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RUGGEDNESS VALIDATION OF MAS-100 SIRIUS®

APPLICATION NOTE

ABSTRACT

The MAS-100 Sirius® is the successor of the microbial air sampler MAS-100 NT®. 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 RUGGEDDNESS of MAS-100 Sirius when operated with different Sirius instruments, interchangeable perforated lids, different lid orientations as well as different agar plate types.

The results show that no statistically significant differences were detected between instruments, perforated lids, lid orientations, or agar plate types, confirming the MAS-100 Sirius as a reliable and robust successor to the MAS-100 NT.

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' 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 four parameters RUGGEDNESS, ROBUSTNESS, EQUIVALENCE and SPECIFICITY. Although MAS-100 Sirius is not classified as an ARMM, these guidelines offer a sound scientific basis for performance validation akin to chemical method validation per ICH Q2(R2).

This application note presents the results of the RUGGEDNESS (also known as intermediate precision) testing, which evaluates the method's tolerance to typical variations in instruments, accessories, or operating parameters. The specific objectives of this part of the study were as follows:

- **Instrument-to-Instrument Reproducibility:** Demonstrate that different MAS 100 Sirius units yield equivalent CFU (colony-forming unit) counts when operated side by side under identical environmental conditions.

- **Lid Interchangeability:** Show that different perforated lids of the same type do not significantly impact CFU recovery, ensuring consistent results regardless of the specific lid used.

- **Lid Orientation Independence:** Confirm that the orientation (angle) of the magnetic perforated lid has no significant effect on microbial recovery, validating the robustness of the design in everyday handling.

- **Agar Plate Type Compatibility:** Validate that various agar plate formats (90 mm settle plates, 55 mm contact plates, and Growth Direct® cassettes) do not significantly influence CFU recovery, allowing users flexibility in media selection for routine monitoring.

MATERIAL & METHODS

TEST ENVIRONMENT

The study was performed in an ISO Class 8 laboratory corridor of the pharmaceutical manufacturer F. 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

- 3 MAS-100 Sirius units (serial nos. 220060, 220062, 220063)
- 3 MAS-100 Sirius perforated lids for sampling at 100 SLPM with 90 mm agar plates (serial nos. ANS830352, ANS830353 and ANS830354)
- 2 MAS-100 Sirius perforated lids for sampling at 100 SLPM with 55 mm contact plates and Growth Direct® cassettes (serial nos. ANR830356, ANR830357)
- Anemometer MAS-100 Regulus® (serial no. 18126) for “as-found” calibration
- Agar media:
  - 90 mm CASO + LT ICR plates (Merck KGaA, Darmstadt, article number 14605000120, batch: 207763)
  - 55 mm CASO + LT ICR contact plates (Merck KGaA, Darmstadt, article number 14619500020, batch: 22724L1)
  - 55 mm Growth Direct® (GD) cassettes (Rapid Micro Biosystems, article number BTSA-048)

STUDY DESIGN

Prior to testing, all air samplers and their respective perforated lids were thoroughly sanitized using 70% isopropanol and sterile wipes. The instruments ran in parallel and were placed approximately one meter apart from each other. To minimize positional bias and to have a balanced distribution, a predefined, randomized experimental layout using a block design was applied. I.e. after each run the instrument or lid/accessory was randomly swapped or adapted. Care was taken to ensure that each position occurred with equal frequency for every instrument or lid/accessory but randomized (for further details, see below).
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.

Test 1 – Instrument-to-Instrument Reproducibility: Air sampling was conducted using a flow rate of 100 SLMP and a fixed sampling duration of 5 minutes per run. Each of the three MAS-100 Sirius instruments (using always the same lid on the same instrument) completed 20 independent sampling runs, yielding a total of 60 data points for analysis. Since the MAS-100 Sirius features a magnetic lid mechanism that allows the lid to be attached in any orientation, both the lid and the instrument were marked to ensure consistent positioning across all runs. For each run, the three Sirius instruments were randomly, yet balanced, assigned to one of the three positions. Throughout the experiment, each instrument occupied each position an equal number of times, but in a random sequence.

