“The Impact of Plate Size on Passive Air Monitoring”

Eric Chenelle[a], Shravani Tadepalli [a], Gediminas Mainelis [b], Taewon Han [b], Owen Griffin [a], Anna Mills [a]

a. Rapid Micro Biosystems, Inc.
b. Rutgers University

Introduction

When developing a contamination control strategy in pharmaceutical industries, measuring microbial air quality is a fundamental step[1]. Microbial air sampling methods are classified into active and passive air sampling. Active air sampling allows a predetermined volume of air and provides a controlled and measurable sampling process. While passive air sampling involves exposing the open growth media filled petri plates to the air for specific duration allowing the natural settling of microorganisms under gravity as illustrated in Figure 1. This method is especially valuable in sensitive environments where employing an active sampling method might disrupt the aseptic operations[2].
When conducting settle plate studies, the plate surface area may impact the number of colonies that settle as Settle plates measure the number of microorganisms settling from air onto a known surface area in a known time[3]. The compendial approach as described in Annex 1, uses a 90mm petri plate with an approximate internal area of 64cm^2 for passive air sampling with a 4-hour exposure time. However, the use of automated microbial detection for colony counting like Growth Direct® System requires strategic replacement of conventional petri plate with a Growth Direct® Cassette that has a diameter of 57mm and surface area of approximately 25cm^2. The use of single consumable like Growth Direct® Cassettes for both air applications in Environmental monitoring will reduce and control the QC inventory.

This study investigates the feasibility of using double or a single Rapid Micro Biosystems® Growth Direct® Cassette in place of a conventional petri plate for settle plate technique by simulating bioaerosol particle deposition and comparing the microbial recovery to conventional environmental settle plates.

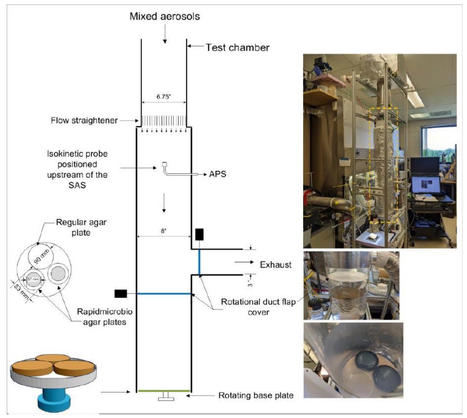
Materials and Methods

Selection of challenge microorganism
Staphylococcus epidermidis (ATCC® 14990™) was chosen as an appropriate challenge microorganism in this study for bioaerosol deposition. The selection was based on its resilience to desiccation during settling and Its size distribution. The bacterial aerosols size distribution with *Staphylococcus 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. The targeted Bacterial concentration in the air was ~5 × 10^3 #/L.

Bioaerosol deposition setup
A Collison nebulizer (CH Technologies, Westwood, NJ) operated at 5 L/min and 20 psi pressure was used to produce aerosols of the challenge microorganism. The resulting aerosols are dried with HEPA-filtered air at an airflow rate of 10 L/min and passed through a charge neutralizer Po-210 to remove the electrical charge, and the neutralized aerosol stream is diluted further with a HEPA-filtered airflow of ~100 L/min. The final aerosol stream was directed downward into the settling chamber as shown in Figure 3. Isokinetic probe is used to measure the bioaerosol concentration and size distribution. Once the desired airborne microorganism concentration is reached, the Growth Direct® Cassettes and a conventional petri plate are exposed to the bacteria above. The Challenge microorganisms start settling under gravity and reach the Growth Direct® Cassettes and traditional petri plate where they are deposited. During the settling, the platform holding the Growth Direct® Cassettes or conventional petri plate is rotated at approximately 1 RPM to minimize the effects of any potentially uneven distribution of challenge microorganism in the air.

