WHITE PAPER

MAS-100 NT Air Sampler Evaluation with Growth Direct® Media Cassettes

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THE COMPANY

Rapid Micro Biosystems® (RMB) creates, sells, validates, and services innovative products for fast, accurate, and efficient detection of microbial contamination in the manufacture of pharmaceuticals, biologics, biotechnology products, medical devices, and personal care products. The company's Growth Direct® is the only fully automated, non-destructive growth-based system for multiple QC test applications: environmental monitoring, water, and bioburden. Automating rapid compendial QC micro testing ensures data integrity, compliance, and operational efficiencies. RMB is dedicated to providing groundbreaking technology and products to support companies in their journey to achieve greater reliability, efficiency, and better predictability, ultimately providing higher quality products for improved patient outcomes.

SUMMARY

The MAS-100 NT has been evaluated for performance compatibility with Growth Direct® Environmental Monitoring (EM) Cassettes. Testing was conducted to determine recovery on 0.45µm pore-size black membrane covered TSA LP80 (Tryptic Soy Agar with Lecithin and Polysorbate 80) and TSA LP80HT (Tryptic Soy Agar with Lecithin, Polysorbate 80, L-histidine and Sodium Thiosulfate) compared to compendial media supplied from Becton Dickinson (BD).BD was used as the control as a global supplier of the standard RODAC™ contact plate with compendial media. Recovery on RMB TSA LP80 media was 87% compared to BD TSA LP80 media. Good recovery was still seen when the media became dehydrated from sampling in the MAS-100 NT. A continuous sampling time of 10-minutes and intermittent sampling over 4-hours, were used to reach a desired sampling volume of $1m^3$ during dehydration testing. *Staphylococcus aureus* recovery on dehydrated RMB TSA LP80 ranged between 78-101%, depending on sampling duration, compared to the TSA LP80 contact plate supplied by BD. *Aspergillus brasiliensis* recovery ranged from 111-116% compared to BD contact plate depending on sampling duration. *Bacillus subtilis*, *Candida albicans* and *Pseudomonas aeruginosa* recovery on dehydrated RMB TSA LP80 media was equivalent to that seen on dehydrated BD TSA LP80 media No difference in recovery was seen between the two available MAS-100 NT lid perforation formats, 400 x 0.7mm and 300 x 0.6mm. The MAS-100 NT is compatible with Growth Direct® EM consumables and produces recovery equivalent to that seen on compendial Petri and contact plates.

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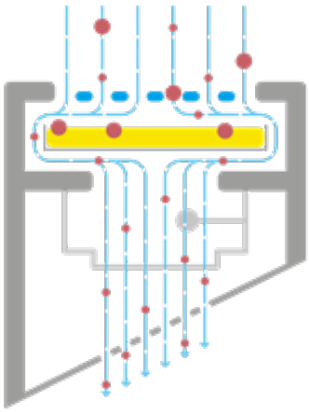
INTRODUCTION

ISO 14698 establishes a methodology for biocontamination control programs, including monitoring air quality in cleanrooms[1]. Monitoring air quality is important for any facility to ensure sterility of products and overall cleanliness. Active air samplers are designed for microbial air monitoring, a majority of which are inertial impact devices. This design draws air into the sampling head by a fan and accelerates it to produce a laminar air flow onto the collection surface. The collection area is controlled by the diameter of the perforation pattern of each lid (Figure 1). Impact air samplers must balance impact velocity, flow rate and total sampled volume[2]. Traditionally 1m^3 is sampled in a short period of time, approximately 10mins. The new Annex 1 for manufacture of sterile products is now requiring air to be sampled over the duration of the manufacturing run, 1m^3 over 4-8 hrs. A d_{50} value is often used to describe physical sampling efficiency of an air sampler, in which it describes the "cut-off" or the particle size where 50% is collected on the media and 50% are too small and continue to follow airflow path[3]. The MAS-100 NT has a d_{50} value of 1.1µm with a 300 x 0.6mm lid and 1.6 µm with a 400 x 0.7mm lid.

