Public Health Agency of Canada | Agence de santé publique du Canada

Re: Final Report for LeVOCC process testing.

Hello Doug

We have finished testing the LeVOCC process, the attached report is the final report of all testing which was carried out. It was interesting testing this piece of equipment as it demonstrated a markedly higher rate of biological removal then any other units tested. LeVOCC's Photocatalytic oxidation technology, which oxidizes biological particles from the air, was able to remove all organism types tested, including spores, mycobacterium, and viral species. I would highly recommend developing a peer reviewed journal publication with both the biological and volatile organic compound testing included.

If any further information is needed please do not hesitate to contact me.

Sincerely

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Canada

Efficacy Testing using the LeVocc Air Purification System

'Final Report'

INTRODUCTION

Air purification has become a very important option for controlling the spread of microorganisms. One only has to look at history of H1N1, SARS, and the common cold which all carry huge costs to the health care system. Commercial air purifiers have the ability to help reduce the amount of microorganism in the air. Utilizing these units in key areas, hospital waiting rooms, schools, and laboratory areas to name a few would help reduce the spread of disease causing organisms.

A commercial air purifying system the LeVocc process was tested to determine the efficacy of reducing microorganisms in both an open and closed area. In testing other air purification units the LeVocc process showed the best testable results. The LeVocc process uses photocatalytic oxidation technology to oxidize particles in the air. This technology has a core surface covered with titanium and platinum nanoparticles, which are activated by UV light, creating a powerful oxidizer. This oxidation technology has been found to inactivate volatile chemicals (VOC) as well as inactivating biological material. Our purpose of experimentation is to define the ability of the LeVocc process to inactivate microbial organisms and remove VOC's. The biological experiments will be carried out in two stages, 1) Defining the efficacy of the LeVocc process to remove and inactivate bacterial, viral, and fungal species when challenged. 2) To determine the ability of the LeVocc process to inactivate viral, bacterial, and fungal species from an enclosed and open area test chambers.

MATERIALS and METHODS

Test Strains:

All strains were chosen to represent a wide range of microorganism. The following test organisms were used MRSA as a vegetative bacteria, *Bacillus atropheaus* as a spore former, Aspirgillus Niger as a fungal strain and HINI as a viral strain for evaluation.

Bacillus atropheaus: A surrogate of *Bacillus anthracis, B.* atropheaus is a Gram positive catalase positive bacterium, carries a rod shape and ahs the ability to form a tough protective endospore allowing the bacterium to tolerate extreme environmental conditions.

Methicillin-resistant *Staphylococcus aureus* (MRSA). This is a designation for any *Staphylococcus aureus* that has developed resistance to beta-lactam antibiotics. *S. aureus* is a facultative anaerobic Gram-positive coccal bacterium. This organism is a common nosocomial transmitted bacteria.

Influenza A virus (H1N1 Puerto Rico 8 strain) is a subtype of influenza virus, which causes the common cold. Influenza A viruses are negative-sense, single stranded, segmented RNA viruses.

Aspirgillus Nigar a fugal strain that causes a disease called black mold. Commonly found in both indoor and outdoor environments, aspirgillus strains have been shown to cause respiratory problems in humans.

Mycobacterium vaccae is a non-pathogenic species from the Mycobacteriaceae family. Used as a surrogate for mycobacterium tuberculosis, *M. vaccae* was tested to evaluate the ability of the LeVocc process to inactivate this organism.

Aerosolization Test Box:

A testing unit (Figure 1) was designed and built internally for the purpose of aerosolization testing. The box is equipped with an aerosolization port as well as a pressure release port that is fitted with a HEPA filter. Virus was aerosolized using a 6 Jet Collision Nebulizer (BGI Inc.). Air samples were taking using a MAS-100 Microbial Air sampler (EMD Chemicals Inc.). Petri plates were placed in the air samplers containing 5ml of virus culturing media, to catch any virus in the air that was sampled. For each test, 1000L of air was sampled with each of the 2 air samplers, which results in sampling the air in the enclosure approximately 3 times per air sampler.

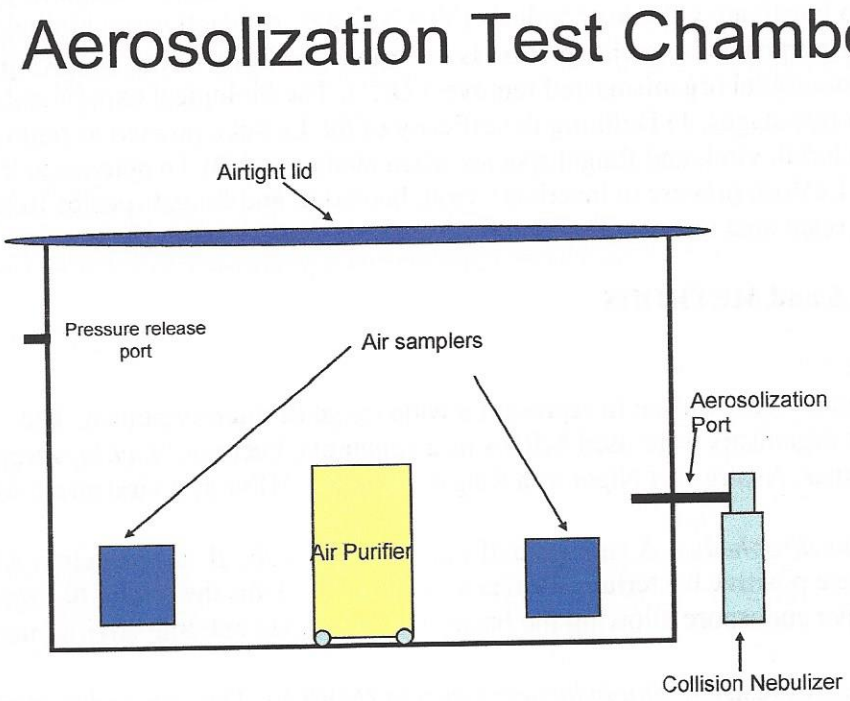

Figure 1. Aerosolization test chamber, this chamber was manufactured to remain airtight during biological testing. Organisms were nebulized and testing conducted according to test organism used.

