AN ASSESSMENT OF ASPERGILLIS FUNGI BYPASS FOR A SMALL COMMERCIAL HVAC UNIT

A Thesis

by

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ABSTRACT

Aspergillus is a commonly found fungus in both indoor and outdoor environments. A common, not so harmful fungi for most people, this fungus can lead to aspergillosis in persons with suppressed immunological systems. This is most likely to occur in a hospital setting with patients of differing levels of immuno-compromised status. Aspergillosis is an upper respiratory infection that is similar to other respiratory diseases such as pneumonia. Once the fungus has invaded the lungs, it can easily spread to other parts of the body. Modern healthcare facilities feature the use of air conditioning systems with filters that capture Aspergillus spores before they can be re-distributed to the building through the system ductwork. In this study, air sampling was performed at the inlet (return air) and outlet (supply) grilles of a small, commercial air conditioning unit serving a university classroom/office building. The difference in Aspergillus counts between inlet and outlet of that unit were sampled over a period of two months. Results showed a consistent pattern of a capture of about 40% of the Aspergillus spores entering the unit filter. This also means that about 40% of the Aspergillus spores were bypassing or passing through the air conditioning filter system and being redistributed into the air of the building. These results, when extrapolated to a healthcare facility setting, demonstrate the importance of proper selection, seating/sealing, and maintenance of this important element of the air conditioning system.

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CONTRIBUTORS AND FUNDING SOURCES

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NOMENCLATURE

CFU	Colony Forming Units
HVAC	Heating, Ventilation, and Air Conditioning
MERV	Minimum Efficiency Reporting Value
CDC	Center for Disease Control and Prevention

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Biological particles such as fungi, bacteria, pollen and viruses, are present in air, either living or originating from living organisms. The collection, culturing and investigation of microorganisms present in indoor air continues to receive attention because of the impact these organisms can have on human health and comfort.

Aspergillus is one of many types of fungi and is commonly found in soil, water, decaying vegetation and in the air we breathe. The spore size for this fungus ranges from 2 microns to 3.5 microns (Reponen et al, 2001). It is commonly found breeding on bread and potatoes, and grows in or on many plants and trees. In short, it has an extensive existence in our environment. Though typically benign to humans, its presence must be controlled in a healthcare environment because Aspergillus can lead to various types of infections such as aspergillosis.

Aspergillosis is a respiratory disease that is the result of an Aspergillus infection. The invasion of fungi into the environment of a healthcare facility can lead to such nosocomial infections. Invasive aspergillosis (IA) is the primary reason of infection leading to loss of life in patients admitted for blood stem cell transplantation, those with cancer or tumors, or patients undergoing organ transplantation (Lortholary et al. 2011). The presence of Aspergillus in an environment that is occupied by humans with weak immune systems is of some concern. Aspergillus spores have been found when sampled from various healthcare sources such as HVAC systems, dust released due to remodeling work or new construction, food, and on flat surfaces like the floor. Aspergillosis related to the lungs is the most common healthcare issue associated with aspergillosis, caused due to inhalation of the Aspergillus spores in air (Weber et

al. 2009). The fungus, upon entering the body, may use the blood to spread inside the body, to reach the various other internal organs. Acute invasion of aspergillosis happens mainly when the immune system is so weak that it can't prevent Aspergillus spores from entering the bloodstream, through the lungs. The weak immune system fails to generate an immune response to fight the spores, the fungal spores spread inside the body and can damage internal body organs.

A study was conducted for Aspergillus presence in hospitals where 200 bone marrow patients were examined (Curtis et al. 2005). The researchers collected practical fungi samples in the healthcare unit every six days and investigated 74 HVAC duct samples for colony forming units (CFU) of Aspergillus. The total of various type of fungi were 257.8 CFU /m^3 in all outdoor environment samples, 53.2 CFU/m^3 in all samples collected inside the building, and 83.5 CFU/m^3 in rooms that had patients undergoing bone marrow treatment. The fungi of concern, Aspergillus, was found to be 6.8 CFU/m^3 outdoors, 12.1 CFU/m^3 in all indoor samples, and 7.3 CFU/m^3 in the bone marrow transplant patient rooms. Interestingly, the study found that Aspergillus propagule concentrations were higher in the indoor environment, compared to the outdoor environment, in all parts of the healthcare unit.

English-language literature has reported more than 60 infection cases related to aspergillus (Weber et al. 2009). A study of 2,496 bone patients in a U.S. cancer hospital reported 143 deaths due to invasive aspergillosis (Arnow et al. 1978). Invasive Aspergillosis is the reason patients admitted with a curable disease might end up losing their life due to a nosocomial infection caused by Aspergillus. It is difficult to relate a particular quantity of colony forming units to be a number than can be considered as a disease hazard among patients, including mainly those having a weak immune system. The Centers for Disease Control and Prevention

(CDC) has not developed or promoted any standards for regular microbiologic air collection before, during, or after the building's construction or remodeling, or for areas in intensive care units (Vonberg and Gastmeier 2006). Invasive Aspergillus is difficult to detect, at an early state, in the hospital environment. Thus, measures to minimize exposure of patients to Aspergillus fungi may be the most effective means of preventing infection and its complications. Understanding the entry of these spores into a building's environment then, is of significant importance. Every day, humans are exposed to an almost infinite number of biological particles, including all microorganisms that can affect humans positively and negatively. The major carriers of biological aerosols inside a building are humans, pets, HVAC and plumbing systems (Prussin and Marr 2015). Each of these potential carriers are discussed below.

Humans

Humans are a large contributor to the population of microorganisms present in air as they carry 1,012 microorganisms on their skin and 1,014 microorganisms in their digestive tract (Luckey 1972). Processes like those of respiration and skin cell shedding contribute towards the population of air borne microorganisms in air. Certain species of fungi may be released as biological particles upon shedding of the epidermis. Researchers have found that on average, 7.3×10^6 fungal genome copies were emitted per person-hour in a built environment. The corresponding mass emission rate was ~30 mg per person-hour (Qian et al. 2012).

Heating, ventilation and air conditioning (HVAC) system

HVAC systems generally provide a mixture of outdoor air and recirculated indoor air to the building or zone being conditioned. The system supplies air, under pressure, through duct systems and usually a portion of this air is recirculated through a return system to the air handler. However, contamination can result in these systems becoming a source of microorganisms

present in the air. (Dondero Jr et al. 1980). During duct cleaning operations, significantly higher concentrations of Aspergillus have been recorded.

The filters in the HVAC system are responsible for keeping a check on the particulates entering the building via the ducts. In hospitals, high efficiency particulate arresting (HEPA) filters are recommended as a preventative measure to keep out harmful microorganisms from entering the facility as it is less expensive when compared to laminar air flow units (MIB 9). In healthcare facilities where HEPA filters are not present, measures such as damp dusting or moving of patients and installation of barriers are recommended. Air sampling is indicated if these measures cannot be implemented, but there are no accepted standards for air sampling and normal air still contains Aspergillus spores.

Filters

Different type of buildings/occupancies have different requirements for filtration of the indoor air. There are various types and sizes of filters available on the market. According to ANSI/ASHRAE Standard 52.2, filters are divided in the categories as shown in Table I. The table shows the common rating for HVAC filters as well as their typical applications. Generally, MERV (Minimum Efficiency Reporting Value) 13 – 16 rated filters are recommended for a hospital, as these filters are effective to capture particles as small as 0.3–1.0 μm whereas a normal commercial building would have a MERV 5 to 8 filter installed.