Figure 1. Air Flow in MAS-100 NT



Air samplers are designed for a standard Petri dish or contact plate to be placed in the sampler and then easily removed, without contamination, to be incubated for microbial growth and detection. Counting the colonies gives a quantitative estimate of the number of colony forming units (CFU) in the sampled air, thus, helping to evaluate air quality. The MAS-100 VT, MAS-100 NT, MAS- 100 VF, MAS-100 Iso MH, and MAS-100 ISO NT from MBV, distributed by SigmaMillipore (US/CA only) and Merck, are Growth Direct® compatible. This paper focuses on MAS-100 NT, but there is no difference in sampling performance expected for the other types. Two air sampler lids are available for the MAS-100 NT, created with perforations of either 300 holes each with a 0.6mm diameter or 400 holes each with a 0.7mm diameter. The 400 x 0.7mm lids have an impaction velocity of 11 m/s, while the 300 x 0.6mm lids have an impaction velocity of 19 m/s. For this reason, it is believed that the 300 x 0.6mm lid is more efficient for traditional media plates[4]. Both sampler lid types are designed either for standard Petri Plate (90-100mm) or contact plate (55mm) dimensions. Additional clips and a modified perforated lid are required for compatibility with Growth Direct® cassettes. These modified lids have been designed to ensure identical diameter, bayonet coupling, and perforation size as the original lid designs. The only difference is the modified height. The modified height of the Growth Direct® perforated lid is required for the larger size on the cassette compared to a contact plate and allows identical distance from the bottom of the perforation to the top of the media (Figure 2)[5]. All heads and adaptors are available from Rapid Micro Biosystems® and Merck Millipore. Part numbers can be found in Appendix A at the end of this paper.

Figure 2. Modified MAS-100 NT Perforated Lid for Growth Direct Consumables

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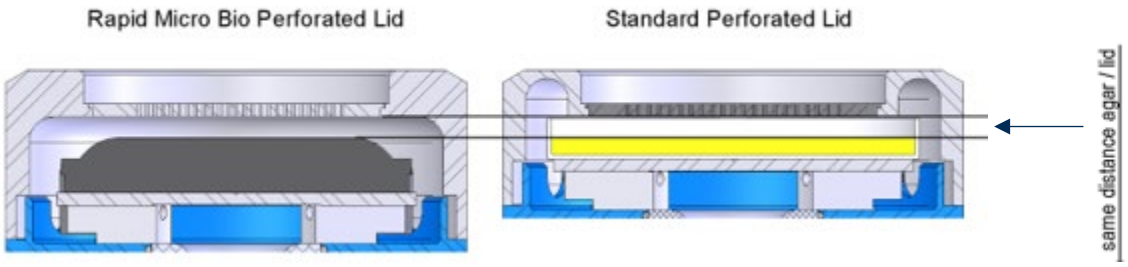

GROWTH DIRECT® SYSTEM TECHNOLOGY DESCRIPTION

The membrane on Rapid Micro Biosystems® EM cassette is essential for the detection of microbial growth by the Growth Direct® System. The Growth Direct® System is a rapid microbial enumeration platform that detects microbial growth based on auto fluorescent emissions. Blue LED lights illuminate the cassettes and cause the microorganisms to auto fluoresce. A CCD camera detects the fluorescence and records growth throughout incubation. Each cassette is supplied with an 0.45-micron pore size membrane placed on top of standard compendial microbiological growth media. The membranes have been dyed black to reduce the background fluorescence of the cellulose esters and the media and generate a better signal to noise ratio. The sampling surface is equivalent to that seen on a standard contact plate shown in Figure 3. The presence of the membrane requires demonstration of the equivalence of capture between the membrane surface and the traditional media surface that is addressed in this study.

