Robustness Validation of MAS-100 Sirius® Microbial Air Sampler

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Abstract

The MAS-100 Sirius® Microbial Air Sampler is the successor of the MAS-100 NT® Microbial Air Sampler. It is designed for reliable monitoring of viable airborne particles in cleanroom environments.

In addition to validation according to ISO 14698 Annex B and EN 17141 Annex E, MBV performed additional testing to ensure comprehensive validation of the instrument's performance.

This application note is part of a series and presents the validation of the parameter ROBUSTNESS of MAS-100 Sirius® Air Sampler when operated at varying flow rates and sampling volumes.

The results show no statistically significant impact of the flow rate or the sampled air volume on microbial recovery, confirming the MAS-100 Sirius® Air Sampler as a reliable and robust successor to the MAS-100 NT® Air Sampler.

Introduction

Reliable monitoring of airborne microbial contamination is fundamental for maintaining GMP-compliant cleanroom environments in pharmaceutical manufacturing.

To go beyond standard requirements of air sampler qualification according to ISO 14698 Annex B and EN 17141 Annex E and ensure the MAS-100 Sirius® Air Sampler's functional reliability, MBV AG applied an extended validation strategy which was inspired by guidelines for alternative and rapid microbiological methods (ARMM), including Ph. Eur. 5.1.6, USP <1223>, and PDA Technical Report No. 33. It included the validation of the 4 parameters RUGGEDNESS, ROBUSTNESS, EQUIVALENCE and SPECIFICITY. Although the MAS-100 Sirius® Air Sampler is not classified as an ARMM, these guidelines offer a sound scientific basis for performance validation akin to chemical method validation per ICH Q2(R1).

To confirm the reliability of the MAS-100 Sirius® Air Sampler in routine cleanroom monitoring, ROBUSTNESS tests were performed using different flow rates and sampling volumes, which is the focus of this application note. The goal was to demonstrate consistent microbial recovery under varying conditions, supporting the instrument's dependable performance in daily use.

Using different flow rates offers practical advantages depending on the sampling objective. The MAS-100 Sirius® Air Sampler offers two air flow rates, the standard flow rate of 100 SLPM and a higher flow rate of 200 SLPM which enables the same volume to be collected in half of the time, ideal for rapid sampling and increased operational efficiency.

Additionally, the sample air volume can be adjusted to exceed the traditional 1000 liters or reduced for higher grade clean rooms, enabling more efficient and tailored sampling.

Figure 1. Comparison of the two different lids for MAS-100 Sirius® Air Samplers used for sampling with 100 SLPM (left) and 200 SLPM (right) with standard 90 mm agar plates.

Material & Methods

Test Environment:

The study was performed in an ISO Class 8 laboratory corridor of the pharmaceutical manufacturer Hoffmann-La Roche AG at Kaiseraugst (Switzerland). The corridor (approximately 3 m wide and 56 m long) was pre-characterized by conducting air sampling at three locations over a period of three days, with microbial concentrations ranging up to 150 CFU/m^3, providing a representative and suitable environment for evaluating air sampler performance.

Materials Used:

- MAS-100 Sirius® Air Sampler: 3 units (serial no. 220060, 220062, 220063) with matching 300 x 0.6 mm perforated lids (ANS830352, ANS830353, ANS830354)
- 3 additional lids with different perforations (serial no. ANS 830353 for 100 SLPM, BNS 830344 for 200 SLPM)
- MAS-100 Regulus® Anemometer (serial no. 18126) for “as found” calibration
- Agar Media: 90 mm CASO + LT ICR plates (Merck KGaA, Darmstadt, article number 14605000120, batch: 207763)

Study Design:

Prior to testing, all air samplers and their respective perforated lids were thoroughly sanitized using 70% isopropanol and sterile wipes. To minimize positional bias, the instruments were placed approximately one meter apart and in accordance with the predefined, randomized experimental layout.

To ensure accurate airflow performance, all instruments were calibrated before and after the measurement series using a MAS-100 Regulus® Anemometer. All calibrations were within the required acceptance criterion.

