The Effectiveness of HEPA filters on DNA

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ABSTRACT

Objectives: Most laboratory techniques utilize biosafety cabinets (BSCs) in order to provide contamination control of the experiments. BSCs depend on airflow and HEPA filters to remove aerosols and particulates from the environment. Aerosols may be created constantly by common lab practices, such as centrifugation, pipetting, and opening tubes. The main principals of HEPA filtration demonstrate that these airborne contaminants are captured and remain adhered by cohesive forces on the fibers of the filter, effectively removing them from the airstream. HEPA filters are very effective at removing various sized particles, but do not prevent gasses and vapors from penetrating through them. At some particular size of particle, HEPA filters will not be effective in their removal. It has been speculated that DNA may not be captured by HEPA filters, allowing for contamination of subsequent experiments by aerosolized DNA. Here we propose to: 1. Determine if DNA is captured by a HEPA filter. 2. Discover if DNA can be dislodged from a HEPA filter. 3. Discern whether Type A2 BSCs will prevent DNA contamination.

Method: Here we have tested this theory in multiple avenues. First, a concentrated solution of plasmid DNA was nebulized directly onto a HEPA filter and the DNA penetrated was measured. Secondly, the DNA was released inside the airflow of a Class II Type A2 BSC and the DNA that has adhered to various surfaces, upstream and downstream of the HEPA filters were measured to determine where DNA deposition occurs, and if it will penetrate through the HEPA filter or not in the context of a BSC. Lastly, we tested whether DNA once stuck to a HEPA filter can become detached through mechanical or electrical forces, allowing for a future unexpected contamination.

Results: These results quantify the capture efficiency of the HEPA filter and the Class II BSC system in reducing the recirculation of airborne DNA, demonstrating HEPA filter capture of the DNA and preventing its release.

Conclusion: HEPA filters provide an excellent method for removing air particulates, including DNA, with a high efficiency.

Outcomes: DNA work should be conducted in any BSC to minimize sample to sample DNA contamination, providing clean and accurate work for any lab using quantitative DNA methods.

Highlights: HEPA filters have limitations, but the extent of these limits is unknown, particularly as it pertains to DNA. Here we probe the outer edges of HEPA filter technology; is DNA captured on a HEPA filter, and whether sensitive, quantitative work with DNA be done in a recirculating BSC.

INTRODUCTION

HEPA filters are the main line of defense against contamination in Biosafety Cabinet (BSC) construction. HEPA (High Efficiency Particulate Air) filters are composed of one continuous pleated sheet of borosilicate fibers woven into a crosshatched design. This sheet has spacers in between the pleats to hold the shape and is completely sealed with two-part polyurethane to a sturdy frame (NSF Standard 49, 2016). This rigid shape and structure shown in **Figure 1B** is critical for directing the air through the filter material to ensure particulate removal.

HEPA filters remove particles by three different mechanisms: Impaction, Interception, and Diffusion. As seen in **Figure 1A**, all three of these methods lead to very high particulate removal of a wide range of sizes, with the most penetrating particle size of 0.21µm. When tested at 0.3µm, near its worst, the HEPA filter has to still remove 99.97% or better of all particulates. NSF requires HEPA filters to be of Type C or better, removing a minimum of 99.99% of 0.3 µm particulates (NSF Standard 49, 2016).

Many common practices within microbiological procedures such as pipetting, opening capped tubes, and centrifugation, have the capacity to create aerosols. These aerosols can contain cross-contaminating DNA that may affect further experimentation. It has long been unknown whether DNA can be captured in a HEPA filter when procedures are conducted within a BSC. Plasmid DNA has been estimated to be 200nm or 0.2µm in diameter when supercoiled (Tsoi *et al.*, Biophys Chem, 2010), however the ability of DNA to exist in multiple conformations leads to uncertainty. If the DNA stays in suspension, it has a greater chance of being captured, however, as the droplets dry, if the DNA will act the same is unknown.

Here we test the ability of DNA to be captured by a HEPA filter in multiple scenarios: direct application to a HEPA filter and circulating in the airstream of a Class II Type A2 BSC (the most common style found in laboratories, a partially recirculating cabinet). We will also test whether DNA can be dislodged from a HEPA filter after a physical shock or an electrical power failure, as well as if DNA is destroyed by a full cabinet decontamination with chlorine dioxide (CD).

