Baker

The effectiveness of HEPA filters to capture DNA

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Presentation agenda

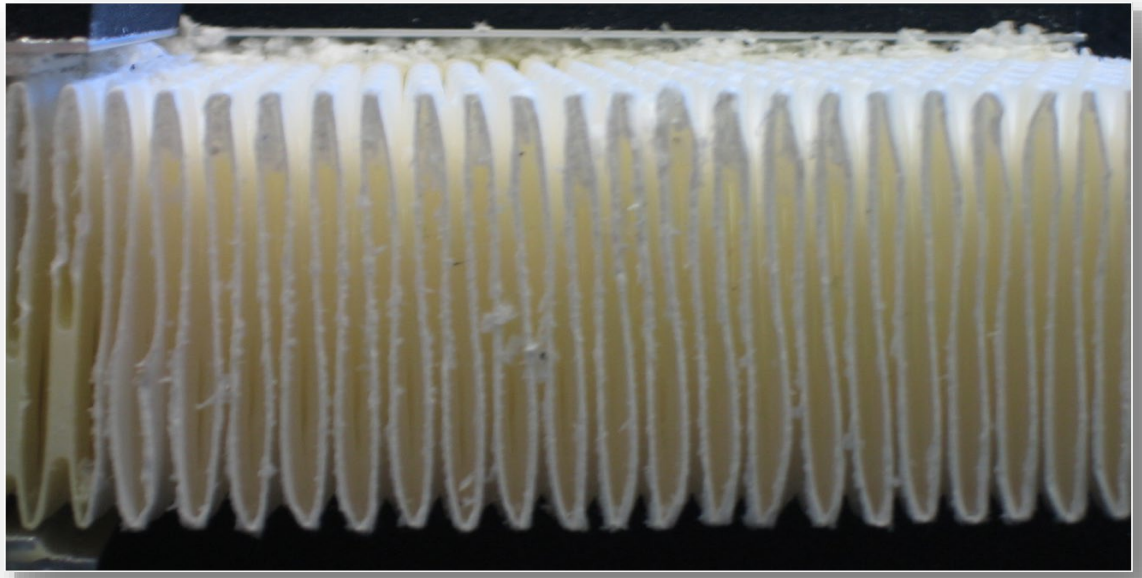
- Types of BSCs and HEPA filters
- Why do we wonder about DNA?
- Experimental Design
- Results
- Conclusions
- Recommendations

Biosafety Cabinets (BSCs)

- A ventilated enclosure for work with biohazard agents assigned to biosafety levels 1 through 4, as per the BMBL.
- Provides 3 types of **CONTAINMENT**:
  - Personnel protection
  - Product protection
  - Environmental protection

HEPA filtration

- **H**igh **E**fficiency **P**articulate **A**ir
  - Filter out 99.97% (or better) of 0.3 µm mass median particles of DOP (Dispersed Oil Particulate)
  - **CANNOT filter out gasses and vapors!**



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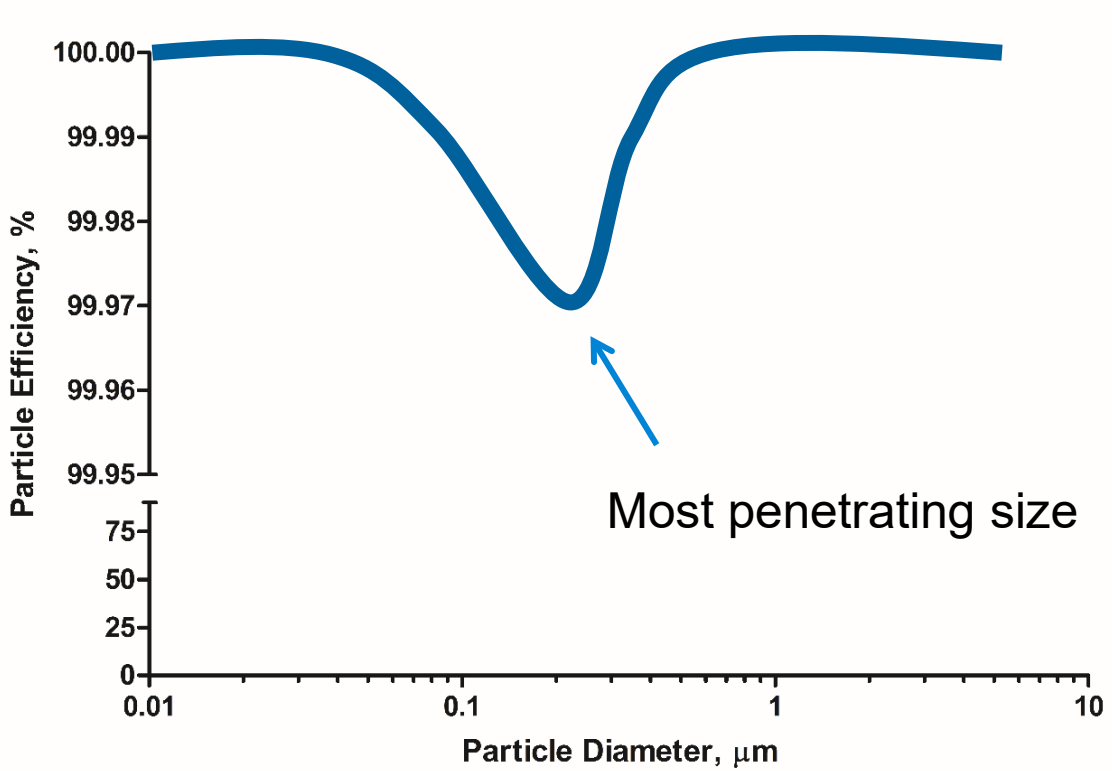
HEPA filtration

- The **worst** filtration occurs at the “most penetrating size” which is 0.22µm. Smaller and larger particles are captured **better**.



HEPA filtration in a BSC

NSF International Standard 49 requires HEPA filters used in BSCs to be a minimum of **99.99% effective** when tested with 0.3µm particulates.

