Available online at www.sciencedirect.com
SCIENCE DIRECT
Aerosol Science 36 (2005) 557–573
Journal of Aerosol Science
www.elsevier.com/locate/jaerosci

# An approach towards characterizing a reference sampler for culturable biological particle measurement

Jim Ho*, Melvin Spence, Scott Duncan

*Defence Research and Development Canada Suffield, Box 4000, Station Main, Medicine Hat, Alberta, T1A 8K6, Canada*

Received 19 February 2004; accepted 8 November 2004

**Abstract**

The first published account of a slit-to-agar sampler was of a system that used 10 cm petri plates. At some point after the 1950s samplers that collected biological aerosol particles on 15 cm plates appeared. More recently published patents describing slit samplers using 10 cm plates have cited economics as the prime motivation. Nonetheless, researchers have cautioned that the smaller plates can become saturated when heavy aerosol clouds are encountered, such as when a slit sampler is used as a reference collector when biological detectors are tested. This report describes how to determine the performance characteristics of slit samplers designed for either 10 or 15 cm plates. It also demonstrates the importance of controlled replicate measurements providing data suitable for rigorous statistical analysis. The results indicate that, for measuring biological clouds of between 5 and 30 agent containing particles per liter of air (ACPLA), the 15 cm plate sampler design is more efficient than those targeted for the smaller collection surface. A statistical method has been employed to test differences between regression slopes.


*Keywords:* Slit-to-agar samplers; STA samplers; Live biological aerosol; Culturable particle measurement; Aerosol chamber; BG spore simulant for anthrax; Median particles size

## 1. Introduction

Due to recent interest in environmental and indoor air quality, slit samplers have been given more attention from workers in aerobiology (Jericho, Ho, & Kozub, 2000; Anderson et al., 1996). In the past decade, two separate patents for slight variations of the slit sampler have been awarded (Kiplinger, 1996;

* Corresponding author. Tel.: +1 403 544 4804; fax: +1 403 544 3388.
*E-mail address:* jim.ho@drdc-rddc.gc.ca (J. Ho).

0021-8502/$ - see front matter 
doi:10.1016/j.jaerosci.2004.11.020

Swenson, 1998). However, very rarely are slit samplers the sole instrument of choice for culturable biological measurements even though its effectiveness has been recommended by a number of workers (Morris, Kokki, Anderson, & Richardson, 2000). More commonly, users deploy a variety of samplers of varying designs, probably as a hedge. Casewell, Fermie, Thomas, and Simmons (1984) compared the Reuter centrifugal air sampler (RCS) with the Casella slit sampler using *Bacillus globigii* spore aerosol. Their results showed that the slit sampler gave counts that were apparently three to tenfold higher than those with the centrifugal sampler. A few years later, the same authors performed more measurements of hospital air and concluded that the RCS could replace the slit sampler primarily due to its convenience of use and portability (Casewell, Desai, & Lease, 1986). Given the diversity of sampler choices, Henningson and Ahlberg (1994) did a very comprehensive review of biological aerosol samplers and in their paper, slit samplers were given good coverage.

Comparative studies on slit samplers appear to be a fairly common scientific activity. Groschel (1980) reported that the Ross Microban sieve sampler and the Biotest Reuter Centrifugal Sampler when tested with the Casella slit sampler, showed comparable results. In fungal studies, Smid, Schokkin, Boleij, and Heederik (1989) reported on the sampling efficiencies of the Andersen N-6 sampler, slit sampler and Reuter centrifugal air sampler (RCS) and concluded that the N-6 and slit sampler performed best. Observations similar to this were confirmed by Verhoeff et al. (1990) who performed studies with the same instruments. In contrast, when Placencia, Peeler, Oxborrow, and Danielson (1982) measured bacterial recovery by a portable Reuter centrifugal air sampler and a standard Mattson–Garvin slit-to-agar air sampler, they found the former yielded significantly higher recoveries.

Heeg and Kanz (1975), in a study that employed a Casella slit sampler located in the middle of an intensive care unit, established that human activities caused bacterial counts to rise by 35–310 percent above the control level. When the dust content of the air was measured with a Royco particle counter, it showed close correlation between changes in the number of particles ($> 5\,\mu m$) with respect to the number of airborne bacteria. These examples serve to illustrate that there is no clear literature consensus for the best sampler when the experimenter needs to collect culturable particle information. Clearly, there is a need for a method that permits replicate measurements as well as a statistical method to objectively evaluate results.

The slit sampler was first described by Bourdillon, Lidwell, and Thomas (1941) over 60 years ago. Since then it has been used as a baseline reference for other types of samplers (Lach, 1985). Commercial versions (Biap Slit-Sampler) have been used for various environmental applications like fungal measurements in Copenhagen (Larsen, 1981) and bacterial contaminations in slaughterhouses (New Brunswick STA 200, Jericho et al., 2000). Tjade and Gabor (1980) mentioned that the Biap slit sampler and the Casella Mk 2 slit sampler, when studied in an orthopaedic operating theatre, showed similar bacterial counts in the range of 74–640 cfu. (colony forming units)/$m^3$ air. Most of these samplers have timing clocks that revolve at 1 or 2 revolutions per hour. In a previous report we described a high resolution slit sampler that was a modification of the New Brunswick Model STA 200. The sampler was capable of resolving a three second-time interval of a passing aerosol cloud (Ho, Spence, & Hairston, 1999). This paper describes a comparison between the aforementioned sampler and a Mattson–Garvin slit-to-agar sampler that also has been modified for high resolution particle collection. In addition, the comparative study also included a sampler that used a 10 cm plate as a particle collection medium. An aerosol chamber has been used to ensure reproducible experiments. We also introduce a statistical approach to objectively compare the performance of slit samplers that have been run under these reproducible experimental conditions.

