WHITE PAPER

An Alternative Environmental Monitoring Plate to Support Passive Air Sampling using the Growth Direct® System

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ABSTRACT

Passive air (i.e. settle plate) sampling is a critical part of the environmental monitoring (EM) program. Historically, regulatory guidelines such as Annex 1 outlined the use of 90mm agar plates for compendial passive air sampling. While this method is effective in capturing airborne contaminants, manual incubation of these traditional 90mm settle plates slows down product release timelines making it less efficient in high-throughput environments. The recent updates to Annex 1 recommend the use of validated rapid microbiological methods (RMM) to assist in the rapid detection of potential contaminants in the environment and the product[1]. This white paper summarizes a study conducted by Rapid Micro Biosystems in collaboration with Rutgers University evaluating the use of the Growth Direct® System EM application as an alternative to the traditional 90mm plate.

INTRODUCTION

When developing a contamination control strategy in pharmaceutical industries, measuring microbial air quality is a fundamental step[2]. Microbial air sampling methods are categorized into active and passive air sampling. In QC microbiology, there is a growing need to adopt RMMs to ensure product safety and decrease sample cycle times. Passive air sampling measures the number of microorganisms settling from air onto a known surface area in a known time[3]. This involves exposing the open growth media-filled plates to the air for specified duration allowing the natural settling of microorganisms under gravity. This method is valuable in sensitive environments where employing an active sampling method might disrupt the aseptic operations[4].

The Growth Direct® System features faster detection, automates the traditional manual QC Microbiology methods, and provides data integrity that provides significant benefits to customers. However, the Growth Direct® EM cassette has a surface diameter of 55mm compared to the traditional 90mm used for passive air sampling. Therefore, it was important to evaluate the effect of the smaller surface area of the Growth Direct® EM cassette on performance compared to the traditional 90mm settle plate surface area.

GROWTH DIRECT® EM CASSETTES

The Growth Direct® EM cassettes are 55mm tryptic soy agar (TSA) with neutralizers. They are designed with specific mechanical and optical features that facilitate the automated handling and imaging processing for rapid microbial enumeration with the Growth Direct® System. This includes a black 0.45-micron pore sized cellulose ester membrane covering the media surface. The Growth Direct® EM cassettes are offered in the following two standard formats:

- TSA with lecithin and polysorbate 80 (TSA-LP80)
- TSA with lecithin, polysorbate 80, histidine, and sodium thiosulfate (TSA-LP80HT)

EXPERIMENTAL DESIGN

Testing was performed by the Department of Environmental Sciences at Rutgers University. The Rutgers laboratory was selected due to its specialty in bioaerosol sampling and advanced microbiological analysis techniques. A joint publication of this study is underway with Rutgers University.

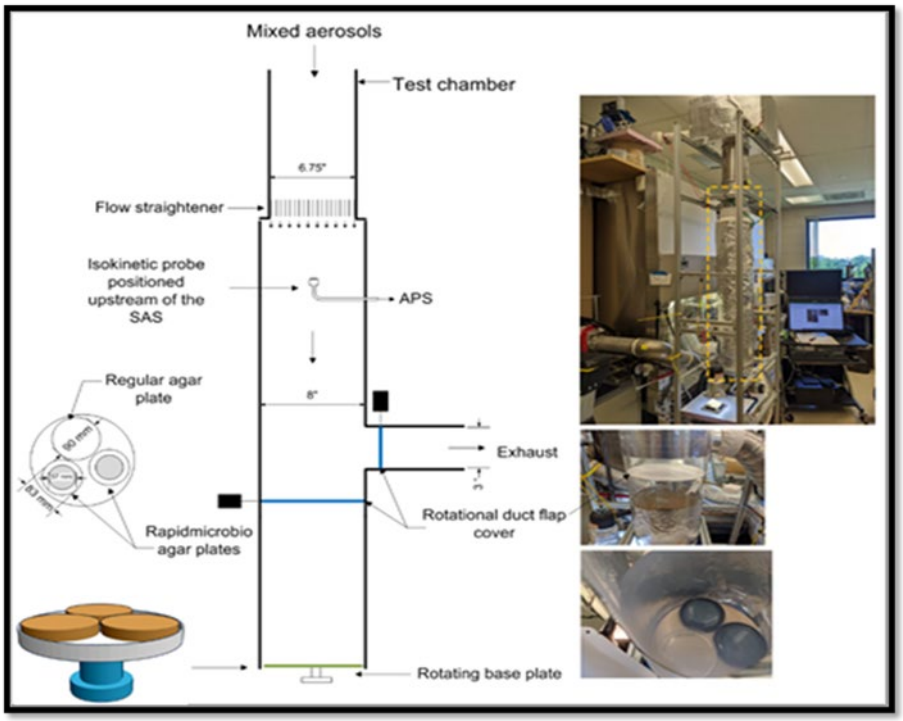
Staphylococcus epidermidis (ATCC® 14990™) was chosen as an appropriate challenge microorganism in this study for its frequency of recovery in pharmaceutical cleanrooms and its bioaerosol distribution

