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VALIDATION OF VHP DECONTAMINATION OF MAS-100 LIBRA® AUTOMATED SETTLE PLATE CHANGER

APPLICATION NOTE

ABSTRACT

This study evaluates the efficacy of vaporized hydrogen peroxide for decontaminating the MAS-100 Libra® and its effect on the functionality of the device. A study using the SKANFOG test unit to simulate a controlled environment was conducted to evaluate decontamination efficacy. A preliminary study using enzymatic indicators was performed to determine the optimal hydrogen peroxide dose required for effective decontamination as well to determine the worst-case positions, thus guiding the placement of biological indicators for subsequent testing. The enzymatic indicators' results demonstrated that a H_2O_2 dose of 30 g combined with 20 minutes of contact time ensured sufficient decontamination across all tested areas. Biological indicators containing *Geobacillus stearothermophilus* spores were then strategically placed at critical positions on and inside the instrument. Results from three identical decontamination cycles confirmed complete microbial inactivation (> 6-log reduction) across all tested positions. The findings demonstrate the effectiveness of vaporized hydrogen peroxide in ensuring the decontamination of the MAS-100 Libra. To evaluate the effect of the decontamination agent on the device, the MAS-100 Libra was exposed to 50 consecutive hydrogen peroxide decontamination cycles. Additionally, the components of the instrument were immersed in concentrated hydrogen peroxide overnight. Both tests showed no negative effects on the components or functionality of the MAS-100 Libra.

INTRODUCTION

Ensuring decontamination in critical devices like the MAS-100 Libra® (see Figure 1) is essential for aseptic applications. Vaporized hydrogen peroxide (VHP) is widely used for efficient and reproducible surface decontamination. In this study, the VHP decontamination process was evaluated for the MAS-100 Libra. The SKANFOG test unit was employed to create a decontaminated environment for testing. The objective was to validate the decontamination efficacy of VHP at achieving a 6-log microbial reduction, providing critical data to ensure the device's operational decontamination.

Decontamination and airflow studies are relevant to prove the impeccable decontamination of a device used in grade A environments (EudraLex Annex 1). For the MAS-100 Libra, a computational fluid dynamics (CFD) study was carried out and showed that the design of the device allows for decontamination via a correct airflow. However, the airflow within the equipment is lower than at its exterior (Figure 2, Application Note MBV 2025). The results of the CFD highlighted the need to test internal areas of the equipment using biological indicators to ensure that VHP could penetrate effectively and eliminate any potential contamination. Since devices in grade A environments are regularly exposed to the decontamination agent, the effect of repeated exposure to hydrogen peroxide (H_2O_2) is relevant to know its effect on the device's components and long-term functionality.