We consider conventional slit samplers that revolve at 1–2 revolutions per hour with an air flow rate of 50 l/min as low-resolution instruments. They are suitable for measurements in environments where few culturable particles are known to exist. In contrast, high resolution sampling is defined as the ability to resolve aerosol cloud concentrations in 1–3 s time slices with the petri plate platform revolving at 1–2 revolutions per minute. High resolution biological aerosol sample collection is not a new concept. Since the 1950s slit samplers that operate at one revolution per minute have been commercially available (Model FD 100, Reyniers & Son, Chicago, IL), but these were driven by mechanical clock works and were not suitable for serial sampling requirements. Serial sampling is a simple concept that describes a set of electronically networked instruments each with an address that can be activated in sequence or any combination thereof.

## 2. Methods and materials

### 2.1. Aerosol chamber

The chamber characteristics have been previously described (Ho, Spence, & Ogston, 2001). Biological aerosol dissemination (spores of *Bacillus subtilis* var *niger*, ATCC 9372, BG, also called *Bacillus globigii* obtained from Dugway Proving Ground, Utah) was accomplished with two Hudson nebulizers (model 1700, Hudson Oxygen Therapy Sales Co., Wadsworth, OH) at 172–206 KiloPascal (25–30 psi). Fans were used to keep the aerosol particles well mixed. A suspension containing BG in the range of 5–30 μg plus 4 mg silica gel (Syloid 245, Davison Chemical, Baltimore, MD) per ml was used as the starting material. For each experiment, the dry BG powder was weighed out from a stock batch. For example, to prepare a 10 μg/ml suspension, 0.1 gm dry powder was weighed out (model AE 200-S, Mettler Instruments, Zurich, Switzerland) and added to 10 ml distilled water. Serial 1:10 dilutions were repeated three times to obtain a final concentration of 10 μg/ml. A working volume of 25 ml was used to fill the aerosol generator. Actual culturable counts in the suspension were determined to obtain the source concentration.

A steady aerosol concentration in a 45 $m^3$ chamber was achieved via a feed back control loop mechanism as previously described (Ho, 1989). In this scheme, a particle sizer was used to measure aerosol concentration at every 5 s interval. The result was compared to a previously defined threshold; custom software performed the control function to regulate switching of the aerosol generator. The monitored particle size range was 0.7–1.0 μm at 1 particle per $cm^3$ concentration threshold. Steady state aerosol concentration was achieved in about 15 min from startup. Syloid was added to BG as a filler to provide sufficient particle counts in the aerosol to maintain efficient operation of this feedback loop.

### 2.2. Sampler collection

Critical to understanding the characteristics of the test aerosol, culturable particles in air must be captured and grown to determine the presence of the "live" fraction. To properly characterize the BG particulate material, a number of standard biological aerosol collectors were used (Chatigny, Macher, Burge, & Solomon, 1989). Biological aerosol particles were impacted onto a 15 cm nutrient agar plate situated in a slit sampler (model STA 203, New Brunswick Scientific, Edison, NJ 08818-4005). The air flow rate was 50 l/min. Each sampler was electronically modified to rotate at 1 revolution/min. A sampler array consisted of 10 devices serially connected to function as a continuous 10 min collector.

After overnight incubation, culturable particles were counted as bacterial colonies by means of a flat bed scanner driven by custom software developed jointly by Defence Research Establishment Suffield (DRES), Dugway Proving Ground (DPG) and Spiral Biotech (model "STAR" version 1.5, Spiral Biotech, Bethesda, MD). Slit sampler data were expressed as agent containing particles per liter of air (ACPLA).

A dichotomous sampler, DS, (model 245, Andersen Samplers Inc., Atlanta, GA) operating at 17 l/min, collected particles on a set of two glass fiber filters representing fine ($< 2.5\,\mu m$) and coarse ($> 2.5\,\mu m$) fractions (Ho, 1991). Sampling was done in 10 min batches, repeated three times. The filters were disrupted in 20 ml water using a wrist action shaker (model 75, Burrel corp. Pittsburgh, PA). Wire gauze disks were used to clarify the supernatant before culturable spore assessment.

### 2.3. *Culturable spore assay*

Culturable organism enumeration was by the spiral plating technique described by Hedges, Shannon, and Hobbs (1978). Liquid samples of the spore containing liquid were applied to standard nutrient agar plates with a spiral platter (model 4000, Spiral Biotech Inc. Bethesda, MD). The plates were incubated overnight at 37 °C and analysed with the CASBA 4 System that combines a high-resolution line scanner and specialised Windows® based software to count microbial colonies on agar plates. Custom software developed by DRES and DPG were used to analyse slit plates. The Colony Image Analysis (CIA) software locates and analyses each colony and provides the plate and sample count. Data output in Excel format can be further processed by conventional software. SigmaStat version 2 (SPSS Inc. 444 N. Michigan Avenue, Chicago, Illinois, 60611) was used for statistical analysis.

### 2.4. *Slit sampler design to achieve high-resolution capabilities*

We consider conventional slit samplers that revolve at 1–2 revolutions per hour with an air flow rate of 50 l/min as low-resolution instruments. They are suitable for measurements in environments where few culturable particles are known to exist. In contrast, high resolution sampling is defined as the ability to resolve aerosol cloud concentrations in 1–3 s time slices with the petri plate platform revolving at 1–2 revolutions per minute. High resolution biological aerosol sample collection is not a new concept. Since the 1950s slit samplers that operate at one revolution per minute have been commercially available (Model FD 100, Reyniers & Son, Chicago, IL), but these were driven by mechanical clock works and were not suitable for serial sampling requirements. Serial sampling is a simple concept that describes a set of electronically networked instruments each with an address that can be activated in sequence or any combination thereof.

