APPLICATION NOTE

AIR OUTLET PARTICLE FILTER FOR VIABLE AIR SAMPLER MAS-100 NT®

SUMMARY

The MAS-100 NT viable air sampler is specified for use in ISO Cleanroom Class 5/GMP Grade A. The quality materials and components used in its construction prevent particle contamination of the environment. If instruments are used in different cleanroom classes particles may be carried over inside an instrument.

The optional HEPA H13 air exhaust particle filter for the MAS-100 NT eliminates this risk because it is fitted at the very end of the air channel. It has passed all tests for filtration efficiency, long-term usage and effect on airflow sensor calibration. The filter module can be retrofitted by users or a certified service centre on all MAS-100 NT. A flow-sensor recalibration using the DA-100 NT digital anemometer is recommended. Filters are specified for at least one-year usage and are user exchangeable without recalibration.

1. INTRODUCTION

Multiple regulatory guidance documents require active viable air monitoring in aseptic production facilities. Microbial air samplers used for this purpose may not contaminate this sensitive environment. The [MAS-100 NT](MAS-100 NT) microbial air sampler is specified for use in ISO Class 5 / GMP Grade A environments. The brushless air blower and the anodized aluminium used for its construction are essentially particle free in operation and very robust. However, if an instrument is used in different grade environments contamination could be carried over inside an instrument and blown out thereafter (Figure 1).

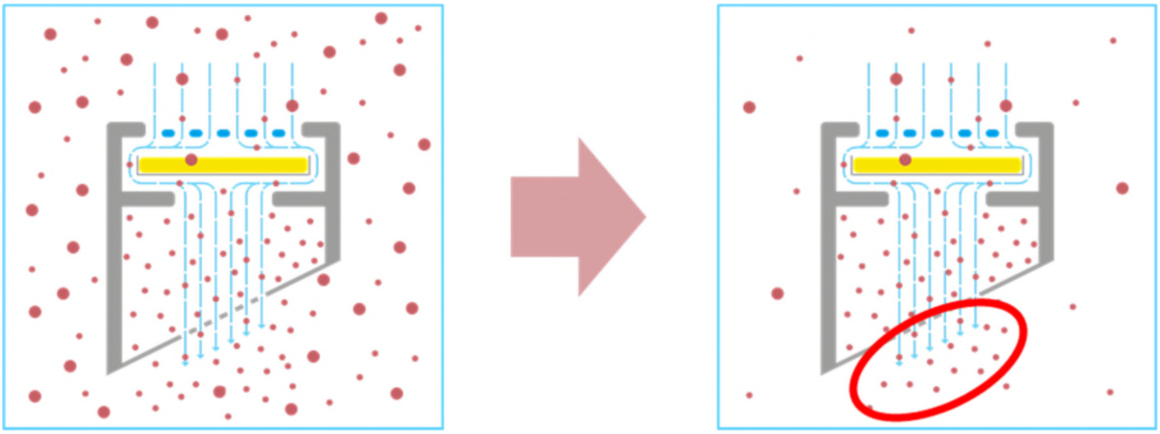
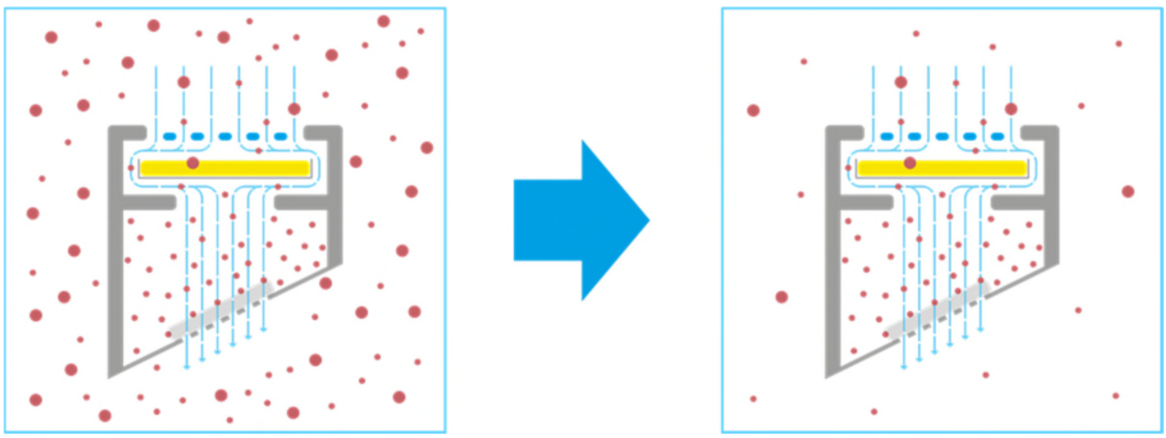
<table>
<tr><th colspan="2">A: POTENTIAL TRANSFER OF PARTICLE CONTAMINATION WITHOUT FILTER</th></tr>
<tr><th>Lower grade cleanroom (C or D)</th><th>Higher grade cleanroom (A or B)</th></tr>
<tr><td colspan="2"></td></tr>
<tr><td>The normal operation of an impaction-based air sampler leads to removal of large particles from the airstream due to impaction into the agar.
Small particles will travel with the airstream around the agar plate and will be exhausted.
Some of the small particles may attach to the inside of the air sampler.</td><td>The instrument has been moved to a higher-grade cleanroom. The air around the air sampler carries much fewer particles.
If the instrument is operated the remaining large particles are impacted. The smaller particles from the ambient air and potentially some of the small particles caught inside the instrument are exhausted and may lead to the contamination of the environment.</td></tr>
<tr><th colspan="2">B: CARRY-OVER OF PARTICLE CONTAMINATION IS PREVENTED BY HEPA FILTER</th></tr>
<tr><th>Lower grade cleanroom (C or D)</th><th>Higher grade cleanroom (A or B)</th></tr>
<tr><td colspan="2"></td></tr>
<tr><td>The environment around the instrument has a high particle count. Large particles impact in the agar, smaller particles are retained by the HEPA filter. The exhausted air is essentially particle free.</td><td>After a transfer to the higher-grade cleanroom the ambient air carries few particles.
These are impacted into the agar. Most of the small aspirated particles and the small particles inside the instrument are retained in the filter. The environment is protected from particle contamination transferred from the lower-grade cleanroom.</td></tr>
</table>