Air Samples:

Air samplers were used to detect microorganisms present in the test chamber during experimentation. Two Andersen-style MAS100 Eco air samplers were used during this experimentation. An Anderson-style air sampler deposits air directly onto the entire surface of the agar/tissue culture plate, which allows for the quantitation of test organisms.

Analysis of Air Samples:

For bacterial testing, TSA plates were incubated at 35^0C to examine the residual bacterial counts, viral testing was carried out on VERO E6 cells, tissue culture plates were incubated at 37°C with 5% CO_2. Air sampling was carried out over 15min intervals, with the Levocc unit running for 15min then sampling of air and surface areas.

Surface Sampling and Analysis:

Surface samples were identified and labelled breaking the test chamber into three areas of testing. 1) Right side of LeVocc unit, 2) left side of LeVocc unit, 3) under the unit once testing is completed. Each area was sampled only once, sponge wipes were placed in sterile bags with 100ml of PBS. The bag was agitated on a bag mixer at speed the highest speed for 90sec. the entire volume was collected, an additional 100ml was added and the process carried out two more times. Serial dilutions of the total volume approximately 300ml were prepared and the dilutions were filtered through a 0.2um filters and grown on the TSA plates or tissue culture plates, the test organism was counted to determine the amount of residual organism.

Controls:

Negative air samples were taken by sampling the air within the test chamber box/room when empty prior to aerosolization of test organisms. Positive control samples were taken by aerosolizing test organisms and sampling of the air inside the test area. Some settling of the aerosolized test organisms was seen, occurring between 10^2-10^4 depending on which test organism was used. Positive controls with out LeVocc unit running demonstrated a loss of 0.5- 2 logs of test organism.

RESULTS

Proof of concept:

To evaluate the functional ability of the LeVocc process we conducted a test experiment to determine the ability of the LeVocc unit to remove and inactivate a viral strain (HINI). To test the virucidal ability, the air purifier was first plugged in and

allowed to warm up for 1 hour to ensure the reaction module was activated. After the air purifier was activated, it was placed in the testing box along with the air samplers. The virus was aerosolized, and then the air purifier was turned on and allowed to run for 30 minutes. After this time the air purifier was turned off and air samples were taken.

Half of each sample was used for TCID50 to determine the amount of viral particles present. Each sample was serially diluted 10 fold up to 10^{-7} and 50ul of each dilution was added to VERO E6 tissue culture cells in pentuplicate. Tissue culture plates were incubated and observed daily for cytopathic effect (CPE) which indicates the presence of test virus.

The remaining half of each sample was processed for real time PCR to determine the amount of RNA (viral genomic material) present. RNA was extracted from the samples using a Qiagen RNA extraction kit. Real time PCR was conducted using an Applied Biosystems platform and reaction kit and a primer/probe set specific for Puerto Rico 8 strain of seasonal H1N1.

Results from real time PCR and TCID50 can be seen in figure 2. A standard curve was used in the real time reaction to determine the amount of RNA present in each sample and this was used to calculate the approximate TCID50 based on the standard curve and viral titer controls. As seen in figure 2 after aerosolization, sampling methods were able to detect approximately 2 logs less than the originally calculated viral titer. This indicates that aerosolization and sampling methods were successful, but some loss of virus was seen on the surface due to settling. After running the photocatalytic air purifier for 30 minutes no virus was detectable in the air. Testing of the LeVocc filter demonstrated the presence of no organism or any genetic material of the test organism.

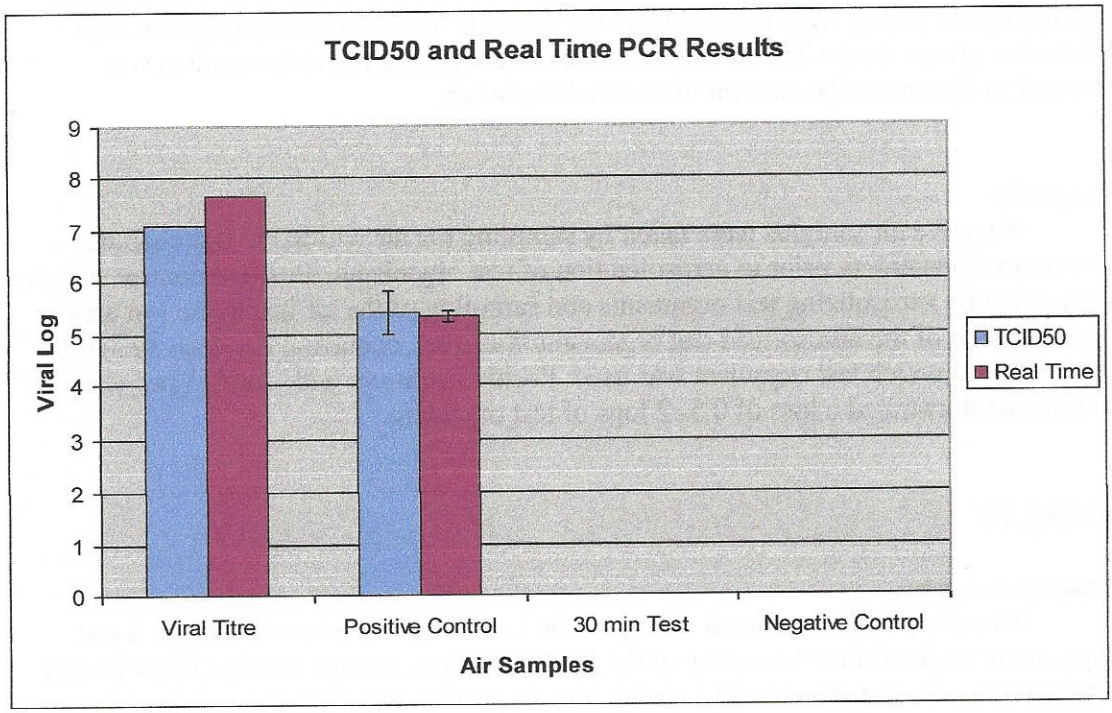

Figure 2. Viral concentration detected (in $\log_{10}$ per ml) based on TCID50 and real time PCR for samples taken on testing day 3. Also included are the TCID50 and real time PCR results determined for the viral culture.