Biosafety Cabinet Classification

Class I

Personnel and Environmental Protection

Class II

Personnel, Product, and Environmental Protection

Class III

For highly infectious agents

Gas-tight, physical barrier

Type A1

Recirculated

Minimum 75 fpm intake velocity

Type A2

Recirculated

Minimum 100 fpm intake

Type B1

Partial recirculation

Minimum 100 fpm intake

Type B2

100% Exhaust

Minimum 100 fpm intake

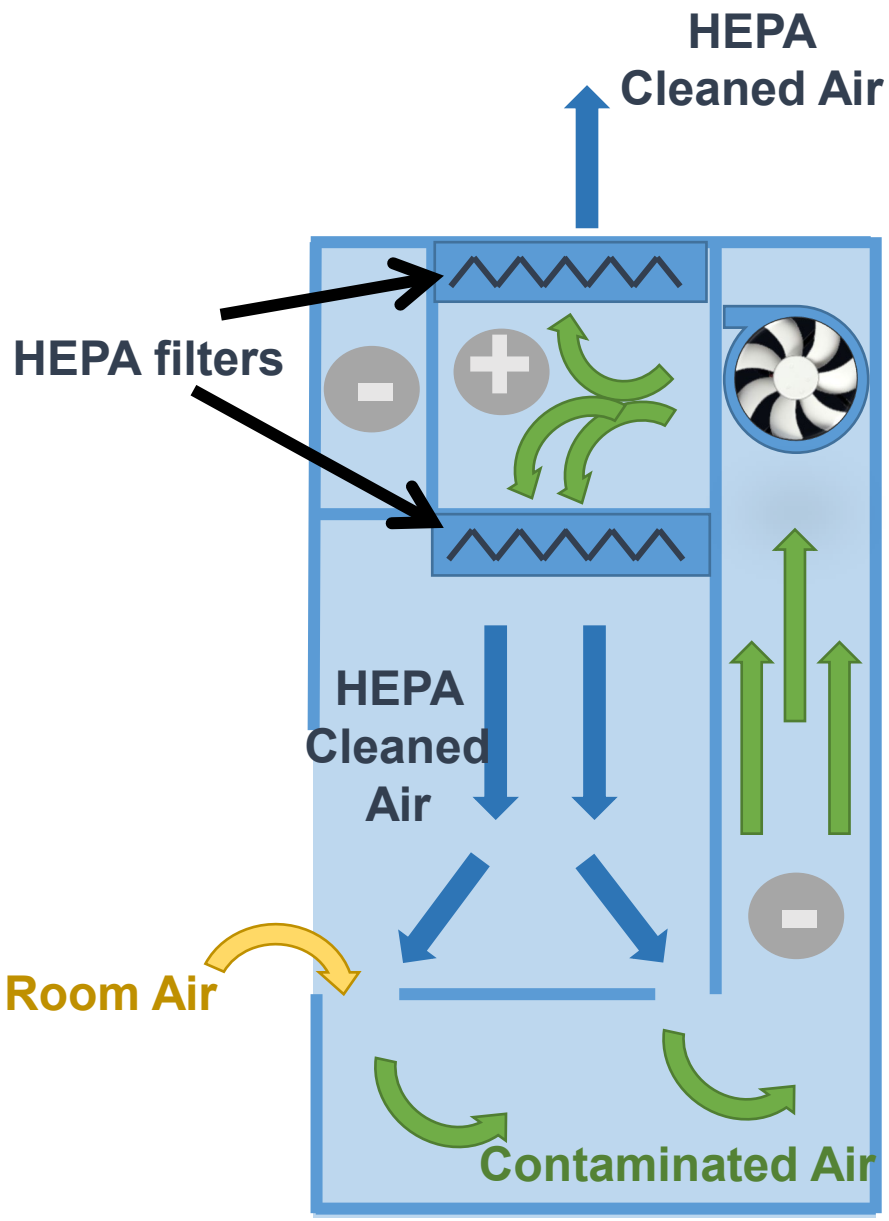
Type C1

Partial recirculation

Minimum 100 fpm intake

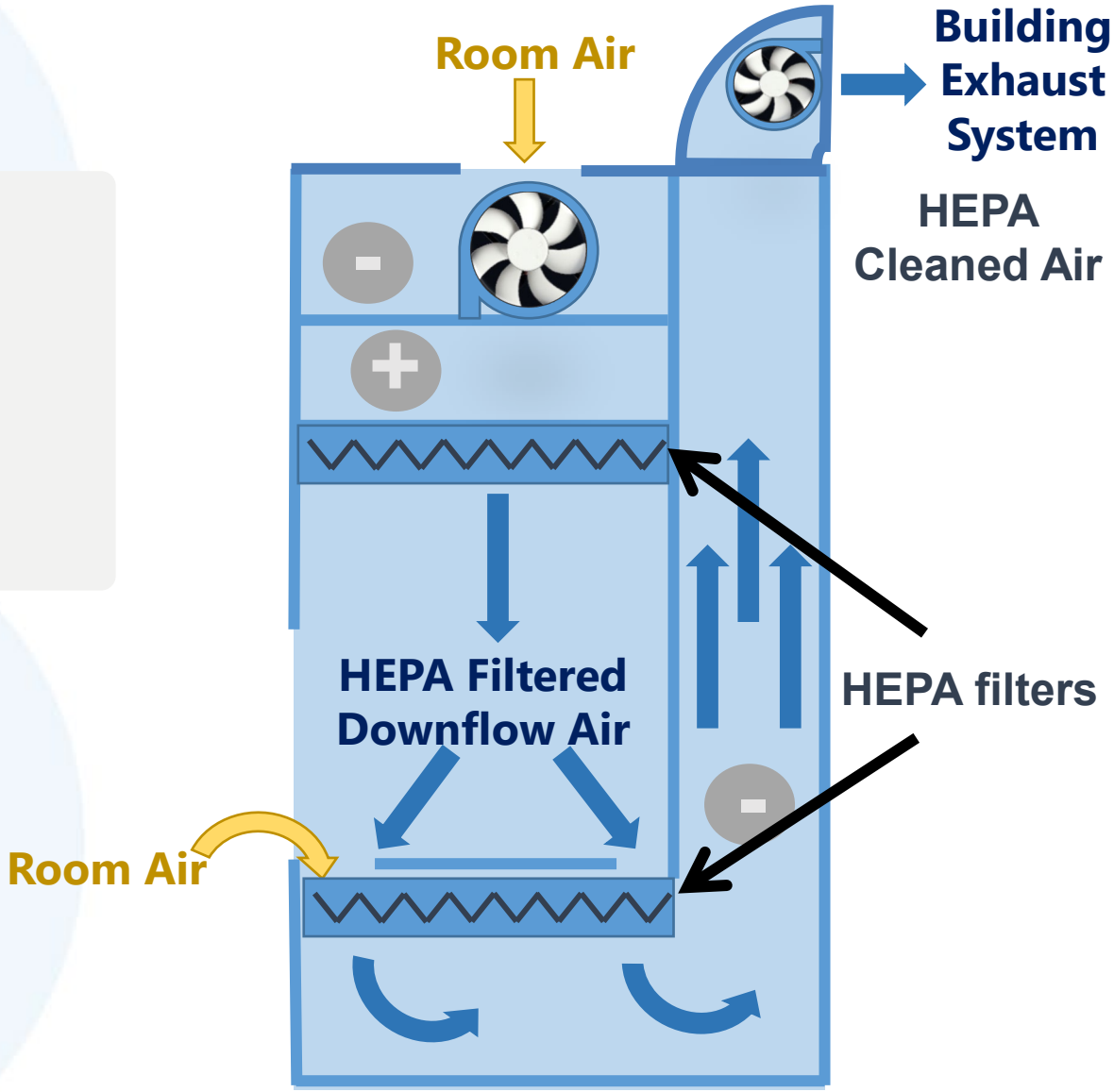
Class II Type A2 BSCs

- Personnel, Product and, Environmental Protection
- Minimum 100 fpm intake air
- Partial recirculation



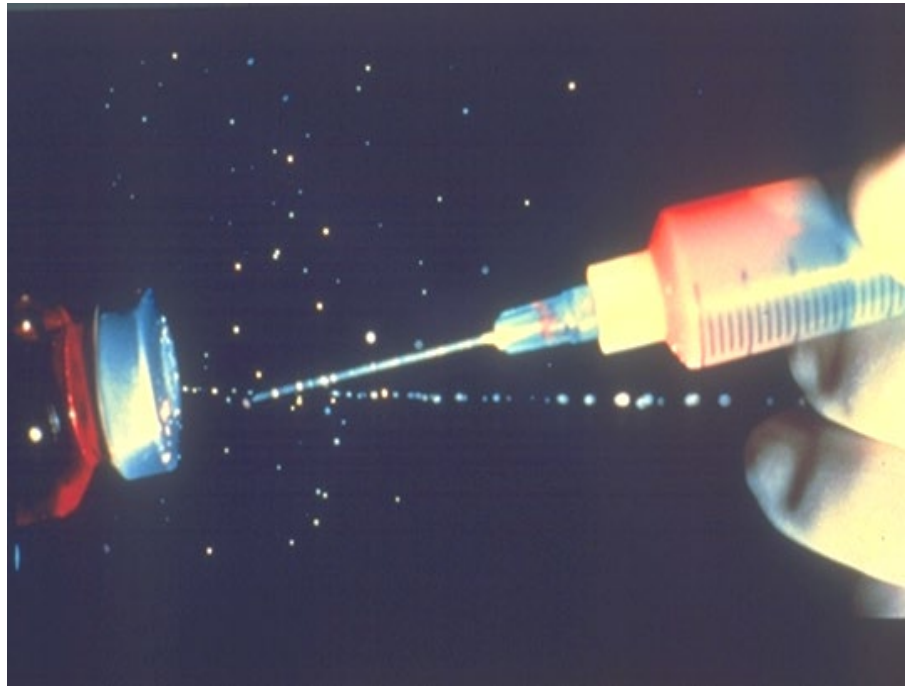
Class II Type B2 BSCs

- Personnel, Product and, Environmental Protection
- Minimum 100 fpm intake air
- 100% Exhausted



