ASSESSMENT OF THE EFFICIENCY OF FOUR AIR BIO-SAMPLERS AFTER AEROSOLIZATION OF *ENTEROCOCCUS FAECALIS* SUSPENSIONS: A PRELIMINARY STUDY

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ABSTRACT

A major challenge for the bio-security within and between livestock farms is the air transmission of pathogens, which plays a key role in the spread of some infectious diseases. In order to study the airborne transmission of diseases efficient air samplers are required. The objective of this study was to investigate the sampling efficiency of four bio-samplers (Andersen six-stage viable bio-sampler "AVB", All Glass Impinger "AGI-30", OMNI-3000, and Airport MD8 with a gelatine filter) using polydisperse aerosols of *Enterococcus faecalis* generated in a HEPA isolator. However, prior to studying their efficiency using aerosols of *E. faecalis* and other bacteria (*Escherichia coli*, *Campylobacter jejuni* and *Mycoplasma synoviae*), the additive used and the influence of air sample processing on the survival of the bacterial involved should be determined. Preliminary experiments were performed to test 1) the effect of fluorescein sodium (uranine), as a physical tracer, on the bacteria survival in their suspensions, 2) the recovery efficiency of bacteria and uranine by rinsing agar plates used in the Andersen bio-sampler, and 3) the recovery efficiency of bacteria and uranine by dissolving gelatin filters used in the Airport MD8. *E. faecalis* suspensions with uranine, *E. faecalis* suspensions without uranine, and uranine suspensions were also aerosolized in duplicate and sampled with four bio-samplers. Results show that the survival of the four bacterial species was not influenced by the addition of 0.02% uranine in the suspensions after an incubation period of 2 hours. The recovery efficiency by rinsing agar plates ranged between 79.8%-123.6% for bacteria, and 33.1%-60.1% for uranine. The recovery rate from gelatine filters after 5 min exposure to ambient air varied between 62.7%-171.7% for bacteria, and 114.2%-123.2% for uranine. Results of preliminary aerosolization tests show that MD8 with a gelatine filter had the highest physical efficiency. The physical efficiency of the AGI-30 and the OMNI-3000 was about 69.8% and 49.4% relative to that of the MD-8. The biological efficiency of all bio-samplers was not significantly different from 100%, implying *E. faecalis* suffered no stress during sampling. Half-life time of airborne *E. faecalis* was on average 8.6 min at 21-23°C and 80-85% RH.

Keywords: sampling efficiency, bacteria, aerosolization, uranine, fluorescein sodium, nebulizing, survival, stress, spray

1 INTRODUCTION

Airborne transmission of pathogens from livestock production poses infection risks to surrounding farms and people living in the vicinity (Hartung, 2005). Measurements of pathogen concentrations and emission from livestock houses have been increasingly performed for hazard evaluation. In order to get precise assessments, bio-samplers with high sampling efficiency are required. However, the sampling efficiency of common air bio-samplers has not been well established so far. The lack of knowledge induces deficiency in reliable evaluation of concentrations of airborne pathogens.

The sampling efficiency of a bio-sampler is determined by both, its physical and biological efficiency. The physical efficiency is determined by how well particles are aspirated by the device's inlet and transported to the collection medium, and how well the bio-sampler retains these particles (Nevalainen *et al.*, 1992; Griffiths and Stewart, 1999). Biological efficiency refers to the ability of a bio-sampler to maintain the microbial culturability

and to prevent cell damage during sampling (Griffiths and Stewart, 1999), e.g. loss of viability due to impaction (Stewart et al., 1995), impingement (Shipe et al., 1959; Tyler and Shipe, 1959; Tyler et al., 1959) and dehydration (Li et al., 1999).

