Comparative Evaluation of Three Active Air Samplers

for the Monitoring of Airborne Microorganisms

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A comparative study of three air samplers used for bioaerosol collection was performed to evaluate the average recovery of colony-forming units and to assess the precision of each device.

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The evaluation of airborne microorganisms is critical in the biopharmaceutical industry. For manufacturers that produce drugs in an aseptic environment, cleanrooms must meet strict standards. Regulatory requirements such as the US Food and Drug Administration's *Guidance for Industry, Sterile Drug Products Produced by Aseptic Processing: Current Good Manufacturing Practice* and internal corporate policies require monitoring to verify that these standards are maintained. Cleanroom monitoring is dependent upon reliable instruments that are suitable for sampling airborne contaminants in an aseptic environment. These instruments must also be calibratable, portable, and easy to use.

In this study, three active air samplers were evaluated for the collection of culturable airborne microorganisms. Bioaerosol collection includes whole microorganisms as well as fragments, toxins, and particulate waste products from all varieties of living things (1).

Materials and methods

Air samplers. The following air samplers were studied:

- The "SAS Super 180" sampler (Bioscience International, Rockville, MD) aspirates air at 180 L/min through a solid 304 stainless steel sampling head with 219 precision-drilled holes positioned over the agar at a prescribed distance. After impaction, air exits through an exhaust screen. The unit can accommodate either a standard 55/84-mm contact plate or a 90-mm standard Petri dish below the sampling head. The sampler is fully programmable for features such as time between aspirations, volume of air for each aspiration, and total air volume to be sampled.
- The "RCS Plus" (Biotest Diagnostics Corp., Denville, NJ) is a centrifugal impactor that draws air through the top of the instrument at 50 L/min. The air exits the unit through exhaust ports. The sampler is designed for unidirectional airflow that minimizes turbulence, thereby permitting sampling in laminar flow environments. The unit uses a proprietary air strip containing 34 wells each measuring ~1 cm^2. The sample volume is programmable between 1 and 1000 L of air.
- The "Air Ideal" sampler (Bio Merieux Inc., Hazelwood, MO) is an air sampler based on air impaction. The air is aspirated

Table I: Estimates from the mixed-effects model of average recovery and standard deviation of the replicate measurements (within day) of the airborne culturable bacteria concentration by air sampler in $\log_{10}$(cfu/ft^3) units.

Air sampler	Average response*			Standard deviation*		
	Lower bound	Estimate	Upper bound	Lower bound	Estimate	Upper bound
SAS 180	–0.66	–0.49	–0.32	0.20	0.25	0.32
Air Ideal	–0.61	–0.46	–0.32	0.19	0.24	0.31
RCS Plus	–0.49	–0.31	–0.14	0.16	0.21	0.27

*Estimates are reported with 95% confidence intervals.

Table II: Estimates from the mixed-effects model of average recovery of the concentration of airborne culturable bacteria by air sampler in units of cfu/ft^3.

Air sampler	Average response*		
	Lower bound	Estimate	Upper bound
SAS 180	0.22	0.32	0.48
Air Ideal	0.25	0.34	0.48
RCS Plus	0.33	0.49	0.72

*Estimates are reported with 95% confidence intervals.

by a turbine at 100 L/min through a perforated surface. The holes form air jets that force the particles onto the agar placed underneath the grid. The unit can accommodate either a 90-mm standard Petri dish or a 65/70-mm contact dish. The sample unit is programmable for delay time and sample volumes.

Methods. When comparing various air samplers, the device properties of each piece of equipment must be taken into account (2). The three air samplers that were evaluated have various flow rates. Therefore, all instruments were run for the same period of time, not the same aspiration volumes. The same period of time was used to obtain each sample because of the varying population of airborne organisms from minute to minute. All sampling was conducted indoors in a nonclassified laboratory area of nonmicrobiological practices. The room's windows remained closed at least 24 h before measurements were taken. The opening of doors was minimal. Sampling times were chosen at random throughout the day and over a period of several days.

Before the study, the three air samplers were checked for flow rates using traceable air flow meters. The samples were checked by the vendor or by in-house metrologists. All instrument surfaces and accessories were wiped clean with filter-sterilized 70% isopropyl alcohol. Each sampling instrument was programmed to sample for 10 min. The RCS Plus and Air Ideal samplers were aseptically loaded with sterile or irradiated trypticase soy agar (TSA) media. The Air Ideal sampler was evaluated with a 90-mm Petri dish media. The SAS 180 sampler was aseptically loaded with irradiated 55-mm contact plates containing TSA and lecithin and polysorbate 80 neutralizers.

