Evaluation of One Aspirating Head Versus Three Aspirating Heads Using a Microbial Air Sampler in a Sterile Medical Product Manufacturing Area

Authors: R. Ligugnana[1], A. Ligugnana[1], K. Spallino[2], A. Klavins[2], R. Malherbe[2], A. Hsiung[2], J. Hardy[2]
1. Orum International, Milan, Italy; 2. Hardy Diagnostics, Santa Maria, CA, United States.

Abstract

The objective of this study is to determine if there is a difference in CFU results when using a microbial air sampler with three aspirating heads instead of one. The regulatory and good manufacturing practice standards applied in a cleanroom setting require that contamination monitoring is performed "at rest", as well as "in operation", during each shift. Collecting samples at rest and during the work shift is important in order to analyze air samples that accurately reflect the microbial load in the air throughout the day. Clean rooms should be evaluated regularly, as mandated by USP <797>, in order to assess the overall air quality by setting a baseline, tracking trends, and taking appropriate actions in the event of high microbial load. Increased bacterial or fungi recovery in a cleanroom may be affected by factors like the number of operators working in the room, air conditioning hygiene, operator manipulations, and cleaning procedures. When using a single head active air sampler, the operator using it is involved several times in the air sampler preparation and culture plate transfer. Additionally, the air sampler can be programmed and prepared before or after operations (at rest) as well as during operations. The operator hands-on time and risk of contamination can be reduced with the use of three aspirating heads instead of one aspirating head on a microbial air sampler.

Glossary

Active air sampler, CFU, Culture plate, Fraction number, Fraction time, Grade D, QA, Settle plate, Shift, SOP, USP<797>.

Introduction

During a typical sample collection using the single head air sampler, the operator must return to the unit multiple times to activate sample collection and transfer culture plates. The air samplers draw in the surrounding air through the aspirating heads at a constant flow rate. The surrounding air will flow over the culture plate mounted inside the aspirating head with controlled velocity (impaction). The impaction of air allows for a larger volume of air to quickly flow over the agar surface while heavier particles, such as viable organisms, make impact with the agar surface in the culture plate. This is considered an active sampling method, and is recommended over passive air sampling methods (settle plates). After the air samples are collected, the culture plates are incubated in the appropriate temperatures for subsequent analysis of organism growth. The standard media used are Tryptic Soy Agar plates for bacteria and Sabouraud Dextrose (SabDex) agar plates for fungi and molds.

Materials

- One aspirating head TRIO.BAS™ model MONO active microbial air sampler
- Three aspirating heads TRIO.BAS™ model TRIO active microbial air sampler
- Three aspirating heads TRIO.BAS™ ISOLATOR RABS unit with 3 independent satellites
- Tryptic Soy Agar Petri plates

Methods

Sampling plans are written prior to beginning routine environmental programs and are developed based on the size of the area, number of hoods, and take into account the risk assessment. In order to document the number and type of microorganisms on a regular basis, it is necessary to follow a Standard Operating Procedure (SOP) each time. Designated sample areas that may be considered include the laminar flow benches, biosafety cabinet, isolator, restricted access barrier systems, buffer zone, gowning areas, and anteroom areas. Different hours of sampling were programmed by applying different "delay", "fraction time", "fraction number" settings. The data from each cycle are stored in the TRIO.BAS air sampler models and can then be transferred to a personal computer. Air samples can be programmed and prepared to start monitoring at a particular time, with culture plates collected at the end of the shift. Each air sampler's aspiration flow rate is 200 liters of air per minute.

The same room, Grade D (similar standard to USP anteroom or an ISO 8, class 100,000), was monitored for 10 days over 5 weeks with the same number of personnel. The working shift was 4 hours and 31 minutes. The TRIO and MONO air samplers (ORUM International, Milan) were programmed to take three aspirating fractions (toal of 1,000 liters of air) one hour before the beginning of working activity.

The 3 culture plates (90mm Tryptic Soy agar) were introduced at the same moment into the 3 aspiration chambers prior to air sample collection for the TRIO model. After the air was sampled, the 3 culture plates were simultaneously collected and incubated at 32°C for 48 hours.

For the MONO model, the culture plate was collected at the end of each fraction cycle and a new plate introduced for the execution of the following cycle until all three samples were collected. All three culture plates were transferred to the incubator after all fractions were collected (32°C for 48 hours).

Results

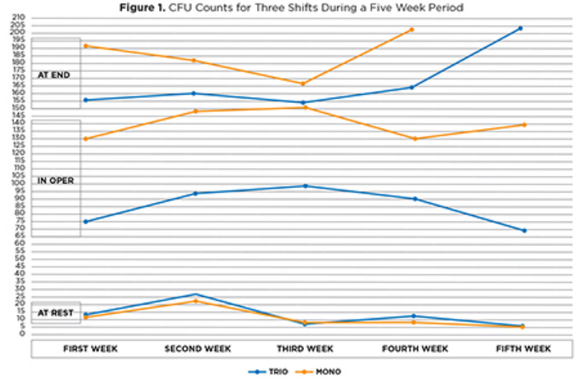
After sampling 1,000 liters of air, there was a difference in the CFU count between the TRIO and MONO air samplers. This discrepancy in CFU count indicates that the MONO air sampler may provide a falsely increased CFU count compared to the TRIO due to removal of the aspirating head by the operator between each air sample. The time and the risk of the contamination can be reduced with the use of a 3 aspirating head sampler like the TRIO.

Table 1. Air Sampler Results

Day of testing[1]	Shift time	CFU counts from total culture plates	
		TRIO	MONO
Day 1 and 2	At rest	13	12
	In operation	65	125
	At the end of activity	155	195
Day 3 and 4	At rest	27	22
	In operation	85	145
	At the end of activity	160	184
Day 5 and 6	At rest	7	8
	In operation	91	148
	At the end of activity	153	167
Day 7 and 8	At rest	12	8
	In operation	81	125
	At the end of activity	164	207
Day 9 and 10	At rest	6	5
	In operation	58	135
	At the end of activity	208	Too numerous to count

[1]TRIO and MONO air samplers were tested on alternating days.

Figure 1. CFU Counts for Three Shifts During a Five Week Period



- TRIO and MONO samples during the "at rest shift" showed similar numbers of CFUs and averaged 13 and 11 CFU per 1,000 liters of air, respectively.
- TRIO samples during the "in operation shift" were lower in comparison with the MONO. The average for the TRIO was 82 CFU per 1,000 Liters of air whereas the MONO resulted in an average of 135 CFU per 1,000 Liters of air.
- TRIO samples during the "end of operation shift" were lower in comparison with the MONO. The average for the TRIO was 128 CFU per 1,000 Liters of air whereas the MONO resulted in an average of 187 CFU per 1,000 Liters of air.
- The comparison of the results for in operation and end of operation shifts show that the CFU counts are lower when the 3 heads air sampler (TRIO) is used.
- The increased operator hands on time with MONO air sampler may lead to falsely increased CFU counts.

Conclusions

This study suggests that the use of three aspirating heads, such as the TRIO model, will deliver more accurate results for airborne microbial detection and monitoring, in order to maintain high accuracy in quality assurance for the manufacture and processing of sterile medicinal products.

References

European Standard Draft prEN17141 – Cleanrooms and associated controlled environments – Biocontamination control.

The SOP – Standard Operating Procedure for the environmental monitoring can be downloaded from **www.TRIOBAS.com** or requested from ORUM International SRL. Milan, Italy.