# Particle Dosimetry Characterization of the Vitrocell AMES 48 In Vitro Exposure System Poster Board P719 Michael J. Oldham<sup>a</sup>, Jingjie Zhang<sup>a</sup>, I. Gene Gilman<sup>b</sup>, Pavel Kosachevshy<sup>b</sup>, Francesco Lucci<sup>c</sup>, Arkadiusz K. Kuczaj<sup>c</sup>, Nicolas D. Castro<sup>a</sup>, Ali A. Rostami<sup>a</sup>, Yezdi B. Pithawalla<sup>a</sup>, K. Monica Lee<sup>a</sup> <sup>a</sup>Altria Client Services, 601 East Jackson Street, Richmond, VA 23261, USA; <sup>b</sup>Enthalpy Analytical, 1470 East Parham Road, Richmond VA 23228; <sup>°</sup>Philip Morris Products S.A., Quai Jeanrenaud 5, CH-2000 Neuchâtel, Switzerland Society of Toxicology 57th Annual Meeting, March 11 - 15, 2018, San Antonio, TX, USA This poster may be accessed at www.altria.com/ALCS-Science



## Abstract

When using multiphase aerosol (particulate and vapor) delivery and exposure with *in vitro* assays, knowledge of the delivered dose and its time course is critical to interpreting and extrapolating biological results. In this study, the particle dosimetry of aerosol exposure of cells was measured using monodisperse fluorescent particles (0.5, 1.1, 2.1, & 3.2 µm in diameter) in each of the 48 wells of the Vitrocell AMES 48 exposure system (Figure 1). Three different flowrates at each of the 48 wells were evaluated (5, 10, and 20 cc/min) in three replicate experiments. Fluorescent particle distribution across each well was photographed using fluorescent microscopy and counted using image analysis software. Particle losses throughout the in vitro exposure system were quantified by repeatedly washing each part with aqueous surfactant and counting the collected fluorescent particles using either a hemocytometer with fluorescent microscopy or image analysis software. Results indicate that overall deposition efficiency is below 1% for each well regardless of flowrate for each particle diameter tested. For each particle diameter, there were characteristic particle distribution patterns across the wells. For multiphase aerosols. such as e-vapor aerosols, combination of our particle dosimetry results with predictions of vapor deposition using computational fluid dynamic techniques would provide a more complete picture of cellular exposure that occurs in the *in vitro* exposure system.



Open in climate controlled chamber. There are eight independent climatic chamber. rows for exposure with six replicate 35 mm Petri dish holders per row. (Picture courtesy of Vitrocell Systems GmbH.)



Figure 1. Front view of AMES 48 In Vitro Exposure System Figure 2. Aerosol generation system and AMES 48 In vitro Exposure System in

### Methods

- Each of the 48 wells was uniquely identified using a row number (1-8) and letter (A-F) for the column
- Petri dishes were marked to indicate orientation for correct representation of particle distribution patterns
- Monodisperse fluorescent particles of different diameters = 0.51, 1.1, 2.1 & 3.2 µm were generated (Figure 2) in pairs(0.51 & 2.1 µm and 1.1 & 3.2 µm)
- Because of nebulizer flow limitations, only four of the eight independent rows of Petri dishes were run simultaneously, each had a flow = 2000 cc/min with the horn height above petri dishes = 2 mm
- Three replicates were performed for each horn flowrate (5, 10, and 20 cc/min) and particle pair
- Fluorescent microscopy was used to photograph particle deposits on Petri dishes and filters
- Image J software used to count and provide coordinates of particles from photographs
- Particle distribution plots were adjusted to compensate for flipping of images within the microscope and Image J software and conversion of pixels to microns
- Particles in the particle distribution plots were enlarged for visualization
- Variability between the number of particles depositing in each Petri (Figure 3) dish was plotted as a function of the average number of particles depositing in the row (for example: # of particles depositing in A1 minus mean # of particles depositing in row 1 divided by the mean # of particles depositing in row 1 expressed as a percentage)
- Particle distribution in microns from the center is middle of Petri dish for four of the eight rows measured for 2.1, 1.1 and 0.51 µm particles at 10 cc/min horn flowrate are shown in Figures 4-6
- Estimates of particle deposition efficiency for the first of three runs were <1% regardless of particle size



Figure 4. Particle Distribution for 2.1 µm particles in 4 of 8 rows of the AMES 48 In vitro Exposure System. Coordinates are in microns. (Main channel airflow is moving from left to right.)