properties. *S. epidermidis* has a resilience to desiccation and has optimal size distribution that allows for its survival and distribution in the bioaerosol chamber. The bacterial aerosol's size distribution with *S. epidermidis* can range from 0.8 to 0.45 µm when produced using a centrifugal generator. This results in slow settling velocities and stimulates natural settling patterns.

For this study TSALP80 was selected as the test medium. Both the selected Growth Direct® System EM 55mm cassette and the traditional 90mm plate were prepared using the same media formulation as catalog # ET80-100.

A collision nebulizer, operated at 5 L/min and 20 psi pressure, was used to aerosolize the challenge microorganism. The resulting aerosols were dried with HEPA-filtered air at an airflow rate of 10 L/min, passed through a charge neutralizer to remove the electrical charge, and then diluted further with a HEPA-filtered airflow of approximately 100 L/min. The final aerosol stream was directed downward into the settling chamber, as shown in Figure 1. An isokinetic probe was used to measure the bioaerosol concentration and size distribution. Once the desired airborne microorganism concentration was reached, the Growth Direct® EM cassette(s) and 90mm plate were exposed for a minimum of 4 hours. The challenge microorganisms settled by gravity onto the Growth Direct® EM cassettes and the 90mm plate, where they were deposited onto the agar surface. During the exposure process, the platform holding the Growth Direct® EM cassette(s) and 90mm plate were rotated at approximately 1 rotation per minute (RPM) to minimize any uneven distribution of the challenge microorganism in the air.

Figure 1. Bioaerosol Chamber Design

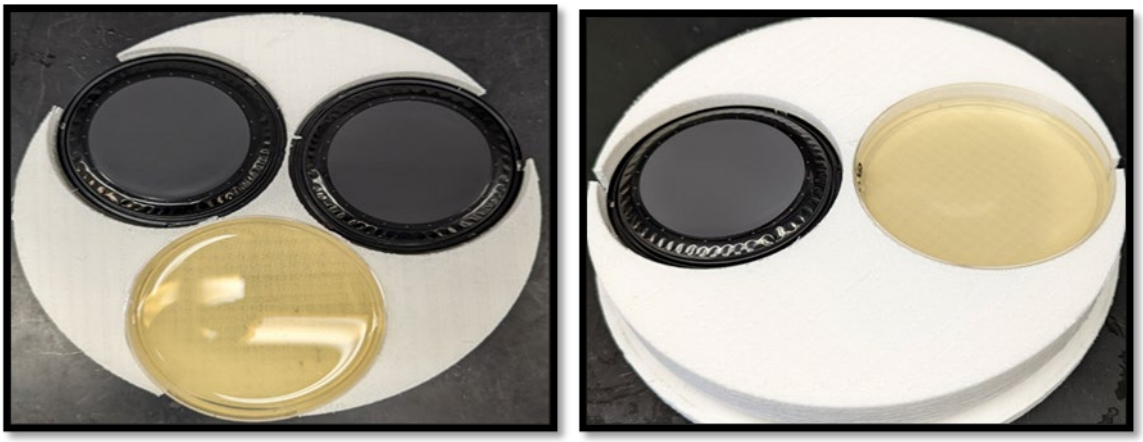


This study explored two test configurations:

a. Double Growth Direct® System EM Cassettes: Two Growth Direct® System EM Cassettes compared against one standard EM 90mm plate.
b. Single Growth Direct® System EM Cassettes: One Growth Direct® System EM Cassette was compared against one standard EM 90mm plate.

Both configurations are outlined in Figure 2.

Figure 2: Test Configurations



Growth Direct® System EM Cassettes and standard EM 90mm plate were placed into the bioaerosol chamber. The growth media was exposed to an average concentration of 200-500 CFU/L in air for a minimum of 4 hours. Testing was repeated until a minimum of 30 configuration replicates were collected for Double Growth Direct® System EM Cassettes and a minimum of 18 configuration replicates for Single Growth Direct® System EM cassettes. The exposed Growth Direct® System EM cassettes and standard EM 90mm plates were incubated at 32.5±2.5°C for ≤72 hours.

