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A COMPARATIVE STUDY: HOLE AND SLIT LID PERFORMANCE IN ACTIVE AIR SAMPLING

WHITEPAPER

ABSTRACT

Air samplers play a crucial role in monitoring the microbial air quality, and their design significantly impacts their performance. Most air samplers rely on the impaction method, where air is collected through a perforated lid featuring either holes or slits.

MBV has always supplied its MAS-100® air samplers with perforated lids using holes. However, in search of the optimum solution, the experiment described in this whitepaper attempts to answer the question of how an alternative approach using slits would perform.

The experiment was conducted with the MAS-100 NT® air sampler using three different lids: the standard lid with 300 holes, and two lids with 20 slits each, differing in slit widths and lengths.

The collection efficiency of both lids with slits was found to be significantly lower compared to the perforated lid. To be able to explain this observation, several factors that have physical and biological effects on the sampling result were examined.

The most likely factor for a lower collection efficiency when using lids with slits was found to be the higher risk of local desiccation, as less of the nutrient plate surface area is used.

INTRODUCTION

Various methods can be used for active microbiological contamination control. These include impaction, membrane filtration, impinger, or online measurement devices (e.g. BFPC, biofluorescence particle counters). The most common method is impaction where the samplers aspirate air through a lid with holes or slits, it is accelerated and undergoes a rapid lateral change of direction; this causes suspended particles to impact onto the detection surface due to their mass inertia (Stewart et al. 1995). Among other factors, the size of the holes (sieve samplers) or the width of the slits (slit samplers) influences the collection efficiency of the air sampler.

There are no standards for the design of air inlets in lids, which has led to several different strategies. However, both the physical and biological collection efficiencies are influenced by the lid design, and ISO 14698-1/Annex B and the new EN 17141 define requirements to be fulfilled.
MBV has always supplied its MAS-100 air samplers with lids with holes, following the established approach of the Andersen sampler (Andersen 1958). However, always looking for the best solution, MBV wanted to quantify how an alternative approach with slits would perform.

This whitepaper is based on data from experiments performed with the MAS-100 NT air sampler using three different lid designs: one lid with holes and two lids with different slits. The results are presented and discussed with regard to the underlying physical and biological principles.

MATERIAL & METHODS

MATERIAL

Test instruments:	3 MAS-100 NT with a flow rate of 100 standard liters per min (SLPM) ± 2.5%. All calibrated with the MAS-100 Regulus® anemometer (± 1% reproducibility, temperature and pressure compensated)
Lids (Figure 1):	Perforated lid: 300 holes with a diameter of 0.6 mm (MBV AG)[1] Slit lid 1: 20 slits with a width of 0.2 mm and a length of 17.0 mm (MBV AG) Slit lid 2: 20 slits with a width of 0.3 mm and a length of 14.0 mm (MBV AG)
Nutrient plates:	Casein Soya Bean Digest (CASO) nutrient plates (90 mm), article number 1.46050.0120, Merck KGaA Darmstadt

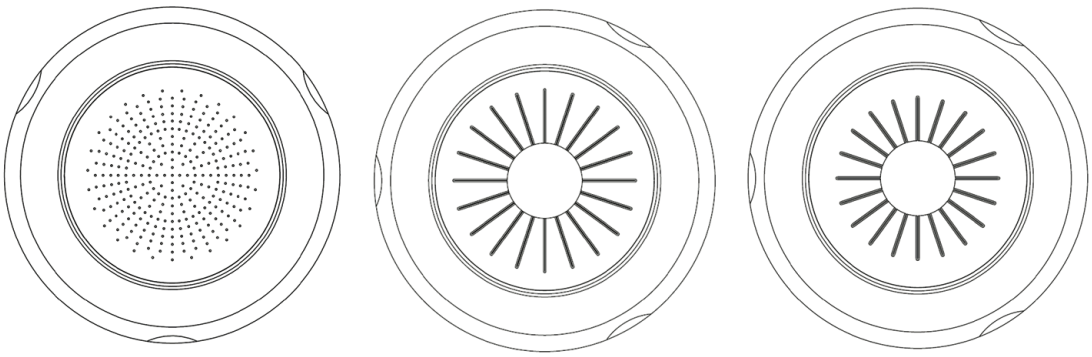

FIGURE 1: Graphical presentation of the three different lids used: Sampling lid with radial arrangement of 300 holes with a diameter of 0.6 mm (left); sampling lid with radial arrangement of 20 slits (0.2 mm width, 17 mm length (middle) and 0.3 mm width, 14 mm length (right)).

