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Research Article

Comparative Evaluation of the Collection Efficiency of Seven Different Air Samplers to Monitor Airborne Viable Microorganisms

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Abstract

The study describes the results of a series of comparative experiments aimed at determining the differences in the ability to collect bacteria and fungi colonies by seven different impaction air samplers. The tests were performed simultaneously under identical environmental conditions in a "clean" room routinely used for cell culture or in a biochemistry room generally used for chemical experiments in the microbiological research laboratory of the University of Milan. The air flow in the rooms was switched-off for all the time of the experiments. The seven different air samplers were positioned on a cart, side by side, and operated simultaneously to collect 1m^3 of atmosphere each. The results demonstrated that the numbers of airborne microorganisms impacted on TSA-containing Petri dishes, and grown as single colonies (CFU/m^3), were different for each air sampler, although the difference was not statistically significant. Head to head tests were also performed with two identical TRIO.BAS apparatuses calibrated to 100 or 200 litres of aspirated air per minute. This test aimed at determining if a shorter aspiration time could negatively influence the cell viability and/or the bacterial concentration in the bioaerosol, as determined by counting the number of CFU/m^3. The data ruled out this possibility and suggest that an aspiration time of 200 litres per minute might save time, especially when a repeated air sampling is mandatory for the control of sterility in virology laboratory "clean rooms", pharmaceutical manufacturing areas and surgical rooms in the hospitals.

Keywords: *Bioaerosol Sampler Comparative Study; Active Impactors; Airborne Microorganisms; Bioefficiency*

Abbreviations

S.B.E.: Sampling Biological Efficiency; TSA: Trypticase Soy Agar; CFU: Colony Forming Units; ANOVA: Analysis of Variation; BSL2/3: Bio-Safety Level 2/3

Introduction

The air samplers used to determine the amount of airborne microbial population in hospitals and laboratory clean rooms draw the airflow and accelerate it through a series of holes or a slit, and direct it to impact the surface of a nutrient agar medium. The plate, containing nutrient agar, is then incubated at a suitable temperature and for an adequate time, and the microbial colonies are counted to ascertain the number of microbes collected from a given volume of air. The first microbial air samplers were already designed and produced in the '40s and '50s [1-3] to efficiently impact single microbial cells with a size of about 1 - 5 µm. This size was chosen since air samplers were

designed by investigators working in the field of bacteriology and mycology, whereas viruses have a very small mass and cannot replicate on acellular synthetic substrates. Furthermore, it was found [4] that the majority of microbes in a laboratory room come from aerosols and fragments of skin cells dispersed by workers in the room. Indeed, the most popular microbial air samplers used to monitor the quantity of viable airborne particles in outdoor and indoor environments are by impact and are called "active". The most relevant source of biological contamination is represented by viable microorganisms as bacteria, fungi and spores, that can be found almost everywhere in the atmosphere. Due to their extremely small size, these viable particles can remain suspended in the air for long periods of time. Ljungqvist and Reinmuller [5] compared a selection of air samplers currently used to monitor the airborne microbial community, and demonstrated that some of them can collect up to 10 times less microbes than others. This result implies that when a low efficiency sampler is used, unhygienic rooms seem to achieve the correct cleanliness standard. More recently [6], a detailed review of bioaerosol sampling devices, mechanisms, bioefficiency and operational considerations was published. This study showed that active single-stage impactor sampling systems are the most suitable for microbial collection and enumeration both in terms of the number of colony forming units (CFU) for a specified volume of aspirated air and for the duration of a specified time-period (i.e. CFU/m^3/10 min). The efficiency of an air biosampler to collect inhalable particles during sampling can be formulated, considering its ability to collect the highest number of viable microorganisms (biological collection efficiency). In the recent European Standard Guideline document [7] the requested volume of aspirated air in a low contamination environment is 1.000 litres (1 m^3) and the time for each cycle of sampling is 10 minutes. For a pharmaceutical industry that performs hundreds of tests, 10 minutes per each sampling means a lot of time for the operator in charge of this duty. By combining the aspiration of 200 litres per minute and air samplers endowed with 2 or 3 aspirating chambers, the time invested by the operator can be drastically reduced. Therefore, to save time, the volume of aspirated air per minute has been doubled from 100 litres to 200 litres per minute in the TRIO.BAS air samplers. The specific aim of this research was to determine the sampling efficiency (SE) of seven different biological samplers by characterising their performance expressed as numbers of CFU grown on the surface of Trypticase Soy Agar (TSA) medium in 9-cm plates [8,9]. The results should enable investigators to make informed decisions about the choice of a bioaerosol sampler and its application in monitoring the quantity and quality of airborne microbial community in laboratory settings.

