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Engineering Individual Solutions

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April 18, 2011

Mr. Michael Funk
Director of Operations
bioBubble, Inc
3024 W. Prospect Rd.
Fort Collins, CO, 08526-1035

RE: UVM Cell Sorter Containment Testing
University of Vermont
Given Complex Room C316
Burlington, Vermont
ATC Project No. 63.41229.0001

Dear Mr. Funk:

This correspondence follows your request for equipment performance consulting services at the above mentioned facility. This report documents the tests ATC Associates Inc performed on March 10, 2011 which included testing the cell sorter containment system under varying conditions of operation. The test conditions and procedures were developed jointly with the University of Vermont, bioBubble Inc, and ATC Associates. Mike Funk of bioBubble was on-site and constructed the cell sorter containment as well as the positive pressure secondary enclosure. All tests were conducted in Given C316, the location of UVM's BD FACS Aria II flow cytometer (cell sorter). The following provides a description of the testing conditions and the results:

Participants:

Jeff LaBossiere	University of Vermont (UVM) Environmental Safety
Collette Charland	University of Vermont (UVM)
Julie Wolfe	University of Vermont (UVM)
Mike Funk	bioBubble, Inc (bioBubble)
Tom Broido	ATC Associates (ATC)
Devin Porter	ATC Associates (ATC)

Sample Locations:

A – At the cell sorter door, within the primary enclosure, closest to the instrument.
B – In the breathing zone of the cell sorter operator with the primary enclosure closed.
C – Inside the positive pressure secondary enclosure
D – In room C316, outside both enclosures

Methodology

ATC utilized several different instruments to collect data regarding effectiveness of the bioBubble enclosures. Prior to beginning other tests, a digital manometer (Dwyer Series 475 Mark III) was used to determine the differential pressures among the various functional spaces (general lab, secondary enclosure, and primary enclosure). This allowed ATC to qualitatively check that the enclosures were operating as expected.

Having established that the enclosures were operating as planned, ATC tested the enclosures with a laser particle counter (Lighthouse Handheld 3016). Readings were taken at the locations referenced above, in order to compare particle levels in and around the enclosures (Tests 1-3). The particle counter is capable

of detecting individual airborne particles (both solid and liquid phase) in the range of 0.3 – 10 µm. Particular attention was paid during the testing to the difference in particle concentrations between locations A and B. These locations are immediately inside and outside (respectively) the cell sorter access door. Location A is where one would expect the highest concentration of aerosol escaping the cell sorter. Location B is in the breathing zone of the cell sorter operator. If the bioBubble primary enclosure is functioning effectively, the results should indicate levels above backgrounds at A, and levels below location A levels at location B. Readings were collected at location C to confirm that ambient particle levels within the secondary enclosure were not affecting the readings taken at B. Readings collected at location D provide a record of the overall particle concentrations being excluded by the enclosures.

Following the particle counter testing, ATC utilized an aerosol photometer (TEC Services PH-4) and aerosol generator (ATI TDA-4B Lite) to test the enclosures under very stringent conditions (Tests 4-5). ATC configured the aerosol generator to introduce an aerosol fog into the primary enclosure. The aerosol generator creates a mist of liquid particles of poly-alpha-olefin (PAO). The photometer was calibrated for use with PAO. The particle size distribution of the aerosol is approximately 0.1 to 3 µm(polydispersed). Once the aerosol was being actively introduced into the primary enclosure, ATC used the aerosol photometer to collect percent penetration readings at the points of interest. Percent penetration represents the ratio of the PAO concentration at the location sampled to the PAO concentration measured "upstream". The upstream sample was taken at the cell sorter door, near location A, in order to represent a large quantity of aerosol being released from the device. This reading fluctuated between 70 and 80 µg/L. If the bioBubble primary enclosure is functioning effectively, the results at B should indicate penetration of less than 0.03%, the standard for HEPA filtered air.

In addition to measuring concentrations of PAO with the aerosol photometer, ATC used the aerosol fog as a visual check of airflow patterns within and around the primary enclosure.