SAMPLING PROCEDURE

The test environment was an unclassified area with the air conditioning system turned off. The air samplers were placed on tables, at a distance of 1 m from each other, approximately 80 cm above the floor. The three lids were used for sampling at the same time. On two different days, a separate series of 15 measurements per lid was performed, resulting in a total sample size of N = 30 for each lid type. To avoid positional effects, the position of the samplers was changed after three consecutive measurements.
Temperature and pressure were monitored with the MAS-100 Regulus calibration unit.

The plate holders and the perforated lids were spray disinfected prior to use. To prevent secondary contamination, the operator wore gloves that were regularly spray disinfected. Immediately before the start of each experiment, the nutrient plates were labeled and inserted into the instruments. All instruments were set up with a 1-minute delay and a 200 L sampling volume. While measuring, the operator remained stationary and stood at least four meters away from the instruments. After sampling, the nutrient plates were removed, sealed with parafilm, and incubated at room temperature (20-25 °C) for a period of 7 days. After incubation, the colony forming units (CFU) were counted manually.

[1] Standard lid for the MAS-100 NT air sampler.

EVALUATION OF SAMPLING RESULTS

Two response parameters were analyzed: the counted colony forming units (CFU) and the Feller-corrected colony forming units (Feller-corrected CFU).
Statistically, there is the probability of multiple microorganisms entering the same hole or slit, forming only one CFU instead of multiple. This leads to an underestimation of CFU. To correct for this a statistical adjustment called Feller-correction is applied to the counted CFU on the nutrient plate.
The Feller-correction for the perforated lid was applied using the table published by MBV (MBV 2023). To apply the Feller-correction to the slit lids, the equivalent number of holes was calculated based on the slit diameter:

- For the 0.2 mm slit, the equivalence of 85 holes of 0.2 mm per slit was assumed, resulting in a total of 1700 holes of 0.2 mm across 20 slits (refer to equation (1)):

$$\text{Number of ‚holes' for the 0.2 mm slit} = \frac{17.0\ \text{mm}}{0.2\ \text{mm}} \times 20 = 1700 \quad (1)$$

- For the 0.3 mm slit lid, the equivalence of 933 holes was assumed (refer to equation (2)):

$$\text{Number of ‚holes' for the 0.3 mm slit} = \frac{14.0\ \text{mm}}{0.3\ \text{mm}} \times 20 = 933 \quad (2)$$

This approach for slit lids is suggested by PMS (2023). However, this is controversial, since the physical properties of slits and holes differ drastically and the correction factor is negligible or not appropriate for slits since a clear separation of nearby impacted colonies in slit lids is not feasible (Climet 2023; PMS 2023).

To obtain normally distributed data and equal variances (homoscedasticity), the data were $\log_{10}$ transformed. An Analysis of Variance (ANOVA) was applied to the log-transformed data, with lid type as main factor and sampling day, sampler position and time as co-variates. The Tukey-Kramer Honest Significant Difference (HSD) test was used for pairwise comparisons between the three lids. Statistical analysis was performed using the statistical software JMP SAS (version 5.1.2).

RESULTS

Overall, the CFU count was within a favorable range for evaluation, without zero counts or significant outliers. The average CFU count for each lid is depicted in Figure 2, revealing a statistically significant higher CFU count for the perforated lid (cyan) compared to the two slit lids (gold and yellow) (F-ratio = 20.82, $p < 0.0001$). This effect is even more pronounced considering the Feller-corrected CFU count (F-ratio = 33.43, $p < 0.0001$) shown in Figure 3. This is attributed to the increased effect of the Feller-correction on the perforated lid compared to the slit lids.