RESULTS

The mean percent CFU recovery for the Growth Direct® System EM cassette(s) was compared to the standard EM 90mm plate for both the double and single cassette test configurations. At a minimum, the mean percent CFU recovery is required to be within 50-200% to meet the specified acceptance criteria in the study. Additionally, common industry practice considers a ≥70% mean percent CFU recovery of a new method compared to a traditional method to demonstrate equivalency between the two methods.

A test for equal variance using the Bonett and Levene methods was performed to compare the individual replicates for both the double and single cassette configurations. The analysis was used to assess the variance between the individual replicates for the Growth Direct® System EM cassettes and standard EM 90mm plate when compared against themselves. A p-value >0.05 demonstrates equal variance and a p-value <0.05 demonstrates unequal variance.

A Mann-Whitney test was performed to assess the equivalency between the Growth Direct® System EM cassette(s) configurations and the standard EM 90mm plate. A p-value >0.05 concludes that the mean of the standard EM 90mm plate is not significantly different from the mean of the Growth Direct® EM cassette(s). Conversely, a p-value of <0.05 means there is a significant difference.

All replicates meeting the acceptance criteria of 30-300 CFU were selected for analysis. This selection was based on the ability to accurately enumerate, reduce inherent microbial variability, and perform statistical analysis.

Double Growth Direct® System EM Cassette Configuration

A total of 31 configuration replicates met the 30-300 CFU acceptance criteria and are outlined in Graph 1. The graph compares the two Growth Direct® System EM cassettes against one standard EM 90mm plate. The mean percent CFU recovery for two Growth Direct® System EM Cassettes compared against the one standard EM 90mm plate was 72%. The Bonett and Levene p-value for two Growth Direct® System EM cassettes replicates compared to the replicates of the one standard EM 90mm plate was >0.05. This met the acceptance criteria for equal variance with a p-value of >0.05. Additionally, the Mann-Whitney p-value for the mean of one standard EM 90mm plate compared to the mean of two Growth Direct® System EM cassettes was >0.05.

Graph 1: Replicate CFU Distribution – Double Growth Direct® EM Cassette Configuration

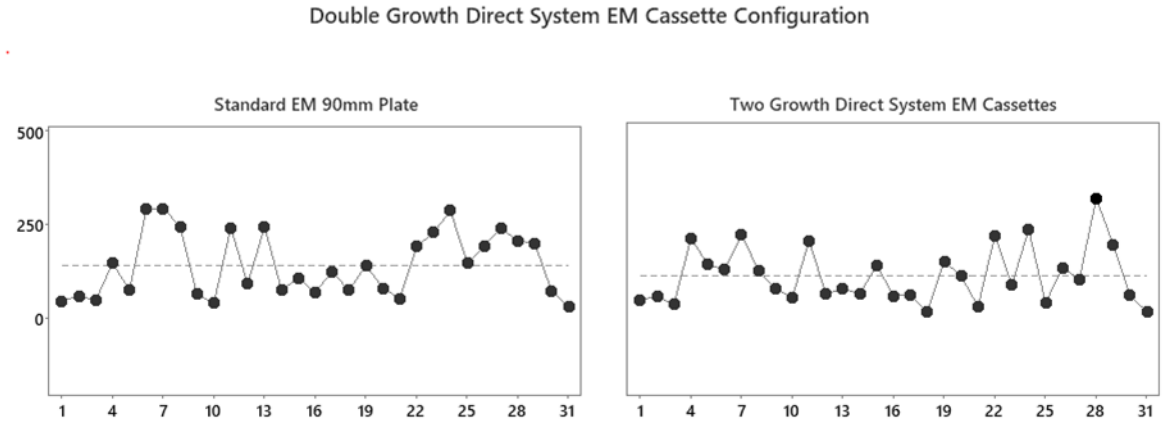


Table 1: Microbial Recovery

Microorganism	Standard EM 90MM Plate Mean (CFU)	Two Growth Direct® System EM Cassettes Mean CFU	Recovery	50-200% Recovery
S. epidermidis	142	102	72%	Yes

Single Growth Direct® System EM Cassette Configuration

A total of 18 configuration replicates met the 30-300 CFU acceptance criteria and are outlined in Graph 2. Graph 2 compares the one Growth Direct® System EM Cassettes against one standard EM 90mm plate. The mean percent CFU recovery for one Growth Direct® System EM Cassette compared against the one standard EM 90mm plate was 36%. The Bonett and Levene p-value for one Growth Direct® System EM cassette replicates compared to the replicates of the one standard EM 90mm plate was <0.05. Additionally, the Mann-Whitney p-value for the mean of one standard EM 90mm plate compared to the mean of one Growth Direct® System EM cassette was <0.05.

