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SASS 3100 high-volume electret filter air sampler

— sampling and recovery efficiency

Gunnar Skogan
Marius Dybwad

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Marius Dybwad, *Research Manager*
Janet M. Blatny, *Research Director*

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Summary

The Norwegian Defence Research Establishment (FFI) and The Netherlands Organisation for Applied Scientific Research (TNO) both have experience in Test and Evaluation (T&E) of Biological Detection, Identification, and Monitoring (BioDIM) equipment. The bioaerosol T&E infrastructure in the two organizations have a high degree of technical similarity, which provide a good starting point for increased procedural harmonisation.

The work described in this report was done in the context of Bioaerosol RDT&E SMART Bioscreen, a CBRN project in the SMART agreement (Strategic Mutual Assistance in Research and Technology) between the Netherlands and Norway.

This report summarize the results from a aerosol chamber-based T&E that was done to assess the sampling and recovery efficiency of the SASS 3100. The test objective was to assess the end-to-end sampling efficiency using different test aerosols and downstream sample analysis techniques. The test aerosols included Gram-positive bacterial spores, Gram-negative vegetative bacteria, fluorescent tracer, and polystyrene latex (PSL) microspheres. The collected samples were analyzed using cultivation for Gram-positive bacterial spores, fluorescence measurement for Uranine AP used as a fluorescent tracer, Flow cytometry (FCM) analysis for PSL microspheres, and quantitative PCR (qPCR) analysis for Gram-negative vegetative bacteria. The end-to-end sampling efficiency was also assessed at different sampling flow rates and particle sizes. End-to-end sampling efficiencies were determined by comparing SASS 3100 samples with well-characterized gelatine filters used for reference sampling.

The SASS 3100 high-volume electret filter air sampler was also used in the now completed SMART Bioscreen project to realize the high-volume air sampling element of an integrated detect-to-treat BioDIM technology demonstrator.

Taken together, the test results showed that very high (~100%) extraction efficiencies can be achived with the SASS 3100 electret filters both for bacterial spores and microspheres. The test results also showed that the SASS 3100 air sampler has a very high (78–100%) end-to-end sampling efficiency, and that the end-to-end sampling efficiency is largely independent of the sampling flow rate, sampled particle size, test aerosol composition and downstream analysis technique.

Sammendrag

Forsvarets forskningsinstitutt (FFI) og the Netherlands Organisation for Applied Scientific Research (TNO) har begge erfaring med test og evaluering (T&E) av ustyr til biologisk deteksjon, identifikasjon og monitorering (BioDIM). Infrastruktur knyttet til T&E av BioDIM-utstyr i de to organisasjonene har store likheter og er et godt utgangspunkt for økt harmonisering av testprosedyrer.

Denne rapporten beskriver en leveranse innenfor delprosjektet Bioaerosol RDT&E (forskning, utvikling, test og evaluering) i samsvar med SMART Bioscreen, et CBRN-prosjekt innenfor SMART-avtalen (Strategic Mutual Assistance in Research and Technology) mellom Norge og Nederland.

Rapporten oppsummerer resultater fra T&E utført i FFIs aerosolkammer for å vurdere SASS 3100s effektivitet som luftprøvetaker og effektivitet ved eluering av luftprøver fra electret-filtrene. Formålet med forsøkene var å finne den totale (ende-til-ende) prøvetakingseffektiviteten for ulike typer aerosol, partikkelstørrelser og nedstrøms analysemetoder. De ulike testaerosolene inkluderte bakteriesporer, gram-negative vegetative bakterier, fluoriscerende sporstoff (uranin) og monodisperse plastkuler (polymerpartikler). Prøvene ble analysert ved bruk av ulike metoder som dyrking av sporer på agarmedie, fluorescensmåling av uranin AP, flowcytometri av monodisperse plastkuler og kvantitativ PCR på vegetative gram-negative bakterier. Den totale prøvetakingseffektiviteten ble også undersøkt ved ulike strømningshastigheter og partikkelstørrelser. Den totale prøvetakingseffektiviteten ble bestemt ved å sammenligne SASS 3100-prøver med referanseprøver fra godt dokumenterte og karakteriserte gelatinfiltre.

SASS 3100 ble også benyttet i det avsluttede SMART Bioscreen-prosjektet innen CBRN-beskyttelse der den ble benyttet som høyvolumluftprøvetaker i en integrert «detect-to-treat»-kapabilitet basert på metagenommetode og haglesekvensering.

Resultatene viste at høy elueringseffektivitet (~100%) ble oppnådd fra electretfiltrene både ved bruk av bakteriesporer og monodisperse plastkuler. Forsøkene viste også at SASS 3100 var en effektiv luftprøvetaker (70–100 %) og at den høye effektiviteten var uavhengig av strømningshastighet, partikkelstørrelse, testaerosol og nedstrøms analysemetode.