Test 2 – Lid Interchangeability: Air sampling was conducted using a flow rate of 100 SLMP and a fixed sampling duration of 5 minutes per run. Each of the three MAS-100 Sirius instruments completed twelve independent sampling runs, yielding a total of 36 data points for analysis. The lid was marked to ensure consistent orientation. The three Sirius instruments remained fixed at their position in the room, and the three lids were randomly assigned to them in each run, balanced so that, over the course of the experiment, each lid appeared equally often on each Sirius instrument.

Test 3 – Lid Orientation Independence: Air sampling was conducted using a flow rate of 100 SLMP and a fixed sampling duration of 5 minutes per run. Each of the three MAS-100 Sirius instruments completed twelve independent sampling runs, yielding a total of 36 data points for analysis. The orientation of the lids was deliberately varied at a rotation of the lid with respect to the instrument. 0° corresponds to the lid handle pointing to the right of the instrument: The first lid was mounted at 0°, the second lid at 90° and the third at 120° (Figure 1). These rotations were chosen to maximize the likelihood of detecting any potential systematic mechanical mismatch between the instrument and the lid.
To prevent systematic bias, the angle of the lid assignments was randomized for each run in a block design. As for Test 2, the three Sirius instruments including its lid remained fixed in their position within the room, while the lid angles were randomly assigned in each run. The assignment was balanced to ensure that, over the course of the experiment, each lid angle appeared equally often but randomly on each Sirius instrument.

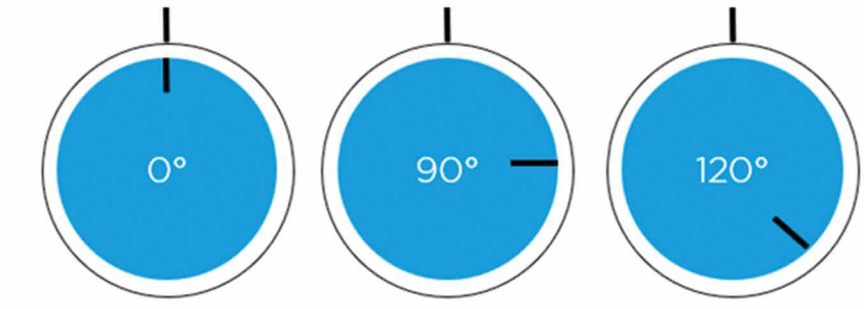

FIGURE 1: Presentation of the different lid orientations for the MAS-100 Sirius®: 0° (left), 90° (middle) and 120° (right).

Test 4 – Agar Plate Type Compatibility: The MAS-100 Sirius supports standard 90mm agar plates as well as alternative formats, including 55mm contact plates and Growth Direct® cassettes. Using another agar plate type requires adjusting the plate holder and selecting the correct perforated lid to maintain a consistent distance between agar and sieve (Figure 2). The potential influence of these adjustments was investigated in the present study by comparing the standard 90mm settle plate with the alternative contact plate and Growth Direct® cassette. Air sampling was performed at a flow rate of 100 SLPM, with a fixed sampling duration of 5 minutes per run. Each plate type underwent 10 independent runs, resulting in 30 valid data points. The lid itself as well as its orientation was kept consistent using position markers.
For each run, the three Sirius instruments along with their corresponding lids were randomly but balanced assigned to one of the three positions, i.e. over the course of the experiment, each instrument and lid appeared equally often at each position, though in a random sequence.

FIGURE 2: Comparison of the three different agar plate types with their corresponding lids: Lid ANS for sampling with standard 90 mm agar plates (left), Lid ANR for sampling either with 55 mm contact plates (middle) or with Growth Direct® cassettes (right).

For all four tests, after sampling, agar plates were incubated in a two-stage protocol under controlled conditions. The plates were first incubated at 20-25°C for 4 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 table and recorded for statistical evaluation.