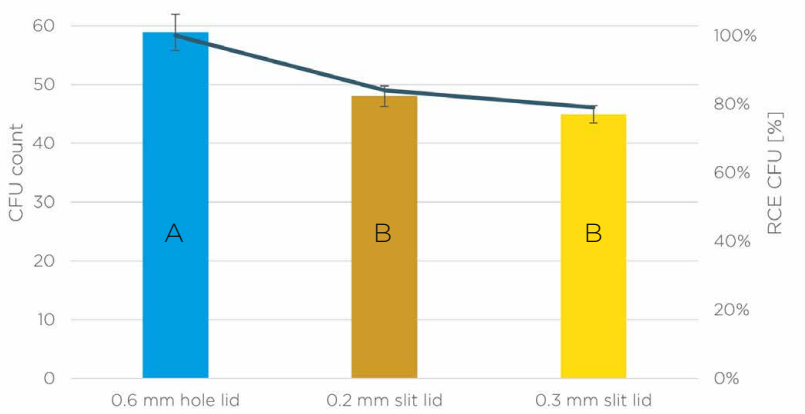

FIGURE 2: On the left y-axis the mean values of CFU counts (uncorrected) with standard error of the mean (SEM) of the two lid types with holes and slits are depicted: Lid with 300 x 0.6 mm holes (cyan), slit lid with 20 x 0.2 mm x 17 mm slits (gold), slit lid with 20 x 0.3 mm x 14 mm slits (yellow). Lids with the same letter do not significantly differ from each other using the Tukey-Kramer HSD test. On the right y-axis the relative collection efficiency (RCE) is indicated and depicted as a dark blue line in the figure.

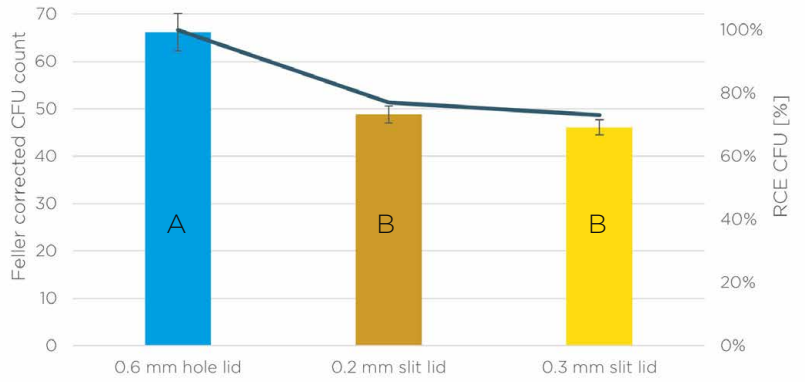

FIGURE 3: On the left y-axis the mean values of Feller-corrected CFU counts with standard error of the mean (SEM) of the two lid types with holes and slits is depicted : Lid with 300 x 0.6 mm holes (cyan), slit lid with 20 x 0.2 mm x 17 mm slits (gold), slit lid with 20 x 0.3 mm x 14 mm slits (yellow). Lids with the same letter do not significantly differ from each other using the Tukey-Kramer HSD test. On the right y-axis the relative collection efficiency (RCE) is indicated and depicted as a dark blue line in the figure.

Examining the relative CFU data of the slit compared to the perforated lids (100%), the 0.2 mm slit lid had a relative collection efficiency (RCE CFU, y-axis on the right) of 84%, while the 0.3 mm slit lid displayed a capture efficiency of 79% (refer to Figure 2, dark blue line). Similarly, when looking at the Feller-corrected CFU data, the difference is even more noticeable: the 0.2 mm slit showed a RCE of 77%, while the 0.3 mm slit exhibited a RCE of 73% (refer to Figure 3, dark blue line).

DISCUSSION

In this experimental setup, a statistically significantly better microbial recovery was observed with the perforated lid compared to the two different slit lids. Several possible explanations are discussed below, divided into physical and biological effects:

PHYSICAL EFFECTS

The lid design can significantly affect the physical collection efficiency. To analyze the CFU results from the experiment and to compare them for the different lids the following calculations were made as shown in Table 1:

- Average air speed and d_{50}: An essential factor for the physical collection efficiency is the speed of the air that is reached while the air is drawn through the lid. The total amount of air passing through the lid per minute is divided by the total cross-sectional area of all openings (300 holes or 20 slits) (refer to Table 1 Line 1.1). This also affects the theoretical physical sampling efficiency d_{50} (refer to Table 1 Line 1.2), which is the size of a particle that the sampler can capture with 50% efficiency and calculated as follows:

$$d_{50} \approx \sqrt[2]{\frac{40Dh}{U}}$$

where

40	is the constant factor for air viscosity [°C];
Dh	is the equivalent hydraulic diameter of sieve holes or 2x slit width [mm];
U	is the impact velocity [m/s]

