atrophaeus* sample, therefore, in some cases, a second elution was performed, 10 µL of water with 0.025% Tween 20 was moved manually by dragging the droplet, which was attached to a pipette tip, across the glass slide after the first elution. The droplet was added to 1 mL PBS 0.025% for analysis by qPCR or culture. On three occasions the slide was swabbed after the first elution to determine sample loss, due to the suspicion that the bacteria was not eluted off the slide completely. The swab was wetted/dipped in 1 mL water with 0.025% Tween 20 and eluted in the same volume containing the 1 mL of water/Tween 20. The tube was closed with the swab inside and vortexed for 30 seconds before culturing. A second elution or a swab was performed when the droplet stopped moving during the EWOD elution.

Three 1 mL aliquots were prepared for each sample. One aliquot was pelleted and stored at -20ºC for deoxyribonucleic acid (DNA) extraction and qPCR analyses. One aliquot was frozen for further analysis in the DMF-TLR biosensor. The final aliquot was stored at 4ºC until culture (same day) and Limulus Amebocyte Lysate (LAL) assay (1–2 weeks post aerosol trial) could be performed.

Endotoxin measurement was performed in duplicate using LAL Kinetic-QCL™ (Lonza, Walkersville, MD USA) according to manufacturer instructions. Samples were diluted, and an inhibition and/or enhancement test was performed prior to measurement.

DNA extraction was performed on 1 mL of each sample using the Qiagen DNeasy® PowerLyzer® PowerSoil® Kit according to manufacturer guidelines. DNA was eluted in 100 µL of elution buffer supplied with the kit.

The qPCR protocols used for the detection of the three microorganisms are presented in Annex A [11]. The specificity of each test was verified against the three microorganisms. Primers, probes, and plasmids for standard curves were ordered from Integrated DNA Technologies (IDT) (Iowa, USA). The qPCR reaction volume was 20 µL, which included 10 µL of BioRad iQSupermix for probes and 2 µL of DNA. Primers and probe concentration were set for each assay (see Annex A). Reactions were conducted using the BioRad CFX-96, with a thermocycling protocol set for each assay (see Annex A).

We compared the air samplers based on the number of microorganisms captured per liter of air sampled. Normalization between experiments was done with the concentration of particles in the chamber during the test as measured by the APS, Equation (1).

$$\text{Relative recovery ratio (APS)} = \frac{\text{Concentration recovered by air sampler (CFU/L or Endotoxin units/L or 16S/L)}}{\text{Particle concentration in the chamber (Particles/L)}} \quad (1)$$

For nebulization with mix of bacteria, normalization was done according to the nebulizer content of each microorganism, Equation (2).

$$\text{Relative recovery ratio (nebulizer)} = \frac{\text{Concentration recovered by air sampler (CFU/L or Endotoxin units/L or 16S/L)}}{\text{Concentration in nebulizer (CFU/mL or Endotoxin units/mL or 16S/mL)}} \quad (2)$$

To compare raw sample concentrations, we used the SKC BioSampler as a reference. Raw sample concentration was determined by dividing the ESP and filter sample concentrations by the SKC BioSampler sample concentrations collected at the same time, Equation (3).

$$\text{Raw concentration ratio} = \frac{\text{Concentration recovered by ESP or filter (CFU/L or Endotoxin units/L or 16S/L)}}{\text{Concentration in SKC BioSampler (CFU/mL or Endotoxin units/mL or 16S/mL)}} \quad (3)$$

Statistical analysis was performed using Graph Pad Prism version 9 (Boston, MA, USA). The log normality of the data was assessed using the Shapiro-Wilk test. Wilcoxon non parametric paired and unpaired T-test were performed on relative recovery ratios.

# 3 Results and discussion

## 3.1 Collection efficiency comparison

The three air samplers efficiently collected *E. coli* and *P. agglomerans* (Figure 3). There was no significant difference between air samplers when the samples were analyzed using LAL assay. It is important to note that many factors (e.g., β-D-glucans, cellulose, Tween, proteins, etc.) could affect the LAL assay results [12]–[13]. The filter support pads are cellulose-based, which can react and give false positive results in the LAL assay. When the results were analyzed using qPCR, relative recovery for *E. coli* was three times lower in filters and four times lower in ESP compared to the SKC Biosampler. While the relative recovery of *P. agglomerans* was nine times lower in filters and 12 times lower in ESP than the SKC Biosampler. The differences in the relative recovery ratio (APS) may be due to the additional extraction steps in the filter and ESP samples. More work needs to be done to determine where we are losing efficiency, either in collection or in extraction. While these relative recovery ratios are lower in the ESP than the SKC Biosampler, there are many parameters that need to be optimized to improve collection efficiency. However, these preliminary results show that ESP has comparable collection and elution efficiency to two standard bioaerosol collectors.