Figure 1: Graphic representation of particle carry-over inside a microbial air sampler. Panel A: Without protective HEPA filter at the air exhaust. Panel B: Instrument equipped with a particle filter at the air outlet.

2. HEPA H13 FILTER MODULE FOR MAS-100 NT

To prevent the potential carry-over of particles inside the instrument an air outlet filter adapter was developed for the MAS-100 NT (Figure 2) and its explosion-proofed version MAS-100 NT Ex®. The filter itself is graded as HEPA H13. It is held in place by an adapter that is fitted to the air exhaust of the MAS-100 NT just below the air blower.

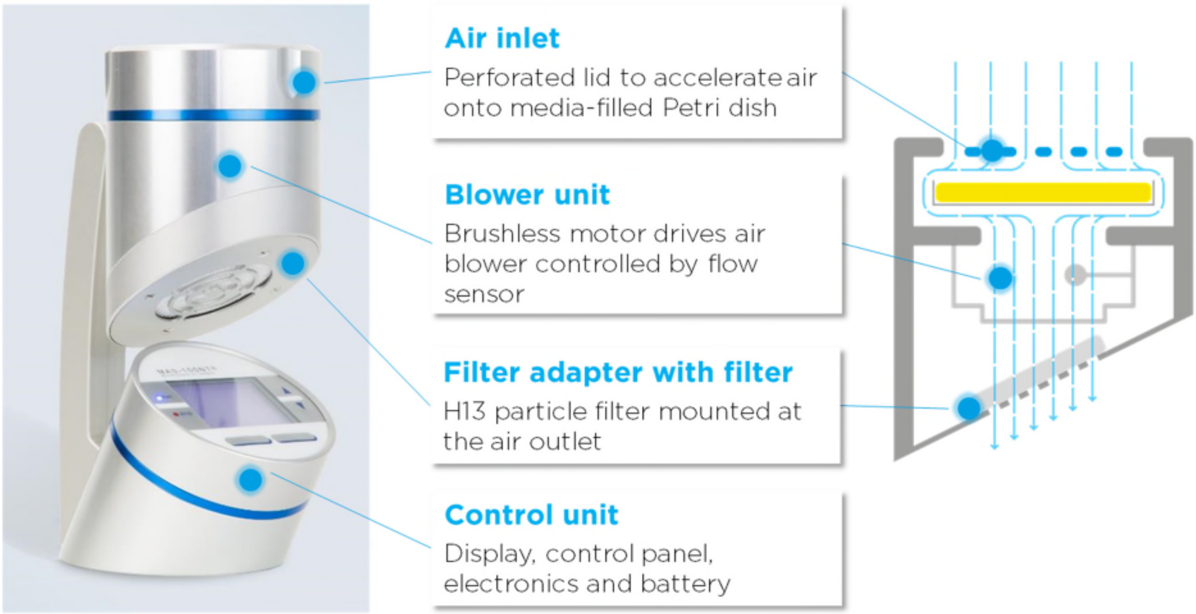


Figure 2: MAS-100 NT including air outlet filter adapter. The filter is mounted at the very end of the air duct. The direction of the air outflow is the same as the direction of air aspiration. This prevents disturbing the unidirectional airflow in cleanrooms.

The particle filter for the MAS-100 NT has the following characteristics:

- The filter is located at the very end of the air channel. This leaves no unfiltered air inside the instrument that may be exhausted later in a higher-grade cleanroom (Figure 1).
- Rated HEPA H13
- The module can be retrofitted to all MAS-100 NT by the user or a certified service center. An airflow recalibration is recommended.
- The filter can be user exchanged (Figure 3). No airflow recalibration is required.
- The filter lifetime is more than one year under intense use.
- All functions (such as swiveling the head) of the MAS-100 NT remain unaffected.

Figure 3: Filter in the adapter of the MAS-100 NT with the exhaust plate removed. The filter can be easily exchanged by the user, if required.

3. EXPERIMENTAL SET-UP AND RESULTS

4.1. MATERIALS AND EQUIPMENT

- Microbial air samplers: Several used standard MAS-100 NT (MBV AG, Switzerland) were equipped with the newly developed filter adapter.
