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Application Note

Specificity Validation of the MAS-100 Sirius® Microbial Air Sampler

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Abstract

The MAS-100 Sirius® Air Sampler is the successor of the MAS-100 NT® 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 SPECIFICITY of MAS-100 Sirius. The study assesses whether the MAS-100 Sirius® Air Sampler and its predecessor, the MAS-100 NT® Air Sampler, differ in the spectrum of microbial species they detect under ISO Class 8 conditions.

The results show no statistically significant differences in the distribution of microbial species, 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 four parameters RUGGEDNESS, ROBUSTNESS, EQUIVALENCE and SPECIFICITY. Although 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(R2).

This application note focuses on the parameter specificity, which in this context refers to the ability to detect a comparable spectrum of airborne microorganisms typically encountered in pharmaceutical cleanrooms.

The objective was to verify that the MAS-100 Sirius® Air Sampler can reliably replace the MAS-100 NT® Air Sampler in routine cleanroom monitoring by demonstrating statistical similarity in microbial detection profiles. Since the new MAS-100 Sirius® Air Sampler uses the same impaction-based sampling technology as the established MAS-100 NT, no differences in specificity are expected. The critical parameter influencing specificity is the impaction velocity of microorganisms. A deviation in this velocity could affect the recovery of more sensitive microbial species and thereby influence specificity. However, both instruments operate with the same impaction velocity, allowing the assumption that specificity remains unaffected.

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:

- MAS-100 Sirius® Air Sampler (100 SLPM): 3 units (Serial Nos. 220060, 220062, 220063) with matching 300x0.6 mm perforated lids (ANS830352, ANS830353, ANS830354)
- MAS-100 NT® Air Sampler (100 SLPM): 3 units (serial Nos. 103549, 103550, 103552) with 300 x 0.6 mm perforated lids
- MAS-100 Regulus® Anemometer (serial no. 18126) for "as-found" calibration
- Agar Medium: 90 mm CASO + LT ICR plates (Article number 1.460500.0120, batch: 207763)
- MALDI-TOF mass spectrometer: Inventory no. B1027, identifier no. 8269944.03033

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. To minimize positional bias, the instruments were placed approximately one meter apart and in accordance with the predefined, randomized experimental layout, i.e. after each run the instrument's position was randomly changed.

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.

For this specificity study, agar plates from the equivalence study (LitCode AN14677EN) were used. In this study, air sampling was performed at a flow rate of 100 SLPM (standard liters per minute) for a fixed duration of 5 minutes per run, resulting in a sampled air volume of 500 liters per measurement.

For the equivalence study a total of 60 agar plates were sampled, however, for the SPECIFICITY the agar plates of the first three runs were used resulting in a total of 18 CASO agar plates (9 plates for each sampler) and 340 identified isolates. The 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.

On each agar plate, morphologically identical colonies were counted and documented. One representative colony from each morphologically similar group was selected for identification using MALDI-TOF. If MALDI-TOF did not yield a conclusive result, Gram staining was performed to allow categorization into the following predefined morphological groups:

- Gram-positive cocci
- Gram-positive rods (non-sporulating)
- Gram-positive rods (sporulating)
- Gram-negative rods
- Gram-negative cocci
- Molds
- Yeasts
- Miscellaneous/not identifiable

Statistical Analysis and Acceptance Criteria:

The statistical analysis was primarily descriptive and based on percentage distributions. For each morphological group, the relative proportion was calculated and visualized in a pie chart.

In addition, a chi-square test (x^2-test) with a significance level of $p = 0.05$ was performed for the three largest morphological groups (Zar, 1999). A hypothesized ratio of 1:1 was used as the expected frequency.

It is important to note that very low detection numbers introduce substantial uncertainty and imprecision (see for example USP chapter <1227>). Therefore, statistical testing was limited to those morphological groups with more than 10 isolates to ensure robustness of interpretation.

Results & Discussion

Comparison of the microbial profiles

A total of 340 isolates were recovered, with 122 individual identifications performed. To enable analysis, all isolates were categorized into their respective morphological groups and are displayed in **Figure 1**. Over 95% of the isolates belonged to the groups of Gram-positive cocci, Gram-positive rods, or Gram-negative rods. As shown in **Figure 1**, no significant differences were observed between the MAS-100 NT® Air Sampler and the MAS-100 Sirius® Air Sampler in terms of distribution among these groups. For the three largest groups, a chi-square (x^2) test was performed (see **Table 1**), and no statistically significant differences were found.