Figure 3. Equivalent Surface Sampling Area

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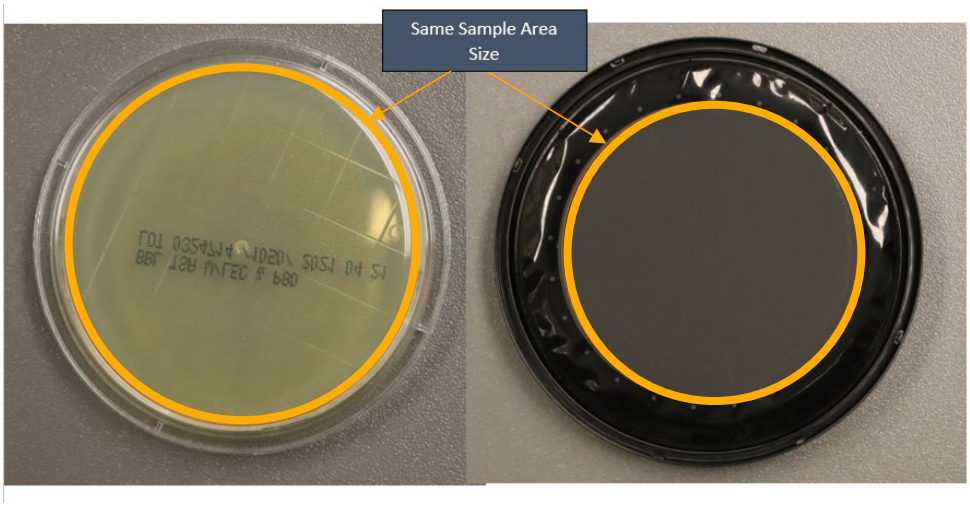

METHODS and RESULTS

All air samplers were calibrated at an aspiration volume of 100L/min and checked using a DA-100 NT Anemometer prior to use. Media was equilibrated to room temperature prior to use.

1. Media Dehydration and Growth Promotion During Normal and Extended Sampling Times

All air samplers were fitted with 400 x 0.7mm sampler lids. Each modified to hold either an RMB cassette or BD contact plate. A biological safety cabinet (BSC) was used to sample the air and remain sterile for a spike and recovery experiment. Two sampling times were used to compare the traditional sampling of 1,000 L in 10 min (short) and the new Annex 1 recommended 1,000L in 4 hours, 50L every 12 mins (long). Cassettes were weighed before and after sampling in the air sampler for both methods. The weight of the empty plastic bases was subtracted from the total sample weight to give initial and final weight of only the media. The media weight was used to determine dehydration of each sample. MAS-100 NT samplers started about 30 seconds apart and were placed on opposite sides of the BSC. Plates were left in the air sampler or uncovered in the BSC between sampling times, creating a worst-case scenario dehydration, Figure 4. With the cover off, RMB media lost 6% more moisture than when samples were left in MAS sampler for full run (14%), an example of real-life method. BD media lost about 9% more moisture when plates were left uncovered between sampling times compared to 27% seen in real life method in the sampler.

Upon sample completion, each plate was aseptically removed from the air sampler and immediately inoculated and spread. This avoids organism rapidly absorbing to the dehydrated application site while inoculating remaining replicates producing an uneven organism dispersal that would be difficult to count. Each sample was spiked with 100µl of either *Staphylococcus aureus* or *Aspergillus brasiliensis*. Organisms were prepared to contain 50-100CFU in 100µl. Samples run for the long method were spiked at the end of the 4-hour sampling period. Spiked cassettes were allowed to dry in the BSC prior to capping with a lid. Three replicates per plate type, organism and sampling method were taken.