Test #1 – Particle Counter, Aerosol Photometer; Background Readings

Conditions and Observations:

- Prior to any work with the cell sorter or inside the enclosures.
- Both the primary and secondary enclosures were constructed and operating.
- One person (tester) was inside the secondary enclosure.
- Repeated with the cell sorter local exhaust fan operating.
- Observed positive pressure between the room and the secondary enclosure. Airflow was visible through the strip door.
- Particle counter was run for a one minute sample. Cumulative particle counts are reported.
- Aerosol photometer was operated in upstream mode (µg/mL). Maximum observed readings are reported.
- No pressure differential was measurable between the room and the secondary enclosure.
- No pressure differential was measurable between the primary and secondary enclosures while the primary enclosure access door was open. When this door was closed, a pressure difference of 0.01 inches of water was measured, the primary enclosure being negative with respect to the secondary enclosure.
- After two sets of readings were taken with the cell sorter fan off, the cell sorter computer was connected to the device, and the fan was activated.

Results:

Test #1 was initiated at approximately 1015 AM and continued until approximately 1140 AM. The purpose was to establish background particle concentration levels. Cumulative particle count results are summarized below:

- A: 9 – 51 Average: 26
- B: 4 – 105 Average: 50
- C: 0 – 1 Average: 0.3
- D: 6620 – 8659 Average: 7384

Maximum observed aerosol concentrations are summarized below:

- A: <5.0 µg/mL
- B: <5.0 µg/mL
- C: <5.0 µg/mL
- D: <5.0 µg/mL

Conclusions:

Particle levels within both enclosures were well below those outside the enclosures (< 0.7 %). Aerosol concentrations were not detected at levels high enough to cause significant interference. These results indicate proper function of both HEPA filters and the secondary (positive pressure) enclosure, both with and without the cell sorter fan operating.

<u>Test #2</u> – Particle Counter, Aerosol Photometer; Cell Sorter in Normal Operation

Conditions and Observations:

- Both the primary and secondary enclosures were constructed and operating.
- Cell sorter fan was operating.
- Tested in the operator's breathing zone (B) with the primary enclosure access door both fully closed and open approximately one-third.
- Particle counter was run for a one minute sample. Cumulative particle counts are reported.
- Aerosol photometer was operated in upstream mode (µg/mL). Maximum observed readings are reported.
- Initially sampled with two people in the secondary enclosure (tester and cell sorter operator). Were unable to account for error introduced by the presence of a second person. This data has therefore been disregarded. Sampled again with the tester seated in the position the cell sorter operator would occupy.

Results and Conclusions:

Test #2 was initiated at approximately 1143 AM and continued until approximately 1208 PM. The purpose was to quantify particle concentrations during normal operation of the cell sorter with the primary enclosure in place. Cumulative particle count results are summarized below:

- A: 11
- B: 14

- B: 0 (Primary enclosure access door open approximately one-third)
- C: 9

Maximum observed aerosol concentrations are summarized below:

- A: <5.0 µg/mL
- B: <5.0 µg/mL (Primary enclosure access door open approximately one-third)
- C: <5.0 µg/mL
- D: <5.0 µg/mL

Particle and aerosol levels within both the primary and secondary enclosures are consistent with background levels. This indicates that with the cell sorter operating normally, particles were not escaping the device.

Test #3 – Particle Counter; Cell Sorter in Failure Modes

Conditions and Observations:

- Both the primary and secondary enclosures were constructed and operating.
- Cell sorter fan was operating.
- One person (tester) was inside the secondary enclosure.
- Test was initially conducted with the cell sorter stream misaligned, simulating a blockage or clog of the mechanism.
- During this phase of the test, the primary enclosure access door was fully open.
- Following initial testing, with the cell sorter stream still misaligned, the operator activated the bulk injection unload mechanism. This procedure has been observed to create a particle release.
- During this phase of the test, the primary enclosure access door was open approximately one-third.

Results and Conclusions:

Test #3 was initiated at approximately 1218 PM and continued until approximately 1245 PM. The purpose was to determine:

a) whether, under commonly encountered conditions of device failure, particles could be released from the cell sorter, and:
b) whether, given these failures, the bioBubble primary enclosure could effectively prevent the particles from entering the breathing zone of the cell sorter operator.