Aerosols

- Many laboratory procedures create aerosols during routine use.
  - Syringes
  - Centrifuges
  - Pop cap tubes
  - Blowing out pipettes
  - Pouring liquids
  - Shaking or vortexing tubes



Is DNA captured in a BSC?

- DNA is small
- “Sticky” due to negatively charged backbone
- “Special” molecule
- Does it become dislodged from HEPA?
- Currently UNKNOWN

Experimental Design

- Test #1: Direct application of DNA aerosol to HEPA filter
  - Can DNA be detected downstream of the HEPA filter? If so, how much?

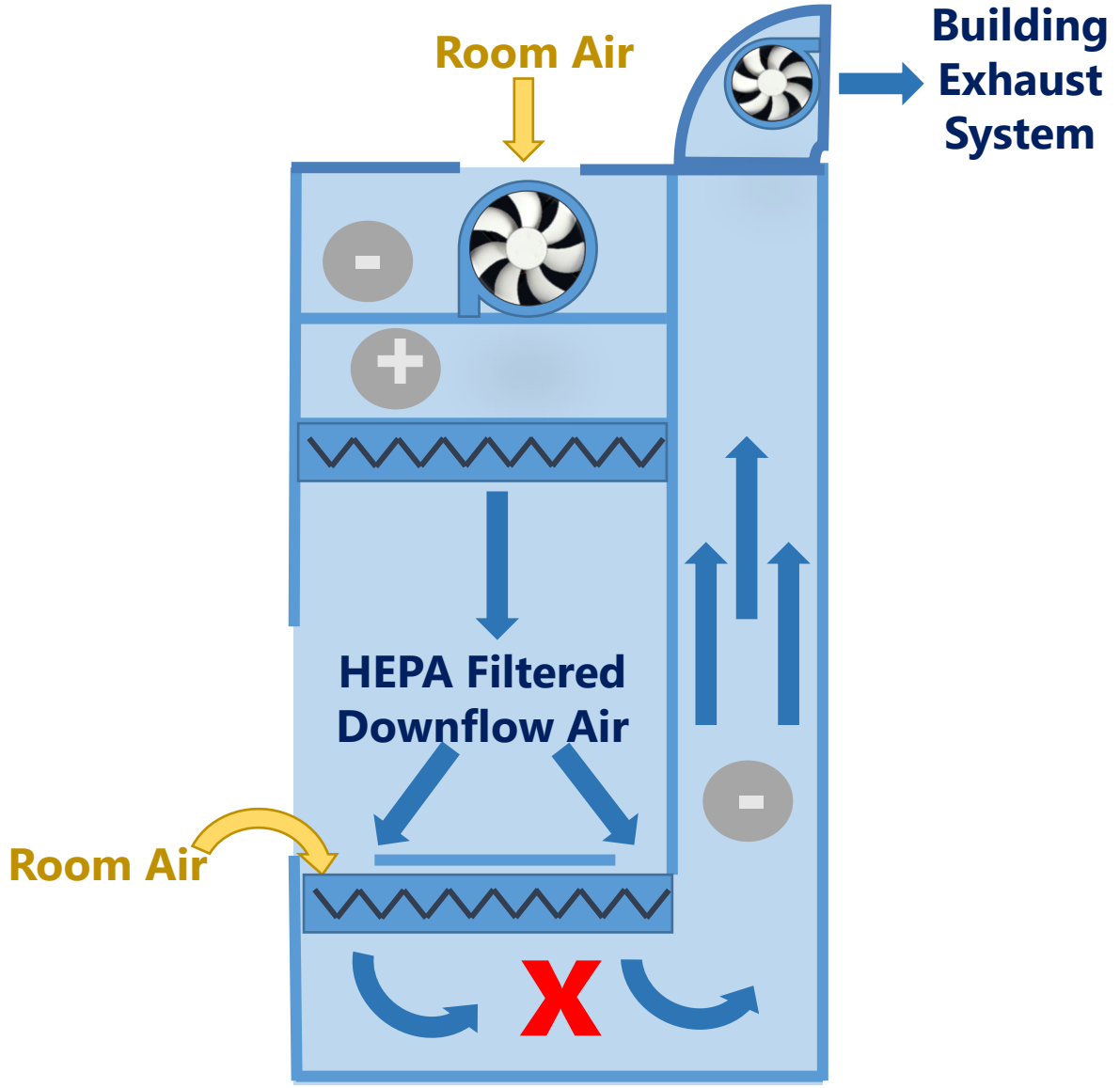
- Test #2: DNA aerosol within a Class II Type A2 BSC (Internally recirculating)
  - Is DNA detected on the internal walls of the BSC?
  - Does it pass through the HEPA filters? If so, how much?
  - Can it be removed with a standard fumigation or bleach and ethanol?