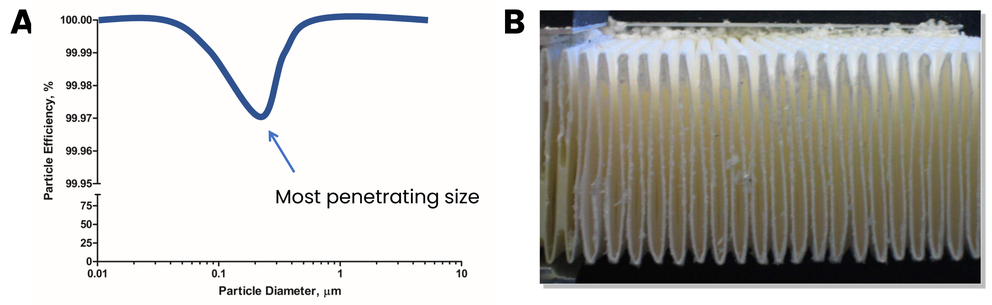

Figure 1. HEPA filter function and design. (**A**) Shows the efficiency of a HEPA filter of removing particles of a particular size. (**B**) Depicts the folded structure of the borosilicate sheets of a HEPA filter cross section. These pleats help direct air through the filter and ensure a greater surface area for filtration.

METHODS

Direct Application to the HEPA filter. Plasmid DNA (60mL, $1x10^9$ copies/mL in TE buffer) was placed in a Collison nebulizer which generates a defined droplet size of ~1µm (May, 1973). A Class II Type B2 BSC (BioChemGARD, Baker) was installed with new HEPA filters, adjusted to setpoint of 105 fpm intake air with an 8-inch viewscreen opening, and cleaned with 10% bleach and 70% ethanol. The worksurface was removed to expose the two side by side exhaust HEPA filters. The nebulizer was placed directly in front of either side HEPA filter at a height of 6, 9, and 12 inches, secured by a ring stand (**Figure 2**). Pre-experiment samples were collected to ensure no background contamination. The nebulizer was then run for 1 minute on each side, with and without the HEPA filters installed (**Figure 2A** and **2B**, respectively). Samples were collected by cotton swab on glass slides and the direct metal surface, and Whatman filter paper. Before and after weight measurements were used to determine how much DNA was introduced in each condition. The BSC was thoroughly cleaned with 10% bleach and 70% ethanol in between samples and replicates.

Mechanical and electrical removal of DNA from HEPA filters. HEPA filters were removed and re-installed jarringly to try to remove any DNA captured within the HEPA filter. Electrical power failure on the BSC and exhaust blower system was simulated three successive times to test for unintentional DNA contamination. In both situations, samples were captured downstream of the HEPA filter.

DNA collection in a BSC airstream. A Class II Type A2 BSC (SterilGARD, Baker) was installed with new supply and exhaust HEPA filters, adjusted to a setpoint of 105 fpm intake air, 50 fpm downflow velocity with an 8-inch viewscreen opening, and cleaned with 10% bleach and 70% ethanol. Plasmid DNA (60mL, $1x10^9$ copies/mL in TE buffer) in a Collision nebulizer was placed 8 inches away from the bottom of the viewscreen and run for 15 minutes (**Figure 3A**). Samples were collected by cotton swab on glass slides or directly on the metal surface, and on Whatman filter paper throughout the BSC: on the worksurface, below the worksurface, upstream from the supply HEPA filter, directly downstream from the supply HEPA filter, and directly downstream from the exhaust HEPA filter (**Figure 3B**). Before and after weight measurements were used to determine how much DNA was introduced. The BSC was thoroughly cleaned with 10% bleach and 70% ethanol in between samples and replicates. Capture efficiency was calculated by **Equation 1**.

Equation 1: $(1 - (\frac{copies\ detected}{copies\ introduced * \%\ sampled})) * 100\%$

DNA destruction by Chlorine Dioxide. After aerosolization of the plasmid DNA into the Class II Type A2 BSC (SterilGARD, Baker) in the previous experiment, the cabinet was prepared for a standard biodecontamination with chlorine dioxide using the DRS Laboratories Mini Chlorine Dioxide System per the manufacturer's instructions.

