# Medical mask using a novel antimicrobial / antiviral biofilter material

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## INTRODUCTION: Statement of problem

Common Personal Protecting Equipment (PPE) are disposable, non-degradable, single use items that not only incur a high cost but are vulnerable to penetration by microorganisms.

The most common type of particulate filtering facepiece respirators are made of non-degradable non-woven polypropylene fiber and removes 95% of airborne particles (N95).

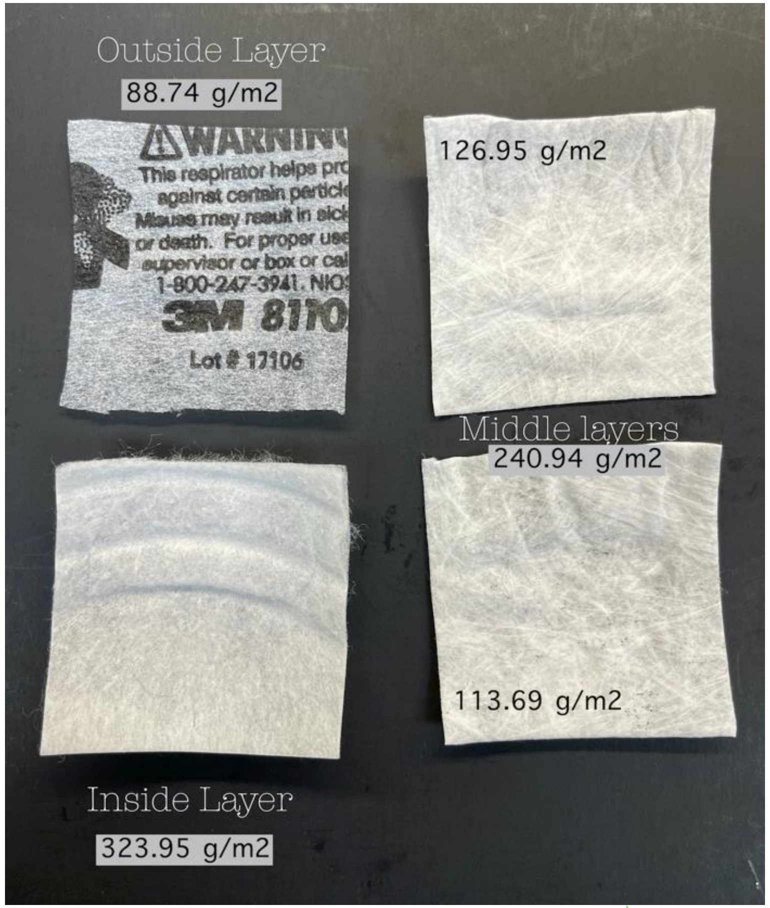
# N95 mask (example)

N95 classification of air filtration, means that it filters at least 95% of airborne particles.

N95 uses **nonwoven polypropylene** fabric.

The novel material consists of both positive and negative charges, which are able to attract particles (such as dust, bacteria and viruses) and trap at least 95% of them by polarization before the particles can pass through the mask.

The N95 **mask** is comprised of four layers of **material**: an outer layer of spun-bond **polypropylene**, a second layer of **cellulose/polyester**, a third layer of melt-blown **polypropylene** filter **material** and an inner (fourth) layer of spun-bound **polypropylene**.

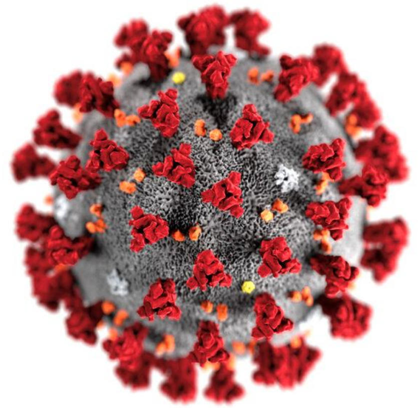



**Grammage > 600 g/m$^2$**

## MAIN GOAL

To produce a Novel **reusable nanoporous biofilter** that protects against microorganisms that have a diameter greater than 50nm, **including COVID-19, which has an approximate 125nm diameter**

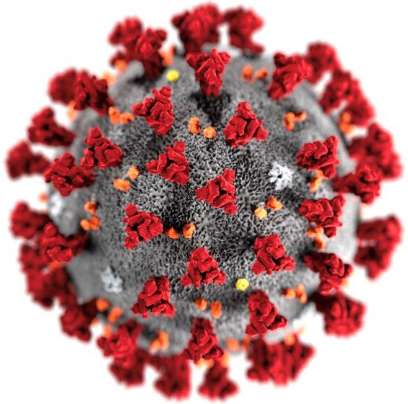
This novel biofilter will be made of biodegradable non-woven Polylactic, cellulose nanofibers (CNF) and copper nanoparticles. The biofilter will remove 99.9% of airborne particles and will have antimicrobial & antiviral properties.



## HYPOTHESIS

1) by producing bio-nanocomposite filaments (polylactic acid (PLA), PLA plus cellulose nanofibrils) with copper nanoparticles, we will reach the effectiveness of these particles towards killing 99.999% of bacteria and viruses.

2) Considering the diameter of cellulose nanofibrils are smaller than the common size of the new coronavirus (COVID-19), which is spherical with a diameter of approximately 125 nm, it is expected that the viruses can be retained in a biofilter prepared with an optimum and well distributed amount of nanocellulosic material.

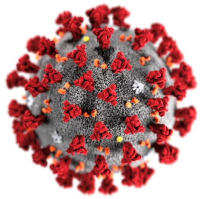


# MATERIALS AND METHODOLOGY

a) Materials [polylactic acid (PLA)*, masterbatch PLA+copper nanoparticles** , cellulose nanofibrillated (CNF)***]

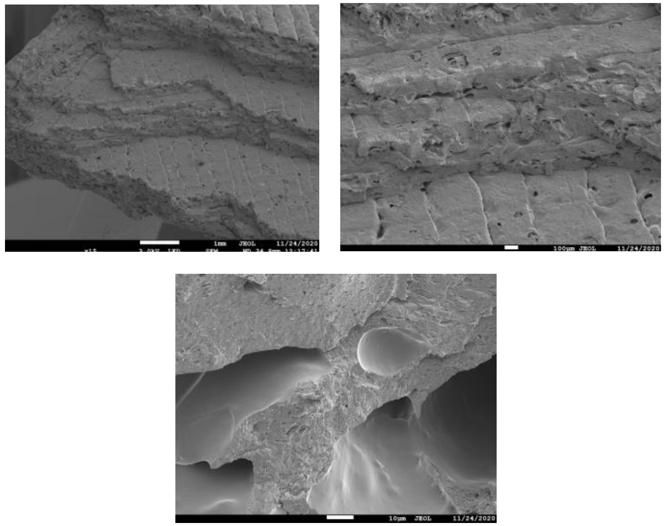
b) Methods to produce the biofilter (electrospinning, forcespinning, dead filtration)

c) Biofilter characterization (breathability, filtration (phage exposure and salts), antimicrobial properties)

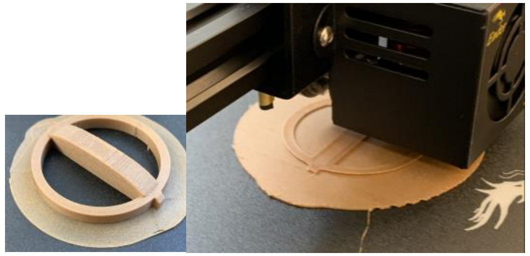


* NatureWorks (https://www.natureworksllc.com/)
** Nanotec Chile (https://www.nanotecchile.com/)
*** Forest Product Laboratory (Dr. Ronald Sabo)

# Mask prototype

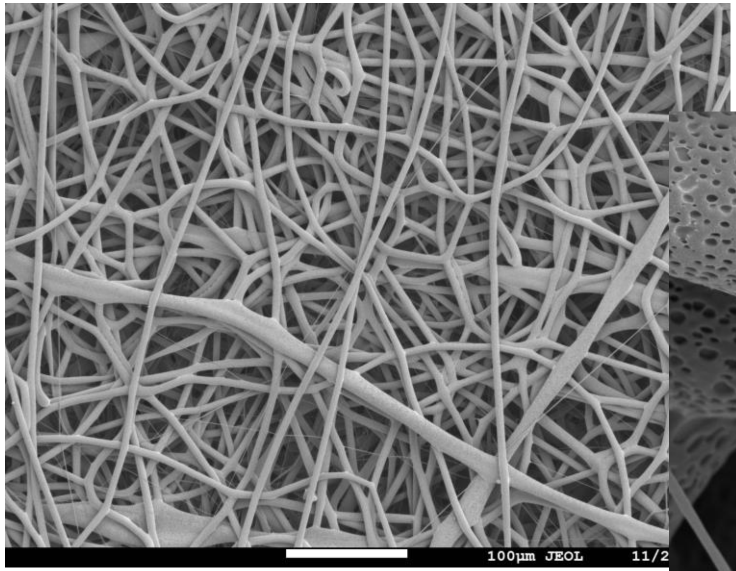
Prototype of a mask 3D printed* in Polylactic Acid (PLA)(white) and in Wood-PLA (light brown)