Creating DNA aerosol

- Collison Nebulizer
  - Creates ~1µm droplets
- 10^9 copies DNA/mL TE buffer
  - Known amount of DNA applied to BSC

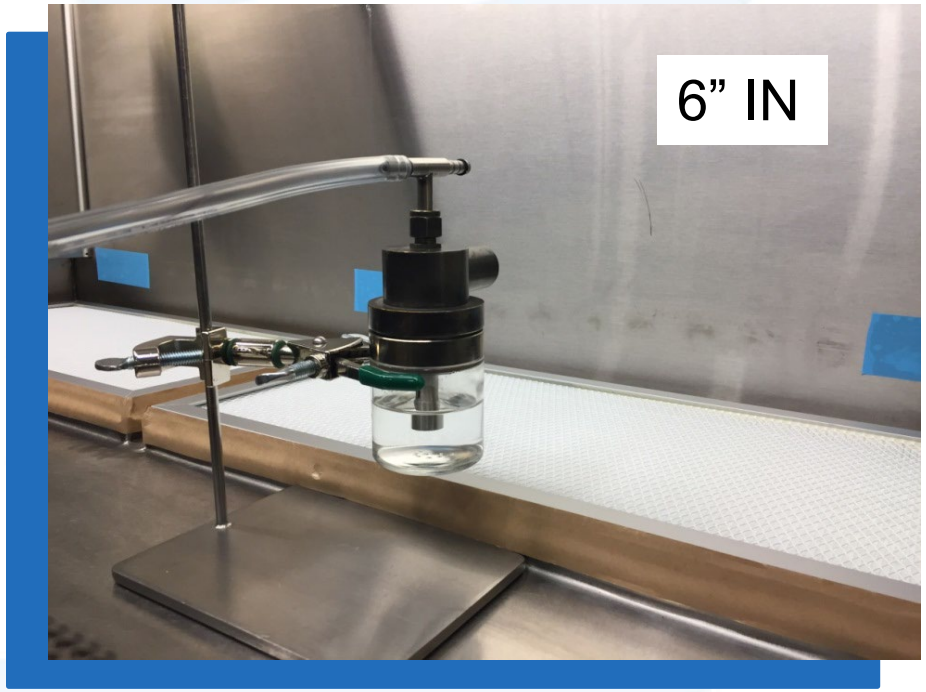
Baker's Class II Type B2 BSC - BioChemGARD

- Remove the worksurface to use the exhaust HEPA filter
- Power Failures to the Building Exhaust system can be simulated
- Filters are easy to remove
- X = sample location



Experimental Setup

- Nebulizers were placed 6, 9, and 12 inches above the HEPA filters
- Experiments tested with filters in place (IN) and removed (OUT)



Test #1: Direct Application Results

Filter IN/OUT	Location	Avg Copies of DNA detected
IN	Left	0
IN	Right	0
OUT	Left	$2.5x10^7$
OUT	Right	$3.3x10^7$
Power Failure	Left	0
Power Failure	Right	0

Summary, Part 1

DNA aerosols applied directly to a HEPA filter are captured with 100% efficiency

Power Failures and Mechanical forces will not dislodge the DNA from the HEPA filter.

