Millipore®
Preparation, Separation,
Filtration & Monitoring Products

Application Note

Growth Properties of Dehydrated ICR Settle Plates Used in Passive Air Monitoring

Investigation on ICR Settle Plates with and without neutralizers

GMP guidance recommends the use of active air samplers as well as settle plates as standard methods for environmental monitoring of air in manufacturing areas. Active air monitoring allows the quantitative determination of microorganisms in the volume of collected air, whereas settle plates, as a semiquantitative method, provide continuous monitoring of the process. The formulation of the chosen culture media should promote the growth of a wide range of microorganisms. Furthermore, it is also stated in the guidance, including ISO 14698, to pay attention to the length of passive air sampling to avoid excessive dehydration of the agar medium.

In this study we investigated the influence of a 5-hour exposure of two different settle plates, TSA w. LTHThio sedi. - ICR (Article No. 146786) and TSA - ICR+ (Article No. 146685) on their growth promoting properties. Following dehydration by 5-hour exposure under a laminar flow hood, we did not carry out a standard growth promotion test (adding specific bacterial strains as a liquid suspension), but instead active air sampling using a MAS-100 NT® air sampler was executed. Using this method, the detection of environmental microorganisms could be compared between pre-dried and fresh plates without re-humidifying the dehydrated plates as in a typical growth promotion test.

The active air sampling was performed in a non-controlled environment with high human movement in order to detect a broad range of real airborne microorganisms and further to achieve higher microbial counts for comparison.

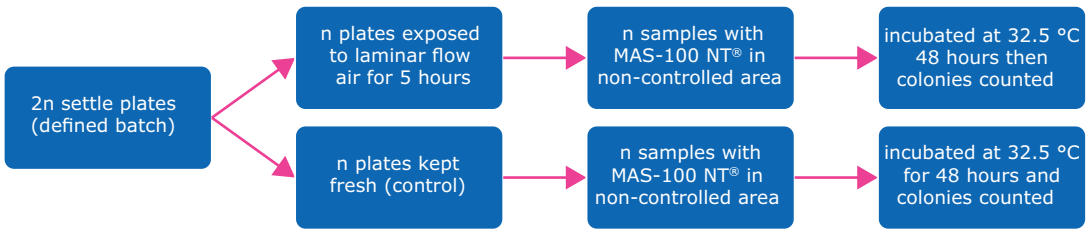
Test Procedure

7 settle plates of each article TSA w. LTHThio sedi. - ICR (Ref. No. 146786) and TSA - ICR+ (Ref. No. 146685) were weighed and then exposed in a laminar flow hood for 5 hours as settle plates. After passive air sampling in a sterile environment, the plates were weighed again, and the actual water loss was calculated.

These pre-dried plates were used for active air monitoring of 1000 liters of air in a non-controlled environment in a side by side test against active air samples from fresh plates of the same culture medium batch. Two air samplers were positioned side by side at a distance of approximately one meter apart. Seven measurements in a row were taken using one pre-dried and one fresh plate from the same batch per run. The position of pre-dried and fresh plates was alternated for every run.

Following sampling, the plates were incubated for 48 hours at 32.5 °C and the colonies were counted and corrected by Feller conversion to CFU/m^3.

Figure 1: Test procedure of growth properties of dehydrated ICR settle plates



MERCK

Results and Discussion

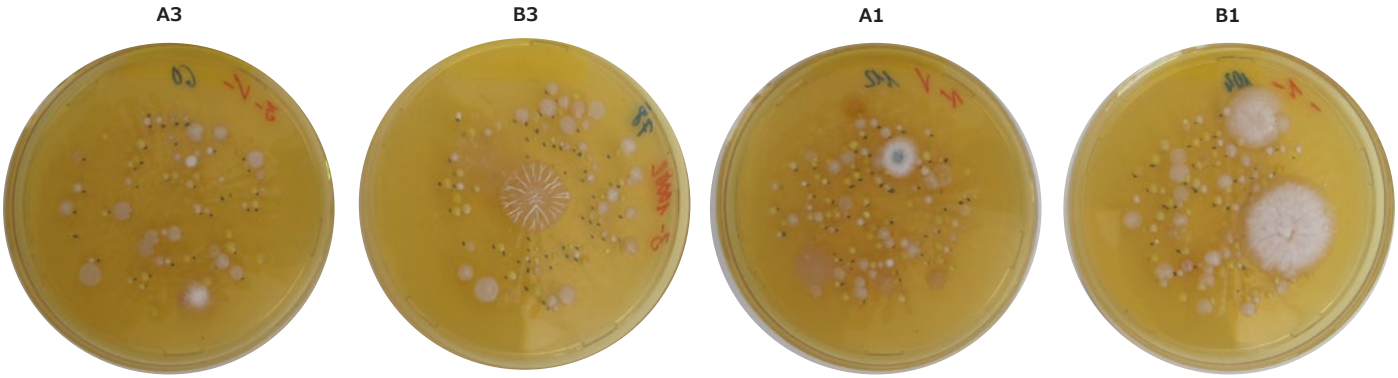
The average water loss after 5 hours exposure under the laminar flow was approximately 3 g for both plate formulations TSA - ICR+ (Ref. No. 146685) and TSA w. LTHThio sedi. -ICR (Ref. No. 146786) with no significant difference.

