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Preparation, Separation,
Filtration & Monitoring Products

Application Note

Suitability of MC-Media Pad® for Hygiene Monitoring

(passive air, personnel and surface monitoring)

This study shows that the MC Media Pad® is suitable for a range of hygiene monitoring applications.

The study focused on usage of MC-Media Pad® in passive air, personnel and surface monitoring in comparison to standard test methods.

The MC-Media Pad® is designed as a convenient method for rapid routine testing of microbial contamination in food and beverage samples throughout production, from raw materials to finished products. The MC-Media Pad® solution is composed of a series of ready-to-use pads for total count and specific detection and enumeration of indicator organisms.

Each MC-Media Pad® consists of a fabric pad coated with a dedicated culture media formulation, placed on an adhesive support with a transparent gas-permeable cover (figure 1). The MC-Media Pad® has been specifically developed for the testing of 1 mL samples.

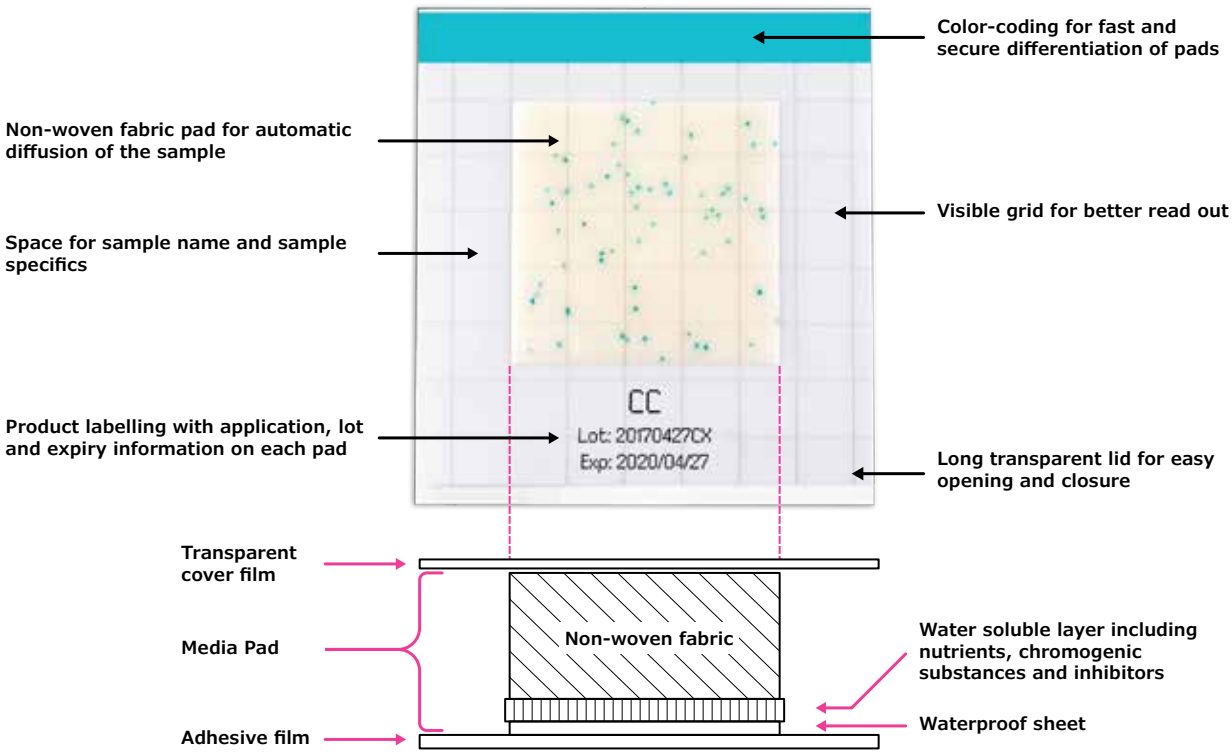


Figure 1: Description of MC-Media Pad®

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

Table 1: Typical incubation conditions for MC-Media Pad®

MC-Media Pad®	Incubation time	Incubation temperature
Rapid Aerobic Count (RA)	24 - 48 hours	35 ± 1 °C
E. coli / Coliform (EC)	24 ± 2 hours	35 ± 1 °C
Yeast & Mold (YM)	48 - 72 hours	25 ± 1 °C

Control of microbial contamination in the food processing environment is a fundamental part of HACCP or HARPC programs under FSMA. Standard procedures include active and passive air monitoring, as well as surface and personnel control using either swabbing techniques or agar devices.

This study was carried out to investigate if MC-Media Pad® can be used as a replacement for agar media, which are traditionally used as settle plates in passive air monitoring, or for pour or spread plate methods after swabbing surfaces.