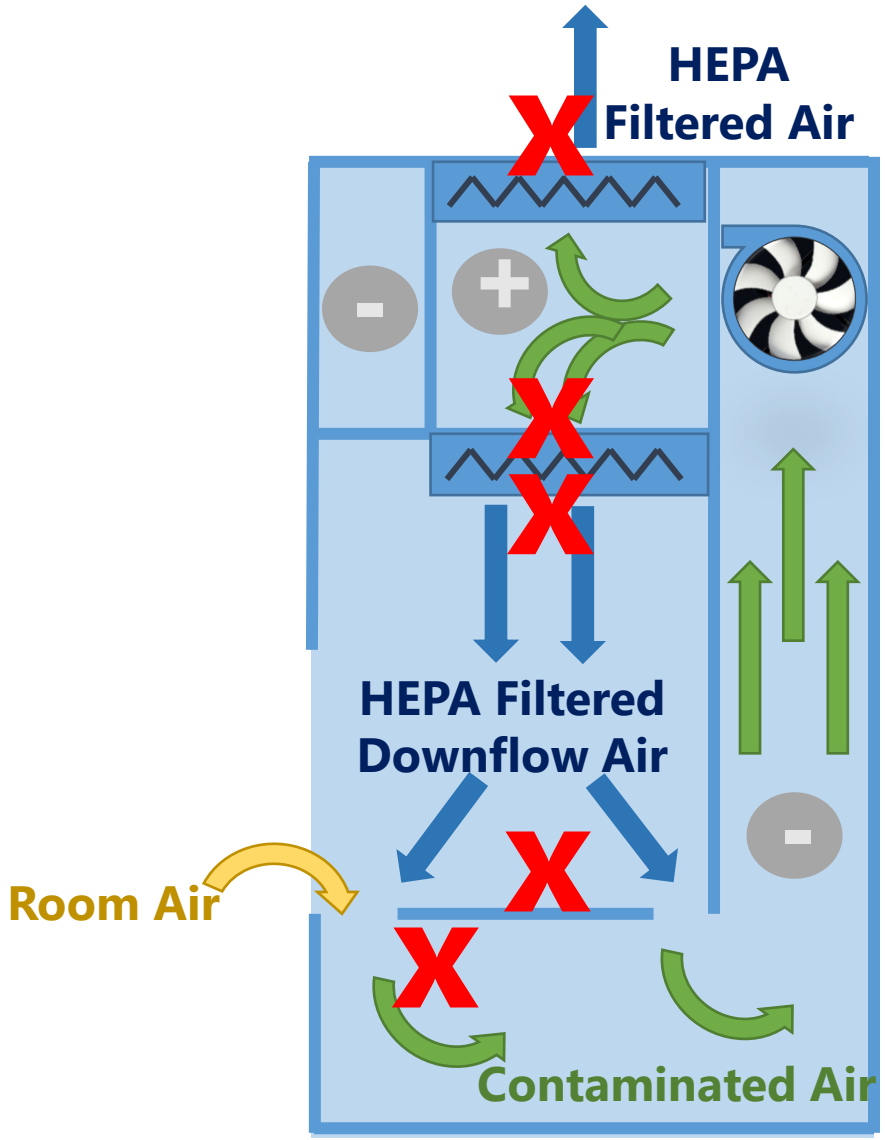
Experimental Design

- Test #1: Direct application of DNA aerosol to HEPA filter
  - Can DNA be detected downstream of the HEPA filter? If so, how much?

- Test #2: DNA aerosol within a Class II Type A2 BSC (Internally recirculating)
  - Is DNA detected on the internal walls of the BSC?
  - Does it pass through the HEPA filters? If so, how much?
  - Can it be removed with a standard fumigation or bleach and ethanol?

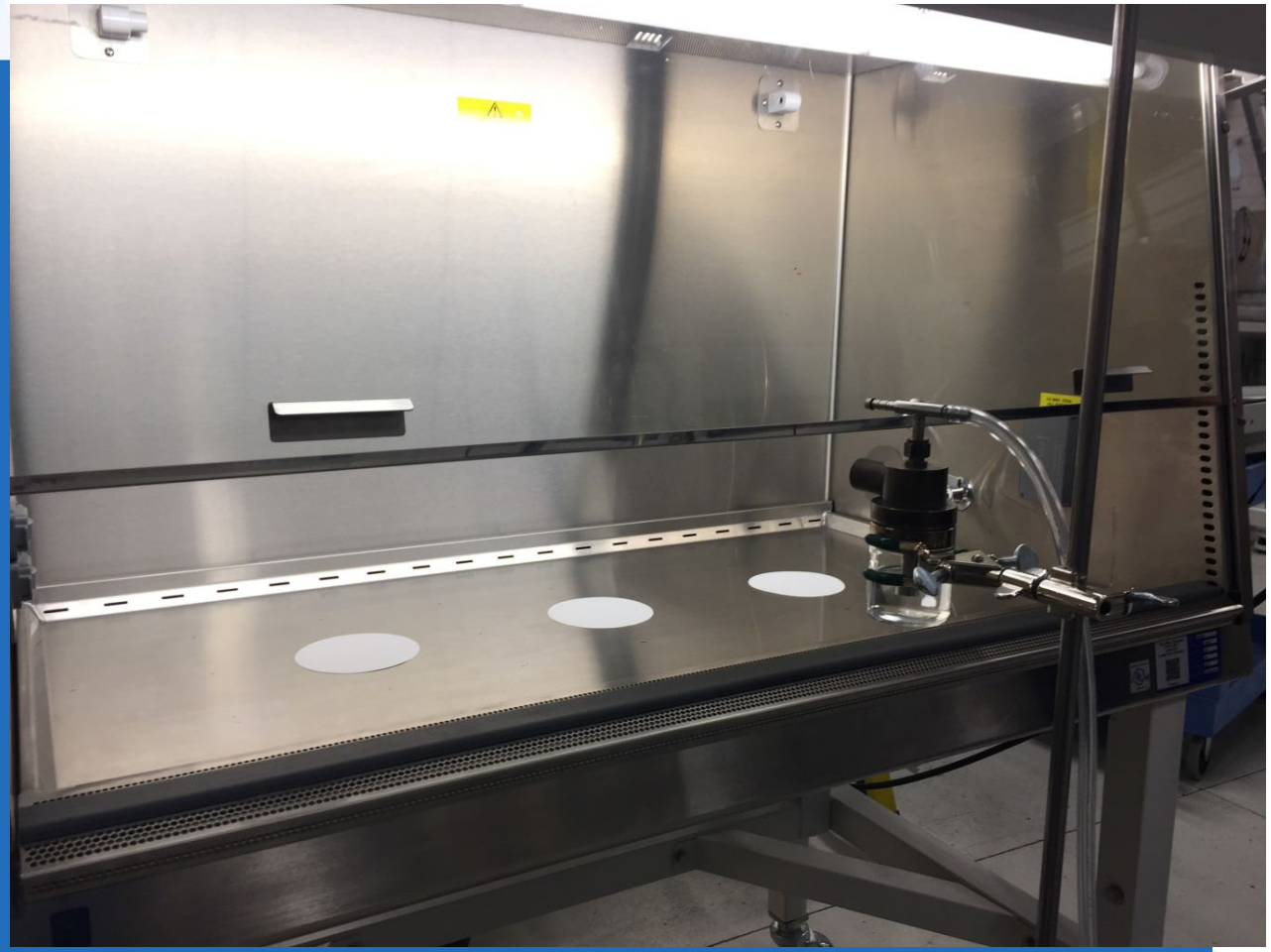
Baker's Class II Type A2 BSC - SterilGARD

- Partially internally recirculating
- May be vented back to the room or outdoors with a canopy connection
- X = sample location



Nebulizer placement

- Samples taken by Whatman filter paper and cotton swabs on the stainless steel surface or glass microscopy slides