On one occasion (numbered test T22), the automated EWOD elution failed due to a sticky region holding the droplet halfway along the surface. The resulting qPCR signal of the half-completed elution was recorded in the same order of magnitude as the fully completed one (see Figure 3(A), ESP 1). The remaining material on the T22 slide was eluted manually into a 10 µL 0.025% Tween 20 droplet and its resulting qPCR outcome included in Figure 3 as ESP 2.

When samples were analyzed by culture, the ESP and filters showed poor to zero recovery of *E. coli* and *P. agglomerans*, as shown in Figure 3 and Table 1. The corona discharge of the ESP could have an effect on microorganisms' viability. The relative humidity in the chamber is very low (below 4%). Therefor, microorganisms could dry out once sampled on filter and ESP. These dry conditions could affect microorganisms' viability compared to the wet cyclone of the SKC BioSampler. For field applications, if molecular genetic assay such as qPCR is the preferred downstream analysis where no further culturing method is necessary, then ESP is comparable to the two standard collectors. The SKC Biosampler is more cumbersome for field applications and filters required manual extraction, the potential of the ESP for automated sample concentration and processing for downstream molecular analyses is an attractive feature for system integration.

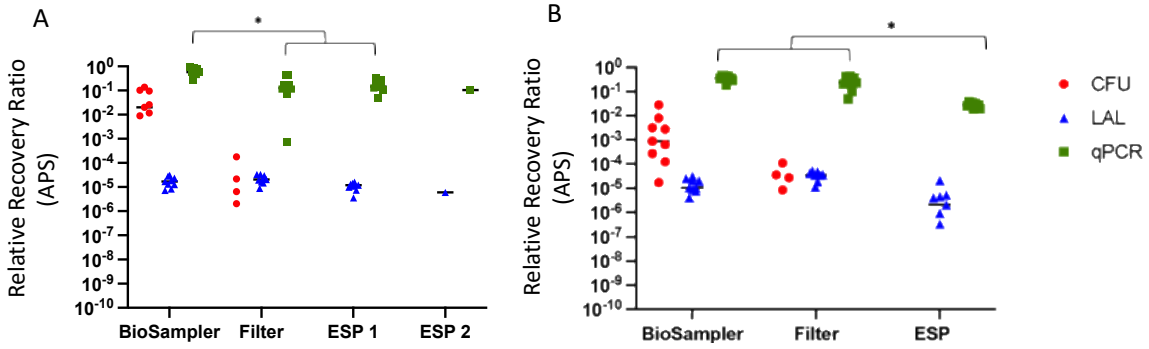


***Figure 3:*** *Collection efficiency of the three air samplers for E. coli (A) and P. agglomerans (B). Nebulization of 3.5 × $10^9$ CFU/mL, 1.5 × $10^6$ endotoxin units/mL, 3.5 × $10^9$ genomes/mL for E. coli and of 2.9 × $10^9$ CFU/mL, 1.2 × $10^5$ endotoxin units/mL, 1.1 × $10^9$ genomes/mL for P. agglomerans. Ratios were performed with particles concentration in the chamber, Equation (1). *Significant difference in qPCR with ESP (p = 0.004 for E. coli and p = 0.004 for P. agglomerans) (Wilcoxon non-parametric paired T-test). ESP 1 and ESP 2 were the first EWOD-actuated and second manually-eluted samples from T22.*

***Table 1:*** *Average sample concentration results from culture (CFU/ml).*

| | SKC BioSampler | Filter | ESP |
|---|---|---|---|
| *B. atrophaeus* spores | $4.5 \times 10^3 \pm 2.5 \times 10^3$ | $1.3 \times 10^3 \pm 5.3 \times 10^2$ | $7.6 \times 10^4 \pm 5.4 \times 10^4$ |
| *E. coli* | $1.0 \times 10^5 \pm 1.2 \times 10^5$ | $5.2 \times 10^1 \pm 1.3 \times 10^2$ | 0 |
| *P. agglomerans* | $5.7 \times 10^3 \pm 1.5 \times 10^4$ | $1.8 \times 10^1 \pm 3.9 \times 10^1$ | 0 |

Since *B. atrophaeus* spores do not contain endotoxins in their structure, the signal detected in filter cassettes with LAL assay is likely a background caused by the filter's cellulose support pad (Figure 4). As reported by Roslansky and Novitsky, the LAL assay used can react with β-D-glucan [12], present in vegetation and wood fibers like cellulose.

The qPCR reaction was not as efficient as culturing in detection of *B. atrophaeus* spores. We hypothesized that the DNA extraction protocol was not optimal for spores. However, we consider that the bias resulting from inefficient lysis is the same for all samples and should not interfere with air sampler comparisons.