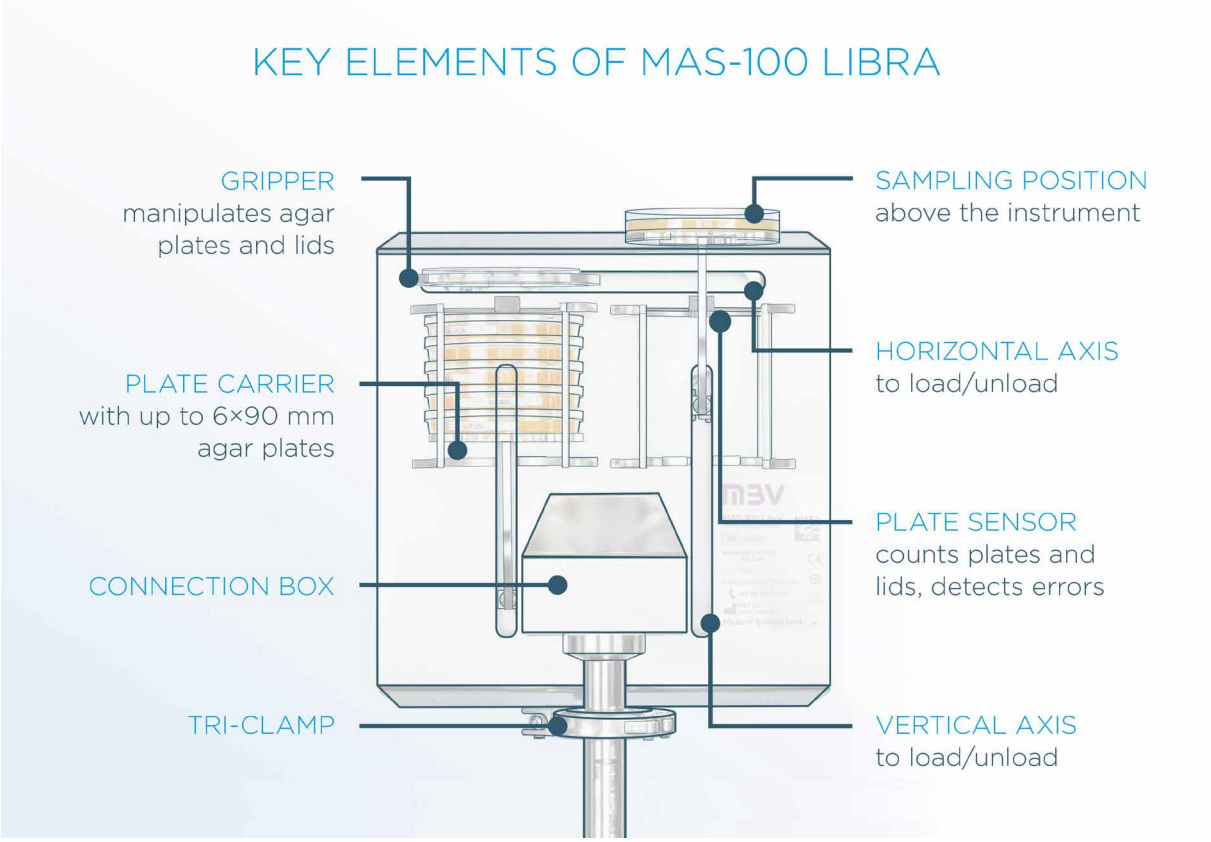

FIGURE 1: Presentation of the MAS-100 Libra with its components.

The objective of this study was to validate the decontamination efficacy of VHP in achieving a 6-log microbial reduction and ensuring the device's functionality after repeated expose to H_2O_2.

H_2O_2 DOSE DETERMINATION

Before conducting VHP decontamination validation with biological indicators, a preliminary study using enzymatic indicators was performed. Enzymatic indicators containing thermostable adenylate kinase (tAK) as a marker for H_2O_2 exposure were chosen for their fast and cost-effective method of determining the optimal H_2O_2 dose and identifying worst-case positions for subsequent testing with biological indicators. The degradation of tAK, quantified via luminescence measurements, enabled precise assessment of the presence of H_2O_2 and activity across various device locations, ensuring the efficiency and accuracy of the validation process.

The enzymatic indicator study was conducted on devices placed inside the SKANFOG test unit, exposing them to a series of decontamination cycles with varying H_2O_2 doses and contact times (Figure 2). The parameters tested included three different H_2O_2 dosed amounts (10 g, 20 g, 30 g) and three different H_2O_2 contact times (10 min., 15 min., 20 min.). Luminescence data from the enzymatic indicators was analyzed to identify the H_2O_2 dose sufficient to achieve high degradation levels, correlating with effective decontamination.

Key findings from the enzymatic indicator study demonstrated that an H_2O_2 dose of 30 g combined with a total contact time of 20 minutes provided uniform decontamination across internal and external device positions (Figure 3). These parameters were then applied to VHP decontamination validation using biological indicators.

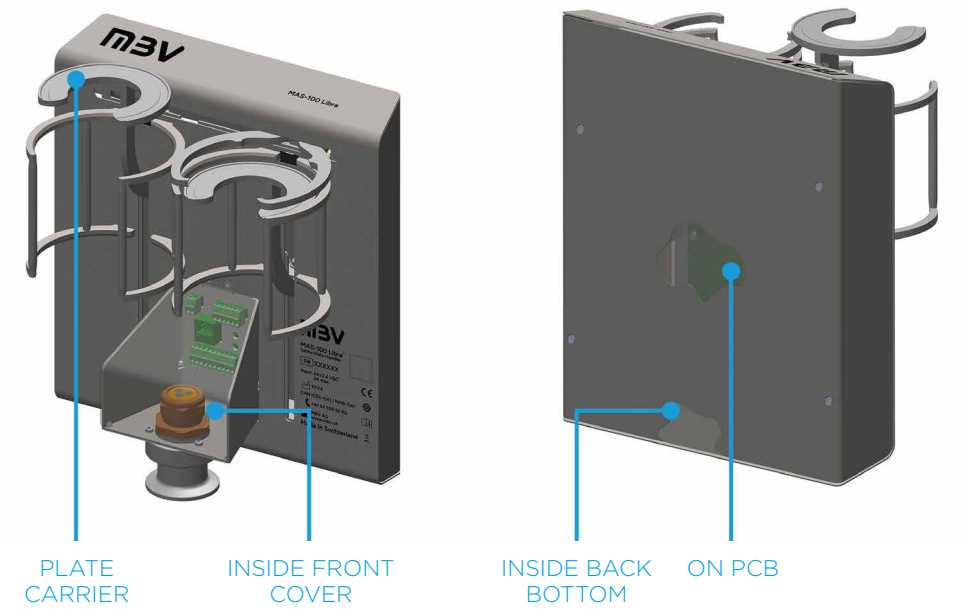

FIGURE 2: Schematic representation of the four positions of enzymatic and biological indicators.