Test #2: DNA in a BSC Results

Location	Avg Copies of DNA detected	HEPA Capture efficiency (%)
Pre-cleaning	0	
Under worksurface	$2.17x10^7$	
Worksurface	65	99.9997%
Upstream Supply HEPA	$6.25x10^4$	
Downstream Supply HEPA	159	99.9991%
Downstream Exhaust HEPA	$2.37x10^3$	99.9945%

DNA removal

The DNA contaminated BSC was tented and subjected to chlorine dioxide fumigation overnight

Bleach (10%) and ethanol (70%) were wiped on the contaminated surface for DNA removal as well

Test #2: DNA removal efficiency

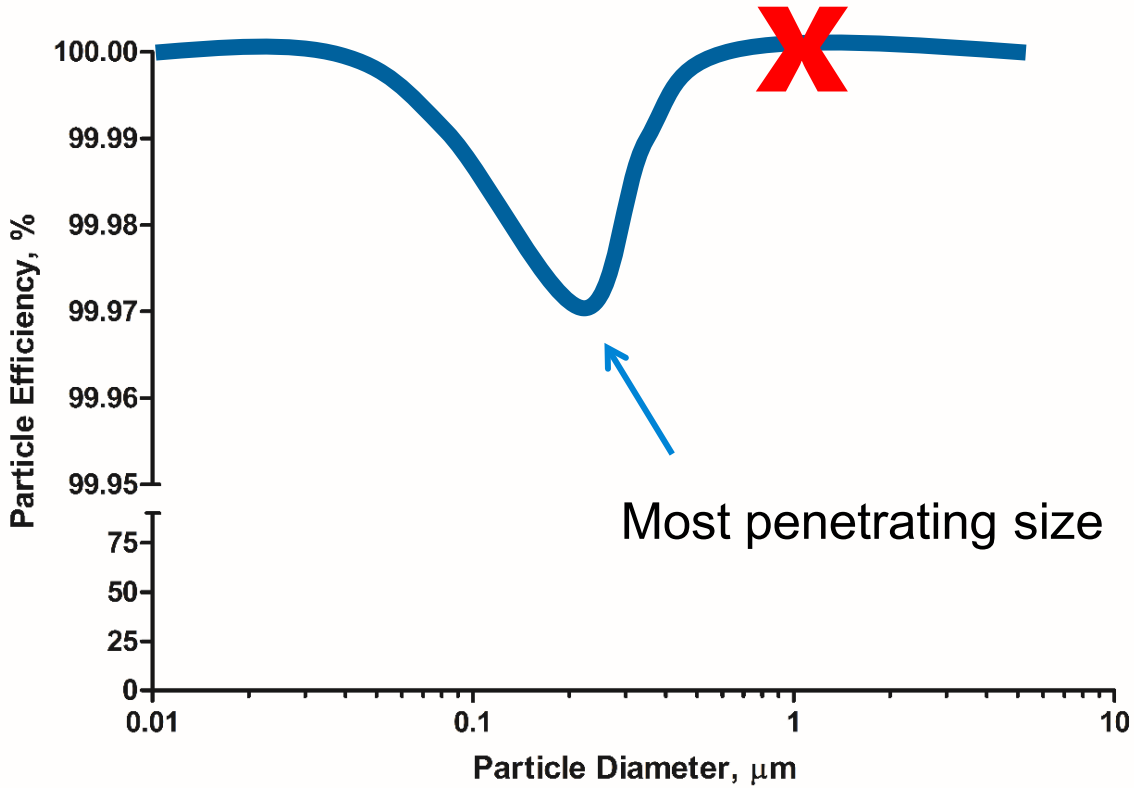
Location	Avg Copies of DNA detected	Removal efficiency (%)
Pre-decontamination	$3.31x10^9$	
Post-decontamination	$1.07x10^7$	99.7%
Post-cleaning	0	100%

Summary

- DNA aerosols applied directly to a HEPA filter are captured with 100% efficiency
- Power Failures and Mechanical forces will not dislodge the DNA from the HEPA filter.
- DNA within the airstream of a BSC is removed with 99.99+% efficiency. Some cross-contamination is possible.
- Chlorine Dioxide decontamination only removes 99.7% DNA. Bleach and ethanol remove 100%.

So, what's going on?

- 1µm droplets are captured 100%
- As the droplets pass through the air, they dry becoming smaller (0.2µm)
- This reduces capture efficiency.