*B. atrophaeus* spore recovery with ESP was lower compared to the SKC BioSampler (38x–135x) and filter cassettes (22x–59x). We observed a sticky effect of the droplet during elution from the ESP collection surface in 3 samples. Overall, 90% of the automated elution tests were completed as expected, leaving room for optimization of the elution efficiency and actuation performance. This effect can be caused by the spores or the slide itself. However, it happened more often with the spores (3/10) compared to the other microorganisms (1/18). In a second (manual) elution (ESP 2 in Figure 4), the recovery was similar to the first; however, when the plate was swabbed after the initial elution of the samples, the recovery was eight times higher compared with the Elutions 1 and 2, indicating that elution from the plate can be improved. This indicates that the EWOD actuated droplet is not removing the collected *B. atrophaeus* spores as efficiently as it removes *E. coli* or *P. agglomerans*. Previous work on this sampler has shown much higher surface removal efficiency for *B. atrophaeus* spores, with efficiency averages of 46% [14] and 93% [3]. Both these tests used deionised water droplets as elution fluid. It is possible that the *B. atrophaeus* spore preparation had some effect on the recovery rate. For these tests, the spores were milled, not washed, spray-dried, and mixed with a fluidizer as described in Section 2.1. For the tests reported in Foat et al. [3] and Jonsson-Niedziolka et al. [14], spores were grown, washed and supplied as a slurry. More work should be carried out to explore the effect of different spore preparation methods on the performance on the ESP-EWOD system.

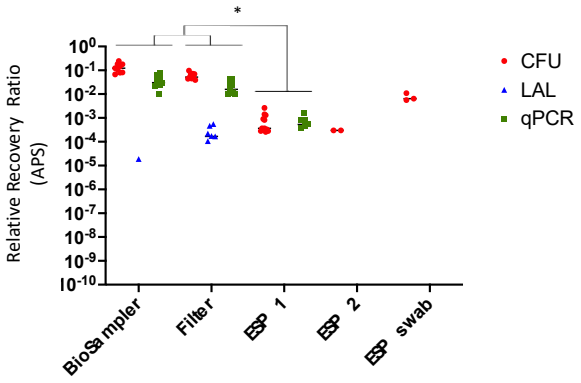


***Figure 4:*** *Collection efficiency of the three air samplers for B. atrophaeus spores. Nebulization of $2 \times 10^6$ CFU/mL, 0.6 endotoxin units/mL, $1 \times 10^5$ genomes/mL. Ratios were performed with particles concentration in the chamber, Equation (1). ESP 1 and ESP 2 were the first EWOD-actuated and second manually-eluted samples, while ESP swab was the sample obtained with a wetted swab.*

We performed an experiment with three concentrations of *B. atrophaeus* spores in the nebulizer to evaluate if the sticky effect was linked to the concentration of spores on the ESP collection surface (Figure 5). In these experiments, the first elution (ESP 1 in Figure 5) was performed with an EWOD actuated 2 µL water droplet (i.e., it was automatically delivered by the system as with the previously-described experiments). The droplet actuation stopped in mid plate from the two experiments with $10^9$ CFU/mL *B. atrophaeus* spores in the nebulizer. These samples were recovered by manual extraction from the sticky region of the plate. The second elution (ESP 2 in Figure 5) was performed with a manually actuated 10 µL water droplet supplemented with 0.025% Tween 20. Previously reported tests [14] of surface elution using EWOD showed that the addition of Tween 20 to the actuated water droplet had no effect on the elution efficiency, although it reduced the surface tension of droplet and improved its actuation performance. From the conclusions by Jönsson-Niedziółka et al., it was decided that only deionised water should be used in this chamber trial until the benefit of a surfactant can be proven. Further unpublished studies have since shown that using a different surfactant (Tween 80) could be beneficial in helping actuation on heavily loaded surfaces and could possibly increase the elution efficiency, with the second elution leading to similar amount of material recovered in the droplet compared to the first elution. Evidence from the current tests indicates that the addition of Tween improves the elution efficiency; however, this is not conclusive since the larger droplet volume used and the manual process during the second elution might have had an impact on the observed material recovery. We did not conduct enough repetitions to perform a statistical analysis of the effect of concentration on the recovery rate. Further, the ESP collection surface, EWOD electrode surface defects, or other factors causing the droplet to stick cannot be ruled out. Nevertheless, the effect of the spore batch and/or spore concentration on the glass slide deserves more investigation.

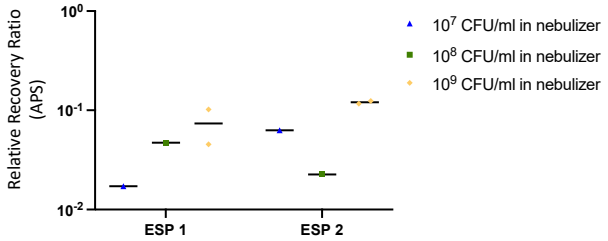


***Figure 5:*** *Effect of B. atrophaeus spore nebulizer concentration on ESP sampling and elution. Samples analyzed using culture. Ratios were performed with particles concentration in the chamber, Equation (1). ESP 1 and ESP 2 were the first EWOD-actuated and second manually-eluted samples.*

We performed co-nebulization of *E. coli* and *B. atrophaeus* spores (Figure 6) to determine the effect of the presence of bacterial spores on the collection and elution of *E. coli*. We used the same concentration of *E. coli* ($10^9$ CFU/mL) in the nebulizer as for the previous experiments, but we increased the concentration of *B. atrophaeus* spores to $10^8$ CFU/mL to bring it closer to the concentration of *E. coli*. We did not want to use a high spore concentration (i.e., $10^9$ CFU/mL) since there was indication of a negative effect on elution from the ESP surface. We maintained the same concentration of *E. coli* as the experiments using *E. coli* alone for comparative purposes.