- Airflow calibration of the air samplers: DA-100 NT certified digital anemometer (MBV AG, Switzerland).
- Filter: Circular 74 mm H13 rated PP HEPA filter.
- Particle counter: ACS Plus with particle sizes from 0.3µm -10 µm (KM OptoElectronik GmbH, Germany)
- Environments:
  - Clean environment: Biosafety workbench Model 1.2 for product protection (Skan, Switzerland).
  - Reference environment: Uncontrolled environment next to biosafety workbench at MBV AG.

Figure 4 gives an impression of the general set-up of the experiments performed.

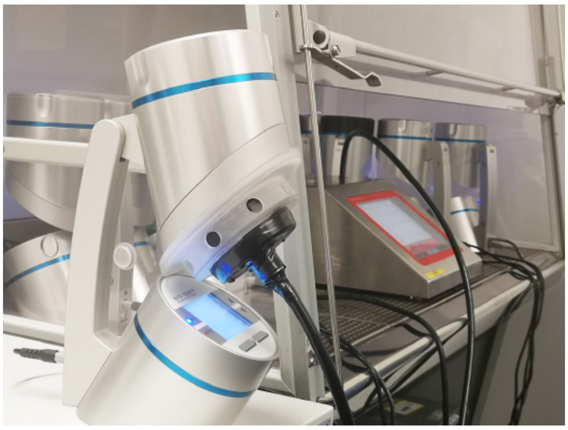


Figure 4: Experimental set-up for testing the HEPA filter on the microbial air sampler MAS-100 NT. Here, the instrument is placed in the reference (uncontrolled) environment outside of the biosafety workbench. The particle counter resides inside the clean environment and is connected to the sampler with an antistatic tube.

4.2. SAMPLING AIRFLOW CALIBRATION AND STABILITY OF AIRFLOW

Introducing a particle filter into the airstream downstream of the blower and flow-sensor of a MAS-100 NT leads to additional small resistance to the airflow. This increased resistance must be compensated by the blower and must not impact the airflow measurement of the flow sensor.

All MAS-100 NT were calibrated and if required adjusted to 100 SLPM (Standard Liters Per Minute) using a DA-100 NT digital anemometer. Thereafter the filter adapter was mounted on the instruments. The instruments were calibrated (but not re-adjusted to 100 l/min) again. Subsequently the filter was inserted into the filter adapter and the replacement exhaust plate was mounted. The instruments were calibrated again, and the results were compared to the instrument with its standard configuration without filter.

