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Investigation on CA-MRSA isolates for eradication measures:

The efficiency of 3000-3600 Air Deluxe and Induct/Dux Deluxe air purifier technology on eradication of bacterial pathogen in the laboratory conditions with special reference to *Staphylococcus aureus*

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Introduction

The air surrounding us plays an extremely important role in our well being and efficiency. Breathing pure and clean air allows us to think more clearly, sleep more soundly, and stay healthier. Studies show that we receive 56% of our energy from the air we breathe, more than from water and food combined. On average we breathe 37 pounds of air a day (equivalent to volume of an Olympic sized pool) (1). In previous experimental studies utilizing the 3000 –3600 air purifier, it has been established that the use of negative ions in a purification system is an effective means of eradicating aeroallergens such as mold and microbes in room air (2). Studies conducted by the United States Department of Agriculture (USDA) have illustrated the ability negative ion air purifiers to significantly reduce the airborne amounts of *Salmonella enteritidis* in rooms containing infecting cage laying chickens (3). The removal of bacterial pathogens from the air, especially in diseases that are transmitted by droplets like *Mycobacterium tuberculosis*, is a promising way to reduce disease transmission in the clinical healthcare setting.

In this study, the efficiency of negative ion purifiers such as the 3000-3600 air purifier and the Induct/Dux Deluxe air purifier were evaluated in the microbiology and mycology room of Baptist Saint Anthony's Hospital (BSA) laboratory in Amarillo, TX. This department processes around 85,000 bacterial and fungal cultures per calendar year, and the staff also performs many various types of serological testing for bacterial pathogens and routine parasitological examinations on various types of clinical specimens. Specifically, this study was concentrated on the efficiency on the net reduction of bacteria in a negative pressure laboratory and the specific effect on isolates indentified to be methicillin resistant *Staphylococcus aureus*, MRSA.

Materials and Methods

The two units we evaluated were the 3000-3600 and Induct/Dux Deluxe Air Purifier. The 3000 – 3600 is designed to sanitize air, as well as kill surface mold, bacteria, and viruses in areas up to 3000-3600 square feet (4). It has been proven to reduce colony growth in petri-dishes in one, two and four foot distances (5). The effectiveness of the 3000 –3600 air purifier was evaluated in the mycology room which is approximately 80 square feet. In this experimental study the re-evaluation

of the square footage of the microbiology room and the layout of the air ducts were taken into consideration. The 3000-3600 air purifier was evaluated in the 80 square foot mycology laboratory. The two Induct/Dux Deluxe unit was evaluated in the microbiology room, which is 960 square feet. These units use an Advanced Hydrated Photocatalytic Oxidation filtration system that is placed directly into the air circulation system via a hole cut into the ventilation system. It is a broad-spectrum high intensity UV light targeted on a quad metallic catalyst in a low-level ozone and moist atmosphere.

The BSA Microbiology department is projected to process approximately 85,000 to 90,000 bacterial cultures by the end of 2008 according to Danis Watson MS MT (ASCP), microbiology supervisor. The peak hours of activity of culture evaluation and clinical specimen setup in the microbiology department are from 0600 to 1630 daily. The BSA Microbiology department handles a large variety of clinical specimens from the inpatient cases, emergency room, two urgent care centers, a dozen long-term care facilities, and many of the area physician offices.

In order to establish growth patterns in both rooms, tryptic soy agar plates with 5% sheep blood were placed out in the microbiology and mycology rooms at 15, 30, 60, 120, 180, 240, 300, 360, 420, and 480 minutes of exposure to room air during peak times of activity. The plates were evaluated for the total number of bacterial and fungal colonies observed at 24 and 48 hrs of incubation at 35^o C in a CO₂ incubator.

Results and Discussions

The following growth patterns were observed. Only bacterial colonies were observed during this initial growth rate evaluation.

