### AN ANALYSIS OF REQUIRED SPACING AT THE FRONT **INTAKE AREA OF CLASS II BIOLOGICAL SAFETY CABINETS** If biological can ett capital

Crosby Ravert, Robert Timer, Lewis Exner, Adam Costa, Anh Huynh, Jason Scrafano, and James T. Wagner

### **Purpose**

The primary method of determining the face velocity of a Class II Biosafety Cabinet (BSC) has been the Direct Inflow Measurement (DIM) device since 1992. This method was confirmed to be the most repeatable method available in 2002. Since 1992, general practice has been to only use this method when there is at least 18 inches of clearance at the leading edge of the DIM. There is no consensus between which we started practitioners as to where that required distance came from; therefore, we aim to determine whether the 18-inch distance from a DIM intake to obstruction is truly integral to accurate measurement of Class II BSC air intake velocity. An additional goal is to determine whether alternative DIM mounting methods, which would decrease the overall DIM length, result in reproducible and comparable intake volume measurements when compared to the traditional mounting method. 28 November 2023

### **Questions:**

- **>** How does the distance between an obstruction and the front intake area of a BSC affect the flow rate of air through the front intake area with a DIM installed?
- **>** Does the skirt used with the DIM device affect its accuracy relevant to the method of DIM installation used by NSF when the listed intake velocities are established? Will the same readings be measured when using a variety of skirts: "biobag" skirt, no skirt, or a 12" x 48" skirt?
- **>** Does the distance between the obstruction and front intake area affect the differential pressure between the interior and exterior of the biosafety cabinet?

### **Hypotheses:**

If the distance between a wall and the front intake area of a BSC decreases to below 18 inches, then airflow rate through the front intake area would decrease due to the obstruction. If that 18-inch clearance can be reduced, the use of a DIM device, which is the primary and most repeatable testing method, would be feasible for more field applications.

If the DIM device were to be assembled with a variety of skirts which help funnel air into the meter, there should be little to no observed difference in the readings in a scenario where all other independent variables are the same. If no difference is observed, this would make the DIM device more feasible and accessible in field applications.

If the distance between an obstruction and the front intake  $\frac{1}{\sqrt{1-\frac{1$ is affected, then the change in differential pressure across If the biosafety cabinet is expected to be directly proportional  $\frac{m}{\sqrt{2}}$  through some square-rooted functional form to the  $\sum_{i=1}^{N}$  change in velocity, that is  $\frac{v_f}{v_i} \sim \int_{P_i}^{P_f}$  which is derived from the  $\frac{1}{2}$  relationship between linear velocity and velocity pressure of air at standard conditions.  $\int$  died of a DJC decreases to Deform  $\int$  $\mu_{\text{2}}$  ine biosalety cabinet is expected to be directly proportional  $\frac{df}{dt}$  which is derived from

### **Experimental Design:**

The experiment was conducted using a NUAIRE NU-540- 400 Class II Type A2 BSC with a Shortridge Instruments flow hood kit attached to the front intake area. A voltmeter was connected to the main blower as a means of measuring the voltage at every reading to be able to determine if voltage variation is present and has any effect on reading variation. Additionally, a hydraulic lift fitted with two 96" x 48" sheets of 1/4" pine plywood fastened together with three pine boards running across the back and drywall screws was used to create a  $96" \times 96"$  artificial wall capable of moving varying distances from the leading edge of the flow hood. The method outlined above was used at CEC to eliminate any potential of perturbing the flow hood or biosafety cabinet, while maintaining a large flush face to avoid air moving from around the back of the obstruction. A series of readings were taken with the artificial wall placed at each of the varying distances from the DIM. The DIM was set to "Auto-Read" mode to allow a smooth collection of data without possible perturbations to the meter setup itself. In addition to the mode, we ran a short process to determine when balanced readings can be obtained through the Auto-Read mode. In addition to airflow measurements, we recorded voltages and the differential of pressure from the inside to outside of the biosafety cabinet at each stage of the data collection.

As a means of process qualification, we recorded a series of readings through the DIM in Auto-Read mode to determine the number of bad reads, or a measurement taken before proper stabilization of the DIM. This process was done five times, and the number of bad reads was averaged and rounded up to be conservative with the meter. Through five of these tests, we found that only the first readings are to be discarded at each stage due to DIM reading stabilization.