MERV Std 52.2	Efficiency (%)	Particle Size (microns)	Typical Applications
1–4	60–80	>10	Residential building Light Commercial building
5–8	80–95	3.0–10.0	Industrial work place Commercial Better Residential Paint booth
9–12	90–98	1.0-–3.0	Superior Residential Better Commercial buildings Better Industrial workplaces
13–16	95–99	0.3–1.0	Smoke removal General Surgery, Hospital and healthcare Superior Commercial buildings

Table I. Filter types and their related efficiencies per ANSI/ASHRAE Standard 52.

The sampling of Aspergillus in air is a problematic procedure because it depends on various physical and biological factors. Biological factors such as the survival capacity of the Aspergillus during aerial transport is as important as germination and growth requirements. The physical size of Aspergillus is the characteristic which helps in understanding its aerodynamic behavior. The diameter of Aspergillus spores is 2 – 3.5 microns, which is therefore an important measure in understanding how this fungus can be trapped in an air sampler (Morris et al. 2000).

There are also difficulties understanding the impact of numerous variables like building occupants, weather, etc. Human traffic in buildings has also been shown to affect the sampling of Aspergillus, but the full extent of this affect has yet to be studied fully (Buttner and Stetzenbach 1993). Also according to earlier studies, it has been difficult to associate the concentrations of Aspergillus with specific seasons or with weather conditions (Lutz et al. 2003).

CHAPTER II

PURPOSE OF STUDY

Health issues related to the presence of Aspergillus in a healthcare facility is a known threat. The presence of Aspergillus can be a source of nosocomial infection in such a facility, and the heating, ventilating, and air conditioning system of a building is a known vector for the movement and growth of fungi. The proposed study is an attempt to develop a repeatable air sampling protocol for an HVAC unit that will be used as a surrogate for a similar unit operating in a healthcare setting. The sampling will provide quantifiable information on the number of Aspergillus colony forming units (CFU) that are entering and exiting this unit. This CFU count for Aspergillus spores that are returned to the indoor air of the building and after filtration will provide an indication of how this fungus might be "held" in the HVAC unit and, by extension, characterize this same behavior in a healthcare operation. In that environment, the supply of Aspergillus into the building's air could contribute to the development of nosocomial aspergillosis infections. This study aims to develop the basis or protocol for further studies on this topic. Ultimately, how changes in Aspergillus bypass or pass-through as related to routine changing of the HVAC filter system also needs to be investigated. Nevertheless, without an understanding of how to sample, where to sample, and how to quantify these samples, further study of the problem cannot move forward. The goal of the current research is that an understanding of Aspergillus passing through an HVAC system and the filtering systems in those systems will lead to improved system operation to minimize the reintroduction of Aspergillus into the building indoor environment.

CHAPTER III

MATERIALS AND METHODS

Field sampling of indoor Aspergillus was performed during the months of March, April, and June 2017. The experiment was conducted in Francis Hall, a building on the campus of Texas A&M University, in College Station, Texas. This building houses the Department of Construction Science and was chosen because of availability and because the configuration of the HVAC system was of a common type. Francis was originally built around 1912 and a complete renovation was completed in early 2015. ALL new interior construction/materials including new HVAC system. The intent was to select an air handling unit (AHU) of a type that might be found in a typical healthcare facility. In this study, the chosen HVAC system serving the first floor of Francis Hall was used as a surrogate for a similar HVAC system in a healthcare facility. The entry lobby/hallway of this building was served by a small central air handling unit (AHU) of a type used in many different applications including healthcare facilities. The AHU was composed of the following sections: a primary fan flowing about 3,000 cubic feet per minute (CFM) (5,097 m3/h) through a down-flow chilled water/air heat exchanger, supply air ductwork located under the floor of the lobby with supply air diffusers at several locations in the lobby/entry area, a single return air grille that directs air through a two inch (50 mm) pleated filter, and then back to the inlet of the primary fan, repeating the cycle. Fresh outdoor air was supplied to this unit from a dedicated outdoor air handler located on the second floor of the building. Refer to Figure I for a plan view of the unit and the area it serves. Figure II shows a representative elevation of the HVAC unit.

This AHU used a standard pleated air filter which was changed on a recurring basis, about every three months, by a third party service subcontractor. Though the AHU had a differential pressure gauge for the pressure drop across the filter, this was not used as an indicator of filter performance. The filter used in this arrangement had a minimum efficiency reporting value (MERV) of 8. The MERV 8 is very common as a "low end" or standard air filter type.

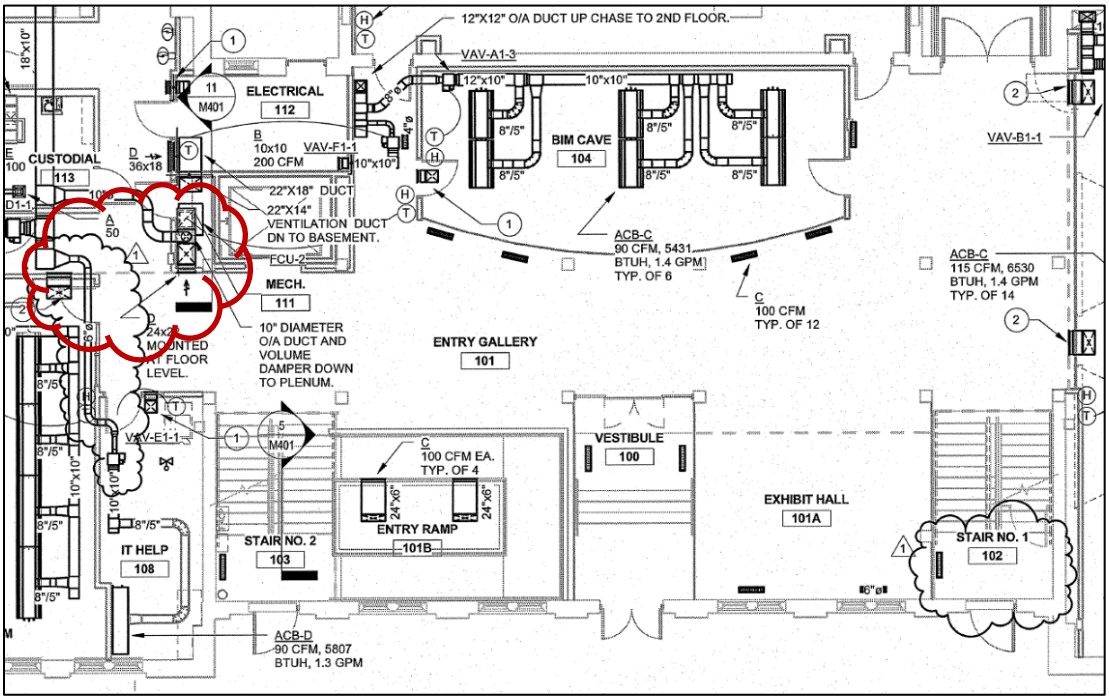

Figure I: Plan view of the lobby of the university classroom/office building and location of the HVAC unit (red "cloud" at left of drawing).