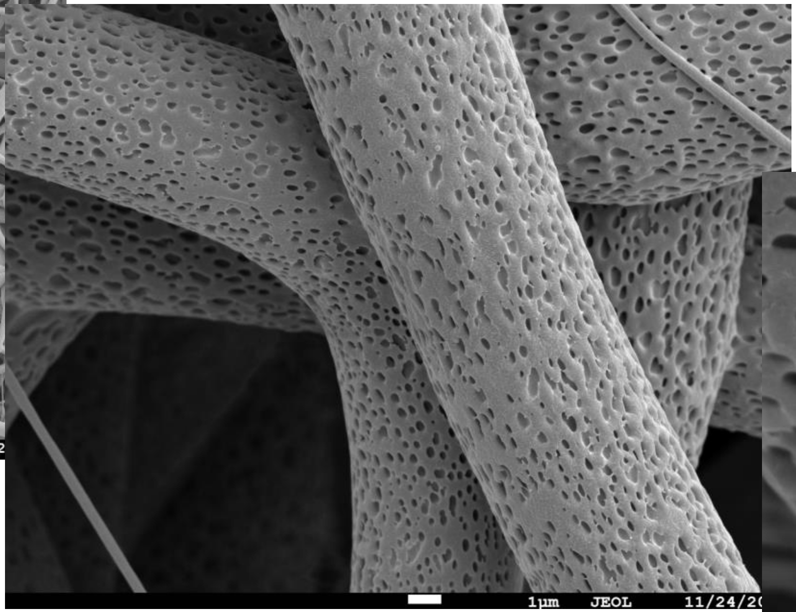


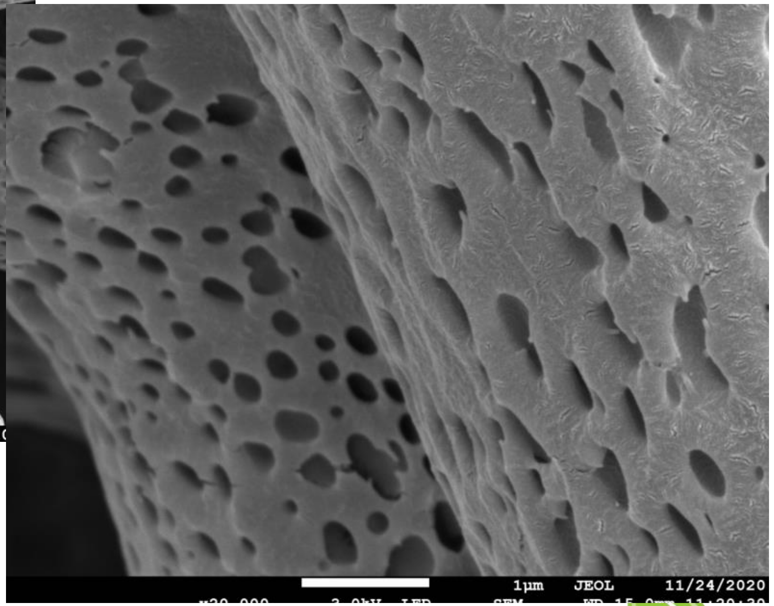
* Design provided by a Chilean company Copper3D (https://copper3d.com/)

# METHODS AND RESULTS: Producing our Biofilter

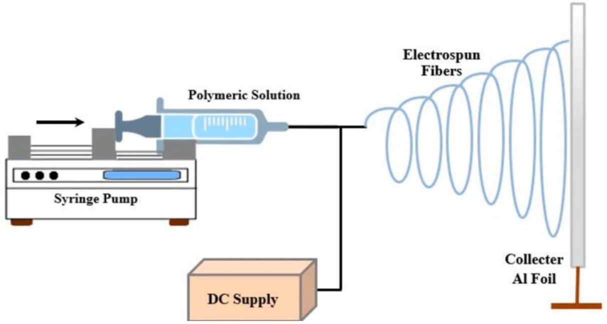
## Electrospinning (Electrostatic Forces)



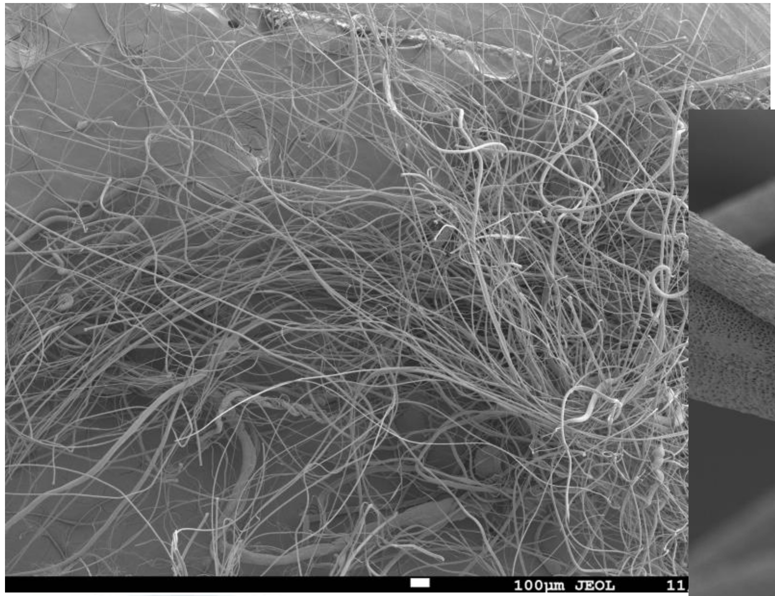



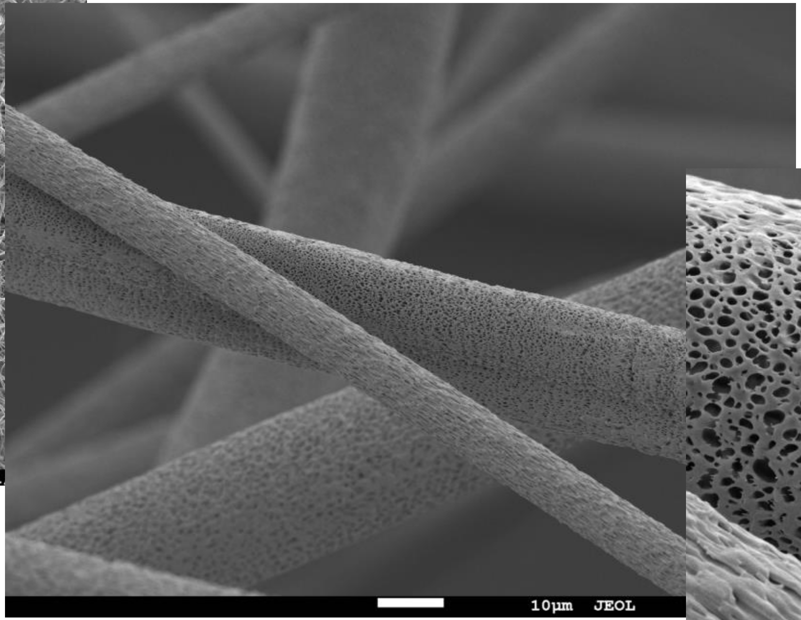

Biofilter will go here!