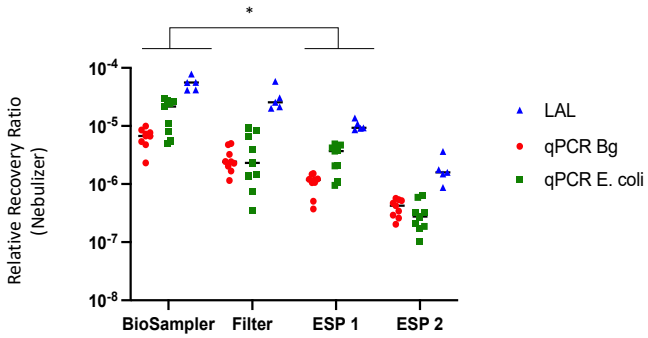


***Figure 6:*** *Co-nebulization of E. coli (1.2 × $10^9$ genomes/mL, 8.5 × $10^4$ endotoxin units/mL) and B. atrophaeus spores (8 × $10^7$ genomes/mL). Ratios were performed with the concentration in the nebulizer, Equation (2). *Significant difference (p = 0.001) between SKC BioSampler and ESP (Wilcoxon non-parametric paired T-test). ESP 1 and ESP 2 refer to the first EWOD-actuated and second manually-eluted samples.*

For this experiment, we normalized air sampler results with nebulizer content for the microorganisms examined (i.e., *E. coli* genomes for *E. coli* qPCR, *Bacillus* genomes for *Bacillus* qPCR, endotoxin units for LAL assay, see Equation (2). This is based on the assumption that nebulization does not introduce bias between *E. coli* and *B. atrophaeus* spores.

Co-nebulization of *E. coli* ($10^9$ CFU/mL) with *B. atrophaeus* spores ($10^8$ CFU/mL) did not have a significant effect on the recovery of *E. coli* compared to nebulization of *E. coli* alone when samples were analyzed using LAL assay (Figure 7). However, the recovery of *E. coli* by the ESP when co-aerosolized with *Bacillus* spores is three times lower than when aerosolized alone as shown in the qPCR analysis (p = 0.0019). It is hypothesised that the presence of *B. atrophaeus,* by exhibiting the previously observed sticky behaviour, inhibits the elution efficiency of the droplet on the ESP surface. We cannot compare the recovery of *B. atrophaeus* spores when aerosolized alone with co-aerosolization with *E. coli* since the aerosolization parameters were different.

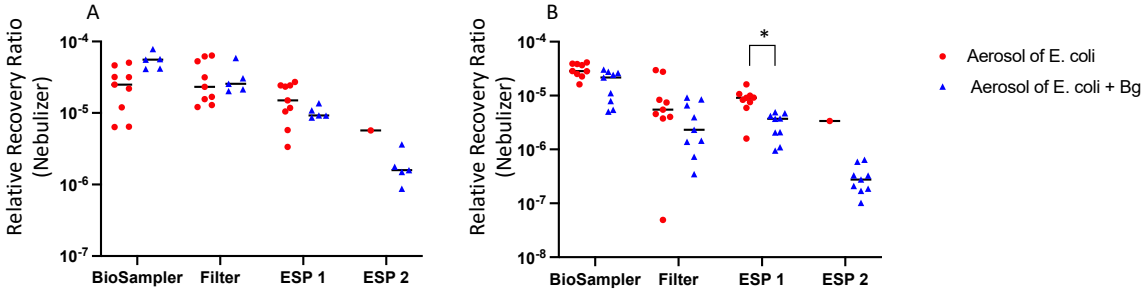


***Figure 7:*** *Co-nebulization of E. coli (1.2 × $10^9$ genomes/mL, 8.5 × $10^4$ endotoxins units/mL) and B. atrophaeus spores (8 × $10^7$ genomes/mL) compared to nebulization of E. coli only (3.5 × $10^9$ genomes/mL, 1.5 × $10^6$ endotoxins units/mL). Samples were analyzed using LAL assay (A) and E. coli-specific qPCR (B). Ratios were performed with E. coli concentration in the nebulizer, Equation (2). *Significant difference (p = 0.0019) between E. coli nebulized alone and E. coli nebulized with B. atrophaeus spores when analyzed by qPCR (Wilcoxon non-parametric unpaired T-test). ESP 1 and ESP 2 were the first EWOD-actuated and second manually-eluted samples.*