LeVocc 100 unit testing:

The LeVocc 100 unit was tested for its efficacy to remove test organisms from the test chamber. Test organisms were aerosolized within the test chamber and an evaluation of residual organisms was tested. All starting concentrations were 10^6 CFU/FFU, this concentration was used to flood the system to evaluate the LeVocc process ability to neutralize the test organism. These experiments were broken up into three parts. 1) Determining test organism in the air 2) Determining test organism on which has settled on the surface of the test chamber 3) Determine the reduction of test organism in the air by the LeVocc unit.

Influenza A analysis (H1N1 Puerto Rico 8 strain)

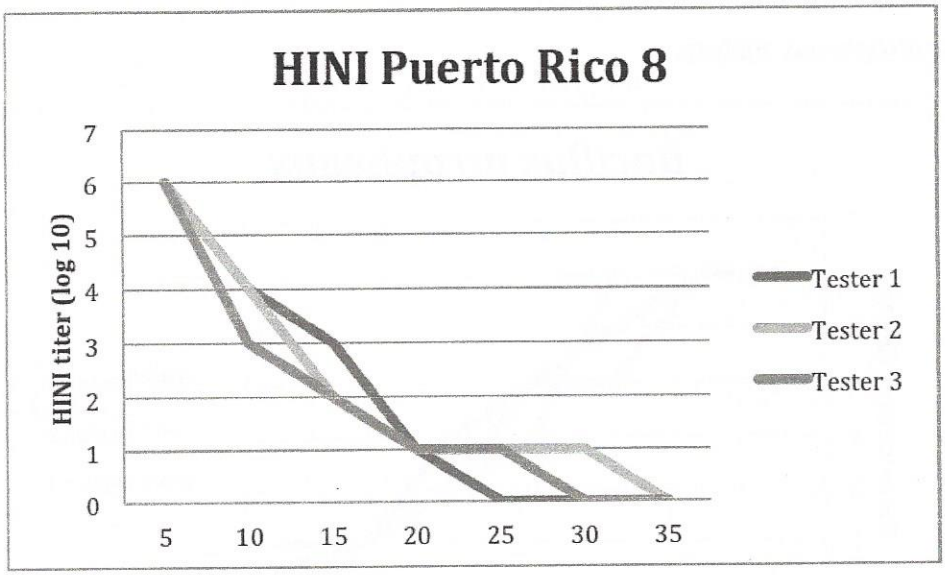

Figure 3, Sampling of circulating air within the test chamber, samples were taken in five-minute intervals to determine the amount of test virus within the air of the test chamber.

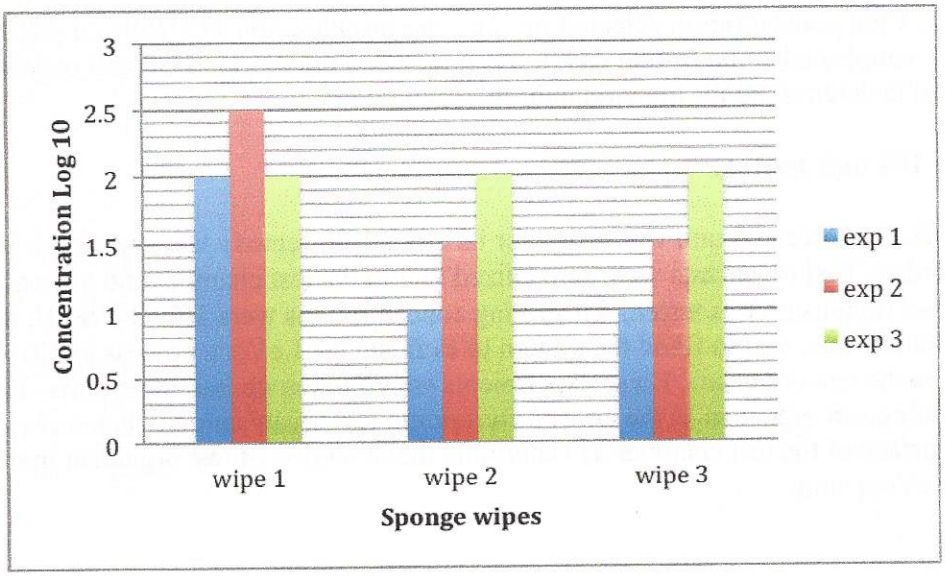

Figure 4, Surface sampling for HINI within the test chamber after experimentation was completed. Residual organism is expected as circulation was dependant on air circulation from both the LeVocc unit and air samplers.

Bacillus atropheaus analysis

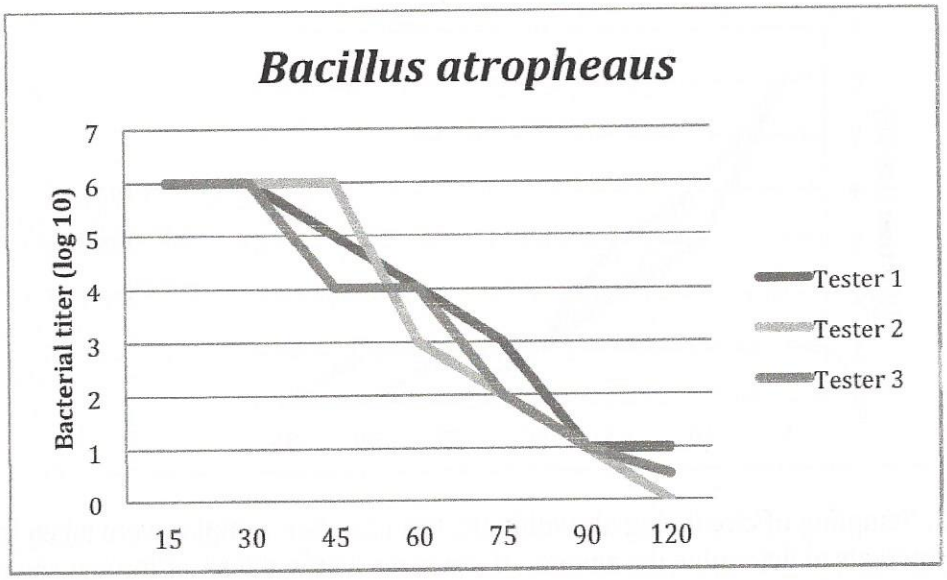

Figure 5, Sampling of air within the test chamber, samples were taken in fifteen-minute intervals to determine the residual amount of *B.atropheaus* within the test chamber.