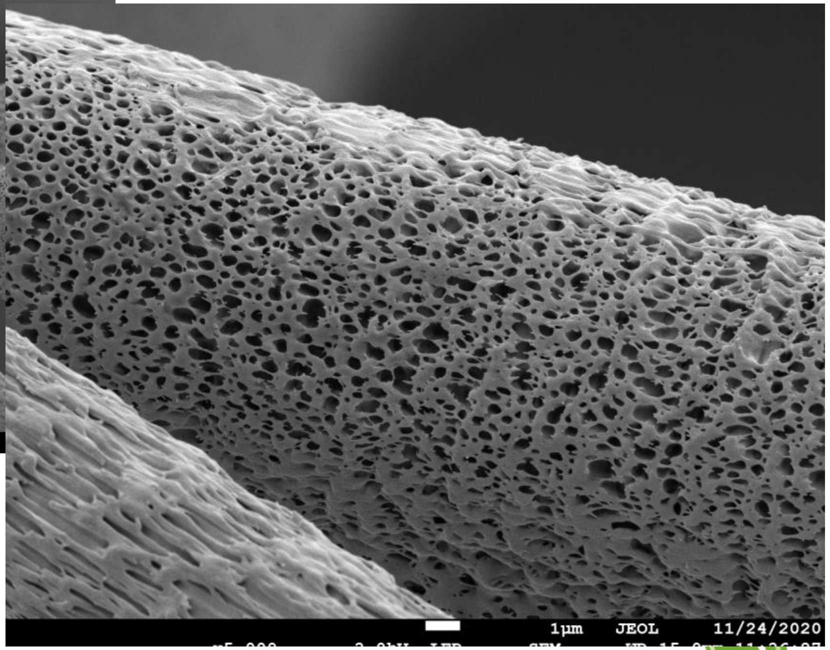


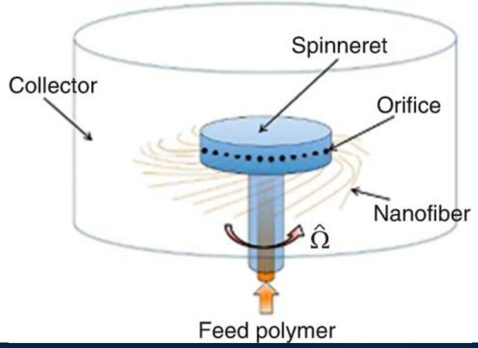
# METHODS AND RESULTS: Producing our Biofilter

## Forcespinning (Centrifugal Forces)



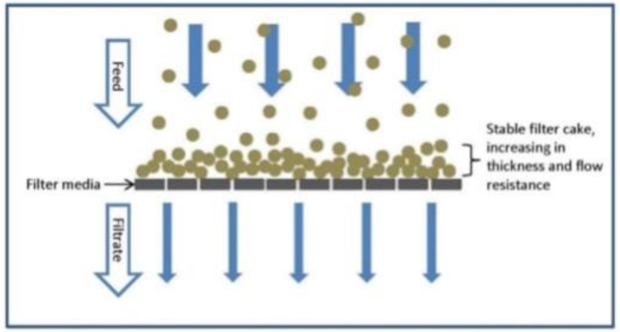



# METHODS AND RESULTS: Producing our Biofilter

**Dead end filtration**

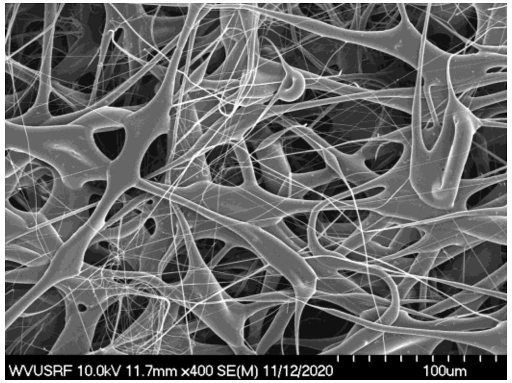


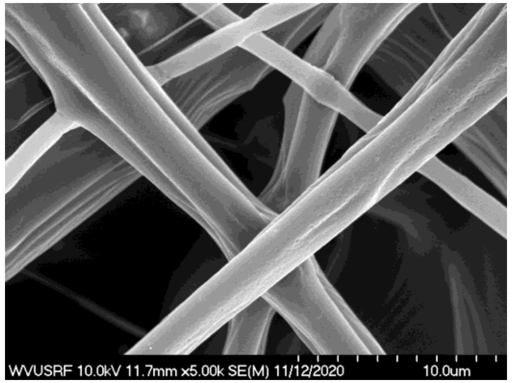

Biofilter will go here!

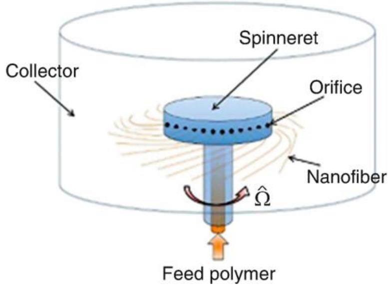
Combinations of hardwood kraft pulp and nanocellulose (NFC and TNFC)

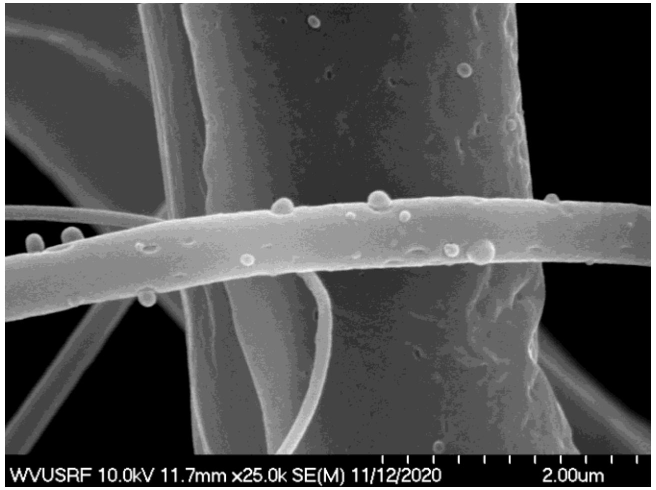
**Grammage: 100, 300, 600 g/m2**

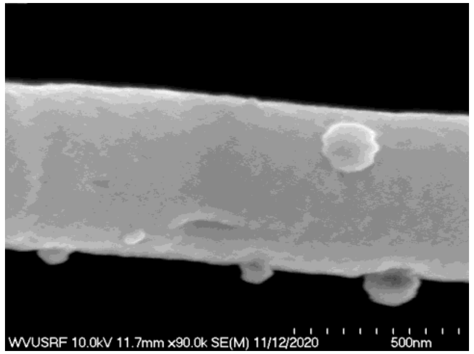
# METHODS AND RESULTS: Producing our Biofilter with copper nanoparticles

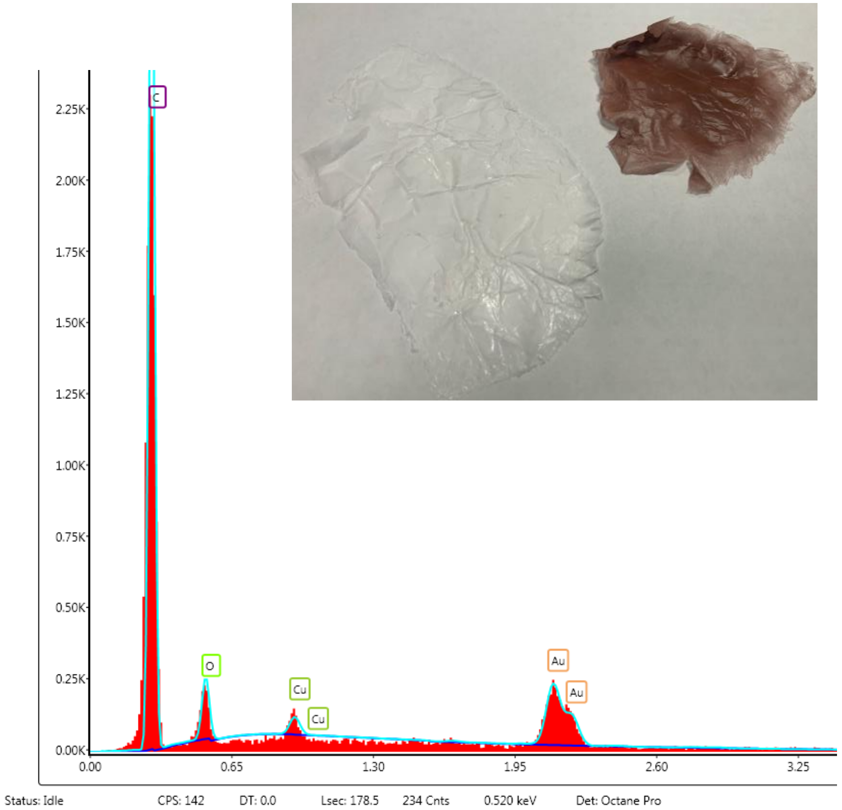



* PLA-Cu Masterbatch from NANOTEC CHILE (https://www.nanotecchile.com/)

# METHODS AND RESULTS: Biofilter production / Forcespinning (examples)

0.3 g

0.5 g

1 g

Grammage: 150 - 600 g/m2

# METHODS AND RESULTS: Biofilter production / Forcespinning (examples)

**Thickness after pressing:**

- **0.5 g (600 g/m²): 1.2 ± 0.05 mm**
- **0.25 g (300 g/m²): ~0.65 ± 0.05 mm**

# METHODS AND RESULTS: Biofilter production / Forcespinning (examples)

**PLA Fibers with copper nanoparticles - Masterbatch**

- 3 wt.% in DCM.
- Rate: 5.1 mL/min.
- Samples: **1-5 (300 g/m$^2$); 6-10 (600 g/m$^2$).**
- Thickness: **1.2 ±0.05 mm; 0.65 ±0.05 mm.**
- Std deviation in fiber wt.: **2 mg (< 1%).**
- Force: 0.25 metric tons.