# Results

Figure 5. Particle Distribution for 1.1 µm particles in 4 rows of the AMES 48 In vitro Exposure System. Coordinates are in microns. (Main channel airflow is moving from left to right.)

Figure 6. Particle Distribution for 0.51 µm particles in 4 of 8 rows of the AMES 48 In vitro Exposure System. Coordinates are in microns. (Main channel airflow is moving from left to right.)

• 10 cc/min horn flowrate resulted in the minimum variability of 0.51, 1.1, 2.1 and 3.2 µm particle deposition • Estimates of particle deposition efficiency were <1% regardless of particle size

 Compare experimental results with Eulerian and Lagrangian particle tracking based CFD predictions Measure dosimetry for prototype e-vapor product formulation (gravimetrically and chemically)

| tion A5 @<br>Flowrate  | 1.1 Micron Distribution B5 @<br>10 cc/min Horn Flowrate  | 1.1 Micron Distribution C5 @<br>10 cc/min Horn Flowrate  | 1.1 Micron Distribution D5 @<br>10 cc/min Horn Flowrate  | 1.1 Micron Distribution E5 @<br>10 cc/min Horn Flowrate   | 1.1 Micron Distribution F5 @<br>10 cc/min Horn Flowrate  |
|--|--|--|--|---|--|
|  | 15750  | 15750  | 15750  | 15750   | 15750  |
|  |  |  |  |   |  |
| 15750  | -15,50 15,750  | -15,50 15750   | -15750 15750   | -15,50 15,750   | -15750   |
|  |  |  |  |   |  |
| •••  | 15750  | 15750  | -15750   | -15750  | -15750   |
| tion A6 @<br>Flowrate  | 1.1 Micron Distribution B6 @<br>10 cc/min Horn Flowrate  | 1.1 Micron Distribution C6 @<br>10 cc/min Horn Flowrate  | 1.1 Micron Distribution D6 @<br>10 cc/min Horn Flowrate  | 1.1 Micron Distribution E6 @<br>10 cc/min Horn Flowrate   | 1.1 Micron Distribution F6 @<br>10 cc/min Horn Flowrate  |
|  | 15750  | 15750  | 15750  | 15750   | 15750  |
|  |  |  |  |   |  |
| 15750  |  | 15750  | 15750  |   |  |
| 13/30  | -13/50   | -13/30   | -13/30   | -13/30  | -13,50   |
|  | 15750  | 15750  | -15750   | -15750  | -15750   |
| tion A7 @  | 1.1 Micron Distribution B7 @   | 1.1 Micron Distribution C7 @   | 1.1 Micron Distribution D7 @   | 1.1 Micron Distribution E7 @  | 1.1 Micron Distribution F7 @   |
| iowidle  | 15750  | 15750  | 15750  |   |  |
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| 15750  |  | -15750 15750   | -15750   | -15750 15750  | -15750 45750   |
|  | 15.75.0  | 15750  | 15750  | 15750   | 15750  |
| tion A8 @  | 1.1 Micron Distribution B8 @   | 1.1 Micron Distribution C8 @   | 1.1 Micron Distribution D8 @   | 1.1 Micron Distribution E8 @  | 1.1 Micron Distribution F8 @   |
| owidte   | 15750  | 15750  | 15750  | 15750   | 15750  |
|  |  |  |  |   |  |
| 3  |  |  |  |   |  |
| 15750  | -15750   |  |  | -15750  | -15750   |
|  | 15750  | 15750  | 15750  | 15758   | -15750   |
|  |  |  |  |   |  |
| bution A5 @  |  |  |  | 1   |  |
| Flowrate   | 0.5 Micron Distribution B5 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution C5 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution D5 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution E5 @<br>5 cc/min Horn Flowrate  | 0.5 Micron Distribution F5 @<br>5 cc/min Horn Flowrate   |
| Flowrate   | 5 cc/min Horn Flowrate   | 0.5 Micron Distribution C5 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution D5 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution E5 @<br>5 cc/min Horn Flowrate  | 0.5 Micron Distribution F5 @<br>5 cc/min Horn Flowrate   |
| Flowrate   | 5 cc/min Horn Flowrate   | 0.5 Micron Distribution C5 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution D5 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution E5 @<br>5 cc/min Horn Flowrate  | 0.5 Micron Distribution F5 @<br>5 cc/min Horn Flowrate   |
| Flowrate   | 0.5 Micron Distribution BS @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution C5 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution D5 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution E5 @<br>5 cc/min Horn Flowrate  | 0.5 Micron Distribution F5 @<br>5 cc/min Horn Flowrate   |
| Flowrate   | 0.5 Micron Distribution BS @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution C5 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution D5 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution E5 @<br>5 cc/min Horn Flowrate  | 0.5 Micron Distribution F5 @<br>5 cc/min Horn Flowrate   |
| Flowrate   | 0.5 Micron Distribution BS @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution C5 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution D5 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution E5 @<br>5 cc/min Horn Flowrate  | 0.5 Micron Distribution F5 @<br>5 cc/min Horn Flowrate   |
| Flowrate   | 0.5 Micron Distribution BS @<br>5 cc/min Horn Flowrate<br>15750<br>15750<br>0.5 Micron Distribution B6 @   | 0.5 Micron Distribution C5 @<br>5 cc/min Horn Flowrate<br>-15750<br>-15750<br>0.5 Micron Distribution C6 @   | 0.5 Micron Distribution D5 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution ES @<br>5 cc/min Horn Flowrate  | 0.5 Micron Distribution F5 @<br>5 cc/min Horn Flowrate   |
