BioCascade Exhaled Breath Sampler (BEBS): Sample Viability

Ansley Chartrand, Anna Pulley, Aditya Srikakulapu

Department of Undergraduate Research, University of Maryland

ABSTRACT:

The viability of samples collected in the BioCasade Exhaled Breath Sampler (BEBS) machine is to be measured by simulating an exhaled breath sampling event via a Collison nebulizer. There are four stages the nebulized stimulants are divided into within the BEBS system, they divide the particles based on size. The stages are labeled large, medium, small, and BioSpot and they collect particles of sizes >8.2µm, 3.3-8.2µm, 1.15-3.3µm, and <1.15µm respectively.

Similar experiments have been done to examine the viability of collected samples in the BEBS system, but all are done at lower collection flow rates, where this test collects at 12 lpm[5]. In addition, this experiment collects the nebulized virus onto a hydrogel surface as opposed to previously used liquid mediums.

PR8 virus will be used as the model to simulate the collection of influenza viruses[1]. The purpose of this experiment is to recreate real-world collection data to ensure the BEBS efficacy for collecting culturable samples prior to clinical use in the EMIT-2 study.

BACKGROUND:

The BioCascade Exhaled Breath Sampler (BEBS) consists of two mains components. The first is the BioCascade that separates the collected particles by size and the second is the BioSpot-Viable Virus Aerosol Sampler (VIVAS) that uses condensation to grow the collected particles.

The BioCascade is separated into four stages of all different sizes adjusted for the flowrate of 12 lpm. The particles are separated by size through gentle impaction onto the hydrogel surface[3]. The stages decrease in size as the air flows through the machine, the final stage empties particles <1.15µm into the BioSpot-VIVAS.

The BioSpot periodically injects water into a condensation column that helps expand the fine aerosols into a size that is optimal for collection[2]. Fine aerosol samples must be grown in order to properly observe the contents of the sample.

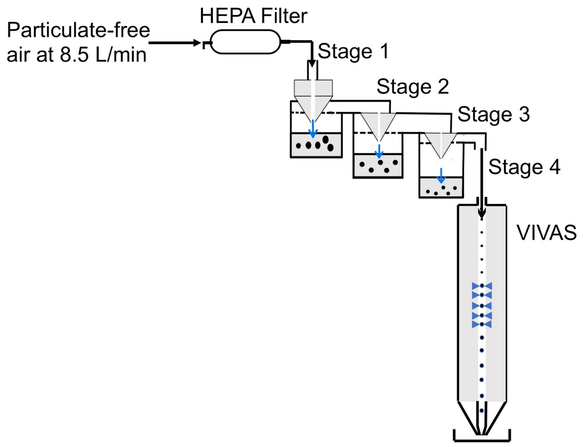

Figure 1: Diagram of the BEBS stages[1]. *This experiment pulled air at 12 L/min instead of 8.5 L/min as stated in the diagram

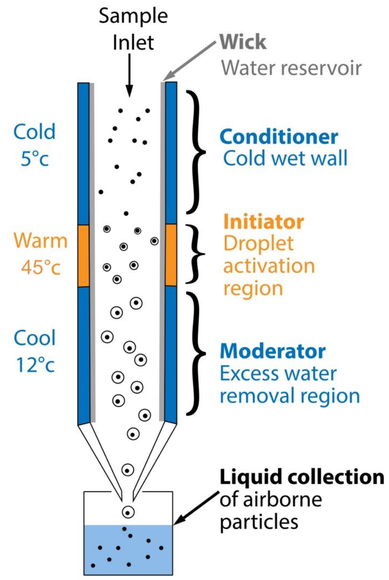

Figure 2: BioSpot-VIVAS simplified condensational system diagram[2].

METHODS:

A 1:10 dilution of PR8 and PBS is made to put into the nebulizer prior to the start of the experiment. A Chest Controller is used to "sneeze" the nebulized solution at 5 second intervals, with a sneeze lasting 2 seconds and a break of 3 seconds. The BEBS uses a vacuum pump to pull the nebulized air into the Biocascade through a cone at 12 liters per minute.