# METHODS AND RESULTS: Biofilter characterization

1) Filtration efficacy test (ASTM F 1671/ISO16604)

Using phi-X174 Bacteriophage Penetration as Test System

2) Filtration efficiency (salt solution) based on OSHA 1910.134 App A

https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_id=9780&p_table=STANDARDS

3) Breathability test – Pressure drop test (~ASTM F 1862)

ASTM F1862/F1862M-17 Standard Test Method for Resistance of Medical Face Masks to Penetration by Synthetic Blood (Horizontal Projection of Fixed Volume at a Known Velocity).

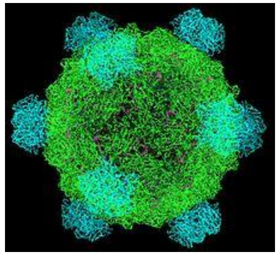
4) Antimicrobial properties according to EPA standards

*Staphylococcus aureus* (ATCC strain 6538, Gram positive opportunist)

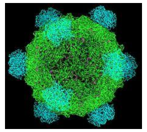
# METHODS AND RESULTS: Biofilter characterization

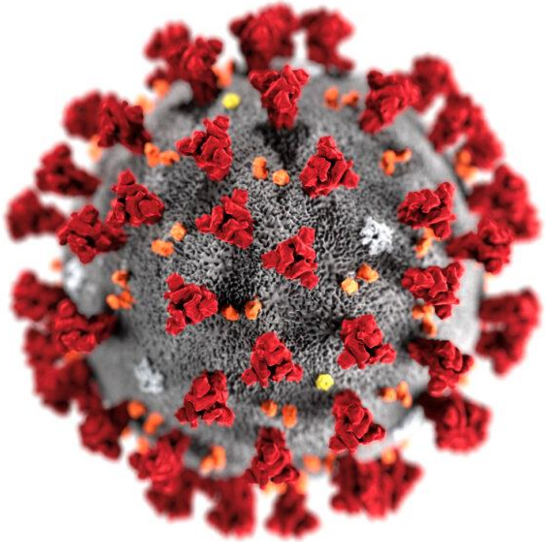
## 1) Filtration efficacy test (ASTM F 1671/ISO16604)

*phi-X174 Bacteriophage Penetration as Test System*

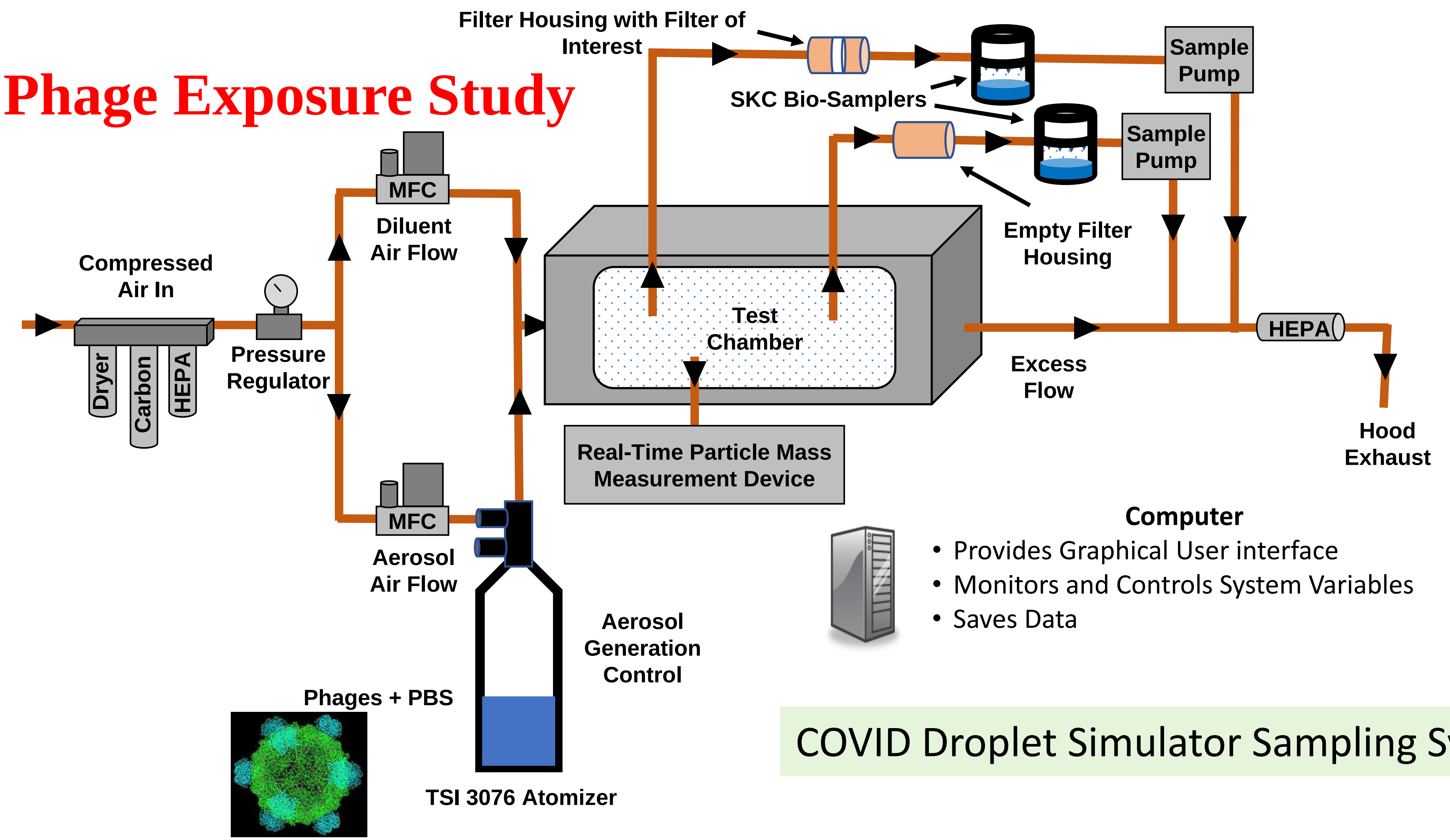


PHI X174 bacteriophage with envelope (similar to the COVID structure). It is a well-established ASTM standardized test; however, the size of the bacteriophage with the envelope is ~25 nm, which is 1/5$^{th}$ of the size of the actual COVID virus, that is 125 nm with envelopes (spikes)





