



# U.S. ARMY COMBAT CAPABILITIES DEVELOPMENT COMMAND CHEMICAL BIOLOGICAL CENTER

Modified Cascade Impactor for Liquid  
Collection and Increased Sampling Time

04 OCT 2023

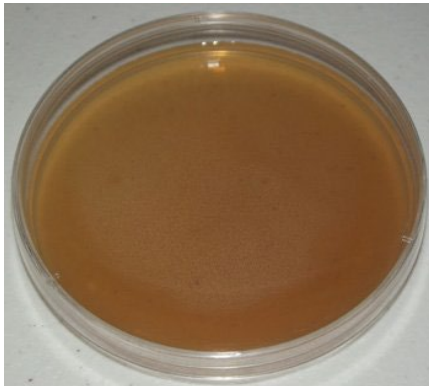
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# INTRODUCTION

Viable cascade impactors utilize aerodynamic forces to separate and collect particles on to agar plates for culture analysis.



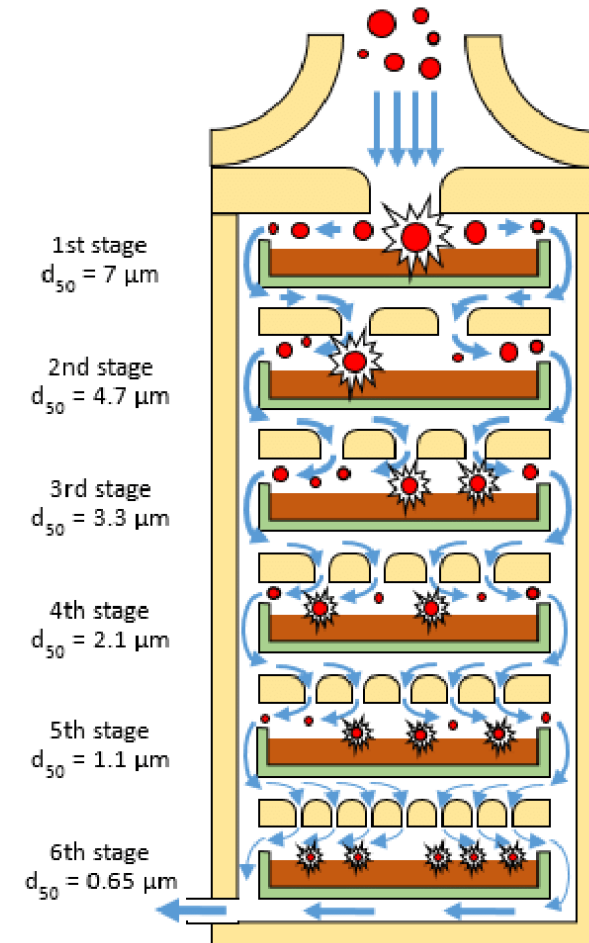
Single Stage cascade impactor



Agar filled plates are loaded into the Biological impactors



Multi-stage cascade impactor



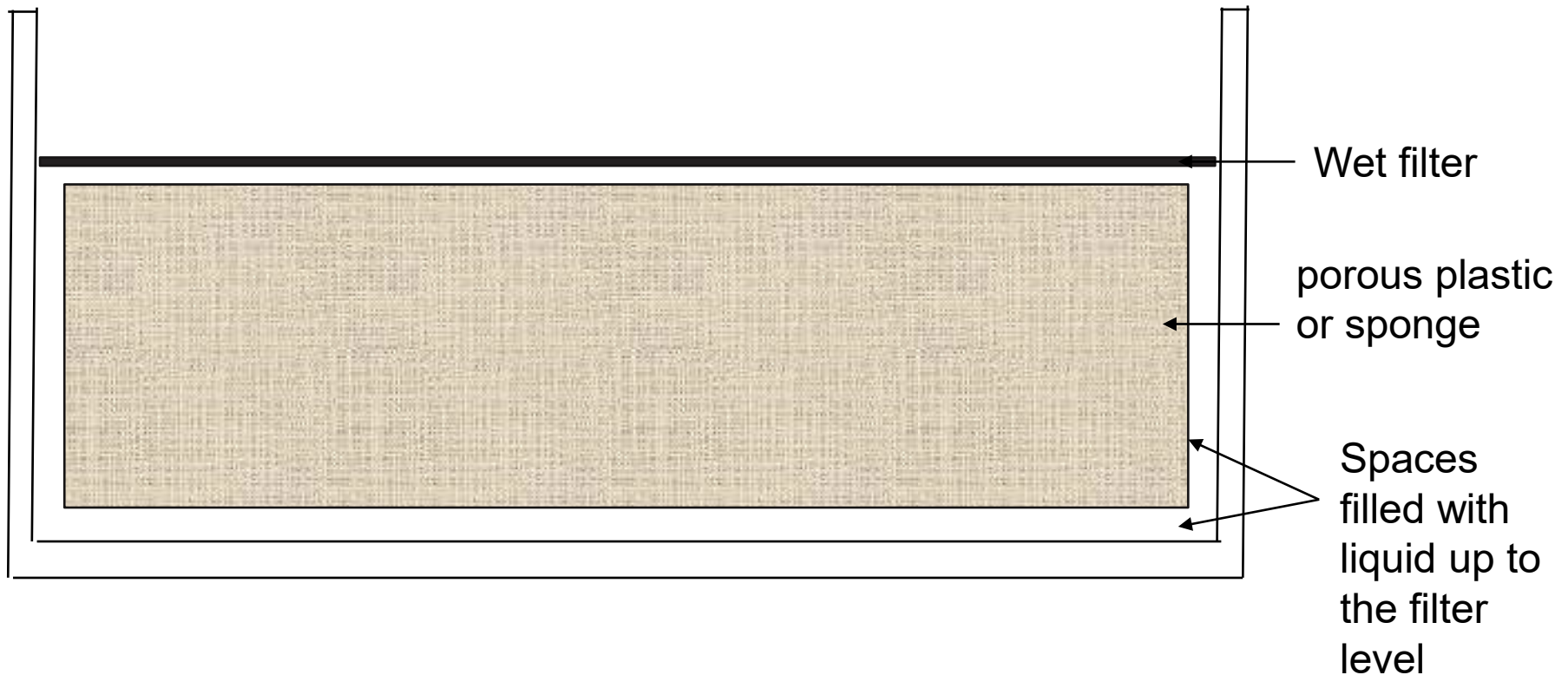
Six stage cascade impactor

# DISADVANTAGES



- Short sample time due to drying of the agar
- Only culture analysis for evaluating the samples
- Fast growing organisms will overgrow
- Cannot detect killed organisms or slow growing organisms
- Some organisms may prevent the growth of others
- Cannot use PCR or Immunoassay analysis
- In most cases, cannot determine organism concentration in air

# IMPROVED COLLECTION METHOD



Place a porous material or a sponge in the petri dish, add a collection liquid, and place a filter on top as an impaction surface.

# OBJECTIVE



- Determine sampling efficiency
- Determine the effect of filter on the collection efficiency
- Length of sampling time for a one stage impactor
- Length of sampling time for a six-stage cascade impactor