In our experiments we found that the airflow sensor detected the lower flow and the blower compensated for the higher resistance. The change in airflow was below the specified repeatability of the air sampler and the DA-100 NT digital anemometer used for instrument calibration. However, it is recommended to re-calibrate and readjust an instrument if it is upgraded with a filter unit.

No recalibration was required between filter changes. The calibration differences between all filters were negligible and significantly below both the airflow precision specification of both the MAS-100 NT and the DA-100 NT.

To test the long-term stability of the airflow two instruments with mounted adapter including a new filter were calibrated and if required adjusted to 100 SLPM. Long-term (simulated 1 year of usage) tests were performed by running the air samplers continuously for 21 days in the uncontrolled reference environment. PWM value (power in % of blower rating used to sample at 100 SLPM) were recorded from time to time to test if filter clogging or wear and tear leads to higher power consumption of the blower. Figure 5 shows the PWM value over the 21 days of testing. The data show that the air blower of the MAS-100 NT has around

40% spare capacity. They also demonstrate that over the 21 days of continuously running and aspirating reference air there is no trend to higher power consumption and therefore the filter is not being clogged significantly. The airflow calibration for both instruments was also within tolerance after the 21 days of operation.

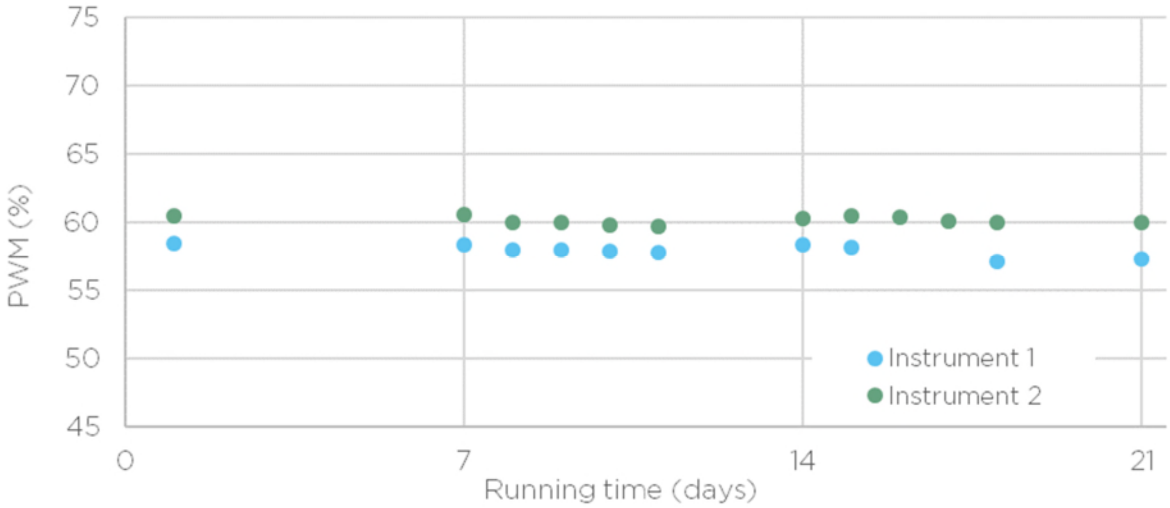


Figure 5: Power consumption (PWM) in % of the maximum power consumption of the blower throughout the long-term airflow stability test of two MAS-100 NT viable air samplers. The instruments were run continuously in the reference (uncontrolled) environment with a filter mounted.

4.3. ESTABLISHING A SUITABLE TEST SYSTEM

A 3D printed particle sampling adapter was fitted to the air exhaust of the MAS-100 NT (Figure 4). The sampling adapter was equipped with additional air outlets as the MAS-100 NT samples and exhausts air with a rate of 100 SLPM while the particle counter ACS Plus samples at 1 ft^3/min (28.32 l/min).

Reference samples (uncontrolled environment, without filter) which had a very high particle load are averages of 10 samples of 10 s each with an intermediate purge time of 20 s in the clean environment and then normalized to 1000 l. The samples were taken with the sampling adapter either directly or from the air exhaust of a running MAS-100 NT.

Sampling in the clean environment or if a filter was mounted in the reference environment consisted of 12 consecutive samples of 3 min each (=1019 l) without intermediate purge.

