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Evaluation of 3D Printer Isolation using a Modified Biosafety Cabinet

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INTRODUCTION

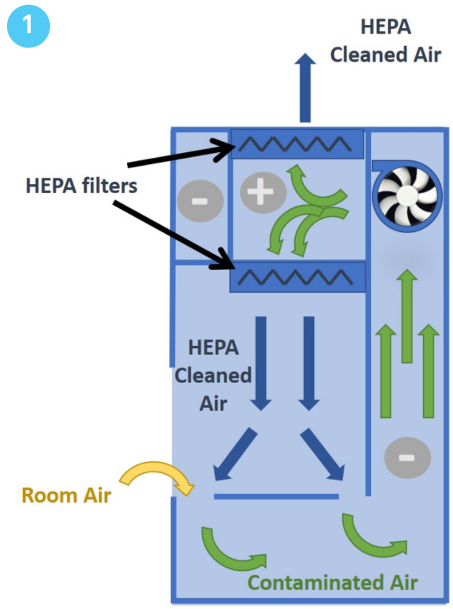
The use of three-dimensional (3D) printing has found application in the tissue and cell culture arena. *In vivo* cell growth is naturally three dimensional and to best represent cell growth *in vitro* the 3D platform has found increasing use and benefits. As with traditional petri dish and multi-well plate cultures, the elimination of unwanted organisms, or contaminants, is essential to the control of experiment. Primary components of the 3D printing process are the biofluid (or bio-ink) jets and the print bed where the 3D structures are built. This architecture limits the use of closed system culture isolation and necessitates primary engineering controls to surround the operation to attain contaminant free cultures and to protect workers from potentially biohazardous bio-inks used.

Class II Type A biological safety cabinet review

The Class II, Type A biological safety cabinet (BSC) is a primary engineering control utilized for traditional tissue and cell culture. The downward flow of HEPA filtered air provides protection to the work area. The air flowing into the front grille, located between the worker and the workspace provides containment of potentially contaminated workspace aerosols. The front grille receives both downflow air and the intake air, and thus, is the interface between the clean suppled and contained workspace air, and the contaminant laden ambient air. The cabinet's front access opening allows user interface with the cultures in the workspace. The air curtain at the access opening is the weakest point of the cabinet in terms of both isolation and containment, as it is an open area dependent on entrainment from the air stream to protect both the workspace and the user. **Figure 1** shows the airflow pattern of the Class II Type A2 BSC. Notice that the particulate laden incoming air enters the front perforated grille adjacent to the worksurface.

FIGURE 1.

Class II, Type A2 BSC Airflow Schematic



Bioprinter user interface

3D printing often requires user interactions with the ink jets, typically during setup and occasionally during the print process itself. This use case introduces turbulence in and out of the workspace and across the air curtain, impacting the containment and product protection properties the cabinet is designed to provide; as a result, it is the primary means of introducing contamination within the cabinet and for the potential escape of aerosols from the cabinet. As such, the primary engineering control needs to contemplate this use case.

FIGURE 2.

3D Printer inside the BSC

Integration with BSC

As with many types of lab automation, 3D printer geometry often exceeds the depth of the BSC workspace requiring custom designs to accommodate the instrument as well as to maintain adequate containment and protection over the work area and the worker. Even when the instrument physically fits within the workspace, the (i) geometry of the critical area, (ii) the location of any aerosol release points, and/or the (iii) user interface requirements to operate the printer may dictate a horizontally deeper BSC. The print bed is located at the very front of the instrument with the ink jets directly above. Thus, the critical clean area and the potential release of biohazardous aerosol are in the weakest area of the cabinet, right next to the front air curtain at the front access opening. Along with relatively frequent user intervention with the instrument, the risk of isolation and containment breach is increased. In anticipation of this potential for deceased performance, a custom horizontally extra deep cabinet was designed to in order to allow for placement of the print bed further away from the critical area, further into the cabinet and away from the front access opening. The greater the distance between the contamination and the location to be isolated, the greater the chance of attaining your control objectives, for containment and product protection. **Figure 2** shows the optimized placement of the printer inside the BSC.

METHODS

The Aspect Biosystems model RX1 3D printer was tested in the Baker 4‘ nominal width and modified extra deep work surface Class II, Type A2 BSC (Model SG404 4XD). The printer was first positioned as if the cabinet had not been modified, simulating a standard dimension work surface and placing the print bed closer to the front grille; the printer was then positioned deeper into the cabinet in order to maximize the distance between the print bed and front grille, which is facilitated via an extra deep work surface in the BSC. **Figure 3** shows the relative positions of the printer with respect to the BSC front grille. These tests were performed to quantify the difference in performance presumed from a theoretically favorable deeper cabinet location. The standard NSF Aerosol test was performed but is limited in its ability to assess dynamic challenges. This method is discussed below, and results are available via the link provided. However, this study focuses on particle counts collected in the critical print bed area during dynamic changes to the access opening airflow.