# METHODOLOGY



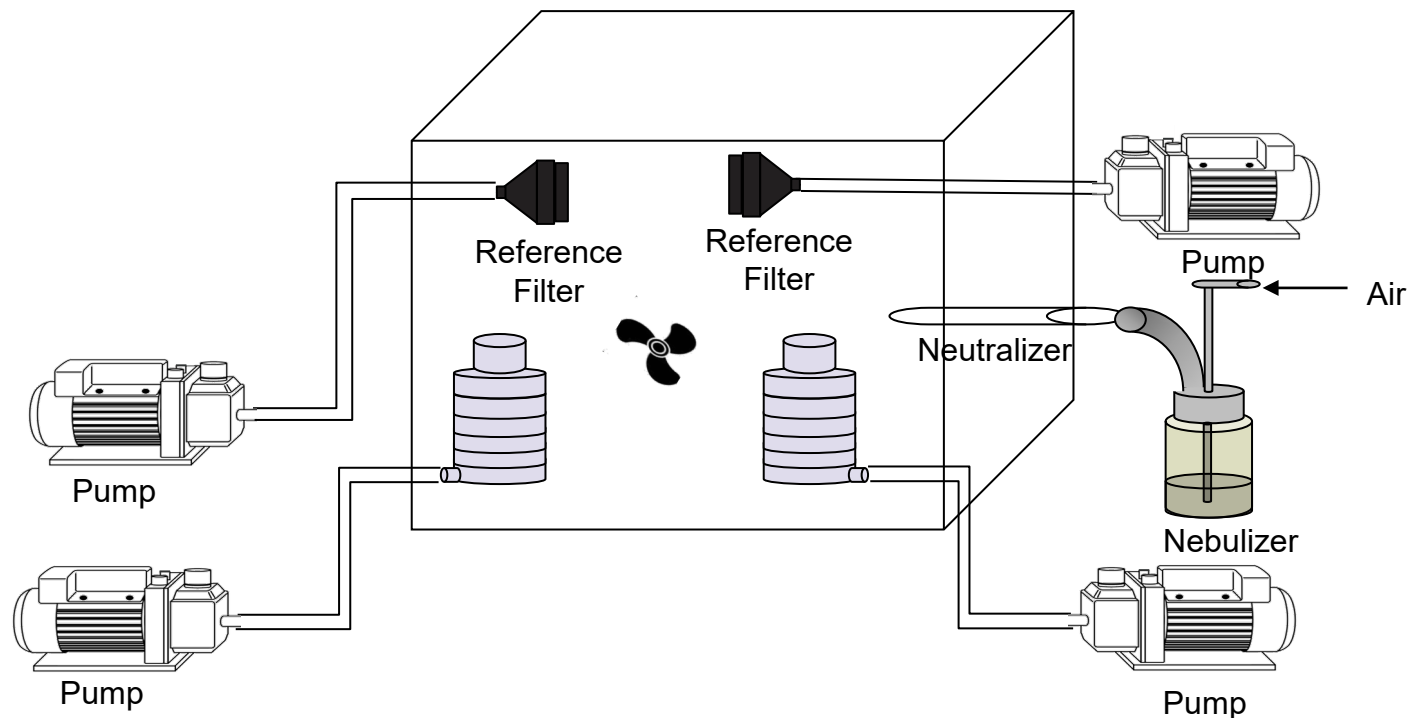
- Tisch Environmental provided Porous Polyethylene plastic disk with hydrophilic treatment. Sponge material were also used in this test.
- Polyethylene disks absorbs ~7 mL of water. A sponge absorbs ~25 mL of water.
- Only 9 mL of additional water can be added to the petri dish with a polyethylene disk without water splashing out during the operation of the cascade impactor. This gives a total of 16 mL of water in each petri dish.
- Decontamination is achieved by autoclaving at 121° C for 45 minutes for the porous disk.
- Place disks flat in the autoclave during decontamination.

# SAMPLING EFFICIENCY TEST SETUP



Sampling efficiency tests conducted with wax filled plates

- Various chambers were used in this test
- PSL: monodispersed 0.5, 1, 3, 5  $\mu\text{m}$
- Biological particles: *Bacillus atrophaeus* var. *globigii* (BG) spores and vegetative cells



# METHODOLOGY



- Polystyrene Latex (PSL) and Biological aerosols were generated into a chamber using a nebulizer or a MAD device.
- Cascade impactors and reference filters sampled the aerosol for the same amount of time.
- Filters and the remaining liquid the petri dish were removed and placed in a 50 mL centrifuge tube and the petri dish was washed 2X with 10 mL of liquid and the wash solution was added to the centrifuge tube.
- Centrifuge tube with the filter was vortexed to remove particles from the filter
- For tests with biological materials, samples were diluted, plated, incubated overnight, and CFUs were quantified.
- For tests with fluorescent PSL material, samples were quantified with a fluorometer.