Contents

1 Introduction

The Minister of Defence of the Kingdom of The Netherlands and the Minister of Defence of the Kingdom of Norway have reached an understanding on information exchange, joint research, exchange of scientific personnel, and exchange of equipment and materials, concerning chemical, biological, radiological and nuclear (CBRN) Protection. This understanding has been formalized as a Technical Arrangement entitled *Strategic Mutual Assistance in Research and Technology* (SMART) on CBRN Protection. Following a successful pilot phase (SMART CBRN Protection Phase 1), SMART CBRN Protection Phase 2 is now running.

As part of the SMART CBRN Protection Phase 1 collaboration, the Bioscreen project was established due to shared interests in the field of Biological Detection, Identification and Monitoring (BioDIM). The objective of the Bioscreen project was to evaluate new BioDIM capabilities for the Norwegian and Netherlands Ministry of Defence (MoD) in order to rapidly detect and characterize biological threats in air (bioaerosol threats). The work of the Bioscreen project is now being continued as part of the SMART Bioaerosol RDT&E (Research, Development, Test & Evaluation) project under SMART CBRN Protection Phase 2.

To efficiently detect and identify airborne biothreats, a method based on high-volume air sampling and shotgun metagenomics was developed in the Bioscreen project (FFI-rapport 18/00707). The Bioscreen method involves efficient air sampling, DNA extraction, shotgun sequencing and a bioinformatics pipeline for rapid detection and identification of potential bioaerosol threats. Because air is regarded as a very low biomass environment, the high-volume air sampler SASS 3100 was selected for this purpose.

The SASS 3100 dry air sampler (Research International Inc., WA, USA) is a compact, ruggedized and highly portable dry air sampler with user-selectable airflow in the range of 50 – 300 lpm. Particles are collected on a 44 mm diameter disc with electric field charged polymer fibers. These fields induce a charge in aerosols passing through the “electret” filter and provide a capture mechanism effective even for sub-micron particles. The SASS 3100 sampling efficiency have previously been tested using bacterial spores and fluorescent particles (Dybwad, Skogan & Blatny 2014).

The end-to-end sampling efficiency of the SASS 3100, which will be the combined product of the collection efficiency and the recovery (filter extraction) efficiency, may depend on several parameters, including but not limited to the bioaerosol composition, particle size and sampling flow rate. The recovery efficiency of the SASS 3100 electret filter was assessed using bacterial spores and microspheres in spiking experiments performed in the lab. The end-to-end sampling efficiency of the SASS 3100 was examined under controlled and reproducible conditions in the Aerosol Test Chamber (ATC) at FFI, using different test aerosol compositions and downstream analysis techniques. The test aerosols and downstream analysis techniques used were cultivation for Gram-positive bacterial spores, fluorescence measurement for Uranine AP used as a fluorescent tracer, Flow cytometry (FCM) analysis for PSL microspheres and quantitative PCR (qPCR) analysis for Gram-negative vegetative bacteria.

2 Methods

2.1 Recovery efficiency

The SASS 3100 has already been used for many years for bioaerosol sampling at FFI, and the samples have been analyzed with a number of different techniques. Because several previous measurements have shown physical end-to-end sampling efficiencies in the range of 90-100% for the SASS 3100, the recovery (filter extraction) efficiency for the SASS 3100 electret filters have been estimated to be ~100%, although this have never been specifically examined. In order to examine and verify that the electret filter extraction efficiency is high, spiking experiments were performed in this study using *Bacillus atrophaeus* (BG) spores and 3.7 μm fluorescent PSL microspheres (ThermoFisher FluoSpheres (FS) F8859). *Bacillus atrophaeus* has historically been known by several other names, including *Bacillus globigii* (the origin of its military nickname "BG") and *Bacillus subtilis* var. *niger*.

Three SASS 3100 filters and gelatin reference filters were spiked by pipetting 100 μl each of solutions containing $1x10^5$ BG spores/ml in MQ water and $5x10^5$ FS/ml (in suppliers stock solution) evenly over the filter surfaces. Positive controls were made by pipetting 100 μl of BG and FS spiking solutions into sterile sample tubes containing 9.9 ml sterile PBSTA (PBS with 0.05% Triton X-100 and 0.005% Antifoam Y-30) buffer. After 1 hour drying at 20 ºC the filters were transferred to sterile 50 ml sample tubes containing 10 ml sterile PBSTA buffer. Gelatin filters were incubated in water bath at 37 ºC for 15 minutes in order to dissolve the gelatin. SASS 3100 filters were shaken by hand to wet with buffer before being vortexed vigorously for 15 seconds. A sterile forceps was use to transfer the filter into a 10 ml syringe to extract (completely) any residual liquid in the filter back into the vial before discarding the filter. FS were counted both in filter samples and positive control using FCM (BD Accuri C6). 100 μl of the filter samples and positive control with BG were plated onto Trypticase Soy Agar (TSA) plates in triplicate followed by incubation at 30 ºC for 20 hours and colony counting. The filter recovery efficiency was calculated relative to the positive control by the following equation:

$$Recovery\ efficiency = \frac{\text{filter sample concentration (\#/ml) x sample volume (ml)}}{\text{pos. ctr. concentration (\#/ml) x pos. ctr. volume (ml)}}$$