Graph 2: Replicate CFU Distribution – Single Growth Direct® EM Cassette Configuration

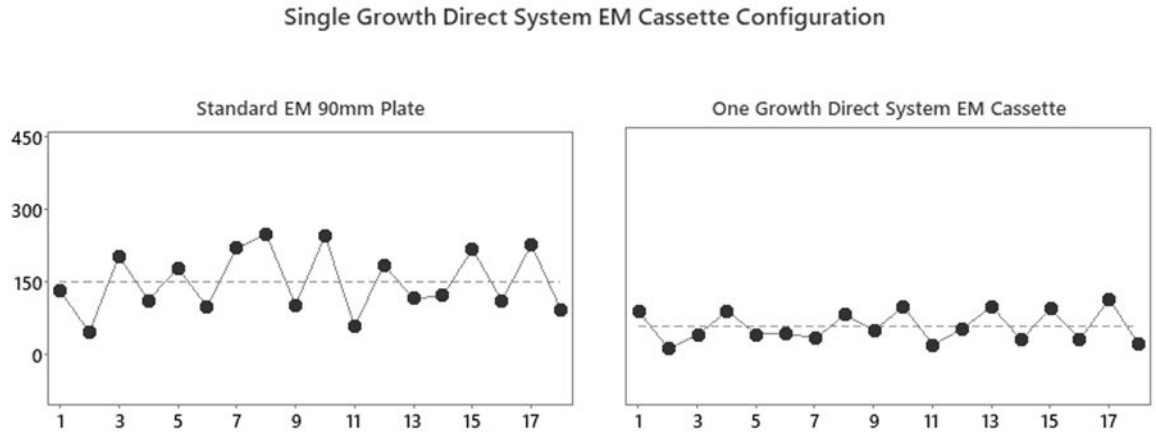


Table 2: Microbial Recovery

Microorganism	Standard EM 90MM Plate Mean (CFU)	One Growth Direct® System EM Cassettes Mean (CFU)	Recovery	50-200% Recovery
S. epidermidis	151	54	36%	No

CONCLUSION

The use of two Growth Direct® System EM cassettes in place of a one standard EM 90mm plate with a 4-hour exposure time to air met the acceptance criteria of 50-200% and was ≥70% recovery needed to demonstrate equivalency. The p-value for the Mann-Whitney test was >0.05 demonstrating no significant difference from the mean of the one standard EM 90mm plate compared to the mean of the double Growth Direct® System EM cassettes. However, the use of one Growth Direct® System EM cassettes in place of a one standard EM 90mm plate did not meet the acceptance criteria of 50-200% or the ≥70% recovery needed to demonstrate equivalency and the p-value for the Mann-Whitney test was <0.05 demonstrating a significant difference from the mean of the one standard EM 90mm plate compared to the mean of the single Growth Direct® System EM cassettes.

If the use of one Growth Direct® EM cassette is the preferred option, then a correction factor could be utilized. When a correction factor of at least 2 is applied to the Single Growth Direct® System EM cassette data the p-value is >0.05 demonstrating no significant difference from the mean of the one standard EM 90mm plate. Additionally, when a correction factor of 2 is added to the one Growth Direct® System EM cassette the 4-hour exposure time to air met the acceptance criteria of 50-200% and was ≥70% recovery needed to demonstrate equivalency. If this approach is taken additional validation effort would be required to demonstrate equivalency with the traditional 90mm plate.

The data and statistical analysis support the use of two Growth Direct® EM Cassettes or one Growth Direct® EM cassette with a correction factor as a viable alternative to traditional 90mm plates for passive air sampling. Additionally, the transition from a standard 90mm plate format to the Growth Direct® System EM cassette can provide an added advantage of rapid detection and automation to the traditional manual method. It is important to explore the use of RMM like the Growth Direct System to enhance the detection capabilities critical parts of your EM program. It is up to the user to select the best method for their facility and environmental monitoring program. Once selected, you will need to design the right validation plan to demonstrate that the passive air method provides the desired microbial recovery and detection compared to your current method.

GROWTH DIRECT® SYSTEM EM CASSETTE IMPLEMENTATION AND VALIDATION

Rapid Micro Biosystems offers a comprehensive validation service for the Growth Direct® System and applications. If you are looking to implement the Growth Direct® System and are interested in the use of EM cassettes for passive air testing, we have a team of experts that can develop and implement a robust and proven validation plan. Use the QR code below to explore what Growth Direct® System can offer your QC Microbiology Laboratory!

www.rapidmicrobio.com

RMB operates a fully automated manufacturing suite in Lowell, MA with administrative offices, demo labs, back-up manufacturing, and a state-of-the-art Innovation Center in Lexington, MA.

REFERENCES

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