Validation of the air collector SASS 2000^{PLUS} in a bioaerosol test (BAT) chamber at TNO

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English summary

This report describes a preliminary evaluation of the air collector SASS 2000^{PLUS} in a BioAerosol Test (BAT) chamber using spores of *Bacillus globigii* (BG) and vegetative *Erwinia herbicola* (EH) cells. The presented work was a collaboration between FFI and TNO Defence, Security and Safety in the Netherlands, and involved a two-week stay of FFI staff at TNO. The main purpose of FFI's visit to TNO was to evaluate the air collector SASS 2000^{PLUS} in the BAT chamber at TNO. During the visit FFI worked with TNO's experts on bioaerosols and FFI gained valuable experience with bioaerosol dissemination experiments including generation and dissemination of bioaerosols, performance of the chamber, and collection of bioaerosols using the SASS 2000^{PLUS} air collector along with the slit-to-agar samplers as reference equipment. TNO was able, for the first time, to connect and test a wetted-wall cyclone liquid air collector, such as the SASS 2000^{PLUS}, to the BAT chamber.

Sammendrag

Rapporten beskriver et samarbeidsprosjekt mellom FFI og TNO Defence, Security and Safety i Nederland. Samarbeidsprosjektet hadde som formål å teste luftprøvetakeren SASS2000^{PLUS} i et bioaerosolkammer ved TNO med *Bacillus globigii* (BG) sporer og *Erwinia herbicola* (EH) celler. Representanter fra FFI arbeidet sammen eksperter fra TNO og FFI fikk unik kunnskap innenfor ulike områder som, hvordan lage en bioaerosol, bruk og vedlikehold av et bioaerosolkammer, prøvetaking av luft ved hjelp av SASS2000^{PLUS} og hvordan oppnå referanseprøver på agarskåler (”slit-samplers”). TNO hadde ikke tidligere utført tilsvarende forsøk. De anså forsøket som et pionerarbeid og samarbeidet som meget nyttig for å oppnå egenkunnskap.

Contents

Preface

Abbreviations

ACPLA	Agent Containing Particles per Liter of Air
BAT	BioAerosol Test
BG	*Bacillus globigii*
cfu	colony forming units
EH	*Erwinia herbicola*
HEPA	High Efficiency Particulate Air (filter)
PCR	Polymerase Chain Reaction
STA	Slit-to-agar (sampler)
TNO	Toegepast Natuurwetenschappelijk Onderzoek (Applied Scientific Research)

1 Introduction

1.1 Air sampling of bioaerosols

Bioaerosols are aerosols of biological origin, and may contain viruses, bacteria, fungi, spores of bacteria or fungi, pollen and other particles from living organisms. Aerosols containing biotoxins (e.g. from a culture supernatant) are also mostly considered to be a bioaerosol. Bioaerosols are naturally found both indoors and outdoors. Pathogenic microorganisms associated with an airborne route of infection are of considerable concern for public health (Stetzenbach et al., 2004). Such microorganisms might be exemplified by *Mycobacterium tuberculosis, Legionella pneumophila*, airborne viruses such as SARS and influenza A, and fungal spores (Blatny et al., 2007 a, b; Booth et al., 2006; Tellier, 2006). Pathogenic microorganisms may also be deliberately dispersed as bioaerosols (Atlas, 2002; Levine and Amorim, 2003) and examples of such agents are *Bacillus anthracis*, *Francisella tularensis*, *Yersinia pestis*, *Brucella* spp. and *Coxiella burnetii* (Klietmann et al., 2001; Atlas, 2002)). A potential air transmission of *C. burnetii* is indicated since airborne *C. burnetii* was detected using PCR in air samples collected near a sheep herd infected with *C. burnetii* (Schulz et al., 2005).

Detection and identification of microorganisms in air require efficient methods for sampling and analysis. Microbiological methods have been widely used for analysis of viable bioaerosols (for review see Stetzenbach et al., 2004). However, bioaerosol sampling studies have previously shown that less than 0.1 % of cells collected were culturable (Radosevich et al., 2002). Real-time PCR has been used to detect and characterize bacterial species in environmental samples (Alvarez et al., 1995; Kuske, 2006; Kuske et al., 2006). Several bioterror agents in air samples containing a variety of commonly found airborne bacteria and contaminants may be easily detected by real-time PCR (Fykse et al., 2008). In these studies, no time-consuming DNA extraction was performed and the analysis was completed within one hour. Rapid sampling using highly efficient sampling methods allowing detection of potential pathogens is of crucial importance for initiating respiratory and clothing protection and medical treatment for both civilian and military personnel. Subsequently, the samples can be further analyzed by more time-consuming methods for verification of live agents.

Some of the available techniques for air sampling may have an impact on the viability of microorganisms harvested. A decrease in viability may be caused by excessive stress on sensitive microorganisms during sampling. The different aerosol collectors need to be evaluated in each case. The viability of microorganisms might also be affected during aerosolization. Some microorganisms might survive in an aerosol, whereas others may not. Spores and Gram-positive bacteria are generally more robust than Gram-negative bacteria and might survive in a harsh environment as well as tolerate more stress. Gram-positive spore forming bacteria are exemplified by the *Bacillus* spp. group (Helgason et al., 2000; Olsen et al., 2007). *Bacillus* species are commonly found in air (Harrison et al., 2005; Griffin et al., 2006; Fykse et al., 2008).