High resolution sampling was the result of a DRES-DPG collaborative effort which also undertook the development of a 15 cm petri plate scanner (Ho et al., 1999). The hardware and software system permitted automated data reduction which was essential for field trials that generate many sample plates that had to be processed within a short time, a restriction due to microbial growth characteristics. In the first iteration, stock New Brunswick slit samplers were modified by a replacement digital motor together with the appropriate driver board interface and serial switching and timing circuitry. This work was done by a contractor (Dycor, Edmonton Ca.) and the instrument was designated the Dycor modified New Brunswick. Unfortunately, the original slit sampler platform went out of production. The requirement for a more efficient replacement sampler resulted in a number of radical design changes. Fortunately, key components such as the original slit cup (slit dimension $0.15 \times 48$ mm) and the plastic chamber


Fig. 1. Instruments used in aerosol chamber measurement studies.

bowl could be obtained from New Brunswick as spare parts. These items were considered necessary to construct a sampler that would perform like the original. While working on the design of the original sampler, it was decided that by putting two slit and bowl units on one base, operational efficiencies could be realized (Fig. 1). The integration and electronics for this dual slit platform was performed by another contractor (HF Research, Medicine Hat, Alberta) and thus the instrument was designated the HF modified slit system. The only major specification deviation from the Dycor modified slit sampler was that the flow rate was changed to 33 l/min due to the solenoid valve flow restriction.

As mentioned in the Placencia et al. (1982) paper, Mattson–Garvin offers a model 220 slit sampler (Barramundi Corporation, Homosassa Springs, FL) with interchangeable drive motors (60, 30, 15, 5 min). In the present comparison, a MG sampler with a 2 min per revolution motor drive was used.

Bourdillon et al. (1941) originally designed their slit dimension at 0.25 by 27.5 mm for a standard 10 cm petri plate. The Casella sampler, also using a 10 cm plate, mentioned by Groschel (1980), had a very similar set of slit specifications ($0.3 \times 28$ mm). For this series of comparison measurements, a 10 cm plate design (Solectron Ltd, Fareham, Hampshire, UK) using a $0.3 \times 17.5$ mm slit was also tested.

In the current experiments, the following sampling systems were located in the chamber and operated simultaneously: (1) Mattson–Gavin (model 220, slit dimension $0.152 \times 48$ mm; www.mattson-garvin.com/models.htm), (2) Dycor modified design, (3) HF modified design and (4) the 10 cm design (custom

Table 1
Flow rate (LPM) characteristics for the 10 cm design and the Dycor modified New Brunswick

| Sampler index | 10 cm design | Conv. $1/x$ | Dycor modified NB |
|---|---|---|---|
| 1 | 16 | 1.07 | 41 |
| 2 | 14 | 0.93 | 22 |
| 3 | 8 | 0.53 | 34 |
| 4 | 12 | 0.8 | 42 |
| 5 | 12 | 0.8 | 37 |
| 6 | 18 | 1.2 | 29 |
| 7 | 18 | 1.2 | 42 |
| 8 | 11 | 0.73 | 24 |
| 9 | | | 40 |
| 10 | | | 44 |

The conversion factors were used to estimate ACPLA values for the 10 cm design, taking into account the different flow rates. For the other instruments, flow rates and conversion factors used for calculation of ACPLA were as follows: HF = 33(1/1.1); Mattson–Garvin = 28.3 (1/1.89). Even though each Dycor modified sampler had different flow rates, for calculation purposes, 50 LPM was used (see text for explanation).

design, not commercially available) operating at flow rates shown in Table 1. As the aerosol in the chamber was well mixed by two sets of circulating fans, placement of the samplers was not critical. In fact, when the same experiment was repeated in the UK (Burke, Platt, Reid, & Whatley, 2001), similar results were obtained.

### 2.5. *Statistical analysis*

Table Curve 2D was used to derive slope and linear regression statistical calculations (Table Curve version 4, SPSS, Chicago, IL). The program produces a graphical plot with all the relevant statistical values. Comparison of slopes was done according to the method described by Armitage and Berry (1995). The routines were rendered in an Excel spreadsheet and tested with sample data provided in their book. Biological data is not normally distributed so the median concentration is used as data transform so that parametric methods is deemed appropriate for data analysis.

### 2.6. *Results and discussions*

Aerosol measurements are subject to great numerical variances influenced by a variety of parameters that are difficult to control. Slight variations in experimental maneuvers can contribute to different results that may appear unacceptable. For this series of chamber experiments, we structured an approach that would account for variations such as the act of weighing the starting material or pipetting errors. It can be seen that overall, larger amounts of dry BG material, when rendered into suspension, gave correspondingly greater culturable counts in each replicate experiment. When the mean culturable counts of the replicate are compared, Fig. 2 shows that there is a log-linear relationship with respect to dry weight.

Before each experimental run, conventional low-resolution slit samples were used to determine if there was any background aerosol particle contamination. As shown in Fig. 3, the chamber was very clean except for when a worker went into the space.

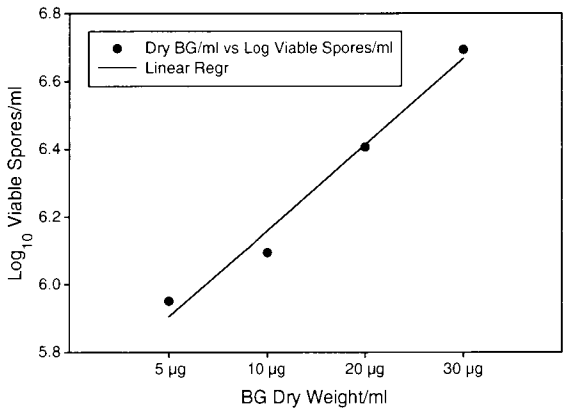


Fig. 2. Plot of dry BG powder by weight vs mean log culturable spores in 1 ml of liquid suspension. Each experimental run starts with the weighing of dry BG powder to take into account weighing errors that may affect final outcome. In practice, 50–100 mg batches of the material were deemed appropriate for weighing accuracy. Working suspensions were serially diluted to the final concentrations that are used in the sprayer. Culturable spore counts were performed on the final preparation.