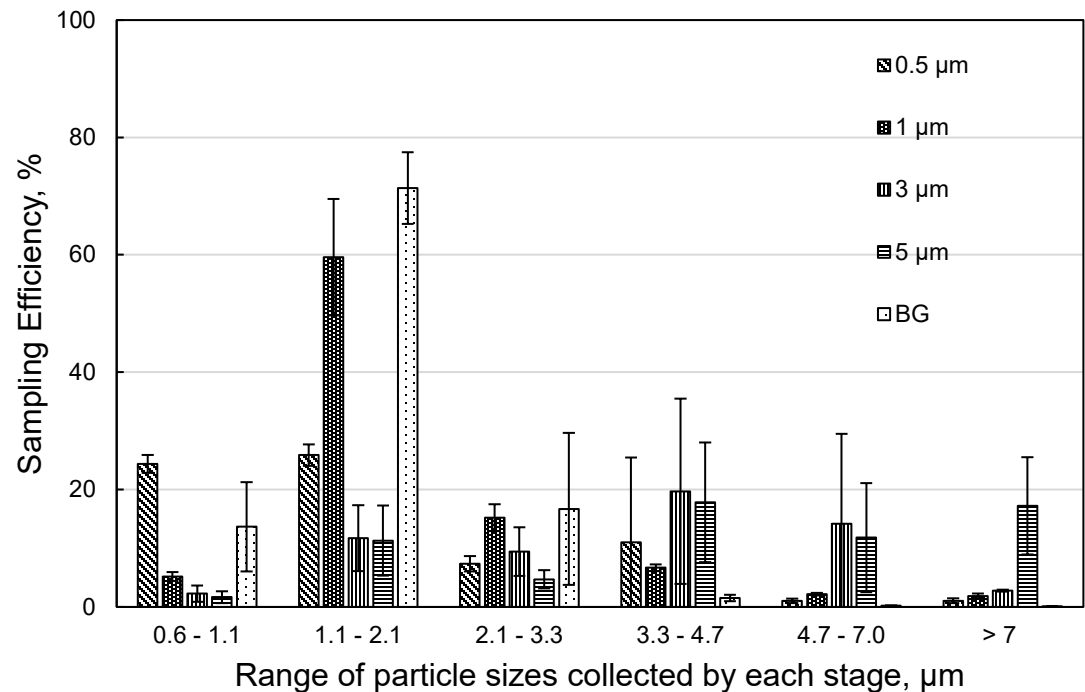
# RESULTS – SAMPLING EFFICIENCY



## Total Sampling Efficiency

Particle size	Efficiency, %
0.5 PSL	71
1 PSL	91
3 PSL	60
5 PSL	64
BG	105

## Sampling Efficiency for Each Stage

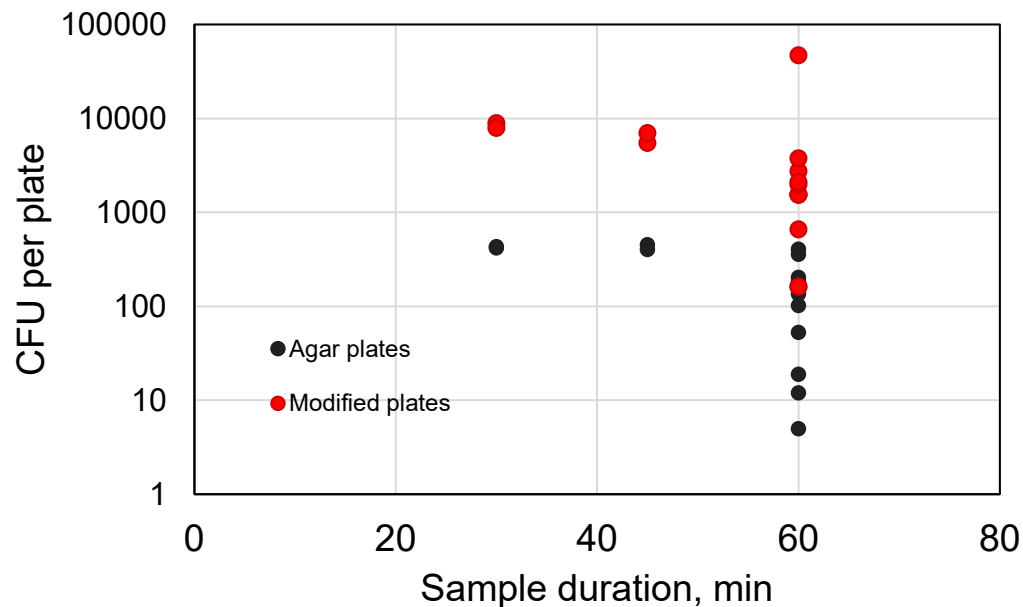


Lower efficiency may be due to particle bounce and inefficient recovery of particles from the collection surface.

# RESULTS



- Agar plate collected multiple organisms on each impactation location resulting in multiple organisms producing one cfu. Maximum of 400 impactation locations.
- The modified impactor collected the particles on filters, placed the filters into liquid, vortexed, diluted the solution, plated, and incubated overnight to quantify the CFUs.
- Clusters are separated by this process so individual organisms are counted.



# RESULTS



- Higher efficiency was observed with filter on top of the porous disk compared to no filter
- 90 mm filter (cut to fit) provided less evaporation compared to smaller filters
- For the one stage impactor, good collection for up to 60 minutes with the disk method.
- For longer sampling time liquid may need to be added as the liquid evaporates
- In the six-stage cascade impactor, liquid in the first stage evaporates faster compared to liquid in the last stage. For example, forth stage had significant amount of liquid after 2 hours of sampling. RH of the laboratory air was 23%.
- Drying of the filter will depend on the RH of the environment and the number of stages above it.

# CONCLUSION



Modified cascade impactor provides

- Long sampling duration
- Viable organisms in the sample
- Organisms are collected on a continuous wet filter.
- Sample can be divided for various analysis methods (PCR, culturing, immunoassay, etc.)
- Killed organisms can still be analyzed using PCR or immunoassay.
- Provides particle size separated sample
- Easy decontamination of material



# THANK YOU.

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