Passive Air Monitoring

90mm settle plates with TSA and/or SDA are used for passive air monitoring. They are exposed to the air for a specific amount of time to collect airborne bacteria or fungi, which may settle onto food contact surfaces. In a side by side test, MC-Media Pad® RA (Rapid Aerobic Count) and MC-Media Pad® Yeast & Mold versus TSA and SDA settle plates respectively were used in parallel. The test runs were performed twice, in environments with both higher and lower levels of contamination.

Test Procedure

Four test runs with 20 MC-Media Pad® solutions (Type RA for total aerobic counts and YM for yeast and mold count) and 20 settle plates (TSA for total count and SDA for yeast and mold count) each in a side by side study were performed in two different standard laboratories. The cover films of the MC-Media Pad® tests were opened and the pads, as well as the bottom side of the cover films, were exposed for 3 hours to the ambient air (see figure 2). The settle plates were exposed without the lid to the air in a side by side pattern for the same amount of time. After exposure, the settle plates were closed with their lids, and the MC-Media Pad® tests were humidified by adding 1 mL of sterile water and the cover films were returned to the pads. Finally, total aerobic count samples were incubated at 35 ± 1 °C for up to 48 hours, and yeast and mold count samples were incubated for up to 7 days at 25 ± 1 °C. The colonies were counted after 24 and 48 hours for total count measurements and after 2, 3 and 7 days for yeast and mold detection.

The following picture shows an opened MC-Media Pad® during passive air sampling.

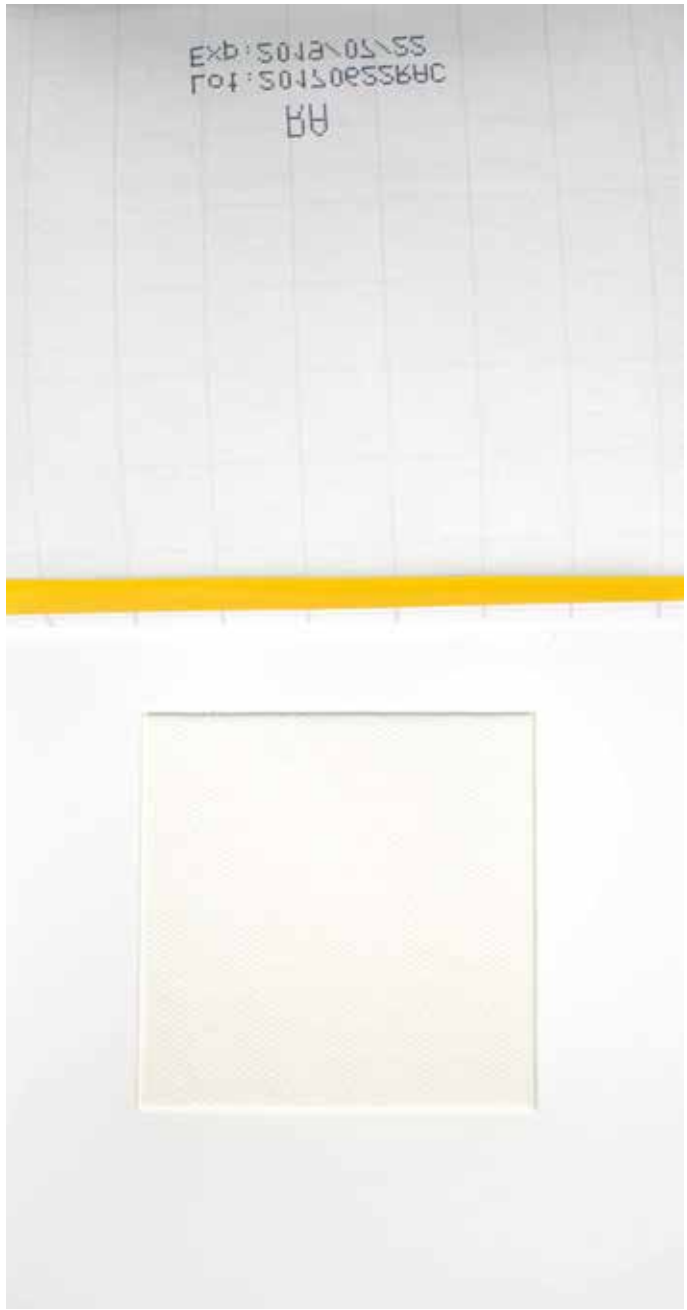


Figure 2: Exposition of an opened MC-Media Pad® for passive air sampling.