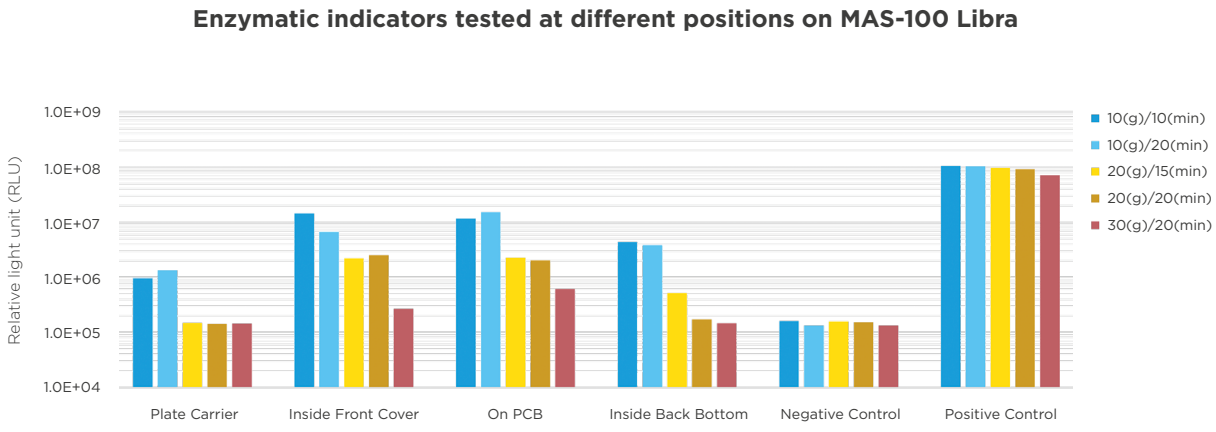

FIGURE 3: Relative light unit (RLU) values on a logarithmic scale as an output of the enzymatic indicators for different positions on the device and different decontamination cycles. Different amounts of H_2O_2 dose (g) and contact time (min) were tested.

VALIDATION OF VHP DECONTAMINATION

MATERIALS AND METHODS

The study was conducted in the SKANFOG test unit with a chamber volume of 1.5 m^3. Biological indicators were strategically placed on the MAS-100 Libra at four critical locations: on the plate holder, inside the front electronics compartment, on the printed circuit board (PCB), and at the bottom of the device body (see Figure 2). These placements were chosen based on the preliminary enzymatic indicator study to include both external surfaces and challenging internal areas and cover the worst-case positions. Three independent decontamination cycles were conducted under identical conditions to ensure reproducibility of results.

TABLE 1: DECONTAMINATION PARAMETERS

Parameter	Setting
H_2O_2 Concentration (weight/weight)	34-37 % (w/w)
Initial Relative Humidity	25 ± 5 %rH
Aeration Time	30 minutes
Dose Amount	30 g
Conditioning Time	15 minutes
Holding Time	5 minutes
Total H_2O_2 Contact Time	20 minutes

TABLE 2: TEST EQUIPMENT

Equipment / Materials	Description
SKANFOG Test Unit	To generate and distribute VHP
Biological Indicators	Containing 2.0 x 10^6 *Geobacillus stearothermophilus* spores
Tryptic Soy Broth (TSB)	Liquid growth medium for yes/no answer of the treated biological indicators
Tryptic Soy Agar (TSA)	Solid growth medium used for the enumeration of viable spores

BIOLOGICAL INDICATOR EVALUATION METHODS

1. **Turbidity analysis:** Exposed biological indicators were incubated in TSB at 55-60 °C for seven days. Any presence of turbidity was considered indicative of microbial survival.
2. **CFU plate counting:** Exposed biological indicators were suspended in sterile water with Tween 80, sonicated, and serially diluted. Aliquots were spread on TSA and incubated at 55-60 °C for 48-72 hours to determine the number of viable spores.

RESULTS

The VHP decontamination process was evaluated by analyzing the presence or absence of microbial growth and quantifying the log reduction of spores across all biological indicator positions.