The independent variables for the experiment were the distance of the artificial wall from the leading edge of the

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flow hood, blower voltage, cabinet mode (run/calibration), and which, if any, skirt is attached. The two dependent variables were the volumetric rate at which air enters the BSC front access opening and the differential in pressure between the workspace of the cabinet and the room. Additionally, all sets of testing were done in both calibration mode and run mode to determine if any difference is observed.

**Pictures documenting the data collection set-up and process are shown here.**



*Front side of the (wall) artificial obstruction, side facing the cabinet.*



*12" x 48" Capture Skirt Configuration*



*Back side of the (wall) artificial obstruction, side facing away from the cabinet.*



*10" x 24" (Biobag) Capture Skirt Configuration*



*The three flow hood configurations used in this experiment, as well as the method they were connected to the biosafety cabinet.*



*The set-up used for measuring the differential of pressure between the interior and exterior of the biosafety cabinet. Arrow indicates across the interface at which the pressure differential was measured.*



*DC Voltages of the blower were measured and recorded alongside the volumetric flow rate and pressure differential at each step in the procedure.*







*How the obstruction was used to simulate various distances between the front intake opening and a wall. The yellow arrow represents where the corresponding distance was measured.*

#### **Materials:**

- 1 Artificial wall made from the following materials:
	- o  $2 1/4$ " x 48" x 96" Construction grade pine plywood sheets
	- o  $3$  Eight foot long I" x 4" Pine boards
	- o  $12$  GripRite  $#6 \times 1 5/8"$  Drywall Screws
	- o 2 National Hardware N100-362 5/16" x 1-1/8" Stainless steel rope loop
	- o 4 Generic Plastic Zip Ties

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- 1 Dayton: 2000lb capacity hydraulic forklift
- 1 Fluke: 323 True RMS Clamp DC Voltage Meter
- 1 Air Intake Measurement Flow Hood:
	- o 1 Shortridge Instruments: Airdata multimeter ADM-870C **|** Electronic micromanometer **|** Model: ADM-870C **|** Serial No.: M19140 **|** Calibrated on: 02 MAR 2023 **|** Calibration due: 02 MAR 2024
	- o 1 Shortridge Instruments: Bio Hood Series 8400 Frame
	- o 1 Shortridge Instruments: Bio Hood Support Kit
	- o 1 Shortridge Instruments: 10" x 24" Capture Skirt **|** Commonly referred to as "Biobag".
	- o 1 Shortridge Instruments: 12" x 48" Capture Skirt
- 1 TSI Manometer **|** Model: 9565P**|** Serial No.: 9565P1729024 | Calibrated on: 16 JUN 2023 **|** Calibration Due: 16 JUN 2024
- 1 NUAIRE: Class II Type A2 BSC | Model: NU-540-400 | Series: 5 | Serial No.: 194499101519
- $\bullet$  4 9"  $\times$  12" Acrylic panels
- Stucco Tape
- Rubber Tube

#### **Procedure:**

- 1. A  $1/4$ " x 96" x 96" artificial wall was assembled by putting two sheets of plywood together and securing from behind with planks using the following materials:
	- a. Two sheets of  $48'' \times 96'' \times 4''$  pine plywood.
	- b. Three boards of eight foot long I" x 4" pine wood.
	- c. Drywall Screws
	- d. National Hardware N100-362 Stainless Steel Rope Loops  $(5/16" \times 1-1/8")$
- 2. The wall is then fastened upright to a hydraulic lift using plastic zip ties and set aside for later.
- 3. Assemble DIM device in desired configuration for current test on biosafety cabinet.
	- a. "Biobag" Skirt: The Shortridge Instruments flow hood with "biobag" skirt and micromanometer were assembled and secured to the front intake area of the NUAIRE BSC. BSC is then further sealed using acrylic panels and stucco tape around the perimeter where the flow hood meets the biosafety cabinet and cabinet sash.
- b. 12" x 48" Skirt: The Shortridge Instruments flow hood with the 12" x 48" skirt and micromanometer were assembled and secured to the front intake area of the NUAIRE BSC. BSC is then further sealed using stucco tape around the perimeter where the flow hood meets the biosafety cabinet and cabinet sash.
- c. No Skirt: The Shortridge Instruments meter frame was propped within the sash opening and further sealed using acrylic panels and stucco tape around the perimeter where the frame meets the biosafety cabinet and cabinet sash.
- 4. The BSC is then turned on and allowed to complete its warmup cycle.
- 5. Set up the required independent variables as desired for current testing set on the biosafety cabinet.
	- a. Make sure biosafety cabinet is in the proper mode for the desired test (Run/Calibration)
	- b. Set blower voltage to desired value (Low blower speed ≈ 6.0 Volts; High Blower Speed  $\approx 8.0$  Volts)
- 6. After powering up the micromanometer, the hydraulic lift is first placed 48 inches from the top legs of the capture hood frame.
- 7. The first reading is discarded as a bad reading due to adjustments and stabilization in the micromanometer.
- 8. Five readings are recorded at each distance.
- 9. Average the five readings taken then round the answer to the nearest integer. This is the final value used for each distance.
- 10. The hydraulic lift is then brought closer to the opening of the capture hood at varying distances (48", 36", 24", 18", 12", 6", 2"). Repeat from step 8 until at 2" from the biosafety cabinet. After collecting data for 2" from the biosafety cabinet, move to step 11.
- 11. Upon completion of the testing set with given independent variables, continue by starting from step 4 as needed.