2.2 Flow rate measurements

The flow rate of the SASS 3100 can be adjusted between 50 and 300 lpm when using the electret filter intended for bioaerosol sampling. The flow rate adjustment must be done when the SASS 3100 is connected to a computer with a serial cable. Flow rate adjustment relies on a calibration table from the manufacturer regarding the relationship between the centrifugal fan speed and the resulting flow rate. The flow rate of the SASS 3100 was measured in this study at six nominal settings (50, 100, 150, 200, 250 and 300 lpm) using a Testo 410-1 vane anemometer mounted on a 3 cm long tube with identical diameter as the electret filter. Flow

rates were calculated from the measured air velocities and the resulting flow rates (Table 3.2) were subsequently used when calculating the sampling efficiency.

2.3 Sampling efficiency

Sampling efficiency tests were performed under controlled and reproducible conditions in FFIs Aerosol Test Chamber (ATC-12, Dycor Technologies Ltd). The ATC is a 12 m^3 stainless steel box (2x2x3 m, HxWxL) positioned on a frame approximately 2 meter above floor level. The ATC is equipped with two internal circulation fans to ensure sufficient mixing to create stirred settling conditions (homogenous aerosol distribution). The test equipment may be placed either inside or under the ATC with their respective air inlets penetrating into the ATC through inside-outside sampling ports. A ventilation system with HEPA filtered air inlet and outlet was used to empty the ATC of particles before aerosol generation and to purge the ATC after each test. A GRIMM 1.108 particle counter serves as feedback element for controlling the amount of particles introduced into the chamber by means of a closed-loop control system algorithm. In addition to the GRIMM 1.108, an Aerodynamic Particle Sizer (APS 3321, TSI Inc.) was used in all tests to monitor the concenctration and size distribution of the test aerosol.

Gelatine membrane filters (Sartorius 12602-37-ALK) with a diameter of 37 mm were used for reference sampling. The physical sampling efficiency of gelatine filters has been benchmarked against other reference samplers (Bøifot 2020) and found to be higher than the sampling efficiency of the SKC BioSampler and similar to isopore membrane filters (Millipore HTTP03700, 0.4 μm pore size). Gelatin filters have also previously been shown to have a physical sampling efficiency of >99.5% for particles larger than 0.5 μm (Koller 1974). The gelatine filters were positioned in a 2-piece conductive filter cassette (SKC 225-308, SKC Inc., PA, USA), resting on a polypropylene support pad (SKC 225-2902). The sampling flow rate was set to 10 lpm using an adjustable nozzle and monitored with a Sierra Top-Trak 820 Series thermal mass flow meter (Sierra Instruments Inc., CA, USA).

The SASS 3100 air samplers and gelatine reference filters were placed inside the ATC. Air sampling was turned on and off through power outlets and a vacuum pump controlled by the ATC operating software. When aerosol generation was finished, the air inside the ATC was homogenized for 1 minute before SASS 3100 and gelatine filter sampling were started. The samplers were turned off after 5 minutes and the ATC was purged until the total particle concentration according to APS 3321 was below 0.1 particles/ml before the access door was opened and filters collected. All aerosol trials were performed with a relative humidity (RH) of 50% and a temperature of 20ºC inside the ATC. The relative sampling efficiency (RSE) was calculated using the following equation:

$$\text{Relative sampling efficiency} = \frac{\frac{\text{sample conc. (\#/ml) x volume (ml)}}{\text{airflow rate (lpm) x duration (min)}}\ \text{(Sampler)}}{\frac{\text{sample conc. (\#/ml) x volume (ml)}}{\text{airflow rate (lpm) x duration (min)}}\ \text{(Reference)}}$$

Figure 2.1 Conductive sampling cassette used for gelatine filters (left) and SASS 3100 air sampler with electret filter mounted on top (right).

2.4 Sample analysis

2.4.1 Filter extraction

SASS 3100 filters were transferred to 10 ml PBSTA, shaken by hand to wet the filter and then vortexed at maximum speed for 15 seconds. A sterile forceps was use to transfer the filter into a 10 ml syringe to extract any residual liquid in the filter back into the vial before discarding the filter. Gelatine filters were transferred to 10 ml PBSTA and dissolved by incubation at 37°C for 15 minutes before analysis.