Different methods have been used in order to sample aerosols and to detect bacteria and viruses in air. A wide variety of air collection devices can be used to collect aerosol samples (Jensen et al., 1992; Juozaitis et al., 1994; Stezenbach et al., 2004; Blatny et al., 2006). Impaction is a frequently used method for collecting particles containing bacterial cells on the surface of petri dishes containing growth medium or collection on filters. However, such a collection requires the presence of viable cells. Sampling of microorganisms might also be performed by impingement. Impingement involves collecting the microorganisms in liquid and the use of different analytical methods for identification (including culture, microscopy, immunoassay, flow cytometry and molecular methods). A third method is virtual impaction, in which the impaction plate is replaced by a collection probe. Particles larger and smaller than the cutoff size are separated from the collected particles. In a virtual impactor the particles can be sampled on a filter or in a liquid (Hinds, 1999). A variety of different collectors have been described that differ in collection efficiency and the survival rate of e.g. *Legionella* spp. (Blatny et al., 2007a). Commonly used impingement samplers, e.g. the SKC Biosampler®, are operated at an air flow of 10-12 l/min. High velocity samplers have also been developed for sampling larger volumes of air over extended sampling time. Examples of such collectors are the SpinCon® (Sceptor Industries, USA), the wetted-wall cyclone SASS 2000^{PLUS} (Research International, USA) and the virtual impactor XMX-2CV (Dycor Technologies, Canada). Sampling time may have an impact on the viability of the bacterial cells collected. It is therefore necessary to perform extensive testing of the air collectors of interest. Today's challenges in detecting aerosolized bacteria include the need for rapid detection, to enable rapid health response. For detection of bioaerosols, a highly efficient collection device such as the SASS 2000^{PLUS} combined with rapid analysis methods such as real-time PCR might be an efficient approach.

1.2 Bioaerosol test chamber (BAT chamber)

A bioaerosol test chamber (BAT chamber) can be used for testing and evaluation of biodetectors and air collectors. The BAT chamber may also be used for the study of bioaerosol behaviour, agent fate, re-aerosolization and decontamination studies. The BAT chamber at TNO (Figure 1.1) is a stainless steel chamber of 12 m^3 designed and installed at TNO by Dycor Technologies (Canada). The actual chamber is 2 m wide, 3 m long, and 2 m high, and is placed upon a steel frame two meters above floor height, enabling easy access to the connection ports on the underside of the chamber. A similar aerosol chamber will be installed at FFI in 2008.

Some properties of the BAT chamber are summarized in Table 1.1. Details are explained in the text below.

Figure 1.1 Bioaerosol test (BAT) chamber at TNO.

Table 1.1 Main features of the BAT chamber.

Feature	Description
Material	14-gauge stainless steel
Size	3 m long, 2 m wide, 2 m high
Volume	12 m^3
Maximum airflow	11750 l/min
HEPA filters	2 (inflow and outflow) with pre-filters
Access	1 door (70 × 70 cm) with window and 2 gloves; 1 extra window and glove; 1 airlock; 7 connection-ports
Maximum number disseminators	2, independently controlled
Bioaerosol concentration range	Minimum 10 - 100 ACPLA
Reference equipment	Grimm 1.108 particle sizer; New Brunswick Scientific STA-203 sampler (2 pieces)
Interface	PC with dedicated chamber software
Sensors	Relative humidity (precision +/- 5%); Temperature (precision +/- 2 ˚C); Air pressure
Air pressure	50 - 150 Pa negative in chamber

1.2.1 Airflow

The volume of the chamber is 12 m^3 and the airflow in the chamber is adjustable, with a maximum of 705 m^3/hour (11750 l/min), resulting in a refreshment of the total air volume approximately once every min (at maximum airflow). Working conditions usually are ~30 %, which corresponds to 210 m^3/h (3.5 m^3/min) and a refreshing time of the chamber of about once in every 3.5 minutes. Both incoming and out-flowing air are filtered through a High Efficiency Particulate Air (HEPA) filter, excluding contamination of the chamber with particles from outside, and preventing escape of experimental microorganisms to the outside.

The chamber is limited in its regulation of temperature and humidity. Most tests are performed at an inside working temperature of 20 °C that should be achievable year round in a Dutch climate. At average environmental temperatures, chamber temperatures between 15 and 30 °C should be achievable. However, this has not been tested, and will probably not be possible under extreme outdoor weather conditions. The relative humidity in the chamber can be regulated upwards to 90%, but not downwards compared to the environmental value. The exhaust fan provides a negative air pressure inside the chamber of 50-150 Pa. Two small fans in opposite corners inside the chamber ensure homogeneous mixture of the aerosol during the experiments.

1.2.2 Reference equipment

A particle sizer model 1.108 Grimm Technologies is connected to the chamber through one of seven connection ports. The Grimm 1.108 measures particle size distribution from 0.3 µm to 20 µm, in 15 channels. It collects particles at a flow rate of 1.2 l/min from the BAT chamber and measures particles every 6 seconds (120 ml). All data from the Grimm are stored in a log file. It is possible to use the Grimm particle sizer in a feedback loop, to keep a stable aerosol concentration. The disseminator is automatically switched on and off to maintain the pre-determined aerosol concentration in the chamber. Alternatively, the disseminator can be manually operated, or even continuously. The chamber has a PID (proportional, integrated, derivative) controlling system for the concentration. The principle of Grimm is laser scattering counting the amount and proportions of particles. In the lower ACPLA range (as used in this study), the Grimm particle sizer might not be sensitive (or reliable) enough to estimate/determine the ACPLA level. Therefore, *Bacillus globigii* spores (BG spores) usually are mixed with silica or siloid (SiO_2).

Two slit-to-agar (STA) samplers (New Brunswick Scientific) with culture-plates are used to measure the concentration of viable particles in the aerosol. These are connected through sample ports underneath the chamber. Airborne particles are impacted on the agar plate in the slit samplers. After sampling, the plates are incubated at 37 °C, overnight. The number of grown colonies can be used to calculate the aerosol concentration in ACPLA values. The STA samplers are switched on manually, at operator chosen time points.

1.2.3 Operation

The BAT chamber is operated through a dedicated software called ATC (for Aerosol Test Chamber), based on Labview. It enables the operator to click on several virtual buttons for various actions, and allows the user to view all conditions and values.