Results and Discussion

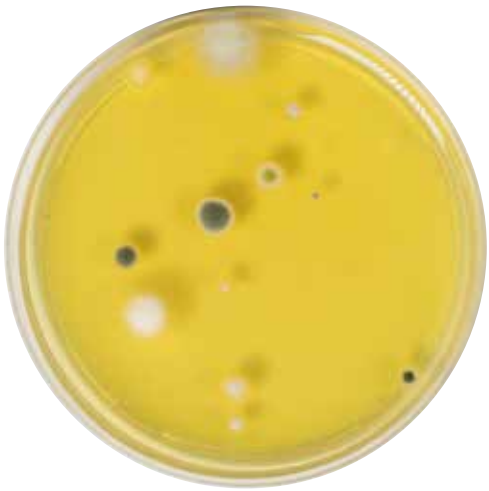
All test runs for settle plates, as well as for MC-Media Pad® tests, resulted in consistent counting results with low variances for all 20 replicates per device in the dedicated environment.

Figure 3 below demonstrates an example of colonies on MC-Media Pad® tests and Settle Plates after passive air sampling, each media pair (RA and TSA, Y&M and SDA) were subjected to the same sampling and incubation conditions.

MC-Media Pad® RA

TSA settle plate

MC-Media Pad® Y&M



SDA settle plate

Figure 3: Colonies on MC-Media Pad® and Settle plates after passive air monitoring and incubation.

The total aerobic counts as well as yeast and mold counts show good comparability for each of the the 20 replicates in the side by side test for both TSA/ SDA plates and MC-Media Pad® Rapid Aerobic Count and Yeast & Mold (see figures 4 and 5). In addition, the level of airborne contamination, meaning a lower or higher contamination grade of the two test environments was well reflected by both methods.

In total count measurements, the MC-Media Pad® did not show significant differences after 24 hours or 48 hours of incubation.

With regards to incubation time for yeast and molds, the MC-Media Pad® did not show significant differences between 3 and 7 days incubation, whereas on SDA settle plates the counts increased after prolonged incubation up to 7 days for low contamination levels.

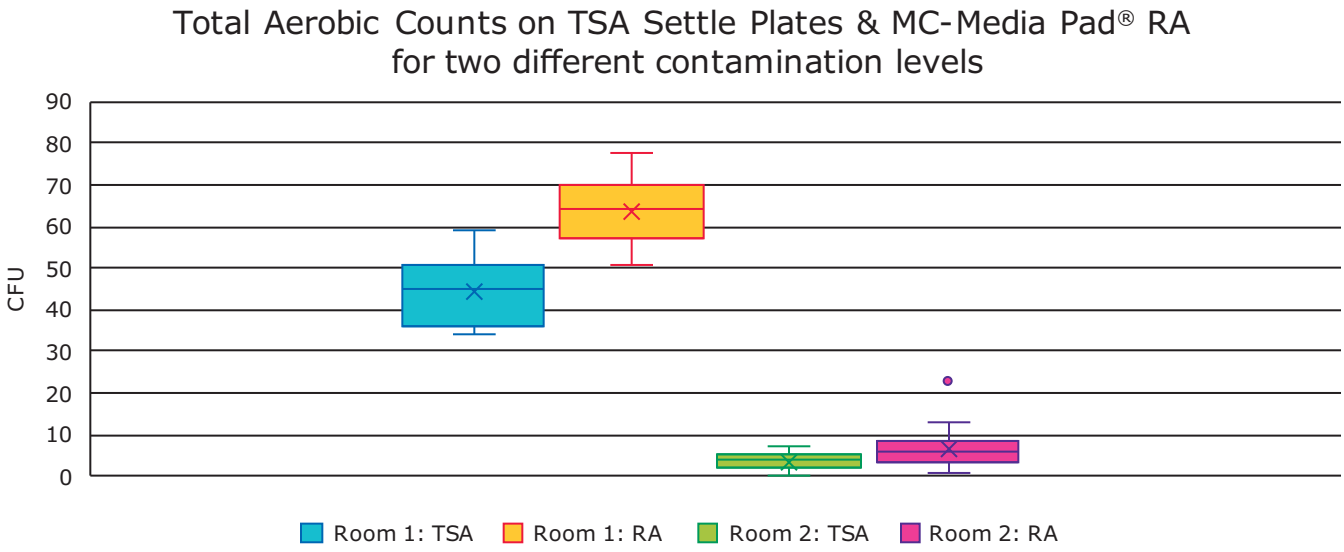


Figure 4: Total aerobic count in 2 different sampling environments after 48 hours incubation using TSA settle plates and MC-Media Pad® Rapid Aerobic Count