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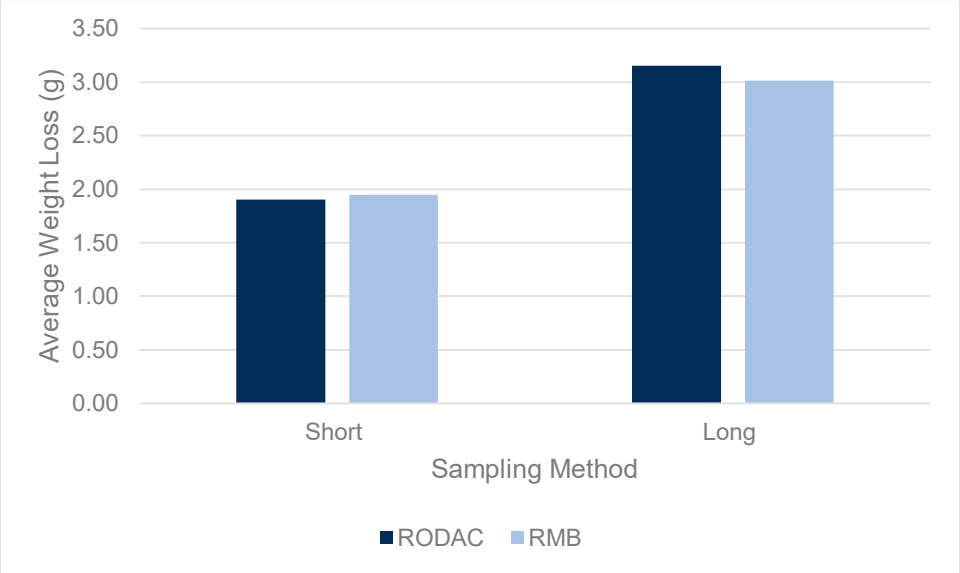
Recovery of *Bacillus subtilis*, *Candida albicans* and *Pseudomonas aeruginosa* were only run on 4-hour dehydrated samples. Plates were incubated for 48 to 72 hours at 30-35°C in Growth Direct or incubator depending on format.

Compendial TSA LP80 contact plates from BD (RODAC™) lost the same amount of weight as RMB TSA LP80 cassettes after both short and long sampling methods (Figure 4). Both plate types lost about 1.9g during the short sample method and 3.0 to 3.1g for the long sample method (plates left in sampler). Dehydration was calculated by the weight lost as a percentage of the total media weight; Growth Direct® cassettes contain more media than RODAC™. In this case, BD TSA LP80 contact plates lost a higher percentage of moisture compared to RMB TSA LP80 contact plates. BD contact plates lost 16% and 27% compared to RMB cassettes which lost 9% and 14%, for short and long sampling methods respectfully. For both methods the plates were left in the sampler for duration of sampling time. The similar weight loss in grams, of both plate types, shows that the 0.45µm MCE membrane does not reduce evaporation (Appendix B).

Figure 4. TSA LP80 Media Weight Loss After Active Air Sampling in MAS-100 NT. Cassettes remain in sampler for duration of sampling time.



Good *S. aureus* and *A. brasiliensis* recovery was seen on dehydrated RMB TSA LP80 with 101% and 111%, respectfully, compared to BD TSA LP80 (short method). *S. aureus and A. brasiliensis* recovery of 78% and 116% respectfully, compared to BD media was seen on the long sampling method. Good