DNA quantification. Samples collected with swabs were extracted by eluting in 150µL of TE. Samples collected using filter papers were hole punched into 150µL of TE. Both types of samples were vortexed, centrifuged and 5µl of sample added to qPCR reaction. DNA quantification was performed by using qPCR with MMQCI's internal assay and plasmid-based standards on Roche LightCycler 2.0.

RESULTS

Direct application of the aerosolized DNA to the HEPA filter (pictured in **Figure 2A**) resulted in a complete capture of all DNA containing particles (**Table 1**) as demonstrated by placing the nebulizers directly upstream of the HEPA filters located directly below the worksurface on the Class II Type B2 BSC and collecting the samples directly downstream of these exhaust HEPA filters (**Figure 2C**). After attempting to remove the DNA captured in the HEPA filter through mechanical and electrical means by simulating a power failure of the cabinet and external exhaust system, no DNA was detected (**Table 1**).

When DNA was aerosolized within a Class II Type A2 BSC as depicted in **Figure 3A**, DNA was detected at significant quantities throughout the cabinet as expected based on airflow: under the worksurface and upstream from the supply HEPA filter that provides clean air to the worksurface (**Table 2**, **Figure 3B**). However, unlike the direct application above, downstream from both the supply and exhaust HEPA filters, low quantities of plasmid DNA were detected. Using airflow calculations previously determined (Stuart *et al.*, 1983; Held *et al.*, 2016), it is known that the mass flow velocity of any compound added to the airstream by the intake air is equal to the concentration of compound in the downstream air and exhaust air. Combined with the known amount of DNA added to the airstream (Volume displaced*DNA density = copies introduced) and the proportion of the surface area tested as compared to the full HEPA face area, the capture efficiency can be calculated by **Equation 1**. All downstream HEPA samples exhibited better than 99.99% DNA removal efficiency (**Table 2**).

Chlorine dioxide (CD) was applied to determine if a gaseous biodecontamination protocol would remove DNA from the metal surfaces within the BSC. As shown in **Table 2**, CD resulted in the removal of 99.7% of the contaminated surface. After further cleaning with bleach and isopropanol, all traces of plasmid DNA were removed.

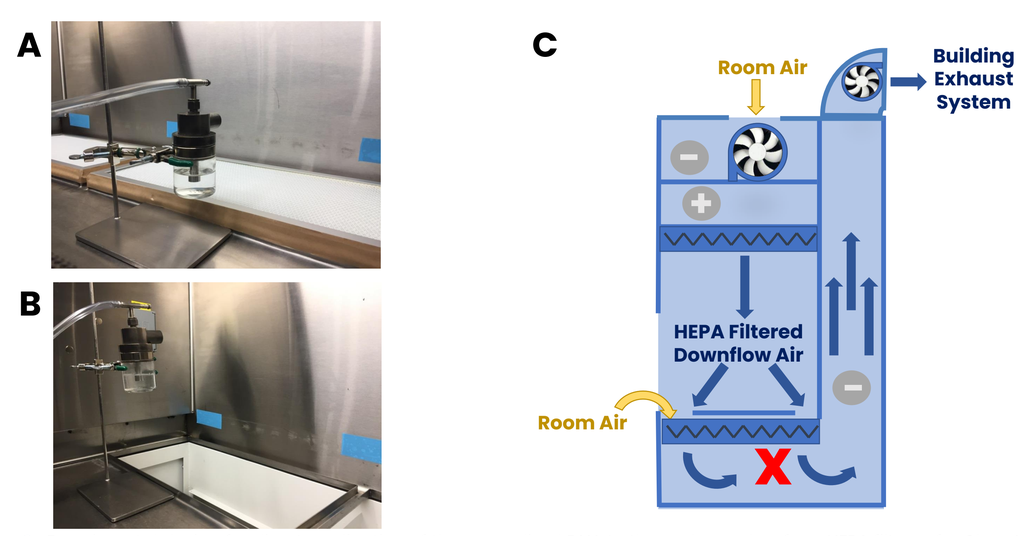

Figure 2. Experimental design for direct application of the aerosolized DNA to the under worksurface HEPA filters of a Class II Type B2 BSC. Depicted are the filters in place (**A**) and removed (**B**). **C.** Schematic of a Class II Type B2 BSC showing airflow arrows, positive and negative pressure areas (+ and - , respectively), HEPA filter locations (zigzag lines) including one below the worksurface, motor/blower, and the sampling locations denoted by red Xs.