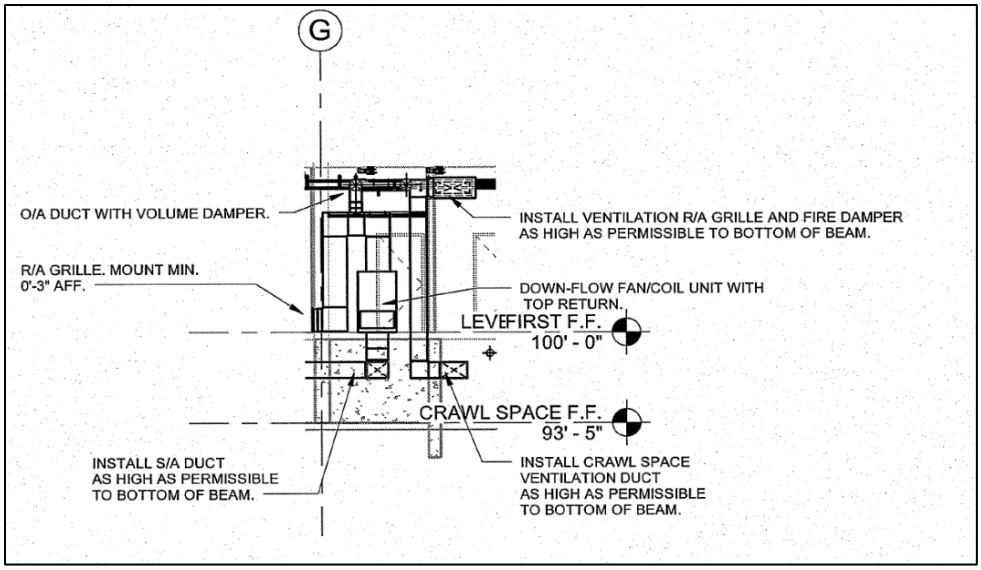

Figure II. Elevation of Francis Hall AHU serving the first floor entry of the building.

Measurement of Sample: A Zefon 37mm (0.8 um spore size) cassette filter was used to obtain air samples. The particulates were caught in the filter mesh present inside the cassette (Zefon 2017). Zefon 37mm Air Sampling Cassette w/ PVC Filters are designed to meet all applicable air sampling standards and provided a preloaded, ready to use and leak free design. Figure III shows two views of these cassettes. Each experiment used two cassettes, one placed at the return air grill of the air handler (Return) and the other at the one of the supply air openings (Supply) on the supply duct distribution system to the lobby of the building. A typical cassette installation is shown in Figure IV. The blue and red pins indicated the inlet and outlet sides respectively, which would only be opened during collection of the air samples.

Figure III. Zefon® 37mm Air Sampling Cassettes. Blue plug is air Inlet, Red plug is air outlet to sampling pump.

The outlet side of the filter cassette was connected to a vacuum pump that was set to a constant airflow rate of 30 liters per minute ± 5 lpm, or about 15 liters per minute for each cassette. The mass of these cassettes was measured before the sampling and immediately after sampling had been completed. The cassettes were carried in a dust free bag to the sampling site and the pins were only removed during the time of the experiment. The Zefon cassettes were mounted into sampling position using a two prong clamp/ring stand at the return and supply opening respectively. Both cassettes were mounted perpendicular to the direction of air-flow and at the centerline of the openings in which they were installed.

Figure IV. The position of the Zefon sampling cassettes at the return air grille (left) and supply air opening (right) for the HVAC system.

The occupants of the building were allowed to carry on their routine movement while the experiment was being conducted. The building occupant population was studied for a week and an average range of human count was considered for both batches of sampling. Sampling was conducted a total of 23 times during the course of these two sampling periods. In batch 1, (March through April) the sampling was conducted for 10 separate four-hour periods on Tuesday and Thursday of a typical week. In sample set 2, the sampling was conducted for 11 separate periods on a Monday, Wednesday, and Friday. These sampling sets were selected to correspond to typical university student schedules/movement. To understand the effect of human population on sampling more thoroughly, one sample was conducted on a Sunday when minimum student

traffic was anticipated. The weather conditions during each of the sampling sessions, including air temperature, humidity, precipitation, and wind speed, were obtained from www.wunderground.com. The sealing pins that were removed from the sample cassettes before the experiment were attached back to the cassette upon completion of the sample collection. During sampling, these pins were stored in a clean, dust-free plastic bag.

Sample Analysis

After air sampling, the filter cassettes were sealed and weighed on a precision gram scale. The Department of Biology at the Texas A&M University processed the filter cassette filters, cultured the samples, and provided counts of the colony forming units (CFU) containing Aspergillus as a fungus but they did not identify a particular Aspergillus species. All filter preparation and handling procedures were performed at this laboratory. Upon receipt of the samples, the laboratory staff would suspend each filter in 10 ml of PBS for 2 hours, vortex them, and finish by centrifuging the sample. The supernatant was discarded and the remaining 1.0 ml of suspension was then plated onto YNB agar medium and incubated at 22°C for 4 days. Colonies grown on the plate (Figure V) were counted and examined under stereoscope. After colony counts, the plates were then autoclaved. This process was carried out for both the Return and the Supply grille samples. The CFU count was then noted for each sample collected. This method was appropriate for counting the number of Aspergillus spores present in the sample but was not useful for the identification of the species of Aspergillus present in the sample.

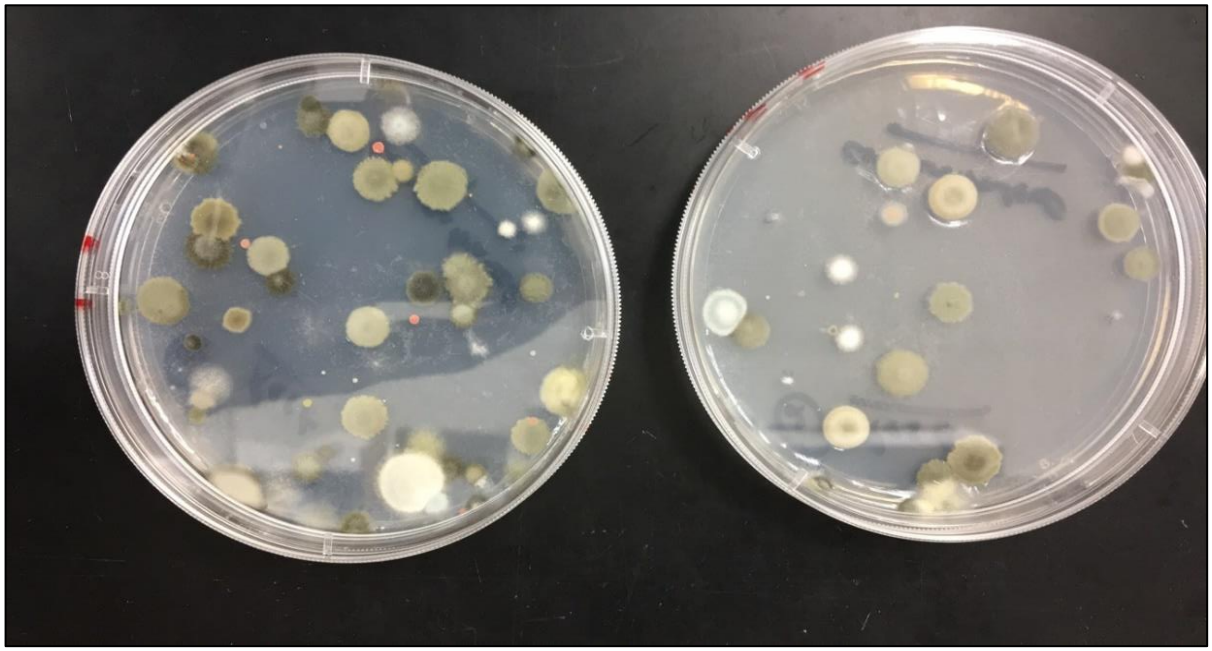

Figure V. Cultured plates after incubation at the Biology Lab at Texas A&M University. The plate on the left is the sample for the return air grille and the plate on the right is the sample from the supply air diffuser.