Studies on sampling efficiency have been carried out by comparing the performance between bio-samplers in the same environment (Thorne *et al.*, 1992; Engelhart *et al.*, 2007). However, in these studies the efficiency of bio-samplers was not compared to defined aerosol concentrations, therefore only the relative efficiency between bio-samplers was calculated. As a solution, Thompson et al (1994) developed a system to investigate sampling efficiency by aerosolizing *Pseudomonas fluorescens* suspensions of known concentrations in isolators. Sampling efficiency was separately determined by investigating physical efficiency, which was the difference of the particle numbers in the upstream and downstream air of a bio-sampler monitored by an aerosol spectrometer, and biological efficiency, which was the difference of ratio (viable bacteria counts towards total particle counts) in the aerosol suspensions and the air samples. However, the viability loss of *Pseudomonas fluorescens* caused by shear force during aerosolization was not determined in their study.

Sampling efficiency can also be determined by aerosolizing aerosol microbial suspensions containing inert tracer compounds. The amount of inert tracer collected by bio-samplers indicates the physical efficiency, and the differences of ratio of viable micro-organisms towards tracer in aerosol microbial suspensions and in air samples indicate the biological efficiency. It is important to ensure that the used tracer is harmless to micro-organisms both in suspension and in the aerosol state. Among the tracer compounds used for the detection of airborne virus, fluorescein sodium (uranine) remains popular because it was reported as virus-friendly in suspensions and was detectable at tiny doses (Ijaz *et al.*, 1985a; Ijaz *et al.*, 1985b; Auckenthaler *et al.*, 2002).

The present study is part of a broader project aiming to investigate the sampling efficiency of four bio-samplers (Andersen Viable Bio-sampler "AVB", All Glass Impinger "AGI-30", OMNI-3000 high volume bio-sampler, and Airport MD8 with a dissolvable gelatine filter) on four species of animal-associated airborne bacteria (*Enterococcus faecalis*, *Escherichia coli*, *Campylobacter jejuni*, and *Mycoplasma synoviae*) by collecting bacterial aerosols containing uranine as the physical tracer in an isolator. Analysis of both bacteria and uranine requires that the air samples are in liquid form. The AGI-30 and OMNI-3000 already collect the bacteria directly into liquid media, however for the other two bio-samplers (the Andersen collects bacteria on agar plates and the Airport MD8 on gelatine filters) further procedures are needed to transfer the samples into liquid. Transfer of samples from the latter two devices is performed by rinsing the surfaces of bacteria-loaded agar plates with buffered peptone water (BPW) for the AVB, and by dissolving gelatine filters into 37°C BPW for Airport MD8. The recovery efficiency of bacteria and uranine of these devices has to be assessed.

The objectives of this preliminary study were to investigate 1) the effect of uranine (as a physical tracer) on the bacteria survival in their suspensions and in aerosol state, 2) the recovery efficiency of bacteria and uranine by rinsing agar plates used in the AVB, 3) the recovery efficiency of bacteria and uranine from gelatin filters used in the Airport MD8, and 4) sampling efficiency of the bio-samplers to detect airborne *E. faecalis*.

2 MATERIALS AND METHODS

2.1 Bio-samplers

The AVB (Andersen, 1958) bio-sampler consists of six stages in each of which a plate with agar is put under a screen with 400 holes. The number of holes is the same for each stage but the diameter of the holes becomes smaller in each stage following downwards direction. When taking samples at the airflow rate of 28.3 l min^{-1}, the air speed in AVB increases from the first stage to the sixth stage (the first stage with lowest air speed, and the sixth stage with highest air speed) due to the hole size. The particles are impacted and ranged by their aerodynamic diameter (largest particles are retained on the plate of the first stage, and smallest particles on the plate of the sixth stage). In this experiment, the bacteria loaded agar plates were rinsed three times (see 2.5), and the rinsing-off fluid was used for bacteria counting following an international standard (ISO 7402:1985).

The AGI-30 (7540, Ace glass Inc., US) impinges airborne micro-organisms into a liquid collection medium. The airflow of AGI-30 is 12.5 l min^{-1}. After sampling, decimal dilutions are made from the collection liquid, which are pipetted onto agar plates for incubation.