## 3.2 Sample concentration

With the filter and the SKC BioSampler, the bioaerosol samples were collected and prepared in larger volumes than necessary, resulting in more diluted samples. Collecting a concentrated sample in a small volume is key to increasing detection sensitivity. We compared the raw sample concentrations and obtained the raw concentration ratio based on Equation (3) (Figure 8). Filters and ESP raw sample concentrations were divided by the SKC BioSampler's sample concentration collected at the same time. Sample concentration with the ESP was 33 times higher than with the SKC BioSampler for *B. atrophaeus* spores and 3-orders of magnitude (1000 times) higher for *E. coli* and *P. agglomerans.* The difference of samples concentration corelate with collection volume for *E. coli* and *P. agglomerans.*

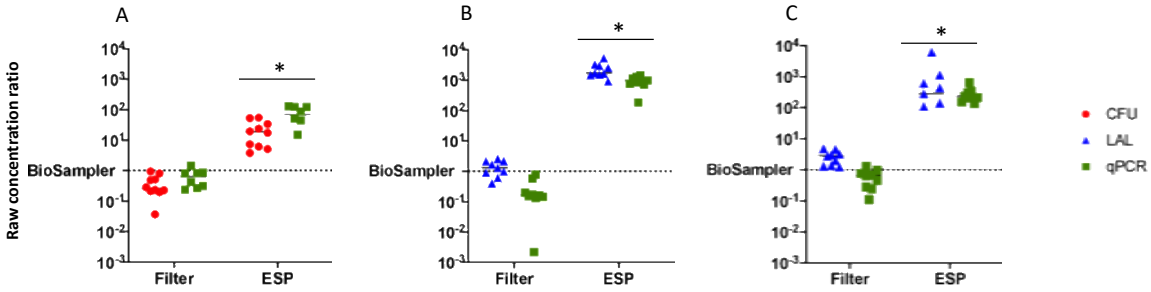


***Figure 8:*** *Comparison of sample raw concentrations for B. atrophaeus spores (A), E. coli (B) and P. agglomerans (C). Ratio of ESP and filter concentration on SKC BioSampler concentration, Equation (3). Dotted line represents SKC BioSampler concentration. *Concentration in ESP significantly ($p<0.05$) higher than SKC BioSampler and filter (Wilcoxon non-parametric paired T-test).*

# 4 Conclusion

This bioaerosol chamber trial tested and compared the ESP-EWOD personal aerosol sampler to two standard bioaerosol collectors. Three bacteria (*Bacillus atrophaeus* spores, *Pantoea agglomerans*, and *Escherichia coli*) were used in the test trial. The collected bioaserol samples were analyzed by culture, endotoxin assay, and qPCR.

Based on our results (e.g., lower *E. coli* recovery when co-nebulized with *B. atrophaeus* spores, higher *B. atrophaeus* spore recovery with the swab, sticky effect with higher nebulized *B. atrophaeus* spore concentration), we postulate that the ESP's lower recovery efficiency for *B. atrophaeus* spores was due to incomplete elution. This could be caused by the sticky effect of the spores on the glass slide. Further investigation could be conducted to determine if the sticky effect is caused by differences in *B. atrophaeus* spore preparation and/or concentration in order to improve *B. atrophaeus* spore elution from the glass slide.

Liquid based air sampling, like the BioSampler, is more appropriate if downstream analysis requires culture. However, the samples are collected in a large volume, which significantly dilute the samples and increase the limit of detection, and its glass components make it cumbersome to use in field applications. Despite the lower collection and elution efficiency of the ESP with *B. atrophaeus* spores, the raw sample concentration was much higher compared to the SKC BioSampler. Therefore, if sample volume required for analysis is 2 µL or lower, the ESP would provide greater sensitivity for detection using qPCR or other methods not involving culture. For automated field applications, filters required manual extraction, the potential of the ESP for automated sample concentration and processing for downstream molecular analyses is an attractive feature for system integration.

More experimental work should be carried out to optimize several parameters (e.g., fan/pump in the outlet, collection surface, EWOD electrode surface defects, detergent effects) to improve ESP collection and elution efficiency. Future field sampling trials would be required to test interference of environmental contaminants (dust, fungi, pollens, etc.) on collection and elution efficiency of the ESP in more realistic environments and to test the possibility of its use on unmanned aerial systems.

Since ESP is as efficient as filters to collect *E. coli* and almost as efficient as SKC BioSamplers and filters to collect *P. agglomerans*, it collects samples in a very small volume suitable for microfluidic downstream molecular analysis, and it can be fully automated, the ESP-EWOD system is a promising technology for integration into a microfluidic biodetection system.

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# Annex A Quantitative polymerase chain reaction protocols

This Annex contains the detailed qPCR protocols used to quantified the microbes collected in the experiments. Each protocol shows the sequence of the target gene of each organism, the primer and probe DNA sequences, the qPCR master mix reagent composition, and the thermocycle steps.