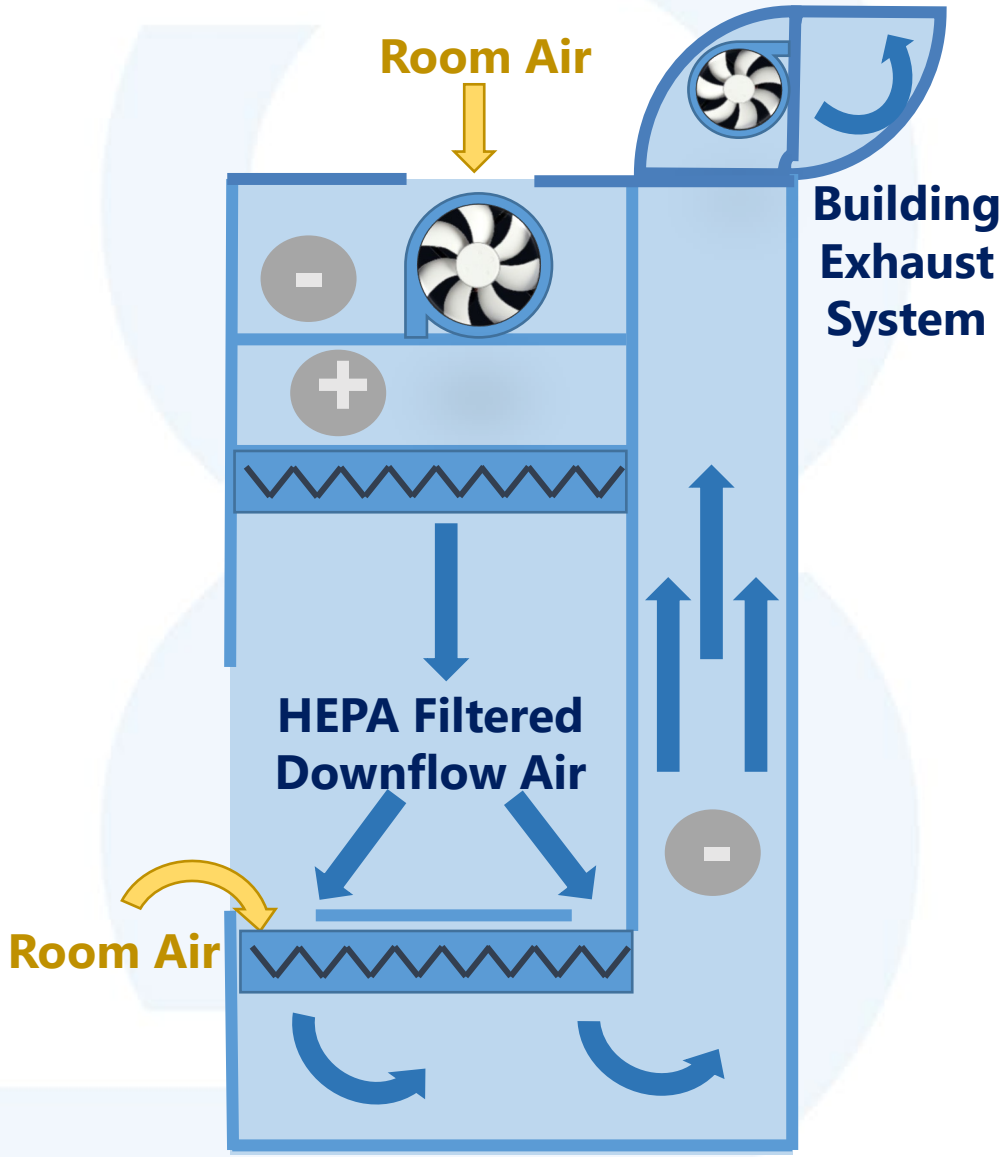
Conclusions

- DNA aerosols may be captured by HEPA filters
  - Size dependent
- DNA dried particles will be removed according to the HEPA's efficiency rating.
- Fumigation decontamination is not enough to remove all DNA. Bleach, ethanol, and physical wiping are required.

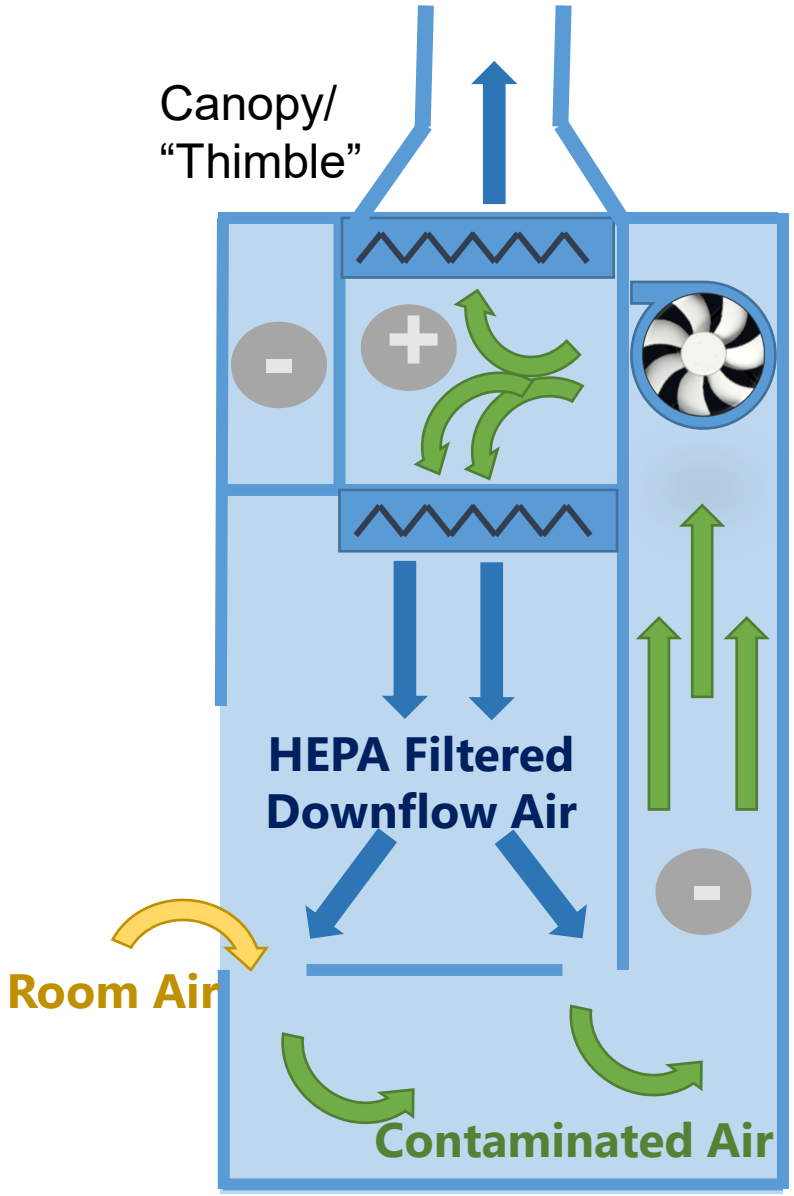
Recommendations

- Use a higher efficiency rated HEPA filter
  - ULPA = 99.999%
  - SULPA= 99.9999%
- BSC selection is critical, dependent on where your contamination concern is