The three air samplers were placed at three designated sites, one meter apart, with the sample ports facing the same direction, at approximately the same level. At most times, activity in the room sampled was kept to a minimum. One minute after placing the units at their respective sampling locations, the air-sampling units were simultaneously started. The SAS 180 and Air Ideal samplers have delay-time programming features that allow for this time lag. This feature was programmed manually for the RCS Plus device. The delay in sampling minimized the variability in results because it minimizes the number of times an operator must enter the sampling environment. When the sampling time was complete, the TSA was removed from each sampler and transferred into a 32 °C (±2.5 °C) incubator. Each sampler surface was wiped with 70% isopropyl alcohol to prepare for the next sampling. This procedure was repeated two more times on the same day, rotating air samplers among the room sites between replicates. A total of nine samples were taken each evaluation day. The evaluation continued for 15 days, with a total of 135 samples taken at the end of the evaluation.

All TSA plates and strips were incubated at least 72 h at 32 °C (±2.5 °C). Unexposed media were also included in the incubation as part of standard laboratory controls. (A positive recovery from any of the unexposed media would have invalidated that day's worth of test results.) After incubation, colonies were enumerated and recorded.

Data analysis. The results were converted to colony-forming units (cfu) per volume (m^3) of air sampled; a statistical correction was made to counts from the SAS 180 and Air Ideal using the positive hole conversion table by Feller (3). When the recovered count increases in sieve-type samplers, the probability increases that more than one contaminant is propelled through the same aperture onto the plate and is counted as a single colony-forming unit. Feller's correction factor adjusts the result for this possibility. The counts were adjusted, recalculated as cfu per cubic foot (cfu/ft^3), and transformed to $\log_{10}$ for data analysis purposes. The logarithm transform was used in past studies (4, 5).

The objectives of the data analysis were to compare the air samplers on their average recovery of colony-forming units and to evaluate their precision. An air sampler with a high recovery may be considered more sensitive than an air sampler with low recovery. Precision measures the variability in replicated measurements. When replicated measurements have low variability, the precision of the air sampler is said to be high and *vice-versa*. The three daily measurements were made in less than an hour while the laboratory was sealed and were considered as replicate measurements in the analysis for the purpose of estimating the precision.

Mixed-effects models, a generalization of an analysis of variance model, were used to analyze the data (6). A mixed-effects model was used to model two sources of variation: the day-to-day variation in the number of colonies in the room and the variation in the within-day measurements. Mixed-effects models were used to estimate the average cfu recovery, the variation in the replicate measurements, and to predict the true cfu in

the laboratory. The assumptions of the mixed-effects model, normality and constant variance, were verified with QQ-plots and residual plots not shown in this article (7). Data analysis was performed using "S-PLUS V.6.2" software (Insightful Corp., Seattle, WA).

Results

A mixed-effects model was made for each air sampler. Estimates from these models, average recovery, and standard deviation of the replicate measurements are listed in Table I in units $\log_{10}(\text{cfu/ft}^3)$ by air sampler with 95% confidence intervals. The recovery data expressed in $\log_{10}(\text{cfu/ft}^3)$ and the average recoveries are plotted in Figure 1 according to air sampler. Estimates of the recovery on the original scale (cfu/ft^3) are given in Table II. The standard deviations were interpretable only within the context of the model in which the logarithm of the data was taken and were not expressed on the original scale.