Table 1A: Number of Colony forming units (CFU) observed during the initial growth rate determination

TIME (min)	MICRO ROOM 24 HR INC	FUNGUS ROOM 24 HRS	MICRO ROOM 48 INC	FUNGUS ROOM 48 INC
15	2	0	2	0
30	2	3	2	3
60	4	2	4	2
120	1	5	1	5
180	7	8	9	9
240	4	8	4	8
300	13	4	13	12
360	10	10	12	13
420	10	7	10	13
480	17	12	20	12

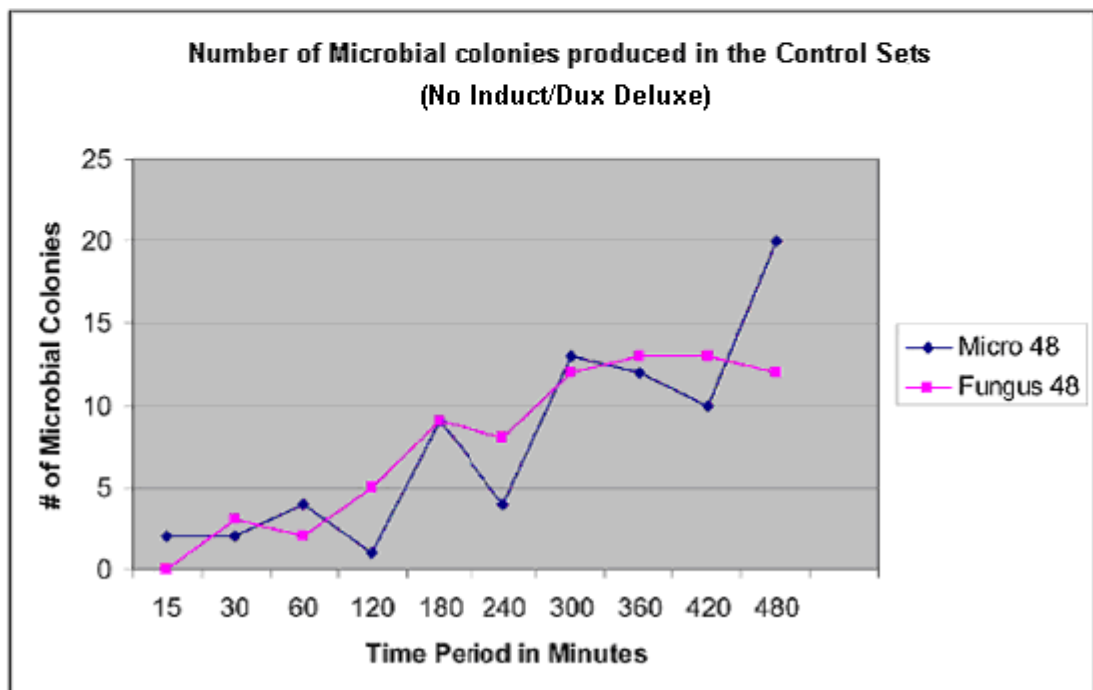


Fig 1A: Graph of initial growth rates in the micro and fungus rooms without any Induct/Dux Deluxe (Control)

Table 1B: Number of Colony forming units (CFU) observed during the initial growth rate determination

TIME (min)	MICRO ROOM 24 HR INC	FUNGUS ROOM 24 HRS	MICRO ROOM 48 INC	FUNGUS ROOM 48 INC
15	1	2	1	0
30	3	3	2	2
60	3	4	4	3
120	4	5	3	5
180	2	4	5	4
240	3	4	3	2
300	9	8	3	4
360	3	3	4	2
420	2	2	4	3
480	1	3	4	3