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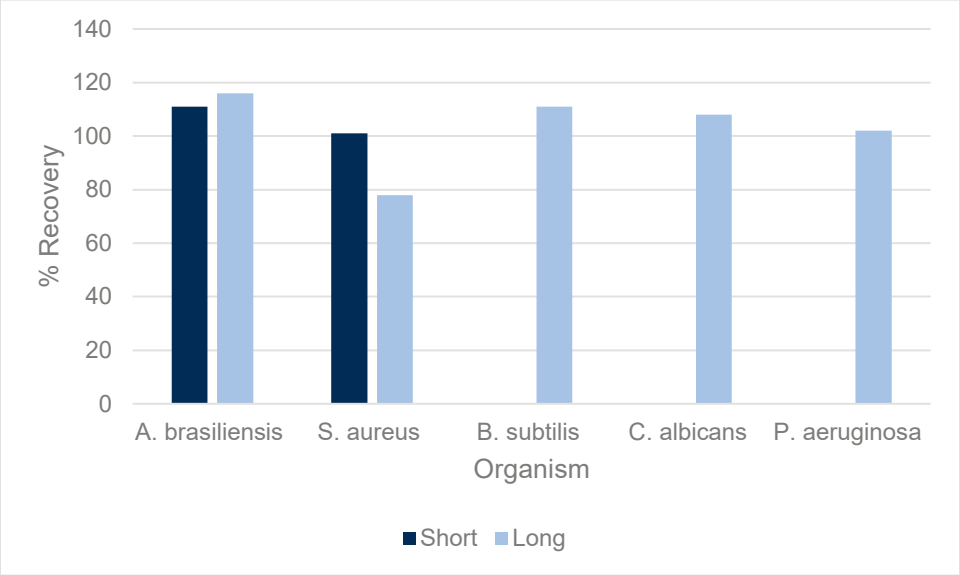
recovery was also seen on 4-hour dehydrated RMB TSA LP80 media for *B. subtilis* at 111%, *C. albicans* at 108%, and *P. aeruginosa* at 102% (Figure 5).

Figure 5. Organism Recovery on Dehydrated TSA LP80 Media



2. Equivalence of capture on Petri plates, contact plates and RMB cassette format

Air samplers were fitted with 400 x 0.7mm lids; each modified to hold either a BD Petri plate or RMB cassette, BD TSA LP80, RMB TSA LP80, and RMB TSA LP80HT. Remaining samplers were fitted with 300x 0.6mm lids and modified to hold either RMB TSA LP80 or BD TSA LP80 contact plates. Plates were loaded into the MAS-100 NT samplers and set to start with a 1-minute delay. Samples were placed 1 meter apart in an uncontrolled environmental area to ensure suitable organism counts for statistical analysis. Each MAS-100 NT was set to aspirate a volume of 100L/min for a total of $1m^3$. Upon sample completion, plates were aseptically removed and covered with a lid. 17 replicates were taken for each plate and media type and incubated for 72 hours at 30-35°C.

As seen in Figure 6, RMB TSA LP80 media had a recovery of 86%compared to the BD TSA LP80 Petri plate control. RMB TSA LP80 media had an organism recovery of 91% compared to RMB TSA LP80HT media (Appendix B). RMB TSA LP80 had recovery of 81% compared to BD TSA LP80 contact plates (Appendix C) Figure 6. Results are not statistically different with a two-sample T-test (Table 1).

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Figure 6. Recovery on different media formats compared to RMB TSA LP80 Media