2.4.2 Cultivation analysis

Samples containing BG and BT spores were diluted 10 – 1000x in PBSTA, based on expected spore concentration. 200 µl of the dilutions were plated on TSA plates (Difco 236950, Becton Dickinson, NJ, USA) in triplicate, followed by incubation for 18 hours at 30ºC and colony counting.

Cultivation of samples containing Gram-negative bacteria *Pantoea agglomerans* and *Escherichia coli* were performed by plating 200 µl of undiluted sample on TSA plates, followed by incubation for 18 hours at 37ºC and colony counting.

2.4.3 Physical fluorescent tracer

Uranine AP, a disodium salt of fluorescein widely used as a non-toxic physical fluorescent tracer, were mixed into the same spray solutions as spores/bacteria. Droplets generated from these spray solutions therefore contain both spores/bacteria and Uranine AP, and which after rapid evaporation of water, leaves droplet nuclei (solid particles) consisting of spores/bacteria and Uranine AP. Uranine AP was used as a physical tracer in all experiments where the test aerosol contained Gram-positive bacterial spores or Gram-negative vegetative bacteria, and fluorescence measurements were used to determine the sampling efficiency for the tracer.

Previous experiments have shown that for reproducible and comparable fluorescein measurements, the samples should have at pH > 8 (Kesavan, 2000). All samples in this study was therefore diluted 2 – 10x, based on expected fluorescein levels, in PBSTA before a final dilution 1:2 with 0.1M Trizma base buffer with a pH of 9.5. Triplicate aliquots of 100 µl were pipetted into 96-well black polystyrene assay plates (Costar 3915) before fluorescence measurements were recorded using a CLARIOstar plate reader (BMG Labtech, Ortenberg, GE).

2.4.4 Flow cytometry (FCM) analysis

Preliminary experiments showed that gelatine filter samples had to be diluted 1:2 with PBSTA in order to reduce the viscosity before FCM quantification (data not shown). The three different sized fluorescent PSL microspheres had no signal overlap/bleeding on the BD Accuri C6 flow cytometer and were therefore quantified together in each of the triplicates.

2.4.5 qPCR analysis

DNA extraction and purification from SASS 3100 and gelatin filter samples was done using the NucliSENS Magnetic Extraction Reagents kit (bioMérieux 200293) and the purified DNA was eluted in 100 µl of the provided elution buffer. Sample concentration of *Pantoea agglomerans* and *Escherichia coli* were measured performing triplicate qPCR analysis using primers targeting the bacterial 16S rRNA gene (933F/1387R). qPCR was performed using the LightCycler 480 SYBR Green I Master mix and a LightCycler 96 instrument (Roche).

2.5 Bacterial spore testing

One of the most commonly used test organisms for T&E of BioDIM equipment is BG spores (Dybwad 2014). BG spores have previously been used for examining the sampling efficiency of the SASS 3100 (Dybwad 2014) and was used in this study in order to examine the sampling efficiency at different particle sizes and sampling flow rates. In addition, experiments were performed to examine whether the sampling efficiency of the SASS 3100 was comparable when using different bacterial spores (different species/strain). The test organism for this experiment was spores from the species/strain *Bacillus thuringiensis* var. *Novosibirsk* (BT spores).

Test aerosols containing BG or BT spores were generated using a 120 kHz ultrasonic atomizer nozzle (Sono-Tek, NY, USA) powered by an ECHO ultrasonic generator (Sono-Tek) operated at 3 W. The ultrasonic atomizer was fed by a dual syringe pump at a flow rate of 500 µl/min. The duration of the dissemination period was 2 min when generating 1 µm particles and 30 sec when generating 3 µm particles.

2.5.1 *Bacillus atrophaeus* (BG)

The spray solutions were made by mixing BG spores (Dugway BG spores LOT# 19076-03268) and Uranine AP (Merck 108462, Merck KGaA, Darmstadt, GE) in MQ water. The final concentration in spray solutions for 1 µm and 3 µm particles were 2E+07 cfu ml^{-1} BG spores/0.15 mg ml^{-1} Uranine AP and 2.8E+08 cfu ml^{-1} BG spores/0.40 mg ml^{-1} Uranine AP, respectively.

Four SASS 3100 samplers were used in each trial at nominal sampling flow rates 50, 100, 200 and 300 lpm. The flow rate of the different samplers were alternated between trials in order to avoid sampling efficiency bias by sampler-to-sampler variation or chamber position, even though the flow rate measurements (Chapter 2.2) did not indicate any flow rate variation. A total of 10 trials were done, resulting in five independent measurements for each particle size and SASS 3100 sampling flow rate.