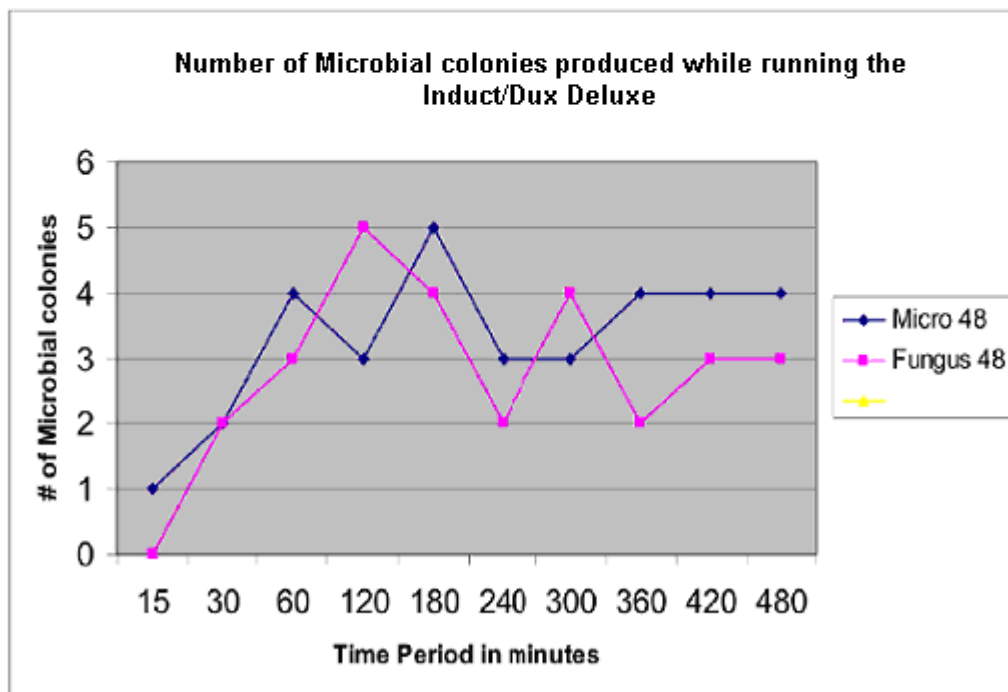


Fig 1B: Graph of microbial growth rates in the microbiology room with the Induct/Dux Deluxe running (Experimental)

The next part of the evaluation was to determine a set time frame for plate exposure to room air to reduce the effects of desiccation on the media, adequate time exposure, and increased sampling runs during the peak times of activity in the adjoining rooms in the microbiology department. From the trend observed in the data from the initial growth rate determination, the number of bacterial colonies isolated did not increase greatly after 300 minutes of exposure to room air.

In order to maximize the number of samples collected during the peak activity in the microbiology department, TSA plates were placed at each of the workstations in the microbiology room and fungus room for four-hour exposure periods at 0600, 1000, and 1400. Control plates were collected on 7/27/08, 8/11/08, and 8/12/08 without the operation of the air purifiers. Experimental plates were collected while operating both air purifiers on three consecutive days of operation on 8/24/08, 8/25/08, 8/26/08. To identify *Staphylococcus aureus* isolates, colonies were evaluated for microscopic morphology using Gram staining, beta hemolysis on TSA agar with 5% sheep blood, and the presence of coagulase by latex agglutination testing. The data were then grouped and compared by the days of collection: Sunday, Monday, and Tuesday respectively to account for the difference in workload during the workweek. The data for the control and experimental setups are summarized by table and graphs as follows:

Table 2: Total number of bacterial colonies observed during control and experimental setups on Sunday, 7/27/08 and 8/24/08.

LOCATION	7/27/08 CONT 0600	7/27/08 CONT 1000	7/27/08 CONT 1400	8/24/08 EXP 0600	8/24/08 EXP 1000	8/24/08 EXP 1400
MICRO BENCH I	1	1	1	0	0	0
MICRO BENCH II	4	1	2	3	0	1
MICRO BENCH III	1	1	1	0	0	0
MICRO BENCH IV	1	1	1	0	0	0
MICROSTREP	2	3	2	1	2	1
MICRO SETUPS	4	1	1	3	0	0
FUNGUS WORKBENCH	1	2	2	0	1	1
FUNGUS SHELF	1	2	1	0	1	0
FUNGUS SINK AREA	1	1	1	0	0	0
FUNGUS CENTRIFUGE AREA	1	3	1	0	2	0