Data Analysis

The CFU difference (delta) for each sample set was checked for statistical significance using a Student t-test. The relationship between CFU delta and weather was determined using an independent sample t-test for independent variable. The relationship between human population and CFU delta was determined using a one-way ANOVA test. All statistical analyses were done using SPSS version 20.0.

CHAPTER IV

RESULTS

Sampling in March and April (Sample Set 1)

Table II. Results obtained after sampling in March and April (SS1).

Trial Number	Date	Human Count Range	Count at Return	Count at Supply	CFU Delta	Duration(hrs)
1	Tuesday, March 28, 2017	210-240	24	13	54.2%	4
2	Thursday, March 30, 2017	210-240	18	6	33.3%	4
3	Tuesday, April 4, 2017	210-240	57	26	45.6%	4
4	Thursday, April 6, 2017	210-240	37	8	21.6%	4
5	Tuesday, April 11, 2017	210-240	26	8	30.8%	4
6	Thursday, April 13, 2017	210-240	65	33	50.8%	4
7	Tuesday, April 18, 2017	210-240	26	8	30.8%	4
8	Thursday, April 20, 2017	210-240	78	21	26.9%	4
9	Tuesday, April 25, 2017	210-240	12	4	33.3%	4

Where,

CFU Delta = % difference in CFU count between return and supply grilles.

There were a total of 10 samples collected from 28th March through 25th April, 2017. The department of biology recommended that duration of the sampling should be at least four hours. At the flow rate of 15 lps and a duration of 4 hours, the volume of the sample was approximately 3,600 liters (3.6 m^3). This recommendation was implemented for the duration of this study. The CFU count at the return grille sample ranged from 12 to 78 CFU (3.33 CFU/m^3 to 21.67 CFU/m^3), and at the supply diffuser sample ranged from 4 to 33 CFU. The CFU difference (Delta %) ranged from 21.6% to 54.2% with a mean of 36.4%. The occupant population data inside the building was recorded for a week, and an average range of 210-240 was considered as the maximum human count for the sampling during this period.

Table III. Weather data for Sample Set 1 (www.wunderground.com)

Trial #	Date	Hi – °F	Low - °F	Dew Point	Precipitation (inch)	Wind Speed(mph)
1	3/21/2017	84	62	61	0	9(S)
2	3/28/2017	87	66	67	0	11(SE)
3	3/30/2017	77	55	49	0	10(W)
4	4/4/2017	87	60	60	0	5(W)
5	4/6/2017	77	48	40	0	1(WSW)
6	4/11/2017	73	59	63	1.62	11(E)
7	4/13/2017	82	66	64	0	6(SE)
8	4/18/2017	77	64	66	0.1	7(E)
9	4/20/2017	86	68	67	0	9(SSE)
10	4/25/2017	86	62	65	0	14(S)

Sampling in June (Sample set 2): In addition to the data obtained in March and April, sampling was conducted in June.

Table IV. Results obtained sampling in Sample Set 2 (SS2) with four hour sample duration**.**

Trial #	Date	Human Count Range	Count at Return	Count at Supply	%CFU
1	Monday, June 5, 2017	90-107	8	2	25.00%
2	Tuesday, June 6, 2017	90-107	6	1	16.67%
3	Wednesday, June 7, 2017	90-107	10	4	40.00%
4	Thursday, June 8, 2017	70-87	13	6	46.15%
5	Friday, June 9, 2017	70-87	4	1	25.00%
6	Sunday, June 11, 2017	44	9	7	77.78%
7	Monday, June 12, 2017	90-107	8	4	50.00%
8	Monday, June 12, 2017	90-107	10	3	30.00%
9	Tuesday, June 13, 2017	90-107	10	1	10.00%
10	Wednesday, June 14, 2017	90-107	8	5	62.50%
11	Wednesday, June 14, 2017	90-107	16	7	43.75%
12	Thursday, June 15, 2017	70-87	10	4	40.00%
13	Friday, June 16, 2017	70-87	13	2	15.38%

Where,

CFU Delta = % difference in CFU count at return and supply

There were a total 13 samples collected in June 2017. The duration of each was 4 hours. The CFU count at the AHU return grille ranged from 4 to 16 CFU and at the supply diffuser it ranged from 1 to 7 CFU. The CFU delta ranged from 10% to 77.8% with a mean of 37.1%. The occupant population data inside the building was recorded for a week, and an average range of 90 - 107 (Monday, Tuesday and Wednesday) and 70 - 87 (Thursday and Friday) and was considered as the maximum human count for the sampling during this period. Samples 1 to 7 were collected when a MERV 8 rated filter was installed in the air handling unit. The samples numbered from 8 to 13 were collected after a MERV 8 rated filter was replaced with a MERV 13. MERV 13 filters are commonly used in non-critical areas of a healthcare facility. Sample number 6 was collected on a Sunday (low human population), to underscore the effect of human population on the Aspergillus count.

Table V. Weather data in sample set 2 (www.wunderground.com)

Trial #	Date	Hi - °F	Low - °F	Dew Point	Precipitation (inch)	Average Humidity	Wind Speed(mph)
1	6/5/2017	84	68	71	0.17	88	4(SSE)
2	6/6/2017	88	69	70	0.00	81	8(N)
3	6/7/2017	91	69	67	0.00	66	5(NNE)
4	6/8/2017	88	70	63	0.00	61	6(E)
5	6/9/2017	84	71	66	0.01	71	7(SE)
6	6/11/2017	91	69	70	0.00	73	9(SSE)
7	6/12/2017	91	73	72	0.00	79	7(SSE)
8	6/12/2017	91	73	72	0.00	79	7(SSE)
9	6/13/2017	91	75	75	0.16	88	9(SSE)
10	6/14/2017	93	77	73	0.00	74	13 (S)
11	6/14/2017	93	77	73	0.00	74	13 (S)
12	6/15/2017	93	75	74	0.00	81	10(S)
13	6/16/2017	93	73	73	0.00	79	9(S)

Statistical analysis was used to describe the relation between the independent variable CFU Delta and the two dependent variables (Human Population and Weather). Measurement of the air sample was continuous and the comparison was made as a ratio type. The relationship of the variables could be investigated by using a parametric test (independent samples t-test for the independent variable weather and one-way ANOVA for the independent variable human population). To determine if there was Aspergillus bypass during both the sets, one-sample t-test was used. The level of significance alpha (α), was set at .05. The significance of ANNOVA test was a corrected significance level of 0.017 (0.05/3 = 0.017).

The assumptions underlying the t-test were checked for normality before applying the independent samples t-test. Normality was determined using Kolmogorov-Smirnov and Shapiro-Wilk statistical tests. The results are presented in Table VI.

Table VI. Results for Kolmogorov-Smirnov and Shapiro-Wilk test.

		Kolmogorov-Smirnov[a]				Shapiro-Wilk		
CFU Delta (%)	Sample	Statistic	df	Sig.(p)		Statistic	df	Sig.(p)
	SS1	0.274	9	**0.06**		0.907	9	**0.295**
	SS2	0.117	13	**.200***		0.959	13	**0.735**

Where,

* this is a lower bound of the true significance.

[a] Lilliefors Significance Correction

df = Degree of Freedom

Sig. = Significance.

The results presented in Table VI for two sample sets reveal that the obtained statistic for both the normality tests are not statistically significant (all p >0.05). For normal data, the points plotted in the QQ plot should fall approximately on a straight line, indicating high positive correlation and that the data follow a normal distribution with no bias. This is the case with these data, so normality in the distribution of CFU Delta (%) can be assumed for both sample set 1 and sample set 2. This normality check is shown in Figure V1 for the sample set 1.