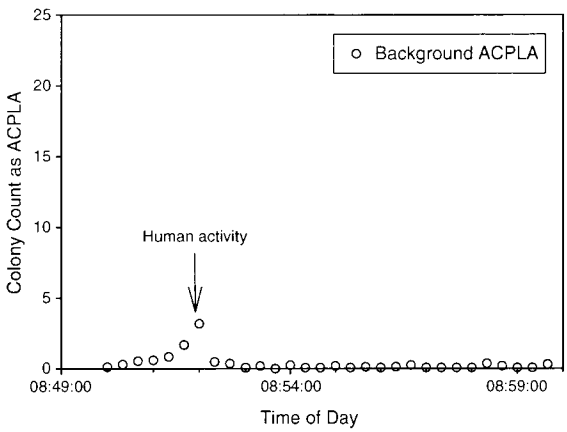


Fig. 3. Typical background aerosol concentration in the chamber before the start of an experiment. Note the slit samplers are capable of registering about 4 ACPLA, the presence of contaminants primarily due to the human activity stirring up residual material on chamber surfaces.

## 3. Aerosol characterization with slit samplers

From observation, approximately 15 min was required for the aerosol to reach an equilibrium concentration in the chamber. Following this the slit sampling sequence was initiated via remote control that activated the first in a series of 10 devices. Concomitantly, the vacuum source was also activated providing the desired air flow for each of the systems. The speed of the revolving sample plate provided a very fine resolution of the instantaneous aerosol concentration encountered during the 20 min experiment. When the resultant colony counts were analyzed for each of the 30 segments per plate, each representing a 4 s time slice, the 300 data points can be depicted first as a scatter plot (Fig. 4) and then

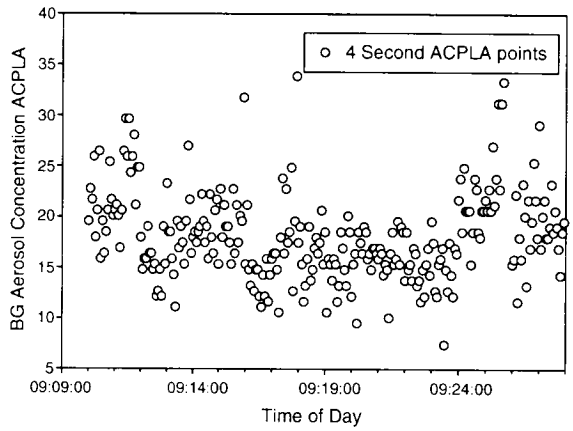


Fig. 4. Typical high resolution profile of the culturable particle concentration measured as 4 s time slices. The whole experimental run was performed in 20 min using an array consisting of 10 slit samplers each sequentially sampling at 2 min each. During colony counting, each plate was divided into 30 sectors each representing 4 s of particle impaction on the agar surface. The operational flow rate (28.3 LPM for Mattson–Garvin design) and the colony counts was used to determine ACPLA.

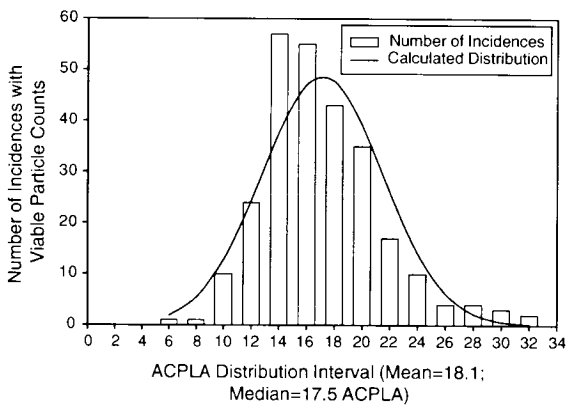


Fig. 5. Typical culturable count frequency distribution showing non-normal characteristics. This is an essential basic test for all microbiological data to determine if non-parametric statistical analysis techniques are required. The median rather than the mean is an appropriate description. Each experimental run produced a median value that described the overall concentration profile that was estimated by the slit sampler design.

as a frequency distribution plot as shown in Fig. 5. Each data set ($n = 300$) for all the sample designs when subjected to normal distribution testing, failed without exception. This confirms similar observation for field trial measurements of biological aerosol using optical methods (Ho et al., 1999). Tillett and Carpenter (1991) also reported that raw epidemiological data in microbiology were not normally distributed, thus recommending the use of non-parametric statistical techniques.

Instead of obtaining the mean over the 20 min sampling period, the appropriate way is to report the median aerosol concentration, expressed in agent containing particles per liter of air (ACPLA). In Fig. 5, the median (17.5) was mathematically derived by the software that performed the distribution calculation. Numerically this was slightly lower than the estimated mean (18.1). For consistency and

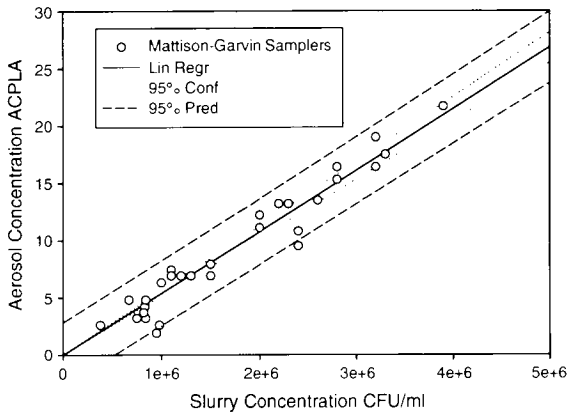


Fig. 6. Typical data plot profile of median values obtained from different experimental runs. Each run was started using a different spore suspension concentration, thus resulting in varying aerosol concentration as shown in this figure. The pooled data when fitted to a linear regression prediction gave normally distributed relationship. This plot demonstrates the results from the Mattson–Garvin sampler.

statistical correctness, all subsequent slit sampler data are reported as median ACPLA, obtained over the sampling period.

Fig. 5 gives a very good illustration of how biological aerosol sampling is best described as a statistical population study where at any given time, the actual concentration can be anywhere between two extremes of the distribution. In this example, there were samples that registered in the low end at 6 while others were in the high at 32 ACPLA. To fully describe biological aerosol characteristics, we recommend that these descriptive treatments should represent minimal requirements.