Fig 2: Graph of control and experimental counts for Sunday, 7/27/08 and 8/24/08

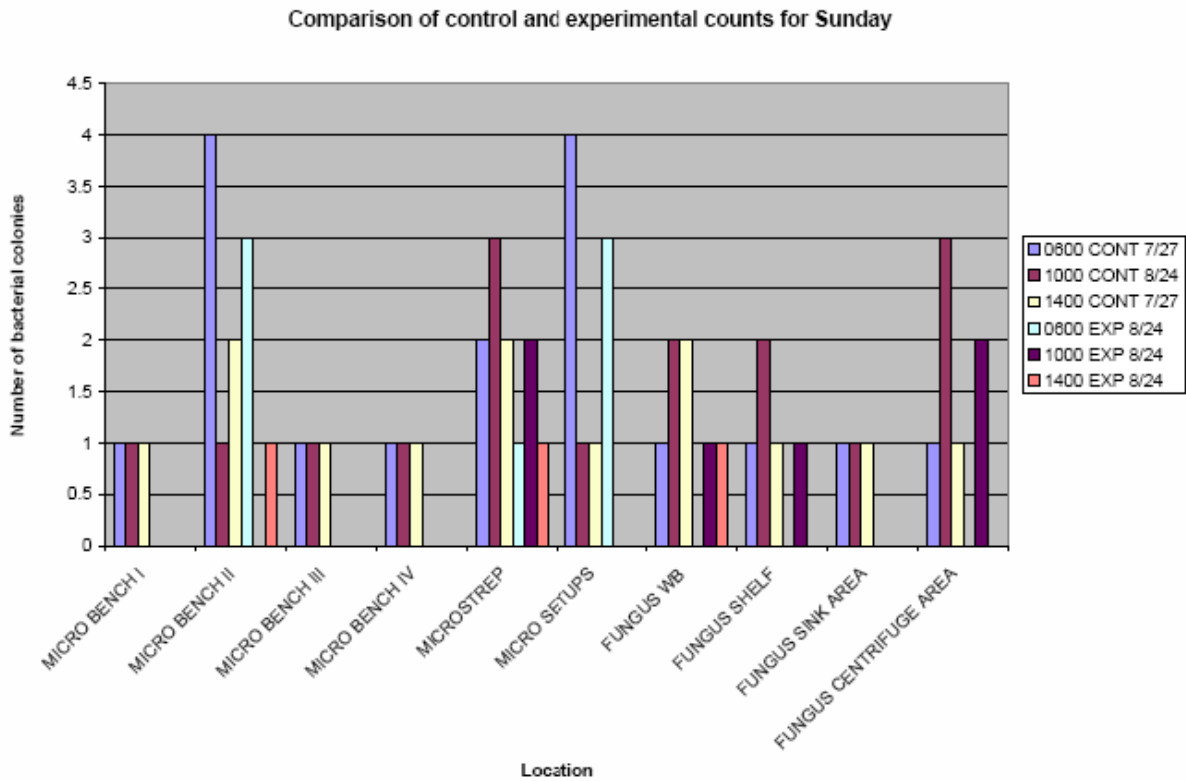


Table 3: Total number of bacterial colonies observed during control and experimental setups on Monday, 8/11/08 and 8/25/08.

