ALL-IN-ONE PMM SWAB

for sampling in critical environments

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1. General description of PMM Swab

PMM Swab (art.-no. 885.0120) is a gamma-sterilized, all-in-one device for sampling in critical environments. It is designed to perform a qualitative presence/absence test for environmental monitoring of dry surfaces, filling needles, tubing and surfaces that are hardly accessible with contact plates in cleanrooms, RABS and isolators.

Growth medium

- The growth medium is a Soybean-Casein Digest Medium (Tryptic Soy Broth, TSB) supplemented with neutralizers to neutralize residues of disinfectants, including Quaternary Ammonium Compounds (QAC). The basic medium is prepared according to the current European Pharmacopeia (2.6.1) and USP <71>.
- The growth medium is protected by a glass ampoule. The ampoule is sealed and autoclaved immediately after filling with the medium (2 mL).
- The double tip glass ampoule enables an easy medium release.

Swab

- The swab head is made of double layer knitter polyester and is thermally bounded to a polypropylene handle.
- Head size: 5.8 (width) x 3 (thickness) x 17 (length) mm; handle size: 3.2 (width) x 75 (length) mm.
- The swab head is flat to facilitate the sampling and guarantee a large swabbing area.
- The swab is pre-wetted with physiological NaCl solution.

Packaging and irradiation

- PMM Swabs are triple wrapped. 6 single-packed swabs are packed in a second and in a third foil.
- All foils contain a high barrier for H_2O_2 as well as for water-vapour.
- Foils are transparent to enable integrity check before use.
- A hole is integrated in the innermost and middle foil to facilitate the sanitization in the isolators.
- PMM Swabs are gamma-sterilized (> 25 kGy).
- Transport and storage at 2 – 25 °C.
- PMM Swabs are delivered in packaging units containing 20 packs of 6 swabs.

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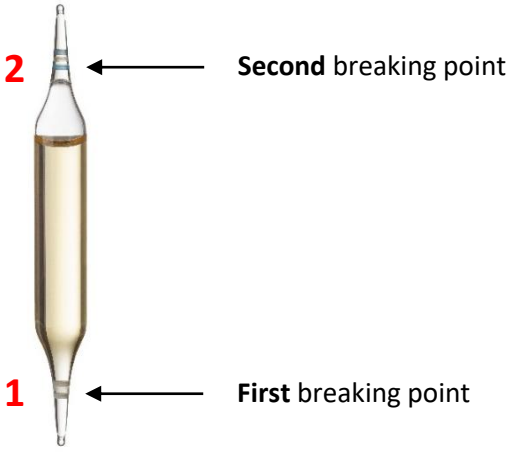
2. How to use PMM Swab

1. Open the PMM Swab and sample the surface according to ISO 14698. To open the swab, gently press on the plastic connector between the upper and lower part of the device and pull to separate the two parts.

2. Insert the swab back into the lower unit of the device and release the broth medium by breaking the ampoule in the device on both tips.
   ATTENTION: break the ampoule first at the bottom and then at the top to optimally release the broth medium and avoid pressing on the ampoule a second time which may cause the formation of glass splinters.



   ATTENTION: It is recommended to transfer the culture medium from the ampoule into the bottom part of the device as soon as possible after sampling. Storage periods of more than 8 hours should be avoided.

3. Incubate the PMM Swab vertically as a whole at the appropriate temperature.
4. Monitor the presence/absence of micro-organisms by checking the turbidity of the growth medium.

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3. Growth Promoting Properties of the Medium

3.1 Quality Control Tests

3.1.1. Procedure

After irradiation, three validation batches of PMM Swabs have undergone quality control tests. To determine the growth promoting properties of the growth medium, the content of the ampoules of 12 swabs was transferred to the lower part of the device and the medium was inoculated with 10 to 100 CFU of following test strains in duplicate:

- *S. aureus* ATCC 6538
- *E. coli* ATCC 8739
- *P. paraeruginosa* ATCC 9027
- *B. spizizenii* ATCC 6633
- *C. albicans* ATCC 10231
- *A. brasiliensis* ATCC 16404

The number of CFU was determined on TSA+LTHT CSG 90 mm plates (art.-no. 200.0060). Good growth was defined by visible turbidity of the growth medium and confirmed by a subculture on the reference medium. The incubation took place in controlled incubators at 30 - 35 °C for bacteria and 20 - 25 °C for yeast and mold (Table 1).

Moreover, the growth of *S. aureus* ATCC 6538 was analyzed upon addition of the disinfectant Aerodesin® 2000 (2 µL in 2 mL of growth medium) to investigate the efficacy of the neutralizers contained in the growth medium. Each validation batch was tested in two replicates. TSB without neutralizer (art.-no. 500.B100) was used as a control.