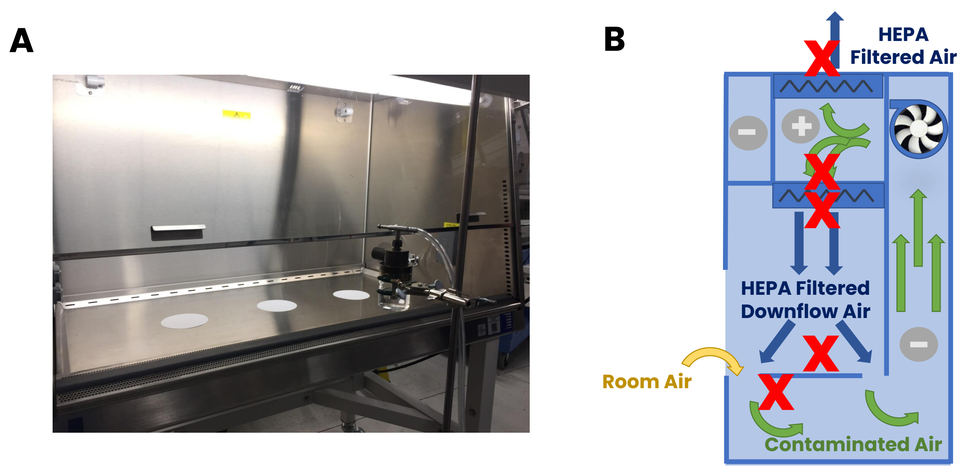

Figure 3. DNA in the BSC airstream. (**A**) The nebulizer layout in front of the BSC. (**B**) Diagram of a Class II Type A2 BSC showing airflow arrows, positive and negative pressure areas (+ and - , respectively), HEPA filter locations (zigzag lines), motor/blower, and the sampling locations denoted by red Xs.

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

Table 1. Direct application of aerosolized DNA to a HEPA filter using a Class II type B2 BSC.

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%
Pre-decontamination	$3.31x10^9$	
Post-decontamination	$1.07x10^7$	
Post-cleaning	0	

Table 2. Aerosolized DNA detected throughout a Class II Type A2 BSC and the capture efficiencies of the HEPA filters.

CONCLUSIONS

Upon direct application of DNA to the HEPA filter by the nebulizer inside the Class II Type B2 BSC, it was observed that all DNA aerosols were captured, with no variation observed when the nebulizer was placed 6, 9, or 12 inches above the exhaust HEPA filters (**Table 1**, **Figure 2A**, **2B**, and **2C**). However, when the DNA was introduced to an entire BSC as simulated in the Class II Type A2 BSC (**Figure 3A**), DNA was detected both upstream and, to a lesser extent, downstream of both the supply and exhaust HEPA filters (**Table 2**). DNA was expected to be detected upstream of the HEPA filter (under the worksurface and pre-HEPA samples, **Figure 3B**), as these samples were exposed to the DNA-contaminated room air introduced by the nebulizer. Controlling for the area sampled as compared to the total amount of DNA introduced into the airstream (**Equation 1**), allows for the determination of the efficiency of DNA removal by the HEPA filter. All three areas where DNA was detected post-HEPA had efficiencies greater than 99.99%, as specified by NSF Standard 49 (NSF, 2016, **Table 2**). The disparity between the results from the Class II Type B2 and Type A2 BSCs may be explained by particle size. Direct aerosolization produces droplets ~1µm/1000nm in size. These particles are an ideal size to be captured by a HEPA filter. However, as the droplets move through the airstream, they will dry, as a droplet has a "wet time" of 0.6s (May, 1973), and this will decrease the DNA aerosol size to the most penetrating size of the HEPA filter, ~0.2µm or 200nm (**Figure 1**).

Chlorine Dioxide was able to remove 99.7% of the surface contaminated DNA, but not all. The most thorough cleaning option was bleach and isopropanol, however this solution only pertains to easily reachable surfaces.

RECOMMENDATIONS

Here we have shown that direct application of DNA aerosols will be captured completely by a HEPA filter, but as the droplets move through the BSC, there can be up to 0.01% contamination. To eliminate this potential contamination, undersurface HEPA filters or a non-recirculating BSC design could be employed. It could also be helpful to use a more stringent filter, such as an ULPA (99.999% effective) or a SULPA (99.9999% effective).

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