The total time for each run is 30 minutes and 2 separate runs were conducted for this experiment. Once the run is complete, the hydrogels are collected from the petri dishes then placed into a tube containing 5mL of PBS+BSA. The collected hydrogels contain the sample that will be used for PCR and cell culture.

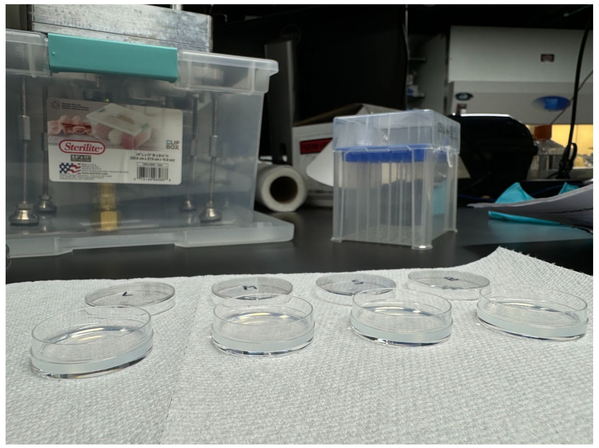

Photo 1: Hydrogels (2mL) in 35 mm petri dishes, all labeled according to their stage (large, medium, small, and BioSpot)

RESULTS:

The main observations drawn from this experiment is the hydrogel loss. There were significant gel losses from three stages during the experiment which can affect the viability of the virus.

The stages with most to least gel loss are the large, medium, small, and BioSpot respectively. The large stage had the most loss in Run #1, during the second run the large stage started with low hydrogel amounts.

The BioSpot stage had a small layer of water sitting atop the gel after each run, this is likely from condensation within the machine. The collection of condensation on top of the BioSpot hydrogel is likely the reason for low gel losses within that stage.

Gel Mass (g): Run #1		
	Before	**After**
Large	4.15	3.85
Medium	4.44	3.56
Small	4.10	3.41
BioSpot	4.06	5.11

Table 1: Gel mass before and after during run #1

Gel Mass (g): Run #2		
	Before	**After**
Large	2.52**	2.53**
Medium	3.79	3.24
Small	4.16	3.31
BioSpot	4.63	5.65

Table 2: Gel mass before and after during run #2. **The large stage had very little gel to begin with so it would be difficult to observe gel loss

CONCLUSIONS:

With the high flow rate of 12 lpm, a more dense hydrogel may be needed to avoid gel losses. With the increased velocity of the airflow and at its current constitution, the hydrogel may be evaporating more quickly. With hydrogel losses the sample viability decreases. For future experiments, we need to use a denser hydrogel to ensure stability across the sampling period. Additionally, PCR and cell culture experiments should be run to test sample viability.

References:

1. Chen, Y., Chen, J., Nannu Shankar, S., Amanatidis, S., Eiguren-Fernandez, A., Kreisberg, N., … Wu, C. Y. (2024). The BioCascade impactor: A novel device for direct collection of size-fractionated bioaerosols into liquid medium. *Aerosol Science and Technology*, *58*(3), 264–275. https://doi.org/10.1080/02786826.2024.2301941
2. Handix Scientific. (2023, July 25). *BioSpot-VIVAS*. Aerosol Devices. https://aerosoldevices.com/biospot-vivas/
3. Handix Scientific. (2023a, April 13). *Biocascade*. Aerosol Devices. https://aerosoldevices.com/biocascade/
4. Pan, M., Eiguren-Fernandez, A., Hsieh, H., Afshar-Mohajer, N., Hering, S. V., Lednicky, J., Hugh Fan, Z., & Wu, C.-Y. (2016, March 1). *Efficient collection of viable virus aerosol through laminar-flow, water-based condensational particle growth*. OUP Academic. https://doi.org/10.1111/jam.13051
5. Shankar, S. N., Vass, W. B., Lednicky, J. A., Logan, T., Messcher, R. L., Eiguren-Fernandez, A., Amanatidis, S., Sabo-Attwood, T., & Wu, C. Y. (2024). The BioCascade-VIVAS system for collection and delivery of virus-laden size-fractionated airborne particles. *Journal of aerosol science*, *175*, 106263. https://doi.org/10.1016/j.jaerosci.2023.106263