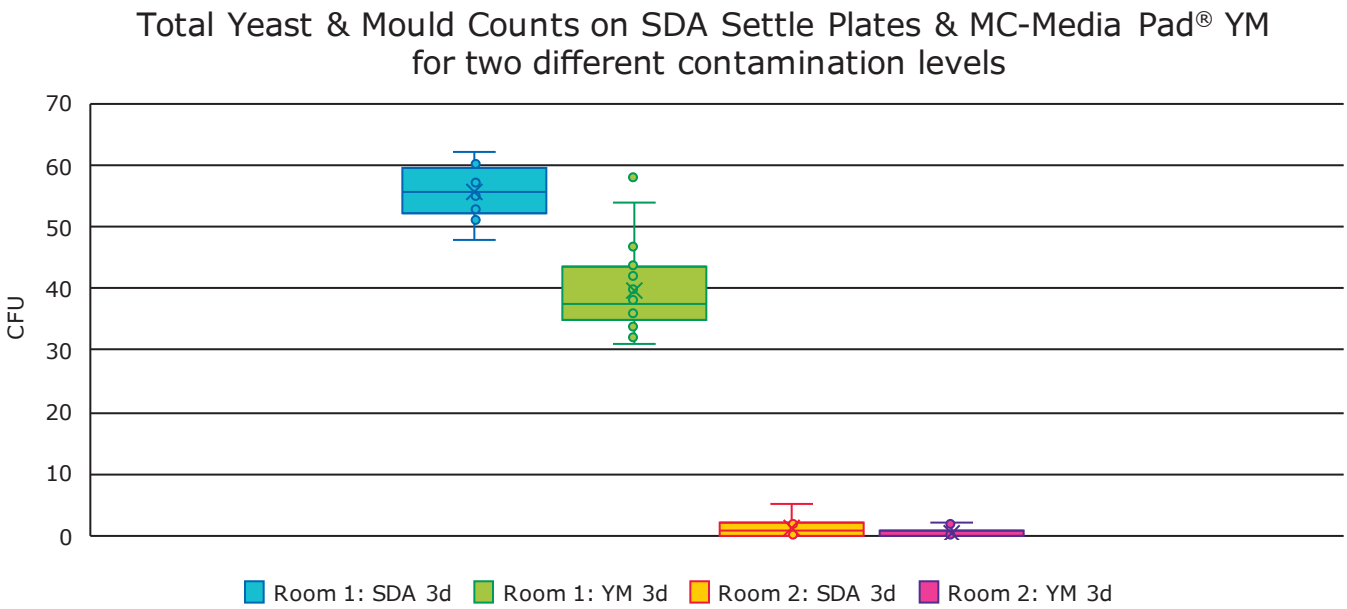


Figure 5: Yeast and mold counts in 2 different sampling environments after 3 days incubation

Overall, the colony count of a single sample does not give an indication of the cleanliness of the environment. In general, air monitoring should be performed on a regular basis at the same critical control points. The comparison of data over a period (e.g. monthly, quarterly) will help to monitor changes in the cleanliness level of the food producing environment.

From the obtained data, it can be concluded that MC-Media Pad® is suitable for passive air monitoring. To achieve consistent trending data, you should maintain the same sampling procedure and sampling device for trend analysis of defined critical control points.

Personnel Monitoring (e.g. direct fingerprint)

Fingerprints are often part of the hygiene monitoring of personnel to avoid cross contamination between production areas, or from personnel to food. To check the suitability of MC-Media Pad® for personnel monitoring, direct fingerprints were performed on MC-Media Pad® (types RA, EC/CC and YM).

Test Procedures

The cover film of MC-Media Pad® was opened, and 1 mL of sterile water was dispensed on the center of the media pad. The cover film was closed and maintained at room temperature for 15 minutes to be moistened. The cover film was then opened and a fingertip of a test person or alternatively a glove finger (pre-inoculated with test strains) was slightly pressed on the moistened pad surface (figure 6). After closing the cover film, the pads were incubated as indicated in table 1. Finally, the colonies were counted.

Results and Discussion

This test was carried out to demonstrate the recovery of microorganisms from finger or glove prints. The results of the pure fingerprint test showed colonies on the various MC-Media Pad® tests. The highest number of CFU (59) on the RA pads were obtained after 48 hours of incubation. Very low counts, mainly pinpoint colonies were detected on MC-Media Pad® E. Coli / Coliform as well as on MC-Media Pad® Yeast and Mold. In both cases, the maximum incubation time, indicated in table 1, is required to detect the colonies.

The pre-inoculated glove fingerprints showed clear growth of the chosen test strains on the dedicated media pads as shown in figure 7. These results indicate that MC-Media Pad® tests can be used for direct fingerprint testing.

Figure 6: Direct fingerprint on a pre-moistened MC-Media Pad® test

References

ISO 18593:2018 Microbiology of the food chain — Horizontal methods for surface sampling.