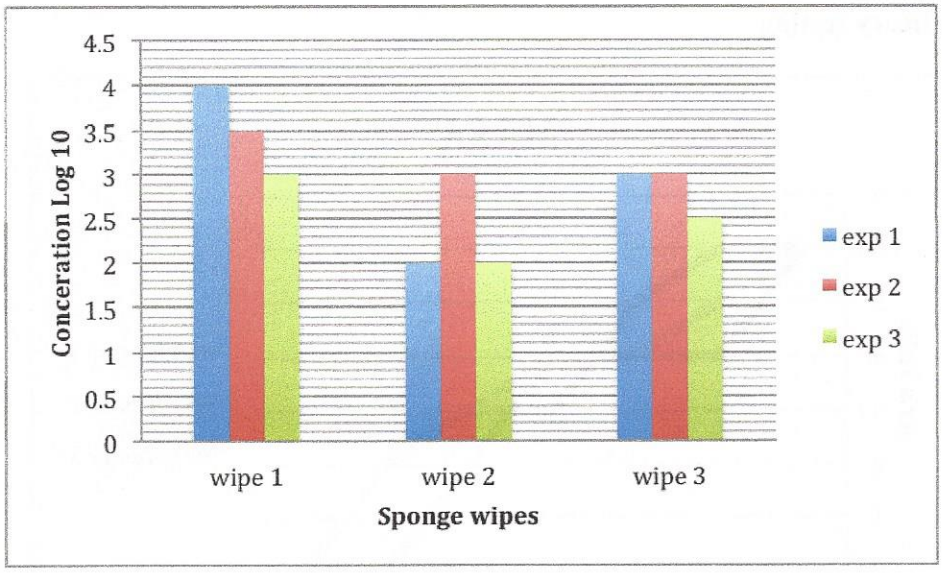

Figure 6, Surface sampling of test chamber after test run was finished. Residual organism is expected as circulation was dependant on fan movement in both the LeVocc unit and air samplers. Spores settled quickly in the area of the aerosolization nozzle (wipe 1).

MRSA efficacy testing

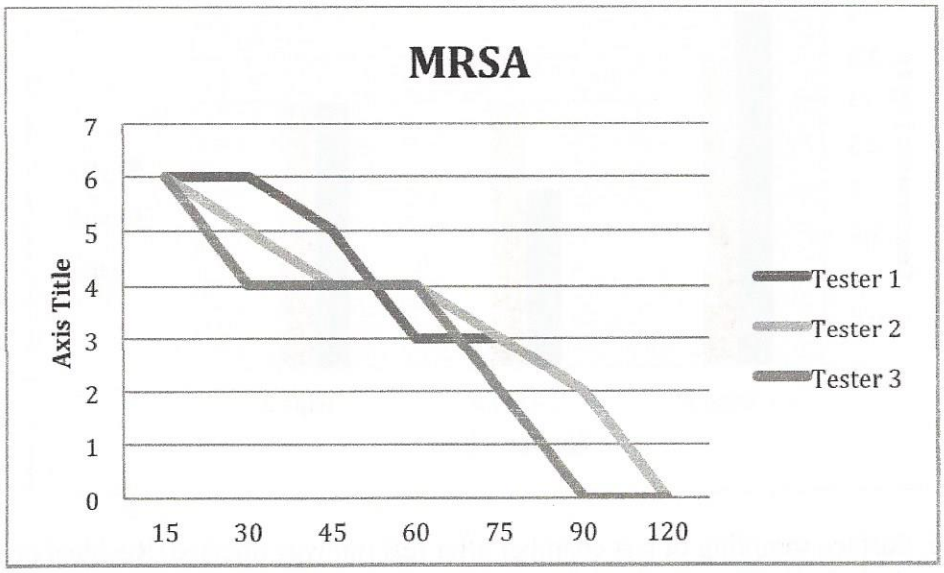

Figure 7, Sampling of air within the test chamber, samples were taken in fifteen-minute cycles to determine the amount of residual test organism within the chamber.

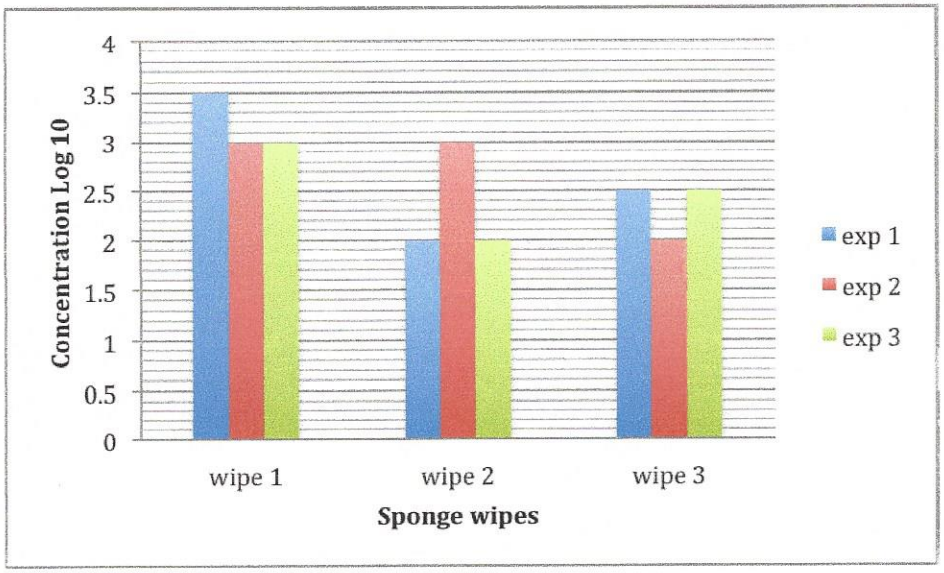

Figure 8, Surface sampling of test chamber after test run was finished. Residual organism is expected as circulation was dependant on fan movement in both the LeVocc unit and air samplers. Higher concentrations were seen at the site of aerosolization.