The OMNI-3000 (Sceptor Industries Inc., US) operates at a high airflow rate of 300 l min^{-1}. The collection fluid consisting of 10 ml Phosphate Buffered Saline (PBS) is sucked from a cartridge into a contactor where PBS

rotates and is exposed to incoming air. After sampling, PBS containing the collected airborne micro-organisms is drained back to the cartridge automatically. Bacterial counts are subsequently performed on a sample from the cartridge.

The Airport MD8 (Sartorius, Goettingen, Germany) collects micro-organisms on a gelatin filter (17528-80-ACD, Sartorius, Goettingen, Germany) by filtration. The loaded filter is dissolved in liquid medium, which is then used to make decimal dilutions and inoculate agar plates for bacteriological analysis.

2.2 Isolator

A stainless steel negative pressure HEPA isolator (Beyer and Eggelaar, Utrecht, the Netherlands) of 1.94 m in length, 0.75 m in width, and 0.95 m in height was used as aerosolization space. Ventilation and temperature were controlled electronically. A temperature and humidity sensor (HygroClip2, ACIN Instrumenten BV, Rijswijk, the Netherlands) installed in the middle of the isolator was used to monitor the climatic conditions during aerosolization.

2.3 Aerosolization, sampling and sample processing

A Walther Pilot I spray head with a nozzle of 0.5 mm diameter (Walther Spritz- und Lackiersysteme, Wuppertal, Germany) was used for the aerosolization of bacterial suspensions. The aerosol spectrum of the spray-head was characterized previously by laser diffraction (Mastersizer-S long bed; Malvern Instruments, Malvern, UK) and the volume median diameter D(v,0.50) was about 10 µm (Figure 1). The Walther Pilot I spray-head was connected to an air compressor (Mecha Concorde type 7SAX, 1001, SACIM, Verona, Italy) which was set at an air pressure of 2 bar.

Twenty ml of bacterial suspension with 0.02% uranine (F/1300/48, Fisher Scientific, Landsmeer, the Netherlands) was aerosolized in about 1.2 min (16.7 ml min^{-1}) in the isolator. All bio-samplers were positioned in duplicate inside the isolator before aerosolization. Four bio-samplers (one of each kind of bio-sampler) took air samples directly after aerosolization, and the rest took samples 20 min after aerosolization. An open plate containing 20 ml BPW, positioned near the nozzle when spraying, was used to evaluate the loss of bacterial viability during aerosolization. A polytetrafluoroethylene (PTFE) filter, which was expected to have a high efficiency for even tiny airborne particles (Burton et al., 2007), was also used to sample uranine particles for comparison. Sampling time was 2 min. During the experiments air temperature and relative humidity were 21-23°C and 80-85%, respectively. The isolator was ventilated for at least 2 h between two aerosolizations. Inlet of the isolator was kept open to allow the entrance of air in order to compensate for the negative pressure induced by the bio-samplers during sampling.

Liquid samples were processed for bacteriology and uranine analysis without further treatment. Samples from the AVB and Airport MD8 were transferred into liquid form according to the procedures described in section 2.5 and 2.6. Uranine was analyzed by a fluorescent detector (HP 1046 A, HP, USA), which had a detection limit of 0.002 µg ml^{-1}.

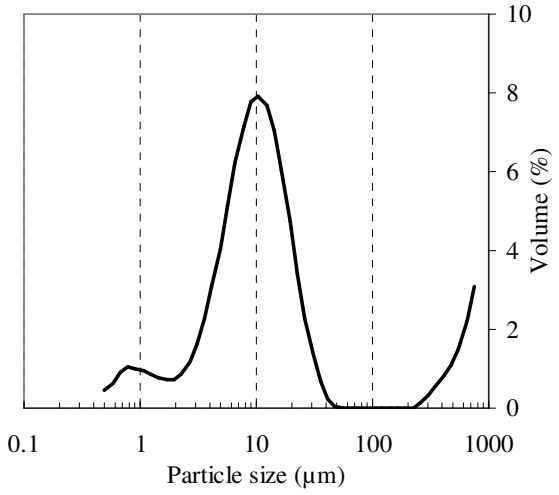

Figure 1. Droplet size distribution measured with a laser diffraction particle size analyser of an aerosol of water generated by the Walther Pilot I spray-head