In the test setup described above, the SASS 3100 samplers were positioned inside the ATC and the open-faced electret filter was therefore exposed to the test aerosol during the dissemination, homogenization and purge phase of each trial. This may cause an additional sampling effect due to gravitational settling and electrostatic interaction/precipitation. In order to quantify the passive sampling effect, additional aerosol trials were performed as described above except that the ATC purge was initiated immediately after the 1-min homogenization phase. Bias caused by a passive sampling effect would be expected to have the highest impact at low sampling flow rates. The passive sampling effect was subsequently subtracted from each SASS 3100 sample before calculating the sampling efficiency. A similar passive sampling effect was not expected for the gelatine reference filters, since they were housed inside narrow-faced sampling cassettes and therefore not passively exposed to the test aerosol in the same way as the SASS 3100 filter.

2.5.2 *Bacillus thuringiensis* (BT)

Spores from the bacterial species/strain *Bacillus thuringiensis* var. *Novosibirsk* (BT spores) were plated on sporulation AK Agar #2 plates (Becton Dickinson) and incubated at 30°C for 1 week to let the bacteria grow and then sporulate. The sporulation was inspected by microscopy before spores were harvested when sporulation efficiency was >95% with a cell scraper and transferred into sterile MQ water. The BT spores were washed thrice with sterile MQ water and incubated at 80 °C for 15 minutes to inactivate remaining vegetative bacteria before quantification by plating on TSA. Spray solutions for 1 µm and 3 µm particles were made with final concentrations of 2E+07 cfu ml^{-1} BT spores/0.15 mg ml^{-1} Uranine AP and 2.8E+08 cfu ml^{-1} BT spores/0.40 mg ml^{-1} Uranine AP, respectively

Three SASS 3100 samplers (300 lpm) were used in each trial, performing three trials each with 1 μm and 3 μm particles.

2.6 PSL microspheres

The sampling efficiency of the SASS 3100 at different sampling flow rates was determined using three different sized PSL microspheres; fluoresecent 1 μm FS (ThermoFisher F8823), fluorescent 3.7 μm FS (ThermoFisher F8859), and non-fluorescent 8 μm PSL beads (Merck 78511). Spray solutions were made by mixing 50 μl 1 μm FS, 1 ml 3.7 μm FS and 2 ml 8 μm PSL beads in MQ water to a final volume of 10 ml. A Hudson medical nebulizer propelled with nitrogen gas was used to generate the test aerosol inside the ATC for 5 min. Three SASS 3100 samplers operated at a nominal sampling flow rate of 50, 100 and 250 lpm, were used in each of three trials.

2.7 Quantitative PCR (qPCR)

The two Gram-negative vegetative bacterial species/strains used in this study were *Pantoea agglomerans* (ATCC 33243) and *Escherichia coli* (MRE-162, Dstl, Porton Down, UK). Since both species/strains are stress-sensitive and not aerostable like bacterial spores, cultivation cannot be used to accurately determine the physical sampling efficiency. Sampling efficiency calculations based on cultivation was therefore only performed for aerostable bacterial spores in this study. On the other hand, DNA extraction from bacterial spores are often ineffective and variable, and which makes it difficult to use qPCR to accurately determine the physical sampling efficiency. Sampling efficiency calculations based on qPCR was therefore only performed for Gram-negative vegetative bacteria in this study.

The two Gram-negative vegetative bacterial strains were inoculated into 50 ml Nutrient Broth in glass Erlenmeyer flasks with baffles and grown in an orbital shaking incubator at 200 rpm and 30ºC for 20 hours. Both bacterial cultures were transferred to 50 ml sterile polypropylene tubes and centrifuged at 2200g for 15 minutes. The supernatants were removed before the bacterial pellets were re-suspended in 50 ml MQ water. 3 ml of the bacterial solutions were mixed with Uranine AP (final concentration 0.15 mg/ml) in MQ water to produce 50 ml spray solutions.

A Sono-Tek 120 kHz ultrasonic atomizer nozzle was used to produce the test aerosol inside the ATC for 3 minutes at 1 ml/min and 3 W nebulizer power. Three trials were performed for *Pantoea agglomerans* and for *Escherichia coli*, each time with three SASS 3100 samplers operated at a nominal sampling flow rate of 300 lpm.

A preliminary test was done to verify that Uranine AP did not interfere with the qPCR analysis after DNA isolation (data not shown).