On one side of the chamber, an access door with a window may be used to enter the chamber, and airtight gloves fitted in the door can be used to manipulate items in the chamber while the door is closed. There is also a small airlock close to the access door.

The properties of the BAT chamber, the operational use, and development of standard procedures for the biosimulants have been described extensively in a TNO report (Broekhuijsen, 2007).

1.3 Aim of the present study

The present work was collaboration between FFI and TNO Defence, Security and Safety, located in Rijswijk, The Netherlands. During the period January 15-26, 2007 Dr Else Marie Fykse (one week) and Gunnar Skogan (two weeks) from FFI stayed at TNO and visited the group of Dr Martien Broekhuijsen. The collaboration with TNO is valuable for FFI since aerosol studies is an emerging and important area of work at both institutes. A similar chamber will be installed at FFI in June 2008.

The aim of the present study was to validate the SASS 2000^{PLUS} air collector in the BAT chamber. The capacity and the collection efficiency of the sampler were evaluated. For TNO, the main goal was to use the BAT chamber for evaluating an air sampler. This was the first time that an external party brought their own device for testing; commonly leading to unexpected circumstances, such as connection to the chamber, exhaust problems, detection sensitivity issues, and more.

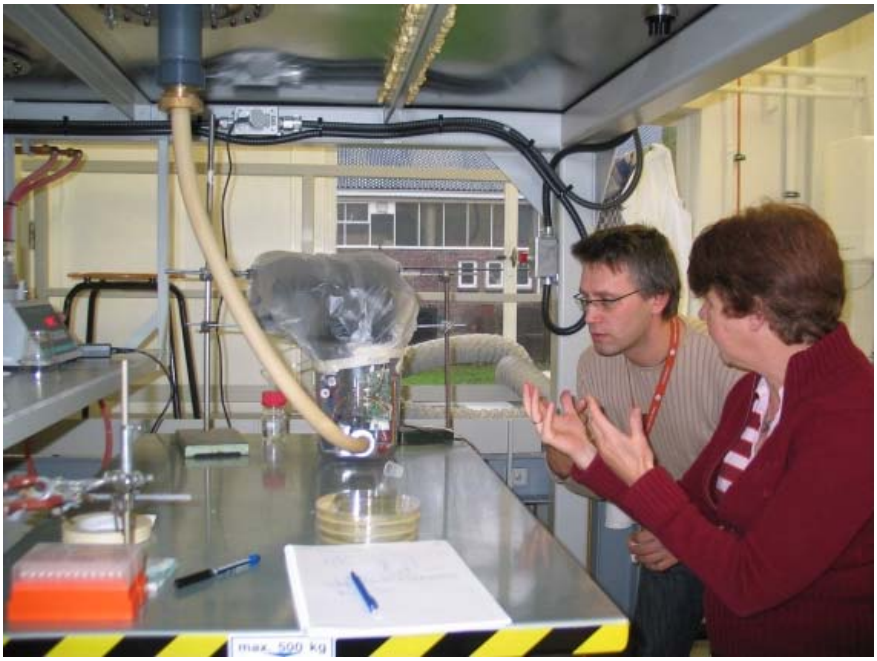


Figure 1.2 Gunnar Skogan (FFI) and Dory van de Meent (TNO) in detailed discussions regarding the connection of the SASS 2000^{PLUS} to the BAT chamber.

2 Materials and Methods

2.1 Operation of the BAT chamber

A controlled concentration of bioaerosols was generated by a Hudson nebulizer inside the BAT chamber. Silica particles or NaCl were present in a constant concentration relative to the bioparticles. Temperature, humidity, and negative pressure inside the chamber were continuously and automatically monitored during the experiments and stored in log files.

Two STA samplers (Figure 2.1) were used for reference samples to indicate the agent containing particles per liter of air (ACPLA) of the sampled cloud. The Grimm detector was used to monitor the number of NaCl or SiO_2 particles as described to keep a constant aerosol concentration.

A disseminator (nebulizer) was filled with a suspension and placed inside the chamber, if necessary through the airlock and using the airtight gloves. Typical wet disseminators (e.g. the Hudson disposable nebulizer) are connected with a tube to a nitrogen gas supply, and the dissemination was regulated by the Grimm particle sizer feedback loop through switching the gas flow on or off.

Figure 2.1 Connection of the slit-to-agar STA-samplers to the BAT chamber.

2.1.1 Standard procedure

A Hudson Up-draft® nebulizer was used for dispersion of BG spores. A mixed suspension of BG spores and SiO_2 was made in ultrapure water. The number of SiO_2 particles was monitored using a Grimm particle counter as described. The BG suspension was made from a stock solution of BG spores (source "Dugway", TNO-code BM341, $1.5x10^8$ spores/mg). A nebulizer-suspension of BG spores of 0.1 mg/ml is suitable for generating an aerosol concentration of 80-100 ACPLA. A lower concentration of BG spores (e.g. 0.01 mg/ml) can be used for generating an aerosol of 10-20 ACPLA. Nebulizer-suspensions of BG always contained SiO_2 in a concentration of 4 mg/ml. The aerodynamical size distribution of the SiO_2 particles is 0.5 – 10 µm (80 % 1-5 µm).

A Collison 3-jet nebulizer was used for dispersion of *Erwinia herbicola* strain ATCC 33243, also referred to as *Pantoea agglomerans*. An *E. herbicola* (later referred to as EH) aerosol was made from EH cells and 0.9 % NaCl (1:5). The EH suspension was made from an overnight culture in Brain Heart Infusion culture medium (20 ml). Of this culture, 15 ml was centrifuged and the supernatant was added to the remaining 5 ml of the culture. To this suspension (20 ml), 80 ml of 0.9 % NaCl was added and this final suspension was applied to the nebulizer.

