Improved Recovery of Airborne Microorganisms

In clean areas using a portable air sampler

Abstract

A new portable air monitoring system, recently developed at Millipore Corporation, provides improved recovery of airborne microorganisms. The M Air T™ system is based on the Anderson principle and uses a sieve with about 1,000 micro-perforations, which reduces the potential for overlapping colonies and minimizes the desiccation of the medium.

The system incorporates a unique cassette design that employs a larger agar volume and depth along with a flat surface, which, in combination with a stepwise increase in air velocity, allows consistent particle size impaction. Up to 1,000 L (1 m^3) of air can be sampled within seven minutes. The cassette surface rapidly rehydrates after impaction thereby renewing the water activity and reducing further effects of drying on organisms present.

The M Air T System was compared to the United States Pharmacopeia's recommended method, Slit-to-Agar (STA) sampling, and two other portable systems in two test areas. Recoveries were equivalent to the Slit-to-Agar impactor and significantly higher than the other portable systems in the test area with a significant microbial population. Recoveries were equivalent for all samplers in the M 3.5 (Class 100) clean room. The M Air T System provides an alternative to other sampling devices for monitoring airborne microorganisms where both portability and sensitivity are needed.

Introduction

Microbiological monitoring of air is a critical component of the environmental monitoring program of any pharmaceutical, biotherapeutic, or medical device manufacturer. Air monitoring is also a Good Manufacturing Practice (GMP) requirement for both the United States and Europe.

Air monitoring provides information on the quality of the processing environment during manufacturing and enables the study of microbiological air quality trends. A critical component of air monitoring is the selection and validation of the microbiological air sampler.

The selection of a sampler typically entails comparison to an "industry standard." Such a comparison is difficult as the mechanisms of particle capture and the particle capture cutoff-size may vary among air samplers. Further, to obtain accurate results the devices must be oriented to minimize positional bias and air turbulence due to sampler exhaust.

The purpose of this study was to compare the performance of a new microbial air sampler (M Air T), to the U.S. industry standard (Slit-to-Agar or STA) and two other widely used air samplers. The samplers were tested in areas with two different levels of cleanliness.

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Materials

- Four active microbiological air samplers were selected for evaluation (refer to Table 1 for operating parameters).
  - –M Air T Air Tester (Millipore Corporation, Bedford, MA)
  - –RCS PLUS Air Sampler (Biotest Diagnostics, Denville, NJ)
  - –RCS High Flow Air Sampler (Biotest Diagnostics, Denville, NJ)
  - –Slit to Agar Biological Air Sampler, STA 203 (New Brunswick Scientific Co., Edison, NJ)
- Air Current Tubes (Dräger, Sicherheitstechnik, Germany)
- Recovery agar was standard Soybean Casein Digest Agar (also known as Tryptic Soy Agar or TSA). Multiple lots of cassettes, strips, and dishes were used.
  - –M Air T TSA Cassettes for M Air T (Millipore Corporation, Bedford, MA)
  - –Biotest TSA Test Strips for RCS PLUS and High Flow (Biotest Diagnostics, Denville, NJ)
  - –TSA dehydrated powder reconstituted and sterilized according to manufacturer's instructions at 65 mL per 150 mm Petri dish for STA 203 (Difco, Detroit, MI)

Method

A two-factor, four-level, full-factorial blocked design was used for this study. The factors were Air Tester (M Air T, RCS PLUS, RCS High Flow, STA) and Test Position (P1, P2, P3, P4). The four samplers were tested simultaneously as a group. After all samplers completed sampling 1 m^3 (1,000 L) of air, the samplers were switched for test position. One test block consisted of all air samplers tested in all test positions for a total of 16 tests in four groups. Six test blocks were completed in a single experiment for a total of 96 tests in 16 groups.

Five experiments each were conducted in a controlled-access, but unclassified, testing room (room volume 87 m^3) and in a Class 100 clean room (room volume 70 m^3). All samplers were placed at least 1 m from the ceiling and 1 m from each other (except between positions 2 and 3 in the clean room, which were 0.76 m apart). While the samplers operated simultaneously, their orientation was tested for exhaust turbulence and other potential air current disturbances using Dräger air current tubes.

All TSA agar plates, cassettes, and strips were weighed before and after testing. All agar was incubated in humidified environments for 48 hours at 35 °C followed by 72 hours at 25 °C. Samples from the unclassified test room were enumerated at both 48 and 72 hours. Samples from the clean room were enumerated only at the completion of the two-step incubation.

Statistical Evaluation

Plate counts were transformed to the square root of the count and analyzed using a general linear ANOVA for a DOE design for all air samplers (α 0.05). An orthogonal pairwise analysis was performed on: M Air T versus STA, RCS High Flow versus RCS PLUS, and grouped M Air T/STA versus grouped RCS High Flow/RCS Plus. The results were also confirmed by a non-parametric analysis using the ranks of the square root of the counts (rank of the count). In addition, a prevalence analysis was performed on the clean room data. In this case, all zero cfu results were coded as "0" and all positive cfu results were coded as "1." All data were analyzed using Minitab release 12.2 (Minitab, Inc., State College, Pennsylvania).