2.4 Calculation of the sampling efficiency

The bacteria survival during aerosolization and present in the aerosol (N), the physical efficiency (E_p), the biological efficency (E_b) and the sampling efficiency (E) were calculated using the equations below.

$$N = \frac{C_{plate,viable} / C_{plate,tracer}}{C_{viable} / C_{tracer}} \times 100\% \quad \text{(Equation 1)}$$

$$E_p = \frac{C_{bio-sampler,tracer}}{C_{ref-sampler,tracer}} \times 100\% \quad \text{(Equation 2)}$$

$$E_b = \frac{C_{bio-sampler,viable} / C_{bio-sampler,tracer}}{C_{viable} / C_{tracer} \cdot N} \times 100\% \quad \text{(Equation 3)}$$

$$E = E_p \times E_b \quad \text{(Equation 4)}$$

C_{tracer}: the tracer concentration in the aerosol bacterial suspension;
C_{viable}: the viable bacteria count in the aerosol bacterial suspension;
$C_{plate,tracer}$: the tracer concentration in the air sample from the open plate;
$C_{plate,viable}$: the viable bacteria count in the air sample from the open plate;
$C_{bio\text{-}sampler,tracer}$: the tracer concentration measured by a bio-sampler;
$C_{ref\text{-}sampler,tracer}$: the tracer concentration measured by PTFE filters;
$C_{bio\text{-}sampler,viable}$: the viable bacteria concentration measured by a bio-sampler.

2.5 Assessment of the effect of uranine on the survival of bacteria in suspension

Bacterial suspensions were prepared in different media, i.e. *E. faecalis* and *E. coli* in BPW, *C. jejuni* in Heart Infusion Broth (HIB), and *M. synoviae* in Mycoplasma Experience Broth (MEB). Two suspensions of 10 ml were prepared for each species, one contained 0.02% uranine, while the other did not. Viable bacteria were counted in these eight suspensions just after adding uranine and 1 h and 2 h later. All countings were preformed in duplicate.

2.6 Assessment of the recovery efficiency of bacteria and uranine by rinsing agar plates

Sheepblood Agar (SBA), MacConkey Agar (MA), Charcoal Cefoperazone Deoxycholate Agar (CCDA) and Mycoplasma Experience Agar (MEA) were used in the AVB for *E. faecalis*, *E. coli*, *C. jejuni* and *M. synoviae*, respectively. 0.1 ml of a bacterial suspension of known concentration with 0.02% uranine was pipetted onto the corresponding agar plate. After 5 min at room temperature, the bacteria-loaded agar was rinsed three times consecutively with 2 ml BPW by carefully scraping its surface with a plastic scraper. The rinsing-off liquid samples were mixed together for bacteriology and uranine analysis. A fourth rinsing was performed in order to assess the amount of remaining bacteria and uranine. The test was done twice for all bacterial species.

2.7 Assessment of the recovery efficiency of bacteria and uranine from gelatin filters

The recovery efficiency of bacteria from gelatine filters was performed as described earlier (Landman et al., 2004). Briefly, 0.5 ml of a bacterial suspension of known concentration with 0.02% uranine was added to a sterile gelatin filter in a petri dish. After 5 min at room temperature, the gelatine filter was dissolved in 20 ml BPW kept at 37 ºC. The liquid sample was pipetted into a small flask containing 30 ml BPW (final sample volume was 50 ml). From the final sample, bacterial counting and uranine analysis were performed. The test was done twice for all bacterial species.

2.8 Assessment of the efficiency of the bio-samplers after aerosolization of *E. faecalis*

A preliminary study was performed in duplicate to assess the efficiency of four bio-samplers at detecting airborne *E. faecalis* after aerosolizing 20 ml of either *E. faecalis* suspensions (in BPW) with 0.02% uranine, *E.*

faecalis suspensions (in BPW) without uranine, or solutions of 0.02% uranine only. The aerosolization protocol, air sampling and sample processing were performed as described before.

