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EFFICACY OF VHP DECONTAMINATION OF MAS-100 SIRIUS®

APPLICATION NOTE

ABSTRACT

The MAS-100 Sirius® is a portable microbial air sampler for cleanroom applications and the successor to the world's best-selling MAS-100 NT®.

This study validated the effectiveness of vaporized hydrogen peroxide (VHP) decontamination using enzymatic indicators (EIs) and biological indicators (BIs). A 30-gram VHP dose with a 15-minute conditioning and 5-minute holding time achieved the highest enzymatic degradation, active (flush) instrument mode yielded the highest internal degradation results.

Using the optimized identical VHP cycle, validation with BIs containing *Geobacillus stearothermophilus* spores showed complete (≥6 log) spore reduction across all test locations when the MAS-100 Sirius was run in flush mode, whereas passive mode (blower off) failed to fully decontaminate the internal sampling head volume. A general correlation between EI and BI results was observed at the extremes (complete kill or survival), though discrepancies appeared in the fractional field of the BIs.

These results show that while passive VHP decontamination in standby mode cleans the outer hull, it does not sufficiently reach the internal sampling head. The new integrated flush mode ensures active decontamination of the complete air flow path and is therefore recommended for critical MAS-100 Sirius applications.

INTRODUCTION

Maintaining strict microbial control is essential in cleanroom environments to ensure product quality and safety. The MAS-100 Sirius is a portable air sampler designed for such environments, with an integrated ISO 35H exhaust filter that minimizes risks of contamination when using the instrument at various sampling locations.

Despite these design features, critical situations – such as transferring the instrument between different cleanroom grades or after service handling – may still require thorough decontamination of the internal flow path. Especially in the context of the new GMP Annex 1:2022, ensuring the contamination-free introduction of portable air samplers into cleanrooms has gained even more importance. Vaporized hydrogen peroxide (VHP) is effective in decontaminating surfaces as well as sensitive equipment such as air samplers. To facilitate the decontamination of the instrument's internal volume between the air inlet and filtered outlet, MBV has integrated an optional instrument flush mode. In this mode, the blower operates at fixed intervals at a reduced speed compared to normal sampling (see cover image).

This application note presents an experimental setup based on skanfog® technology that was used to evaluate the efficacy of vaporized hydrogen peroxide (VHP) decontamination of the MAS-100 Sirius, using enzymatic as well as biological indicators, in accordance with regulatory guidance on sterilization (Ph. Eur. 5.1.2 and USP <1229.5>).

MATERIAL & METHODS

The following table summarizes the used equipment and materials (Table 1):

TABLE 1: OVERVIEW OF USED EQUIPMENT AND MATERIALS USED IN THIS DECONTAMINATION STUDY

Equipment / Materials	Description
Test instruments	- MAS-100 Sirius (serial number 220044) without perforated lid mounted. - Separate MAS-100 Sirius 300 x 0.6 mm perforated lid, placed next to instruments.
Instrument modes	- Passive: MAS-100 Sirius is turned on, but blower is not running. - Active: MAS-100 Sirius running in flush mode. Timeout cycles of 10 seconds activated blower at low blower speed followed by 50 seconds with the blower at rest.
Calibration instruments	Anemometer MAS-100 Regulus® (serial no. 18126) for as-found calibration.
VHP procedure	Skanfog® test unit with a chamber volume of 1.5 m^3. Operating parameters: VHP concentration: 34.0-37.0% (w/w) Initial relative humidity: 25 ± 5% rH Initial temperature: 22 ± 4 °C Dosing rate: 2.0 ± 0.4 (g/min.) Airflow through nozzle: 70 ± 7 l_n/min. Aeration time: 1800 s Dose amount: 10 g, 20 g, 30 g Conditioning time: 5 min., 10 min., 15 min. Holding time: 5 min., 10 min., 15 min.
Indicators	- Enzyme indicators (EI) containing thermostable adenylate kinase (tAK) from Protak Scientific LTD. - Biological indicators (BI) with 2.0 x 10^6 *Geobacillus stearothermophilus* spores from Mesa Laboratories, Inc.