The average number of CFUs for pre-dried plates was similar to fresh plates for TSA w. LTHThio (same mean CFU) and for pure TSA (only 8% more CFUs compared to fresh plates), even if the variance of microbial counts was high in the non-controlled environments. The results are indicated in figure 3.

For 7 parallel runs with fresh and pre-dried plates of TSA w. LTHThio sedi. - ICR, the detection ranged between 56 and 102 CFU/m^3. The average of the counts was 69 CFU/m^3 for both pre-dried and fresh plates. That means that water loss of 3 g per plate did not have any influence on the growth promoting properties of the culture medium. The type and colony morphology of collected microorganisms on pre-dried plates were comparable to fresh agar plates. A prolongation of incubation time was not performed in this comparison study as the counts were high and even molds grew on the agar after 48 hours (see **Figure 2**).

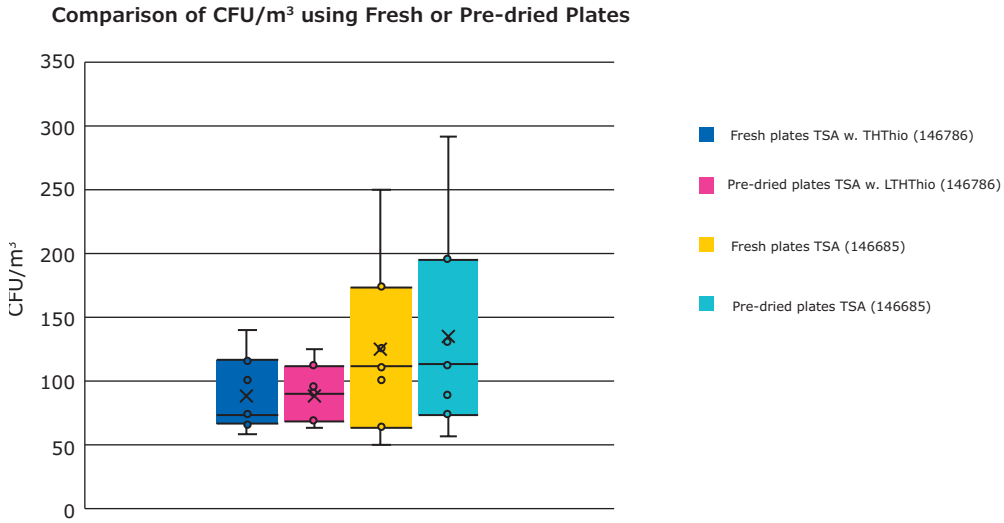
For the seven parallel runs using fresh and pre-dried plates of TSA - ICR+, the countable CFU for 1000-liter sampling volume ranged between 48 and 187 CFU/m^3. The average value was 136 CFU/m^3 for pre-dried and 125 CFU/m^3 for fresh plates. No influence on growth promoting properties of the culture medium after 5 hours exposure under a laminar flow was observed. In addition, the type of microorganisms collected were comparable according to colony morphology even with a longer incubation time of 48 h.

Figure 2: Colonies grown from the collection of 1000 liters of air in a non-controlled environment after 48 hours incubation [A3 and B3 = TSA - ICR+]



(A = pre-dried plates; B = fresh plates)

Figure 3: Results of the comparison between pre-dried and fresh plates of TSA-ICR+ and TSA w. LTHThio sedi. - ICR (7 runs per plate type) using a MAS 100-NT® active air monitoring system



Conclusion

The growth promotion abilities of the two ICR settle plates—containing pure TSA (TSA - ICR+, article number 146685) or TSA with four neutralizers (TSA w. LTHThio sedi - ICR, article number 146786)—were documented by using them as settle plates after drying in a sterile laminar flow hood for 5 hours with an average water loss of 3 g per plate (pre-dried plates) compared to fresh agar plates (same batch). There was no detectable difference in the ability to promote the growth of a broad range of airborne microorganisms.

Literature

- FDA Guidance for Industry (2004): Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice.
- ISO 14698-1(2003): Cleanrooms and associated controlled environments - Biocontamination control - Part 1: General principles and methods
- United States Pharmacopoeia 40 NF 35: <1116> Microbiological Control and Monitoring of Aseptic Processing Environments

SigmaAldrich.com/settle-plates

Merck KGaA
Frankfurter Strasse 250
64293 Darmstadt, Germany

To place an order or receive technical assistance

Order/Customer Service: **SigmaAldrich.com/order**
Technical Service: **SigmaAldrich.com/techservice**

SigmaAldrich.com

MK_TN4909EN Ver. 2.0
59176
10/2024