Table 1.

Properties of the Four Microbial Air Samplers

	M Air T (Millipore)	STA-203 (New Brunswick Scientific)	RCS High Flow (Biotest)	RCS PLUS (Biotest)
Particle capture mechanism	Impaction	Impaction	Centrifugal impaction	Centrifugal impaction
Sampling volume (maximum)	1,000 L	3,000 L	1,000 L	1,999 L
Time to sample 1,000 L (1 m^3)	6.5 min (140 L/min 1st 500 L, 180 L/min 2nd 500 L)	20 min (50 L/min)	10 min (100 L/min)	20 min (50 L/min)
Water loss after sampling 1 m^3*	4.2%	4.3%	10%	13%
Particle diameter cutoff size (d_{50})	3.5 µm	0.5 µm	2-5 µm	2-5 µm
Aspiration mechanism	Impeller below agar cassette	Vacuum required	Impeller perpendicular to agar strip	Impeller perpendicular to agar strip
Agar volume	34 mL	65 mL	8 mL	8 mL

*In an unclassified environment with 53% relative humidity. Sample size was 120 per tester.

Results

Overall results for the unclassified room studies showed a significant difference among samplers at both incubation enumeration points and a significant difference among test positions. The P-values for these analyses are shown in Tables 2 and 3.

Overall results for the clean room showed a significant difference in test position but no significant difference in recovery among air samplers. Because of the high number of zero cfu results, the rank of the square root of the counts (rank of the counts) was used in the clean room analysis. The orthogonal analysis was performed on the final time-point counts for the clean room. As expected, the clean room results indicated no significant difference among pairs of samplers (Table 4 and Figure 1).

The overall analysis for the unclassified room suggested a significant difference in recovery for certain pairs of samplers. An orthogonal analysis performed on the 48-hour and 72-hour plate counts demonstrated no significant difference between the M Air T and the STA samplers and no significant difference between the RCS PLUS and RCS High Flow samplers. There was a significant difference in recovery between the grouped M Air T/STA and the grouped RCS PLUS/RCS High Flow data (Figure 2).

Table 2.

P-Values for DOE Overall Analysis

	Unclassified Room 48 hr at 35 °C SQRT* of count (n=479)	Unclassified Room 72 hr at 25 °C SQRT of count (n=478)	Clean Room Rank of count (n=480)
P-value tester	0.000	0.000	0.628
P-value position	0.032	0.035	0.000
MSE	1.385	1.499	10778

*SQRT denotes square root.

Table 3.

P-Values for Orthogonal Analysis

		M Air T vs. STA	High Flow vs. PLUS	M Air T/STA vs. High Flow/PLUS
Clean Room (rank of count)				
	Sample size	240	240	480
	P-value tester	0.243	0.598	0.714
	P-value position	0.033	0.004	0.000
	MSE	2675	2586	11049
Unclassified room, 48 hr at 35 °C (square root of count)				
	Sample size	239	240	479
	P-value tester	0.772	0.675	0.000
	P-value position	0.078	0.250	0.032
	MSE	1.495	1.359	1.388
Unclassified Room, 72 hr at 25 °C (square root of count)				
	Sample size	239	239	478
	P-value tester	0.166	0.552	0.000
	P-value position	0.043	0.282	0.035
	MSE	1.848	1.190	1.507

Table 4.

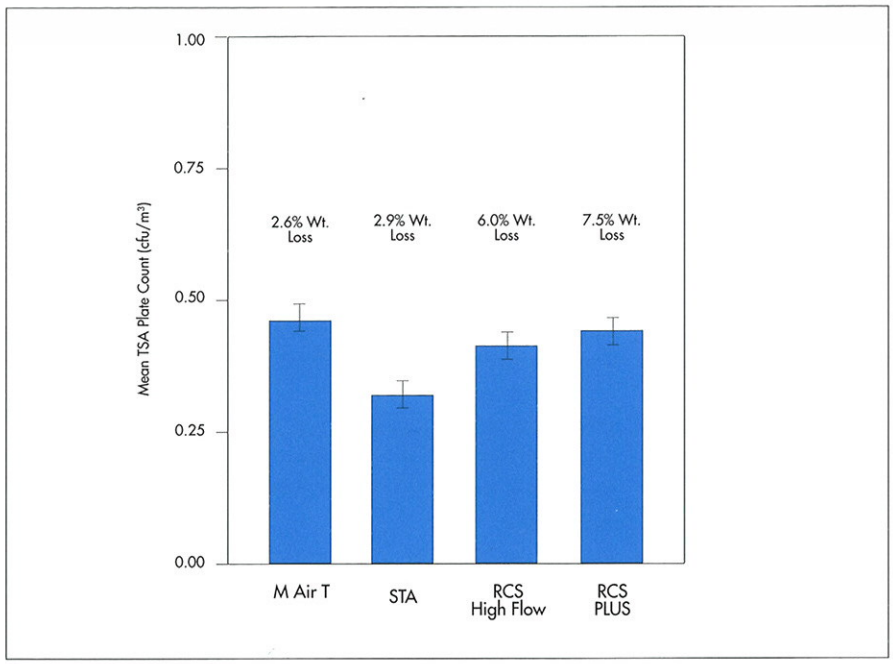
Prevalence of Positive Results for Three Microbial Air Samplers Against the Industry Standard Slit-to-Agar in a Clean Room Environment (Class M3.5)*

