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Laboratory Certification
AIHA LAP #183867
CDC Elite since 2009
NY State Legionella Certified #12050
State of TN #03147
EPA license #801-14

# Experimental trials conclude that the CAZ-100B System is 99.99% effective at removing Airborne Mold Spores.

**Product Testing Lab**
**February 5th 2019**

## Experimental Overview

- Assured Bio Labs, LLC was contracted by Clean Air Zone, Inc. (CAZ) to conduct time series analysis to determine the capacity of the CAZ-100B System to capture airborne mold spores.

- Two experimental time-series trials were conducted to determine the capacity of the CAZ-100B system to remove mold spores from the air.

- Two mold species were used in each experimental trial. Both species are common contaminants of the built-environment when water intrusion, elevated humidity or "sick building syndrome" issues are reported. Molds species were cultured on sterilized corn kernels for maximum spore production (see table 1, figure 1).

- An air recirculation attachment was designed and fitted to the CAZ-100B system. The attachment allowed for delivery of dry mold spores into the CAZ-100B, and provided inlet and outlet ports to measures the airborne spore concentrations of recirculating air over time (See figure 2).

- Spore concentrations were measured immediately following release into the CAZ-100B system at two inlet ports, and at regular intervals up to 30 minutes following spore release using two outlet ports. M-TRAP® capture cassettes were used to measure spore concentrations via DNA analysis, using high-fidelity, quantitative PCR technology.

- In experimental trial I, 139 million spores were released into the CAZ-100B system. In experimental trial II, 85 million mold spores were released into the CAZ-100B system.

## Key Findings

- In both experimental trails the CAZ-100B removed 99.99% of mold spores from the airstream within 30 minutes of spores being release into the system.

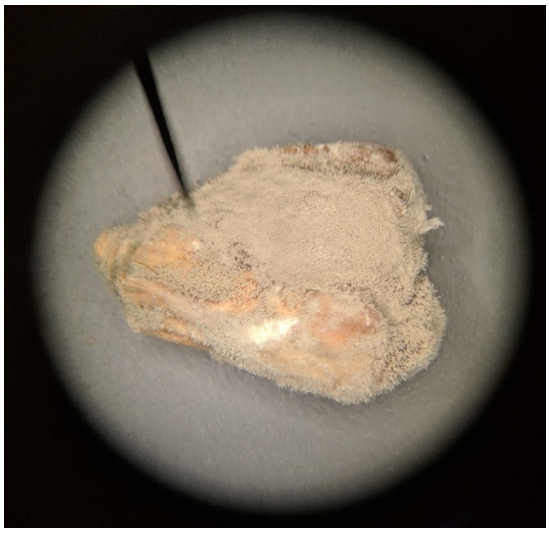
- In trial I, the concentration of the airborne *Penicillium brevicompactum* spores was reduced from a starting concentration of 137 million spores to 572 spores within 30 minutes (see figure 3). The concentration of *Aspergillus flavus* spores was reduced from a starting concentration of 957 thousand spores to non-detectable levels within 15 minutes (see figure 4).

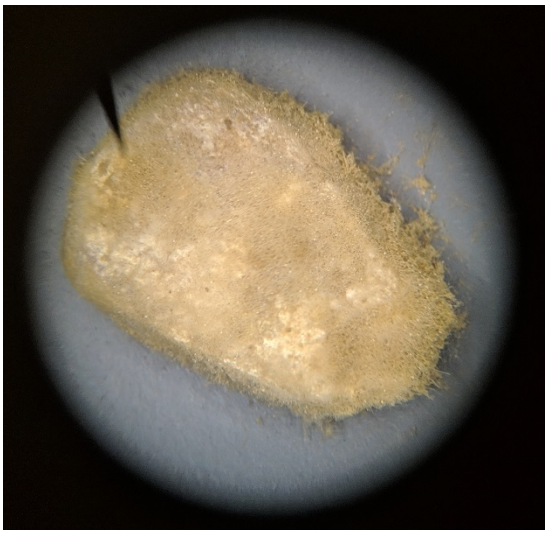
- In trial II, the concentration of the airborne *Penicillium brevicompactum* spores was reduced from a starting concentration of 85 million spores to 540 spores within 30 minutes (see figure 3). The concentration of *Aspergillus flavus* spores was reduced from a starting concentration of 28 thousand spores to non-detectable levels within 5 minutes (see figure 4).