Experimental Set-up

Figure 3. The schematic picture shows Bioaerosol deposition setup



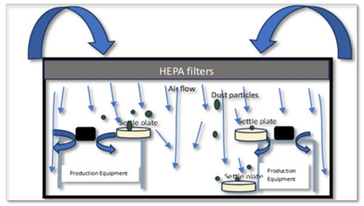


Figure 1. Demonstrates natural settling of microorganisms on to an exposed growth media filled petri plate in clean room

Microbial recovery rate with Double Growth Direct® Cassette setup

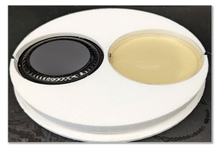
In this experiment setup, same media formulation of TSA LP80 was employed for both Growth Direct® Cassettes and conventional petri plates. Data was gathered from a total of 31 replicates, each consisting of two Growth Direct® Cassettes and one conventional petri plate positioned on a rotating base platform during each testing cycle. This arrangement facilitated the deposition of bioaerosols from the challenge microorganism onto the settle plates. The average concentration of bioaerosols used was 3.7 × 10^1 CFU/liter in air. The settling time was set to 4 hours and later the Growth Direct® Cassettes and conventional petri plate are removed and incubated at 32± 2.5°C for 48 and 72 hours. The combined CFU (Colony-forming unit) counts obtained from two Growth Direct® Cassettes were then compared to the CFU counts on the conventional petri plate at each testing cycle. All replicates included in this analysis adhered to the 30-300 CFU acceptance criteria for the conventional petri plate. Figure 6 illustrates the correlation between two Growth Direct® Cassettes combined and conventional petri plate for all replicates. Around 12 replicates exhibited a recovery rate of ≥90% when compared to conventional settle plate. Whereas only 3 replicates displayed a recovery rate of ≤30%. It’s worth noting that about 83% of replicates fell within the recommended recovery range of 50-200% as defined in both the US and European Pharmacopeia.

Figure 4. Double Growth Direct® Cassette and a traditional petri plate are placed side-by-side on a rotating base platform for Bioaerosol deposition.

Microbial recovery rate with single Growth Direct® Cassette setup

In this setup, a Single Growth Direct® Cassette, which possessed approximately 2.5 times less surface area than the conventional petri plate, was positioned side-by-side with a conventional petri plate on a rotating base platform during each testing cycle. All plates were prepared with the same formulation of TSA LP80 growth media. The average bioaerosol concentration of challenge microorganism in settling chamber was 4.5 × 10^1 CFU/liter in air.
A total of 18 replicates were analyzed, and all of them met the predefined acceptance criteria of 30-300 CFU for the conventional petri plate. After 4 hours of settling time, both the Growth Direct® Cassette and conventional petri plate were incubated at 32± 2.5°C for 48 and 72 hours. The CFU counts obtained from single Growth Direct® Cassette are compared to conventional petri plate at each testing cycle. CFU counts from Growth Direct® Cassette for all replicates were approximately 2.5 times lower compared to conventional petri plate (Figure 9). As seen in Figure 10, only 3 replicates demonstrated microbial recovery within 50-200% while 9 replicates exhibited recovery ≤30% when compared to conventional petri plate.

Figure 5. Single Growth Direct® Cassette and a traditional petri plate are placed side-by-side on a rotating base platform for Bioaerosol deposition.



Results

Figure 6. Microbial CFU counts from two Growth Direct® Cassettes compared to conventional petri plate in a Double Growth Direct® Cassette setup.

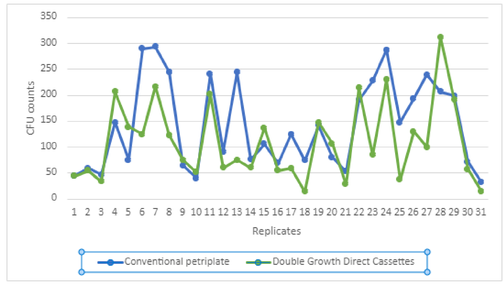


Figure 7. Microbial recovery percentage of two Growth Direct® Cassettes compared to conventional petri plate and compliance with 50-200% acceptance criteria in a Double Growth Direct® Cassette setup.

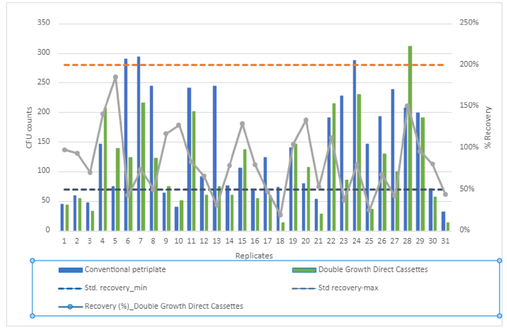


Figure 9. Microbial CFU counts of all replicates from single Growth Direct® Cassettes setup compared to conventional petri plate.