After sampling, CASO agar plates were incubated in a two stage protocol under controlled conditions. The plates were first incubated at 20–25 °C for 3 days, followed by a second incubation phase at 30–35 °C for an additional 3 days. Colony forming units (CFU) were subsequently counted, corrected using the Feller tables and recorded for statistical evaluation.

Test 1 – Flow Rate Robustness: Air sampling was conducted using flow rates of 100 SLMP and 200 SLPM over a fixed sampling duration of approximately 10 minutes per run, resulting in a sampled air volume of 500 L per measurement. The sequential mode was used in order to carry out the measurement over (almost) the same period of time. Each of the two MAS-100 Sirius® instruments completed twelve independent sampling runs, yielding a total of 24 data points for analysis.

Test 2 – Sampling Volume Robustness: All instruments ran at 100 SLPM. Target volumes of 500 L, 1000 L or 1500 L were collected in continuous mode (i.e. the instruments were running for 5 min., 10 min. or 15 min. respectively; **Figure 2**). Each of the three MAS-100 Sirius® instruments completed twelve independent sampling runs, yielding a total of 36 data points for analysis.

Figure 2. Study design for the sampling of different air volumes using three different MAS-100 Sirius® instruments.

Statistical Analysis and Acceptance Criteria:

For each sampling run, CFU recovered on the agar plates were corrected using Feller’s table to account for multiple particle impaction and then normalized to CFU per m^3 for the air volume study. Statistical analysis was performed using Analysis of Covariance (ANCOVA), with “Flow Rate” (100 SLPM, 200 SLPM) or “Sample Volume” (500 L, 1000 L, 1500 L) as the fixed factor of interest and “Instrument” as well as “Run” as a covariate to account for temporal variability in corridor bioburden.

To confirm the validity of the ANCOVA model assumptions as well as to check homogeneity among the test conditions, homoscedasticity was assessed using Bartlett’s Test, and the normality of residuals was verified using the Shapiro Wilk test. Statistical power of the ANCOVA was calculated in MATLAB based on the methodology described by Zar (1999), enabling quantitative assessment of the ability to detect meaningful differences between flow rate or volume levels.

The following predefined acceptance criteria were applied to determine the robustness of the MAS-100 Sirius® Air Sampler:

- No statistically significant difference in CFU recovery between flow rates or between sampling volumes (ANCOVA, $p \geq 0.05$).
- No significant difference in variance between the different flow rates and sampled air volumes, respectively.
- A statistical power of at least 80% for each ANCOVA model.

Meeting all three acceptance criteria demonstrates that the MAS-100 Sirius® Air Sampler delivers robust measurements independent of the flow rate or air volume measured under ISO Class 8 conditions.

Results & Discussion

4.1 Flow Rate Robustness

Mean microbial recoveries at the two different flow rates were 24 CFU per 500 L for 100 SLPM and 22 CFU per 500 L for 200 SLPM (Figure 4), indicating a negligible difference in performance. This was confirmed by the statistical analysis applying the ANCOVA model (**Table 1**). No statistical difference for the factor “flow rate” could be found. Furthermore, the interaction between the flow rate and the instrument was also not significant. These results confirm that the MAS-100 Sirius® Air Sampler performs equivalent when measuring at different flow rates.

Using Bartlett’s test, it was confirmed that there is no significant difference in variance among the two different flow rates ($F = 0.0465$, $df = 1$, $p = 0.830$) fulfilling the predefined acceptance criterion for homoscedasticity and confirming that all two flow rates deliver consistent measurement variation.

The calculated statistical power was 84%, well above the 80% acceptance criterion, confirming the reliability of the conclusion.

The MAS-100 Sirius® Air Sampler maintains consistent collection efficiency at both certified flow rates when used with the appropriate perforated lids. This means that the MAS-100 Sirius® instruments deliver identical results regardless of the flow rate.