COMPLETE MICROBIAL INACTIVATION

All biological indicators placed on the MAS-100 Libra demonstrated complete inactivation of *Geobacillus stearothermophilus* spores, achieving a minimum log reduction of 6.3 at every tested position (see Figure 4).

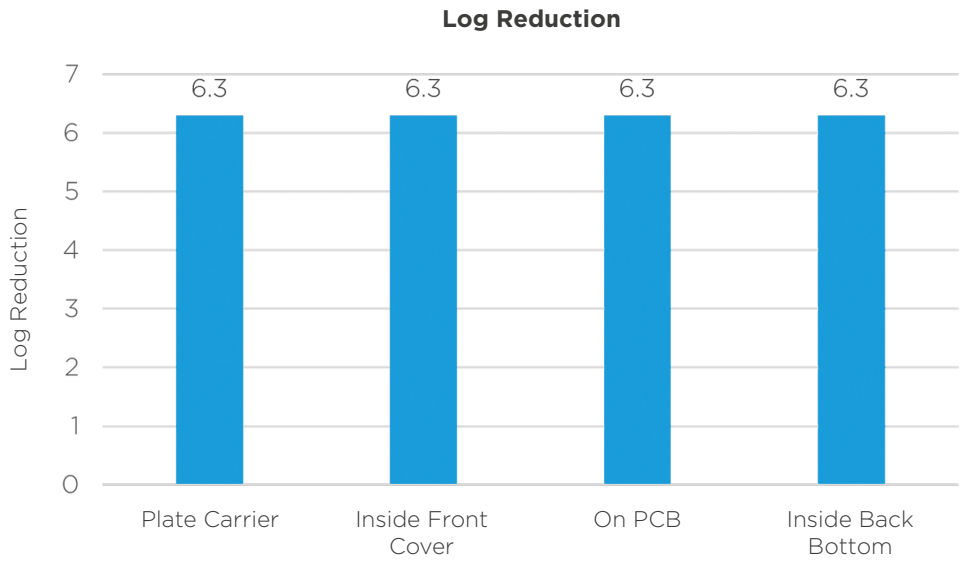

FIGURE 4: Log reduction as an output of the biological indicators for different positions on the device (average of the three decontamination cycles).

UNIFORM DISTRIBUTION OF VHP

The decontamination process proved effective on external surfaces, such as the plate holder, as well as in internal areas like the printed circuit board and electronics compartment. Even in challenging internal positions, complete spore inactivation was observed, confirming the thorough distribution and penetration of VHP.

QUANTITATIVE OBSERVATIONS

For each biological indicator, the initial spore population was 2.0 x 10^6 spores. Following exposure to VHP, no viable spores were detected, indicating a reduction exceeding the detection limit of 6-log.

RESIDUAL H_2O_2 EVALUATION

While direct analysis for residual H_2O_2 was not performed, the findings from the CFD study as well as those from the biological indicator log reduction suggest that air entering the equipment during decontamination exits through defined pathways, facilitating the removal of H_2O_2 during the aeration phase. This natural airflow dynamic minimizes the risk of residual H_2O_2, ensuring the device's safe use. Furthermore, MAS-100 Libra is largely composed of materials such as stainless steel 1.4404 and POM that are well known for their ability to retain only minimal amounts of H_2O_2.

H_2O_2 COMPATIBILITY

In addition to verifying the efficacy of VHP decontamination, the resistance of the MAS-100 Libra to repeated H_2O_2 exposure was assessed in two different approaches. The first tests were performed at SKAN in a dedicated test chamber using a specific system. This system generates nebulized H_2O_2, designed to represent a more rigorous challenge compared to ex-posure with non-condensing volatile H_2O_2. In a second set of experiments, relevant parts of MAS-100 Libra were directly inverse in 35% liquid H_2O_2.

Thus, the instrument was subjected to two types of compatibility assessments:

- **Persistence testing:** The MAS-100 Libra was exposed to 50 consecutive decontamination cycles using the SKAN system (see Figure 7). Upon completion of these cycles, a comprehensive analysis of the instrument revealed no optical or functional effects. All components of the MAS-100 Libra maintained their integrity and performance (plate handling, particle emission, electrical communication), demonstrating excellent compatibility with repeated VHP exposure.

- **Material compatibility:** Device and individual components of the MAS-100 Libra were immersed in a 35% H_2O_2 solution overnight. Following immersion, no visual or structural degradation was observed on the tested materials.

These findings confirm the robustness of the MAS-100 Libra against prolonged and repeated use of H_2O_2, making it suitable for environments requiring frequent decontamination cycles.