| Flowrate<br>19750<br>Dution A6 @<br>Flowrate   | 0.5 Micron Distribution BS @<br>5 cc/min Horn Flowrate<br>-15750<br>0.5 Micron Distribution B6 @<br>5 cc/min Horn Flowrate<br>-15750   | 0.5 Micron Distribution C5 @<br>5 cc/min Horn Flowrate<br>-15750<br>-15750<br>0.5 Micron Distribution C6 @<br>5 cc/min Horn Flowrate<br>-15750   | 0.5 Micron Distribution D5 @<br>5 cc/min Horn Flowrate<br>-15750<br>-15750<br>0.5 Micron Distribution D6 @<br>5 cc/min Horn Flowrate<br>-15750   | 0.5 Micron Distribution ES @<br>5 cc/min Horn Flowrate<br>-15750<br>-15750<br>0.5 Micron Distribution E6 @<br>5 cc/min Horn Flowrate<br>-15750  | 0.5 Micron Distribution F5 @<br>5 cc/min Horn Flowrate<br>-15750<br>-15750<br>0.5 Micron Distribution F6 @<br>5 cc/min Horn Flowrate<br>-15750   |
| Flowrate   | 0.5 Micron Distribution BS @<br>5 cc/min Horn Flowrate<br>15750<br>15750<br>0.5 Micron Distribution B6 @<br>5 cc/min Horn Flowrate<br>15750  | 0.5 Micron Distribution C5 @<br>5 cc/min Horn Flowrate<br>-15750<br>-15750<br>0.5 Micron Distribution C6 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution D5 @<br>5 cc/min Horn Flowrate<br>15750<br>15750<br>0.5 Micron Distribution D6 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution ES @<br>5 cc/min Horn Flowrate  | 0.5 Micron Distribution F5 @<br>5 cc/min Horn Flowrate<br>-15750<br>-15750<br>0.5 Micron Distribution F6 @<br>5 cc/min Horn Flowrate   |
| Flowrate   | 0.5 Micron Distribution BS @<br>5 cc/min Horn Flowrate<br>-15750<br>0.5 Micron Distribution B6 @<br>5 cc/min Horn Flowrate<br>-15750   | 0.5 Micron Distribution C5 @<br>5 cc/min Horn Flowrate<br>-15750<br>-15750<br>0.5 Micron Distribution C6 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution D5 @<br>5 cc/min Horn Flowrate<br>-15750 15750<br>0.5 Micron Distribution D6 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution ES @<br>5 cc/min Horn Flowrate<br>-15750<br>-15750<br>0.5 Micron Distribution E6 @<br>5 cc/min Horn Flowrate  | 0.5 Micron Distribution F5 @<br>5 cc/min Horn Flowrate<br>-15750<br>-15750<br>0.5 Micron Distribution F6 @<br>5 cc/min Horn Flowrate   |
| Flowrate   | 0.5 Micron Distribution BS @<br>5 cc/min Horn Flowrate<br>-15750<br>0.5 Micron Distribution B6 @<br>5 cc/min Horn Flowrate<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-15750<br>-157 | 0.5 Micron Distribution C5 @<br>5 cc/min Horn Flowrate<br>-15750<br>-15750<br>0.5 Micron Distribution C6 @<br>5 cc/min Horn Flowrate<br>-15750<br>-15750<br>-15750<br>-15750   | 0.5 Micron Distribution D5 @<br>5 cc/min Horn Flowrate<br>-15750<br>-15750<br>0.5 Micron Distribution D6 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution ES @<br>5 cc/min Horn Flowrate<br>-15750<br>-15750<br>0.5 Micron Distribution E6 @<br>5 cc/min Horn Flowrate<br>-15750<br>-15750<br>-15750  | 0.5 Micron Distribution F5 @<br>5 cc/min Horn Flowrate<br>-15750<br>-15750<br>0.5 Micron Distribution F6 @<br>5 cc/min Horn Flowrate<br>-15750<br>-15750<br>-15750<br>-15750   |
| Flowrate   | 0.5 Micron Distribution B5 @<br>5 cc/min Horn Flowrate<br>-15/50<br>0.5 Micron Distribution B6 @<br>5 cc/min Horn Flowrate<br>15750<br>15750<br>15750<br>15750<br>15750  | 0.5 Micron Distribution C5 @<br>5 cc/min Horn Flowrate<br>15750<br>15750<br>0.5 Micron Distribution C6 @<br>5 cc/min Horn Flowrate<br>15750<br>15750<br>15750  | 0.5 Micron Distribution D5 @<br>5 cc/min Horn Flowrate<br>-15750<br>-15750<br>0.5 Micron Distribution D6 @<br>5 cc/min Horn Flowrate<br>-15750<br>-15750<br>-15750   | 0.5 Micron Distribution ES @<br>5 cc/min Horn Flowrate<br>-15750<br>0.5 Micron Distribution E6 @<br>5 cc/min Horn Flowrate<br>-15750<br>-15750<br>-15750<br>-15750  | 0.5 Micron Distribution F5 @<br>5 cc/min Horn Flowrate<br>-15750<br>0.5 Micron Distribution F6 @<br>5 cc/min Horn Flowrate   |
| Flowrate   | 0.5 Micron Distribution BS @<br>5 cc/min Horn Flowrate<br>-15750<br>0.5 Micron Distribution B6 @<br>5 cc/min Horn Flowrate<br>-15750<br>-15750<br>-15750<br>-15750   | 0.5 Micron Distribution C5 @<br>5 cc/min Horn Flowrate<br>-15750<br>0.5 Micron Distribution C6 @<br>5 cc/min Horn Flowrate<br>-15750<br>-15750<br>-15750<br>-15750   | 0.5 Micron Distribution D5 @<br>5 cc/min Horn Flowrate<br>-15750<br>0.5 Micron Distribution D6 @<br>5 cc/min Horn Flowrate<br>-15750<br>0.5 Micron Distribution D6 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution ES @<br>5 cc/min Horn Flowrate<br>-15750<br>0.5 Micron Distribution E6 @<br>5 cc/min Horn Flowrate<br>-15750<br>-15750  | 0.5 Micron Distribution F5 @<br>5 cc/min Horn Flowrate<br>-15750<br>0.5 Micron Distribution F6 @<br>5 cc/min Horn Flowrate<br>-15750<br>-15750   |