STATISTICAL ANALYSIS AND ACCEPTANCE CRITERIA

For test 1 to 4, statistical analysis was performed using Analysis of Covariance (ANCOVA), with “instrument”, “lid” or “angle” as the fixed factor of interest and “run” and “position” or “instrument”, respectively, as covariate to account for temporal or special variability in bioburden. To compare the CFU recovery of the contact plates and Growth Direct® cassettes with that of the 90mm settle plates, a non-inferiority test was implemented in MATLAB using the two one-sided test (TOST) approach, with an 80% margin and a 95% confidence interval.

To confirm the validity of the ANCOVA model assumptions as well as to check homogeneity among the test conditions, homoskedasticity was assessed using Bartlett’s Test, and the normality of residuals was verified using the ShapiroWilk test. Statistical power of the TOST was calculated in MATLAB as well as for the ANCOVA, for the latter, the methodology described by Zar (1999) was used. This enabled quantitative assessment of the ability to detect meaningful differences between instruments, lids and lid position.

The following predefined acceptance criteria were applied to determine the RUGGEDNESS of the MAS 100 Sirius:

- No statistically significant difference in CFU recovery between instruments, lids or lid angles, respectively (ANCOVA, $p \geq 0.05$)
- The CFU recovery from the contact plate or the Growth Direct® cassette, respectively, is non-inferior to that of the settle plates, with a non-inferiority margin (δ) of 80% and a 95% confidence interval
- No significant value for homoscedasticity (variance)
- A statistical power of at least 80% for each statistical model

Meeting all acceptance criteria demonstrates that the MAS 100 Sirius delivers reliable measurements independent of instrument, perforated lid, perforated lid orientation (lid angle) and agar plate type under ISO Class 8 conditions.

RESULTS & DISCUSSION

INSTRUMENT-TO-INSTRUMENT REPRODUCIBILITY

Microbial recoveries from the three different MAS-100 Sirius instruments resulted in a mean of 18 CFU/500 L for all three instruments (Figure 3), indicating no difference in performance. This was confirmed by the statistical analysis applying the ANCOVA model (Table 1). No statistical difference for the factor “instrument” could be found, which confirms that the three MAS-100 Sirius instruments with their assigned lid (fixed) demonstrate equivalent performance.

The covariate “run” showed a highly significant effect, which is attributable to the fact that the airborne microbial count in the corridor changed over the course of the measurements. This was expected, as people occasionally entered the corridor. Therefore, the microbial load in the air varies throughout the day. The covariate “position” was also significant, indicating that randomization of the instruments is critically important to avoid systematic effects.

Using Bartlett's test, it was confirmed that there is no significant difference in variance among the three different instruments ($F = 0.215$, $df = 2$, $p = 0.801$) fulfilling the predefined acceptance criterion for homoscedasticity and confirming that all three instruments deliver consistent measurement variation.

The calculated statistical power was >99%, well above the 80% acceptance criterion, confirming the reliability of the conclusion.

In summary, the MAS 100 Sirius maintains consistent collection efficiency and comparable variation across its three instruments, meaning that the MAS-100 Sirius delivers identical results regardless of the instrument used (Figure 3).