Aspirgillus Niger efficacy testing:

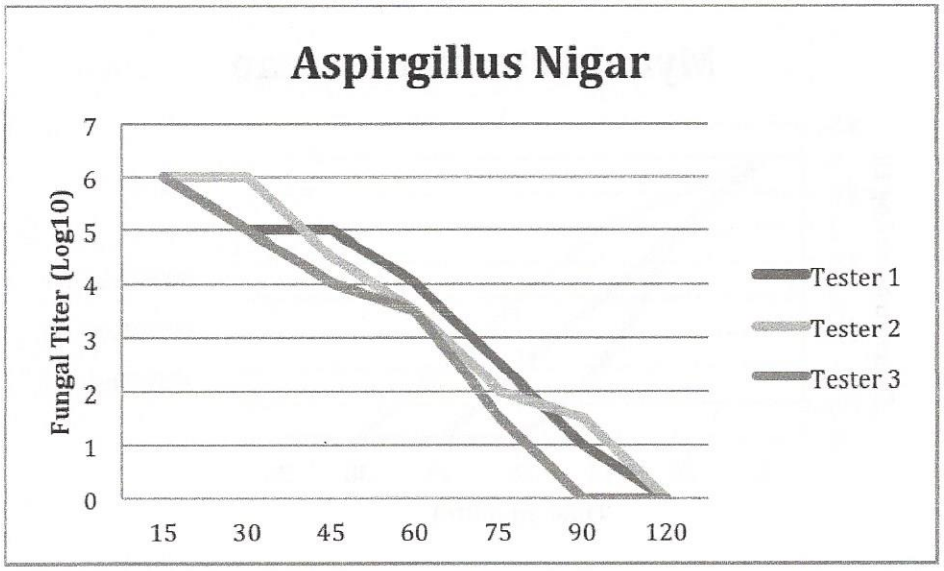

Figure 9, Sampling of air within the test chamber, samples were taken in fifteen-minute cycles to determine the amount of test organism in the circulating air within the test chamber.

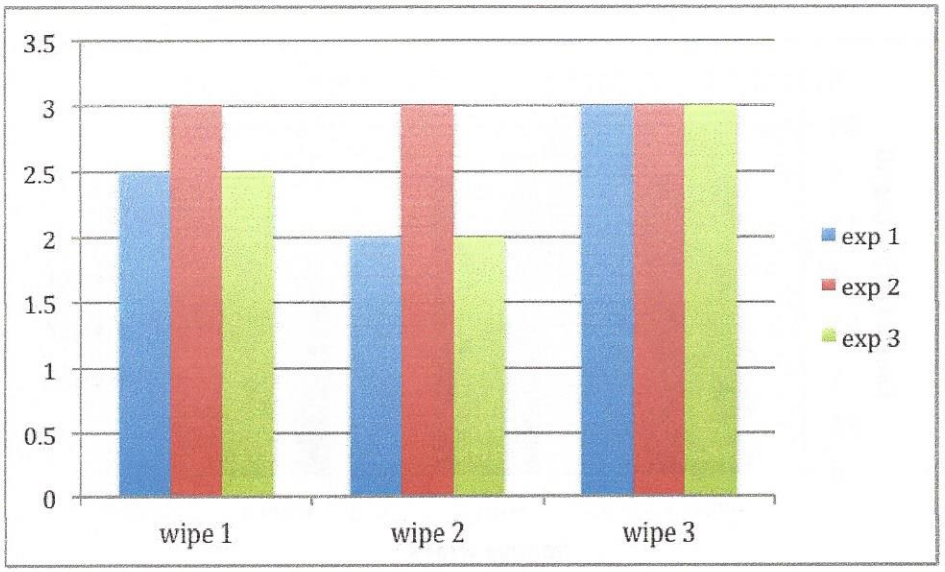

Figure 10, Surface sampling for aspergillus niger within the test chamber after test run was completed. Residual organism is expected as circulation was dependant on fan movement in both the LeVocc unit and from air samplers. Higher concentrations were seen at the site of nebulization

***Mycobacterium vaccae* testing**

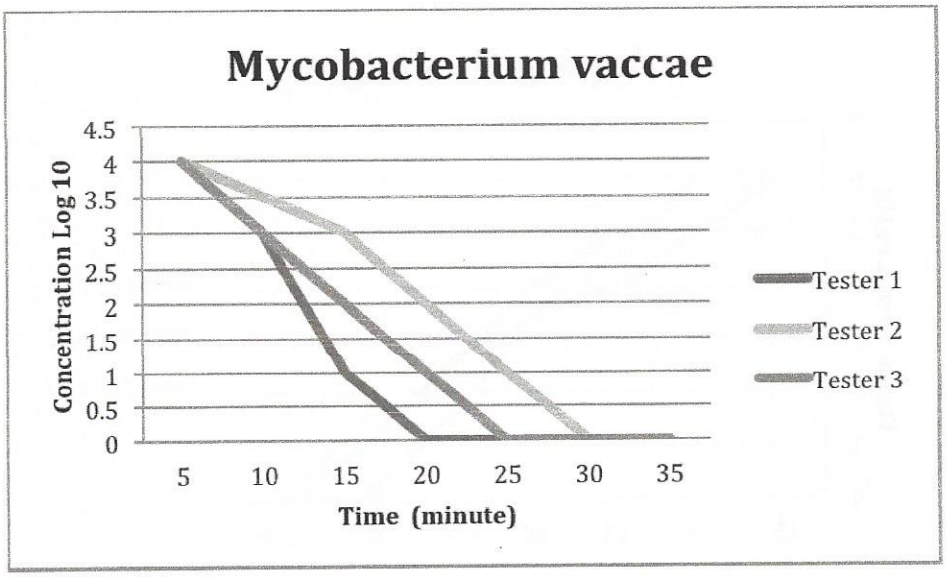

Figure 11. Sampling of the air within the test chamber, samples were taken in five-minute intervals to determine the amount of live *Mycobacterium vaccae* within the test chamber. Settling occurred very quickly which resulted in greater settling