In all cases the particle sizes 0.3 µm, 0.5 µm, 1 µm, 3 µm and larger than 5 µm were recorded.

It was verified that this set-up representatively samples the size distribution of particles in the reference and clean environment by comparing reference samples with the sampling adapter only and the sampling adapter mounted to a running MAS-100 NT without filter (Table 1, Figure 6). The sampling adapter was connected to the particle counter using the 3 m antistatic tube that is part of the particle counter.

The data in Table 1 show that the biosafety cabinet provides a very low particle environment and that the particle adapter does not produce a relevant number of particles. Due to the very low background particle count we have not corrected our filtration efficiency results for them (the results would be even better).

[Counts/m³]	0.3µm	0.5µm	1.0µm	3.0µm	>5.0µm	Total counts
Permissible ISO Class 5 particle concentration	10200	3520	832	n.a.[1]	n.a.[1]	n.a.[1]
Clean air[2]	15	8	5	2	7	37
Reference air without MAS-100 NT[3]	762'314	186'569	45'432	4'462	5'923	1'004'700
Reference air incl. MAS-100 NT[4]	701'746	163'906	42'185	4'109	4'956	916'901

Table 1: Particle numbers and size distribution of the testing system. For reference also the permissible particle concentrations of ISO Class 5 are given. [1]Technical and statistical limitations make classification of particles > 1 µm inappropriate. [2]Air inside the clean environment sampled through the particle sampling adapter. Data are averages of 2 1000 l samples. [3]Air sampled in the uncontrolled environment through the particle sampling adapter. Data for reference air are averages of 10 samples of 10 x 10 s sampling time each and then standardized to 1000 l. [4]Air sampled from the exhaust of a running MAS-100 NT without particle filter. No media-filled Petri dish was placed in the air sampler as this would essentially capture all particles ≥1 µm.

Figure 6 demonstrates that the size distribution of the particles that are collected from the air exhaust (without filter) of a running MAS-100 NT is essentially the same as the size distribution that was directly measured in ambient air. The slight decrease in particle number and the relative shift to smaller particles is to be expected as some particles are impacted on the sampling plate of the MAS-100 NT.

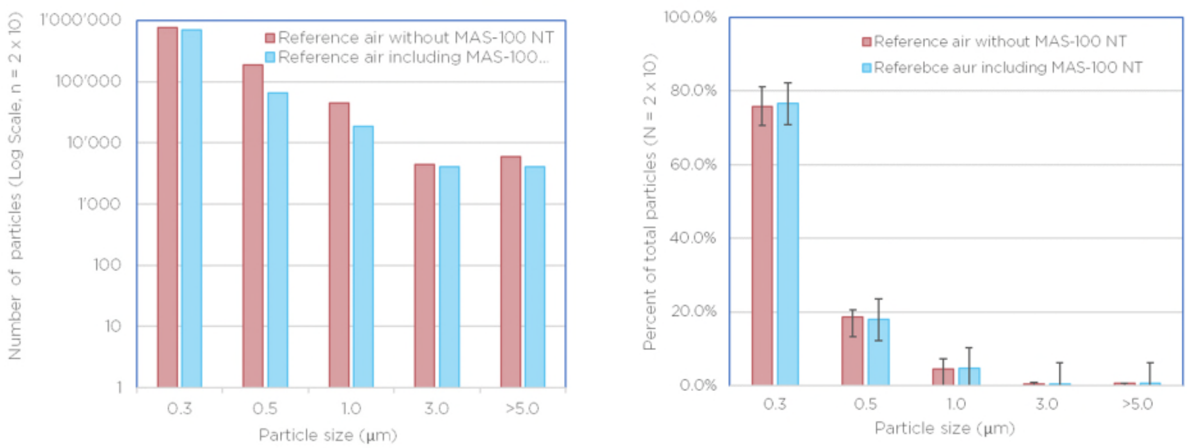


Figure 6: Distribution of particle size of air that is directly collected from ambient air through the sampling adapter versus particle size distribution of air that is exhausted by a running MAS-100 NT.

4.4. CROSS-CONTAMINATION OF CLEANROOMS

Once the test system was established the basic assumption was to be tested: Is there really a need for a filter to prevent the transfer of particle contamination between different environments?