LOCATION	8/11/08 CONT 0600	8/11/08 CONT 1000	8/11/08 CONT 1400	8/25/08 EXP 0600	8/25/08 EXP 1000	8/25/08 EXP 1400
MICRO BENCH I	11	9	2	5	9	25
MICRO BENCH II	2	8	1	6	9	3
MICRO BENCH III	13	6	3	6	25	2
MICRO BENCH IV	9	10	6	16	9	3
MICROSTREP	14	7	8	9	10	2
MICRO SETUPS	7	7	4	8	10	1
FUNGUS WORKBENCH	9	2	11	12	8	4
FUNGUS SHELF	7	2	10	6	2	2
FUNGUS SINK AREA	6	2	3	5	4	1
FUNGUS CENTRIFUGE AREA	4	2	6	2	4	2

Fig 3: Graph of control and experimental counts for Monday, 8/11/08 and 8/25/08

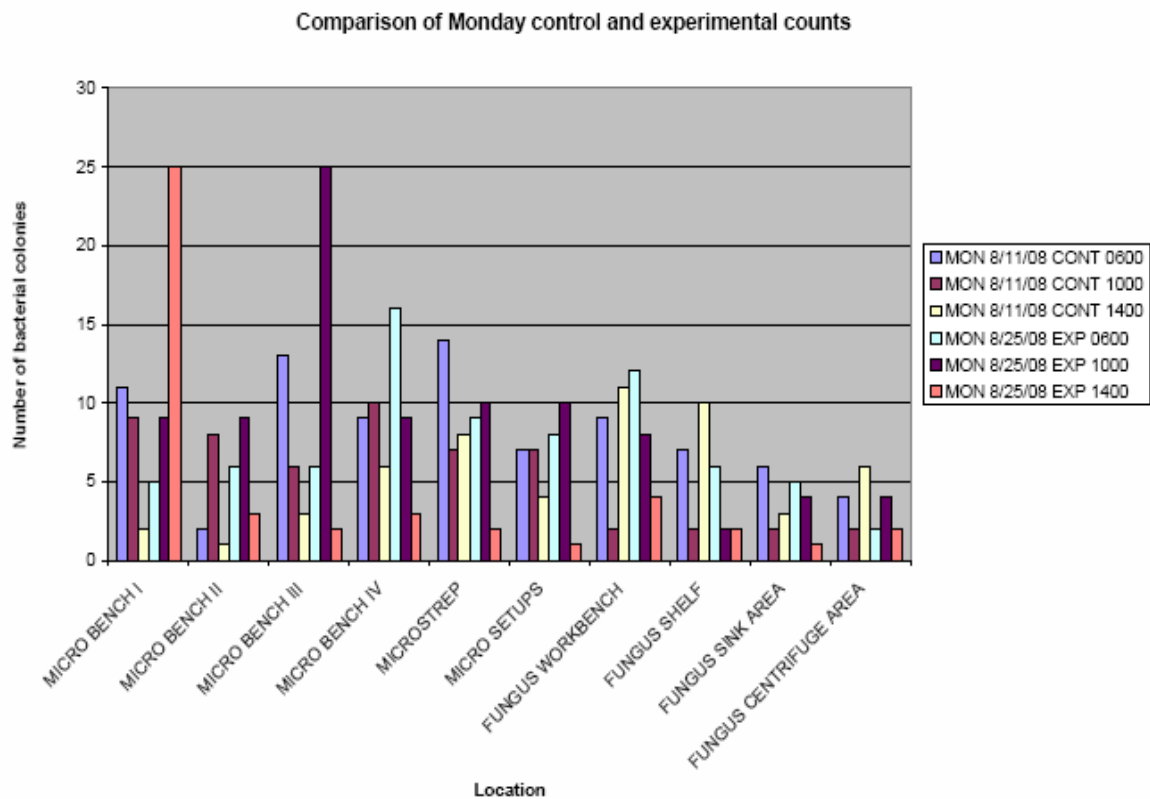


Table 4: Total number of bacterial colonies observed during control and experimental setups on Tuesday, 8/12/08 and 8/26/08.