Sporulating Gram-positive rods were rare; only a single colony was identified from a MAS-100 Sirius® Air Sampler. Mold detections were also infrequent. Due to the low number of such isolates, no meaningful conclusions can be drawn, and the observed differences are likely incidental.

All isolates could be assigned to one of the predefined morphological groups and there were no organisms in the “Miscellaneous/not identifiable” category. Furthermore, no yeasts were found, which aligns with expectations, as yeasts are seldom detected in cleanroom environments.

Parameter	Gram-positive cocci	Gram-negative rods (without spores)	Gram-negative rods
MAS-100 NT® Sampler	131	29	8
MAS-100 Sirius® Sampler	124	30	11
x2	0.1922	0.0169	0.4737
p	0.661	0.896	0.491

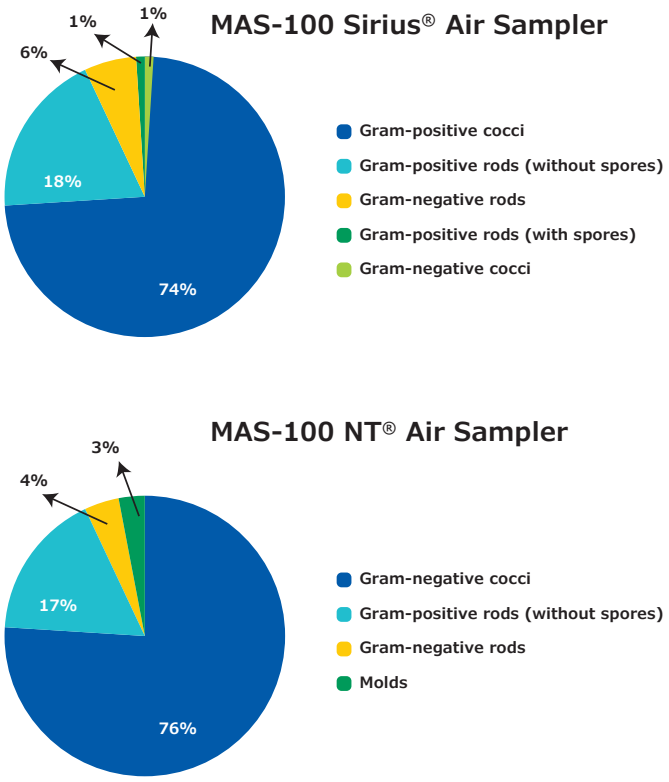

Figure 1: Percentage of morphological groups found during microbial air sampling using the MAS-100 Sirius® Air Sampler(top) and the MAS-100 NT® Air Sampler (bottom).

Conclusion

Experiments were conducted to evaluate the specificity of the MAS-100 Sirius® Air Sampler, focusing on the spectrum of airborne microorganisms.

With a total of 340 isolates from three runs, a large number of microorganisms were identified. No appreciable divergence was observed between instruments in the percentage distribution of the three most represented morphological groups. Spore-forming Gram-positive rods as well as molds were rare on both instruments; yeasts were absent. The results detected are consistent with typical ISO 8 or pharmaceutical cleanroom flora dominated by personnel-borne bacteria (see for example Sandle 2011, Goverde 2018).

In conclusion, the MAS-100 Sirius® Air Sampler showed similar spectrum of airborne microorganisms compared to the MAS-100 NT® Air Sampler, with no significant differences. These results confirm that the MAS-100 Sirius® Air Sampler is well suited for quantitative monitoring of airborne viable particles in pharmaceutical cleanrooms and can be confidently adopted as a direct replacement for the MAS-100 NT® Air Sampler.

References

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Abbreviations

Abbreviation	Term
ARMM	Alternative or Rapid Microbiological Method
CASO	Casein Soya Bean Digest
x^2	Chi-square, test parameter of the chi-square-test
EN	European Norm
ICH	International Conference on Harmonization
ISO	International Organization for Standardization
CFU	Colony Forming Unit
p	Significance level
PDA	Parenteral Drug Association
Ph. Eur.	European Pharmacopoeia
SLPM	Standard Liter per Minute
TR	Technical Report
USP	United States Pharmacopeia

Further Information

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 else. 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.

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