3 RESULTS AND DISCUSSION

3.1 Assessment of the effect of uranine on the survival of bacteria in suspension

Uranine was added as a tracer to the aerosol fluid in order to assess the physical efficiency of the bio-samplers by comparing the amount of uranine collected in the air samples to the amount of uranine collected by high efficient filters (the gelatine filter and PTFE filter). A prerequisite was that the tracer itself should not affect the bacteria, either negatively or positively, during the experimental period.

Table 1 shows the results of bacterial survival in suspensions with and without addition of 0.02% uranine. Differences $\leq$1 $\log_{10}$ in the concentration of *E. faecalis*, *E. coli* and *M. synoviae* in both suspensions at three counting moments were observed. These differences were regarded as not significant and within the variation common to this technique. *C. jejuni* concentrations in HIB at three counting moments were also within 1 $\log_{10}$ (3.75×10^6 to 1.32×10^7). However, after adding 0.02% uranine *C. jejuni* concentrations notably decreased from 1.04×10^7 to 1.73×10^5 in 2 h.

Statistical analysis (Generalized Linear Model, SAS) was performed to test the effect of three fixed factors, i.e. uranine, time (0, 1, 2 h after adding uranine) and bacterial species, on the bacterial concentrations of suspensions. The analysis showed that only the factor bacterial species ($P < 0.01$) was responsible for differences in concentration. Both, the P values of uranine ($P = 0.61$) and time ($P = 0.17$) were non significant. It indicates that the addition of 0.02% uranine has no significant effect on all four bacterial species at least with a 2 h period after its addition. It should be noted, however, that the power of the analysis was low, caused by the low number of repetitions. Especially, for *C. jejuni* a negative effect of uranine can not be excluded.

Table 1. Bacterial survival in suspensions with or without 0.02% uranine (each value is the average of at least one sample, which was counted in duplicate), and the result of GLM analysis of the factors uranine, time and bacterial species on bacterial survival

Species	Suspension	Concentration (± se, cfu ml^{-1})			GLM analysis	
		0 h after adding Uranine	1 h after adding uranine	2 h after adding uranine	Factor	P[2]
E. faecalis	BPW[1]	$7.70 (\pm 0.10) \times 10^8$	$1.02 (\pm 0.26) \times 10^9$	$7.80 (\pm 0.40) \times 10^8$	Uranine	0.61
	BPW + uranine	$1.07 (\pm 0.19) \times 10^9$	$5.93 (\pm 0.63) \times 10^8$	$8.17 (\pm 1.20) \times 10^8$	Time	0.17
					Bacteria	< 0.01
E. coli	BPW[1]	$8.40 (\pm 1.60) \times 10^8$	$1.60 (\pm 0.32) \times 10^9$	$7.20 (\pm 1.20) \times 10^8$		
	BPW + uranine	$1.07 (\pm 0.13) \times 10^9$	$1.05 (\pm 0.15) \times 10^9$	$7.65 (\pm 0.56) \times 10^8$		
C. jejuni	HIB[1]	$1.32 (\pm 0.69) \times 10^7$	$3.75 (\pm 1.55) \times 10^6$	$7.60 (\pm 3.40) \times 10^6$		
	HIB + uranine	$1.04 (\pm 0.16) \times 10^7$	$2.15 (\pm 0.25) \times 10^6$	$1.73 (\pm 0.78) \times 10^5$		
M. synoviae	MEB[1]	$1.30 (\pm 0.10) \times 10^8$	$1.55 (\pm 0.15) \times 10^8$	$1.40 (\pm 0.00) \times 10^8$		
	MEB + uranine	$1.23 (\pm 0.28) \times 10^8$	$1.40 (\pm 0.10) \times 10^8$	$1.65 (\pm 0.15) \times 10^8$		

[1] Original culture medium. BPW: Buffered Peptone Water; HIB: Heart Infusion Broth; MEB: Mycoplasma Experience Broth.
[2] Probability that there was no effect of the factor on bacterial survival.