## A.1 *B. atrophaeus* spores

- **Target**: *recF* gene
- **Primers and probe**
- recFFor: accagacaatgctcgacgtt
- recFRev: ccctcttgaaattcccgaat
- recFProbe: FAM-actgaacagctgatcgagacagctgca-BHQ
- **recF plasmid insert**
- Genomic Sequence: NZ_CP007640.1
- GeneID:23412130

TTGTATATCCAAAATTTGGAATTAACGTCTTATCGTAATTACGAGCGTGTTGAACTTCAATTTGAAAATAAAGTGAATGTC
ATCATCGGTGAGAACGCTCAAGGGAAGACAAATCTCATGGAAGCGATCTATGTTTTGTCGATGGCGAAATCACATCGCACG
TCAAATGACAAAGAACTTATACGGTGGGACAAAGACTATGCTAAAATAGAGGGAAGAGTGATGAAGCAAAACGGGTCGATC
CCGATGCAGCTTGTCATTTCCAAGAAGGGGAAAAAGGGCAAGGTCAATCATATCGAACAGCAAAAACTGAGCCGGTATGTA
GGAGCGCTGAATACAATTATGTTCGCGCCCGAGGACTTAAATCTCGTAAAGGGCAGCCCGCAAGTAAGACGGCGGTTTCTT
GACATGGAAATCGGCCAGGTATCTCCAGTCTATTTGTACGATCTTTCTCTGTACCAGAAAATTCTTACGCAAAGAAATCAT
TTTTTGAAACAGCTGCAAAGCAGGAAACAGACTGACCAGACAATGCTCGACGTTCTGACTGAACAGCTGATCGAGACAGCT
GCAAAGGTTGTCGTAAAACGCCTGCAATTTACAGCGCAGCTTGAAAAATGGGCGCAGCCGATCCATTCGGGAATTTCAAGA
GGGCTTGAAGAACTGACGCTGAAGTATCAGACGGCTCTTGAGGTATCAGATCCCGAAGACTTGTCGAAAATAGGAGATAGT
TACCAAAGGGCGTTTTCAAAGCTAAGAGAAAAAGAAATTGAGCGTGGTGTAACGCTGTCAGGACCTCATCGGGATGATGTT
CTTTTTTATGTGAACGGACGCGATGTGCAAACATATGGTTCTCAGGGGCAGCAGCGGACAACGGCATTGTCACTCAAGCTG
GCGGAAATTGACTTGATTCATGAAGAAATCGGAGAATACCCTATCTTACTTTTGGATGATGTGCTGAGTGAGCTGGATGAC
TATCGGCAGTCGCATTTGCTTCATACGATCCAAGGCCGTGTTCAAACGTTTGTCACTACGACAAGTGTAGATGGCATTGAT
CACGAAACCTTACATCAAGCAGGAATGTTCCGTGTGCAAAACGGTGCGTTAGTGAAGTGA

- **Master mix reagent preparation**

| Reagent | Volume (µL) for each reaction |
|---|---|
| iQ supermix | 10 |
| recAFor + recARev (50 µM) | 0.13 |
| recAProbe (10 µM) | 0.4 |
| Sigma water | 7.47 |
| DNA | 2 |
| Total volume per reaction | 20 |

- **Thermocycling protocol** [15]

  95°C for 3 min
  95°C for 15 sec
  60°C for 60 sec
  Fluorescence reading
  Return to Step 2 and repeat 39x.

## A.2 *P. agglomerans* ATCC 33243

- Target: aroQ gene
- Primers and probe
- aroQFor: gctgcaaaacgcacaaca
- aroQrev: cgtgaacaaacggctcca
- aroQProbe: FAM-ccgggcttgaaccccactcc—BHQ
- aroQ plasmid insert
- Sequence ID: M95628.1

ATGACGCACTTTGTGGCAATTTTTTTCTCTTCACTGTTTATGTGCAGTAACGTTTTTGCAGGTTCGGTTTCATCTGTTTCT
CTTGGATCACTCTCTTCTGCGCTCAATGAACGGATGCAGGTGATGAAAGCGGTGGCGGGTTATAAAGCACTGCATCATTTA
CCTATTGAGGATCTCCCACGGGAGCAGGTGGTGCTGGATCATATGCTGCAAAACGCACAACAGGCCGGGCTTGAACCCCAC
TCCGTGGAGCCGTTTGTTCACGCTTTGATGAACGCCAGCAAGACGATCCAGTATCGCTATCGGGCTGACTGGCTCTCATCA
CCAGACAGCGCTGTTCCTGTCAGGGATCTGACCGAGACCAGACAGCAGATACAACAGCTGGATACCCAGCTCCTGACGGCG
ATCAGCCAGCGCCTGATGACTGGCGCCTTCTCGCAGGAGGACAAAGAATTTCTGATGTCACACCTCACGGCACCTCACCTC
AGTGAAAGTGATAAAAACAGCCTGTTCGCTTCCCTCTCCCGCATTCAGCGCCAGCACTAA

- **Master mix reagent preparation**

| Reagent | Volume (µL) for each reaction |
|---|---|
| iQ supermix | 10 |
| aroQFor + aroQRev (50 µM) | 0.12 |
| aroQProbe (10 µM) | 0.4 |
| Sigma water | 7.48 |
| DNA | 2 |
| Total volume per reaction | 20 |

- **Thermocycling protocol** [16]

  95°C for 3 min
  95°C for 15 sec
  60°C for 60 sec
  Fluorescence reading
  Return to Step 2 and repeat 39x.