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FIGURE 3.
Aspect Biosystems model RX1 locations within the BSC

Aerosol Microbiological Challenge

Aerosol Microbiological testing, per NSF International Standard 49, releases bacterial spores from a nebulizer for a 5 minute period of time from one side of the air curtain while collection plates, vacuumed impingers and slit samples are used to sample air streams on the opposite side of the air curtain, collecting any spores that have traversed the air curtain into a growth media. The spores are incubated and counted to quantify escape. While this method utilizes a static stainless steel cylinder laying across the front perforated grill to simulate a user arm and break the front access air curtain, this method introduces no dynamic activities (such as the use case described above). The testing apparatus is spread across the front access opening limiting any variations to the standard test that would include dynamic testing. In addition, the colony forming units collected over the five minute testing period, are counted after a 48 hour incubation period, limiting the ability to associate a specific user activity with a breach of the air curtain. **Figure 4** shows the test set up for the NSF containment testing of BSCs.

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FIGURE 4.
NSF Containment Test

FIGURE 5.

Sampling Nozzle Location, Aspect Biosystems printer at 2” depth within the BSC

Dynamic particle count challenge

Particle counters collect and display the presence of viable and non-viable particles in real-time and can be used for dynamic testing of BSCs. The room ambient background particle burden provides the challenge to the cabinet when performing product protection or print bed isolation challenge, as the outside air contains many more particles than the inside of the HEPA filtered BSC work area. The particle counter sample nozzle is placed in the print bed area to determine the number of particles that breach the air curtain and reach the critical area. **Figure 5** shows the sample nozzle location. This test methodology allows real-time dynamic testing. Specific dynamic activities can be associated immediately with increases in particulate in the print bed area.

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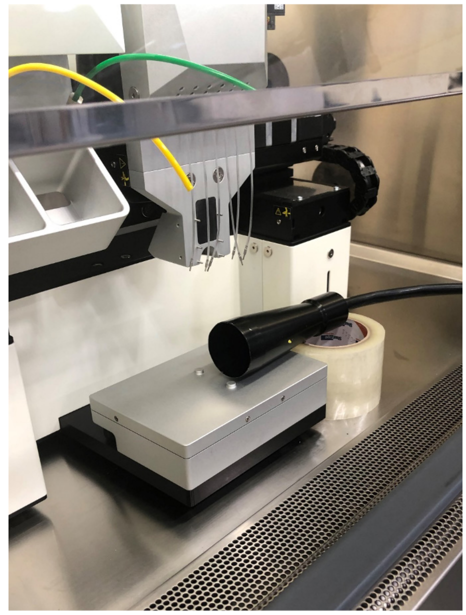

Since the particle count testing is real-time with no lag between an activity that causes a breach and the recording of particles by the instrument, dynamic challenges to the air barrier were performed. A repeated hand motion into and out of the BSC was performed throughout the 1-minute particle collection period. This resulted in approximately 25 to 30 cycles of the hand motion. This dynamic challenge was used as it simulates an operator intervention. The 30 cycles are much more than typical use but offers a rigorous challenge to the BSC air curtain. Initially, a hand motion at the center of the access opening was used. During the dynamic challenge, however, it was noted that a hand entering the access opening towards the top of the access opening and further from the front grille proved more challenging than a hand entering the bottom of the opening closer to the front grille. This was conducted with the printer 2” and then 7” from the front grille showcasing a printer in a standard BSC and a horizontally extra deep BSC. After the initial planned testing with a mid-level hand motion, a second dynamic challenge was performed with the printer 2” from the front grille, as it was shown to demonstrate the greatest difference in data. A high and low hand entry was tested to quantify the effects of hand elevation during user interface with the printer. **Figure 6** shows the hand entry challenge.

FIGURE 6.

Showing dynamic hand motion challenge

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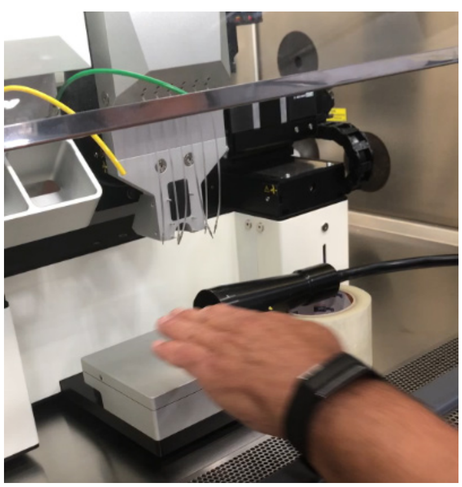

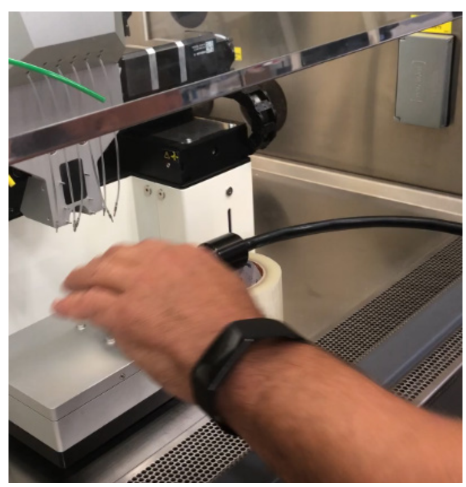

FIGURE 7.