Cumulative particle count results are summarized below:

Cell Sorter Stream Misaligned:

- A: 198 – 403 Average: 301
- B: 6 (Primary enclosure access door fully closed)
- B: 0 (Primary enclosure access door open approximately one-third)
- C: 0

Cell Sorter Stream Misaligned and Bulk Injection Unload Activated:

- A: 62 (At end of test)
- B: 35 – 64 Average: 50
- C: 7

The particle levels measured at location A were 18 – 37 times higher than those measured during normal operation. These results clearly indicate that, while in an induced failure, the cell sorter causes particles to become airborne outside of the device. Repeating the test during the bulk injection unload did not produce such clear-cut results. Since the operator's breathing zone was considered the highest priority, the readings at B were collected during the bulk injection unload event. Particle levels at A were therefore measured approximately 3-4 minutes after the event. Due to this delay, the measurement at A may not represent the true levels present at A during bulk injection unload. Despite this, particle results in the breathing zone (location B) were consistent with those collected during Test 1 (Backgrounds), indicating proper function of the bioBubble primary enclosure. These results also confirm instrument response to the aerosol created by the cell sorter.

Test #4 – Photometer; Both Enclosures Operational

Conditions:

- Both the primary and secondary enclosures were constructed and operating.
- Cell sorter fan was operating.
- The cell sorter was operating in normal mode.
- The challenge agent was introduced in the lower left corner of the primary enclosure.
- An upstream sample at the cell sorter chamber door (location A) read between 70 and 80 µg/mL PAO

Results and Conclusions:

Test #4 was initiated at approximately 1406 PM and continued until approximately 1415 PM. The purpose was to verify the primary enclosure's effectiveness when challenged with a very high concentration of particulate. Average percent penetration results are summarized below:

- A: 0.03 – 0.5% (Highly variable)
- B: 0.0045 (Primary enclosure access door open approx. one-third)
- B: 0.004 (Primary enclosure access door fully open)
- C: 0.012
- HEPA Exhaust: 0.006

The results of Test 4 indicate a concentration at location B that is 10-100 times lower than the concentration at A. This is consistent both with the primary enclosure access door fully open and mostly closed. The air at B tested slightly cleaner than the exhaust from the HEPA filter outflow, which was itself within the HEPA specification (<0.03 % penetration). These results confirm that the primary enclosure is effective at containing aerosol generated within the primary enclosure. Visual observation of aerosol movement patterns also indicated airflow upwards across the cell sorter front wall and away from the operator.

Test #5 – Photometer; Secondary Enclosure Disabled

Conditions:

- Only the primary enclosure was constructed and operating.
- Cell sorter fan was operating.
- Cell sorter was operating in normal mode.
- The challenge agent was introduced in the lower left corner of the primary enclosure.
- An upstream sample at the cell sorter chamber door (location A) read between 70 and 80 µg/mL PAO

Results and Conclusions:

Test #5 was initiated at approximately 1434 PM and continued until approximately 1441 PM. The purpose was to verify the primary enclosure's effectiveness under typical lab conditions, when challenged with a very high concentration of aerosol. The secondary enclosure was shut down and opened up to more closely represent lab conditions during actual cell sorter use. Average percent penetration results are summarized below:

- A: 0.05 – 5% (Highly variable)
- B: 0.012 (Primary enclosure access door open approx. one-third)
- B: 0.019 (Primary enclosure access door fully open)
- C: 0.012

The results of Test 5 are consistent with those of Test 4. Even without the support of the secondary enclosure, results indicate a concentration at location B that is 2 – 417 times lower than the concentration at A, with the access door both open and mostly closed. These results confirm that the primary enclosure is effective at containing aerosol generated within the primary enclosure without the secondary enclosure. Visual observation of aerosol movement patterns indicated airflow upwards across the cell sorter front wall and away from the operator.

Test #6 – Photometer, Anemometer; bioBubble Equipment Check

Conditions:

- Both the primary and secondary enclosures were constructed and operating.
- Cell sorter fan was operating.
- Cell sorter was operating in normal mode.
- Primary enclosure access door was fully open.
- Aerosol fog was introduced in the lower left of the primary enclosure.
- Photometer was set to 100% upstream at the primary enclosure exhaust duct.
- Filtered air was probed with the photometer (scanning was not feasible due to configuration).