Materials and Methods

Active sampling devices

The comparative study was conducted indoor by simultaneous air sampling using seven different impaction microbial air samplers. The different performance of the samplers was evaluated by counting the numbers of CFU grown on agar plates after aspiration of 1 m^3 of ambient atmosphere. The seven air samplers we compared are the following:

- SENNON SW1 (SENNON Technologies, China), 400 holes sampling head
  (Figure a)
- MAS-100™ (MBV AG, Merck Millipore, CH), 400 holes sampling head
  (Figure b)
- VWR® (PBI, Italy), 400 holes sampling head
  (Figure c)
- TRIO.BAS™ 100 (ORUM, Italy), 400 holes sampling head
  (Figure d)
- TRIO.BAS™ 200 (ORUM, Italy), 400 holes sampling head
  (Figure e)
- AWEL BC100 (AWEL International. France), 400 holes sampling head
  (Figure f)
- Air IDEAL® (BioMérieux, France), 400 holes sampling head
  (Figure g)

Figure a

Figure b

Figure c

Figure d

Figure e

Figure f

Figure g

All of the sampling units were battery operated and fully portable. The instruments were factory calibrated to aspire 100 litres of air per minute and were equipped with a single aspiration head. However, the TRIO.BAS (ORUM) air sampler was used in two different versions, calibrated for 100 liters (TRIO.BAS™ 100) or 200 litres (TRIO.BAS™ 200) of aspirated air per minute, respectively. The experimental protocol was the same for all of the devices and was performed according to the instruction manual and operated following the most recent European Standard Guidelines Draft prEN17141. Before starting, the air sampler heads were disinfected with 70% ethyl alcohol, and loaded with TSA-containing plates. The culture Petri dishes inserted into the air samplers were 9 cm in diameter and contained Trypticase Soy Agar (TSA) with lecithin and polysorbate 80 (BD, Becton Dickinson, Germany) standard growth medium. The seven samplers were positioned in a BSL2/3 high-containment room, in which a negative pressure air flow is usually present . The BSL2/3 is generally devoted to the manipulation of virus-infected cell cultures. Alternatively, the samplers were used in a biochemistry room, where only non-HEPA filtered and conditioned air flow is insufflated. After sampling, the TSA plates were aseptically removed from the samplers and transferred to an incubator for 48 h at the temperature of 30°C. The CFU were counted manually. The purpose was to evaluate the total bacterial colony counts of the microbial population in the air of different rooms of the laboratory (biochemistry rooms and cell culture rooms). To evaluate the viable airborne particles of the natural bacterial population of the air, the tests were performed in the Microbiology Laboratory at the Dept. of Medical Biotechnology and Translational Medicine of the State University of Milano, only in the presence of the operator. During all of the tests, the air conditioning was switched-off to limit the HEPA-filtered air movements. During the experiments, the temperature and the relative humidity of the air in the laboratory rooms were at 20°C and 50%, respectively. All of the instruments were positioned on a cart table and the sampling starting time was simultaneous.

The statistical analyses of the data (CFU/m^3) were performed using the one-way ANOVA parametric tests [10] and Bonferroni analysis of variance [11], with the GraphPad Prism software, version 2.0 [12].

Results and Discussion

In the present study, the seven different impactors were evaluated and compared side-by-side, for their ability to collect the highest number of microorganisms growing as colonies on TSA plates under identical environment conditions and sampling. Although other studies have already been performed to comparatively evaluate the S.B.E. of different bioareosol samplers [13,14], the rationale of our experimental approach was based on the different structure of the seven air samplers, which were manufactured to collect bioaerosol particles and give rise to a proportional number of CFU on TSA plates to determine the total count of microorganisms. The results of ten different tests are reported in Figure 1, where the total CFU/m^3 of aspirated air is cumulatively expressed together with their relative standard deviation (S.D.) values for each impact sampler. The CFU values per m^3 are based upon the positive-hole Count Correction Equation [15].