## *A.3 E. coli*

- **Target**: *uidA* gene, position 1286–1376
- **Primers and probe**
- uidAFor: CGGAAGCAACGCGTAAACTC
- uidArev: TGAGCGTCGCAGAACATTACA
- uidAProbe: FAM- CGCGTCCGATCACCTGCGTC --BHQ
- **uidA plasmid insert**
- GenBank: CP047127.1

ATGTTACGTCCTGTAGAAACCCCAACCCGTGAAATCAAAAAACTCGACGGCCTGTGGGCATTCAGTCTGGATCGCGAAAACTG
TGGAATTGATCAGCGTTGGTGGGAAAGCGCGTTACAAGAAAGCCGGGCAATTGCTGTGCCAGGCAGTTTTAACGATCAGTTCG
CCGATGCAGATATTCGTAATTATGCGGGCAACGTCTGGTATCAGCGCGAAGTCTTTATACCGAAAGGTTGGGCAGGCCAGCGT
ATCGTGCTGCGTTTCGATGCGGTCACTCATTACGGCAAAGTGTGGGTCAATAATCAGGAAGTGATGGAGCATCAGGGCGGCTA
TACGCCATTTGAAGCCGATGTCACGCCGTATGTTATTGCCGGGAAAAGTGTACGTATCACCGTTTGTGTGAACAACGAACTGA
ACTGGCAGACTATCCCGCCGGGAATGGTGATTACCGACGAAAACGGCAAGAAAAAGCAGTCTTACTTCCATGATTTCTTTAAC
TATGCCGGGATCCATCGCAGCGTAATGCTCTACACCACGCCGAACACCTGGGTGGACGATATCACCGTGGTGACGCATGTCGC
GCAAGACTGTAACCACGCGTCTGTTGACTGGCAGGTGGTGGCCAATGGTGATGTCAGCGTTGAACTGCGTGATGCGGATCAAC
AGGTGGTTGCAACTGGACAAGGCACTAGCGGGACTTTGCAAGTGGTGAATCCGCACCTCTGGCAACCGGGTGAAGGTTATCTC
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GGGCGAACAGTTCCTGATTAACCACAAACCGTTCTACTTTACTGGCTTTGGTCGTCATGAAGATGCGGACTTGCGTGGCAAAG
GATTCGATAACGTGCTGATGGTGCACGACCACGCATTAATGGACTGGATTGGGGCCAACTCCTACCGTACCTCGCATTACCCT
TACGCTGAAGAGATGCTCGACTGGGCAGATGAACATGGCATCGTGGTGATTGATGAAACTGCTGCTGTCGGCTTTAACCTCTC
TTTAGGCATTGGTTTCGAAGCGGGCAACAAGCCGAAAGAACTGTACAGCGAAGAGGCAGTCAACGGGGAAACTCAGCAAGCGC
ACTTACAGGCGATTAAAGAGCTGATAGCGCGTGACAAAAACCACCCAAGCGTGGTGATGTGGAGTATTGCCAACGAACCGGAT
ACCCGTCCGCAAGGTGCACGGGAATATTTCGCGCCACTGGCGGAAGCAACGCGTAAACTCGACCCGACGCGTCCGATCACCTG
CGTCAATGTAATGTTCTGCGACGCTCACACCGATACCATCAGCGATCTCTTTGATGTGCTGTGCCTGAACCGTTATTACGGAT
GGTATGTCCAAAGCGGCGATTTGGAAACGGCAGAGAAGGTACTGGAAAAAGAACTTCTGGCCTGGCAGGAGAAACTGCATCAG
CCGATTATCATCACCGAATACGGCGTGGATACGTTAGCCGGGCTGCACTCAATGTACACCGACATGTGGAGTGAAGAGTATCA
GTGTGCATGGCTGGATATGTATCACCGCGTCTTTGATCGCGTCAGCGCCGTCGTCGGTGAACAGGTATGGAATTTCGCCGATT
TTGCGACCTCGCAAGGCATATTGCGCGTTGGCGGTAACAAGAAAGGGATCTTCACTCGCGACCGCAAACCGAAGTCGGCGGCT
TTTCTGCTGCAAAAACGCTGGACTGGCATGAACTTCGGTGAAAAACCGCAGCAGGGAGGCAAACAATGA

- **Master mix reagent preparation**

| Reagent | Volume (µL) for each reaction |
|---|---|
| iQ supermix | 10 |
| uidAFor + uidARev (50µM) | 0.25 |
| uidAProbe (10µM) | 1.25 |
| Sigma water | 6.5 |
| DNA | 2 |
| Total volume per reaction | 20 |

- **Thermocycling protocol** [9]

  95°C for 3 min
  95°C for 20 sec
  60°C for 60 sec
  Fluorescence reading
  Return to Step 2 and repeat 39x.