RISK ASSESSMENT FOR INDICATOR POSITIONS

Table 2 summarizes the selection of different indicator locations. The EIs and BIs were positioned at the same locations on and in the instrument (see Table 2 and Figure 1). It was anticipated that it would be difficult for the passive mode to achieve complete decontamination because the BI within the instrument at the filter might not receive enough VHP by means of diffusion. Therefore, two experimental setups were used to evaluate decontamination, one in passive mode and one in active mode (flush) of the MAS-100 Sirius.

Indicators no. 1 to 3 are assigned to the VHP decontamination of the MAS-100 Sirius in passive mode, whereas indicators no. 6 to 8 are assigned to the test utilizing the flush mode tested at equivalent positions as in the passive mode. Decontamination of the lid (indicators no. 4 and 5) is valid for both modes because the lid was removed from MAS-100 Sirius and placed next to the unit to allow the VHP to reach the contact surface between the instrument and lid.

TABLE 2: RISK ASSESSMENT FOR THE POSITIONING OF THE DIFFERENT EI AND BI INDICATORS.

Indicator no. for each instrument mode		Position of EI and BI	Probability of contamination	Risk of contamination		Accessibility of the VHP	Representative position
Passive	Active (Flush)			Agar Plate	Cleanroom		
1	6	On the plate holder	High: During preparation and sampling, microbes will access the plate holder.	High: Although there is no backflow of air to the agar, this position is very close to the most critical area.	Medium: Operator handling may transfer microbes to surrounding surfaces or gloves.	Good	For the sampling head and the inner side of the perforated lid surrounding the agar plate during sampling.
2	7	Within the instrument, before the HEPA filter	High: Some microbes might get to the filter during sampling of the air.	Very low: There is no backflow to the agar. Additionally, there is a HEPA filter that prevents microbes from leaving the inner part of the sampler.	Very low: Contamination is contained within the instrument and filtered before reaching the cleanroom.	Poor	For the complete inner part of the sampling head volume. Relevant to compare Sirius in passive and active mode.
3	8	On the outside of the instrument on the upper housing body	High: The instrument is carried by hand by the operator and touched e.g. on the touchscreen.	Very low: The air is sampled isokinetically from above, and the position is far from the air entry.	Medium: Operator handling may transfer microbes to surrounding surfaces or gloves.	Good	For all of the instrument's outer parts.
4		Lid: in the center of the perforated sieve, bottom side	Medium: In the event of an error the operator touches the lid at this position.	Very high: any contamination will be transferred to the agar plate during sampling.	Low: contamination would not affect cleanroom environment.	Good	For the inner part of the lid and outer parts of the instrument.
5		Lid: On the outside of the lid	Medium: The operator might touch the lid; however, this is unlikely since there is a handle.	Low: The air is sampled isokinetically from above.	Medium: Accidental contact may lead to indirect contamination of gloves or surfaces.	Good	For all of the instrument's outer parts.

Regarding passive decontamination, indicator 4 is seen as the most representative for all outer instrument parts. Although the risk was assessed as very low, position 2/7 is considered the most critical indicator to assess active decontamination, due to its limited accessibility to VHP.