Reference air samples were taken by the STA-samplers, using 30 l/min air intake and 2 min rotation time. Samples were collected several times during the experiments. Before switching on the nebulizer, a sample was taken to monitor the background in the chamber to confirm that no bio particles were present. The loss of cell viability in the nebulizer was tested by plate counting of the suspension added to the nebulizer prior to and after aerosolization. The slit sampler plates

were incubated aerobically at 37°C overnight and the numbers of colony forming units (CFU) were determined. The number of ACPLA was calculated by dividing the CFU number by 60 liter of air.

Taking into account the design (specifications) of the nebulizers and the expected size of particles containing BG spores and EH cells, it is assumed that most agent containing particles consist of one spore or cell per particle.

2.2 SASS 2000^{PLUS} (Smart Air Sampler System)

2.2.1 The SASS 2000^{PLUS} and sampling procedures

The SASS 2000^{PLUS} (Research International, USA) is a highly efficient, multiple-effect, wetted-wall cyclone collector that extracts and transfers pathogens from sampled air to a liquid volume.

According to the manufacturer, the air collection rate is 265 – 375 liter of air per min (LPM) (used at 350 LPM in the present experiments), and the collection range of particles is 1-10 µm. However, the collection efficiency is low for particles of 1 µm in size or below (http://www.resrchintl.com/SASS2300-air-sampler.html). The SASS 2000^{PLUS} has previously been used for sampling *Legionella* spp. (Blatny et al., 2007a, b), for bacterial diversity studies at FFI (personal communication) and sampling the release of biological simulants in a field trial in Sweden (FOI) in 2006.

The SASS 2000^{PLUS} was connected through a sample port underneath the chamber (Figure 2.2). During sampling, air was collected in 5 ml of distilled water. In the SASS 2000^{PLUS} evaporating water was replenished continuously. In one experiment with EH 0.9 % NaCl was used instead of water.

In general, SASS 2000^{PLUS} was washed after use with chlorine solution (10 fold-dilution of a 5 % chlorine solution) and sterile distilled water as the final wash[*]. The cleaning process was performed after removal of the fan of SASS 2000^{PLUS} and with a circulation of the distilled water (i.e. turning on the SASS 2000^{PLUS}). The washing buffer was analyzed for the presence of BG or EH by plating on Luria-Bertoni broth (LB) or BHI agar, respectively. No growth of BG or EH was observed after washing. When the air collector was used repeatedly during the same day, cleaning between each sampling was performed three times by flushing the cyclone from the top with water and discarding the buffer through the sample port.

[*] Unpublished results by FFI have shown that the washing procedure described here is sufficient for washing SASS 2000^{PLUS} after sampling of BG spores.

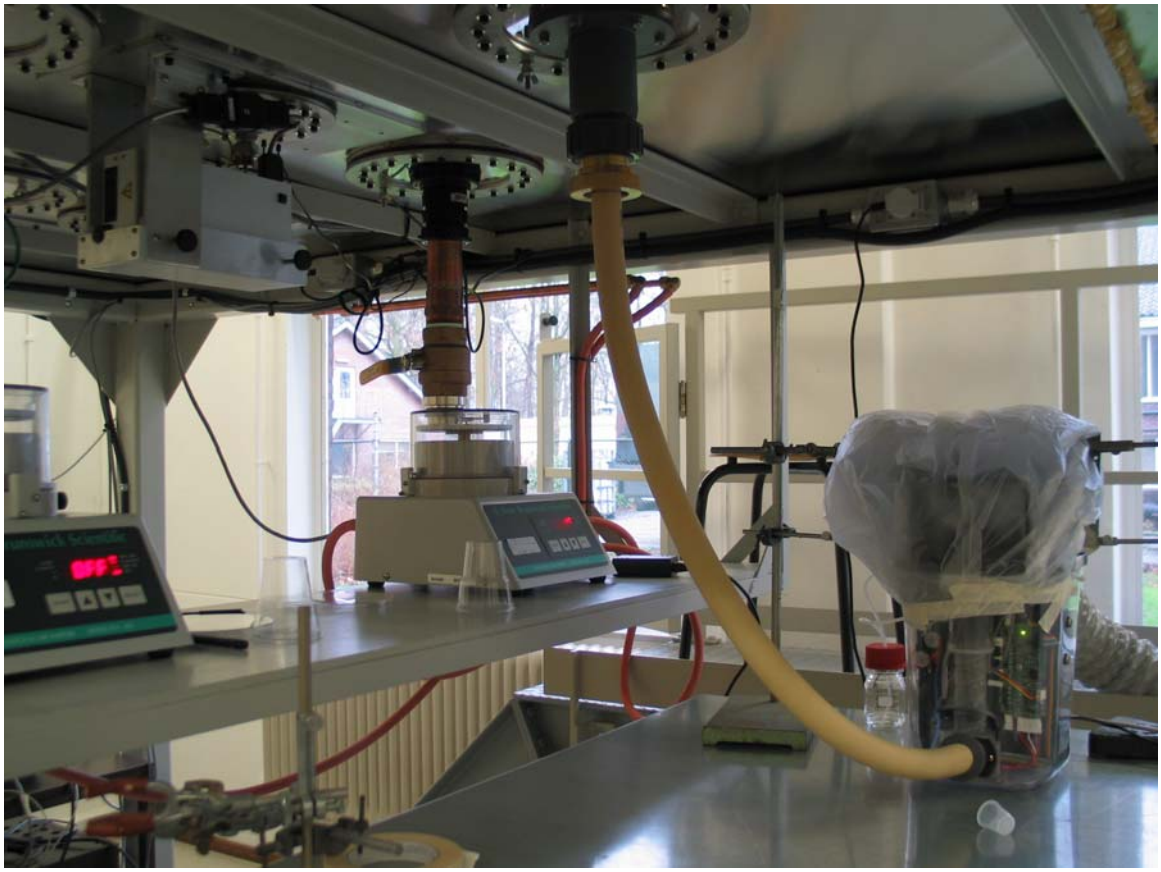


Figure 2.2 Connection of the SASS 2000 PLUS air sampler to the BAT chamber.