E. coli
ATCC 11775
on MC-Media Pad® EC

Candida albicans
ATCC 10231
on MC-Media Pad® YM

Staphylococcus epidermidis
ATCC 12228
on MC-Media Pad® RA

Figure 7: Selected microorganisms grown after glove print on various MC-Media Pad® tests:

Surface Monitoring of Dry Surfaces (Swab Test)

Swabs are a well-known device for microbial monitoring of surfaces. Pre-moistened swabs are used for monitoring of dry surfaces, whereas dry swabs are used for wet surfaces. The overall swabbing efficacy is impacted by several parameters like the absorbing capacity of the swab tips, the release of collected microorganisms from the swab tips and finally by the growth performance of the microorganisms in the appropriate culture media.

The material of the swab and the swabbing procedure have significant influence on the recovery rates (Moore and Griffith). In this study, we did not investigate the efficacy of the chosen swab type for removal or release of microorganisms, but instead we investigated the overall recovery rate of microorganisms from a dry surface using MC-Media Pad® instead of typical 90 mm agar media.

The recovery rates are calculated as follows:

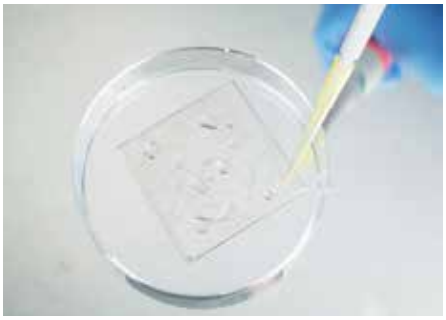
$$\text{Recovery Rate (\%)} = CFU_{\text{test A or B}} \times 100 / CFU_{\text{control}}$$

Test Procedures

Table 2: Procedure of Swab Method A

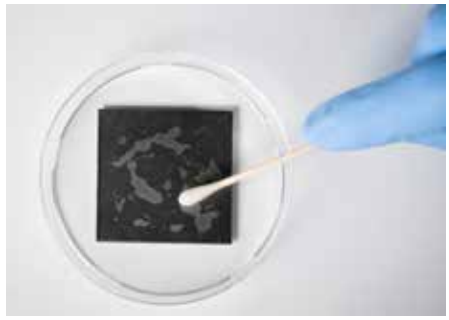
Swab Method A	Reference of viable microorganisms
(triplicate samples)	(triplicate samples)
Stainless steel coupons are inoculated with 100 uL bacterial suspension (coupons in triplicate)	
Coupons dried for 3 hours in a safety cabinet (blower off)	
Stainless steel surface of coupon was swabbed in a zig-zag pattern	Stainless steel coupon was overlaid with molten TSA/SDA (like pour plate method)
Swab was transferred to 2 mL Letheen Broth and vortexed for approx. 30 seconds	
1 mL of Letheen Broth was transferred onto a dry MC-Media Pad®	
Samples are incubated as indicated in table 1	
Colonies were counted, and average of triplicate determined (CFUtestA)	Colonies were counted, and average of triplicate determined (CFUcontrol)

Figure 8: Test procedure for determination of the recovery rates using MC-Media Pad® as an alternative to pour plates (Swab Method A):



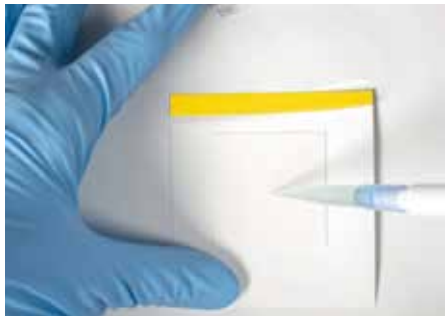
Inoculation of stainless steel coupon with specified test strain

3 hours drying at room temperature



Swab the complete surface horizontally and vertically while rotating the swab

Transfer swab into 2 mL dilution broth (Letheen Broth) and vortex



Transfer 1 mL of dilution broth to MC-Media Pad® RA

Results and Discussion

The recovery rates of 1 mL of the swab rinsing solution transferred to the MC-Media Pad® ranged from 38% for *E. coli* to 124% for *S. aureus* compared to the viable CFU on the stainless steel surface (see figure 9).

Compared to the original inoculum, the recovery rates for the tested microorganisms are within a range which corresponds to the variances reported by Moore and Griffith.

Low recovery rates compared to the original inoculum for the stainless steel coupon are mainly influenced by the high variance of the death rate during drying of the microorganisms on the surface. Therefore, we determine the viable microorganisms on the stainless-steel surface after drying by an agar overlay method as a 100% control (except for spores of *A. brasiliensis*).

The recovery rates for the different test strains and MC-Media Pad® types are shown in figure 9. For surface monitoring using swabs in combination with MC-Media Pad® (Swab method A), it can be concluded, that the recovery rates are comparable to previously reported ranges. In the present study design, a cotton swab was used, which provides the highest recovery rates when vortexed in Letheen Broth and 1 mL of the Broth is then transferred to the MC-Media Pad®.