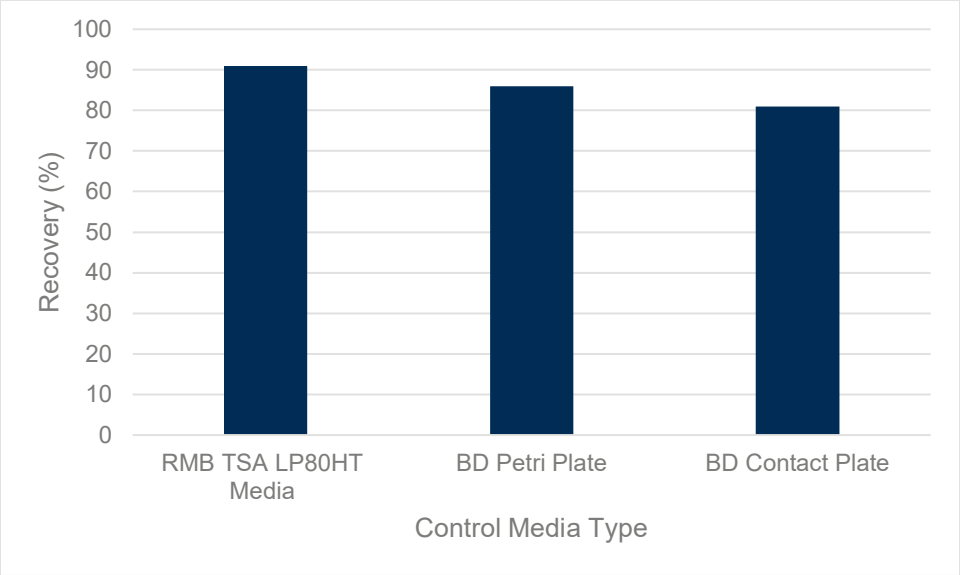

Table 1. *Equivalence Testing P-values*

Media	RMB TSA LP80
RMB TSA LP80HT	0.543
BD TSA LP80 Petri Plate	0.284
BD TSA LP80 Contact plate	0.955

3. Sampler Lid Hole Pattern Efficiency

Two MAS-100 NT air samplers were fitted with 400 x 0.7mm sampler lids and two were fitted with 300x 0.6mm sampler lids. One of the 300x 0.6mm lidded samplers was set for contact plates. The remaining three were set for Growth Direct® cassettes. Each air sampler was set to sample at an aspiration volume of 100L/min for a total of 1m^3. Samples were placed 1 meter apart in an uncontrolled environmental area to ensure suitable organism counts for statistical analysis. Upon sample completion, plates were aseptically removed and covered with a lid. 17 replicates were taken for each plate and media type and incubated for 72 hours at 30-35°C.

No significant difference in recovery for the 400x 0.7mm lid compared to the 300x 0.6mm lid was seen. Organism recovery from two days of testing, on RMB TSA LP80 using the 400x 0.7mm lid, was 110% compared to the same media using the 300x 0.6mm lid. Organism recovery was also high on RMB TSA LP80 media with 300x 0.6mm lid at 81% compared to BD TSA LP80 contact plates with the 300x 0.6mm lid. Lot to lot variation of RMB TSA LP80 was investigated on day two using two TSA LP80 lots, listed in

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Table 2, with 400 x 0.7mm lid. Recovery was 100% and no significant difference was seen (Table 3). Full data set can be found in Appendix C.

Table 2. *Recovery using Different Lid Hole Pattern Sizes*

Lid Size	Media	Average Colony Count (CFU)			% Recovery
		Day 1	Day 2	Overall	
400 x 0.7mm	RMB TSA LP80 (lot 18320L1)	N/A	17	19	110%
	RMB TSA LP80 (lot 13920L1)	22	17		
300 x 0.6mm	RMB TSA LP80	20	14	17	81%
	BD TSA LP80 Contact Plate	21	21	21	

Table 3. *P-values for MAS-100 NT Sampler Lid Efficiency Testing*

Plate Type	RMB 400 x 0.7mm Lot 13920L1		RMB 300 x 0.6 Lot 18320L1
	Day 1	Day 2	Day 2
RMB 400 x 0.7mm Lot 18320L1	0.338	0.214	0.955
RODAC 300 x 0.6mm	0.719	0.110	

4. Time to results (TTR)

To evaluate whether the speed of impaction affected the detection time of the Growth Direct® System (GD) the data generated for the environmental organisms captured by the samplers was evaluated. From the two days of test points an 85% detection level was obtained using interval counts from the Growth Direct® System. TTR 85% refers to the time it takes for organism detection to reach 85% for all samples tested on the GD. Samples run with 300x 0.6mm lids were obtained 4 hours faster at 28 hours compared to 32 hours seen on samples run with 400x 0.7mm lids (Figure 6). This difference is not significant.