DISCUSSION

The results of this study confirm the effectiveness of VHP in achieving microbial decontamination for the MAS-100 Libra. The VHP process consistently ensured complete microbial inactivation across all tested positions, including both external and internal components.

Preliminary study with enzymatic indicators
The preliminary study using enzymatic indicators played an important role in optimizing the validation process. By identifying critical positions and determining the appropriate H_2O_2 dose, the enzymatic indicator study laid the foundation for targeted and effective biological indicator testing.

Effectiveness of VHP decontamination
The uniformity of microbial inactivation across the MAS-100 Libra highlights the robustness of the VHP process. The ability of VHP to penetrate internal compartments of the instrument, such as the electronics housing, is a significant advantage for devices with complex geometries.

Reproducibility of results
The identical outcomes across three independent decontamination cycles demonstrate the reliability of the process. This reproducibility is essential for ensuring microbial decontamination in routine operational settings.

Compatibility with repeated VHP exposure
Compatibility testing demonstrated that the MAS-100 Libra can withstand frequent and prolonged exposure to H_2O_2 without compromising its structural integrity or functional performance. This durability ensures the device's long-term reliability in high-frequency decontamination processes.

Justification for residual safety
The airflow inside the MAS-100 Libra is conservatively estimated at 1 cm/s (Figure 5, Application Note MBV 2025). Given the device's height of 30 cm, an air exchange can be assumed to occur at least every 30 seconds, even in hard-to-reach areas within the housing. The aeration phase within the decontamination cycle, lasting several minutes, will result in multiple air exchanges within the MAS-100 Libra instrument. The CFD evaluation and biological indicator validation demonstrated that air entering through perforations during decontamination exits the device efficiently. This natural airflow dynamic minimizes the risk of residual H_2O_2 within the equipment. This approach ensures both operator safety and compatibility with sensitive environments.

CONCLUSION

This validation study demonstrates that VHP is a highly effective decontamination method for the MAS-100 Libra. Key findings include:

- **Comprehensive decontamination:** The VHP process achieved a >6-log microbial reduction at all tested positions, including challenging internal compartments.
- **Airflow risk mitigation:** Despite the potentially reduced air pathways in the device identified through CFD analysis, the VHP process ensured decontamination throughout the device.
- **Consistent performance:** The decontamination results were reproducible across multiple cycles, highlighting the reliability of the method.
- **Compatibility with H_2O_2:** The MAS-100 Libra maintained its structural and functional integrity after exposure to 50 consecutive decontamination cycles and overnight H_2O_2 contact, demonstrating excellent resistance to repeated use of H_2O_2.
- **No residual H_2O_2:** In addition, no detectable residues of H_2O_2 remained on the MAS-100 Libra, ensuring safety for subsequent use.

ABOUT THE AUTHORS

Dr. Lucas Armbrecht, Project Leader

Lucas Armbrecht is a microsystems engineer and holds a PhD in bio-analytics from ETH Zurich.
At MBV AG, he is responsible for the development of new products and technologies for microbial contamination control and accurate air flow sensing solutions.

Dr. Miriam Schönenberger, Product Manager

Miriam Schönenberger is a microbiologist and holds a PhD in cancer research from ETH Zurich. She has many years of experience in business development and after sales of laboratory equipment and channeled customer needs into concrete product portfolio strategies.
At MBV AG, she is responsible for the products for microbial air monitoring in isolators and RABS and develops convincing solutions for aseptic production together with interdisciplinary teams.

REFERENCES

- EudraLex (2022). Annex 1 – Manufacture of Sterile Medicinal Products. The Rules Governing Medicinal Products in the European Union, Volume 4, EU Guidelines to Good Manufacturing Practice, Medicinal Products for Human and Veterinary Use. 2022

- MBV (2025). Airflow Visualization with the MAS-100 Libra. Application note.

FURTHER INFORMATION

We offer additional information on our products on these channels:

- Our product page on the MAS-100 Libra
- Tutorial for filter adapter mounting and filter exchange: MBV Youtube channel.
- Installation manual for the filter adapter and filter exchange: MBV download page.
- Additional information 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

MBV article number	Description
201120	MAS-100 Libra settle plate changer The following accessories are included in the scope of delivery for the MAS-100 Libra: 1 x Tri-clamp connector 1 x Tri-clamp sealing gasket 1 x Safety sheet 1 x Quick start guide 1 x Test report

MORE INFORMATION ABOUT THE MAS-100 LIBRA

SUBSCRIBE TO OUR NEWSLETTER

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