**Table 1. Target Mold Species.**

| Organism | Strain | Inoculum Substrate |
|---|---|---|
| *Penicillium brevicompatum* | DAOMC 192262 | Sterilized Corn Kernels |
| *Aspergillus flavus* | NRRL AF36 | Sterilized Corn Kernels |

**Figure 1. Mold sporulation on sterilized corn kernels. *Penicillium brevicompactum* is colonizing the kernel in the left photograph and *Aspergillus flavus* is colonizing the kernel in the right photograph. Spores were physically removed from kernels before introduction into the CAZ-100B system.**





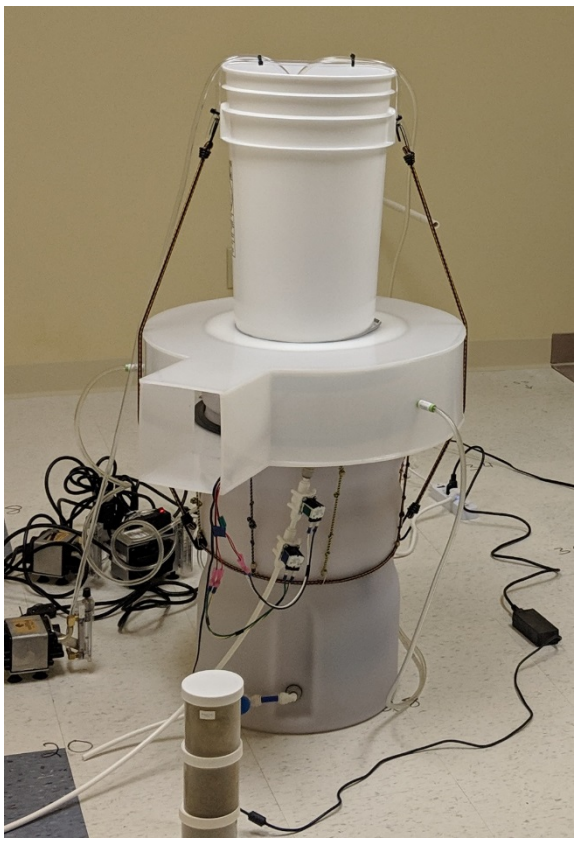


**Figure 2. Air Recirculation attachment mounted on an CAZ-100B System for experimental trials. System is completely sealed off from external air during trials. Sampling ports on the sides, top and back allow for measuring spore concentrations.**

**Figure 3. Time series for trial I (blue line) and trial II (orange line) for the mold species *Penicillium brevicompactum*. The CAZ-100B removed 99.99% of mold spores from the air in both experimental trials.**