Results and Conclusions:

Test #6 was initiated at approximately 1445 PM and continued until approximately 1457 PM. The purpose was to document the operating conditions of the primary enclosure. The exhaust HEPA filter was probed using the aerosol photometer (set to read % of upstream concentration). The maximum observed penetration was 0.03%. Measurements taken using a thermal anemometer (TSI Model AVM 430-A) on a

2" exhaust grid on the upstream side of the HEPA filter indicate an average flowrate of 241 fpm. Over the 10"x10" exhaust opening, this equals a volumetric flow of 167 CFM.

Summary of Results:

During each of the tests, the results from the various instruments used were consistent with values that would be expected of an adequate containment. In Test 1, the particle concentrations indicate that the secondary enclosure was successfully controlling the ambient dust levels in the lab, allowing for very sensitive testing of the primary enclosure. This test also established a baseline for comparison of later results. Test 2 established that, under normal operation of the cell sorter and bioBubble enclosures, particles were not escaping either the cell sorter or the primary enclosure. Several important conclusions can be drawn from Test 3: First, when operated with the stream misaligned, the cell sorter does release significant levels of airborne particulate. Second, the particle counter is capable of detecting those particles. Finally, Test 3 shows that, despite those higher levels immediately outside the cell sorter, the particle levels in the operator's breathing zone were not elevated above the background levels. This combination of results indicates that the primary enclosure is functioning as intended. Test 4 demonstrates the same result given a much higher dose of particulate, and Test 5 similarly confirms the previous results, accounting for normal conditions in the lab. Test 6 successfully documented the current performance of the primary enclosure.

Based on the results of these tests, it appears that the bioBubble primary enclosure fulfills its intended purpose of preventing cell sorter operator exposure to aerosol particulates generated by the BD FACS Aria II cell sorter.

Limitations

ATC provided these services consistent with the level and skill ordinarily exercised by members of the profession currently providing similar services under similar circumstances at the time the services were provided. This statement is in lieu of other statements either expressed or implied. This report is intended for the sole use of bioBubble, Inc. The scope of services performed in execution of this evaluation may not be appropriate to satisfy the needs of other users, and use or re-use of this document, the findings, conclusions, or recommendations is at the risk of said user.

As with all such assessments, the results of the sampling represent conditions found on the date of the survey and may not represent conditions found at other times. Additionally, this assessment was limited with respect to the specific parameters indicated above and should not be construed to be a comprehensive evaluation or a definitive representation of conditions within the facility. The information presented in this report is intended to be used as a guide to evaluate the need for further investigation or the need for modifications to the processes or procedures surveyed.

The Client recognizes and agrees that all testing and remediation methods have reliability limitations, no method nor number of sampling locations can guarantee that a condition will be discovered within the performance of the services as authorized by the Client. Additionally, the passage of time may result in a change in the environmental characteristics at this site. This report does not warrant against future operations or conditions that could affect the recommendations made. The results, findings, conclusions, and recommendations expressed in this report are based only on conditions that were observed during ATC's inspection of the site.

A diagram of the lab and enclosure setup is included in **Appendix A**. **Appendix B** contains photographs of the enclosures and the test procedures. Thank you for selecting ATC Associates Inc. for your environmental management needs. If you have any questions concerning this correspondence, please feel free to contact us at 802-862-1980.

Sincerely,

ATC ASSOCIATES INC.