LOCATION	8/12/08 CONT 0600	8/12/08 CONT 1000	8/12/08 CONT 1400	8/26/08 EXP 0600	8/26/08 EXP 1000	8/26/08 EXP 1400
MICRO BENCH I	3	7	5	13	7	7
MICRO BENCH II	11	13	7	9	13	6
MICRO BENCH III	8	12	1	12	9	8
MICRO BENCH IV	15	16	5	8	8	13
MICROSTREP	24	13	13	13	8	6
MICRO SETUPS	7	6	2	15	11	9
FUNGUS WB	22	10	11	14	7	12
FUNGUS SHELF	7	8	10	12	3	7
FUNGUS SINK AREA	14	4	3	5	3	15
FUNGUS CENTRIFUGE AREA	9	6	6	9	1	2

Figure 4: Graph of control and experimental counts for 8/12/08 & 8/26/08.

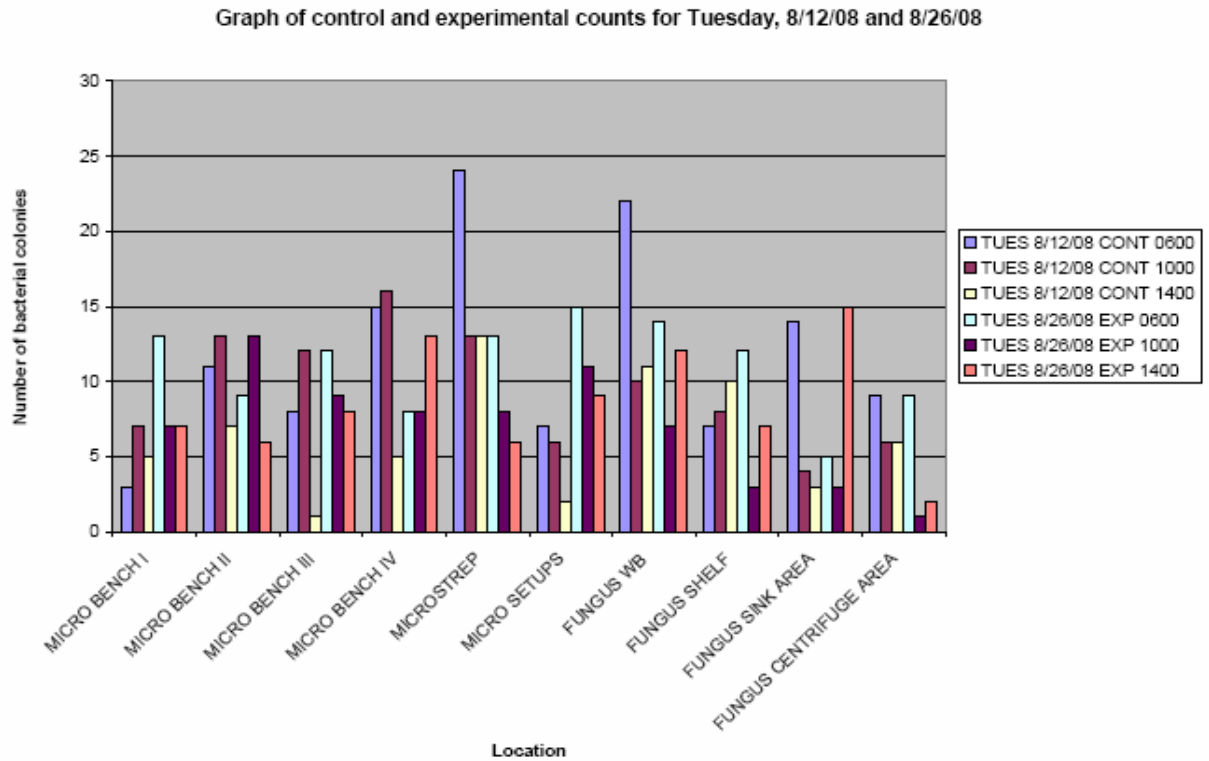


Table 5: Single factor analysis of variance for control and experimental data collected on 7/27/08 and 8/24/08