# 1) Filtration efficacy test (ASTM F 1671/ISO16604)

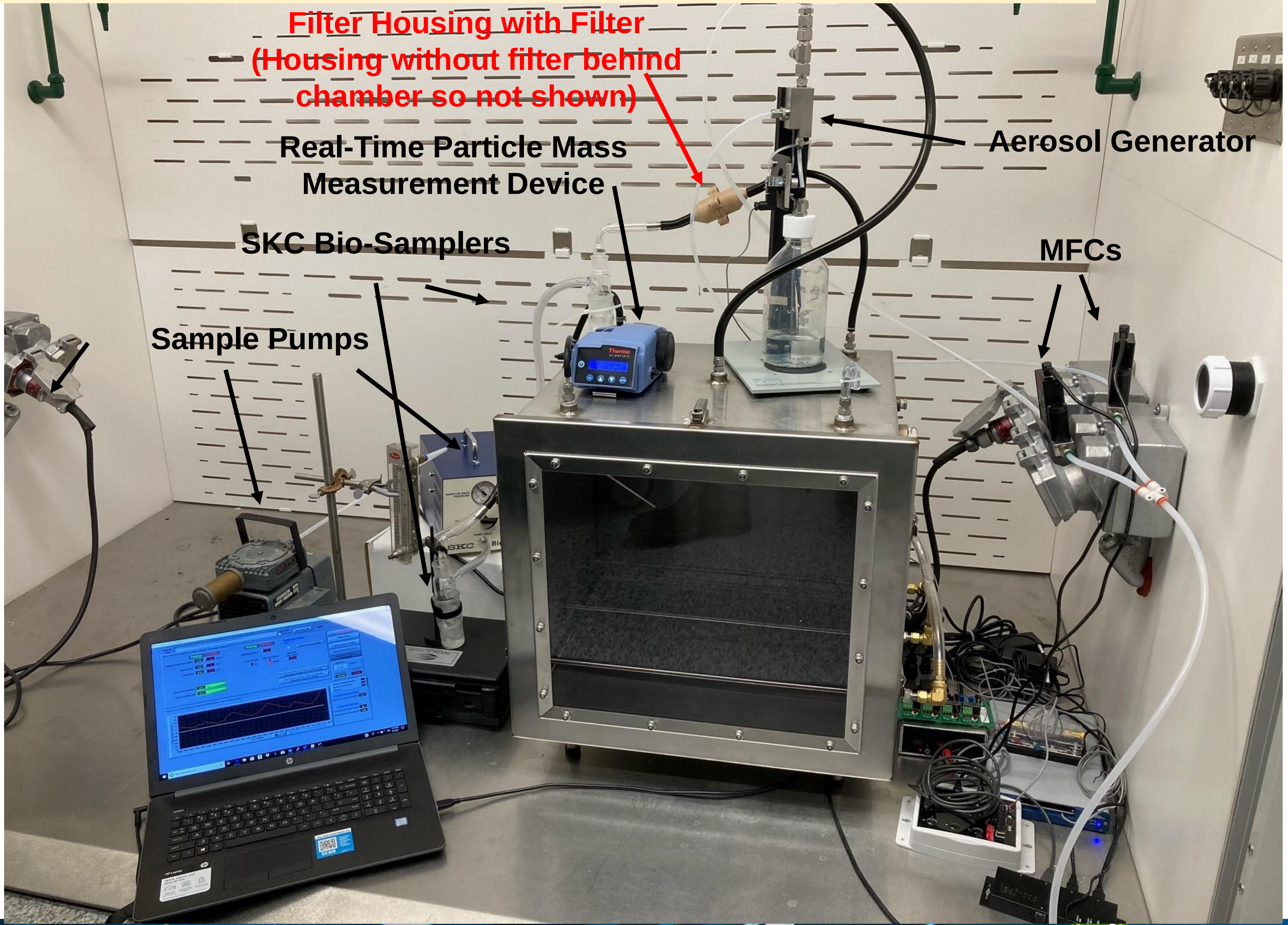



COVID Droplet Simulator Sampling System

# 1) Filtration efficacy test (ASTM F 1671/ISO16604)

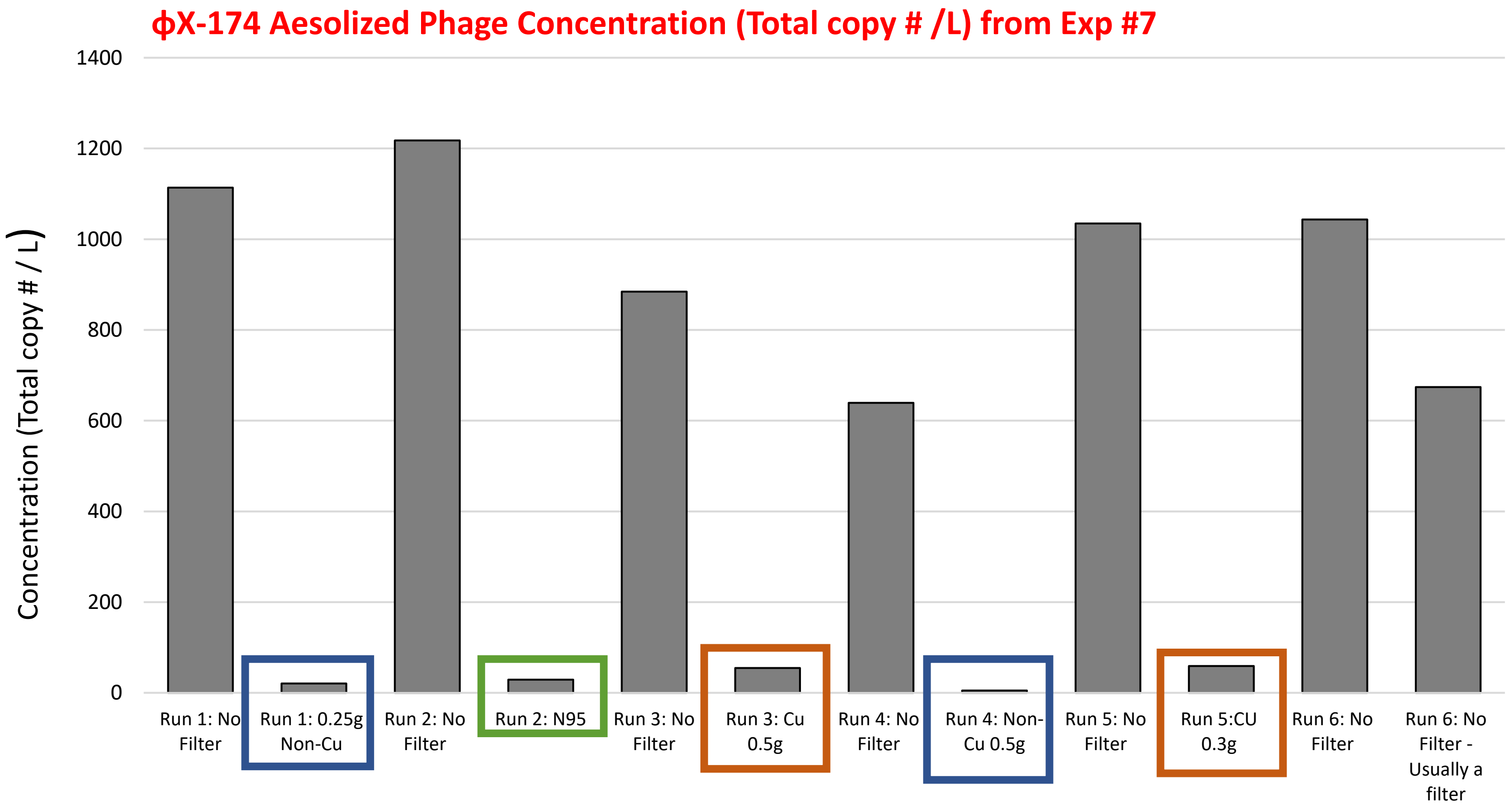
COVID Droplet Simulator Sampling System

**Filtration test using the phage**



# 1) Filtration efficacy test (ASTM F 1671/ISO16604)

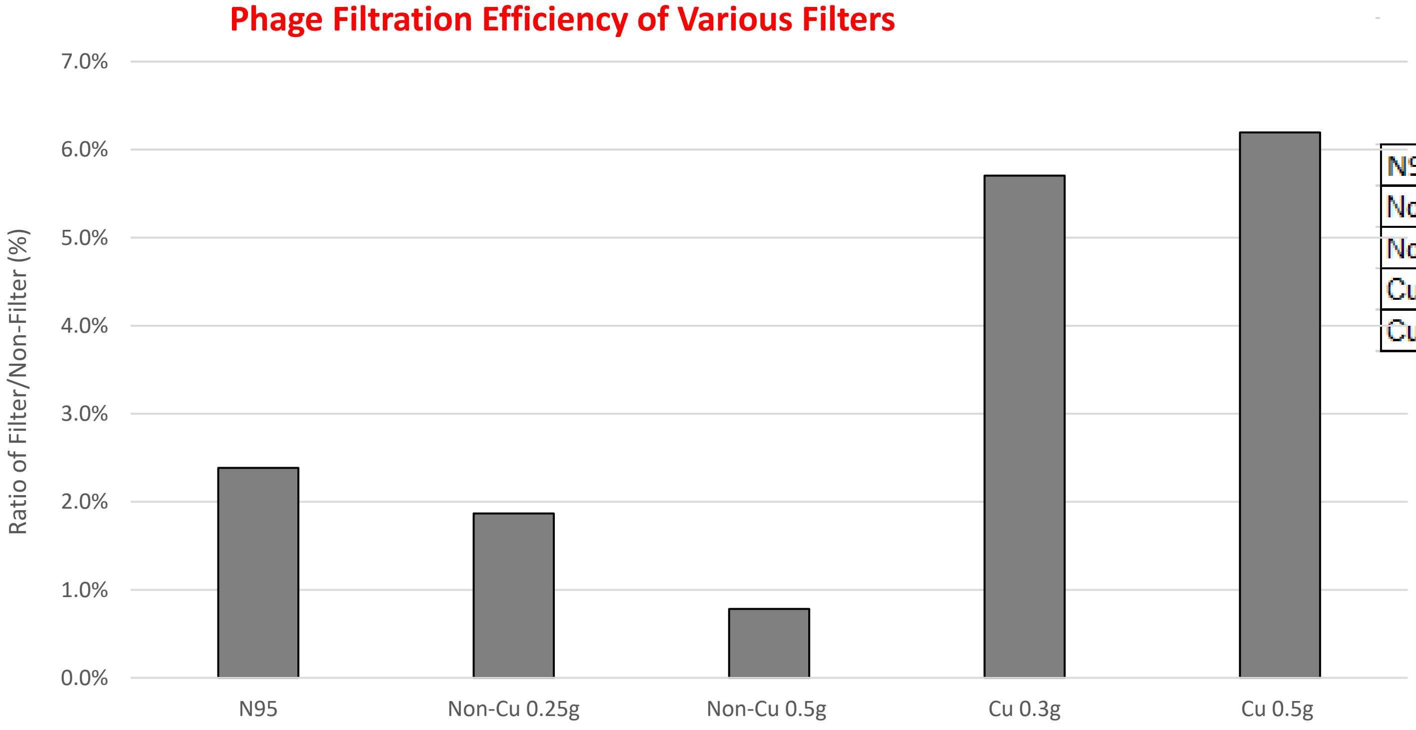
## RESULTS: Forcespinning biofilter




**Filtration Efficiency – Phage Exposure**

# 1) Filtration efficacy test (ASTM F 1671/ISO16604)

## RESULTS: Forcespinning biofilter




| | Ratio of Filter/Non-Filter |