3.2 Assessment of the recovery efficiency of bacteria and uranine by rinsing agar plates

The results in Table 2 show that most of the bacteria were recovered (79.8% to 123.6%). Recovery of *C. jejuni* (123.6%) was higher than 100%. This was explained either by bacteria growth during rinsing or by the variation in analysis of bacteria count or both. An additional (fourth) rinsing step recovered 0.4% *E. faecalis*, 0.6% *E.coli*, 4.4% *C. jejuni* and 1.0% *M. synoviae*. It indicates that the number of bacteria remaining after three rinsing steps was low. Therefore, the fourth time rinsing was considered redundant.

The uranine recovery (33.1% to 60.1%) from all the four types of agar plates was lower than the bacterial recovery. This was explained by the fact that uranine probably binds much stronger with the agar, which was confirmed by the fact that after the fourth rinsing 7.5% to 10.1% uranine could still be recovered from the agar

plates. The recovery obtained in this test was used for correcting the amount of uranine collected in samples of the AVB in the aerosolization test.

Table 2. Percentages of bacteria and uranine recovered by rinsing the agar surfaces with 2 ml of BPW three times consecutively (each value is an average value of at least two measurements)

Species	Agar type[1]	Recovery % (± se)	
		Bacteria	Uranine
E. faecalis	SBA	79.8 (± 1.7)	60.1 (± 4.4)
E. coli	MA	87.2 (± 13.4)	52.9 (± 0.9)
C. jejuni	CCDA	123.6 (± 6.3)	33.1 (± 2.7)
M. synoviae	MEA	98.2 (± 9.4)	48.3 (± 4.2)

[1] SBA: Sheepblood Agar; MA: MacConkey Agar; CCDA: Charcoal Cefoperazone Deoxycholate Agar; MEA: Mycoplasma Experience Agar

3.3 Assessment of the recovery efficiency of bacteria and uranine from gelatin filters

Table 3 shows the results of the recovery of bacteria and uranine after dissolving the gelatine filters in BPW kept at 37°C. Bacterial recovery ranged from 62.7% to 171.7%. The uranine recovery was all higher than 100% (114.2 - 123.2%). This might be caused by the positive effect of BPW on uranine analysis, which was confirmed by a former test that uranine was still detected in pure BPW by the fluorescent meter.

Table 3. Percentages of bacteria and uranine recovered from gelatine filters (each value is an average value of at least two measurements)

Species	Recovery % (± se)	
	Bacteria	Uranine
E. faecalis	62.7 (± 5.3)	123.2 (± 2.2)
E. coli	87.4 (± 11.5)	120.5 (± 1.7)
C. jejuni	171.7 (± 29.8)	123.1 (± 1.2)
M. synoviae	72.5 (± 6.4)	114.2 (± 6.6)

3.4 Assessment of the efficiency of the bio-samplers after aerosolization of *E. faecalis*

Table 4 shows the concentration of airborne bacteria and uranine after aerosolization of *E. faecalis* with uranine, *E. faecalis* without uranine, and uranine solution only. All bio-samplers collected similar amounts of *E. faecalis*, about $\log_{10}$ 9 cfu m^{-3} at 0 min after aerosolization. Twenty min after aerosolization, the *E. faecalis* concentrations decreased to one tenth. There were large variations in recovery of airborne uranine by different bio-samplers. Concentrations of uranine ranged from 190 to 1680 µg m^{-3} at 0 min, and from 36 to 223 µg m^{-3} at 20 min after aerosolization.