Figure 7. *Time to Results 85% for GD TSA LP80 Samples Run with 400x 0.7mm and 300x 0.6mm Lids*

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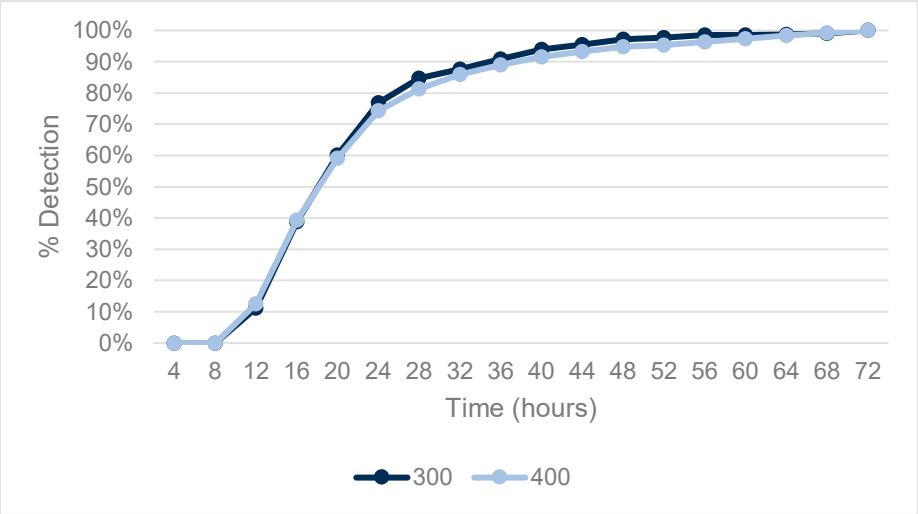

CONCLUSION

Documentation from the suppliers of MAS air samplers confirm that the capture of organisms is independent of the size of the media plate being run. The complete $1m^3$ air sample is focused on the active media and all organisms should be captured in this area. The media from BD should be equivalent to RMB media for growth promotion properties. Recovery of organisms on RMB contact plate vs. BD Petri plate is 86%. The high recovery shows that within experimental sampling error, no capture difference occurred between the 90mm Petri dish and RMB contact plate sized capture areas. Recovery on RMB TSA LP80 was equivalent to recovery on compendial BD TSA LP80 contact and Petri plate media. Recovery of RMB TSA LP80 was also equivalent to recovery seen on RMB TSA LP80HT. This was true for lot-to-lot variation on RMB media as well. No significant difference was seen.

Active air sampling can cause organism stress leading to low recovery because of media dehydration. That is why it is important to test dehydrated media to verify good organism recovery. RMB media lost the same amount of weight as compendial contact plate media supplied by BD. Thus, the 0.45-micron pore size MCE membrane on Growth Direct® consumables does not minimize evaporation, allowing the media to be exposed to the same speed of dehydration as compendial contact plates. *S. aureus*, *A. brasiliensis*, *B. subtilis*, *C. albicans* and *P. aeruginosa* recovery on dehydrated RMB TSA LP80 media was equivalent to that seen on dehydrated BD TSA LP80 media. Colony morphology was similar on both plate types and colonies were well distributed.

There is no significant difference in sampler lid efficiency for organism recovery on RMB EM Media. Recovery on RMB TSA LP80 media sampled with the 300x 0.6mm lid was equivalent to RMB TSA LP80 media sampled with 400x 0.7mm lid and BD TSA LP80 media sampled with 300x 0.6mm lid.

The MAS-100 NT air sampler is compatible with Growth Direct® EM cassettes. Recovery was seen to be equivalent to the compendial method for all tests and the 0.45-micron pore size, MCE membrane poses no disadvantage for active air sampling.