Each experimental run, using a known concentration of spore suspension, provides one data point that is a statistical derivative of the culturable particle concentration, expressed as the median ACPLA. When the suspension strength was changed, one would expect the median ACPLA to behave correspondingly and the results are summarized in Fig. 6. Given the complexity of the data handling process, we were gratified to see that median ACPLA values appear to increase with increasing suspension concentration. Moreover, when the data points were fitted to a linear regression routine, a respectable coefficient of 0.938 was obtained for the Mattson–Garvin array. Due to the clean background conditions in all experiments (Fig. 3) we have mathematically forced the extrapolated curve to pass through the origin. Another observation was that most of these points were within the 95% prediction boundary and that the transformed data plot was normally distributed. By inspecting the curve, it can be seen that a number of experiments yielded data points close to the 5 ACPLA mark, suggesting that it is possible to repeatedly establish such low biological aerosol concentration in the chamber. Indeed, Fig. 6 provides useful information as the starting point to permit the user to select a desired ACPLA level for detector challenge work. By picking an appropriate suspension concentration, there is reasonable probability that the desired median APCLA can be obtained.

## 4. Performance by other samplers

The results for the other sampler systems are shown in Fig. 7 (Dycor modified), Fig. 8 (HF modified) and Fig. 9 (10 cm design). As mentioned before, the Dycor modified samplers had varying flow rates

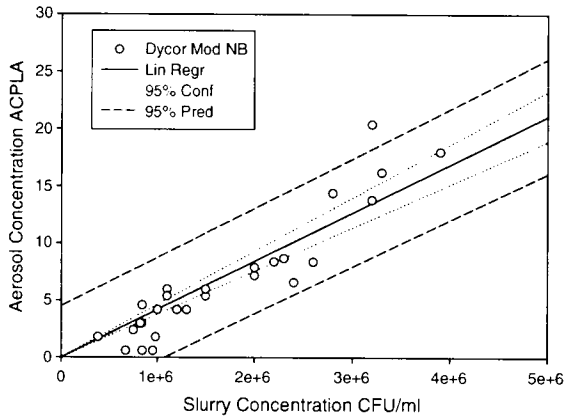


Fig. 7. Plot profile of the Dycor modified slit sampler. In Table 1, it was noted that although 50 LPM was used for calculating the ACPLA values, the actual flow rates varied widely due to dirty slits.

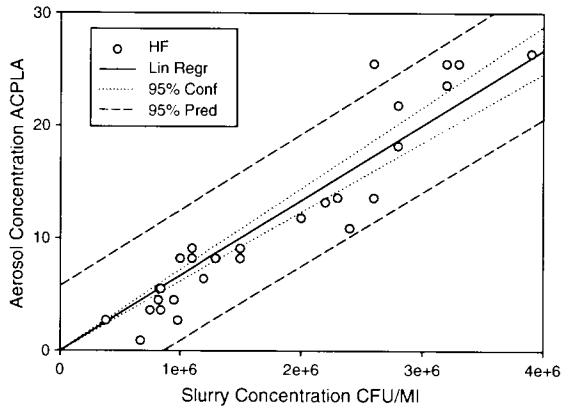


Fig. 8. Plot profile of the HF modified samplers.

(Table 1) but that fact was not known when the calculations were done to produce the plot in Fig. 7. We decided to show the plot unaltered to demonstrate the results of a poorly maintained sampler system. It was rather surprising to note that the composite results did not appear too out of line when compared to the Mattson–Garvin instrument. In particular, Fig. 7 revealed that the Dycor instrument array passed both the normality and variance test. Both the regression coefficient and the standard error were fairly acceptable. The only poor mark was the slope that appeared lower when compared to the HF design.

It was difficult to predict the performance characteristics of a slit sampler designed for a smaller particle capture surface as in the 10 cm plate design when compared to the large surface. One positive observation seen in Fig. 9 was that the regression coefficient was very good (0.918) and that the standard error was very low (0.588). However, when comparing the linear regression slopes of all the systems (Fig. 10), the 10 cm design sampler had the lowest value.

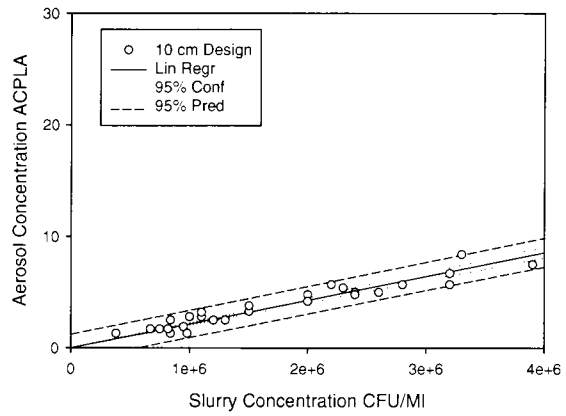


Fig. 9. Plot profile of the 10 cm design samplers.

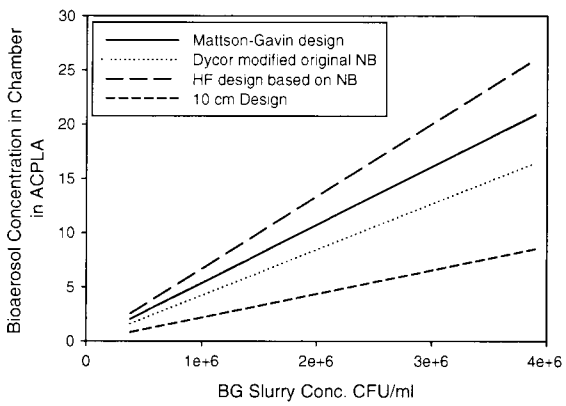


Fig. 10. Summary of the linear regression results from all the samplers under test.

## 5. What does the regression slope mean

The experiments were designed with the expectation that an increase in spore suspension concentration in the generator should lead to a corresponding increase in culturable aerosol particles in the chamber. A perfect slit sampler with 100% collection efficiency would give a straight line plot similar to the data represented in Fig. 10 but its regression slope would have the greatest value. Due to indefinable factors like particle interaction and aggregation, wall losses and fluctuation in airflow patterns in the chamber, real samplers will not collect aerosol at 100% efficiency and consequently, their slopes will be less than this hypothetically perfect device. By the same rationale, the device with the highest slope could be considered the best performer. There is a need to statistically compare the slopes derived from the various instruments and also to determine if there is one that is significantly better than the others. Regression slope values are summarized in Table 2. On inspection, it appears that the HF design produced the highest slope value. Clearly there is a need for an objective way to determine if indeed it is significantly better.