	STA +	STA –	Total
M Air T +	10	26	36
M Air T –	19	65	84
Total	29	91	
RCS PLUS +	13	23	36
RCS PLUS –	16	68	84
Total	29	91	
RCS High Flow +	5	27	32
RCS High Flow –	24	64	88
Total	29	91	

*Sampler recovery agar with colony forming units was scored as a positive. Sampler recovery agar without growth was scored as a negative.

Figure 1.

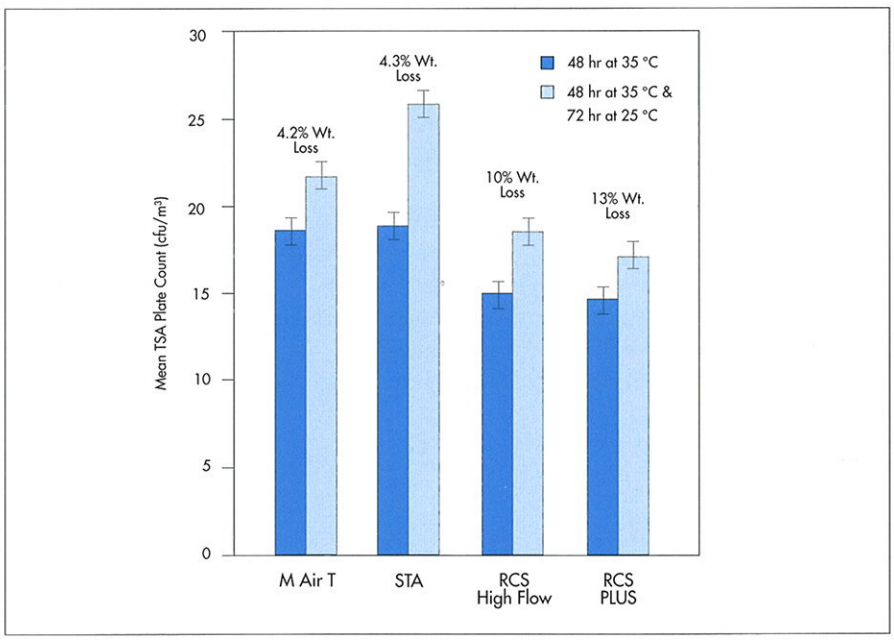
Mean Recovery and Standard Error for Four Different Microbial Air Samplers Operated Simultaneously in a Clean Room (M 3.5).



Conditions: 53% relative humidity; 48 hr incubation at 35 °C followed by 72 hr incubation at 25 °C; 120 samples per sampler.

Figure 2.

Mean Recovery and Standard Error for Four Different Microbial Air Samplers Operated Simultaneously in an Unclassified Test Room.



Conditions: 53% relative humidity; 48 hr incubation at 35 °C versus 48 hr incubation at 35 °C followed by 72 hr incubation at 25 °C.

Discussion

A significant increase in plate counts was observed during the two-stage incubation of 48 hour at 35 °C followed by 72 hour at 25 °C for the unclassified area. Since there were so few colonies recovered in the clean room, the plates were only counted at the end of the two-stage incubation.

The percent weight loss in the unclassified room appeared to be related to recovery. M Air T and STA had lower percent water loss and higher microbial recovery as compared to RCS PLUS and RCS High Flow. However, this relationship was not established in the clean room environment. While the RCS PLUS and the RCS High Flow had higher percent weight loss, the four samplers were not significantly different for recovery.

The lack of relationship between percent weight loss and recovery in the clean room is due to the low incidence of contamination. The mean untransformed plate count for the samplers in the clean room ranged from 0.32 to 0.45 cfu (n=120 per sampler). The mean final incubation plate count (untransformed) in the unclassified room ranged from 18 to 26 cfu. The clean room trials resulted in a significant amount of zero cfu results. The low counts, coupled with the zero cfu results, may not enable the prediction of differences in recovery among samplers or establish a relationship between recovery and percent weight loss in the clean room environment.

Conclusions

- Test position was found to have a significant effect upon microbial recovery. By using a proper test design with switching of air samplers among positions, this bias was eliminated from the test results.
- The low incidence of microbial colonies in the clean room study contributed to no significant difference among samplers in this environment.
- When higher counts are encountered, a statistically significant difference in sampler recovery is observed. In this environment, M Air T is equivalent to STA and more sensitive than RCS PLUS and RCS High Flow.
- Increased sensitivity is important, particularly if the sampler is used in a variety of testing environments or if a test area encounters spikes in microbial counts.

Authors

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