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Appendix A

List of vendor part numbers used in testing for MAS-100 NT

1. 400x 0.7mm Petri Plate Lid (Millipore part # 1090880001)
2. 400x 0.7mm Contact Plate Lid (Millipore part # 1092130001)
3. 400 x 0.7mm GD Cassette Lid (Rapid Micro Biosystems part # EMMA-001)
4. 300x 0.6mm Petri Plate Lid (Millipore part # 1091950001)
5. 300x 0.6mm Contact Plate Lid (Millipore part# 1191490001)
6. Contact Plate Adaptor (Millipore part# 1092140001)

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Appendix B Colony Counts for Equivalence Testing

Media	Replicate	GD Count (CFU)	Visual Count (CFU)	Average (CF)
RMB TSA LP80HT	1	13		15
	2	11		
	3	18		
	4	4		
	5	12		
	6	15		
	7	9		
	8	14		
	9	21		
	10	14		
	11	17		
	12	26		
	13	18		
	14	18		
	15	8		
	16	22		
	17	12		
	negative control	0		
RMB TSA LP80	1	15		14
	2	13		
	3	14		
	4	6		
	5	8		
	6	3		
	7	9		
	8	5		
	9	17		
	10	16		
	11	28		
	12	17		
	13	24		
	14	8		
	15	14		
	16	20		
	17	13		
	negative control	0		
BD TSA LP80 Petri Plate	1		11	16
	2		16	
	3		11	
	4		7	
	5		11	
	6		8	
	7		13	
	8		19	
	9		23	
	10		16	
	11		24	
	12		18	
	13		25	
	14		21	
	15		19	
	16		12	
	17		15	
	negative control		0	

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Appendix C *Colony Counts Day 1 of Sampler Efficiency Study*

Media	MAS Lid	Replicate	GD Count (CFU)	Visual Count (CFU)	Average (CFU)
RMB TSA LP80	400x 0.7mm (RMB Media lot 13920L1)	1	35		17
		2	23		
		3	19		
		4	33		
		5	34		
		6	17		
		7	8		
		8	8		
		9	13		
		10	16		
		11	9		
		12	9		
		13	9		
		14	10		
		15	22		
		16	16		
		17	12		
		Negative Control	0		
	400x 0.7mm (RMB Media lot 18320L1)	1	22		17
		2	26		
		3	23		
		4	32		
		5	34		
		6	20		
		7	14		
		8	8		
		9	23		
		10	3		
		11	7		
		12	13		
		13	11		
		14	15		
		15	19		
		16	16		
		17	10		
		Negative Control	0		
	300x 0.6mm (modified to fit RMB cassettes)	1	29		14
		2	13		
		3	Failed Height Check		
		4	20		
		5	28		
		6	18		
		7	10		
		8	7		
		9	11		
		10	9		
		11	10		
		12	13		
		13	10		
		14	9		
		15	19		
		16	12		
		17	8		
		Negative Control	0		
RODAC TSA LP80	300x 0.6mm (modified to fit RMB cassettes)	1		46	21
		2		25	
		3		28	
		4		36	
		5		52	
		6		44	
		7		15	
		8		6	
		9		9	
		10		5	
		11		9	
		12		13	
		13		6	
		14		7	
		15		15	
		16		28	
		17		13	
		Negative Control		0	

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Media	MAS Head	Replicate	GD Count (CFU)	Visual Count	Average (CFU)
RMB TSA LP80 lot 13920L1	400x 0.7mm	1	38		22
		2	19		
		3	29		
		4	23		
		5	18		
		6	14		
		7	12		
		8	21		
		9	28		
		10	19		
		11	23		
		12	23		
		13	18		
		14	19		
		15	23		
		16	24		
		17	25		
		Negative Control	0		
	300x 0.6mm (modified to fit RMB cassettes)	1	28		20
		2	33		
		3	25		
		4	25		
		5	17		
		6	13		
		7	13		
		8	16		
		9	24		
		10	19		
		11	17		
		12	15		
		13	16		
		14	18		
		15	23		
		16	23		
		17	18		
		Negative Control	0		
RODAC TSA LP80	300x 0.6mm (modified to fit RMB cassettes)	1		31	21
		2		20	
		3		21	
		4		20	
		5		19	
		6		15	
		7		17	
		8		14	
		9		24	
		10		15	
		11		17	
		12		21	
		13		30	
		14		22	
		15		22	
		16		21	
		17		25	
		Negative Control		0	

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