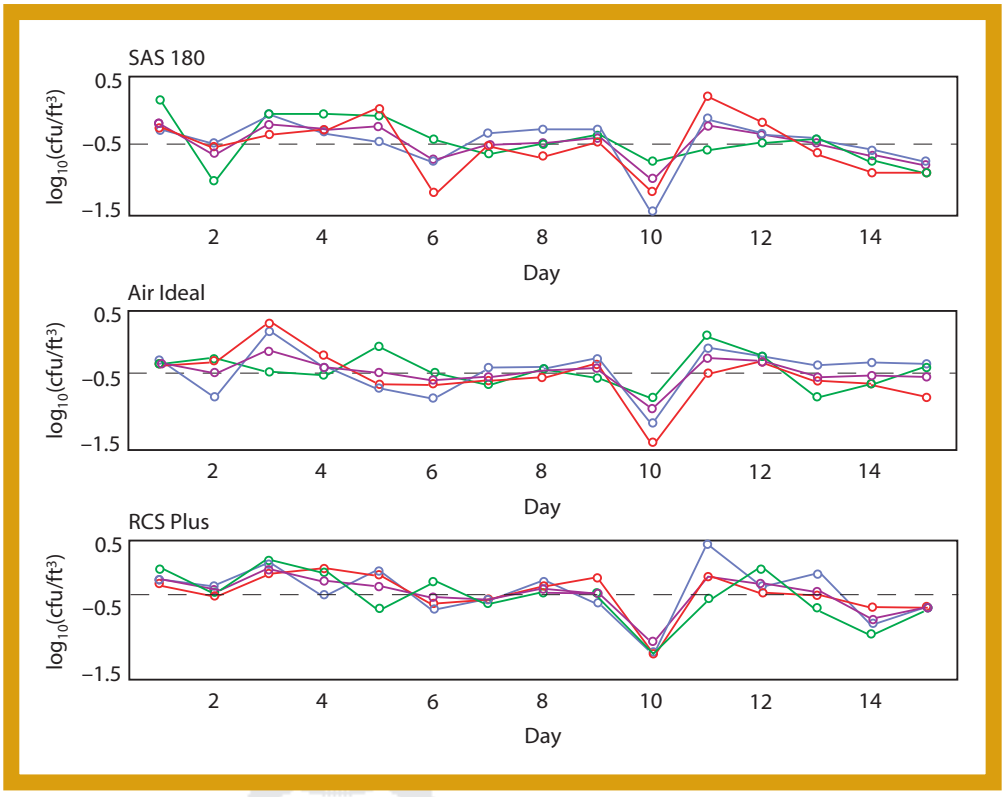

Figure 1: $\log_{10}(\text{cfu/ft}^3)$ by day. Replications are indicated by 1, 2, and 3. The dotted line is the estimated average recovery from the mixed model.

The average recovery of the SAS 180 and the Air Ideal samplers were very similar: 0.32 and 0.34 cfu/ft^3, respectively. The recovery of the RCS Plus air sampler was 0.49 cfu/ft^3. The difference between the RCS Plus and the SAS 180 was 0.17 cfu/ft^3 and the difference between the RCS Plus and the Air Ideal was 0.15 cfu/ft^3. These differences were statistically significant ($p = 0.0003$ and $p = 0.0021$) in a mixed-effects model that incorporated the data from all the air samplers.

The standard deviations of the within-day measurements of the three air samplers were very similar, thus indicating that the air samplers had a very similar level of precision. The daily predictions of the true cfu/ft^3 ranged from 0.06 to 1.18 cfu/ft^3. Assertions made here concerning recovery and precision are valid over this range of colony-forming units.

Conclusion

Distinct patterns are apparent from the evaluation of this study's results:

- The Air Ideal and SAS 180 samplers had similar recoveries of colony-forming units per standard volume.
- All three samplers demonstrated a high level of precision based on the replicates.

Two of the air samplers (Air Ideal and SAS 180) had similar recoveries of colony-forming units per standard volume. On average, the SAS 180 and the Air Ideal produced recoveries of 0.32 and 0.34 cfu/ft^3, respectively. Given this similarity, the Air Ideal and SAS 180 instruments may give companies leeway and flexibility in determining the purchase and implementation of air samplers. Businesses can choose between two samplers that, from this study, produce relatively interchangeable cfu/ft^3 results.

The RCS Plus sampler produced slightly higher amounts of colony-forming units than the Air Ideal and SAS 180 instruments, with a recovery of 0.49 cfu/ft^3 (0.17 and 0.15 cfu/ft^3 greater than the recovery of the SAS 180 and Air Ideal, respectively). Although these variations make little difference when choosing an air sampler for monitoring in less environmentally stringent areas that do not directly influence product quality (*e.g.,* nonclassified laboratories, manufacturing hallways, or breezeways), these variations were statistically significant, are worth noting, and must be taken into account when monitoring in a classified or regulated manufacturing areas. Moreover, areas that directly influence product manufacturing (*e.g.,* Class 100A parenteral drug filling areas, open processing systems) may need special consideration when choosing an air sampler.

Air temperature, pressure, moisture content, wind speed, and turbulence are a few of the environmental factors that can affect the overall performance of an air sampler (1, 8). An air sampler's ease of use, reliability, functionality with a widely available collection media, overall cost, durability, portability, battery life, ability to be cleaned and sanitized, diagnostics, and ability to maintain consistent flow rates while avoiding leaks also should be evaluated when determining which air sampler to use.

Although the three air samplers in this study monitored various levels of cfu/ft^3 on the average when compared with each

other, all three samplers produced similar variance within daily test sessions. The variance among each set of three replicates from the RCS Plus, Air Ideal, and SAS 180 units are similar. This result is significant because it shows that all three samplers were equally affected by each day's environment. Even though the nonclassified laboratory room contained unknown amounts of cfu/ft^3 that prevented the determination of the accuracy of each air sampler, the results from the replicates of each air sampler had little deviation, indicating that each sampler demonstrated a high level of precision.

The true cfu/ft^3 results have been validated for an environment with a 0.06–1.18-cfu/ft^3 range. Depending on the true cfu/ft^3 level of an environment, results may vary and may not be extrapolated from this study. Environmental monitoring provides the most accurate results when they are performed over long periods of time with large volumes of air being sampled (2). Consistent use of an air sampler provides more accurate results than a single snapshot from multiple air samplers. Accurate environmental monitoring is dependent upon trending and not individual data points. A business or company looking to implement a new environmental-monitoring program should not sacrifice consistency for technology.

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