Table 2
Summary of statistical analysis

| Slit sampler | Normality $P$ value | Variance $P$ value | Standard error of estimate ACPLA | Regression coefficient $R^2$ | Slope ACPLA per log slurry |
|---|---|---|---|---|---|
| Mattson–Gavin | 0.325 | 0.922 | 1.413 | 0.938 | 5.4 |
| Dycor mod New Brunswick | 0.670 | 0.632 | 2.075 | 0.853 | 4.2 |
| DRES HF | 0.532 | 0.047 | 2.736 | 0.881 | 6.7 |
| 10 cm design | 0.462 | 0.268 | 0.588 | 0.918 | 2.2 |

## 6. Statistical methods to compare regression slope

Armitage and Berry (1995) described a method to compare two regression lines to determine if the slopes are significantly different. Zar (1998) extended this technique to include a way to compare more than two regression lines at the same time. In this report we applied Armitage and Berry's procedure because it was easier to implement as an Excel spreadsheet function, and standard $t$ distribution tables were available (Fisher & Yates, 1963). The results of comparison between pairs of instruments are shown in Table 3. Some conclusions can be drawn from inspecting this table; it shows that the HF slope is significantly different from that of the 10 cm design at 99.9% certainty. As these instruments registered the highest and the lowest slope values, we interpret the data as representing the two extremes of sampler performance. Similarly, the results in Table 3 also suggest that the HF design performed better than the Mattson–Gavin and the Dycor modified devices. There was no difference between the Mattson–Garvin and the Dycor modified systems.

However, it was noted in Table 1 that the Dycor modified samplers were operating at below the rated flow rates but the ACPLA estimates were done using full flow values. Perhaps with the correct application of the measured flow rates for each instrument in the array, the performance characteristics could be different. We proceeded to apply the proper flow values to determine if its system performance would show up differently. It is interesting to note that when the Dycor modified sampler ACPLA results were recalculated using the correct flow rates from Table 1, the new slope that was obtained (Fig. 11) was the same as that for the HF design (6.7). This made perfect sense because we had used the same physical dimensions as well as the original slit and bowl for construction of both of these instruments.

When these samplers were tested under a different setting in another laboratory, it was confirmed that the 10 cm design was the least efficient (Burke et al., 2001). Moreover, increasing the flow rate from 8 to 25 l/min caused a decrease in performance. The authors explained that the low performance could be due to the smaller impingement surface, hence, resulting in errors related to overlapping of impacting particles. Chang et al. (1995) came to the same conclusion in their studies comparing the Mattson–Gavin versus the Casella samplers. They noted that the collection areas were 112.9 and 46.3 $cm^2$ respectively, making the Casella more susceptible to colony overlap errors. It is obvious that the 10 cm plate design is most suitable for clean air environments that have sparse culturable particle populations.

Overlap error was most apparent when comparing low- and high-resolution samplers. This is shown in Fig. 12 where *Erwinia herbicola* aerosol was disseminated and measured in the field as previously described (Ho et al., 1999). In this figure, the high resolution samplers resolved sharp peaks when brief puffs of aerosol appeared. In contrast, the low resolution plot was represented as broad low-yield curves.

Table 3
Comparison of slopes for the regression lines from various slit sampler designs

| | Mattson–Garvin | Dycor modified | HF | 10 cm design |
|---|---|---|---|---|
| Mattson–Garvin | Not applicable | No difference | 0.02 | 0.001 |
| Dycor modified | No difference | Not applicable | 0.01 | 0.001 |
| HF | 0.02 | 0.01 | Not applicable | 0.001 |
| 10 cm design | 0.001 | 0.001 | 0.001 | Not applicable |

Values given represent significant difference at $P$ taken from standard $t$ distribution tables (Fisher & Yates, 1963). For example, comparing the Dycor modified vs HF designs, a $P$ value of 0.01 means there is a 99% probability that the slopes from each linear regression plot are significantly different. Note that each pair of regression data set was tested to derive a $P$ value (Armitage & Berry, 1995).

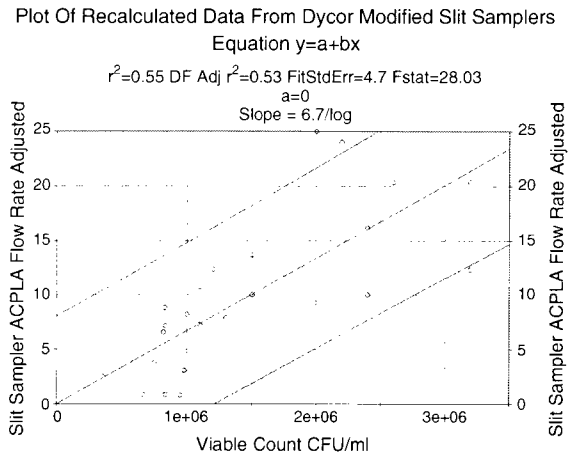


Fig. 11. Replot of recalculated data for Dycor modified slit samplers. Flow rate values for each sampler indicated in Table 1 were applied to readjust the ACPLA results to give better representation of sampler performance. The linear regression line is shown with accompanying 90% prediction boundaries. Graph and statistics were generated by using TableCurve 2D fitting the data to a straight line equation.

Most of the cloud events appeared to be coincident time-wise. However at some events, for example 14:22:30, 14:24:00 and 14:28:30 where extremely narrow puffs were present, the low-resolution sampler hardly registered.

Most surprisingly, when they did register, the ACPLA levels were only about 20% of those from the high-resolution equivalent (to be used later as a correction factor for low-resolution instruments). This has very serious implications in that much of present and historical knowledge on biological aerosols have been obtained from interpreting observations with low-resolution slit samplers. We have discovered that sampling environmental microbial content in the tropics of northern Australia could easily overwhelm a low-resolution slit sampler (data not shown). This became a major problem when some of the bacterial colonies contained "swarming" organisms that cause rapid spreading of boundaries making accurate counting difficult.