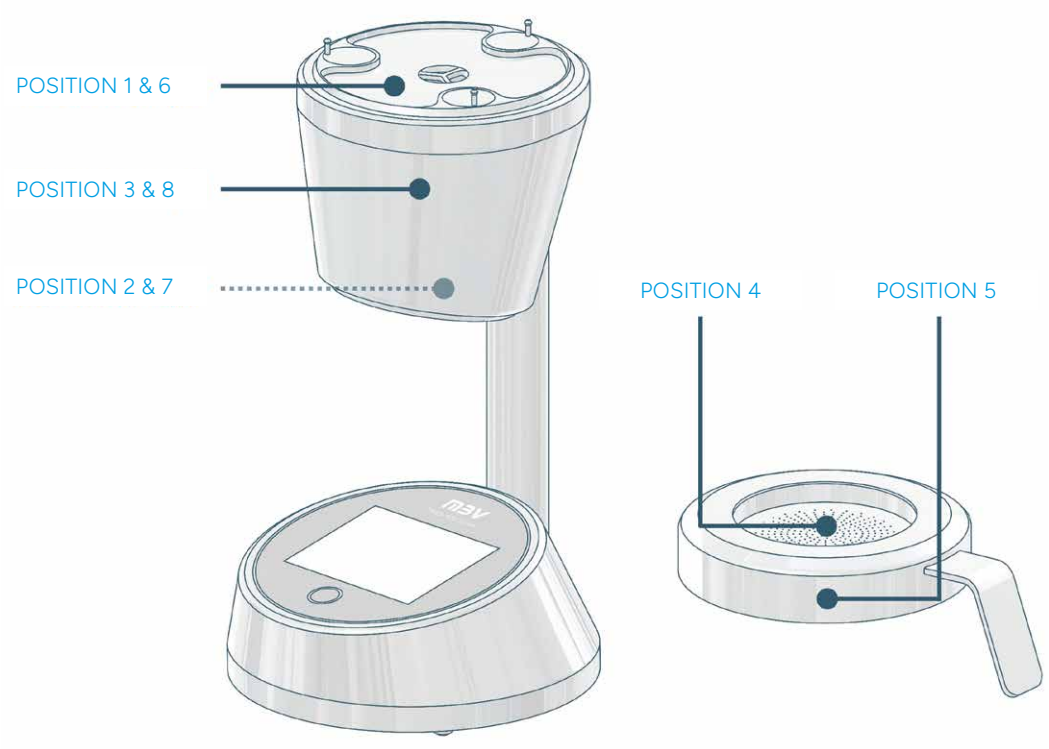

FIGURE 1: Positions of the indicators (EIs or BIs) on the MAS-100 Sirius to test efficacy of VHP decontamination. The line type provides information about the positioning: solid/dashed lines correspond to positions on the outside/inside of the instrument.

EXPERIMENT 1 – EVALUATION OF LOG REDUCTION

The first experiment aimed to determine log reductions at various VHP concentrations and exposure times. EIs were used to achieve the most efficient results possible. The advantage of EIs is that they can be evaluated immediately after VHP treatment and provide log reduction estimates with higher reproducibility compared to traditional bioindicators. This method is much faster than BIs and offers a larger measurement range rendering it especially useful for comparing different locations or decontamination processes within VHP concentrations that produce fractional results (see McLeod et al. 2017).

Five EIs were positioned on and inside the instrument (Figure 1), and five decontamination cycles were run in total (see Table 3).

TABLE 3: SUMMARY OF THE DIFFERENT VHP DECONTAMINATION CYCLES USED.

Cycle number	Dose amount	Conditioning time	Holding time	Total exposure time
1	10 g	5 min.	5 min.	10 min.
2	10 g	5 min.	15 min.	20 min.
3	20 g	10 min.	5 min.	15 min.
4	20 g	10 min.	10 min.	20 min.
5	30 g	15 min.	5 min.	20 min.

For run number five, three independent cycles (replicates) were performed. Both MAS-100 Sirius instruments were placed without the perforated lid, a lid was placed beside the two instruments during the test. One instrument was turned on but not running its blower (passive decontamination setting), the other instrument was on and running the instrument flush mode (active decontamination setting).

After VHP exposure, the indicators were carefully removed, and the remaining enzyme activity was quantified using a Protak Scientific luminometer. The data were recorded as raw relative light units (RLU), and the software provided a log reduction.

EXPERIMENT 2 – VALIDATION OF VHP DECONTAMINATION

The optimal VHP cycle parameters were established based on the findings of Experiment 1, which used a 30-gram dose of VHP with conditioning and holding times of 15 and 5 minutes, respectively. To verify the efficacy of the decontamination process and to validate it, BIs containing *Geobacillus stearothermophilus* spores were used, as recommended for gas sterilization in the European Pharmacopoeia chapter 5.1.2 and United States Pharmacopeia chapter <1229.5>. They were positioned in the same locations as in Experiment 1, the instrument settings were equivalent. They were used for a quantitative log-reduction determination. Three independent cycles (replicates) were performed in total.