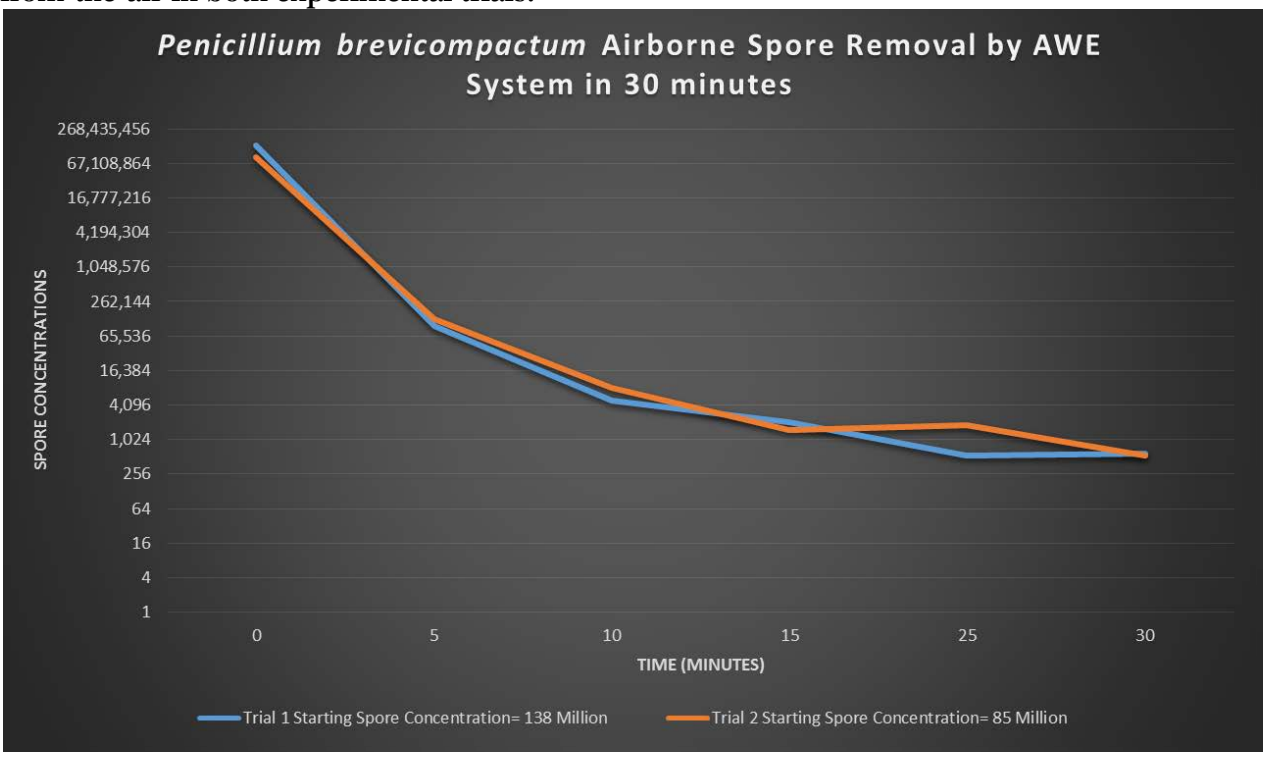
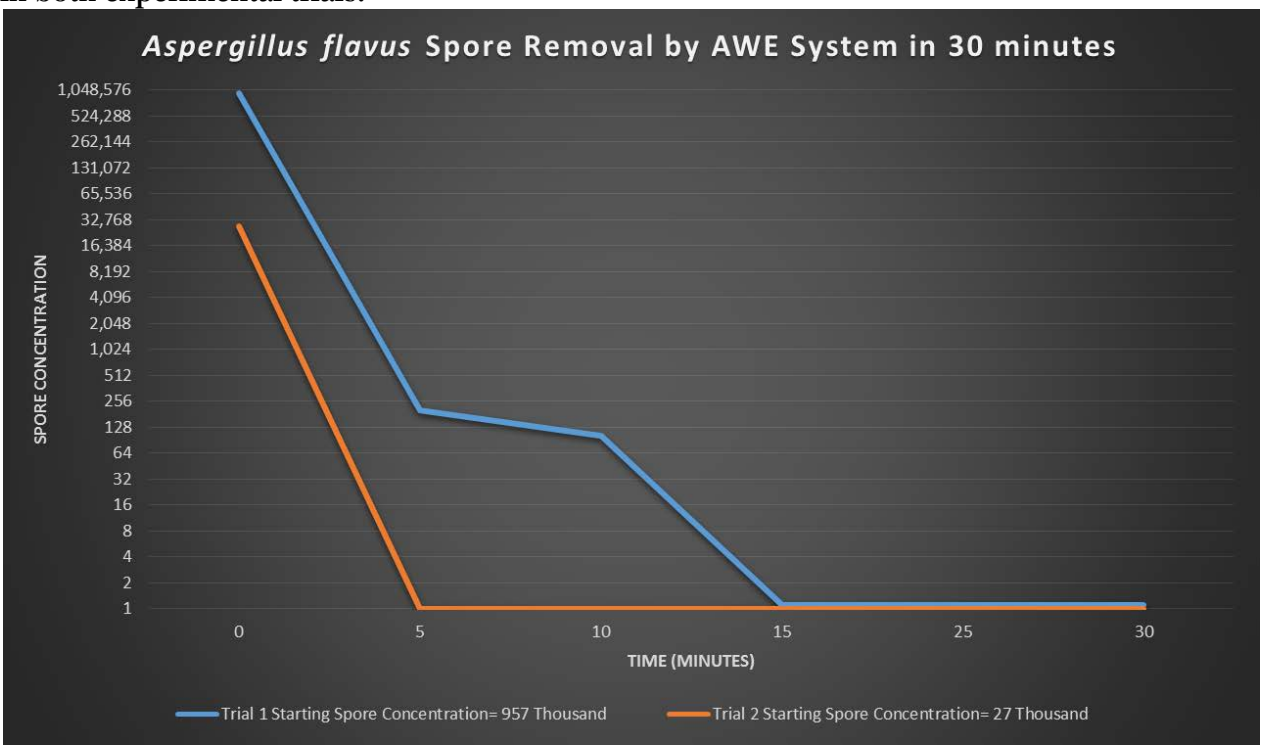