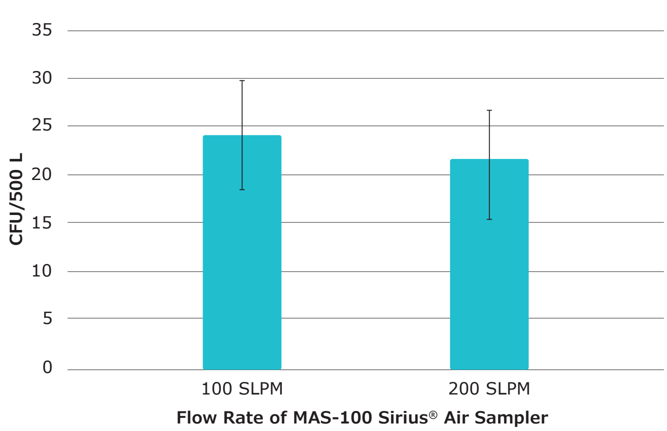

Figure 3: Summary of the microbial count (CFU/500 L, mean ±SEM, N = 24) at two different flow rates using the MAS-100 Sirius® Air Sampler.

Table 1: Summary of the full-factorial ANCOVA and the Power Test comparing the two different flow rates at 100 SLPM and 200 SLPM

Factor	DF	SS	MS	*F-Ratio or θ*	P or Power
Modell	16	7728.167	483.010	10.573	0.002
Error	7	319.792	45.685		
Total	23	8047.958			
Run	11	7579.458	689.041	15.083	<0.001
Instrument [I]	2	5.083	2.542	0.056	0.946
Flow rate [F]	1	35.042	35.042	0.767	0.410
I x F	2	58.583	29.292	0.641	0.555
Power				2.188	84%

4.2 Sampling Volume Robustness

Microbial recoveries at air volumes of 500 L, 1000 L and 1500 L ranged between 13 and 17 CFU/m³ (**Figure 4**). These differences were not statistically significant (**Table 2**). Neither the air volume itself nor its interaction with the instrument ($p = 0.446$, data not shown) had any measurable effect on the results.

The initial statistical model had a relatively low test power with 41%, which means it was less sensitive in detecting small differences. To improve this, a simplified ANCOVA model was created, increasing the power to 64% (Table 2). With this improved model, air volume still had no significant effect on microbial counts. One reason for the lower power is the difference in sampling times: while all tests started at the same time, the 500 L sample finished after 5 minutes, and the 1500 L sample took 15 minutes (**Figure 2**). It is known that air sampling reduces the number of microbes in the air over time due to a mild 'filtration effect' resulting from their collection. This may explain why longer sampling (e.g. 1500 L) tends to result in slightly lower counts compared to shorter sampling (e.g. 500 L), as seen in **Figure 2**. Natural changes in air quality over time may also contribute to this trend.

To check whether variability in the results differed across volumes, Bartlett’s test was used. With a p-value of 0.210, the test confirmed that variability was consistent across all tested volumes.

In summary, it was shown that the air volume has no significant effect on airborne microbial counts; however, the statistical power was below the acceptance criterion as a result of the ‘filtration effect’ caused by the sampling itself.

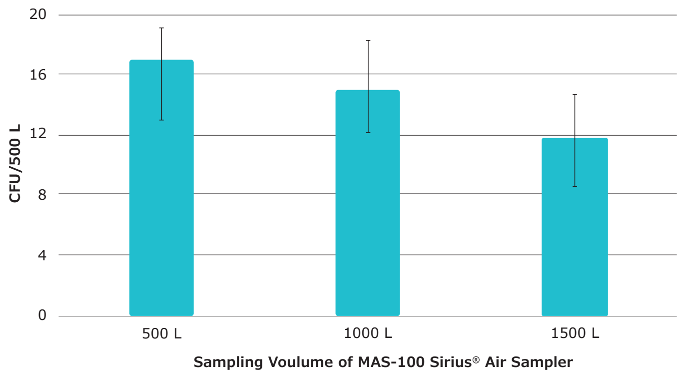

Figure 4: Summary of the bacterial count (CFU/m³, mean ± SEM, N = 36) of three instruments that measured three different air volumes.