EXPERIMENT 3 – VALIDATION OF INSTRUMENT PERSISTENCE

MAS-100 Sirius instruments were exposed to 50 back-to-back cycles using the optimal VHP cycle parameters established based on the findings of Experiment 1. While Experiments 1 and 2 focused on the success of the decontamination itself, this experiment evaluated the instrument's appearance and functionality after repeated decontamination events. Two instruments were used for testing. One instrument was turned on but not running its blower (passive decontamination setting), the other instrument was on and running the instrument flush mode (active decontamination setting). A lid for MAS-100 Sirius was placed beside the two instruments during the test.

ACCEPTANCE CRITERIA

- No acceptance criterion was set for Experiment 1 since the goal of this study was to evaluate and compare the different VHP cycles and positions of the EI and BI. Although EI and BI correlation is suggested by McLeon et al. (2017), the testing laboratory recommended against basing the VHP efficacy tests on a virtual log reduction based on EI testing.
- For Experiment 2, all BIs should demonstrate a reduction of 6 logs.
- For Experiment 3, the instruments and their components as well as the separate perforated lid have no visual defects and are fully functional before and after VHP treatment.

RESULTS & DISCUSSION

EXPERIMENT 1 – EVALUATION OF LOG REDUCTION

Before validating VHP decontamination using biological indicators (BI), a preliminary study was conducted with enzymatic indicators (EI) with thermostable adenylate kinase (tAK) to determine the appropriate VHP dose. In this study, tAK degradation was quantified through luminescence measurements. These measurements provided a precise evaluation of the presence and activity of VHP across various locations on the instrument. The resulting luminescence data helped identify the minimum VHP dose required to achieve a high level of tAK degradation, serving as a proxy for effective VHP decontamination.

Figure 2 shows the RLU for the different cycles on a logarithmic scale and Table 4 the EI converted log reductions. As expected, the highest dose of 30 grams, combined with a conditioning time of 15 minutes and a holding time of 5 minutes, produced the greatest tAK degradation. Based on these results, these parameters were selected for the subsequent VHP decontamination validation using BIs.

The low tAK degradation with EI no. 2, which was placed in front of the filter in the instrument, was expected. The difference between passive (EI no. 2 in Figure 2) and active treatment (EI no. 7 in Figure 2) is clearly visible with this EI. A significantly better log reduction was achieved in active mode (flush mode) (Table 4). All other EIs on the instrument and lid showed a strong log reduction regardless of the instrument mode (Table 4).