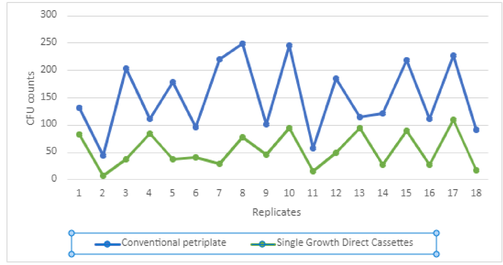
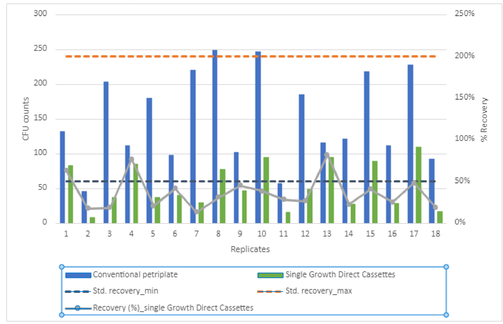


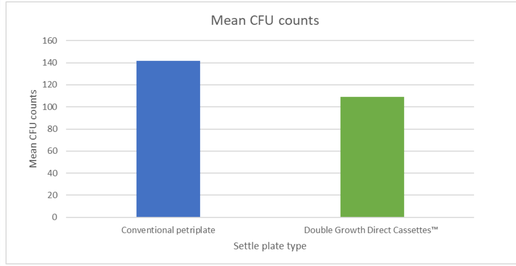
Figure 10. Microbial CFU counts of all replicates from single Growth Direct® Cassette setup compared to conventional petri plate.



Discussion

Per recommendations in Annex 1, passive air monitoring is most often conducted using 90mm agar plates. However, recent updates in Annex 1 also gives the end user the option of utilizing other qualified methods and encourages users to rapid methods whenever possible. Continuing to use to the standard 90mm petri dish prevents manufacturers from improving their environmental monitoring by adopting a rapid method. The demonstrated equivalence between 2 Growth Direct® Cassettes and a single 90mm petri dish provides strong support for manufactures to move away from the traditional method to the 2 Growth Direct® Cassette method without the need for any sort of correction factor across all cleanroom grades.

Figure 8. Mean CFU counts of two Growth Direct® Cassettes and conventional petri plate with mean microbial recovery percentage in a Double Growth Direct® Cassette setup.



Conclusion

The use of two Growth Direct® Cassettes in place of a single petri plate with a 4-hour exposure time to air demonstrated a microbial recovery with efficiency of ≥80%. Data strongly supports the use of Rapid Micro Biosystems® two Growth Direct® Cassettes in place of a single conventional petri plate for passive air sampling. These data also allow manufacturers to develop a correction factor for the use of a single Growth Direct® Cassette instead of a 90mm petri dish. A quantifiable advantage of this approach is the utilization of single consumable for both settle plate and surface sampling purposes of Environmental monitoring. Additionally, it enables the use of Rapid microbial enumeration with Growth Direct® System.

So, what is the right answer?

It depends on the end user to make this choice. The 2 Growth Direct® Cassette option allows for a direct transition away from the standard 90mm petri plate without any sort of correction factor. However, the change in footprint of the 2 cassettes vs 1 petri dish will require additional consideration when validating the method. Alternatively, the single Growth Direct® Cassette method would require a correction factor to directly compare the results with a 90mm petri dish but can be readily used in the existing validated locations, thereby simplifying the adoption process.

References

1.Sandle, T. (2012). Environmental Monitoring: a practical approach. In Moldenhauer, J. Environmental Monitoring: a comprehensive handbook, Volume 6, PDA/DHI: River Grove, USA, pp. 29-54.
2.US Pharmacopeial Convention. US Pharmacopeia Chapter <1116> Microbial Control and Monitoring of Aseptic Processing Environments. USP 37-NF32. Rockville, MD, USA: US Pharmacopeial Convention; 2014, pp. 931–942.
3.Whyte W. In support of settle plates. PDA Journal of Pharmaceutical Science and Technology 1996;50(4):201–204.
4.Samson. A, Sage. A, Jones. D.L, Use of contact plates to perform environmental settle plate testing. European Journal of Parenteral and Pharmaceutical Sciences, 2017, 22(1), 13-19.
5.Courtney Russell, Niloufar Parsaei, David L Jones, Validation of the Growth Direct System for Microbial Environmental Monitoring and Define Optimal Incubation Conditions. EJPPS | 252, (2020).

Acknowledgements

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