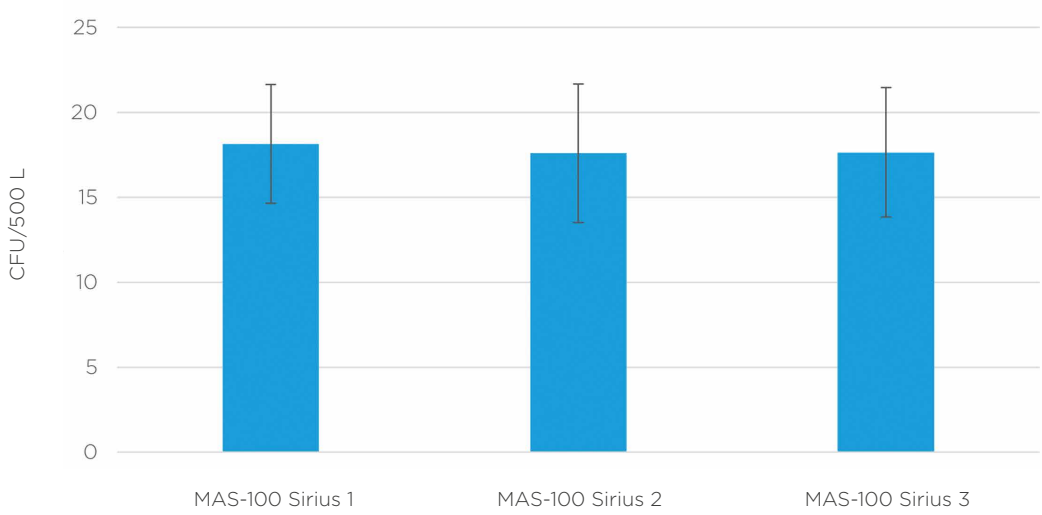

FIGURE 3: Summary of the microbial count (CFU/500 L, mean ± SEM, N = 60) of all three MAS-100 Sirius instruments used.

TABLE 1: SUMMARY OF THE ANCOVA AND THE POWER TEST COMPARING THE THREE MAS-100 SIRIUS INSTRUMENTS

Factor	DF	SS	MS	F-Ratio or θ	P or Power
Model	26	7711.503	296.596	17.351	<0.001
Error	33	564.097	17.094		
Total	59	8275.600	140.264		
Run	19	6368.653	335.192	19.609	<0.001
Position	5	386.203	77.241	4.519	0.003
Instrument	2	13.134	6.567	0.384	0.684
Power				3.302	>99%

NOTE: The factor "position" has 5 degrees of freedom (df), as six different positions were used. This setup resulted from combining the instrument comparison for ruggedness with the parameter "equivalence" in which three MAS-100 Sirius instruments were compared to three MAS-100 NT instruments (see the corresponding application note "AN 66 Equivalence Validation MAS-100 Sirius" for details).

PERFORATED LID INTERCHANGEABILITY

Microbial recoveries for the three identical MAS-100 Sirius perforated lids ranged between 27 and 29 CFU/500 L (Figure 4). These differences were not statistically significant (Table 2). No statistical differences for the factor "lid" as well as the interaction of lid and instrument ("I x L" in Table 2) could be found. These results confirm that the perforated lids of the MAS-100 Sirius perform equivalent independent on the instrument used.

As in the first experiment, the covariate "run" had a significant effect due to fluctuations in airborne microbial counts throughout the day, caused by varying activities in the corridor.

Using Bartlett's test, it was confirmed that there is no significant difference in variances among the three different instruments (F = 0.286, df = 2, p = 0.751) fulfilling the predefined acceptance criterion for homoscedasticity and confirming that all three lids deliver consistent measurement variation. The calculated statistical power was 99%, well above the 80% acceptance criterion, confirming the reliability of the conclusion.

In summary, these results confirm that the perforated lids of the same type perform equivalently independent of the MAS-100 Sirius instrument they are paired with.