Devin Porter
Environmental Technician

Thomas J. Broido
Branch Manager

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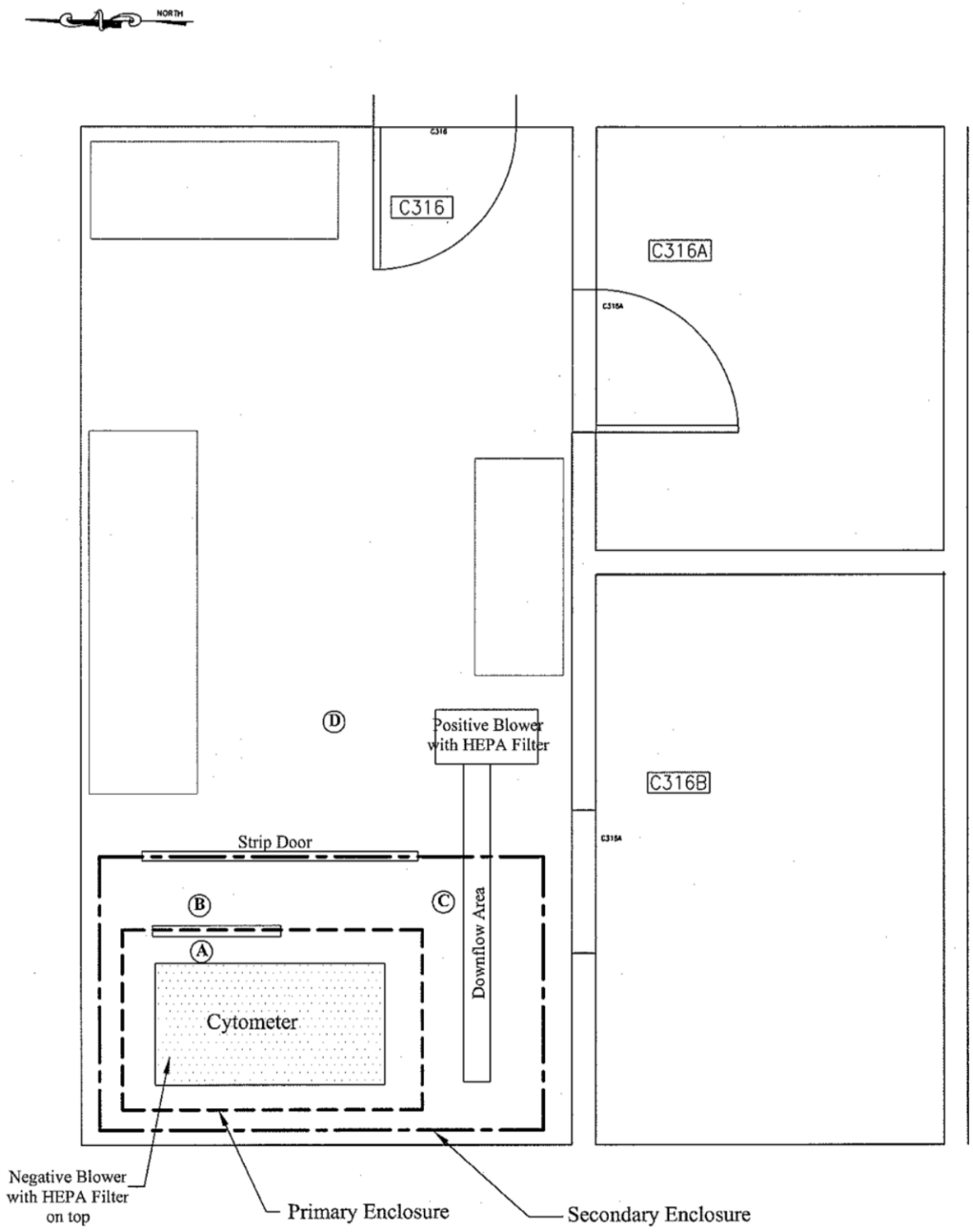
Appendix A