| Flowrate   | 0.5 Micron Distribution B5 @<br>S cc/min Horn Flowrate   | 0.5 Micron Distribution C5 @<br>S cc/min Horn Flowrate<br>-15750<br>0.5 Micron Distribution C6 @<br>S cc/min Horn Flowrate<br>0.5 Micron Distribution C7 @<br>S cc/min Horn Flowrate   | 0.5 Micron Distribution D5 @<br>5 cc/min Horn Flowrate<br>15750<br>-15750<br>0.5 Micron Distribution D6 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution D7 @<br>5 cc/min Horn Flowrate  | 0.5 Micron Distribution E5 @<br>5 cc/min Horn Flowrate<br>15750<br>15750<br>0.5 Micron Distribution E6 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution E7 @<br>5 cc/min Horn Flowrate  | 0.5 Micron Distribution F5 @<br>5 cc/min Horn Flowrate<br>-15<br>50<br>-15<br>50<br>-15<br>50<br>-15<br>750<br>0.5 Micron Distribution F6 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution F7 @<br>5 cc/min Horn Flowrate  |
| Flowrate   | 0.5 Micron Distribution B5 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution C5 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution C6 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution C7 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution C7 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution D5 @<br>5 cc/min Horn Flowrate<br>15750<br>-15750<br>0.5 Micron Distribution D6 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution D6 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution D7 @<br>5 cc/min Horn Flowrate  | 0.5 Micron Distribution E5 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution E6 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution E7 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution E7 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution F5 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution F6 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution F7 @<br>5 cc/min Horn Flowrate<br>15750  |
| Flowrate   | 0.5 Micron Distribution B5 @<br>5 cc/min Horn Flowrate<br>-15 50<br>0.5 Micron Distribution B6 @<br>5 cc/min Horn Flowrate<br>-15 750<br>0.5 Micron Distribution B7 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution B7 @<br>5 cc/min Horn Flowrate  | 0.5 Micron Distribution C5 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution D5 @<br>5 cc/min Horn Flowrate<br>-15750<br>0.5 Micron Distribution D6 @<br>5 cc/min Horn Flowrate<br>-15750<br>0.5 Micron Distribution D7 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution ES @<br>5 cc/min Horn Flowrate<br>-15750<br>0.5 Micron Distribution E6 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution E7 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution E7 @<br>5 cc/min Horn Flowrate  | 0.5 Micron Distribution F5 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution F6 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution F7 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution F7 @<br>5 cc/min Horn Flowrate  |
| Flowrate   | 0.5 Micron Distribution B5 @<br>5 cc/min Horn Flowrate<br>-15 50<br>0.5 Micron Distribution B6 @<br>5 cc/min Horn Flowrate<br>-15 50<br>0.5 Micron Distribution B6 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution B7 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution B7 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution C5 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution D5 @<br>5 cc/min Horn Flowrate<br>-15750<br>0.5 Micron Distribution D6 @<br>5 cc/min Horn Flowrate<br>-15750<br>0.5 Micron Distribution D7 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution D7 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution ES @<br>5 cc/min Horn Flowrate  | 0.5 Micron Distribution F5 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution F6 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution F7 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution F7 @<br>5 cc/min Horn Flowrate  |
| Flowrate   | 0.5 Micron Distribution BS @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution C5 @<br>5 cc/min Horn Flowrate<br>-15750<br>0.5 Micron Distribution C6 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution C6 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution C7 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution C7 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution D5 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution D6 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution D7 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution D7 @<br>5 cc/min Horn Flowrate  | 0.5 Micron Distribution E5 @<br>5 cc/min Horn Flowrate<br>-15750<br>0.5 Micron Distribution E6 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution E7 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution E7 @<br>5 cc/min Horn Flowrate  | 0.5 Micron Distribution F5 @<br>5 cc/min Horn Flowrate   |