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#### **Presenting of Data:**

The data was recorded and organized into the tables on the next several pages, grouped by a variety of parameters for clarity. Additionally, some figures were assembled using statistical properties derived from each data set:

### **Data collected using the 10" x 24" skirt (Biobag): Data collected using the 10" x 24" skirt (Biobag):**



Table 1.A







Table 1.C

Table 1.B

Table 1.D

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#### **Presenting of Data:**

The data was recorded and organized into the tables on the next several pages, grouped by a variety of parameters for clarity. Additionally, some figures were assembled using statistical properties derived from each data set:

### **Data collected using the 12" x 48" skirt: Data collected using the 12" x 48" skirt:**



Table 2.A



Table 2.B





Table 2.C

Table 2.D

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#### **Presenting of Data:**

The data was recorded and organized into the tables on the next several pages, grouped by a variety of parameters for clarity. Additionally, some figures were assembled using statistical properties derived from each data set:

### **Data collected using no skirt: Data collected using no skirt:**





Table 3.B

Table 3.A





Table 3.C

Table 3.D

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# **Comparing the airflow volume averages by flow hood configuration: Comparing the airflow volume averages by flow hood configuration:**



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#### **Data Analysis:**

The experiment went as expected with no unusual events that would have introduced error. The volumetric flow rate of air entering the biosafety cabinet was recorded in cubic feet per minute on Tables 1-3. The average intake volume is an arithmetic mean across all readings at the same distance from the wall. These averages are the values that were further used in the remaining figures. Aside from the intake volume, two accessory readings were taken to document the DC voltage across the blower at each stage, as well as the differential of pressure from the workspace of the biosafety cabinet to the exterior. All this data was taken from the original twelve tables and further used to draw an analysis on the effects of an obstruction at various distances from the front access opening of the biosafety cabinet.

Arguably the most important metric to determine whether a difference occurs at various distances of an obstruction is the average airflow intake volume for the cabinet under a variety of circumstances. This is obvious as it is the property one is directly interested in when using a flow hood for testing intake velocity on a biosafety cabinet. This data was assembled into Figures 1-3 based on the flow hood skirt configuration used for testing. In all three figures, there is a fair consistency in airflow volume until the obstruction comes within six inches of the biosafety cabinet. In the cases when a skirt was used, an upward trend is observed at six inches, but then takes a sharp drop at two inches to levels below the previous average. This behavior is not observed in the case when no skirt was used; From six inches and closer, a strictly increasing monotonicity can be observed in the data indicating a constant increase in the rate of change for the data starting at two inches. By only considering the case most applied by field technicians will apply (Biobag), there is no overwhelming evidence to indicate that a biosafety cabinet needs more than six inches of clearance at the front access opening for proper function.

The averages were grouped by the configuration of the flow hood used for taking readings, and further separated by the set voltage of the blower and the operation mode which

the biosafety cabinet was set to: Calibration or Run. In Figures 1-3, these values were all regrouped to visualize how they compare with the rest of the testing of similar configurations. Through application of the continuity

equation, it can be determined that there must be an increase in linear velocity at the flow hood, and subsequently at the intake of the biosafety cabinet since the crosssectional area remains constant throughout the duration of the experiment.

### **Q=V\*A (Continuity Equation) Fluid Volume Rate=Linear Velocity \*Cross-Sectional Area**

It can be determined that the linear velocity of air entering the biosafety cabinet must be affected when considering this equation with our results, specifically increasing as the wall is brought closer. Due to the fixed cross-sectional area programmed in the flow hood, there is only one logically relevant reason this could have occurred; an increase in the linear velocity of the air entering the cabinet. There is data that shows an undeniable increase in the differential pressure, which theoretically would encourage air to pass through the flow hood at an increased rate. However, we could not find any proportionality between the increase in differential pressure and the increase in intake volume to confirm this to be the entire cause of increase.