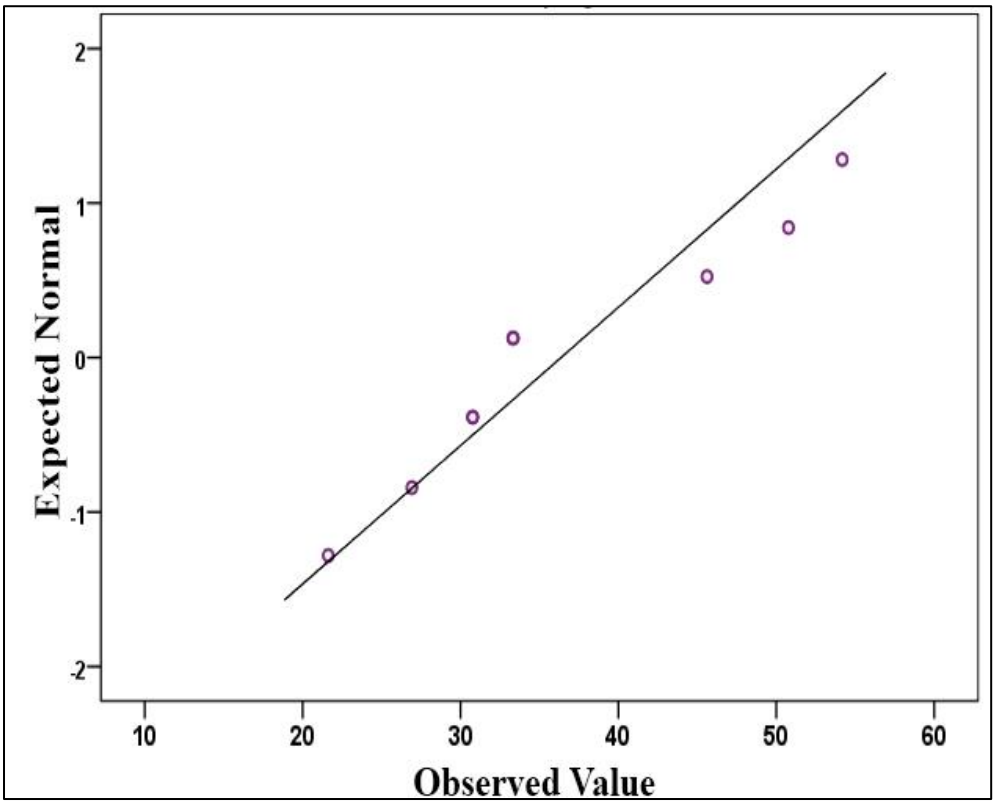

Figure VI. Normal Q-Q plot of CFU Delta for sample set 1.

In similar fashion, a normality check is presented in Figure V11 for the sample set 2.

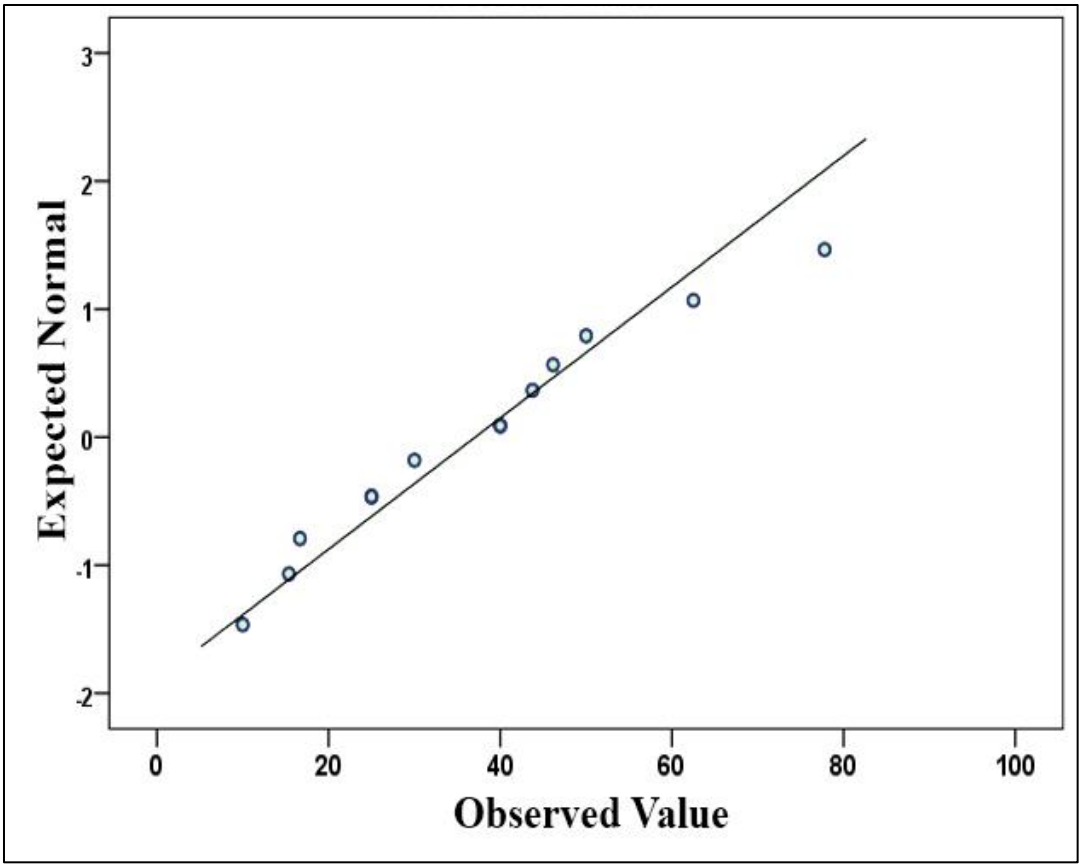

Figure VII. Normal Q-Q plot of CFU Delta for sample set 1.

As with the sample set 1 data, the sample set 2 data shows a strong correlation for normal distribution.

With the confirmation of normality for the sampled data, the assumption for equality of variances was to be checked before t-test could be run. This was performed using Levene's F test. The result of this test showed that the Levene's F statistic of 2.44 at a significance of 0.05 that the two sample populations, sample set 1 and sample set 2 come from populations that have equality of variances. Now that the required assumptions for normality and equal variances for the data sets were met, independent samples t-tests could be applied to the data. Descriptive statistics for CFU Delta (%) are presented for two sample sets in Table VII.

Table VII: Descriptive statistics for the CFU Delta: Independent Variable Weather.

	Sample	N	Mean	Std. Deviation	Std. Error Mean	Min	Max	Percentiles		
								25	50	75
CFU Delta	SS1	9	36.366	11.16717	3.72239	21.62	54.17	28.845	33.33	48.19
	SS2	13	37.095	19.5281	5.41612	10	77.78	20.835	40	48.075

The descriptive statistics presented in Table VII reveal that the mean CFU Delta for sample set 1 (M = 36.37) is lower than that for sample set 2 (M = 37.09). The standard deviation for 'sample set 2' is higher than that for the sample set 1, indicating comparatively less homogeneity in the distribution. This is because data for sample set 2 had lower minimum score and higher maximum score. The comparative differences in the distribution between the two sample sets are presented in Figure VIII.