3 Results

3.1 Recovery efficiency

A set of spiking experiments was performed to determine the recovery (filter extraction) efficiency of SASS 3100 and gelatin reference filters. The observed recovery efficiencies for BG spores and 3.7 μm FS from both filter types are shown in Table 3.1. The results showed with statistical significance (P = 0.011) that recovery efficiency was slightly better for BG spores than for FS. However this might be attributed to the use of different analytics and the associated measurement uncertainty (cultivation v. FCM).

Table 3.1 Recovery efficiencies for BG spores and 3.7 μm FS from SASS 3100 filters and gelatine filters.

	BG spores (N=3)	FS (N=3)
SASS 3100 filter	1.02 ± 0.12	0.97 ± 0.01
Gelatine filter	1.04 ± 0.04	0.94 ± 0.01

3.2 Flow rate measurements

The results from the flow rate measurement showed that the SASS 3100 samplers delivered higher sampling flow rates than the nominal rating/setting (Table 3.2) and that the deviation was proportionally higher at lower flow rates. The potential impact on the sampling efficiency calculations would be large when the SASS 3100 was operated at low sampling flow rates. Therefore the measured SASS 3100 sampling flow rates were used instead of the nominal rating when calculating the sampling efficiencies.

Table 3.2 SASS 3100 sampling flow rate measured at different nominal flow rate settings.

Nominal flow	**50 lpm**	**100 lpm**	**150 lpm**	**200 lpm**	**250 lpm**	**300 lpm**
Measured flow (lpm)	70 ± 1.1	123 ± 1.1	171 ± 3.8	220 ± 2.2	268 ± 3.8	316 ± 2.2
Deviation	+40%	+23%	+14%	+10%	+7%	+5%

3.3 Sampling efficiency

Particle statistics for the different test aerosols in this study, based on APS 3321 measurements from all ATC trials and expressed as Count Median Aerodynamic Diameter (CMAD) and Geometric Standard Deviation (GSD), are shown in Table 3.3.

Table 3.3 Test aerosol particle statistics (targeted particle size, CMAD and GSD).

Spray solution	Target size	CMAD	GSD
BG/BT spores *	1 μm	1.14 μm	1.36
BG/BT spores *	3 μm	3.02 μm	1.26

PA *	1 µm	1.13 µm	1.44
EC *	1 µm	1.16 µm	1.47
FS	1 µm	0.99 µm	1.06
FS	3.7 µm	3.64 µm	1.03
PSL beads	8 µm	7.93 µm	1.06

* and Uranine AP added as physical (fluorescent) tracer

3.3.1 *Bacillus atrophaeus* (BG) spores

Test aerosols containing BG spores, with Uranine AP as a physical fluorescent tracer, were used to determine the sampling efficiency for the SASS 3100 sampler at four different sampling flow rates (50, 100, 200 and 300 lpm) and two particle sizes (1 µm and 3 µm).

The results showed a statistical significant difference in physical sampling efficiency at flow rate 300 lpm compared to 100 lpm and 200 lpm when using fluorescence measurements of Uranine AP for analysis. No significant difference in sampling efficiency was found when using data from cultivation of BG spores. There was no statistical difference in the observed physical sampling efficiency comparing 1 µm and 3 µm particles or BG spore counting and Uranine AP fluorescence measurements.

Table 3.4 SASS 3100 sampling efficiencies at different sampling flow rates for 1 and 3 µm particles containing BG spores (BG) and Uranine AP as a fluorescent tracer (FL).

	50 lpm (N=5)	**100 lpm (N=5)**	**200 lpm (N=5)**	**300 lpm (N=5)**
BG 1 µm	0.93 ± 0.06	0.96 ± 0.14	0.95 ± 0.14	0.86 ± 0.09
BG 3 µm	0.92 ± 0.11	0.88 ± 0.08	0.94 ± 0.12	0.88 ± 0.10
FL 1 µm	0.90 ± 0.05	0.96 ± 0.08	0.93 ± 0.16	0.87 ± 0.02
FL 3 µm	0.91 ± 0.09	1.03 ± 0.07	1.00 ± 0.11	0.89 ± 0.08

The passive sampling effect caused by e.g. gravitational settling and electrostatic interation/precipiation onto the open-faced SASS 3100 electret filter was found to be low and in the range of 0.3 – 0.7% of the total sample concentration when the SASS 3100 was operated at the lowest flow rate (50 lpm). When the SASS 3100 was operated at higher sampling flow rates the passive sampling effect was even lower.

3.3.2 *Bacillus thuringiensis* (BT) spores

All tests with BT spores were performed as described for BG spores except that three trials were performed and only with a nominal sampling flow rate of 300 lpm. The results showed no statistical difference in the observed physical sampling efficiency comparing BT spores (Table 3.5) and BG spores (Table 3.4). No significant sampling efficiency was found comparing 1 µm and 3 µm BT spore particles or BT spore and Uranine AP fluorescence.