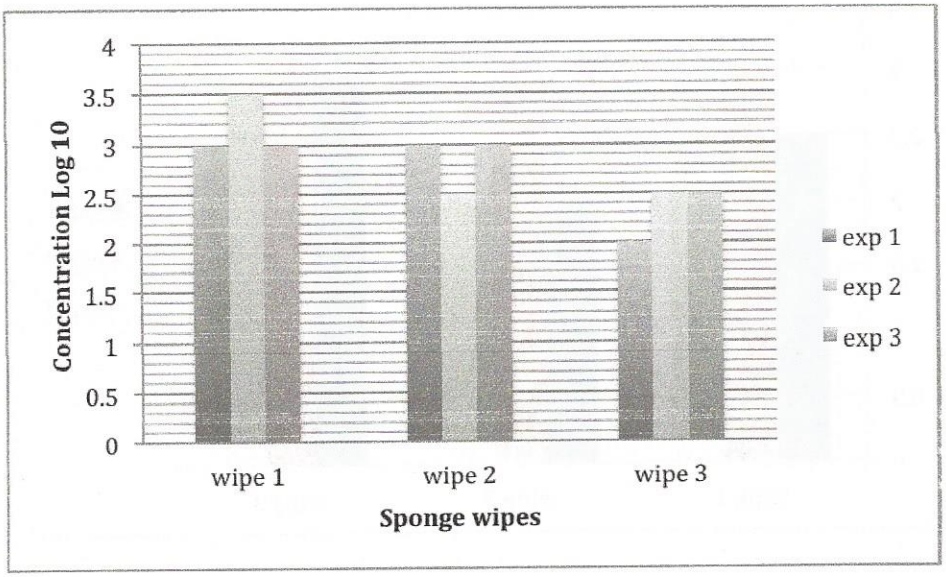

Figure 12. Surface sampling for *Mycobacterium vaccae* within the test chamber after the test run was completed. Residual organism is expected as circulation was dependant on fan movement in both the LeVocc unit and from air samplers. Higher concentrations were seen at the site of nebulization. *Mycobacterium vaccae* was difficult to aerosolize and thus gave varied results as well as lower concentration of starting material.

LeVocc 500 unit Testing:

The LeVocc 500 unit is a larger unit with the capacity to circulate large amounts of air in an enclosed area. To demonstrate the ability of the LeVocc 500 unit testing was carried out within an enclosed decontamination room (12x12 room), two fans were set-up in opposite corners of the room to help facilitate air movement. Air samples were placed through out the room to determine any residual test organism in the air during testing.

Influenza A testing (H1N1 Puerto Rico 8)

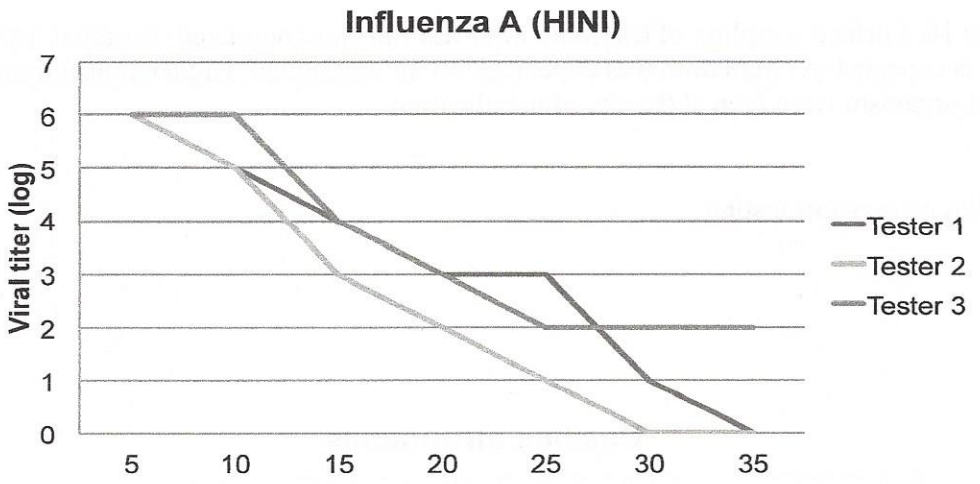

Figure 13, Sampling of air within the test chamber, samples were taken in five-minute intervals to determine the amount of live virus within the test chamber.

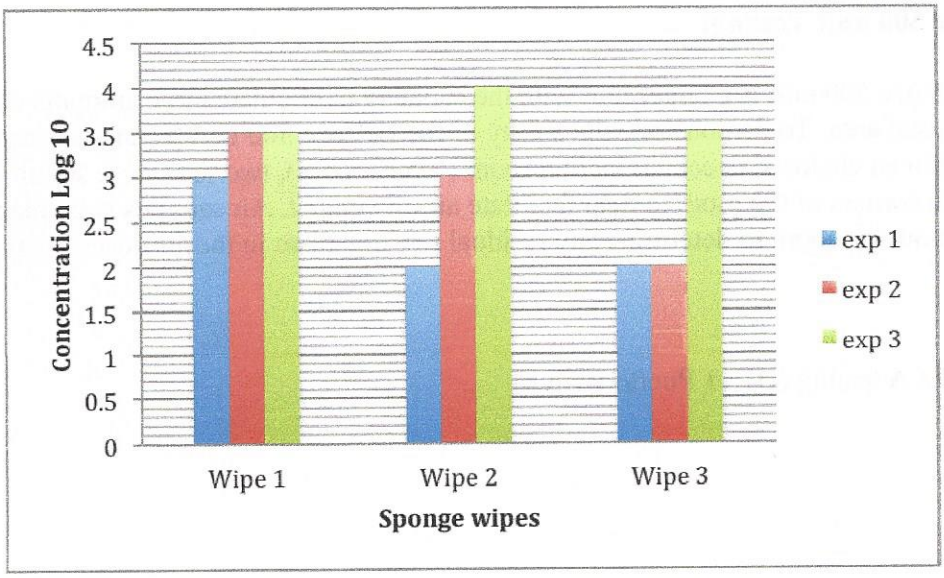

Figure 14, Surface sampling of test room after test run was completed. Residual influenza virus is expected as circulation was dependant on air circulation. Higher concentrations of test organism were seen at the site of nebulization