Table 2: Summary of the full-factorial ANCOVA and the Power Test comparing the two different flow rates at 100 SLPM and 200 SLPM

Factor	DF	SS	MS	*F-Ratio or θ*	P or Power
Modell	13	1836.361	141.259	4.036	0.002
Error	22	769.944	34.997		
Total	35	2606.306			
Run	11	1718.972	156.270	4.465	0.001
Volume	2	117.389	58.695	1.677	0.210
Power				1.423	64%

Conclusion

This ROBUSTNESS validation study confirms that the MAS-100 Sirius® Air Sampler delivers consistent microbial re-covery across the two flow rates (100 SLPM, 200 SLPM) and different air volumes (500 L, 1000 L, 1500 L). Statistical analysis confirmed no significant impact of either parameter on performance, with high model reliability for flow rate and acceptable consistency for sampling volume.

These results confirm that the MAS-100 Sirius® Air Sampler is well suited for quantitative monitoring of airborne viable particles in pharmaceutical cleanrooms. Users can choose the flow rate or adjusting the sampling volume to best fit their environmental monitoring program. This flexibility comes without compromising data integrity or measurement reliability.

Abbreviations

Abbreviation	Term
ANCOVA	Analysis of Covariance
ARMM	Alternative or Rapid Microbiological Method
CASO	Casein Soya Bean Digest
DF	Degree of Freedom
EN	European Norm
ICH	International Conference on Harmonization
ISO	International Organization for Standardization
CFU	Colony Forming Unit
MS	Mean Squares
N	Sample Size
p	Significance level
PDA	Parenteral Drug Association
Ph. Eur.	European Pharmacopoeia
SEM	Standard Error of the Mean
SLPM	Standardliter per Minute
SS	Sum of Squares
TR	Technical Report
USP	United States Pharmacopeia

References

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- ICH Q2(R1) (2005). Validation of Analytical Procedures: Text and Methodology. ICH Harmonised Tripartite Guideline.
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- PDA Technical Report No. 33 (Revised 2013). Evaluation, Validation and Implementation of Alternative and Rapid Microbiological Methods. ISBN: 978-0-939459-63-6, Parenteral Drug Association, Inc.
- Ph. Eur. Chapter 5.1.6 (current edition). Alternative Methods for Control of Microbiological Quality. European Pharmacopeia.
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- Zar J.H. (1999). Biostatistical Analysis. Fourth Edition, PHIPE, Prentice Hall.

Ordering Information

Article	Article Number
MAS-100 Sirius® Air Sampler (calibrated for 100 SLPM, incl. perforated lid type A (for 90 mm agar, 100 SLPM))	**1178800001**
MAS-100 Sirius® Flex Air Sampler (calibrated for 100 and 200 SLPM flow rates, without perforated lid)	**1178810001**
Perforated lid type ANS for 90mm agar and 100 SLPM flow rate	**1178830001**
Perforated lid type BNS for 90mm agar and 200 SLPM flow rate	**1178840001**

Speak to our specialists for more information or request a demo of the MAS-100 Sirius® Air Sampler: **SigmaAldrich.com/sirius-contact**

Many thanks to our partner MBV AG for providing content and graphics in collaboration with MGP Consulting and F. Hoffmann-La Roche AG.

About MBV AG MBV AG stands for air - nothing eise. The family-run company is the global market leader in air samplers and has been a reliable partner to the pharmaceutical, cosmetics and food industries as weil as research laboratories and medical device manufacturers for nearly 40 years. The MAS-100 microbial air samplers are synonymous with innovation, quality and excellence. MBV researches, develops and produces all its instruments in Switzerland. The headquarters are in Stäfa on Lake Zurich, where also R&D, the accredited calibration laboratory and customer service are located.

For more information, please visit: https://www.mbv.ch/en/

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