(European Committee for Standardization 2020)

- Edge effects: When the intake air can flow through the openings unobstructed, it generates a velocity profile that is uniformly distributed across the opening. However, in reality, these openings are finite and constrained by walls, resulting in friction and the emergence of a phenomenon known as the edge effect. This phenomenon causes the air to decelerate along the walls, leading to a higher air velocity along the center of the duct and resulting in a parabolic velocity profile with a higher maximum velocity (Table 1 Line 1.3). The factor embedded in the mathematical formula used to calculate the maximum velocity provides insight into the maximum strength of the edge effect, with a value of 2 for hole openings and 1.5 for slit openings:

$$v_{\text{max hole}} = 2 * v_{\text{mean hole}} \quad \text{(BYJU's 2024)}$$

$$v_{\text{max slit}} = \frac{3}{2} * v_{\text{mean slit}} \quad \text{(BYJU's 2024)}$$

- More accurate theoretical physical sampling efficiency d_{50}: Edge effects result in a constriction of the effective hole or hydrodynamic diameter and lead to an increased air speed with a lower d_{50} value than predicted by normal theoretical calculations[2]. In this whitepaper it is called more accurate theoretical d_{50} (refer to Table 1 Line 1.4).

[2] According to EN 17141, the physical collection efficiency can be determined using the d_{50} value. The d_{50} value defines the aerodynamic equivalent particle diameter size at which the sampler collects 50% of the particles in the air. A d_{50} value smaller than 2 µm is considered appropriate.

TABLE 1: COMPARISON OF CALCULATED PHYSICAL PARAMETERS WHICH ARE CHARACTERISTIC FOR HOLE AND SLIT LIDS

Line		holes 0.6 mm	slits 17x0.2 mm	slits 14x0.3 mm
1.1.1	Number of holes/slits	300	20	20
1.1.2	Airflow [L/min]	100	100	100
1.1.3	Total cross-sectional area of holes/slits [mm^2]	84.8	68.0	84.0
1.1	Average air speed in hole/slit [m/s]	19.7	24.5	20.0
1.2	Theoretical d_{50} [µm]	1.1	0.8	1.1
1.3	Maximum air speed in hole/slit [m/s]	39.3	36.8	30
1.4	More accurate theoretical d_{50} [µm]	0.78	0.66	0.89
2.1	Total approximated sampling area on nutrient plate [mm^2] (see Figure 4)	2376	2172	1647

COMPARISON OF HOLE VERSUS SLIT LIDS

Comparing the hole with the 0.3 mm slit lid, the cross-sectional areas of 84.8 mm^2 and 84.0 mm^2 are almost identical, as are the average velocities (19.7 m/s versus 20.0 m/s). Accordingly, the values for the theoretical physical efficiency d_{50} are also comparable (1.1 µm in each case). In contrast, the 0.2 mm slit lid has a much smaller cross-sectional area of only 68 mm^2, a correspondingly higher average velocity of 24.5 m/s and the lowest theoretical d_{50} of 0.8 µm. In terms of edge effects, holes exhibit stronger edge effects, leading to a higher V_{max} for this lid type and a lower more accurate theoretical d_{50} value and thus a higher physical collection efficiency (more accurate theoretical d_{50}, 0.78 µm vs. 0.89 µm for the 0.3 mm slit lid; Table 1 Line 1.4).

One could argue that this could also explain the higher CFU count on the nutrient plate in the experiment with the perforated lid. However, this explanation does not hold true for the 0.2 mm slit lid, which even has a higher physical collection efficiency (0.66 µm; Table 1 Line 1.4). Since fewer CFU were found on the nutrient plate with the 0.2 mm slit lid, physical parameters are therefore not sufficient to explain the difference to the perforated lid.

BIOLOGICAL EFFECTS

The CFU result on the nutrient plate is ultimately based on the growth of microorganisms, which is why the consideration of influencing biological factors is also crucial:

- Desiccation: A well-moistened environment is a prerequisite for microorganisms to grow on a nutrient plate. Increased desiccation of the nutrient plate decreases microbial viability (see e.g. Hassel 2020, Sandle 2015, Stewart et al. 1995, Whyte et al. 2007), thereby reducing the CFU count. Desiccation of the nutrient plate is contingent upon the level of stress it has encountered and can be mitigated by optimizing the distribution of sampled air across the nutrient plate surface. This distribution is influenced by the size of the total sampling area utilized on the nutrient plate and the lateral spacing between two openings (refer to Table 1 Line 2.1 and Figure 4).