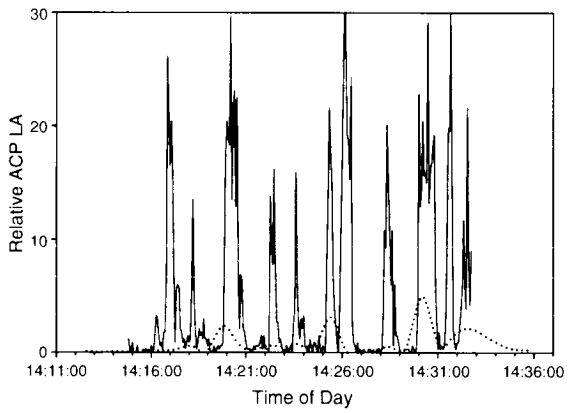


Fig. 12. Measurement of a biological aerosol cloud using two kinds of slit sampler design. The low resolution instrument (dots) is a conventional New Brunswick sampler running at the standard 24 min per revolution. The high resolution instrument (solid) was running at 2 min per revolution. Both ran at similar flow rates.

## 7. Towards deriving an instrument transfer function

The preceding discussions give the impression that the various slit samplers have definable performance characteristics and that each one can be summarized by its linear regression and slope (Fig. 10). If indeed each instrument should actually follow the slope function throughout the range of aerosol concentration then by dividing the slope of one candidate instrument by that of the others, a transfer function is obtained. For example, using the HF and the 10 cm design to illustrate, and using the slopes from Table 2 we get (6.7/2.2) 3.1 as the transfer function. By multiplying any performance value from the 10 cm design by the transfer function, the resultant value would be equivalent to that obtained by the HF instrument.

Similarly, a transfer function can be derived for other instruments, thus allowing corrections to be made to instrument performance under ideal conditions. Perhaps this might lead to a way to correct for the data obtained from historical slit samplers that operated at less than optimal efficiency. Indeed, even for modern instruments like the Mattson–Garvin, a transfer function of 1.24 can be used to estimate "true" ACPLA values. Although not strictly recommended, one could use the data from Fig. 13 to derive a slope value if only the slit length of a sampler is known. By this exercise, it may be possible to extract a transfer function to apply to correcting historical data. To compensate for low-resolution characteristics, another correction factor of 5 should be included to take care of colony overlap errors. Thus the final formula for correcting for a short slit length sampler running at slow turntable speeds would take this form:

"True ACPLA" = measure ACPLA × transfer function × 5.

As an application illustration we use the Casella slit sampler that has a slit length of 28 mm. From Fig. 13, an estimate of the slope is 3.7. This will give a transfer function of 1.8 (6.7/3.7). Assuming it has a low-resolution turntable, a correction factor of 9.05 (1.8 × 5) is derived. In biological work, a measurement change by factor of about 10 is a significant occurrence. It would appear that when reviewing biological aerosol literature concerning Casella data, a conversion factor of this magnitude might be called for. Future chamber trials should include a Casella sampler to verify this prediction. One caveat that must be stated is that the assumptions made above are for instruments of 0.15 mm slit width. Larger slit widths would require slight adjustments to take into account differences in inlet velocity. In final analysis, this

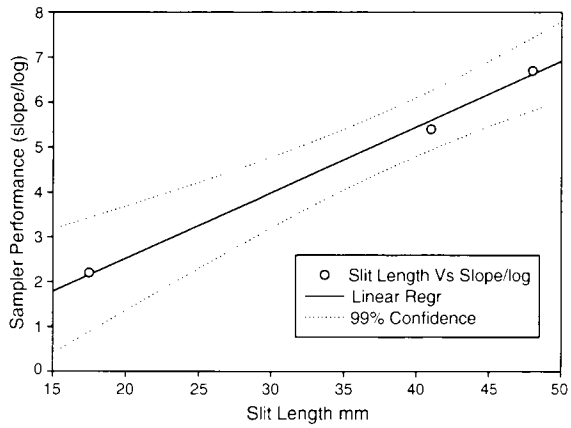


Fig. 13. Relationship between slit length and sampler performance. The figure is derived from 4 data points with identical entries for the 48 mm instrument representing the two New Brunswick design modifications.

transfer function methodology approach may have very narrow applicability in that different instrument designs are expected to have unique efficiency characteristics. Thus what is introduced here should serve as a guideline for workers who require a way to interpret historical slit sampler data.

## 8. Conclusion

We have examined the performance characteristics of different slit sampler designs, primarily ones with different slit length and impaction surface area. Having a short slit length design allows the use of smaller diameter petri plates for low cost particle collection. Although this may have economic advantages, there are definite compromises in terms of performances as depicted in Fig. 13. Here it can be seen that there is a relationship between slit length and regression slope, the latter previously defined as a measure of sampling efficiency. We have also introduced a method to objectively compare regression slopes as means to grade sampler performance. We recommend that future work with slit samplers should include verification that data collection has not been distorted by colony overlap errors. Previous workers (Upton, Mark, Douglass, Hall, & Griffiths, 1994) have been concerned with sampling errors while using the slit sampler in horizontal laminar air flow like in a wind tunnel. Fortunately, in field application, horizontal laminar flow conditions are rarely encountered in nature. We suggest that colony overlap errors may pose real problems when working in natural settings where biological content density can be difficult to predict. Finally, we have defined the concept of transfer functions as a way to correct for slit sampler design deficiencies, which may provide a means to correct either historical or current data. The data obtained here demonstrated that the HF design performs better than the others. We attribute this to better quality control in the assembly of the units more so than inherent design characteristics. Lastly, we would like to mention that the Armitage and Berry (1995) method of slope comparison was extremely helpful in objectively defining which instrument performed better than another. It is surprising that the method has not been used more widely in science since much of scientific measurements can be described as linear regressions.

