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SPECIFICITY VALIDATION OF THE 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 SPECIFICITY of MAS-100 Sirius. The study assesses whether the MAS-100 Sirius and its predecessor, the MAS-100 NT, 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 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 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 can reliably replace the MAS-100 NT in routine cleanroom monitoring by demonstrating statistical similarity in microbial detection profiles. Since the new MAS-100 Sirius 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 (100 SLPM): 3 units (Serial Nos. 220060, 220062, 220063) with matching 300x0.6 mm perforated lids (ANS830352, ANS830353, ANS830354)
- MAS-100 NT (100 SLPM): 3 units (Serial Nos. 103549, 103550, 103552) with 300x0.6 mm perforated lids
- Anemometer MAS-100 Regulus® (serial no. 18126) for "as-found" calibration
- Agar Medium: 90 mm CASO + LT ICR plates (Merck KGaA, Darmstadt, article number 14605000120, 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 (see application note "AN 66 Equivalence Validation of MAS-100 Sirius") 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 (χ^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 and the MAS-100 Sirius in terms of distribution among these groups. For the three largest groups, a chi-square (χ^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 instrument. 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.

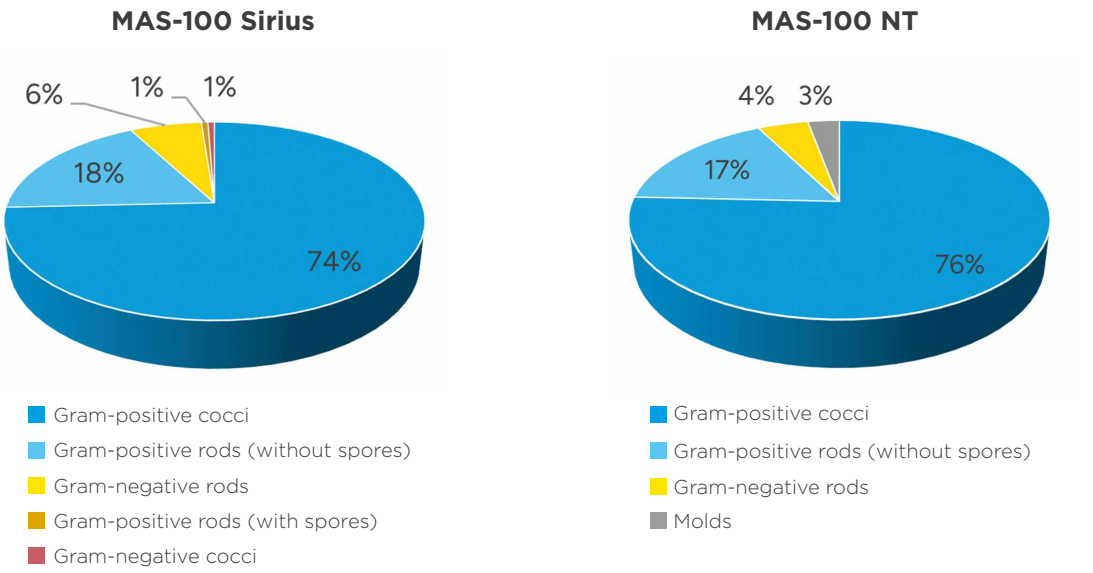

FIGURE 1: Percentage of morphological groups found during microbial air sampling using the MAS-100 Sirius (left) and the MAS-100 NT (right).

TABLE 1: TABULAR SUMMARY OF THE NUMBERS FOUND FOR EACH MORPHOLOGICAL GROUP AND THE RESULTS OF THE X^2-TESTS FOR THE THREE LARGEST MORPHOLOGICAL GROUPS

Parameter	Gram-positive cocci	Gram-negative rods (without spores)	Gram-negative rods
MAS-100 NT	131	29	8
Sirius	124	30	11
X^2	0.1922	0.0169	0.4737
p	0.661	0.896	0.491

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 showed similar spectrum of airborne microorganisms compared to the MAS-100 NT, with no significant differences. These results confirm that the MAS-100 Sirius 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.

ABOUT THE AUTHOR

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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
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

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

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 90mm agar and 100 SLPM flow rate	201139	1178830001
Perforated lid type BNS for 90mm agar and 200 SLPM flow rate	201263	1178840001

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