2.2.2 Analysis of air samples

The number of CFU/ml of the SASS 2000 PLUS air samples were determined by spread plating of serial dilutions of the samples. The BG air samples were plated onto LB agar (Fluka), and the EH air samples were plated onto BHI agar (Sigma). Plates were incubated aerobically at 37°C overnight and the numbers of CFU were determined.

Real-time PCR was performed with a LightCycler® (Roche Diagnostics) using the Light Cycler® Faststart DNA Master PLUS SYBR-Green I kit (Roche Applied Science) as described in Fykse et al., (2008). Briefly, the PCR reaction mixture contained $MgCl_2$ (5 mM), dNTPs, Taq DNA polymerase, reaction buffer, primers (1 µM [each]), and template (2 µl of the SASS 2000 PLUS sample). Distilled water was added to a final volume of 20 µl. The following amplification program was used: 95 ºC for 10 min, 45 cycles of 95 ºC for 0 sec, 58 ºC for 10 s and 72 ºC for 15 s. The primers for EH were agc tgg taa ttg aag att ccg t **(**11031F078; forward) and ggt ctg ttg cag cag atc gta **(**11031R078; reverse). The amplified product (296 bp) was evaluated by the relative fluorescence levels and a specific amplification was indicated by the Threshold-cycle (C_T). The C_T value representing the cycle number at which the fluorescence intensity crossing a fixed threshold that is ten times the standard deviation of the baseline intensity, and it is inversely proportional to the concentration of the initial DNA template. Distilled water was used as a negative control in all real-time PCR runs.

2.3 Performance testing of SASS 2000^{PLUS}

The capacity of the SASS 2000^{PLUS} was investigated in the BAT chamber. The collector was connected to the sample port through a tube as shown (Figure 2.2). The outlet air from the SASS 2000^{PLUS} was captured to avoid contamination in the room. In some experiments, SASS 2000^{PLUS} was also used for collection inside the chamber. The sampling efficiency (according to the manufacturer) was expected to be low due to small aerodynamically particle size (0.8 µm). Therefore, it was decided to collect samples during different times (Table 3.1), to evaluate the required air volume to detect spores and cells in the SASS 2000^{PLUS} samples. It was also investigated if a possible saturation point of sampling was obtained, i.e. a point where the re-aerosolization rate was equal to the collection rate.

The re-aerosolization and microbial survival were determined by spiking EH cells ($1x10^8$ cells/ml) and BG spores ($5x10^2$ CFU/ml; $1x10^3$ CFU/ml; $1x10^4$ CFU/ml ; $1x10^8$ CFU/ml) into the SASS 2000^{PLUS}, and run for different times.

Table 3.1 The SASS 2000 ^{PLUS} collection time for the BG and EH experiments.

Sampling time (350 Liter/min)	Liter of air sampled	Cubic meter of air sampled
5 min	1750	1.75
10 min	3500	3.5
15 min	5250	5.25
20 min	7000	7
40 min	14000	14
60 min	21000	21
90 min	31500	31.5
120 min	42000	42

3 Results

3.1 Performance testing of the SASS 2000^{PLUS} using BG spores and *EH* cells

The performance of the SASS 2000^{PLUS} was investigated in a BAT chamber using aerosols of BG spores and EH vegetative cells. The SASS 2000^{PLUS} was connected through rubber tubing to the chamber from the outside (Figure 2.2). It was investigated if the connection tube reduced the air collection rate of the SASS 2000^{PLUS}, but no reduction was observed. A maximum air collection rate of 350 l/min was used in all experiments since the efficiency of the SASS 2000^{PLUS} sampling was expected to be low due to the small aerodynamically particle size of the BG spores (according to the manufacturer, http://www.resrchintl.com/SASS2300-air-sampler.html). BG spores and EH cells were made from stock solutions as described and when a stable aerosol concentration was obtained in the BAT chamber (usually within about 20-30 min) the SASS 2000^{PLUS} was started. Samples were collected at different times (Table 3.1) and the last sample

was collected after two hours which corresponds to 42 m^3 of air collected. The SASS 2000^{PLUS} samples were plated on LB or BHI agar and CFU counted as described.

Figure 3.1 shows the results of two different BG experiments. No saturation of the sampled bioaerosols was observed when using the SASS 2000^{PLUS} air collector. Reference samples were collected by the slit samplers as described in the material and methods and the number of ACPLA in the chamber varied between 10 and 15 ACPLA during the experiments (Table 3.2). The number of ACPLA collected by the SASS 2000^{PLUS} collector was calculated from the CFU numbers (assuming one CFU = one ACPLA). The primary data is shown in appendix A. Figure 3.2 shows a typical slit sampler plate with 30 sections and representative SASS 2000^{PLUS} agar plates from the BG experiment described. The number of CFU in the nebulizer solution was calculated before and after the aerosolization experiments, and no loss of viability of the BG spores (inside the nebulizer) during aerosolization was observed.

Similar experiments using EH vegetative cells were performed. The results of a single EH experiment is shown in figure 3.3 A. However, in the first experiment the SASS 2000^{PLUS} samples were harvested in 0.9 % NaCl. Due to evaporation of water during sampling and refilling of 0.9 % NaCl, the salt concentration increased in the collection vial. This resulted in decreased viability of the cells and CFU counting was not possible. The EH experiment was repeated using water in the SASS 2000^{PLUS} and this experiment is shown in Figure 3.3 A. Only two slit sampler tests were performed that day due to technical problems (January 24) measuring 9 and 20 ACPLA (Table 3.2). The EH air samples were also analyzed using real-time PCR. Real-time PCR was run directly on the samples without further sample preparation (Figure 3.3 B). The plate counting and real-time PCR results of EH are equivalent and show that no saturation of the sampling is obtained.

BG and EH were sampled for two hours and samples were collected several times during that time period (Table 3.1). The re-aerosolization rate never exceeded the sampling rate, which means that no saturation was obtained.