As far as how the distance between an obstruction and the front access opening of the biosafety cabinet affects the differential pressure across the biosafety cabinet, our data from Tables 1-3 clearly indicates an increase in the pressure differential as the obstruction got closer to the front access opening. This is evident in every single testing set-up that was performed. While there was minor variability as the obstruction came closer, the minimum increase in pressure observed at two inches was 80% whereas the maximum increase was a staggering 625%. However, a chart detailing the correlation coefficient between the airflow volume and the pressure was assembled and included below as Figure 7.:



*Figure 7: Coefficients of correlations across data sets.*

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A coefficient of correlation is a numeric value between -1 and 1 which indicates how similarly two sets of data change, where 1 means the sets trend identically, and -1 implies the two sets trend in opposing directions, and 0 means no common trend. Intuitively, one would expect to see all the correlation coefficients very close to 1, indicating a high correlation, because of a fluid's affinity to flow from higher to lower pressure regions, given that the interior of the cabinet is at a lower pressure than the environment outside of the cabinet. However, only the flow hood without a skirt has a high correlation, indicating that the set-up without a skirt was the only setup in direct noncompliance with Bernoulli's Principle which states an increase in the speed of a fluid occurs with the increase in static pressure. While Bernoulli's Principle is commonly applied to a closed fluid duct, consider the entirety of the cabinet and flow hood set-up to act as the hypothetical fluid duct since the cabinet should be completely contained everywhere between the air intake point and the air exhaust point. Instead of coefficients close to 1, most of the relevant points have a negative correlation, otherwise implying that the air intake rate and the differential pressure across the cabinet are inversely proportional.

Another aspect of our data that can be analyzed is the standard deviation across each series of testing. These values were all collected and presented in Figures 4-5. Figure 4 simply shows the standard deviation across all data collected, whereas Figure 5 shows the same, but with all data from two inches omitted. Standard deviation can be thought of as a metric for how similar, or tight a set of data is. In our application, a higher standard deviation means a larger variation in the readings, whereas a lower standard deviation means all the readings were very close to the average. For our sake, as low of a standard deviation as possible is desired, which correlates to all our readings being tight. Looking at Figure 4., an observed low standard deviation in the experiments using the various skirt configurations. However, when the skirt was removed our standard deviation took a significant rise. This indicates to us that the measurements are much more stable and vary less when a skirt is used to funnel the airflow into the biosafety cabinet. Although there is no current metric to determine when the standard deviation is too high, a configuration with a skirt would statistically perform more favorably compared to one without the skirt.



*Figure 4: Standard Deviations of each cabinet mode/configuration.*



*Figure 5: Standard Deviations of each cabinet mode/configuration excluding all data from two inches.*

Finally, in Figure 5., all data from two inches was omitted because of the amount of outlying data recorded out of curiosity to see how the standard deviation curves change. When comparing Figures 4 and 5, the curves fit much more tightly together in the figure excluding the data from two inches, as well as a noticeably lower standard deviation across the board. This figure denotes that the data collected at two inches does not fit our set well at all, implying that the next closest distance (six inches) is where the accuracy in readings is maintained at a variety of distances.

An initial hypothesis regarding this testing was that as the blower speed increased, the standard deviation of the testing session would increase allowing for acceptance of a larger range of readings. However, Figure 4 directly contradicts this hypothesis. The figure shows a beginning trend of increasing the standard deviation as blower speed increased, but the trend became inconsistent as there are multiple tests done at 6.0 Volts which return a standard deviation closer to those returned with a blower set at 8.0 Volts. However, as

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mentioned above, it seems to be the skirt which had the biggest effect on standard deviation. This figure does well to refute the previous conjecture, as well as invalidate any notion of acceptance with a two-inch clearance.

#### **Conclusion:**

The set of experiments conducted yielded a variety of interesting results. When it comes to using a skirt for airflow intake volume measurement, our data concluded that the skirts are much more favorable in recording data than any configuration without the skirt. Additionally, it can be concluded that the differential of pressure across the biosafety cabinet definitively increases when an obstruction is present at the front access opening, the effects of the change in pressure does not directly affect the airflow as observed in the cases above. In the results, there was no notable change in the air intake rate until the wall came within less than six inches from the biosafety cabinet. In conclusion, a cabinet with an obstruction at six inches would perform similarly enough to a cabinet with an obstruction at eighteen inches to continue safe operations.