COMPARISON OF HOLE VERSUS SLIT LIDS

The cumulated area of all 300 holes in the perforated lid is nearly identical to the area covered by the 20 slits in the 0.3 mm slit lid (refer to Table 1 Line 1.1.3). However, there are disparities in how effectively the available nutrient plate surface is used and in the arrangement of the openings on the lids. The perforated lid facilitates a more efficient utilization of the nutrient plate surface during sampling compared to the slit lid (refer to Figure 4). With the perforated lid, the 100 L/min of air is dispersed over a total approximate area of 2370 mm^2, while with the 0.3 mm slit lid, it is distributed over only 1647 mm^2, representing a 31% reduction compared to the perforated lid (refer to Table 1 Line 2.1).
When comparing the lateral distances between two individual 0.3 mm slits to two neighboring holes, it becomes apparent that the slits are spaced much farther apart than the holes (refer to Figure 4). This indicates that the actual sampling area on the nutrient plate for the 0.3 mm slit lid may be smaller than initially calculated, leading to an even poorer utilization of the nutrient plate surface than our initial estimation suggested (refer to Table 1 Line 2.1).

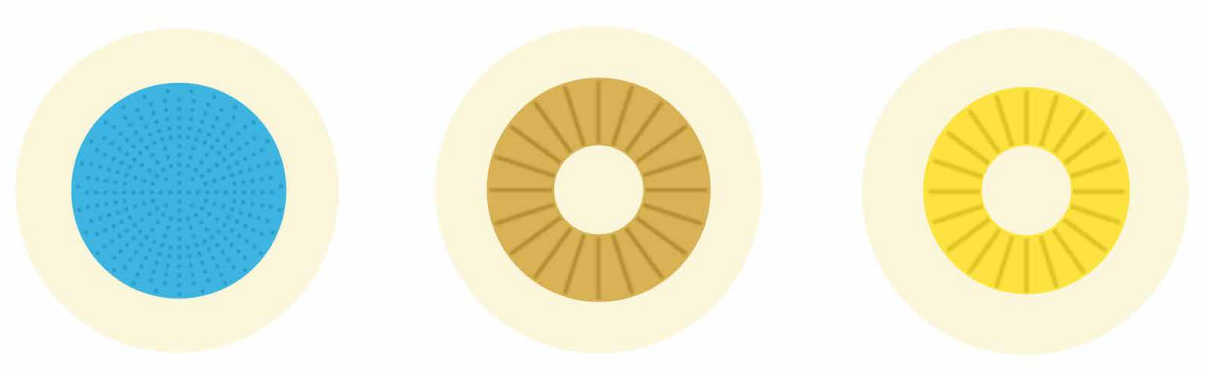

FIGURE 4: Schematic representation of the sampling area with distribution and arrangement of hole (left) and slit (0.2 mm middle and 0.3 mm right) patterns on the nutrient plate surface.

In summary, this indicates that the air distribution across the surface of the nutrient plate is less uniform with the 0.3 mm slit lid compared to the perforated lid. Consequently, greater localized stress occurs with the 0.3 mm slit lid, resulting in increased desiccation which counteracts the growth of CFUs.

If we look at the 0.2 mm slit lid, it is noticeable that the total sampling area of 2172 mm^2 is higher than that of the 0.3 mm slid lid and in a similar range as the perforated lid with 2376 mm^2. Based solely on the total sampling area, one would anticipate a higher CFU count on this nutrient plate compared to the 0.3 mm slit lid.

However, the slits on the 0.2 mm slit lid exhibit an even greater lateral distance between individual openings compared to those on the 0.3 mm slit lid. Considering both, the area and the lateral distance, these two effects likely counterbalance each other. Consequently, the desiccation for the 0.2 mm slit lid is likely to be as pronounced as that for the 0.3 mm slit lid, thereby adversely impacting the number of CFUs on the nutrient plate.

CONCLUSION

This whitepaper compares three different lid designs for active microbial air sampling applications. The perforated lid showed a statistically significantly higher collection efficiency than the two slit lids.