Table 3.2 ACPLA values determined by plate counting using two slit samplers.

Time		Reference[†]	11:40	12:30	13:40	15:44	16:37			
16. Jan	BG	**0**	**27**	**17**	**18**	**22**	**18**			
Time		Reference	11:47	12.01	12.27	13:47	15:17			
17. Jan	BG	**0**	**10**	**15**	**12**	**13**	**10**			
Time		Reference	11:03	14:49						
18. Jan	BG	**0**	**15**							
Time		Reference	11:23	12:22	13:20	14:20				
22. Jan	BG	**0**	**16**	**16**	**16**	**16**				
Time		Reference	9:56	10:21	10:51	11:20	11:51	13:48	14:12	14:39
23. Jan	EH	**0**	**16**	**9**	**10**	**8**	**10**	**6**	**8**	**11**
Time		Reference	10:16	12:14						
24. Jan	EH	**0**	**20**	**9**						

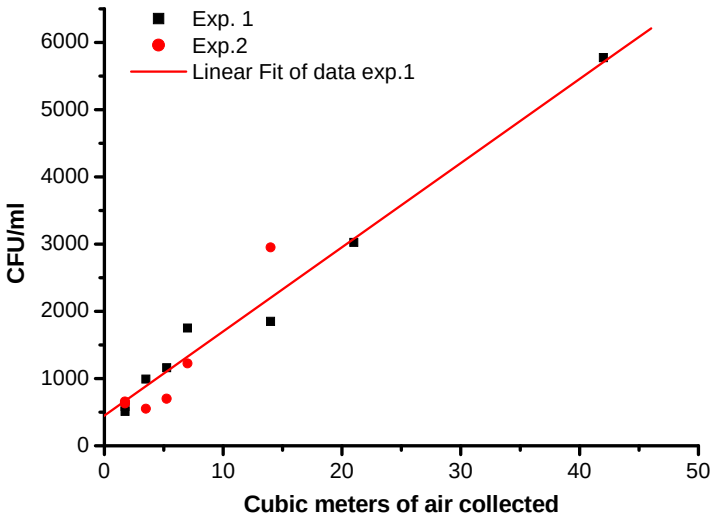


Figure 3.1 Determination of the number of BG spores (CFU) collected according to air volume collected. Time of collection: 5-120 min. Air volume collected: 1 750 - 42 000 liters.

[†] A reference sample was taken before and after finishing of the experiments.

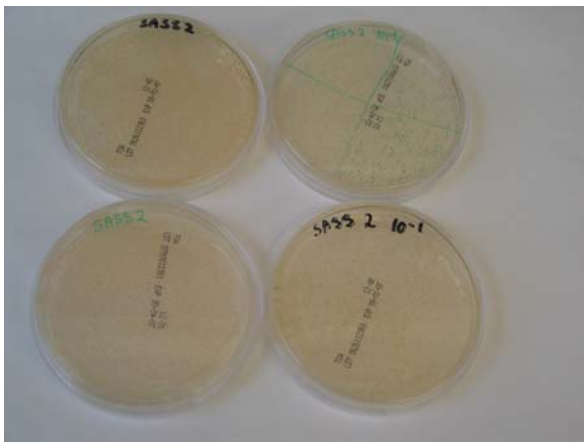
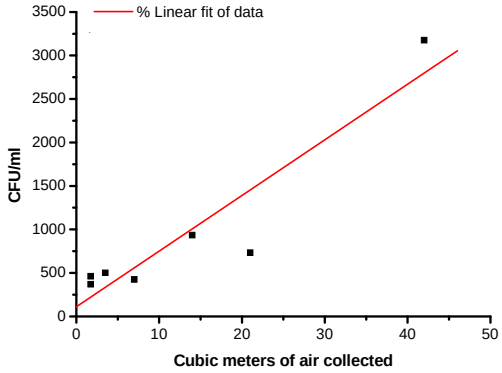
A  B 

Figure 3.2 A: An example of a slit sampler plate showing the 30 sections on the plate, each four seconds sampling. B: Representative plates with the BG SASS 2000^{PLUS} samples.

A 

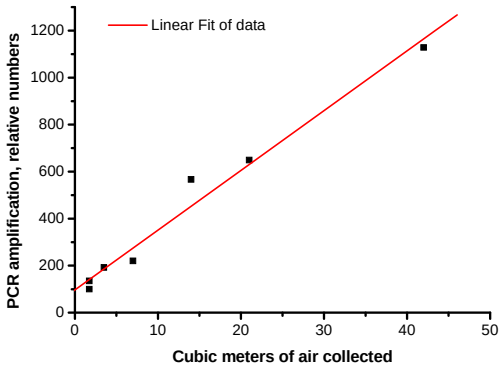
B 

Figure 3.3 Determination of the number of EH cells collected according to air volume collected. Time of collection: 5-120 min. Air volume collected: 1 750 - 42 000 liters. A: Bacterial CFU/cubic meters of air. B: PCR amplification/cubic meters of air.

3.2 Estimation of collection efficiency of the SASS 2000^{PLUS}

Preliminary experiments were done to estimate the collection efficiency of the SASS 2000^{PLUS} for BG spores. Estimation of the collection efficiency is based on CFU values and 100 % survival of the collected BG spores (a microbial growth rate of approximately one). Equation number one is used for estimation of the collection efficiency (Juozaitis et al., 1994).

Equation (1): $Ec^* = \frac{C_{up} - C_{down}}{C_{up}}$

E^*: Collection efficiency based on CFU values.

C_{up}: Upstream aerosol concentration, NTotal (number of CFU in the chamber)

C_{down}: Downstream aerosol concentration; NTotal – NCFU (SASS2000)

E*=1- 626 259 CFU = 0.25x100 % =25 %
840 000 CFU

Air flow: 350 l/min (LPM)
Collection: 120 min
NCFU in 5 ml SASS2000 sample: 213 750 CFU
NTotal: 20 ACPLA[1] x 350 LPM x 120 min= 840000 CFU/l
Cup = 840 000 CFU
Cdown = NTotal – NCFU = 840 000-213 750=626 250 CFU

[1]Average ACPLA value during four hours aerosol dissemination.