References

ISO 18593:2018 Microbiology of the food chain — Horizontal methods for surface sampling.

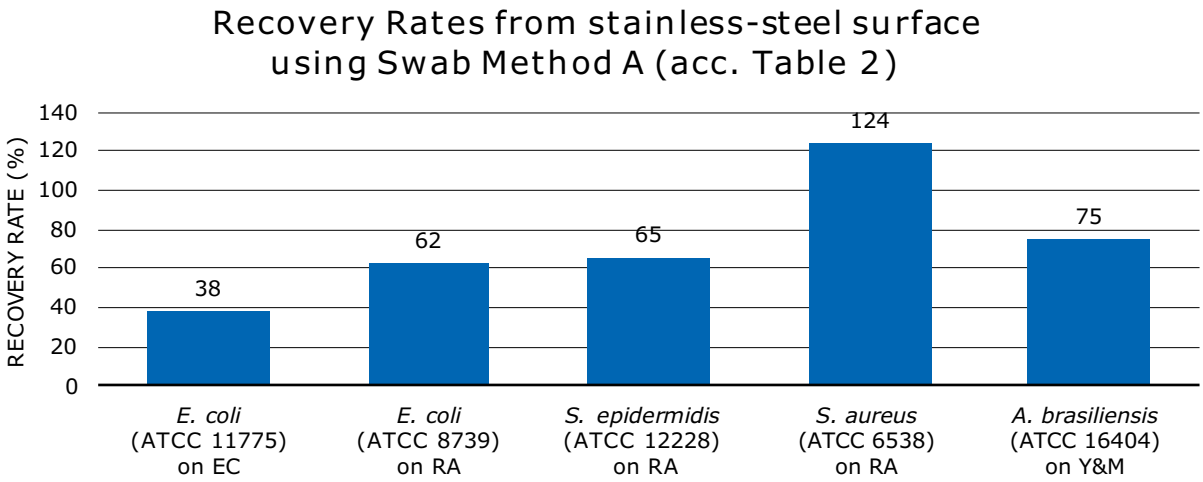


Figure 9: Recovery Rates of different test strains from stainless steel surfaces using swab method A (see Table 2)

E. coli ATCC 11775 | *Staphylococcus aureus* ATCC 6538 | *Staphylococcus epidermidis* ATCC 12228

Figure 10: Examples of MC-Media Pad® used for transfer of 1 mL swab dilution broth

Surface Monitoring of Flat Surfaces (Contact Test)

Test Procedures
Flat surface sampling: bacteria pure culture

Staphylococcus aureus WDCM00032
MC-Media Pad® RA vs. 55 mm Contact Plate TSA

Pure bacteria culture, *Staphylococcus aureus* WDCM00032, was incubated for 18-24 h at 36 ±1 °C in TSB Broth.

A 10-fold serial dilution in sodium chloride peptone broth was carried out. Three different volumes of dilution 10-5 (dilution 1, 2, 3) were selected for the drying process.

All MC-Media Pad® tests and contact plates were tested fourfold.

Area sizes on sterile stainless-steel tablets were inoculated with a defined volume of each dilution.

Inoculated surfaces were dried for 80-90 minutes at room temperature in a safety cabinet w/o blower.

At the same time, MC-Media Pad® RA were hydrated with 1 mL of 0.9% sodium chloride solution for a minimum of 15 minutes (**table 3**).

After drying of bacteria, the hydrated MC-Media Pad® tests were pressed for 10 seconds with a contact pressure of 500 g on corresponding areas. The same procedure was carried out with the TSA Contact Plates on the area adjacent (acc. ISO 18593).

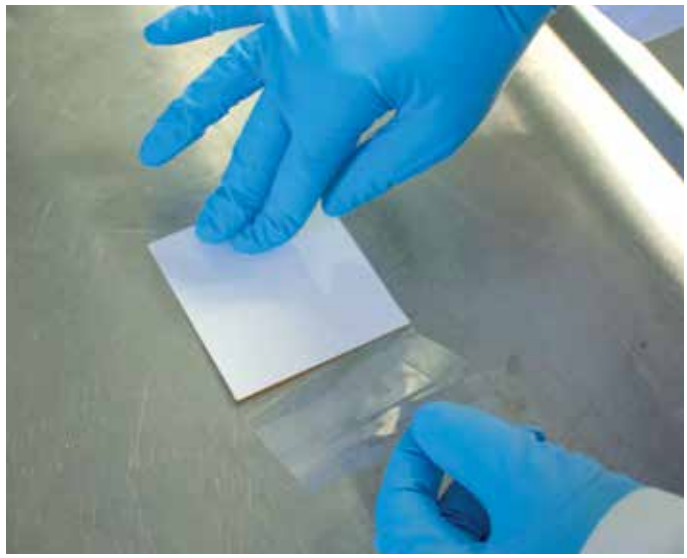


Figure 11: MC Media Pad® pressed on the surface

Incubation of Contact Plates TSA: 22 ±2 h at 30-35 °C (**table 3, figure 12**)