A 1000 l air sample was collected with a MAS-100 NT in the reference environment with or without filter. Thereafter the instrument was transferred into the clean environment and started again. Particle counts were taken from the exhaust air at the outlet every 5 s. The test was repeated 5 times for both setups In tests without filter we found a total of 126 – 296 particles in the first 5 seconds after starting the blower and between 0 and 3 particles for the second 5 seconds and no particles thereafter (Figure 7 A). A total of 1042 particles were detected between all 5 repetitions, about 12% of them larger than 1 µm (Figure 7 B). The data demonstrate that there is a minimal transfer of particles between different environments. The fact that there were no particles detected after 10 seconds proves that the particles were not generated by the blower motor or other instrument components.

If the MAS-100 NT was equipped with a HEPA air outlet filter 0 – 6 particles were counted in the first 5 seconds and 0 thereafter (Figure 7 A). 15 particles, all of them smaller than 1 µm were counted (Figure 7 B). The filter dramatically reduced the transfer of particles and virtually eliminated the risk of them being CFU as those tend to be larger than 1 µm.

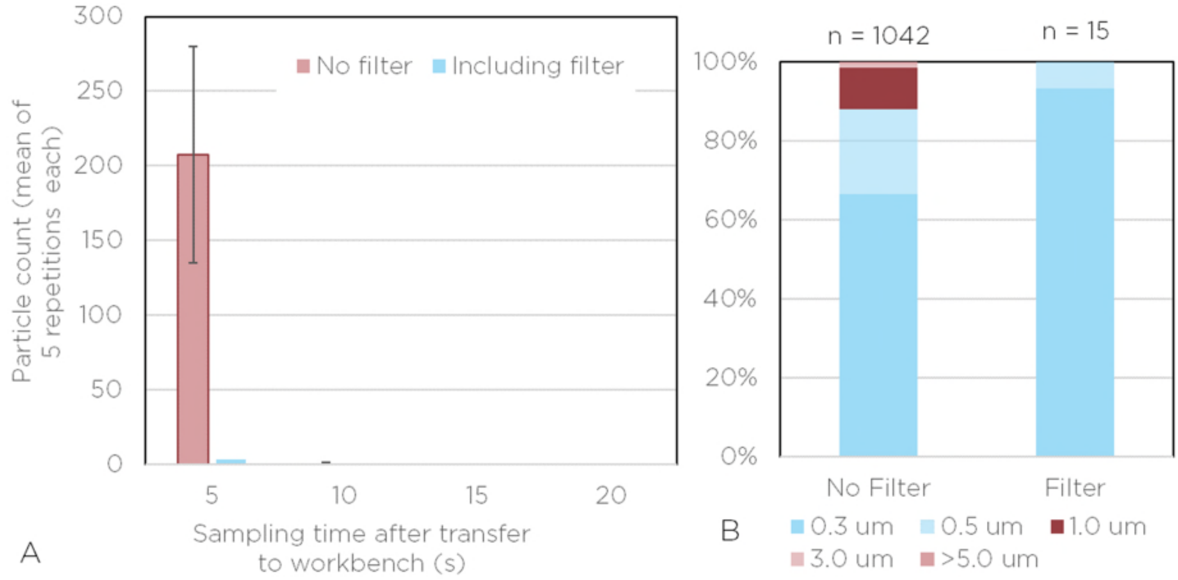


Figure 7: Particles sampled from the air exhaust of a MAS-100 NT with or without air outlet HEPA filter after transfer from reference environment into a biosafety workbench. Panel A: Time resolved total particle count after transfer. Panel B: Size distribution of all particles counted during 5 repetitions of the experiment.

4.5. FILTRATION EFFICIENCY OF THE COMPLETE ASSEMBLY

10 filters on two different units of MAS-100 NT (5 filters each) were tested for filtration efficiency of the complete assembly of air sampler, filter adapter and HEPA filter. The filter itself is rated H13 and we expected therefore a filtration efficiency of 99.95% or better for particle sizes of 0.3 µm or larger.

For every test we took a reference sample by measuring the particle count of the exhaust air of a MAS-100 NT running in the reference environment. As the particle load was very high, we took 10 samples of 10 s each and extrapolated the particle count to 1000 l. This reference count was compared to a 1000 l sample taken from the identical MAS-100 NT with particle filter running in the same environment. Every 3 minutes the particle count was recorded to assess if there was a trend.