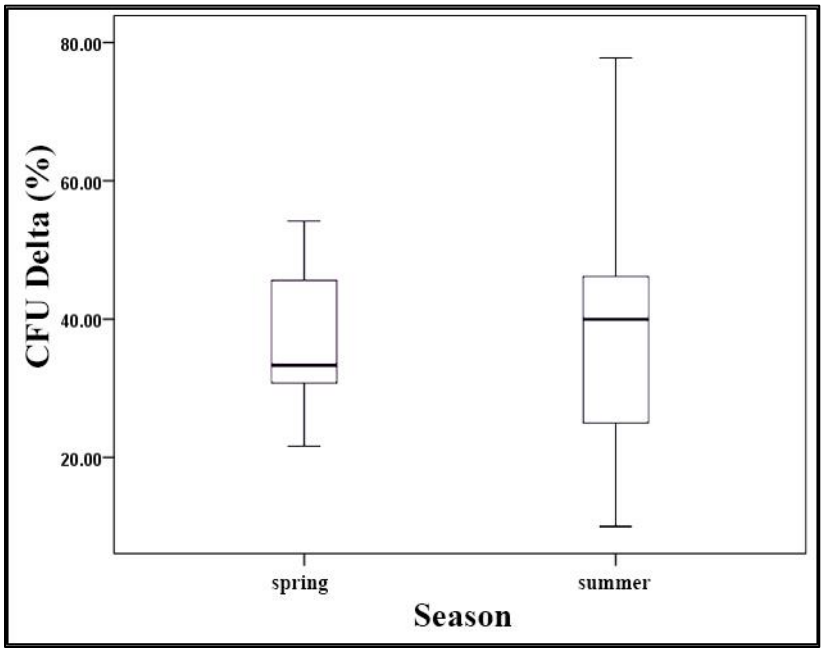

Figure VIII. Boxplot for CFU Delta by season.

The boxplots in Figure 8 are overlapping, indicating small differences in the overall distributions. The medians (indicated by heavy line inside the boxes) are approximately 7 points apart. Both the distributions have small positive skewness. This is checked using the results of the independent t-tests are presented in Table VIII.

Table VIII. Results of Independent Samples t-test.

t	df	p-value (2-tailed)	Mean Diff	Std. Err Diff	95% C.I of the Difference	
					Lower	Upper
-0.101	20	0.921	-0.72906	7.23901	-15.82938	14.37126

The t-value associated with the mean difference of -0.72 is not statistically significant ($t_{(20)}$ = -0.101, p >0.017). In other words, the difference in the means of sample set 1 and sample set 2 is only due to chance. The 95% CI is from -15.83 to 14.37, which crosses value zero. This again shows that the difference in the two means is not significant. The mean CFU Delta for sample set 1 (M = 36.37) and sample set 2 (M=37.09) are comparable, for the present study. A t test for each sample set can be now initiated.

Sample set 1: The descriptive statistics for sample set 1 presented in Table VII indicates that the mean CFU Delta (%) was 36.37. To check if it really differs from the desirable 0% CFU Delta, a one-sample t-test was applied. Results of the same are presented in Table 9.

Table IX. One Sample t-test for sample set 1.

Test Value = 0

	t	df	p-value (2-tailed)	Mean Diff	95% C.I of the Difference	
CFU Delta (%)					Lower	Upper
	9.769	8	0	36.36556	27.7817	44.9494

The results of the One-sample t-test is statistically significant ($t(8) = 9.77$, $p < 0.017$, 95% CI 27.78 to 44.95). The 95% CI does not cross the value zero, indicating significance of the t-value. Hence, the CFU Delta (%) observed for sample set 1 (M = 36.37%) is significantly higher than the desirable level of 0%.

Sample set 2: The descriptive statistics for sample set 1 presented in Table VII indicates that the mean CFU Delta (%) was 37.095. To check if it really differs from the desirable 0% CFU Delta, a one-sample t-test was applied. Results of the same are presented in Table X.

Table X. One Sample t-test for sample set 2.

Test Value = 0

	t	df	Sig. (2-tailed)	Mean Diff	95% C.I of the Difference	
CFU Delta (%)					Lower	Upper
	6.849	12	0	37.09462	25.2939	48.8953

The results of the One-sample t-test is statistically significant ($t(8) = 6.849$, $p < 0.017$, 95% CI 25.30 to 48.90). The 95% CI does not cross the value zero, indicating significance of the t-value. The CFU Delta (%) observed for sample set 1 (M = 37.09%) is significantly higher than the desirable level of 0%. This shows that there is fungal bypass through the HVAC system, even after passing through a filter. This indicates that, on average 37%, of the fungal spores

reentered the indoor environment even after being filtered. The analysis also shows that the CFU Delta is independent of weather.

The second independent variable is Human population. Human population was divided into three major groups according to the maximum number of occupants inside the building during sampling. The three group population ranges were 210-240, 90-107, and 70-87. To check if the difference in human population had any effect on the CFU Delta count, a one-way ANOVA test was conducted. To assure correct interpretation of the obtained statistic, the assumptions underlying one-way ANOVA test were checked before applying the test.

Assumption of normality of the distributions of three levels of Human Count. This was checked using Kolmogorov-Smirnov and Shapiro-Wilk tests. The results are presented in Table XI.

Table XI. Results for Kolmogorov-Smirnov and Shapiro-Wilk test

Tests of Normality

Human Count		Sample	Kolmogorov-Smirnova			Shapiro-Wilk		
			Statistic	df	Sig.	Statistic	df	Sig.
210-240	CFU Delta (%)	Sample set 1	0.274	9	0.06	0.907	9	0.295
90-107		Sample set 2	0.117	8	.200*	0.983	8	0.974
70-87		Sample set 2	0.213	5	.200*	0.946	5	0.711

* This is a lower bound of the true significance.

a Lilliefors Significance Correction

The results presented in Table XI for two sample sets reveal that the obtained statistic for both the normality test is not statistically significant (all $p > 0.05$). It indicates that the normality assumption is valid.

The normality check is also presented in Figure IX, X, and XI for the three different human count average ranges, through the normal Q-Q plot.