| Flowrate   | 0.5 Micron Distribution BS @<br>5 cc/min Horn Flowrate<br>-15/50<br>0.5 Micron Distribution B6 @<br>5 cc/min Horn Flowrate<br>-15/50<br>0.5 Micron Distribution B7 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution B7 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution C5 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution D5 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution D6 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution D7 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution D7 @<br>5 cc/min Horn Flowrate  | 0.5 Micron Distribution E5 @<br>5 cc/min Horn Flowrate<br>-15750<br>0.5 Micron Distribution E6 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution E6 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution E7 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution E7 @<br>5 cc/min Horn Flowrate  | 0.5 Micron Distribution F5 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution F6 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution F6 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution F7 @<br>5 cc/min Horn Flowrate  |
| Flowrate<br>Dution A6 @<br>Flowrate<br>Dution A7 @<br>Flowrate<br>Dution A8 @  | 0.5 Micron Distribution BS @<br>5 cc/min Horn Flowrate<br>-15 50<br>0.5 Micron Distribution B6 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution B7 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution B8 @   | 0.5 Micron Distribution C5 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution D5 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution E5 @<br>5 cc/min Horn Flowrate  | 0.5 Micron Distribution F5 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution F6 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution F7 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution F7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution F7 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution F7 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution F8 @   |
| Flowrate<br>Dution A6 @<br>Flowrate<br>Dution A7 @<br>Flowrate<br>Dution A8 @<br>Flowrate                            | 0.5 Micron Distribution B5 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution B6 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution B7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution B8 @<br>5 cc/min Horn Flowrate<br>15750  | 0.5 Micron Distribution C5 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution C6 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution C7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution C7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution C7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution C8 @<br>5 cc/min Horn Flowrate  | 0.5 Micron Distribution D5 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution D6 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution D7 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution D7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution D7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution D7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution D8 @<br>5 cc/min Horn Flowrate | 0.5 Micron Distribution ES @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution E6 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution E7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution E7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution E7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution E8 @<br>5 cc/min Horn Flowrate | 0.5 Micron Distribution F5 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution F6 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution F6 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution F7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution F7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution F7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution F8 @<br>5 cc/min Horn Flowrate |
| Flowrate<br>19750<br>19750<br>Dution A6 @<br>Flowrate<br>Dution A7 @<br>Flowrate<br>Dution A8 @<br>Flowrate          | 0.5 Micron Distribution B5 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution B6 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution B7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution B8 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution C5 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution C6 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution C7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution C7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution C7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution C8 @<br>5 cc/min Horn Flowrate  | 0.5 Micron Distribution D5 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution D6 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution D7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution D7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution D8 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution D8 @<br>5 cc/min Horn Flowrate  | 0.5 Micron Distribution ES @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution E6 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution E7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution E7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution E8 @<br>5 cc/min Horn Flowrate  | 0.5 Micron Distribution F5 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution F6 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution F7 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution F7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution F7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution F8 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution F8 @<br>5 cc/min Horn Flowrate |