3.1.2. Results

A good growth of all tested strains was observed in all investigated PMM Swab samples (Table 1).

Table 1: Growth promoting properties of the medium of three PMM Swab validation batches

Test strain	Incubation conditions	Microbial growth			
		Batch 1	Batch 2	Batch 3	Reference (CFU)
S. aureus ATCC 6538	≤ 3 d, 30 - 35 °C	Good growth	Good growth	Good growth	31
E. coli ATCC 8739	≤ 3 d, 30 - 35 °C	Good growth	Good growth	Good growth	31
P. paraeruginosa ATCC 9027	≤ 3 d, 30 - 35 °C	Good growth	Good growth	Good growth	35
B. spizizenii ATCC 6633	≤ 3 d, 30 - 35 °C	Good growth	Good growth	Good growth	31
C. albicans ATCC 10231	≤ 5 d, 20 - 25 °C	Good growth	Good growth	Good growth	17
A. brasiliensis ATCC 16404	≤ 5 d, 20 - 25 °C	Good growth	Good growth	Good growth	16

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A good growth of *S. aureus* ATCC 6538 was observed in all investigated PMM Swab samples also upon addition of the disinfectant Aerodesin® 2000, while no growth was observed in the control medium, confirming the efficacy of the neutralizers contained in the growth medium of the PMM Swabs (Table 2).

Table 2: Growth of *S. aureus* ATCC 6538 in the presence of Aerodesin® 2000

Medium	Incubation conditions	Specifications	Microbial growth			
			Batch 1	Batch 2	Batch 3	Reference (CFU)
TSB in ampoule	20 - 24 h, 30 - 35 °C	Good growth	Good growth	Good growth	Good growth	42
Control TSB	20 - 24 h, 30 - 35 °C	No growth	No growth	No growth	No growth	

3.2 Investigation of further test strains

3.2.1. Procedure

The growth promoting properties of the growth medium of three validation batches of PMM Swabs was tested for 11 further strains by analyzing growth curves using a Bioscreen instrument:

- *S. epidermidis* ATCC 12228
- *S. aureus* ATCC 29213
- *S. aureus* ATCC 25923
- *S.* Typhimurium ATCC 14028
- *M. luteus* ATCC 4698
- *K. rhizophila* ATCC 9341
- *E. coli* ATCC 25922
- *S. epidermidis* ATCC 14990
- *B. pumilus* ATCC 14884
- *C. pseudodiphteriticum*, isolate PMM
- *B. cepacia* ATCC 25416.

For each batch and test strain, the growth medium of two PMM Swabs was used and inoculated with 10 – 100 CFU. The number of CFU was determined on TSA+LTHT 90 mm CSG plates (art.-no. 200.0060). The test was performed according to the manufacturer instructions and each sample was tested on three replicates. An already released batch of TSB (art.-no. 500.B100) was used as a comparative medium. The lag-phase of the microbial growth in swab´s growth medium in comparison to the comparative medium and the reproducibility of the read-out among the three batches were considered to determine the growth promoting properties of the growth medium for the investigated micro-organisms. Good growth was confirmed by a subculture on the reference medium.

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3.2.2. Results

With the exception of *M. luteus* ATCC 4698, an easy and reliable read-out was obtained for all micro-organisms after incubation at 30 – 35 °C for 48 hours. *M. luteus* ATCC 4698 showed a weak growth after 2 to 3 days and a good growth after 4 to 6 days-incubation at 30 – 35 °C (Table 3).

Table 3: Growth promoting properties of PMM swab´s growth medium for further test strains

Test strain		Good growth after…
S. aureus	ATCC 29213	2 days
S. aureus	ATCC 25923	2 days
S. epidermidis	ATCC 12228	2 days
S. epidermidis	ATCC 14990	2 days
S. Typhimurium	ATCC 14028	2 days
M. luteus	ATCC 4698	4 -6 days
K. rhizophila	ATCC 9341	2 days
E. coli	ATCC 25922	2 days
B. pumilus	ATCC 14884	2 days
C. pseudodiphteriticum	isolate	2 days
B. cepacia	ATCC 25416	2 days

4. Capability to retrieve *B. spizizenii* and *S. aureus* from different surfaces

4.1 Procedure

Stainless steel and polycarbonate plates with a size of 25 cm^2 were inoculated with either a spore suspension of *B. spizizenii* ATCC 6633 or *S. aureus* ATCC 6538 (10 – 100 CFU) and then dried under a laminar flow. The number of CFU was determined on TSA+LTHT CSG 90 mm plates (art.-no. 200.0060). The inoculated plates (4 plates per strain, per surface) were then sampled with PMM Swabs and the swabs were incubated at 30 – 35 °C after transfer of the growth medium to the lower part of the device. PMM Swabs of three validation batches were used for the test. Growth medium was analyzed for visible turbidity as sign for microbial growth and good growth was confirmed by a subculture on the reference medium (TSA+LTHT 90 mm CSG plates, art.-no. 200.0060).