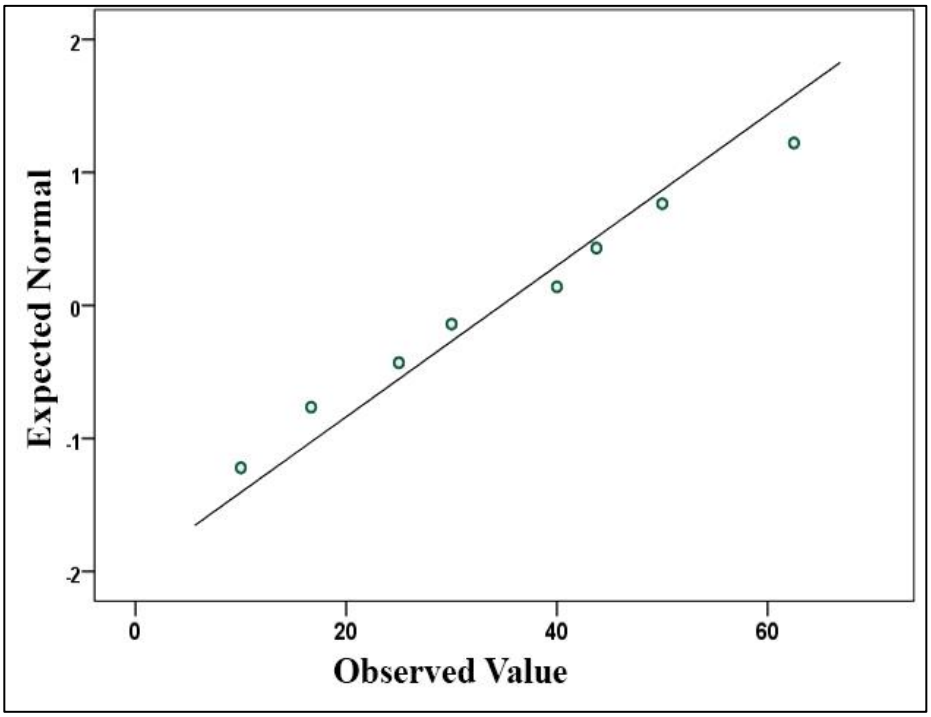

Figure IX. Normal Q-Q plot of CFU Delta for Human count 210-240

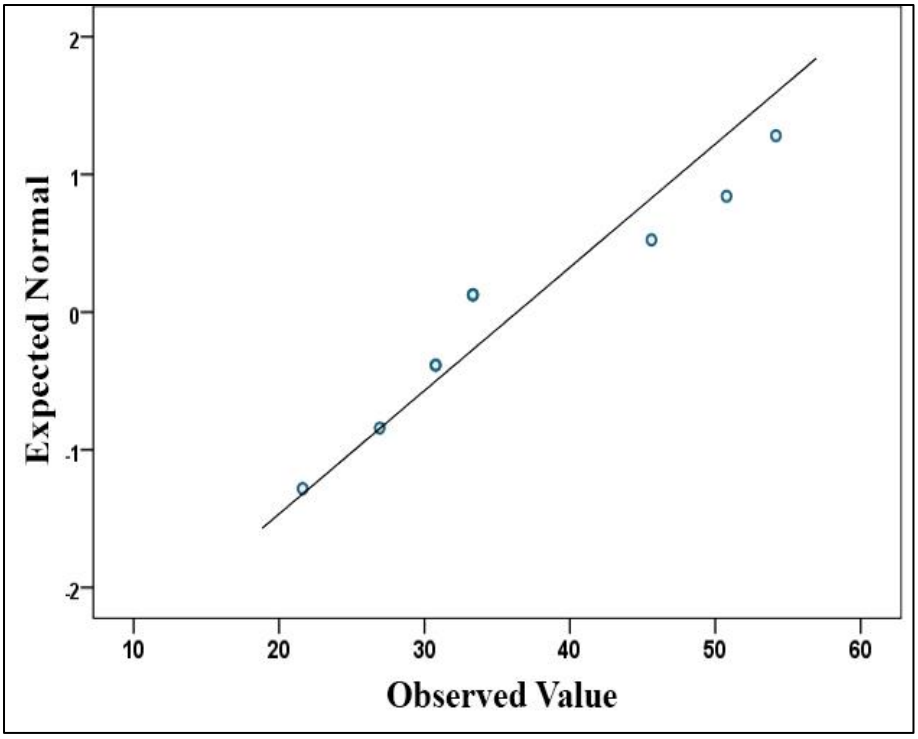

Figure X. Normal Q-Q plot of CFU Delta for Human count 90-107.

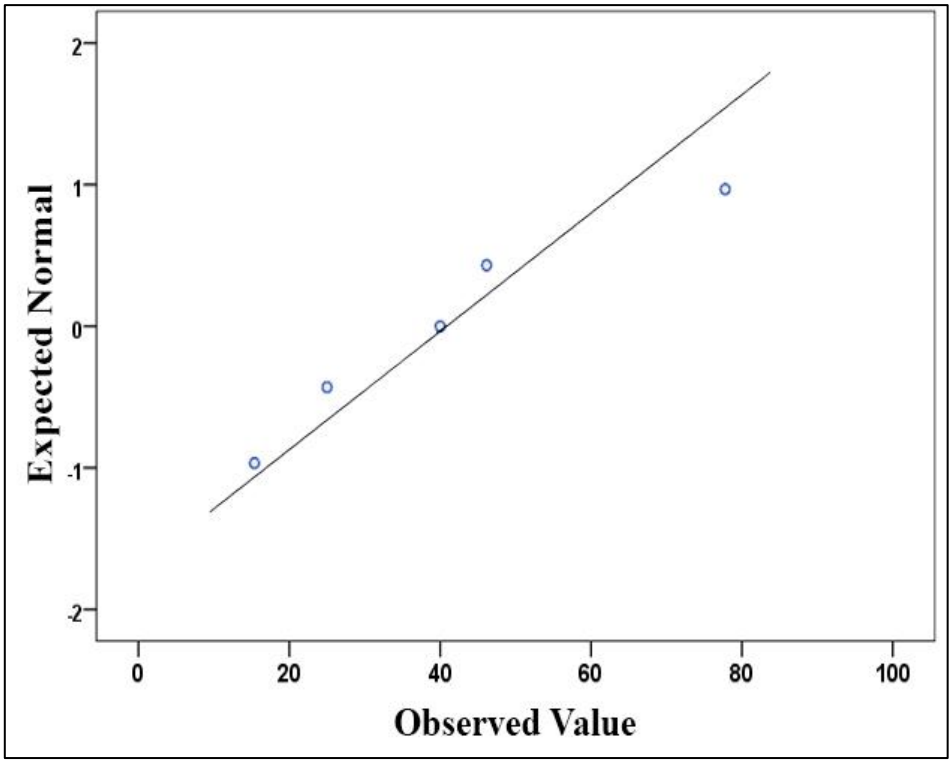

Figure XI. Normal Q-Q plot of CFU Delta for Human count 70-87.

In all three figures, there is slight deviation from the perfect normal distribution model, but there are no outliers or other deviations from the base assumption of normality. Having met the normality assumption, the assumption for homogeneity of variances was evaluated before the ANOVA test was run. This was checked using Levene's test which was shown not to be statistically significant (Levene's Statistic $_{(2, 19)}$ = 1.27, p >0.05). The assumption of homogeneity of variances was therefore valid as well.

Table XII. Descriptive statistics for the CFU Delta (%): Independent Variable Human count.

Human Count	N		Mean	Std. Error	SD	Min	Max	Percentiles		
	Valid	Missing						25	50	75
210-240	9	0	36.36	3.7223	11.16	21.6	54.2	28.845	33.3	48.19
90-107	8	0	34.74	6.2224	17.59	10	62.5	18.752	35	48.44
70-87	5	0	40.86	10.706	23.94	15.3	77.7	20.2	40	61.97

The descriptive statistics presented in Table XII reveal that the mean CFU delta % for 90-107 (M = 34.74) is lower than that for the other two. The standard deviation for a population of 70-87 is greater than that for the sample set 1, indicating comparatively less homogeneity in the distribution. This is because that data had a lower minimum and higher maximum score. These comparative differences in the means are presented in Figure XII.