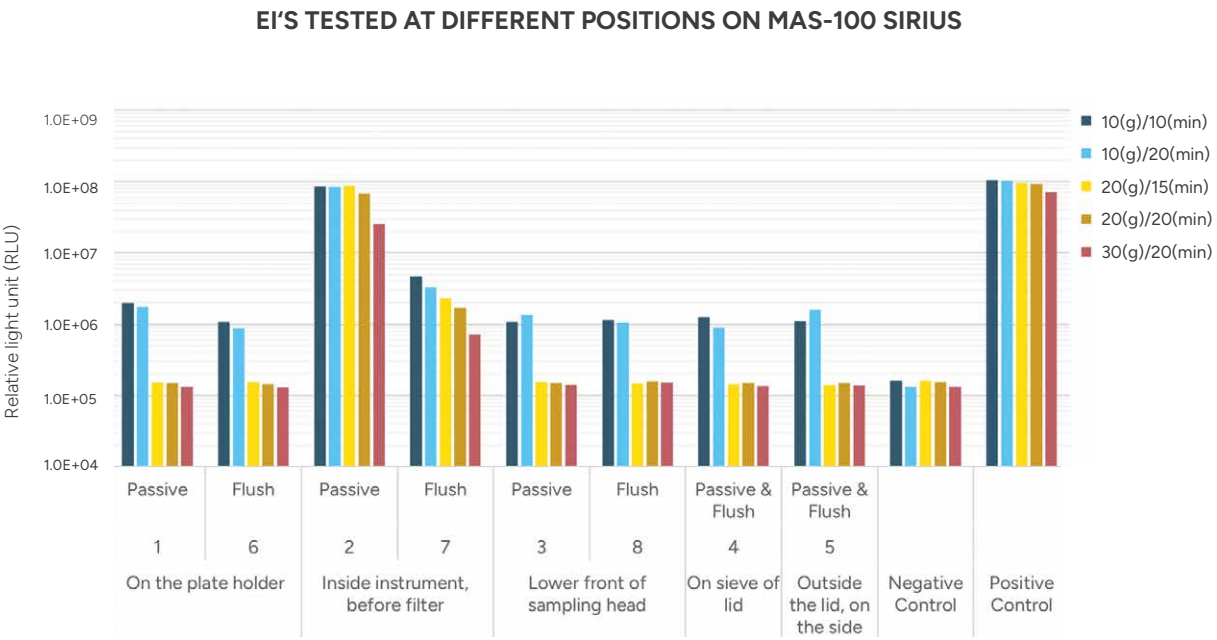

FIGURE 2: RLU values on a logarithmic scale as an output of the EIs for different positions on the MAS-100 Sirius instruments in passive (standby, EI 1 to 5) and active (flush, EI 4, 5 and 6-8) modes for the different decontamination cycles according to Table 3.

TABLE 4: ENZYME LOG REDUCTIONS FOR THE DIFFERENT EI POSITIONS ON AND IN THE MAS-100 SIRIUS IN PASSIVE MODE AND FLUSH MODE (ACTIVE). PC=POSITIVE CONTROL, NC=NEGATIVE CONTROL

		Passive					Active				
VHP Dose	BI no.	10(g)/ 10(min)	10(g)/ 20(min)	20(g)/ 15(min)	20(g)/ 20(min)	30(g)/ 20(min)	10(g)/ 10(min)	10(g)/ 20(min)	20(g)/ 15(min)	20(g)/ 20(min)	30(g)/ 20(min)
1	6	6	6	14	14	14	8	8	14	14	14
2	7	0	0	0	0	0	3	4	5	9	9
3	8	8	7	14	14	14	8	8	14	14	14
4		7	8	14	14	14	7	8	14	14	14
5		8	7	14	14	14	8	7	14	14	14
NC		14	14	14	14	14	14	14	14	14	14
PC		0	0	0	0	0	0	0	0	0	0

EXPERIMENT 2 – VALIDATION OF VHP DECONTAMINATION

Using BIs and the decontamination parameters of cycle number 5 from Table 3, the VHP decontamination results using bioindicators are summarized in Table 5.

In passive mode, all BIs except no. 2 (the BI within the instrument in front of the filter) achieved a reduction of at least 6 logs. BI no. 2 was found to be unaffected by the VHP with the spore population not significantly decreased compared to the untreated BI (control). In contrast to this finding in passive mode, all BIs showed no growth in flush mode, thus achieving a reduction of at least 6 logs (see Table 5).

In summary, according to the aforementioned acceptance criterion, all BIs achieved a 6-log reduction using the flush mode, indicating appropriate decontamination of even the internal air flow path.

TABLE 5: SPORE COUNT FOR THE DIFFERENT BI POSITIONS ON AND IN THE MAS-100 SIRIUS IN PASSIVE MODE AND FLUSH MODE (ACTIVE). PC=POSITIVE CONTROL, NC=NEGATIVE CONTROL

Mode		Passive					Active				
					Cycle Average					Cycle Average	
BI no.		Cycle 1 [spores/ carrier]	Cycle 2 [spores/ carrier]	Cycle 3 [spores/ carrier]	[spores/ carrier]	Log reduction	Cycle 1 [spores/ carrier]	Cycle 2 [spores/ carrier]	Cycle 3 [spores/ carrier]	[spores/ carrier]	Log reduction
1	6	<2.0	<2.0	<2.0	<2.0	**6.3**	<2.0	<2.0	<2.0	<2.0	**6.3**
2	7	2.1×10^6	2.0×10^6	1.9×10^6	2.0×10^6	**0**	<2.0	<2.0	<2.0	<2.0	**6.3**
3	8	<2.0	<2.0	<2.0	<2.0	**6.3**	<2.0	<2.0	<2.0	<2.0	**6.3**
4		<2.0	<2.0	<2.0	<2.0	**6.3**	<2.0	<2.0	<2.0	<2.0	**6.3**
5		<2.0	<2.0	<2.0	<2.0	**6.3**	<2.0	<2.0	<2.0	<2.0	**6.3**
NC		0	0	0	0	**14**	0	0	0	0	**14**
PC		2.2×10^6	1.9×10^6	1.8×10^6	2.0×10^6	**0**	2.2×10^6	1.9×10^6	1.8×10^6	2.0×10^6	**0**