A 25 % sampling efficiency of the SASS 2000^{PLUS} based on CFU values was estimated for BG aerosol sampling. This preliminary result is based on one aerosol experiment only where a two hours sampling was performed.

A sampling efficiency of approximately 15% was estimated in two different experiments. In one of these experiments the SASS 2000^{PLUS} was located inside the chamber during sampling and in the other it was connected from the outside. In both experiments, the sampling time was 60 min. These studies show that further investigations regarding sampling efficiency of the SASS 2000^{PLUS} are necessary.

3.3 Re-aerosolization and survival rate

The re-aerosolization and microbial survival were determined by spiking cells into the SASS 2000^{PLUS}, and subsequently running for different time periods (Table 3.3; 3.4). These experiments were mainly performed at FFI, but a preliminary experiment was performed at TNO. The number of CFU was determined before and after the experiments by plate counting.

The re-aerosolization rate of BG was dependent on sampling time. After one hour sampling the re-aerosolization was approximately 50 %. Longer sampling time resulted in more re-aerosolization whereas shorter sampling time lead to lower re-aerosolization (Table 3.3).

Table 3.3 The re-arosolization rate of BG spores in SASS 2000 PLUS.

Date	**BG spores added to the SASS 2000^{PLUS}**, CFU/ml	**% BG spores[1] retaining in the SASS 2000^{PLUS}**			
		0 min	**30 min**	**60 min**	**120 min**
12.02.07	500	100 %	63 %	35 %	ND[2]
14.02.07	1000	100 %	75 %	52 %	ND
20.02.07	10000	100 %	62 %	48 %	31 %

[1] Number of BG spores is determined by plate counting (CFU).
[2] Not determined

The survival rates of BG and EH were estimated in preliminary experiments using CFU, total counting of bacterial cells in microscope and measuring of optical density (OD_{600}) that decrease with decreasing number of cells. In order to perform microscopic counting a high number of cells or spores ($1x10^8$) were applied. The SASS 2000^{PLUS} spiked with BG spores or EH cells was run for 60 min and the numbers of CFU, total number (microscopic counting) and the OD_{600nm} were determined before and after the experiments. For BG, the percentage of spores retained after one hour based on CFU, total count and OD_{600} was similar, about 40 %. This means that the re-aerosolization rate was about 60 %. However, the survival rate of the remaining spores was approximately 100 %. Similar results were obtained for EH cells. However, to confirm these results more experiments needs to be done.

Table 3.4 Determination of microbial cell viability of BG spores and EH cells after spiking the SASS 2000^{PLUS} with BG spores and EH cells.

Date	**Number of cells/spores added to the to the SASS 2000^{PLUS}**	**% cells/spores retained in the SASS 2000^{PLUS} after 60 min**		
		CFU	**Total count (microscope)**	**OD_{600nm}**
05.03.07	BG – $1x10^8$ spores/ml	41 %	43 %	39 %
12.03.07	EH – $1x10^8$ cells/ml	45 %	57 %	49 %

4 Discussion

This report describes a preliminary evaluation of the use of SASS 2000^{PLUS} air collector in a BAT chamber using BG spores and EH vegetative cells. The SASS 2000^{PLUS} has successfully been used for sampling of *Legionella* spp. and *L. pneumophila* from ambient air (Blatny et al., 2007). *R*eal-time PCR was used to identify the *L. pneumophila*. Viable *L. pneumophila* was also identified from SASS 2000^{PLUS} samples collected for one or two hours. In that case the SASS 2000^{PLUS} proved to be an efficient air collector for collecting viable bacterial cells. In the present work the collection efficiency for BG spores was estimated to 15-25 %. A reason for the low efficiency might be that aerodynamically particle size of the BG aerosol was only 0.8 µm. This is in agreement with other studies testing different air collectors (An et al., 2004; Hogan et al., 2005). The present investigation showed that SASS 2000^{PLUS} successfully sampled viable vegetative cells and spores. Spores are robust and are able to survive lots of stress. In contrast, EH cells are more fragile and might lose their viability. In the present experiments the EH survived the sampling using the SASS 2000^{PLUS} when sampling in water. When NaCl was used as the collecting liquid the salt concentration became too high due to continuously refilling of water evaporating from the SASS 2000^{PLUS}. This caused a reduction in the cell viability of EH cells. The evaporating rate from the SASS 2000^{PLUS} was high, 40 ml was refilled every hour. The present work indicated that within two hours sampling the collection rate was linearly increasing, indicating that no saturation was obtained. Preliminary experiments showed that the re-aerosolization rate of both BG spores and EH cells were approximately 50 % after one hour sampling.

5 Conclusion

- FFI gained valuable experience with the BAT chamber. This includes gaining knowledge of operational and functional use of the chamber, making two different aerosols (BG and EH) and reference samples (particle monitoring and slit sampler tests).
- The work was performed as a joint venture project between FFI and TNO. This contact is valuable for FFI since aerosol studies is a preferred scientific research area of work. The establishment of a bioaerosol test chamber at FFI is currently in progress.
- FFI obtained valuable experience in performing air sampling experiments using the SASS 2000^{PLUS}. The SASS 2000^{PLUS} is able to collect viable microorganisms. However, we were not able to get reliable values for the collecting efficiency of the SASS 2000^{PLUS}. More experiments need to be done.
- Connecting the SASS 2000^{PLUS} collector to the BAT chamber and using the chamber for testing of an external device (in this case an air sampler) was a novel and valuable experience for TNO.

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Appendix A

Data from the experiments performed at TNO.
Samples collected during all experiments are labeled continuously starting from SASS 1. Each plate was counted twice and both figures are presented in the tables.