| Flowrate<br>19750<br>19750<br>Dution A6 @<br>Flowrate<br>Dution A7 @<br>Flowrate<br>Dution A8 @<br>Flowrate          | 0.5 Micron Distribution B5 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution B6 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution B7 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution B7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution B7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution B8 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution B8 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution C5 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution C6 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution C7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution C7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution C7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution C8 @<br>5 cc/min Horn Flowrate  | 0.5 Micron Distribution D5 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution D6 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution D7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution D7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution D7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution D8 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution ES @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution E6 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution E7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution E7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution E7 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution E8 @<br>5 cc/min Horn Flowrate          | 0.5 Micron Distribution F5 @<br>5 cc/min Horn Flowrate   |
| Flowrate<br>Dution A6 @<br>Flowrate<br>Dution A7 @<br>Flowrate<br>Dution A8 @<br>Flowrate<br>15750<br>15750          | 0.5 Micron Distribution BS @<br>S cc/min Horn Flowrate<br>0.5 Micron Distribution B6 @<br>S cc/min Horn Flowrate<br>0.5 Micron Distribution B7 @<br>S cc/min Horn Flowrate<br>0.5 Micron Distribution B8 @<br>S cc/min Horn Flowrate   | 0.5 Micron Distribution CS @<br>S cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution C6 @<br>S cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution C7 @<br>S cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution C7 @<br>S cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution C8 @<br>S cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution C8 @<br>S cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution C8 @<br>S cc/min Horn Flowrate | 0.5 Micron Distribution D5 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution D6 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution D7 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution D7 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution D7 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution D8 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution D8 @<br>5 cc/min Horn Flowrate                            | 0.5 Micron Distribution E5 @<br>5 cc/min Horn Flowrate  | 0.5 Micron Distribution F5 @<br>5 cc/min Horn Flowrate<br>15750<br>0.5 Micron Distribution F6 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution F7 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution F7 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution F7 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution F8 @<br>5 cc/min Horn Flowrate<br>0.5 Micron Distribution F8 @<br>5 cc/min Horn Flowrate                            |
| Flowrate<br>Dution A6 @<br>Flowrate<br>Dution A7 @<br>Flowrate<br>Dution A8 @<br>Flowrate<br>Dution A8 @<br>Flowrate | 0.5 Micron Distribution BS @<br>S cc/min Horn Flowrate<br>0.5 Micron Distribution B6 @<br>S cc/min Horn Flowrate<br>0.5 Micron Distribution B7 @<br>S cc/min Horn Flowrate<br>0.5 Micron Distribution B7 @<br>S cc/min Horn Flowrate<br>0.5 Micron Distribution B7 @<br>S cc/min Horn Flowrate<br>0.5 Micron Distribution B8 @<br>S cc/min Horn Flowrate   | 0.5 Micron Distribution C5 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution D5 @<br>5 cc/min Horn Flowrate   | 0.5 Micron Distribution E5 @<br>5 cc/min Horn Flowrate  | 0.5 Micron Distribution F5 @<br>5 cc/min Horn Flowrate   |

# Conclusions

# Next Steps