The test was repeated using an inoculum of 5 – 15 CFU. In this case, a contact sample of the swabbed surfaces was performed as well using TSA+LTHT contact CSG plates (art.-no. 100.0100). In addition to the analysis of microbial growth in the swabs´ growth medium, the micro-organism recovery rate on the contact plates used to sample the surfaces after swabbing was calculated. Again, PMM Swabs of three validation batches were used for the test.

4.2 results

Growth of both test strains was observed after one-day of incubation at 30 – 35 °C in the growth medium of all PMM Swabs upon swabbing of both stainless steel and polycarbonate surfaces inoculated with 10 – 100 CFU (Table 4).

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Table 4: Growth of test strains upon uptake from different surfaces (inoculum: 10 – 100 CFU)

Strain	Surface	Growth (n/n sample)	Time required for growth
B. spizizenii ATCC 6633	Stainless steel	12 / 12	1 day
	Polycarbonate	12 / 12	1 day
S. aureus ATCC 6538	Stainless steel	12 / 12	1 day
	Polycarbonate	12 / 12	1 day

Growth of both test strains was observed after one to two-days of incubation at 30 – 35 °C in the growth medium of all PMM Swabs also upon swabbing of both stainless steel and polycarbonate surfaces inoculated with 5 – 15 CFU (Table 5). Moreover, the recovery rate of both test strains on the contact plates used to sample the swabbed surfaces was less than 10 % (Table 5). These data support the ability of PMM Swab to efficiently retrieve micro-organisms from different surfaces even in case of a low bioburden.

Table 5: Growth of test strains upon uptake from different surfaces (inoculum: 5 – 15 CFU)

Strain	Surface	Growth (n/n sample)	Time required for growth	Recovery rate on contact plates – Ø of 12 plates
B. spizizenii ATCC 6633	Stainless steel	12 / 12	1 – 2 days	< 10 %
	Polycarbonate	12 / 12	1 day	< 10 %
S. aureus ATCC 6538	Stainless steel	12 / 12	1 – 2 days	< 10 %
	Polycarbonate	12 / 12	1 day	< 10 %

5. Survival of micro-organisms on the swab pad material

5.1 Procedure

PMM Swab pads were inoculated with one of the following strains (5 – 15 CFU, determined on TSA+LTHT 90 mm CSG plates, art.-no. 200.0060):

- *S. aureus* ATCC 6538
- *E. coli* ATCC 8739
- *P. paraeruginosa* ATCC 9027
- *B. spizizenii* ATCC 6633 (spore suspension)
- *C. albicans* ATCC 10231

and then stored at 20 – 25 °C for 24 or 8 hours before transferring the growth medium to the lower part of the device. After that, the swabs were incubated at 30 – 35 °C for a maximum of 7 days. Growth medium was then analyzed for visible turbidity as sign for microbial growth. Good growth was confirmed by a subculture on the reference medium (TSA+LTHT 90 mm CSG plates, art.-no. 200.0060). For each strain the test was performed in triplicates and using three validation batches of PMM Swabs.

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5.2 results

Four of the five test strains (*E. coli* ATCC 8739, *B. spizizenii* ATCC 6633, *S. aureus* ATCC 6538 and *C. albicans* ATCC 10231) showed visible growth (turbidity) in all units after storage of the inoculated swab for 24 hours and subsequent incubation with growth medium for 2 days (Table 6). In contrast, no growth of *P. paraeruginosa* ATCC 9027 was observed after storage of the inoculated swab pad for 24 hours. Reducing the storage time of the inoculated swab before release of growth medium to 8 hours allowed visible growth of *P. paraeruginosa* ATCC 9027 in all test units (Table 6).

Table 6: Survival of test strains on the swab pad material before transfer of the growth medium to the lower part of the device

Strain	Holding time	Time required for growth
S. aureus ATCC 6538	24 h	2 days
E. coli ATCC 8739	24 h	2 days
P. paraeruginosa ATCC 9027	8 h	2 days
B. spizizenii ATCC 6633	24 h	2 days
C. albicans ATCC 10231	24 h	2 days

These results show that the survival of micro-organisms on the pre-moistured swab during the time between inoculation of the swab and transfer of the growth medium from the ampoule into the lower part of the device is highly dependent on the tested strain. Sensitive micro-organisms may not be able to survive if they are stored on the swab pad for a longer period without growth medium. Therefore, it is recommended to transfer the growth medium from the ampoule into the bottom part of the device as soon as possible after sampling. Storage periods of more than 8 hours should be avoided.

Leimen, 20.02.2025

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