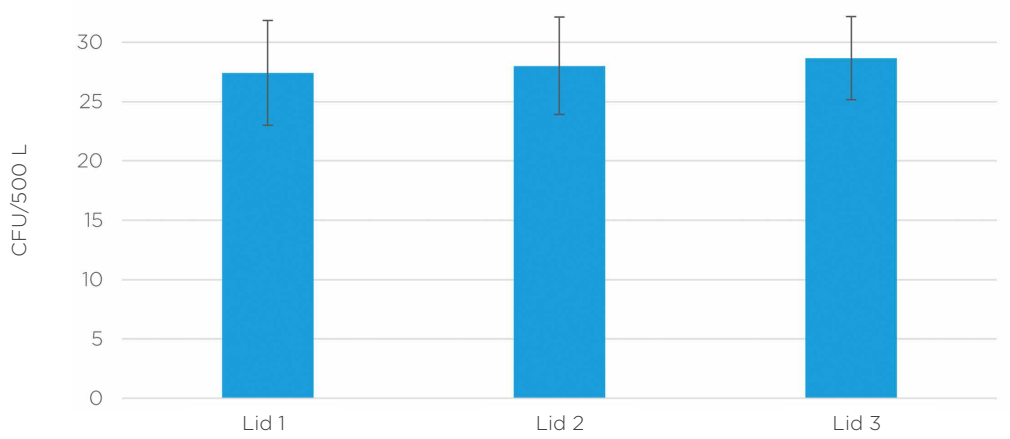

FIGURE 4: Summary of the microbial count (CFU/500 L, mean ± SEM, N = 36) of three MAS-100 Sirius instruments with three different perforated lids of the same lid type ANS (for 100 SLPM, 90 mm Agar).

TABLE 2: SUMMARY OF THE ANCOVA AND THE POWER TEST COMPARING THE THREE DIFFERENT LIDS TYPE ANS

Factor	DF	SS	MS	F-Ratio or θ	P or Power
Model	19	4762.046	250.634	6.673	<0.001
Error	16	600.926	37.558		
Total	35	5362.972	153.228		
Run	11	4524.824	411.348	10.952	<0.001
Instrument [I]	2	32.722	16.361	0.436	0.654
Lid [L]	2	9.389	4.695	0.125	0.883
I x L	4	232.296	58.074	1.546	0.236
Power				1.945	99%

LID ORIENTATION INDEPENDENCE

Microbial recoveries for the three different lid orientations (0°, 90°, 120°) of the MAS-100 Sirius standard lid type ANS ranged between 16 and 18 CFU/500 L (Figure 5). These differences were not statistically significant. Using the full-factorial ANCOVA model, it showed a low power of 50%. Therefore, a reduced model excluding non-significant interactions was applied, increasing power to 71% (Table 3), though still below the 80% threshold.
Given the minimal differences in microbial counts, additional experiments were not conducted and the conclusion can be considered to be valid.

To evaluate whether the variances differed among the three lid positions, a test for homoskedasticity was performed. The results were not statistically significant (Bartlett's test, F-ratio = 0.712, df = 2, p = 0.490), indicating that the variance of microbial counts is not affected by the lid's orientation.

In summary, the lid orientation (angle) does not significantly impact microbial recovery. Although statistical power was slightly below the target, the small observed differences are microbiologically negligible. The system demonstrates consistent performance regardless of the lid orientation.

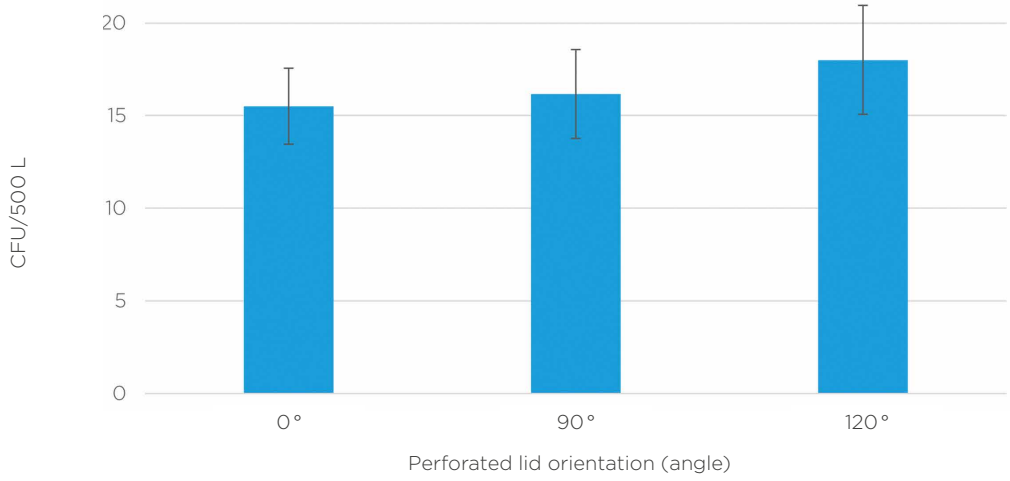

FIGURE 5: Summary of the microbial count (CFU/500 L, mean ± SEM, N = 36) of the three MAS-100 Sirius perforated lids of the same type ANS (for 100 SLPM, 90mm settle plate) with different orientations (angles).