Several factors that have physical and biological effects on the sampling result were discussed. In summary, the 0.3 mm slit lid has a lower physical efficiency than the perforated lid and is more susceptible to desiccation because less nutrient plate surface is used for sampling the 100 L/min. Accordingly, it seems plausible that fewer CFU were found on the nutrient plate for the slit lid.

However, when applying the same rationale to the 0.2 mm slit lid, which exhibited a 5% higher CFU count compared to the other slit lid, this logic no longer holds as the 0.2 mm slit lid demonstrates the highest physical efficiency among the three lids. Moreover, the surface area of the nutrient plate utilized is nearly equivalent to that of the perforated lid. If solely considering physical factors, one would expect a higher CFU count on the nutrient plate for this lid. Nonetheless, this is not observed, suggesting that these physical factors alone are insufficient for elucidating the difference between the lids, and that the biological impact of desiccation might be the decisive factor.

This study demonstrates that the perforated lid used on the MAS-100 NT air sampler has superior collection efficiency in direct comparison with different slit lid types. Therefore, MBV will continue to use exclusively perforated lids to equip its MAS-100 air and compressed gas samplers. This assures customers that they are employing an air sampler with optimized collection efficiency for their active microbiological contamination control. Ultimately, it supports them to keep their production and working environment well protected.

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ORDERING INFORMATION

Description	Article number MBV	Article no. Merck[3]
MAS-100 NT air sampler	101.020.01	1.09191.0001
300 x 0.6 mm perforated lid	101.214	1.09195.0001

[3] Outside Switzerland, the MAS-100 NT® air sampler is distributed by our partner Merck KGaA, Germany, Darmstadt. For inquiries quote the following article numbers

REFERENCES

- Andersen A.A. (1958). New sampler for the collection, sizing and enumeration of viable airborne particles. Journal of Bacteriology, 76(5), 471-484.
- BYJU's (2024). Velocity profile of a fully developed laminar flow in a straight circular pipe. Last visited on April 3rd 2024.
- BYJU's (2024). Parabolic velocity distribution profile of a fully developed laminar flow between two parallel, stationary and identical plates. Last visited on April 3rd 2024.
- Climet (2023). Feller correction explained. www.climet.com/toolbox/feller-correction-calculator/index.html. Last visited on April 3rd 2024.
- European Committee for Standardization (2020). EN 17141:2020 Cleanrooms and associated controlled environments - Biocontamination control.
- Hassel T. (2020). Agar Desiccation – The causes and how to address them. European Journal of Parenteral and Pharmaceutical Sciences. www.ejpps.online/agar-desiccation-the-causes-and-how. Last visited on April 3rd 2024.
- Henningson E.W., Ahlberg M.S. (1994). Evaluation of microbiological aerosol samplers: A review. Journal of Aerosol Science, 25(8), 1459-1492.
- MBV (2023). Feller Table 300 & 400 holes. MBV, Version: 6.0. 06.6051.02. www.mbv.ch/media/06.6051.02_feller_table_300x0.6_400x0.7_mbv.pdf. Last visited on April 3rd 2024.
- Meier R., Zingre H. (2000). Qualification of air sampler systems: The MAS-100. Swiss Pharma, 22(1-2), pp. 15-21.
- PMS (2023). Statistische Korrekturtabelle nach Feller. archive.pmeasuring.com/ch/application-notes/table-of-statistical-corrections-according-to-fell/. Last visited on April 3rd 2024.
- Sandle T. (2015). Settle plate exposure under unidirectional airflow and the effect of weight loss upon microbial growth. European Journal of Parenteral & Pharmaceutical Sciences, 20(2), 45-50.
- Stewart S.L., Grinshpun S.A., Willeke K., Terzieva S., Ulevicius V., Donnelly J. (1995). Effect of impact stress on microbial recovery on an agar surface. Applied and Environmental Microbiology, 61(4), 1232-1239.
- Strauss-Goller S. (2019). A novel laminar-flow-based bioaerosol test system to determine biological sampling efficiencies of bioaerosol samplers. Aerosol Science and Technology, 53(4), 355-370.
- Temprano G., Garrido D., D'Aquino M. (2004). Comparative study of airborne viable particles assessment methods in microbiological environmental monitoring. PDA J Pharm Sci and Tech 2004, 58, 215-221.
- Whyte W., Green G., Albisu A. (2007). Collection efficiency and design of microbial air samplers: Journal of Aerosol Science, 38(1), 101-114.

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