Bacillus atropheaus testing

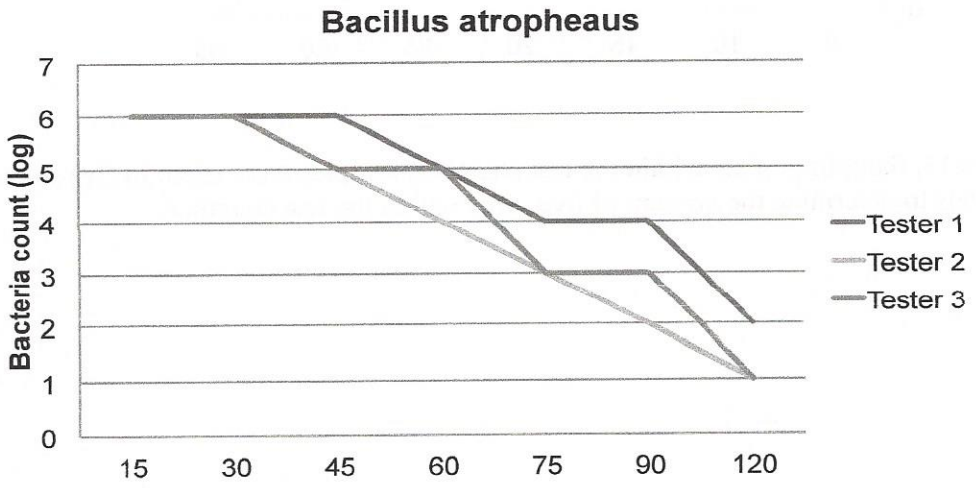

Figure 15, Sampling of circulating air within the test chamber, samples were taken in fifteen-minute intervals to determine the amount of residual bacteria within the test chamber.

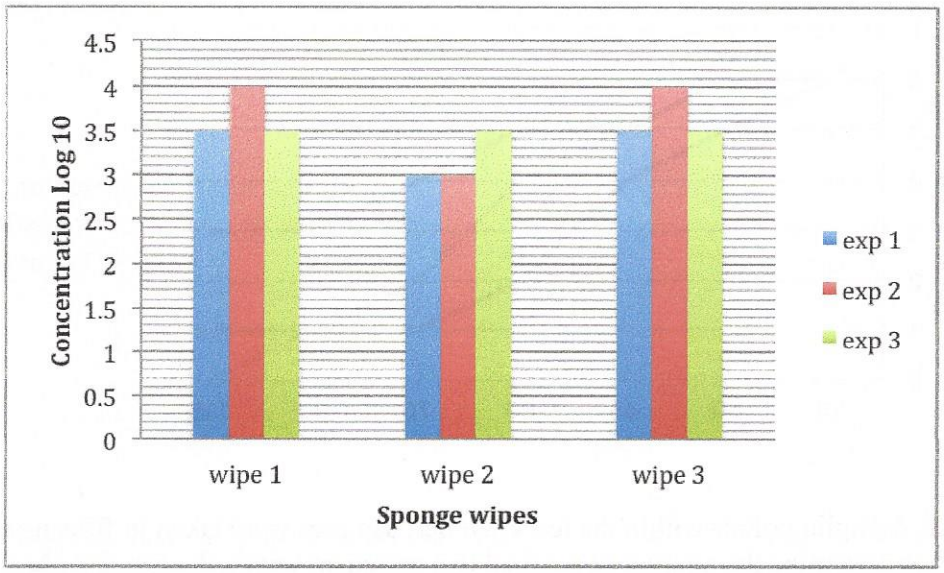

Figure 16, Surface sampling of test chamber after test run was finished. Residual organism is expected, as circulation was dependant on fan circulation. Higher concentrations of test organism were seen at the site of aerosolization.

MRSA efficacy testing

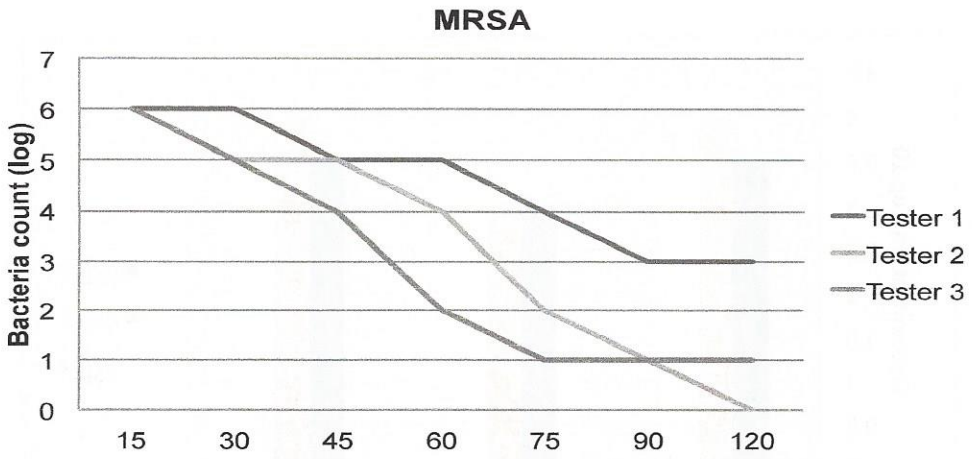

Figure 17, Sampling of air within the test chamber, samples were taken in fifteen-minute intervals to determine the amount of residual test organism within the test chamber.

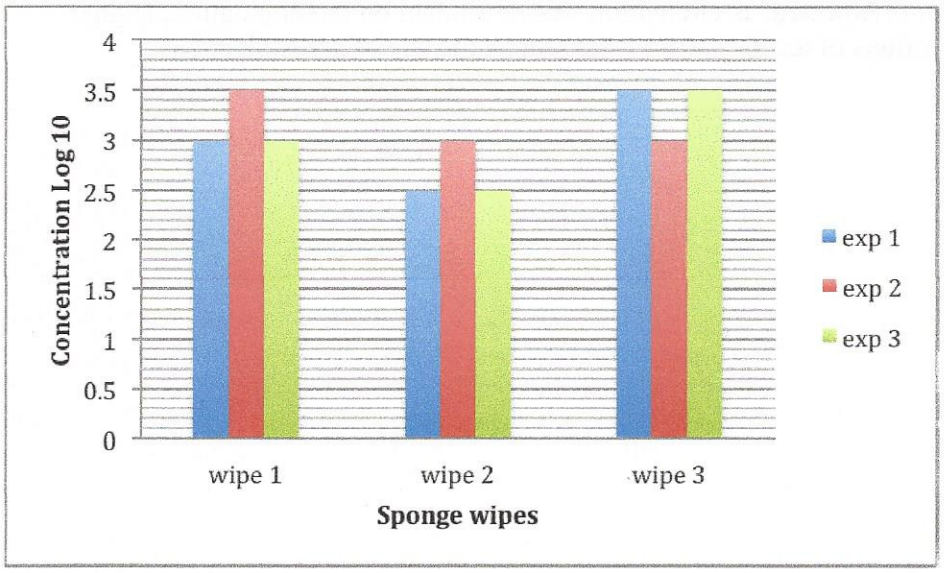

Figure 18, Surface sampling of MRSA within the test chamber after the test cycle was completed. Residual organism is expected as circulation was dependant on fan circulation. Higher concentrations were seen at the site of aerosolization.