|---|---|
| N95 | 2.4% |
| Non-Cu 0.25g | 1.9% |
| Non-Cu 0.5g | 0.8% |
| Cu 0.3g | 5.7% |
| Cu 0.5g | 6.2% |

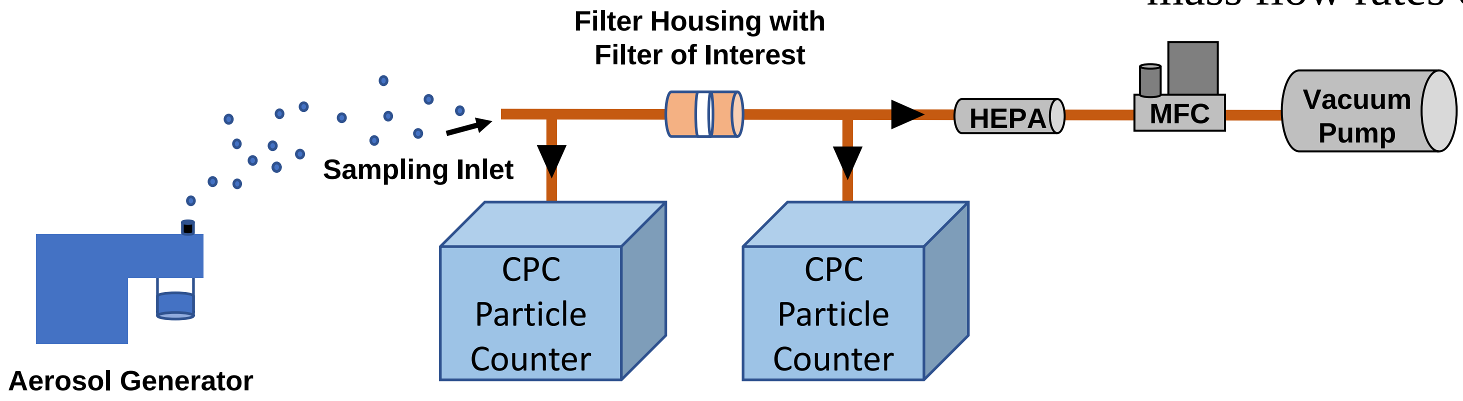
Lower values are best!

**Filtration Efficiency – Phage Exposure**

# 2. Filtration efficiency test (salt solution)

## Filter Efficiency Testing System

mass flow rates of 1, 5 and 10 LPM

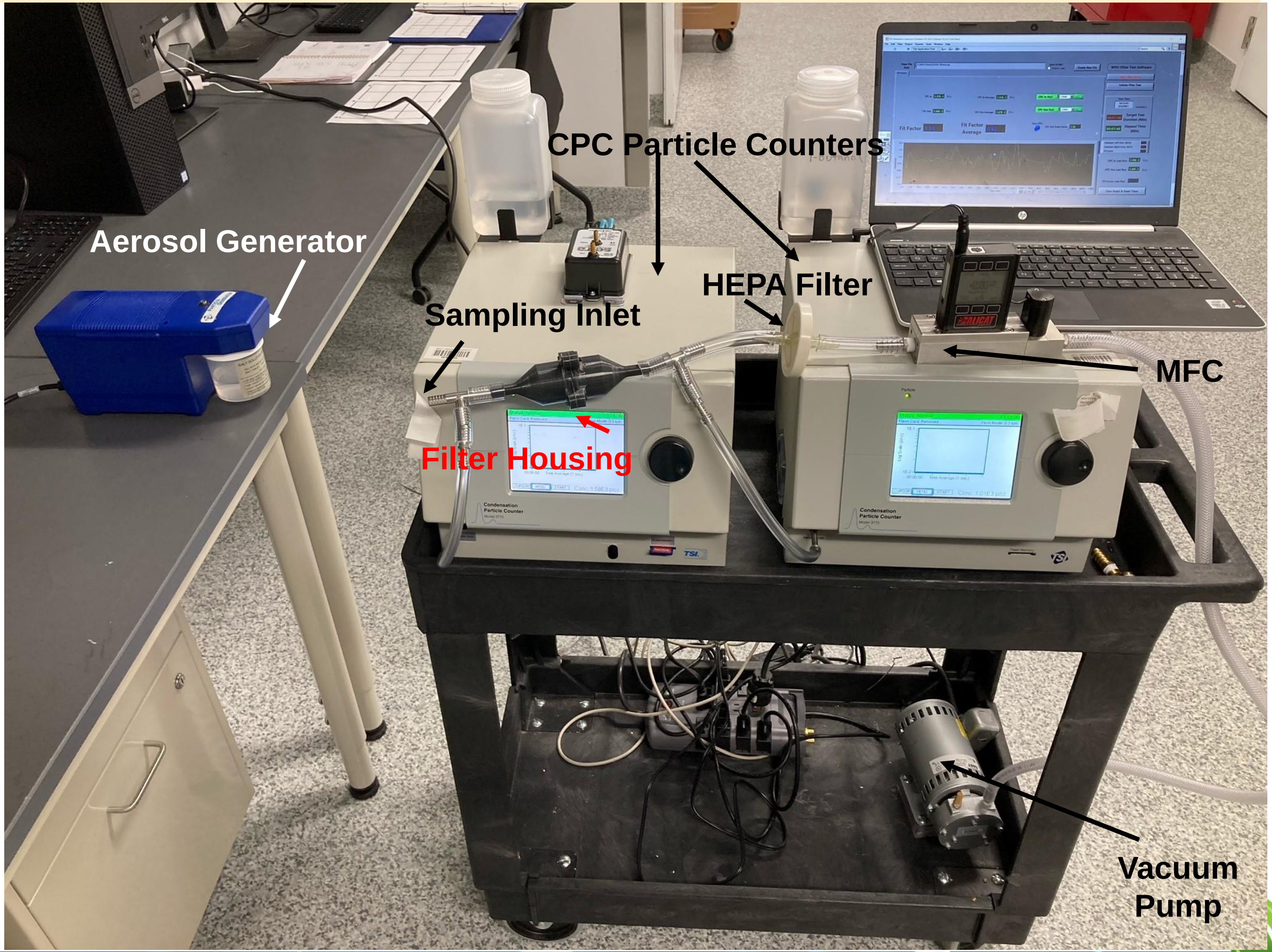



NaCl aerosol generator (Model 8026, TSI) was used to add supplemental particles to those naturally occurring particles in the air.