## References


Anderson, K., Morris, G., Kennedy, H., Croall, J., Michie, J., Richardson, M. D., & Gibson, B. (1996). Aspergillosis in immunocompromised paediatric patients: associations with building hygiene, design, and indoor air. *Thorax, 51*, 256–261.

Armitage, P., & Berry, G. (1995). *Statistical methods in medical research*. (3rd ed.), Blackwell Scientific.

Bourdillon, R. B., Lidwell, C. M., & Thomas, J. C. (1941). A slit sampler for collecting and counting airborne bacteria. *Journal of Hygiene, 14*, 197–224.

Burke, J. S., Platt, S. D., Reid, K., & Whatley, P. R. (2001). Slit sampler trial report phase 2, Porton Down. DERA/DSTL/TR00959/1.0, UK. (copy of this paper is available by writing to: Dstl Detection Porton Down Wiltshire SP4 0JQ jsburke@dstl.gov.uk)

Casewell, M. W., Desai, N., & Lease, E. J. (1986). The use of the Reuter centrifugal air sampler for the estimation of bacterial air counts in different hospital locations. *Journal of Hospital Infection, 7*, 250–260.

Casewell, M. W., Fermie, P. G., Thomas, C., & Simmons, N. A. (1984). Bacterial air counts obtained with a centrifugal (RCS) sampler and a slit sampler—the influence of aerosols. *Journal of Hospital Infection, 5*, 76–82.

Chang, C. W., Grinshpun, S. A., Willeke, K., Macher, J. M., Donnelly, J., Clark, S., & Juozaitis, A. (1995). Factors affecting microbiological colony count accuracy for bioaerosol sampling and analysis. *American Industrial Hygiene Association Journal, 56*, 979–986.

Chatigny, M. A., Macher, J. M., Burge, H. A., & Solomon, W. R. (1989). Sampling airborne microorganisms and aeroallergens. In S. V. Hering (Ed.), *Air sampling instruments for evaluation of atmospheric contaminants* (pp. 199–200) (7th ed.) publ. American Conference of Governmental Industrial Hygienists, Inc.

Fisher, R. A., & Yates, F. (1963). *Statistical tables for biological, agricultural and medical research*. (6th ed.), Edinburgh: Oliver and Boyd.

Groschel, D. H. (1980). Air sampling in hospitals. *Annals of the New York Academy of Sciences, 353*, 230–240.

Hedges, A. J., Shannon, R., & Hobbs, H. D. (1978). Comparison of the precision obtained in counting viable bacteria by the Spiral Plate maker, the droplette and the Miles & Misra methods. *Journal of Applied Bacteriology, 45*, 57–65.

Heeg, P., & Kanz, E. (1975). A bacteriological study of the air in a surgical intensive care unit. *Praktische Anaesthesie, Wiederbelebung Und Intensivtherapie, 10*, 125–135.

Henningson, E. W., & Ahlberg, M. S. (1994). Evaluation of microbiological aerosol samplers: a review. *Journal of Aerosol Science, 25*, 1459–1492.

Ho, J. (1989). Design of a chamber for CBW aerosol studies with relative humidity and particle concentration control (U). Suffield Memorandum No. 1271.

Ho, J. (1991). Characteristics of Simulant Aerosols for Study of the BCD Inlet Nozzle. DRES Suffield Report No. 543.

Ho, J., Spence, M., & Hairston, P. (1999). Measurement of biological aerosol with a fluorescent aerodynamic particle sizer (FLAPS): correlation of optical data with biological data. *Aerobiologia, 15*, 281–291.

Ho, J., Spence, M., & Ogston, J. (2001). Characterizing biological aerosol in a chamber: an approach to estimation of viable organisms in a single biological particle. *Aerobiologia, 17*, 1–12.

Jericho, K. W., Ho, J., & Kozub, G. C. (2000). Aerobiology of a high-line speed cattle abattoir. *Journal of Food Protection, 63*, 1523–1528.

Kiplinger, D. V. (1996). Air sampler. United States Patent 5,500,369.

Lach, V. (1985). Performance of the surface air system air samplers. *Journal of Hospital Infection, 6*, 102–107.

Larsen, L. S. (1981). A three-year-survey of microfungi in the air of Copenhagen 1977–79. *Allergy, 36*, 15–22.

Morris, G., Kokki, M. H., Anderson, K., & Richardson, M. D. (2000). Sampling of Aspergillus spores in air. *Journal of Hospital Infection, 44*, 81–92.

Placencia, A. M., Peeler, J. T., Oxborrow, G. S., & Danielson, J. W. (1982). Comparison of bacterial recovery by Reuter centrifugal air sampler and slit-to-agar sampler. *Applied and Environmental Microbiology, 44*, 512–513.

Smid, T., Schokkin, E., Boleij, J. S., & Heederik, D. (1989). Enumeration of viable fungi in occupational environments: a comparison of samplers and media. *American Industrial Hygiene Association Journal, 50*, 235–239.

Swenson, E. A. (1998). Remote sampling device for determining air borne bacteria contamination levels in controlled environments. United States Patent 5,831,182.

Tillett, H. E., & Carpenter, R. G. (1991). Statistical methods applied in microbiology and epidemiology. *Epidemiology and Infection, 107*, 467–478.

Tjade, O. H., & Gabor, I. (1980). Evaluation of airborne operating room bacteria with a Biap slit sampler. *Journal of Hygiene (London), 84*, 37–40.

Upton, S. L., Mark, D., Douglass, E. J., Hall, D. J., & Griffiths, W. D. (1994). A wind tunnel evaluation of the physical sampling efficiencies of three bioaerosol samplers. *Journal of Aerosol Science, 25*, 1493–1502.

Verhoeff, A. P., van Wijnen, J. H., Boleij, J. S., Brunekreef, B., van Reenen Hoekstra, E. S., & Samson, R. A. (1990). Enumeration and identification of airborne viable mould propagules in houses. A field comparison of selected techniques. *Allergy, 45*, 275–284.

Zar, J. H. (1998). *Biostatistical analysis*. (4th ed.), Englewood Cliff, NJ: Prentice-Hall.

#523766

CA026008