Table 3.5 SASS 3100 sampling efficiencies for 1 and 3 μm particles containing BT spores (BT) and Uranine AP as a fluorescent tracer (FL).

	300 lpm (N=3)
BT 1 μm	0.84 ± 0.08
BT 3 μm	0.88 ± 0.07
FL 1 μm	0.87 ± 0.01
FL 3 μm	0.91 ± 0.11

3.3.3 Microspheres

Test aerosols containing microshperes were used to determine the sampling efficiency for the SASS 3100 sampler at three different sampling flow rates (50, 100, 250 lpm) and three particle sizes (fluorescent 1 μm FS, fluorescent 3.7 μm FS and non-fluorescent 8 μm PSL beads).

The results showed a statistical significant decrease in the sampling efficiencyiy for 3.7 μm FS when the sampling flow rate increased to 250 lpm (Table 3.6) and also a significant lower sampling efficiency for 3.7 μm FS particles than for 1 μm FS particles at 250 lpm. A significant increase in sampling efficiency was observed for 8 μm PSL compared to 1 μm FS at flow rate 50 lpm. No other significant differences were observed based on particle size or sampling flow rates.

Table 3.6 SASS 3100 sampling efficiencies at different flow rates and particle sizes for FS and PSL beads.

Size (μm)	**50 lpm (N=3)**	**100 lpm (N=3)**	**250 lpm (N=3)**
1 μm FS	0.86 ± 0.07	0.90 ± 0.04	0.95 ± 0.06
3.7 μm FS	0.97 ± 0.09	0.99 ± 0.08	0.78 ± 0.02
8 μm PSL	1.09 ± 0.04	1.09 ± 0.11	0.97 ± 0.10

3.3.4 Quantitative PCR (qPCR)

Test aerosols containing *Pantoea agglomerans* (ATCC 33243, PA) and *Escherichia coli* (MRE-162, EC), with Uranine AP as a physical fluorescent tracer, were used to determine the sampling efficiency for the SASS 3100 sampler, both in terms of the biological sampling efficiency based on cultivation and the physical sampling efficiency based on qPCR. All experiments with PA and EC were done at a nominal SASS 3100 sampling flow rate of 300 lpm. Six trials were performed for each organism and only for 1 μm test aerosols.

The results showed that the biological sampling efficiency (retained viability/culturability) of the SASS 3100 based on PA and EC cultivation was very low (~5-6%) relative to the gelatine reference filters (Table 3.7). The results also showed that the physical sampling efficiency based on fluorescence measurements of Uranine AP (Table 3.7) was highly similar to the physical sampling efficiency observed when Uranine AP was used together with BG and BT spores

(Table 3.4-3.5). The results also indicated that the physical sampling efficiency estimates for PA and EC based on sample analysis using qPCR was ~6% higher than the estimates based on fluorescence measurements of Uranine AP in the same samples. These results were however not statistically significant ($p>0.05$), and the possibility that the observed difference could be due to random sampling and measurement variability cannot be excluded.

Table 3.7 SASS 3100 sampling efficiencies for 1 μm particles containing PA or EC and Uranine AP as a fluorescent tracer, based on three different downstream analysis methods; cultivation for PA or EC (biological sampling efficiency), fluorescence measurement for Uranine AP (physical sampling efficiency) and qPCR for PA or EC (physical sampling efficiency).

	PA (N=6)	**EC (N=6)**
Cultivation	0.05 ± 0.01	0.06 ± 0.01
Fluorescence	0.85 ± 0.02	0.85 ± 0.02
qPCR	0.90 ± 0.06	0.92 ± 0.11

4 Discussion and conclusions

A comprehensive T&E campaign has been realized in this study in an effort to determine and better understand the end-to-end sampling efficiency of the SASS 3100 high-volume electret filter air sampler.

Aerosol T&E were performed using a diverse range of test aerosols covering different particle sizes (1, 3, 3.7 and 8 μm) and compositions (Gram-positive bacterial BG and BT spores, Gram-negative vegetative bacterial PA and EC cells, Uranine AP as a fluorescent tracer, and fluorescent FS and non-fluorescent PSL microsheres). The SASS 3100 sampler was also tested at different nominal sampling flow rate ratings/settings (50 lpm, 100 lpm, 200 lpm, 250 lpm and 300 lpm) in order to examine how this affected the observed sampling efficiency.

Taken together, the T&E results showed recovery (filter extraction) efficiencies of ~100% and end-to-end physical sampling efficiencies in the range of 78% – 109% for the SASS 3100. The overall conclusion was that the SASS 3100 air sampler was capable of delivering a very high end-to-end sampling efficiency, and that the sampling efficiency could be expected to be relatively consistent (stable/universal) and largely independent of variables such as sampling flow rate, particle size and particle composition.