Test setup

Test procedure summary

- Two positions of 3D printer
- Particle sampling in the middle of the print bed
- Continuous in and out hand motions to the print bed
- Three elevations of hand motions evaluated
- Three repetitions of dynamic challenge
- Ambient background greater than 100,000 particles/cubic foot
- Instrument Sample rate 50 liters/minute
- Sample time per test: 1 minute
- Particle size sampled: 0.5 micron
- Particle Counter: Climet CI-450t
- **Figure 7** shows the test setup

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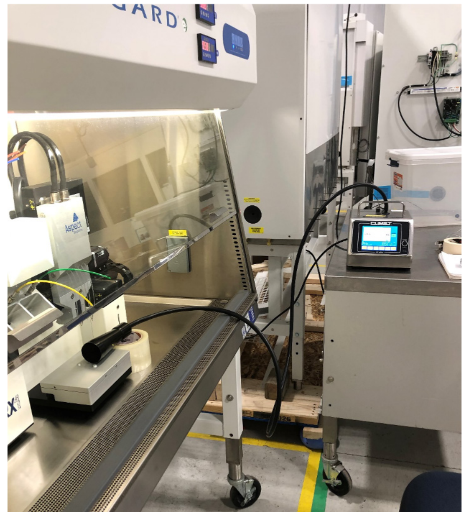

RESULTS

Table 1 summarizes the test results for the particle count testing. A static baseline test for each location of the printer was performed. For both the 2" and 7" distances from the front grille, no particles were detected at the print bed location. Operation of the printer is not static, and the hand motion procedure employed created a challenge to the BSC air curtain. Particles are detected during the dynamic challenge. The location 2" behind the grille resulted in particle counts greater than 500 during each test. With the objective of lowering the particle counts during the challenge the 7" position, allowable with the modified extra deep BSC, was established. Each repetition in this position resulted in less than 40 particle counts.

The high and low hand entry challenges, added to the test procedure, quantified the effect of the entry gradient of the particulate laden ambient. These tests were performed with the instrument in the closer, 2" position from the front grille, to accentuate the effect. The lower hand entry position resulted in no particle entry for the one trial, while the high position resulted in a count over 300. These results are intuitive, considering the airflow dynamics of the BSC. Negative pressure under the worksurface creates a flow of air into the grille. The velocity into the grille and therefore the ability to entrain particulate, falls off exponentially with distance. While the BSC downflow works in combination with the pull from the grille, the bottom edge of the BSC sash is the weakest point of entrainment for the system.

TABLE 1.

Particle counts from the 1-minute sampling tests

NOTE:

The NSF Biotest was performed with the instrument in the 7" position from the grille. The results for both containment and product protection were passing. A separate test report can be made available upon request.

Test #	Distance from front grille	Description	Hand level	# of hand motions	Particle count (total/cm)
1	7"	Baseline static	Middle	0	0
2	7"	Dynamic	Middle	24	0
3	7"	Dynamic	Middle	25	0
4	7"	Dynamic	Middle	28	40
5	2"	Baseline static	Middle	0	0
6	2"	Dynamic	Middle	31	1040
7	2"	Dynamic	Middle	29	520
8	2"	Dynamic	Middle	25	580
9	2"	Dynamic	Low	29	0
10	2"	Dynamic	High	29	314

CONCLUSIONS

The modified Baker Class II, Type A2 biosafety cabinet (BSC) model SG404 4XD provides better than ISO 4 conditions in the static condition per the ISO 14644-1 Cleanroom Standard. However, use of the instrument is not static and the investigation under dynamic conditions produced several findings and confirmations.

- Hand motions (dynamic condition) into the cabinet can increase ambient room particle counts at the printer bed.
- Placement of the instrument further away from the front grille, 7” verse 2”, provides lower counts at the printer bed under dynamic conditions.
- Hand motions into the BSC at the top of the access opening introduce greater particle counts than from the bottom of the opening.
- Better than class ISO 5 conditions are provided at the print bed during the dynamic hand motion challenge with the printer placed 7” from the front grille.

The modified, extra deep BSC allows placement of the printer further back from the front access opening and away from the particulate laden ambient air entering the grille. This increased distance helps prevent the particles from reaching the print bed area, significantly reducing the chance of contaminating the 3D print structure.

Further, the modified BSC exceeded all safety requirements for microbiological product and personnel protection against aerosols containing potential biohazards in accordance with the NSF/ANSI – 49:2019 National and International Standards for biosafety cabinetry.

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