Diagrams



(B) = Sample Number and Location

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Sample Location Diagram

Given Complex Third Floor- Room C316
University of Vermont
Burlington, Vermont

Proj. No.	63.41229.0001
Proj. Mgr.	DP
Date	3/29/11

Figure 1

Appendix B

Photo Log

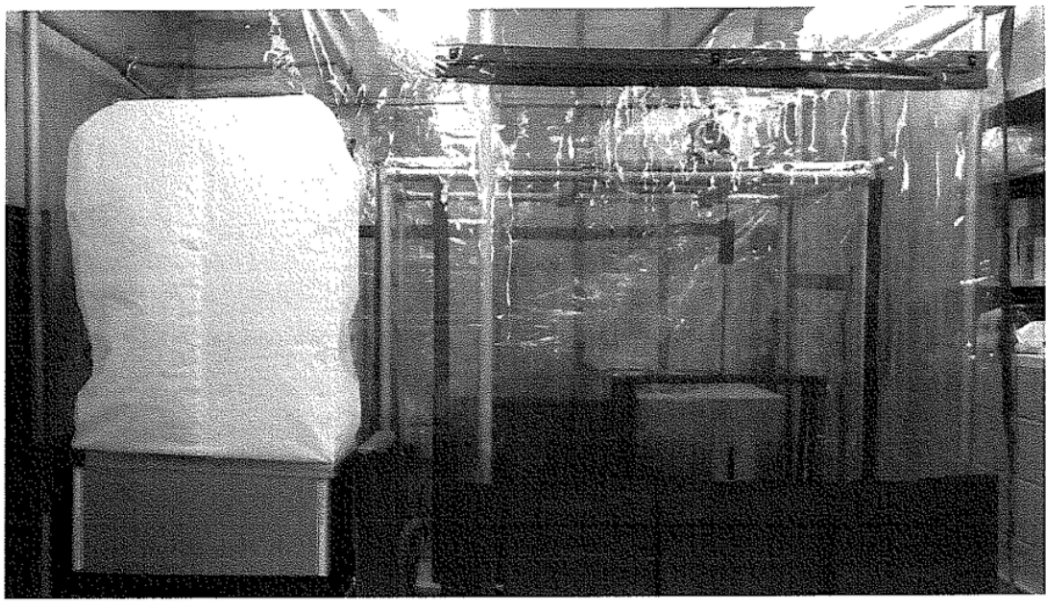

PHOTO 1
Primary and Secondary Enclosures, with Cell Sorter

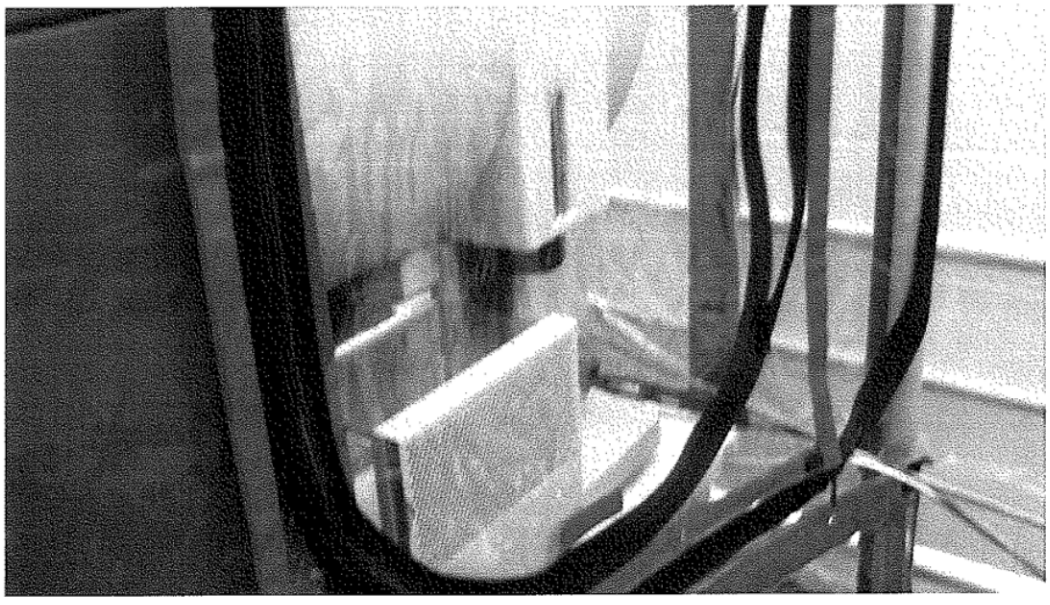

PHOTO 2
Primary enclosure access door (approximately 1/3 open), and cell sorter components.