Table 4. Concentrations of airborne *E. faecalis* and uranine collected by the bio-samplers at 0 and 20 min after aerosolization (each value is the mean of a duplicate test)

Bio-sampler	*E. faecalis* with uranine				*E. faecalis* without uranine		Uranine (µg m^{-3})	
	E. faecalis (cfu m^{-3})		Uranine (µg m^{-3})		*E. faecalis* (cfu m^{-3})			
	0 min	20 min	0 min	20 min	0 min	20 min	0 min	20 min
AVB	2.07×10^9	2.52×10^8	194	36	2.48×10^9	3.26×10^8	190	38
AGI-30	9.44×10^8	1.33×10^8	930	148	1.78×10^9	2.52×10^8	1000	173
OMNI-3000	9.83×10^8	1.43×10^8	783	86	1.23×10^9	1.72×10^8	766	107
MD8	1.63×10^9	1.75×10^8	1590	197	2.33×10^9	2.74×10^8	1680	223
PTFE	-	-	-	-	-	-	845	150

All four bio-samplers recovered comparable uranine concentrations from aerosols of *E. faecalis* with uranine and from aerosols of uranine, both at 0 and 20 min (e.g. 190 vs 194 µg m^{-3} at 0 min, and 38 vs 36 µg m^{-3} at 20 min by AVB). The bio-samplers would give similar results of tracer concentrations with or without bacteria. Therefore, aerosolizing separately uranine suspensions and bacteria suspensions is an option to investigate the sampling

efficiency by avoiding negative effect of uranine on bacteria in aerosol state, especially for those which were suspected to be vulnerable to uranine (*C. jejuni*).

3.4.1 Sampling efficiency

Table 5 shows the result of physical and biological efficiency of all air bio-samplers.

3.4.1.1 Physical efficiency
Uranine recovery from AVB was low (190 – 194 ug m^{-3} at 0 min and 36 – 38 ug m^{-3} at 20 min; Table 4). This was probably caused by the fact that uranine binds strongly to the agar surface, making it difficult to rinse it off (Table 2). Moreover, the 60.1% recovery of uranine from SBA was obtained by rinsing the droplets which were softly pipetted onto the agar surface. When taking air samples, the droplets are impacted on the agar in AVB at a high speed, causing the uranine to be bound on the agar surface even more firmly.

The Airport MD8 with gelatine filters showed the best recovery of uranine (1590 to 1680 µg m^{-3} at 0 min, and 197 to 223 µg m^{-3} at 20 min; Table 4), indicating the highest physical efficiency. Burton (2007) found both gelatine filter and PTFE filter could recover more than 94% of airborne particles. However, our results show that the amount of uranine collected with PTFE was about half compared to that collected by the Airport MD8. This might be due to the non-uniform distribution of uranine aerosols in the isolator. The four bio-samplers were positioned inside the isolator, while the PTFE filter was installed on the side wall. In Table 5, the efficiency of the different bio-samplers and PTFE filter are given relative to the Airport MD8 with a gelatin filter. T-test shows that the physical efficiency of all other samplers was significantly lower than the efficiency of the Airport MD8 (all P-values < 0.05).

Table 5. Physical and biological efficiency of all air bio-samplers

	Physical efficiency % (± se)	P[1]	Biological efficiency % (± se)	P[2]
AVB	13.9 (± 1.0)	0.00	2052.3 (± 1321.6)	0.38
AGI-30	69.8 (± 4.7)	0.00	151.0 (± 98.8)	0.70
OMNI-3000	49.4 (± 4.0)	0.00	117.9 (± 53.4)	0.79
MD8	100 (± 6.4)	-	128.3 (± 75.6)	0.77
PTFE	56.5 (± 6.0)	0.00	-	-

[1] Probability that the physical efficiency of bio-samplers was not different with that of MD8 (a P-value < 0.05 meant there was significant difference).
[2] Probability that the biological efficiency of bio-samplers was not different from 100% (a P-value < 0.05 meant there was significant difference).

3.4.1.2 Biological efficiency
Survival during aerosolization was measured by comparing the bacteria/uranine ratio in an open plate to the ratio in the aerosol suspension (Equation 1). It was found that 88.1 % of *E. faecalis* were alive just after aerosolization with a standard error of 29.1%. The T-test showed that the loss of *E. faecalis* bacteria was not statistically significant (P = 0.75).