# List of symbols/abbreviations/acronyms/initialisms

| | |
|---|---|
| APS | aerodynamic particle sizer |
| ATCC | American Type Culture Collection |
| CAF | Canadian Armed Forces |
| CANSOFCOM | Canadian Special Operations Forces Command |
| CB | chemical and biological |
| CFU | colony-forming unit |
| CRIUCPQ | l'institut universitaire de cardiologie et de pneumologie de Québec |
| DMF | digital microfluidic |
| DNA | deoxyribonucleic acid |
| DRDC | Defence Research and Development Canada |
| Dstl | Defence Science and Technology Laboratory |
| EC | electrochemical |
| ESP | electrostatic precipitator |
| EWOD | electrowetting-in-dielectic |
| IDEaS | Innovation for Defence Excellence and Security |
| IDT | Integrated DNA Technologies |
| ITO | indium tin oxide |
| LAL | Limulus Amebocyte Lysate |
| NB | nutrient broth |
| NRC NANO | National Research Council Nantotechnology Research Centre |
| PBS | phosphate-buffered saline |
| qPCR | quantitative polymerase chain reaction |
| TLR | Toll-like receptor |
| TRL | technology readiness level |
| TSB | trypticase soy broth |
| UH | University of Hertfordshire |

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Bioaerosols; Bioaerosol Collection; Test and Evaluation; Chamber Trial; Biodetection

13a. ABSTRACT (When available in the document, the French version of the abstract must be included here.)

The aim of the project was to test a personal aerosol sampler based on electrostatic precipitation and electrowetting on dielectric (ESP-EWOD). A bioaerosol chamber trial was conducted to compare ESP-EWOD to two standard bioaerosol collectors (a SKC BioSampler and a 37 mm filter cassette hosting 0.8 µm polycarbonate filters). Three bacteria (*Bacillus atrophaeus* spores, *Pantoea agglomerans,* and *Escherichia coli*) were aerosolized, individually and in a mixture, into an aerosol chamber to introduce bacterial samples into the samplers simultaneously. The samples were collected and eluted for culture, endotoxin and quantitative polymerase chain reaction (qPCR) assays. The semi-quantitative comparison showed that ESP has comparable collection and elution efficiency as the BioSampler and filter cassette for *E. coli* and *P. agglomerans*, but reduced elution efficiency with *B. atrophaeus* spores. Sample concentration with the ESP was 1000x higher for *E. coli* and *P. agglomerans* and 33x higher than with the SKC BioSampler for *B. atrophaeus* spores. This first of a series of planned trials showed that ESP-EWOD is a promising technology for bioaerosol detector system integration. More work should be done to optimize ESP parameters to improve collection and elution efficiency and explore the effects of Tween detergent and different spore preparation methods on the ESP performance. Future test plans include field trials in easily accessible locations with complex environmental background, such as animal barns or wastewater treatment plants, and comparison to other low burden sampling systems would further demonstrate the use of the ESP-EWOD system.

13b. Résumé (when available in the document, the French version of the abstract must be included here)

Le but de ce projet est de tester un échantillonneur d'air personnel basé sur la précipitation électrostatique et l'élution sur diélectrique (ESP-EWOD). Un essai en chambre de bioaérosols a été mené pour comparer (ESP-EWOD) à deux échantillonneurs de référence (SKC BioSampler et cassette 37 mm avec un filtres d'une porosité de 0,8 µm). Trois microorganismes (spores de *Bacillus atrophaeus*, *Pantoea agglomerans* et *Escherichia coli*) ont été aérosolisés individuellement et en mélange, dans une chambre d'aérosol de sorte à exposer les trois échantillonneurs simultanément au même aérosol. Les échantillons prélevés ont été élués et analysés par culture, mesure d'endotoxines et de réaction en chaîne par polymérase quantitative (qPCR). La comparaison des concentrations relatives récoltées a montré que l'ESP a une efficacité de collecte et d'élution comparable à celle du SKC BioSampler et de la cassette avec filtre 0,8 µm pour *E. coli* et *P. agglomerans*. L'efficacité d'élution est cependant réduite avec les spores de B. atrophaeous. La concentration des échantillons obtenus avec l'ESP était 1000 fois plus élevée pour *E. coli* et *P. agglomerans* et 33 fois plus élevée pour les spores de *B. atrophaeus* qu'avec le SKC BioSampler. Ce premier essai d'une série d'essais planifiés a montré que l'ESP-EWOD est une technologie prometteuse pour l'intégration avec des systèmes de détection de bioaérosols. Des essais seront effectués pour améliorer la collecte avec l'ESP et l'élution avec EWOD, notammant pour vérifier l'effet de différentes préparations de spores et de détergeants comme le Tween. Des essaient comparatifs sur le terrain, avec d'autres appareils de détection à faible charge de travail, dans des environnements fortement chargés en microorganismes, comme des fermes et des usines de traitement des eaux usées, permettront de valider les performances de ESP-EWOD.