(MFC: mass flow controller )

# 2. Filtration efficiency test (salt solution)

**Filter Efficiency Testing System**



# 2. Filtration efficiency test (salt solution)

## RESULTS: Forcespinning biofilter

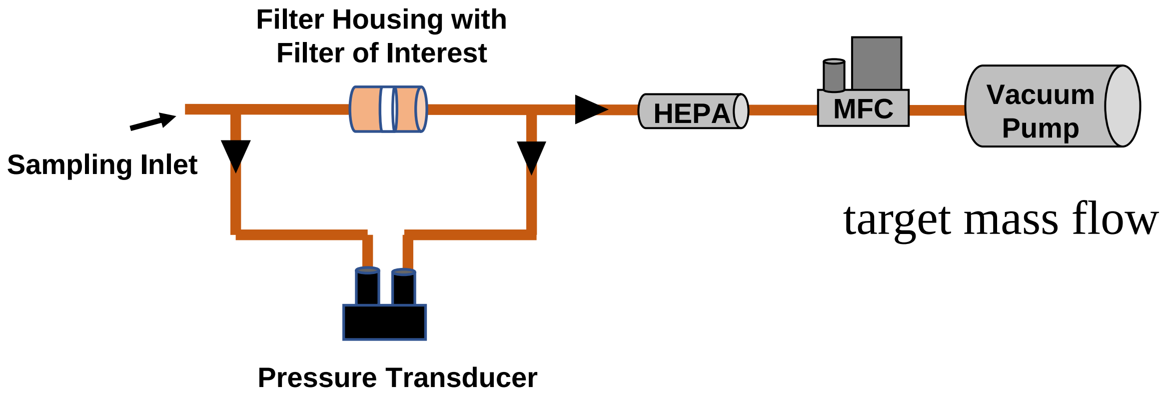
**Filter Efficiency (salt)**

| Filter | Percentage of Particle Penetration |
|---|---|
| None | 91%<br>98%<br>96% |
| Biofilter without copper nanoparticles (300 g/m2) | 11%<br>24%<br>27% |
| Biofilter with copper nanoparticles (300 g/m2) | 5%<br>13%<br>21% |
| Biofilter without copper nanoparticles (600 g/m2) | 1%<br>4%<br>4% |
| Biofilter with copper nanoparticles (600 g/m2) | 12%<br>25%<br>25% |
| N95 | 2%<br>9%<br>15% |

# 3. Breathability test – Pressure drop test

## Filter Resistance Testing System

In order to measure the resistance of various filters, air was pulled across filter material at a constant flow rate.

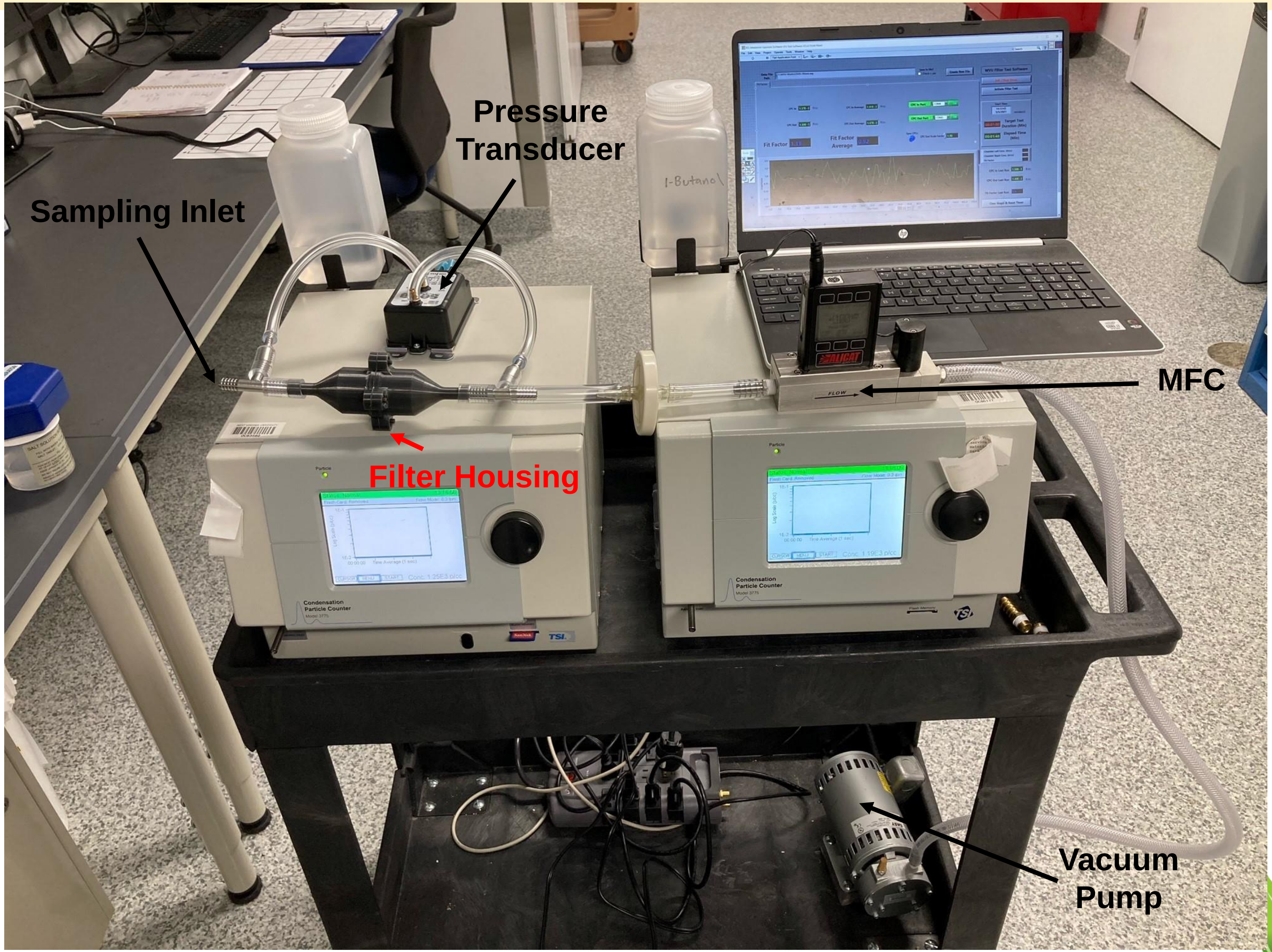



target mass flow rates of 1, 5 and 10 LPM

(MFC: mass flow controller )

# 3. Breathability test – Pressure drop test

Filter Resistance
Testing System



# 3. Breathability test – Pressure drop test

## RESULTS: Forcespinning biofilter

## Filter Resistance Testing System

| Sample ID | Test # | Volumetric flow (LPM) | Pressure ("H2O) |
|---|---|---|---|
| N95 | 1 | 1.032 | 0.15 |
| | 2 | 5.282 | 0.729 |
| | 3 | 10.571 | 1.503 |
| Forcespinning PLA Filter (300 mg/m2) | 1 | 1.055 | 0.522 |
| | 2 | 5.282 | 2.4 |
| | 3 | 10.585 | 4.83 |
| Forcespinning PLA Filter w/copper NP (300 mg/m2) | 1 | 1.046 | 0.612 |
| | 2 | 5.266 | 2.814 |
| | 3 | 10.58 | 5.661 |

# 3. Breathability test – Pressure drop test

| Sample ID | Test # | Pressure ("H2O) |
|---|---|---|
| N95 (Higher than 600 mg/m2) | 1 | 0.037 |
| | 2 | 0.037 |
| | 3 | 0.037 |
| Electrospinning PLA Filter (100 mg/m2) | 1 | 0.09 |
| | 2 | 0.09 |
| | 3 | 0.09 |
| Electrospinning PLA Filter w/ copper NP (100 mg/m2) | 1 | 0.136 |
| | 2 | 0.137 |
| | 3 | 0.137 |
| Electrospinning PLA Filter (200 mg/m2) | 1 | 0.155 |
| | 2 | 0.154 |
| | 3 | 0.154 |
| Electrospinning PLA Filter w/ copper NP (200 mg/m2) | 1 | 0.179 |
| | 2 | 0.179 |
| | 3 | 0.179 |
| Dead-End Filtration (Control) Hardwood pulp (300 mg/m2) | 1 | 0.155 |
| | 2 | 0.157 |
| | 3 | 0.157 |
| Dead-End Filtration Hardwood pulp + CNF lower conc (300 mg/m2) | 1 | 0.143 |
| | 2 | 0.144 |
| | 3 | 0.144 |
| Dead-End Filtration Hardwood pulp + CNF (300 mg/m2) | 1 | 0.845 |
| | 2 | 0.847 |
| | 3 | 0.847 |
| Dead-End Filtration Hardwood pulp + CNF higher conc (300 mg/m2) | 1 | 6.019 |
| | 2 | 6.093 |
| | 3 | 6.109 |

**Filter Resistance Testing System**

At a flow of 0.3 LPM

# METHODS

Organisms used:

*Staphylococcus aureus* (ATCC strain 6538, Gram positive opportunist)

~~*Pseudomonas aeruginosa* (ATCC strain 15442, Gram negative opportunist)~~

4) Antimicrobial properties according to EPA standards

Materials:

Trypticase Soy Broth (5 ml tubes); Trypticase Soy Agar (in 100 mm plates)

Equipment:

UV/VIS Spectrophotometer; Autoplater; Plate scanner with analysis software

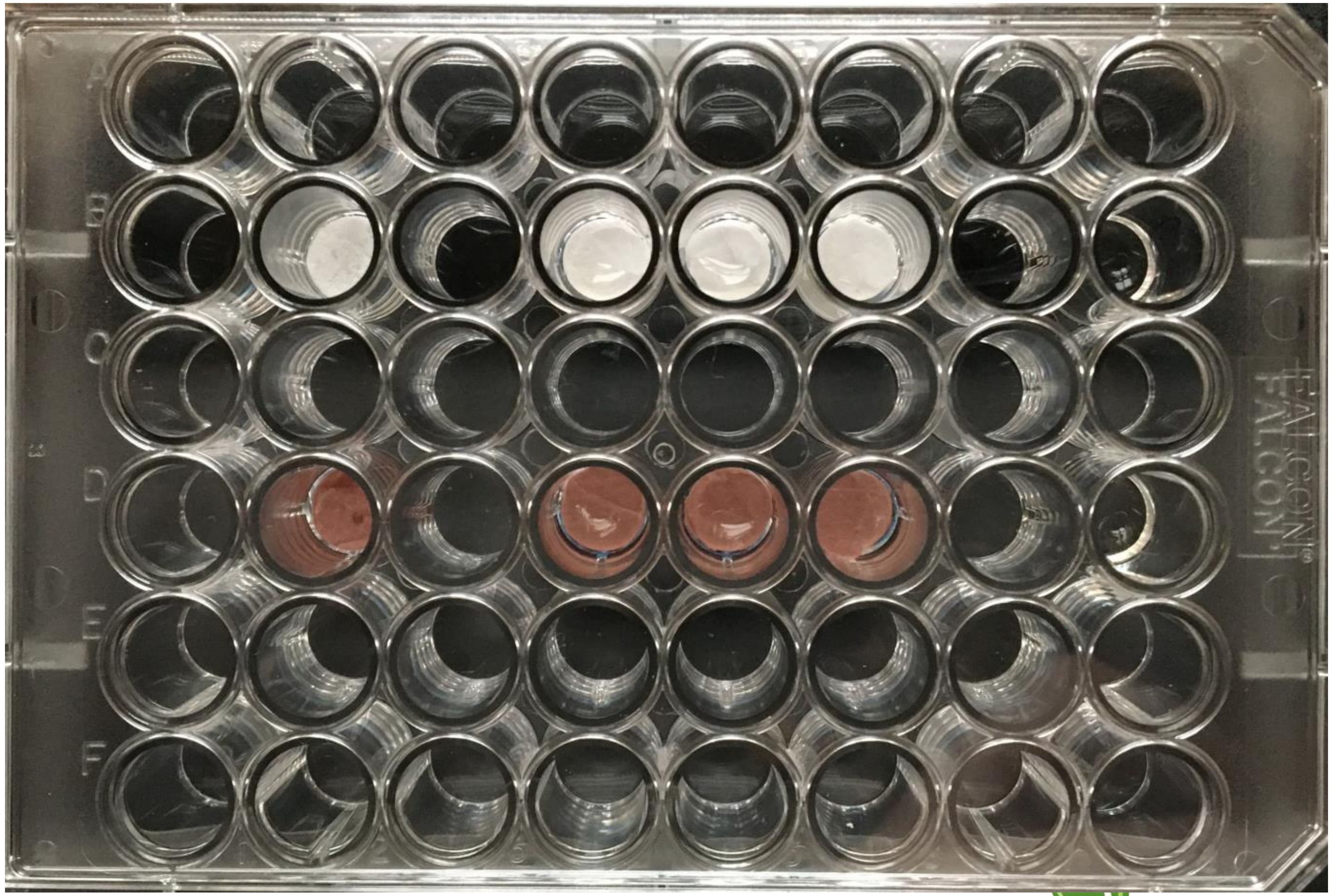
# 4. Antimicrobial properties

## METHODOLOGY

- Samples were sterilized using 254 nm radiation for 5 min and placed in wells of a sterile 48-well TC plate
- Samples were inoculated with $1x10^3$ CFU of *Staphylococcus aureus* ATCC 6538 grown to mid-log phase in 100 µl of TSB and plate is incubated at 37˚C for 16-20 hr. Controls include samples w/ no bacteria, TSB alone and TSB w/ bacteria, in separate wells
- TS broth containing growth was recovered from all wells and following serial dilution; appropriate dilutions were plated in triplicates on 100 mm Trypticase Soy Agar (TSA) plates employing an autoplater
- Growth (Colony forming units - CFU) were counted employing a plate scanner and associated software

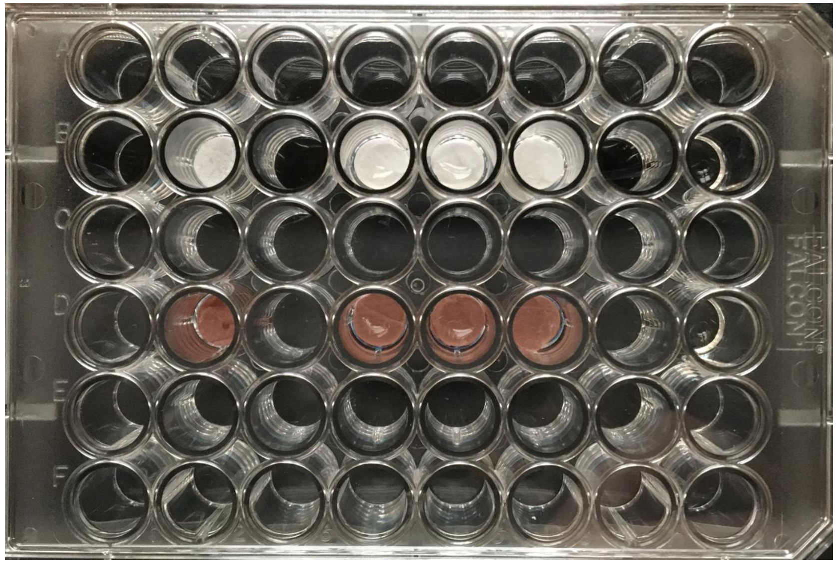
## RESULTS: Electrospinning samples (w/ and wo copper) in 48-well plate

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| A | | | | | | | | |
| B | | 95g/ $m^2$ | | 144g/ $m^2$ | 133g/ $m^2$ | 128g/ $m^2$ | | TSB no bacteria |
| C | | | | | | | | |
| D | | 94g/ $m^2$ | | 120g/ $m^2$ | 117g/ $m^2$ | 141g/ $m^2$ | | TSB with bacteria |
| E | | | | | | | | |
| F | | | | | | | | |



# RESULTS: Biofilter characterization

## Electrospinning samples (w/ and wo copper) in 48-well plate




| Material ID | inoculum (CFU *S. aureus*) | volume inoculated (μl/well) | CFU recovered |
|---|---|---|---|
| B2 - 95 g/m2 | 0 | 100 | 0 |
| B4 - 144 g/m2 | 1.00E+03 | 100 | 1.63E+07 |
| B5 - 133 g/m2 | 1.00E+03 | 100 | 1.10E+07 |
| B6 - 128 g/m2 | 1.00E+03 | 100 | 6.63E+06 |
| D2 - 94 g/$m^2$ | 0 | 100 | 0 |
| D4 - 120 g/$m^2$ | 1.00E+03 | 100 | 0 |
| D5 - 117g/$m^2$ | 1.00E+03 | 100 | 0 |
| D6 - 141 g/$m^2$ | 1.00E+03 | 100 | 0 |
| B8 | 0 | 100 | 0 |
| D8 | 1.00E+03 | 100 | 1.14E+07 |

**CFU: colony forming unit**

# CONCLUSIONS

- A promising biofilter has been developed using a forcespinning, electrospinning and a dead-end filtration process that include less material per unit, and promising properties in terms of filtration capability, breathability and antimicrobial properties.

- So far, an electrospinning process demonstrated the best properties in terms of breathability, filtration capability, and antimicrobial properties compared to forcespinning and dead-end filtration processes most probably due to the diameter of the fibers produced.

# Acknowledgments

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National Institute of Food and Agriculture

NSF



TAPPI NANO DIVISION