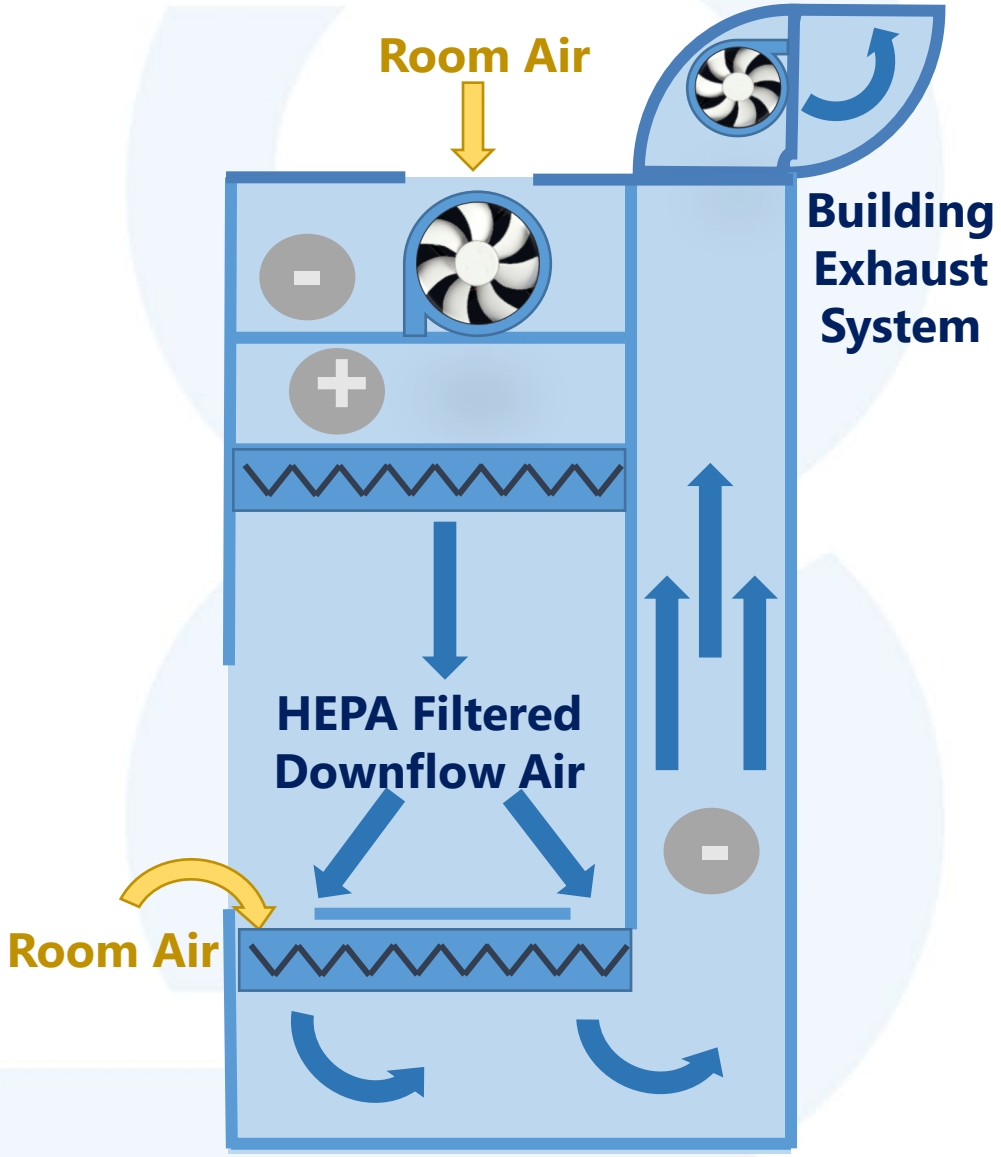
B2 – BioChemGARD



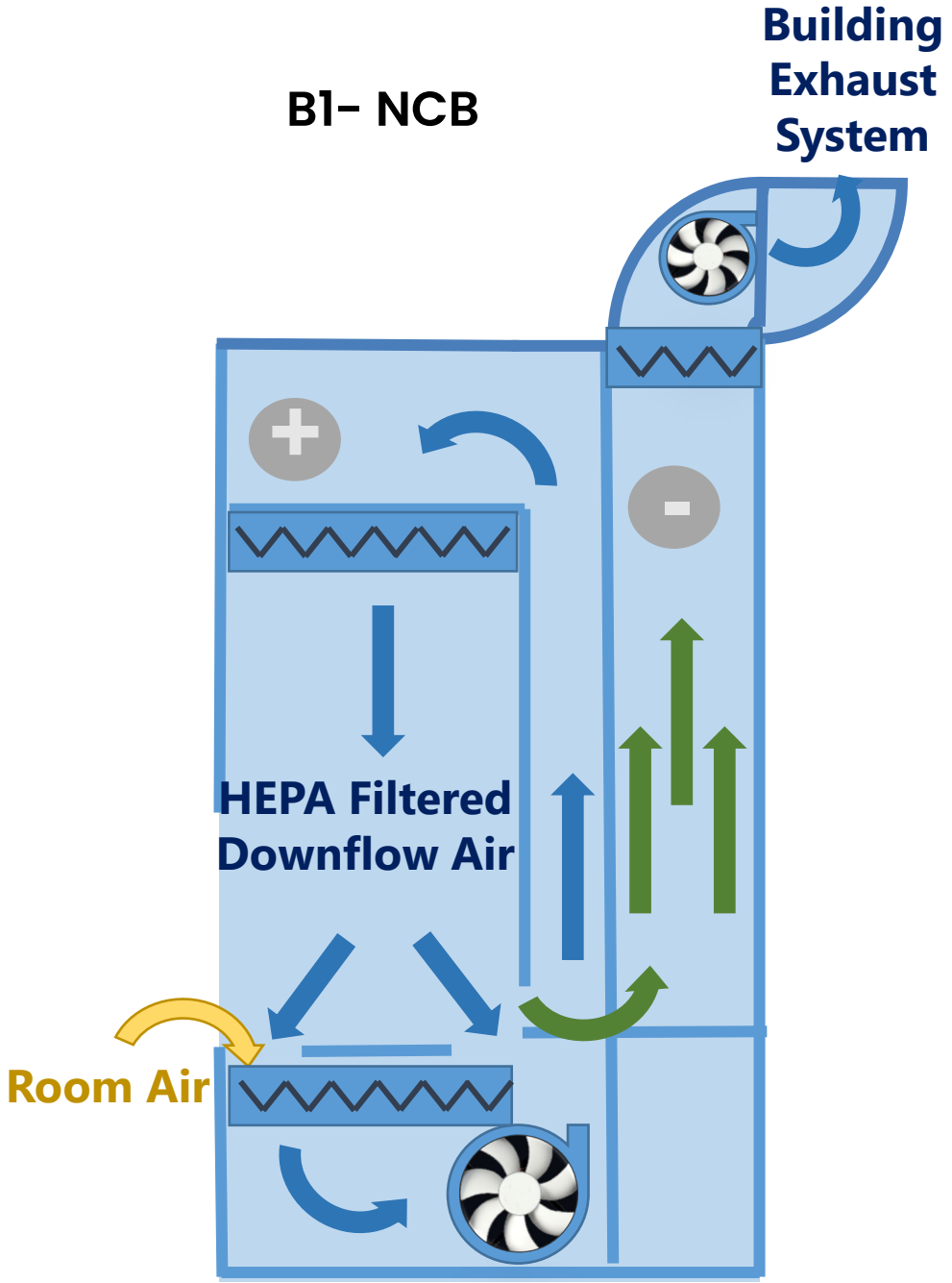
A2– SterilGARD



B2 – BioChemGARD



B1– NCB



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