Incubation of MC-Media Pad® RA: 24 ±2 h at 35 ±1 °C (**table 3, figure 13**)

Table 3: Typical incubation conditions for MC-Media Pad®

MC-Media Pad®	Incubation time	Incubation temperature
Rapid Aerobic Count (RA)	24 - 48 hours	35 ± 1 °C
E. coli / Coliform (EC)	24 ± 2 hours	35 ± 1 °C
Yeast & Mold (YM)	48 - 72 hours	25 ± 1 °C

Staphylococcus aureus WDCM00032

Figure 12

Figure 13

Results and Discussion

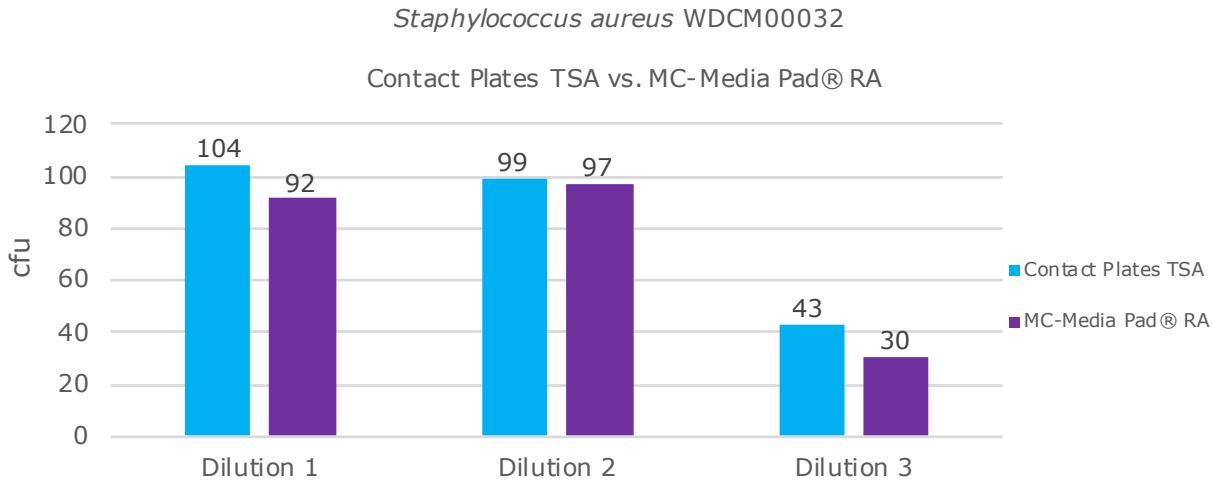


Figure 14: Each column depicts the mean of fourfold sampling

The diagram shows similar performance for the detection of *Staphylococcus aureus* in contact plate TSA and MC-Media Pad® RA for surface monitoring.

Test Procedures
Flat surface sampling: Molds

***Aspergillus flavus* WDCM00141 and *Aspergillus brasiliensis* WDCM00053 MC-Media Pad® Y&M vs. 55 mm Contact Plate SDA**

Aspergillus flavus WDCM00141 was incubated for 6 days at 25 ±1 °C in SABOURAUD 2% Dextrose Broth. *Aspergillus brasiliensis* WDCM00053 was used from reference material (prepared acc. package insert).

From both prepared molds, a 10-fold serial dilution in sodium chloride peptone broth was done.

One dilution of each was selected for the drying process.

All MC-Media Pad® tests and contact plates were tested four times each.

Area sizes on sterile stainless-steel tablets were inoculated with a defined volume of each mold.

Inoculated surfaces were dried for 80-90 minutes at room temperature in a safety cabinet w/o blower.

At the same time, MC-Media Pad® RA were hydrated with 1 mL of 0.9% sodium chloride solution for a minimum of 15 minutes.

After drying of molds, the hydrated MC-Media Pad® was pressed for 10 seconds with a contact pressure of 500 g on corresponding areas. The same procedure was used with the SDA Contact Plates on the area besides (acc. ISO 18593).