TABLE 3: SUMMARY OF THE REDUCED ANCOVA AND THE POWER TEST COMPARING THE THREE DIFFERENT ORIENTATIONS (ANGLES) OF THE LID TYPE ANS

Factor	DF	SS	MS	F-Ratio or θ	P or Power
Model	15	2001.500	133.433	5.344	<0.001
Error	20	499.389	24.969		
Total	35	2500.889	71.454		
Run	11	1830.222	166.383	6.664	<0.001
Instrument	2	131.056	65.528	2.624	0.097
Angle	2	40.222	20.111	0.806	0.461
Power				1.702	71%

AGAR MEDIA TYPE COMPATIBILITY

Microbial recoveries from the three different agar plates yielded mean values of 24 CFU/500 L for the standard settle plate and 19 CFU/500 L for the contact plate and 20 CFU/500 L for the Growth Direct® cassette (Figure 6). For statistical evaluation, the CFU recovery of the alternative plate (contact plate or Growth Direct® cassette) was compared to that of the settle plate using a TOST for non-inferiority. The analysis indicated no significant differences since for both agar plates the Lower TOST is above the Lower TOST Margin (Table 4).

To confirm the robustness of the results, a statistical power analysis was performed, yielding a power of 85% for the comparison between contact plates and settle plates, and 74% for the comparison involving the Growth Direct® cassettes (Table 4). These findings indicate that the number of replicates for the contact plates was sufficient to support the conclusion. In contrast, the Growth Direct® cassettes would require additional replicates to achieve the desired power of 80%. However, due to the small differences observed in microbial counts and the power being close to 80%, no further experiments were conducted, and the conclusion is considered valid.

To assess whether the variances differed between the three agar plate types, a test for homoskedasticity was conducted. The results were not statistically significant (Bartlett's test: F-ratio = 0.072, df = 2, p = 0.930), indicating that the variance in microbial counts is not influenced by the agar plate type or its associated instrumental modifications.

It can therefore be concluded that the CFU recovery of the 55 mm contact agar plates as well as of the Growth Direct® cassettes is not inferior to that of the 90 mm settle plates, i.e. there is no statistically significant difference in microbial recovery between the two alternative plate types and the standard 90 mm settle plate.

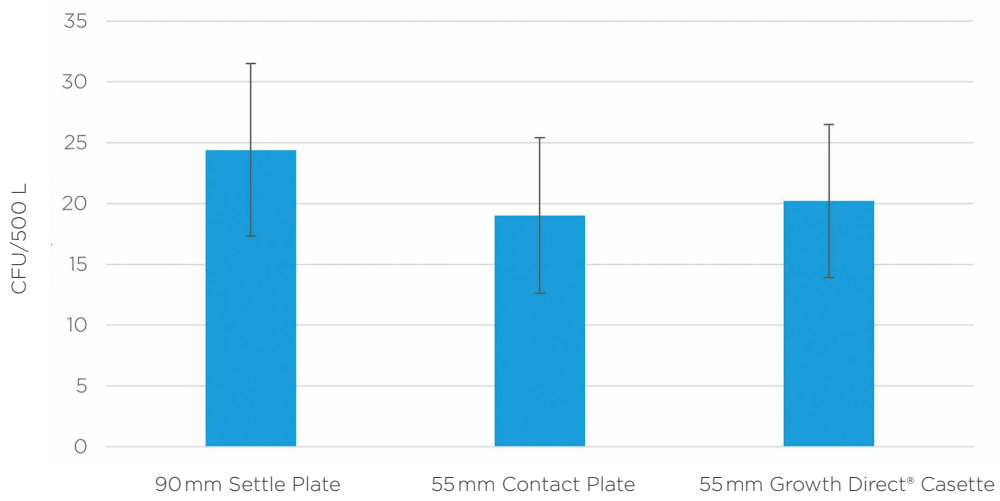

FIGURE 6: Summary of the microbial count (CFU/500 L, mean ± SEM, N = 30) of the three different agar plates used.