In light of these findings, it can at least in general terms be reasonably concluded that an end user/operator of a SASS 3100-based air sampling capability can expect a consistent and high

end-to-end physical sampling performance for most or all sizes and compositions of airborne particulate matter, as a minimum in the 1-10 μm size range.

Results indicated that particle recovery efficiency from the electret filters were slightly better for BG spores than for 3.7 μm FS. However this difference could be a result of different downstream analysis methods used for BG spores and FS (cultivation and FCM, respectively).

The physical sampling efficiency measured with BG spores and Uranine AP was reduced by approximately 2 – 7% when increasing nominal flow rate from 50 lpm to 300 lpm (measured flow rates 70 lpm to 316 lpm), which indicates that the collection principle is efficient throughout the entire flow rate range. An explanation for the small decrease in physical sampling efficiency at 300 lpm could be the increased particle velocity when passing the electret filter, which may require larger electrostatic force to capture the particles. The significantly reduced physical sampling efficiency for 3 μm Uranine AP particles at 300 lpm may in part also be explained by the increased particle velocity when sampling at 300 lpm.

Tests performed using FS/PSL microspheres and FCM for sample analysis in general displayed more variable sampling efficiencies, some of them with statistical significance, than results obtained using bacterial spores, fluorescence measurements and quantitative PCR. Due to several factors (large particles are more expensive, harder to aerosolize, settle faster) the aerosolized concentration of 8 um PSL particles were much lower than 1 μm and 3.7 μm FS particles. Together with the significant lower flow rate (10 lpm) for the gelatine filters (compared to SASS 3100) the collected samples contained significant lower concentration of the 8 μm PSL particles. This might have resulted in larger measurement uncertainty on the gelatine reference samples and some bias. Somewhat increased viscosity of PBSTA buffer after dissolving the gelatine filters may also have affected the FCM measurements. Although these samples were diluted before FCM measurement in order to avoid measurement bias the viscosity of SASS 3100 samples were not increased to match the gelatine reference samples. Notice also that the low number of test experiments (N=3) would contribute to more uncertainty of the results. Despite of this the overall conclusion remains that the SASS 3100 sampling efficiency is high and consistent in the whole flow rate range and at all particle sizes tested.

Although air sampling for cultivation-based downstream analysis was outside the scope of this test effort, the results obtained for Gram-negative bacteria in combination with culture analysis is consistent with our previous results (Dybwad 2014) and confirm that the SASS 3100 is not well suited for air sampling of stress-sensitive Gram-negative bacteria when microbiological culture analysis is the intended downstream assay technique.

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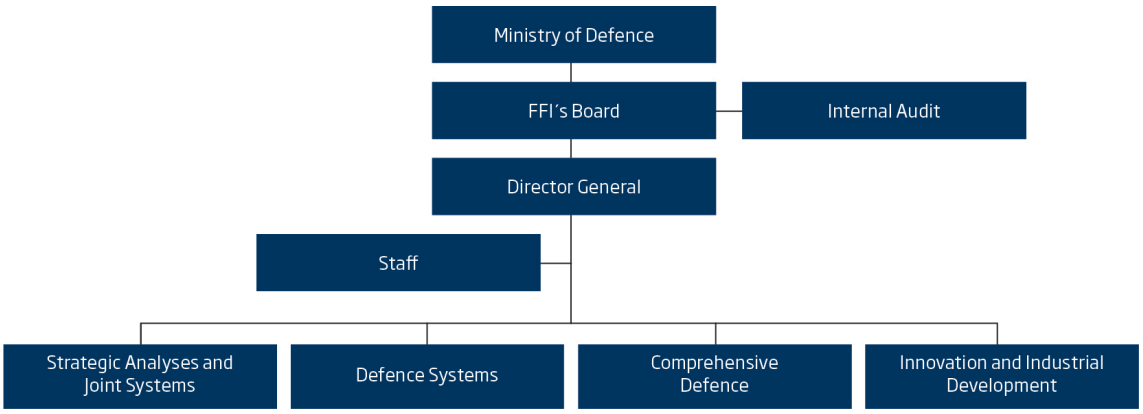

Forsvarets forskningsinstitutt
Postboks 25
2027 Kjeller

Besøksadresse:
Instituttveien 20
2007 Kjeller

Telefon: 63 80 70 00
Telefaks: 63 80 71 15
Epost: ffi@ffi.no

Norwegian Defence Research Establishment (FFI)
P.O. Box 25
NO-2027 Kjeller

Office address:
Instituttveien 20
N-2007 Kjeller

Telephone: +47 63 80 70 00
Telefax: +47 63 80 71 15
Email: ffi@ffi.no

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