Aspergillums Nigar efficacy testing

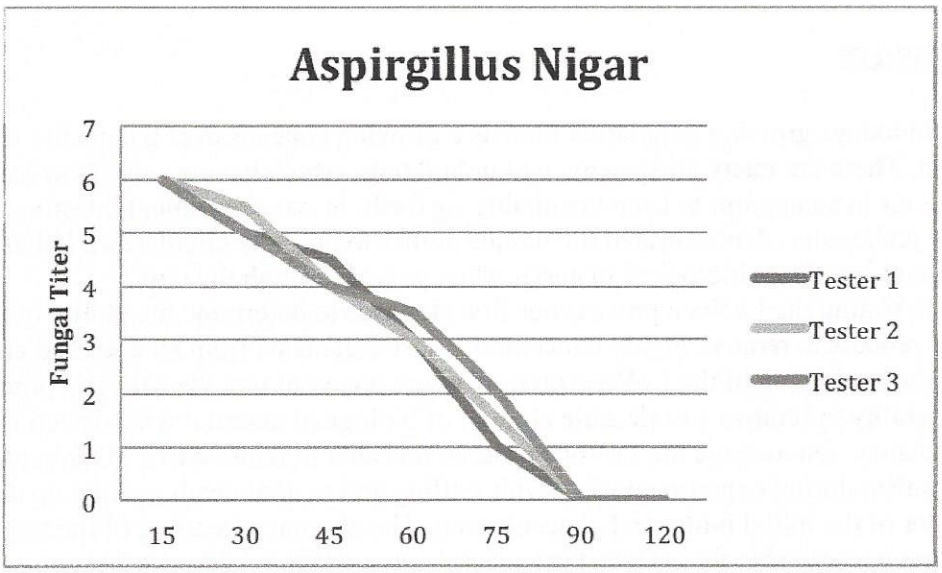

Figure 19, Sampling of air within the test chamber, samples were taken in fifteen-minute intervals to determine the amount of residual test organism in the circulating air within the test chamber during experimentation.

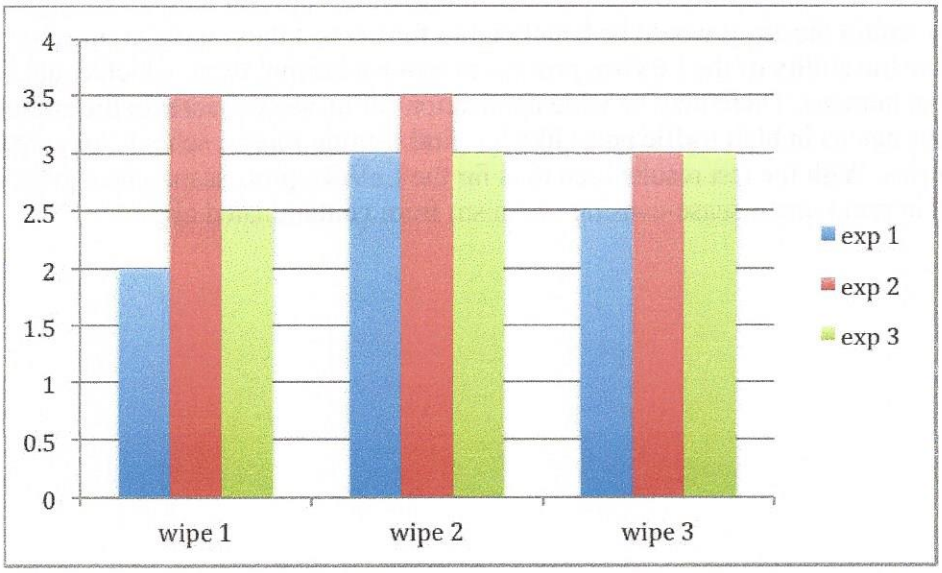

Figure 20, Surface sampling for aspergillus niger within the test chamber after test run was completed. Residual organism is expected as circulation was dependant on fan movement in both the LeVocc unit and from air samplers. Higher concentrations were seen at the site of nebulization.

DISCUSSION

In todays growing population there is a growing concern over the quality of air we breath in. There are many air systems within buildings which have an ability to remove or circulate air in an attempt to keep breathable air fresh. In our experimental testing the LeVocc process has demonstrated the unique ability to not only circulate air but also to remove and inactivate biological material when passed through the unit..

In testing the LeVocc process our first step was to determine the ability of the LeVocc process to remove highly concentrated test organisms from an enclosed area. During the evaluation of the LeVocc process it was apparent that the catalytic process had the ability to remove a noticeable amount of biological material when tested in an enclosed area. On average the LeVocc process was able to remove two to three logs of test organism during experimentation, with settling and natural death making up the remainder of the initial biological concentration. The amount of settling of the test organisms was variable for each test organism on average 0.5 – 2 logs of test organism was lost due to natural death and other settling. Since testing utilized a very high concentration of biological material we pushed the limits of the LeVocc process. As seen in the above figures the LeVocc process demonstrates the ability to remove and inactivating biological material.

The LeVocc process demonstrates the ability to remove variable types of micro-organisms, spores, vegetative bacteria, mycobacterium, and negative stranded viruses were tested. With the ability of the LeVocc unit to inactivate and remove biological material within the air, it would be beneficial to further test these units in open areas to determine the ability of the LeVocc process to remove normal flora, which could cause disease in humans. There may be wide application for its use to decrease the amount of infectious agents in high traffic areas like hospital waiting rooms, schools, or containment laboratories. With the test results seen thus far the LeVocc process may be a great step forward in removing disease-causing organism from contaminated air.