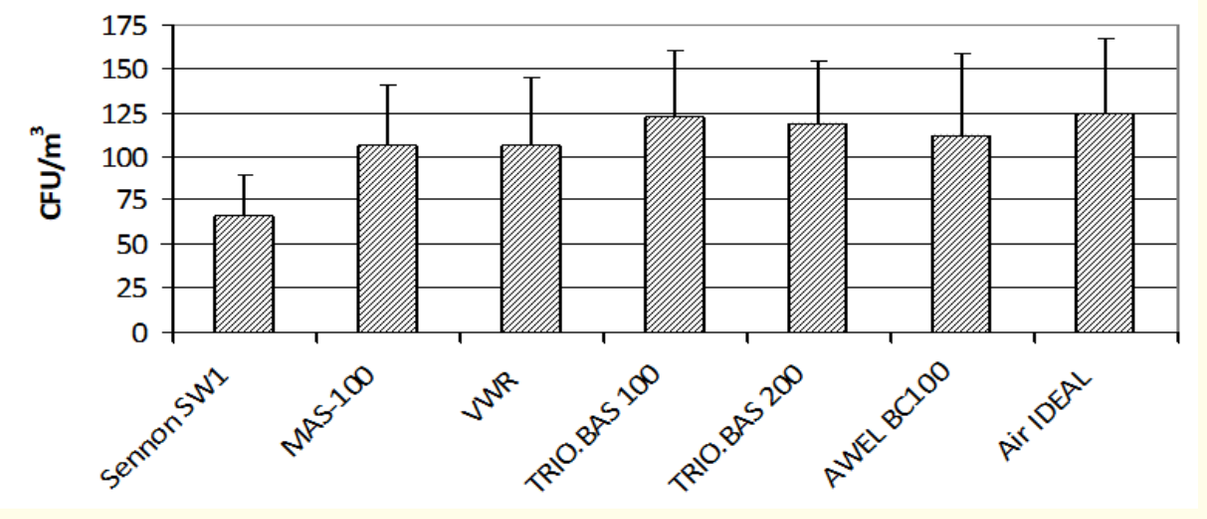

Figure 1: *Average of total CFU/m^3 of aspirated air during ten different samplings.*

Comparison of the sampling efficiency among seven different air samplers

With the exception of the Sennon SW1 sampler, which showed the lowest number of colonies/plate (66,4 ± 22,8), all the others samplers, MAS-100 (106,7 ± 33,7), VWR (106,1 ± 38,9), TRIO.BAS 100 (122,6 ± 37,0), TRIO.BAS 200 (118,9 ± 35,3), AWEL BC100 (111,7 ± 47,1) and Air IDEAL (124,7 ± 42,7), displayed a higher, but statistically non-significant (p value > 0.05), number of colonies/plate, expressed as CFU/m^3 of aspirated air.

Comparison of the S.B.E. of TRIO.BAS samplers at 100 *vs* 200 litres/minute

This test was performed with two identical TRIO.BAS samplers (ORUM). The TRIO.BAS™ 100 (100 litres per minute) and TRIO.BAS™ 200 (200 litres per minute) were separately compared. This experiment is a part of the one performed with the seven air samplers. In this specific test the two TRIO.BAS samplers were positioned in a cell-culture room (cleanroom) or in a biochemistry room in the presence of the operator and the air conditioning switched-off. The results are shown in Table 1 and expressed as CFU/m^3 ± S.D.

Experiment no.	TRIO.BAS™ 100	TRIO.BAS™ 200
1 (Clean Room)	33	36
2 (Clean Room)	51	80
3 (Biochemistry Room)	119	105
4 (Biochemistry Room)	137	115
5 (Clean Room)	67	69
6 (Clean Room)	80	87
7 (Biochemistry Room)	122	107
8 (Biochemistry Room)	136	147
9 (Biochemistry Room)	150	169
10 (Biochemistry Room)	169	140
Mean CFU/m^3 ± S.D.	106.4 ± 45.6	105.5 ± 39.7

Table 1: *Total CFU/m^3 of air aspirated by TRIO.BAS air samplers calibrated to 100 (TRIO.BAS™ 100) or 200 (TRIO.BAS™ 200) litres/minute.*

For each sampling point, the statistical analyses showed that the variability among the total number of CFU/m^3 detected by the different air samplers is not statistically significant. In this study, the seven single-stage inertial impaction samplers, commonly used for microbiological sampling and manufactured by different companies, were evaluated and compared in side by side tests using natural bioaerosols in a real environment setting of a laboratory "cleanroom" or biochemistry room.