Incubation of Contact Plates SDA: 46 ±2 h at 20-25 °C (**table 3, figures 15, 17, 19, 21**)

Incubation of MC-Media Pad® Y&M: 48-72 h at 25 ±1 °C (**table 3, figures 16, 18, 20, 22**)

***Aspergillus flavus* WDCM00141 after 2 days incubation**

Figure 15

Figure 16

***Aspergillus flavus* WDCM00141 after 3 days incubation**

Figure 17

Figure 18

***Aspergillus brasiliensis* WDCM00053 after 2 days incubation**

Figure 19

Figure 20

***Aspergillus brasiliensis* WDCM00053 after 3 days incubation**

Figure 21

Figure 22

Results and Discussion

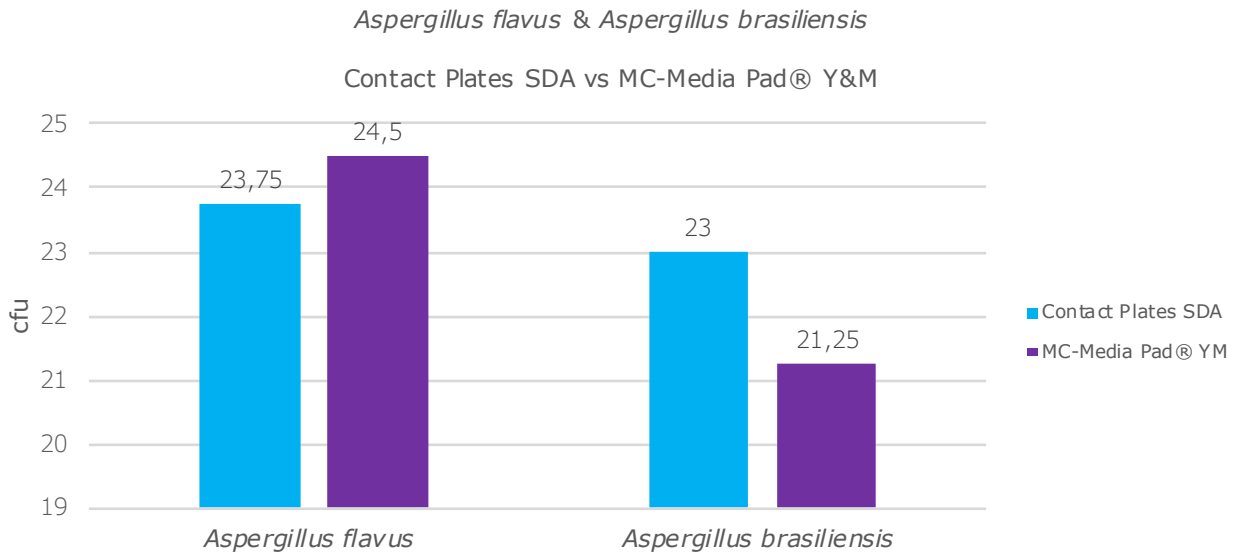


Figure 23: Each column corresponds to the average of four-fold sampling

The diagram shows similar performance for the detection of Aspergillus flavus and *Aspergillus brasiliensis* in contact plate SDA and MC-Media Pad® YM for surface monitoring.

This study shows that MC-Media Pad® is a convenient alternative method for surface monitoring. Bacteria and molds detection using respectively MC-Media Pad® RA and MC-Media Pad® YM give similar results compared to traditional TSA and SDA contact plates.

Summary and Conclusion

The MC-Media Pad® is suitable for hygiene monitoring applications, e.g. passive air sampling, personnel and surface monitoring.

The results generated in this study are comparable with standard reference methods. In addition to their suitability for hygiene monitoring, the MC-Media Pad® provides some benefits in terms of color-coding, handling, time to results for yeast and molds in 48 hours and improved read-out using chromogenic substances / specific dyes to allow fast and convenient control of microbial contamination.

References

ISO 18593:2018 Microbiology of the food chain — Horizontal methods for surface sampling.

References

1. Moore G; Griffith C (2007): Journal of Applied Microbiology 103; 1090-1103

Ordering Information

Product Description			Qty	Cat. No.
MC-Media Pad® E. coli / Coliform	Convenient culture media for simultaneous enumeration of *Escherichia coli* and coliform bacteria	AOAC, cert.-no.070901	100 pads	**1.32357.0001**
MC-Media Pad® Rapid Aerobic Count	Convenient culture media for rapid enumeration of aerobic microbial contamination	AOAC OMA 2019.02, cert.-no.091702 Microval, cert.-no. 2015LR52	100 pads	**1.32359.0001**
MC-Media Pad® Yeast and Mold	Convenient culture media for enumeration of total yeast and mold count	AOAC OMA 2018.02, cert.-no. 111401 Microval, cert.-no. 2015LR51	100 pads	**1.32360.0001**
Letheen Broth Base, modified			500 g	**1.10405.0500**
TSA-LI 30ml EP + USP 03074e			20 plates	**1.46004.0020**
SDA-LI acc. EP 03300e			20 plates	**1.46028.0020**
Tween® 80			500 mL	**8.22187.0500**

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Lit. No. MS_AN3343EN Ver. 2.0
2020-30723
03/2020