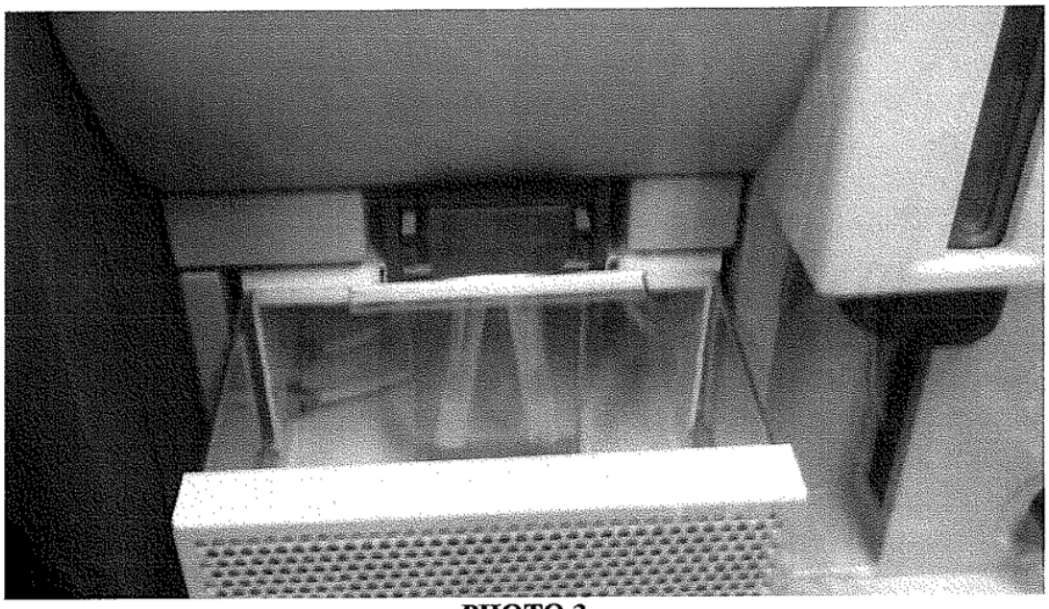

PHOTO 3
Cell sorter collection tubes (site of aerosol generation).

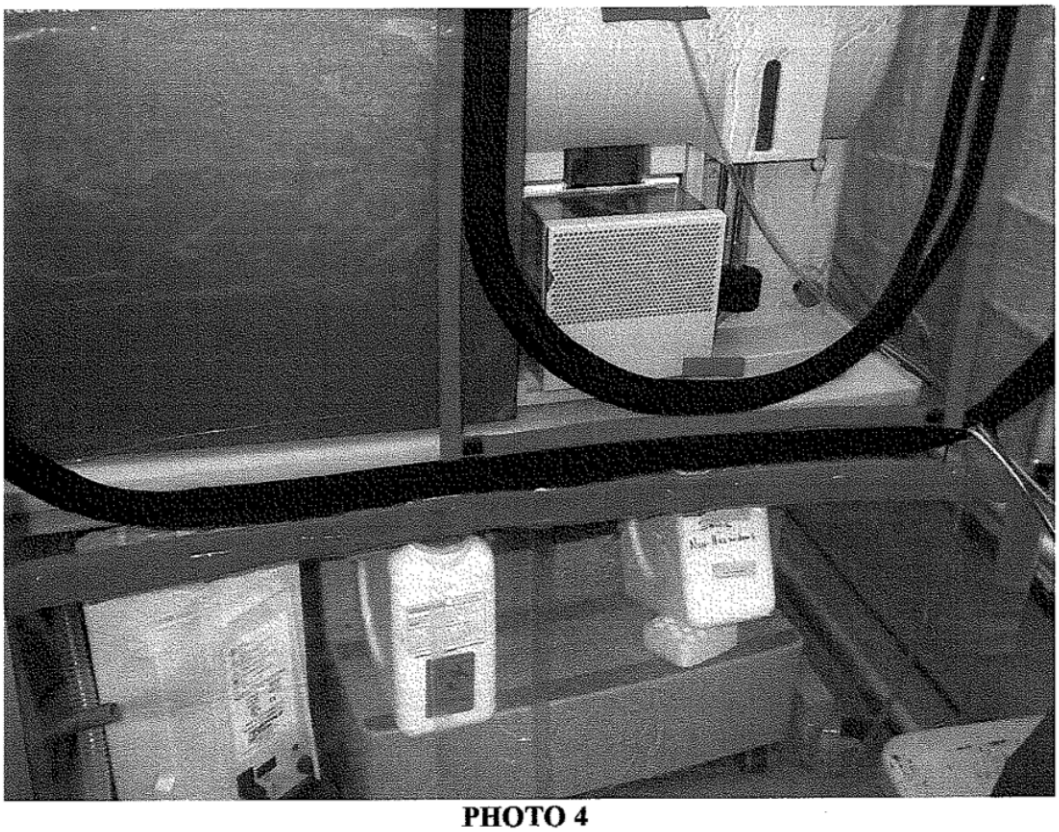

PHOTO 4
Cell sorter with aerosol introduced (lower left corner).