Conclusions

The determination of the microbiological quality of the air is vital in sites where pharmaceuticals and medical devices are manufactured, surgical rooms and other critical areas in hospitals and food processing facilities. In many cases, in these locations only low concentrations of airborne microorganisms are present, which means that large air volumes (1 m^3) have to be sampled to collect sufficient numbers of microorganisms for a proper quantitative assessment to be made. Losses can occur either due to the sampler failure in capturing particles containing microorganisms (physical loss) or due to inactivation of viable microorganisms during collection, so that the formation of visible colonies on agar surfaces will not occur (biological loss). The seven air samplers we compared are impactor instruments based on the principle described by Andersen [3], in which air is aspirated through a plate, perforated with a pattern of 400 small

holes. The resulting air streams containing microbial particles are directed onto the agar surface of a standard Petri dish (9 cm diameter). When the sampling cycle is completed, the Petri dish is removed and incubated. Viable organisms, which form visible colonies, will be counted. In this study, seven portable high flow single-stage impaction-based air samplers were compared for airborne bacteria sampling.

The statistical evaluation of the data, obtained in two separate experiments, showed that:

1. The numbers of CFU/m^3 impacted on TSA growth plates are higher in TRIO.BAS 100, TRIO.BAS 200, AWEL BC100 and Air-IDEAL air samplers than in Sennon SW1, MAS-100 and VWR samplers, although this difference is not statistically significant.
2. The impact of the microorganisms of a laboratory indoor environment on TSA growth agar surface at two different aspiration speeds of 100 or 200 litres/minute does not influence the bacterial cell viability and the numbers of CFU/m^3.
3. The air samplers with an aspiration volume of 200 litres per minute can also be employed without compromising the correct number of microbial colonies and the viability of the impacted airborne microorganisms.
4. The TRIO.BAS microbial air samplers can be considered qualified active impaction devices according to the updated ISO Standard prEN 17141: 2017 for Cleanroom and Associated Controlled Environment.

Conflict of Interest

The authors declare no conflicts of interest.

Bibliography

1. May KR. "The cascade impactor: An instrument for sampling course aerosols". *Journal of Scientific Instruments* 22.10 (1945): 187-195.
2. May KR and Harper GJ. "The efficiency of various liquid impinger samplers in bacterial aerosols". *British Journal of Industrial Medicine* 14.4 (1957): 287-297.
3. Andersen AA. "New sampler for the collection and enumeration of viable airborne particles". *Journal of Bacteriology* 76.5 (1958): 471-484.
4. Davies RR and Noble WC. "Dispersal of bacteria on desquamated skin". *Lancet* 2.7269 (1962): 1295-1297.
5. Ljungqvist B and Reinmuller B. "Airborne viable particles and total number of airborne particles: comparative studies of active air sampling". *Journal of Pharmaceutical Science and Technology* 54.2 (2000): 112-116.
6. Haig CW., *et al*. "Bioaerosol sampling: sampling mechanisms, bioefficiency and field studies". *Journal of Hospital Infection* 93.3 (2016): 242-255.
7. European Standard Draft prEN17141. Cleanrooms and associated controlled environments-Biocontamination control. ICS 13.040.35 (2017).
8. Benbough JE., *et al*. "Determination of the collection efficiency of a microbial air sampler". *Journal of Applied Bacteriology* 74.2 (1993): 170-173.
9. EPA/600/R-12/646 Determination of the sampling efficiency of biosamplers to collect inhalable particle (2012).
10. Kim HY. "Statistical notes for clinical researchers: Sample size calculation 3. Comparison of several means using one-way ANOVA". *Restorative Dentistry and Endodontics* 41.3 (2016): 231-234.

11. Armstrong RA. "When to use the Bonferroni correction". *Ophthalmic Physiological Optics* 34.5 (2014): 502-508.

12. Graph Pad Prism. GraphPad Software.

13. Lach V. "Performance of the SAS air sampler". *Journal of Hospital Infection* 6.1 (1985): 102-107.

14. Jensen PA., *et al*. "Evaluation of eight bioaerosol samplers challenged with aerosols of free bacteria". *American Industrial Hygiene Association Journal* 53.10 (1992): 660-667.

15. Macher JM. "Positive-hole correction of multiple-jet impactors for collecting viable microorganisms". *American Industrial Hygiene Association Journal* 50.11 (1989): 561-568.

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