TABLE 4: SUMMARY OF THE TOST ANALYSIS FOR COMPARING THE ALTERNATIVE AGAR PLATES WITH THE 90 MM SETTLE PLATE AND ITS STATISTICAL POWER.

Plate	Difference	Lower TOST	Lower TOST Margin	Power
55 mm contact plate	-5.4	-10.31	-12	85%
Growth Direct® cassette	-4.2	-11.40	-13	74%

CONCLUSION

Four experiments were conducted to evaluate the RUGGEDNESS of the MAS-100 Sirius air sampler system, focusing on the comparability and reliability of its components:

- The first two tests assessed the influence of different MAS-100 Sirius instruments and perforated lids of the same type on microbial air sampling. Three instruments were compared and showed no significant differences, confirming consistent performance across instruments. Similarly, three interchangeable perforated lids of the type ANS were evaluated, with results indicating no impact on microbial counts. This demonstrates that instruments as well as perforated lids can be exchanged without affecting CFU recovery or increasing variability.

- The third test examined whether the orientation (0°, 90° or 120° angle) of the magnetic perforated lid affected the sampling. While the test power was slightly below the desired threshold, the minimal differences observed were deemed microbiologically irrelevant, showing that the lid orientation will not affect the outcome of the microbial counts or its variability.

- Since the MAS-100 Sirius can be used with different agar plates, the CFU recovery of contact plates as well as Growth Direct® cassettes was compared to that of the standard 90mm settle plates. A non-inferiority test with an 80% margin was performed, and no statistically significant difference was found. This shows that the CFU recovery from the contact plates or the Growth Direct® cassettes is statistically equivalent to that from the 90mm settle plates.

In summary, the MAS-100 Sirius provides reliable and reproducible data under the tested conditions, unaffected by the choice of MAS-100 Sirius instrument, perforated lid of the same type, lid orientation or agar plate type.

This confirms its suitability for flexible and robust quantitative monitoring of airborne viable particles in pharmaceutical cleanrooms, enabling users to adjust air sampling to best fit their environmental monitoring program.

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Corina Keller holds a master's degree in biochemistry from the University of Zurich and an MBA from the Lucerne University of Applied Sciences and Arts. She has many years of experience in product management, focusing on translating customer needs into targeted portfolio strategies and collaborating with interdisciplinary teams to develop effective solutions for microbial air monitoring in pharmaceutical cleanrooms.

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ABBREVIATIONS

Abbreviation	Term
ANCOVA	Analysis of Covariance
ARMM	Alternative or Rapid Microbiological Method
CASO	Casein Soya Bean Digest
CI	Confidence Interval
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
TOST	Two One-Sided Test
USP	United States Pharmacopeia

FURTHER INFORMATION

We offer you additional information on our products on these channels:

- Our product page about MAS-100 Sirius: www.mbv.ch/en/mas-100-sirius
- Answers to the most frequently asked questions can be found on our FAQ page.

We love to hear from you. Write to us: welcome@mbv.ch or call: +41 44 928 30 80.

ORDERING INFORMATION

Article	Article number MBV	Article number Merck KGAA, Darmstadt, Germany
MAS-100 Sirius® air sampler (calibrated for 100 SLPM, incl. perforated lid type ANS (for 90 mm agar, 100 SLPM))	200515	1178800001
MAS-100 Sirius® Flex air sampler (calibrated for 100 and 200 SLPM flow rates, without perforated lid)	201371	1178810001
Perforated lid type ANS for 90 mm agar and 100 SLPM flow rate	201139	1178830001
Perforated lid type ANR for 55 mm agar and Growth Direct® cassettes and 100 SLPM flow rate	201152	1178850001
Perforated lid type BNS for 90mm agar and 200 SLPM flow rate	201263	1178840001
Perforated lid type BNR for 55 mm agar and Growth Direct® cassettes and 200 SLPM flow rate	201267	1178860001

MORE INFORMATION ABOUT MAS-100 SIRIUS

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