EXPERIMENTS 1 AND 2 – EI AND BI CORRELATION

To a certain degree, a correlation can be established between the EI and the BI test results. In general, when EI shows high tAK degradation, BI also shows a strong log reduction. However, the behavior looks different in the fractional field. For the EI, a certain tAK degradation occurred in passive mode for EI no. 2, while no spore reduction was observed for the BI in the same position (BI no. 2). Additionally, for the same indicator position in flush mode (EI no. 7), medium tAK degradation occurred, while BI no. 7 showed a 6-log reduction. Thus, in this experiment, the correlation between the EI and the BI only works at the extremes (survival or kill), but not in the fractional field.

EXPERIMENT 3 – VALIDATION OF INSTRUMENT PERSISTENCE

No visual or structural changes could be detected after performing 50 cycles of VHP decontamination on either the instrument or the lid. Functionality of both the instruments and lid was verified based on the sampling flow rate, with as-found calibration results remaining within specifications.

CONCLUSION

This study evaluated the efficacy of vaporized hydrogen peroxide (VHP) decontamination for the MAS-100 Sirius portable air sampler using both enzymatic indicators (EIs) and biological indicators (BIs), tested in passive and flush (active) instrument modes.

In Experiment 1, log reduction performance was assessed across five VHP cycles using EIs positioned at different instrument locations. A VHP cycle with a 30-gram dose, 15 minutes conditioning, and 5 minutes holding time achieved the highest enzymatic degradation, particularly in flush mode. This optimized cycle was selected for subsequent BI validation.

In Experiment 2, the optimized cycle was validated using *Geobacillus stearothermophilus* BIs. In passive instrument mode, the BI placed inside the instrument near the filter showed no log reduction, indicating poor VHP penetration. In flush mode, all BIs achieved a full 6-log reduction, meeting the required acceptance criteria and demonstrating reliable and consistent decontamination.

Moreover, the data suggests that while EIs and BIs correlate at kill and survival extremes, this relationship breaks down in the intermediate (fractional) range.
These findings are specific to the Skanfog® configuration and VHP parameters tested. For other setups, re-validation is recommended. However, the data showed that the number of BIs could be reduced, because the most critical position is the internal volume of the sampling head; if complete decontamination is achieved at that location, it ensures that all other areas are covered.

This study confirms that the optional flush mode and a sufficiently high VHP dose provide effective VHP decontamination by achieving a complete 6-log decontamination inside the instrument. From a GMP and data-integrity perspective, it is essential to clearly distinguish flush cycles from sampling events to maintain an accurate audit trail as implemented in the instrument application software. The described method therefore offers a robust, compliant, and efficient approach for validated VHP decontamination of the MAS-100 Sirius portable air sampler.

REFERENCES

- McLeod N. P., Clifford M. and Sutton J. M. (2017). Evaluation of Novel Process Indicators for Rapid Monitoring of Hydrogen Peroxide Decontamination Processes. PDA Journal of Pharmaceutical Science and Technology September 2017, 71 (5) 393-404.
- United States Pharmacopeia (USP) chapter <1229.5> (latest edition). Vapor-Phase Hydrogen Peroxide Sterilization.
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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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At MBV AG, he is responsible for the development of new products and technologies for microbial contamination control and accurate air flow sensing solutions.

ABBREVIATIONS

Abbreviation	Term
BI	Biological Indicator
CFU	Colony Forming Unit
EI	Enzyme Indicator
et al.	*et alteri*
GMP	Good Manufacturing Practice
log	Logarithmic
Ph. Eur.	European Pharmacopoeia
RLU	Relative Light Units
SLPM	Standard Liter per Minute
tAK	thermostable Adenylate Kinase
USP	United States Pharmacopeia
VHP	Vaporized Hydrogen Peroxide

FURTHER INFORMATION

Additional information on our products is available 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 and 200 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

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