**Figure 4. Time series for trial I (blue line) and trial II (orange line) for the mold species *Aspergillus flavus*. The CAZ-100B removed 99.99% of mold spores from the air in both experimental trials.**



## Experimental Methods

**Spore Preparation.** *Penicillium brevicompactum* (192262) and *Aspergillus flavus* (AF36) were obtained from the Canadian Collection of Fungal Cultures (DAOMC), and the Agriculture Research Services Culture Collection (NRRL), respectively. They were cultured on malt extract agar for 10 day. Spores were harvested and suspended in sterile distilled water. Corn kernels were sterilized by autoclaving for 1 hour in 500 ml polypropylene containers. Following sterilization, 6 containers were inoculated with 10 ml of the *P. brevicompactum* suspension, and 6 containers were inoculated with 10 ml of the *A. flavus* suspension. Containers were mixed for 30 minutes on a platform shaker to evenly distribute the spore inoculum. All containers were incubated at 27 degrees centigrade for 14 days.

**CAZ-100B Setup**

A custom top was designed and fabricated for the CAZ-100B system. Two inlet ports were built into the top to measure the concentrations of spores released into the system, and two outlet ports to measure the spore concentration of spores that was expelled by the CAZ-100B during air scrubbing. A recirculation duct connected the air intake and air exhaust of the custom top in order to measure airborne spore removal over time. The CAZ-100B was completely sealed from external air during recirculation by rubber gaskets.

**Time Series Trials**

For each trial, one container of corn kernels of *P. brevicompactum* and *A. flavus* was shaken to remove spores and an aliquot of dry spores was released into a side door on the CAZ-100B air intake. The door was immediately sealed following spore release to prevent any spore leakage from the system to external air. The concentration of spores entering the CAZ-100B was measured using M-TRAP® capture cassettes, which were inserted into the two intake ports. At regular intervals, M-TRAP® capture cassettes were collected from the two outlet ports to measure the concentration of airborne spore removal by the CAZ-100B over time. The time series was run for 30 minutes in both trials.

**Analysis & Reporting**

M-TRAP® capture cassettes were processed according to Assured Bio Labs, American Industrial Hygiene accredited DNA mold analysis methods (AIHA LAP #183867). Quantitative PCR analysis was run for two DNA probe and primer sets that corresponded to calibrations standards for *P. brevicompactum* and *A. flavus*. Data was reported in spore equivalents or total spore concentration from the mean of the two inlet port samples at the beginning of each time series trial and the mean of the two outlet port samples for each sampling point in each series trial during recirculation.