January 16 2007. BG aerosol experiments
Efficiency.

SASS 1: Pre washing
No CFU detected after plating the washing solution.

SASS 2: 2 h, 1ml sample

	Numbers of CFU/plate
Non-diluted:	full plate + full plate
10^{-1}.:	450 + over growth
10^{-2}.:	103 + 68
10^{-3}.:	7 + 9

SASS 3: 4 h. The SASS 2000 failed and no sample was collected.
SASS 4: Washing
Plating of non-diluted sample: 125 CFU/plate
The results were used to estimate the counting efficiency.

January 17 2007. BG aerosol experiment

Time course of sampling

One ml sample was collected in water at different time point, 5, 10, 20, 40, 60, 90 min. At 120 min the remaining sample in the cyclone was collected.

Sample	**Sampling time (min)**	**CFU/ 200 µl**	**10^{-1} dilution**	**10^{-2} dilution**	**CFU/ml**
SASS 5	0 min	100 + 107	-	-	515
SASS 6	5 min	95 + 140	-	-	585
SASS 7	10 min	90 + 105	-	-	510
SASS 8	15 min	170 + 226	-	-	990
SASS 9	20 min	235 + 229	-	-	1160
SASS 10	40 min	328 + 322	45 + 30	3 + 5	1750
SASS 11	60 min	-	40 + 34	3 + 4	1850
SASS 12	90 min	-	55 + 66	3 + 4	3025
SASS 13	120 min	-	117 + 114	17 + 14	5775
Experiment 2					
SASS 14	0 min	0 + 0	-	-	5
SASS 15	5 min	130 + 122	5 + 10	-	630
SASS 16:	10 min	128 + 135	12 + 13	-	657
SASS 17	15 min	-	11 + 11	0 + 1	550
SASS 18	20 min	-	13 + 15	2 + 2	700
SASS 19	40 min	-	19 + 26	4 + 4	1225
SASS 20	60 min	-	62 + 56	2 + 3	2950

January 23, 2007. EH aerosol experiment

Time course of sampling

One ml sample was collected in 0.9 % NaCl at different time point, 5, 10, 20, 40, 60, 90 min. At 120 min the remaining sample in the cyclone was collected.

Sample	**Sampling time (min)**	**CFU/200 µl**	**10^{-1} dilution**	**10^{-2} dilution**
SASS 30	Washing solution	0 + 0	-	-
SASS 31	5 min	39 + 42	-	-
SASS 32	10 min	50 + 57	-	-
SASS 33	15 min	62 + 72	-	-
SASS 34	20 min	59 + 45	11 + 12	-
SASS 35	30 min	64 + 56	6 + 11	-
SASS 36	40 min	20 + 16	11 + 4	0 + 1
SASS 37	60 min	19 + 26	2 + 4	0 + 0
SASS 38	90 min	25 + 40	2 + 0	0 + 0
SASS 39	120 min	22 + 19	1 +1	0 + 0
SASS 40	0 min	0 + 0	-	-
SASS 41	5 min	112 + 121	-	-
SASS 42	10 min	107 + 120	-	-
SASS 43	15 min	106 + 134	-	-
SASS 44	20 min	137 + 133	12 + 22	-
SASS 45	30 min	155 + 150	16 + 11	-
SASS 46	40 min	145 + 139	12 + 14	0 + 0
SASS 47	60 min	137 + 132	12 + 12	1 + 0

January 24, 2007. EH aerosol experiment

Time course of sampling

One ml sample was collected in water at different time point, 5, 10, 20, 40, 60, 90 min. At 90 min the remaining sample in the cyclone was collected.

Sample	**Sampling time (min)**	**CFU/200 µl**	**10^{-1} dilution**	**CFU/ml**
January 24				
SASS 48	0 min	0 + 0	-	0
SASS 49	5 min	110 + 75	-	462
SASS 50	10 min	55 + 93	-	370
SASS 51	20 min	116 + 85	-	502
SASS 52	40 min	80 + 90	6 + 4	425
SASS 53	60 min	187 + 187	6 + 9	935
SASS 54	90 min	150 + 143	11 + 10	732
SASS 55	120 min	Over growth	57 + 70	3175
January 25				
SASS 58	0 min	0 + 0		
SASS 59	5 min	7 + 1		
SASS 60	10 min	6 + 1		
SASS 61	20 min	8 + 16	1 + 0	
SASS 62	60 min	6 + 6	5 + 0	

EH samples from January 23 and 24 analyzed by real-time PCR

	Real-time PCR Cp values	Sampling time (min)	Estimated concentration
January 23			
SASS 30	0	0	0,00
SASS 31	37,23	5	100,00
SASS 32	36,54	10	152,88
SASS 33	33,39	15	1061,55
SASS 34	33,01	20	1341,11
SASS 35	35,77	30	245,51
SASS 36	31,96	40	2558,56
SASS 37	34,51	60	532,98
SASS 38	31,97	90	2542,86
SASS 39	29,36	120	12666,06
SASS 40	0	0	0,00
SASS 41	36,65	5	100,00
SASS 42	37,29	10	67,45
SASS 43	35,72	15	177,20
SASS 44	34,94	20	286,33
SASS 45	35,64	30	186,14
SASS 46	33,99	40	513,66
SASS 47	31,94	60	1812,92
January 24			
SASS 48	0	0	0,00
SASS 49	31,12	5	100,00
SASS 50	30,63	10	135,18
SASS 51	30,06	20	191,96
SASS 52	29,84	40	219,78
SASS 53	28,3	60	566,79
SASS 54	28,08	90	648,94
SASS 55	27,18	120	1128,90
January 25			
SASS 58	0	0	0,00
SASS 59	31,16	5	100,00
SASS 60	30,9	10	117,35
SASS 61	28,89	20	404,09
SASS 62	27,03	60	1268,88