[Particle counts/m³]	0.3µm	0.5µm	1.0µm	3.0µm	>5.0µm	Total counts
Clean air in workbench (n=2)	15	8	5	2	7	**37**
Reference (mean, n = 10)	701'746	163'906	42'185	4'109	4'956	**916'901**
Filter (mean, n = 10)	69	6	1	0	0	**76**
Filtration efficiency [%]	99.99%	100.00%	100.00%	100.00%	100.00%	**99.99%**
Max particle count with filter	119	14	6	0	1	**140**
Min particle count with filter	6	1	0	0	0	**8**

Table 2: Filtration efficiency for the HEPA H13 filter assembly for the MAS-100 NT. Tests were performed with 10 filters. All filters passed the 99.95% efficiency requirement for filtration performance.

In all cases the filter assembly passed, and even exceeded, the required performance threshold (Table 2) for H13 filters. Throughout the 36 minutes (approx. 1 m^3) sampling interval there was no trend for any filter (data not shown). The filter assembly therefore meets the need to protect an environment from carry-over of particles from another environment with lower particle rating.

4.6. LONG-TERM FILTRATION EFFICIENCY

It is recommended to recalibrate a MAS-100 NT annually. Therefore, under normal usage a filter should be able to be used for a complete calibration interval. We assumed that 10 samples of 1000 l per day would be taken under intense usage for 300 days per year. This amounts to about 21 days permanent operation.

Two MAS-100 NT with filter were run continuously for 21 days in the uncontrolled reference environment. From time to time 1 m^3 of the exhausted air were sampled for particles from both devices and compared to the reference sample taken immediately before or after without filter.

The data in Table 3 show that there was no detectable deterioration of filtration efficiency during this time. All filters were meeting or exceeding H13 filter specification. Also, there was no detectable filter clogging (Figure 5 in Chapter 4.2) and airflow calibration remained within specification.

Filtration efficiency	Instrument 1		Instrument 2	
	Day 1	Day 21	Day 1	Day 21
0.3 µm	99.980%	99.976%	99.955%	99.984%
0.5 µm	99.995%	99.994%	99.959%	99.999%
1.0 µm	100.000%	100.000%	99.937%	100.000%
3.0 µm	100.000%	100.000%	100.000%	100.000%
>5.0 µm	100.000%	100.000%	100.000%	100.000%
Overall	**99.985%**	**99.981%**	**99.955%**	**99.988%**

Table 3: Filtration efficiency over a simulated 1-year usage lifetime for two MAS-100 NT.

The MAS-100 NT Ex is the explosion proofed version of the MAS-100 NT. The results described in this document can be extended to that product (depicted here without mounted filter adapter).

4. CONCLUSIONS

In this document we present data on preventing the transfer of particle contamination between different cleanroom classes or more generally between environments with different particle load.

With the experimental data we support the following findings:

- There is a risk of minimal transfer of particle carry-over between different environments from air trapped inside a microbial air sampler.
- Particle carry-over was essentially eliminated by the insertion of a HEPA filter at the air outlet of the MAS-100 NT viable air sampler.
- The filter had no significant influence on the airflow calibration and therefore on the microbial sampling efficiency.
- Stressing the filter for a simulated year of heavy usage in an uncontrolled environment did not reduce the filtration efficiency and did not clog the filter.

5. FURTHER INFORMATION

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

- Our product page on the HEPA filter of the MAS-100 NT
- 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.

6. ORDERING INFORMATION

Outside Switzerland MBV products are also distributed and supported by our partner Merck. For inquiries quote the following article numbers.

Article	Description	Article number MBV	Article Number Merck
MAS-100 NT with HEPA filter	MAS-100 NT with filter in transportation case.	06.5020.01	1.17274.0001
MAS-100 NT Ex with HEPA filter	MAS-100 NT Ex (explosion-proofed) with filter in transportation case.	06.5040.01	1.17275.0001
Set filter bracket compl. w/HEPA filter	Filter adapter and filter for retrofitting your MAS-100 NT or MAS-100 NT Ex.	06.5000.01	1.17276.0001
HEPA H13 filter Ø74mm	Replacement filter. Requires a filter adapter to be mounted on the MAS-100 NT (Ex)	06.6068.01	1.17278.0001