The biological efficiency of all bio-samplers, calculated by Equation 3, was above 100%. The efficiency of AVB was extremely high. The high recovery of *E. faecalis* and low recovery of uranine by the rinsing procedure were probably the cause of this high value. The efficiency of other bio-samplers ranged from 117.9% to 151.0%. The P-values of the different samplers were >0.05, indicating that the biological efficiency was not different from 100%, i.e. all the four bio-samplers could recover the airborne *E. faecalis* without significant loss of viability.

3.4.2 Survival of airborne E. faecalis

Equation 5 was used to calculate the half-life time,$t_{1/2}$ (Weesendorp et al., 2008).

$$t_{1/2} = \frac{(\log_{10} 2) \times T}{\log_{10}(C_0 / C_{20})} \qquad \text{(Equation 5)}$$

T: time interval in min. In this experiment, T=20 min;
C_0: bacteria concentration at 0 min after aerosolization;
C_{20}: bacteria concentration at 20 min after aerosolization.

Due to the diluting effect of entering air during air sampling, C_{20} had to be corrected before calculation of half-life time. Assuming the dilution was linear without wall loss, the correction was calculated with Equation 6.

$$C_{20} = C_{bio-sampler,viable} \times \frac{V_{isolator} + V_e}{V_{isolator}} \qquad \text{(Equation 6)}$$

V_{isolator}: volume of the isolator (1.38 m^3);
V_e: extracted amount of air (0.76 m^3).

All bio-samplers gave similar half-life time results of bacteria (7.7 to 11.3 min). This result was in agreement with earlier research by Landman and van Eck (2001), who reported a half-life time for airborne *E. faecalis* of 2-15 min. Comparing results from aerosol experiments with and without uranine showed that *E. faecalis* survival was not affected by the uranine (P = 0.24-0.68).

Table 6. Half-life time of *E. faecalis* aerosolized with uranine and without uranine (each value is the mean of a duplicate test)

Bio-sampler	Half-life time (min)		P[1]
	***E. faecalis* with uranine (± se)**	***E. faecalis* without uranine (± se)**	
AVB	7.7 (± 0.6)	8.7 (± 0.0)	0.24
AGI-30	7.7 (± 1.4)	11.3 (± 4.3)	0.51
OMNI-3000	8.2 (± 1.1)	9.3 (± 1.9)	0.68
MD8	7.6 (± 0.1)	7.8[2]	0.55

[1] Probability that the survival of *E. faecalis* was not affected by the uranine in aerosol state.
[2] Half-life time obtained from one sample. The other sample was contaminated with bacteria other than *E. faecalis*.

4 CONCLUSIONS

The results of this preliminary study can be summarized as follows:

1. Statistical analysis showed that uranine had no negative effect on *E. faecalis*, *E. coli*, *C. jejuni* and *M. synoviae*. However, the power of the analysis was low. Especially, *C. jejuni* might still be affected by uranine.
2. Bacteria were fully recovered by rinsing the surface of agar plates, while uranine was not. Both bacteria and uranine were fully recovered from the gelatine filters.
3. *E. faecalis* concentrations measured by four bio-samplers were similar. However, uranine concentrations were significantly higher in air samples obtained with the Airport MD8, indicating it has the highest physical efficiency. The physical efficiency of the AGI-30 and OMNNI-3000 was 69.8% (± 4.7%) and 49.4% (± 4.0%), respectively, compared to that of the Airport MD8. The low physical efficiency of AVB was probably due to the low recovery of uranine from the agar plates. There was no significant difference in biological sampling efficiency for *E. faecalis* between the AVB, AGI-30, OMNI-3000 and Airport MD8.
4. The average calculated half-life time of airborne *E. faecalis* was 8.6 min at 21-23°C and 80-85% RH, assuming the dilution due to air sampling was linear and no wall loss occurred.

Acknowledgements
We thank Ms. Tao Nan, Mr. Machiel Esmann, Ms. Constance Reugebrink, Mrs. Thea von Bannisseht, Mr. Jean Slangen, Mr. Frans Putirulan, Dr. Klaas Frankena, Mr. Eef Lovink, and Mr. Johan Ploegaert for their contributions to this study.

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