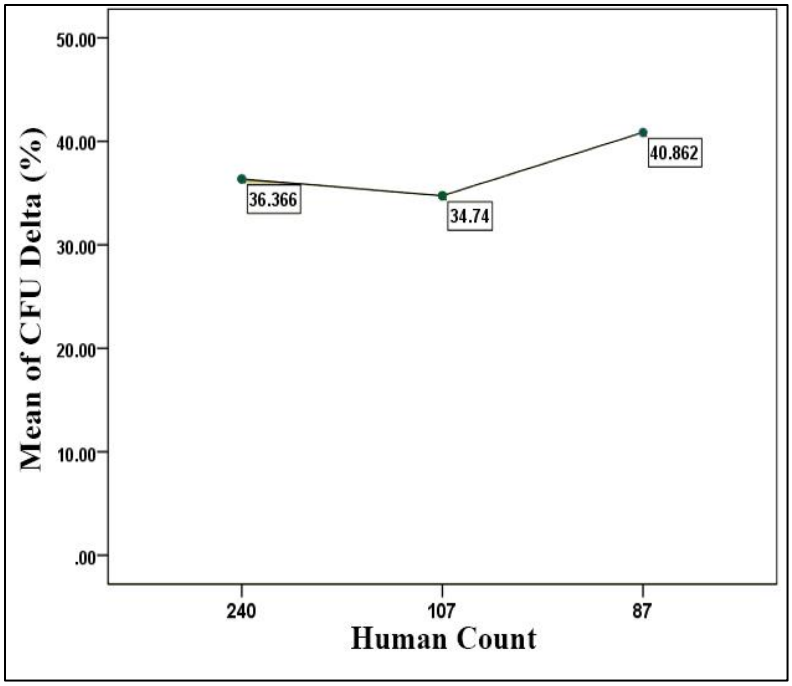

Figure XII. Differences in Means between different maximum human counts.

Comparisons of three means presented in Figure XII shows that they are close to each other and the differences in them are from 1.63 to 6.12. These are small compared to their respective standard deviations and unlikely to be significant. This was checked using one-Way ANOVA and the results are presented in Table XIII.

Table XIII. Results of One-way ANOVA: Independent variable Human Count

CFU Delta (%)					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	118.146	2	59.073	0.206	**0.816**
Within Groups	5458.486	19	287.289		
Total	5576.633	21			

The F-value associated with the mean CFU Delta (%), presented in Table VII, for three Human Counts is not statistically significant ($F_{(2,19)} = 0.21$, $p > 0.017$). The differences obtained in the three means are only due to chance. The mean CFU Delta for human count 210-240 (M=36.37), human count 90-107 (M= 34.74), and human count 70-87 (M = 40.86) are comparable. The effect of human count on the CFU Delta was checked by a t-test. A t-test was run for each of the human counts recorded. The mean for group 210-240 (36.4) and group 90-107 (34.7) was significantly different from zero. The mean for group 70-87 was 40.86 which was significantly different from zero, but it had only 5 samples in the data. The group 70-87 requires more sampling to be statistically significant.

In summary, for the data collected in this study and under the conditions stated earlier, the weather or the human population count did not affect the mean CFU Delta (%). The Mean CFU Delta (%) was significantly above the acceptable level of 0%, irrespective of the weather or human count.

CHAPTER V

DISCUSSION

The genesis of this project was a question about the possible effect of HVAC filter maintenance on the quality of conditioned air delivered to the conditioned spaces in a healthcare facility. A study of nosocomial infection data from a French data set revealed a periodic spike in Aspergillus related infections.(Reboux 2014). It was posited that these periodic episodes might be linked to systematic filter or other maintenance work on hospital HVAC systems. As presented in the literature review, there seem to be no specific studies on this particular issue in HVAC system maintenance. There are many sources citing information about filter system design, operations, placement, effectiveness, etc., but no references have been found with details on the change in biological contamination in a typical HVAC system as a result of filter change.

The CDC recommends that, during an HVAC filter change operation, the systems under maintenance should remain operational. The primary reason for this operational recommendation is to maintain positive/negative pressure relationships within the hospital conditioned space. For example, room pressure should be slightly positive in spaces where burn victims might be recovering. The positive pressure reduces likelihood of infiltration into the space of infectious agents which would put the immunocompromised patient at increased risk of a nosocomial infection. However, turning HVAC systems off during filter change operations is also common, though not well documented.

In commercial HVAC systems, it is quite common to turn fan systems off during filter maintenance. The facility managers know that dust/debris can be dislodged from the dirty

filters during this change and do not want to send those contaminants into the duct distribution system.

Stepping back, it became quite obvious that there were essentially no data on the population change of Aspergillus traversing through an HVAC system much less the added effect of a filter change operation. As stated in the Introduction, the goals of this study became: 1) to develop a consistent sampling protocol for Aspergillus, 2) to investigate some common influences on the Aspergillus CFU count in a public institutional building, and 3) to characterize the difference in Aspergillus CFU counts between the entry into and exit from an operational HVAC unit

Although there are a number of studies advocating sampling techniques, many are particular to a specific piece of sampling equipment (settling plate or impact sampler) or specific location (mine safety). After initial zero CFU samples in this study, the sampling time and volume of air collected were increased so that a sample cassette had about 3.6 m^3 of air passed over the collection filter. Subsequent Asp. counts were very comparable to other studies (Curtis et al. 2005). The sampling period was timed to coincide with high student traffic in the building, especially at class-change times.

The statistics in the previous section revealed that there was no statistically significant impact on Aspergillus CFU delta counts because of influence of the number of people in the building during the sample period or because of the weather at any time of the sampling. This was an important finding for this study because it allowed a focus on the filter/HVAC unit and outside influences on Aspergillus across the unit could be ignored.

Importantly, even when the pleated filter MERV rating was improved from an 8 to 13, there was no reduction in the Asp. CFU exiting the air handler. This result has to have been caused either by imperfections in the filter itself or, more likely, bypass of air around the filter membrane. Again the impact of this result is that even when using "improved" filter media, the opportunity for bypass in the air handling system is a distinct possibility.

The data for Aspergillus obtained in this study showed a consistent average reduction in Aspergillus pass-through of about 40% (Table II and IV). That is, of the total Aspergillus CFU entering the air handler/filter of the HVAC unit, about 60% was held in the unit. The unfortunate corollary is that about 40% of the Aspergillus passed through the unit and was re-introduced into the building air. In addition, this reintroduction was at a velocity of about 0.762 – 1.27 m/s. If this unit were operating in a healthcare facility under those conditions, there would be quite an increased probability of a nosocomial infection.

As with any research, more questions are usually raised than are answered. That was certainly the case in this study. For example, the consistent 40% pass-through of Aspergillus for this unit, even with improved filter media was the case for a "dry coil." A dry cooling coil was consistently observed (no condensate draining from the unit) during all of the sampling periods. The primary HVAC system in this building is a chilled-beam type and has a dedicated outdoor air handler system (DOAS) that provided neutral/cool, filtered, and dehumidified air to the majority of the spaces in the building. As a result, the dew point of the air entering the HVAC system in this study was rarely above the dew point of the chilled water supplied to that unit. The result was little or no condensate on the surfaces of chilled water heat exchanger exposed to that room air flow. Further studies using a system with a

heat exchanger that could be operated wet or dry would need to be undertaken to examine the effect on Asp. CFU counts and the effect of that heat exchanger on reduction (or increase) in the amount of Aspergillus held up in the unit.

Additional work could be done to understand the behavior of different buildings simultaneously. Two or three such buildings can be examined to understand the bypass in each. This experiment is just a small effort towards achievement of the final goal – an Aspergillus free healthcare environment.

CHAPTER VI

CONCLUSIONS

The presence of Aspergillus in hospitals, has been well established. Sampling of a consistently large volume of air improved the detection of Aspergillus fungi. A wide range of the colony forming units were detected though there was no influence from human traffic or weather. The difference for Aspergillus CFU counts at the return grille and supply diffuser, revealed that approximately 40% of the Aspergillus passed through the HVAC system and back into the building environment. Filter media improvement from MERV 8 to MERV 13 did not improve the Aspergillus CFU bypass in this HVAC unit. CFU Delta% was not affected by differing weather and human population conditions inside the building. Further study is needed on potential downstream duct system biological contamination as a result of HVAC filter media change. The effect of a wet vs. dry cooling heat exchanger should be investigated for possible impact on retention of Aspergillus during normal operations.

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