ANOVA ANALYSIS OF DATA COLLECTED ON SUNDAY, 7/27/08 AND 8/24/08

Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance
MICRO BENCH I	6.00	3.00	0.50	0.30
MICRO BENCH II	6.00	11.00	1.83	2.17
MICRO BENCH III	6.00	3.00	0.50	0.30
MICRO BENCH IV	6.00	3.00	0.50	0.30
MICROSTREP	6.00	11.00	1.83	0.57
MICRO SETUPS	6.00	9.00	1.50	2.70
FUNGUS WB	6.00	7.00	1.17	0.57
FUNGUS SHELF	6.00	5.00	0.83	0.57
FUNGUS SINK AREA	6.00	3.00	0.50	0.30
FUNGUS CENTRIFUGE AREA	6.00	7.00	1.17	1.37

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	16.27	9.00	1.81	1.98	0.06	2.07
Within Groups	45.67	50.00	0.91			
Total	61.93	59.00				

Table 6: Single factor analysis of variance for control and experimental data collected on 8/11/08 and 8/25/08

ANOVA ANALYSIS OF DATA COLLECTED ON MONDAY, 8/11/08 AND 8/25/08

Anova: Single Factor

SUMMARY

<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>
MICRO BENCH I	6.00	61.00	10.17	63.37
MICRO BENCH II	6.00	29.00	4.83	10.97
MICRO BENCH III	6.00	55.00	9.17	74.97
MICRO BENCH IV	6.00	54.00	9.00	16.80
MICROSTREP	6.00	50.00	8.33	15.17
MICRO SETUPS	6.00	37.00	6.17	10.17
FUNGUS WB	6.00	46.00	7.67	15.17
FUNGUS SHELF	6.00	29.00	4.83	11.37
FUNGUS SINK AREA	6.00	21.00	3.50	3.50
FUNGUS CENTRIFUGE AREA	6.00	20.00	3.33	2.67

ANOVA

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	334.93	9.00	37.21	1.00	0.13	2.07
Within Groups	1123.67	50.00	22.47			
Total	1458.60	59.00				

Table 7: Single factor analysis of variance for control and experimental data collected on 8/12/08 and 8/26/08

ANOVA ANALYSIS OF DATA COLLECTED ON TUESDAY, 8/12/08 AND 8/26/08

Anova: Single Factor

SUMMARY					MICRO BENCH I
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>	
MICRO BENCH I	6.00	42.00	7.00	11.20	
MICRO BENCH II	6.00	59.00	9.83	8.97	
MICRO BENCH III	6.00	50.00	8.33	16.27	
MICRO BENCH IV	6.00	65.00	10.83	19.77	
MICROSTREP	6.00	77.00	12.83	38.97	
MICRO SETUPS	6.00	50.00	8.33	19.87	
FUNGUS WB	6.00	76.00	12.67	26.27	
FUNGUS SHELF	6.00	47.00	7.83	9.37	
FUNGUS SINK AREA	6.00	44.00	7.33	31.47	
FUNGUS CENTRIFUGE AREA	6.00	34.00	5.67	9.87	

ANOVA

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
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Between Groups	313.73	9.00	34.86	1.82	0.09	2.07
Within Groups	960.00	50.00	19.20			
Total	1273.73	59.00				

Table 7: Two factor analysis of variance with replication for all control and experimental data collected

TWO FACTOR ANOVA WITH REPLICATION FOR ALL DATA COLLECTED

Anova: Two-Factor With Replication

	MICRO BENCH I	MICRO BENCH II	MICRO BENCH III	MICRO BENCH IV	MICRO STREP	MICRO SETUPS	FUNGUS WB	FUNGUS SHELF	FUNGUS SINK AREA	FUNGUS CENTRI-FUGE AREA	Total
<i>SUN 7/27/08 CONT 0600</i>											
Count	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	30.00
Sum	3.00	7.00	3.00	3.00	7.00	6.00	5.00	4.00	3.00	5.00	46.00
Average	1.00	2.33	1.00	1.00	2.33	2.00	1.67	1.33	1.00	1.67	1.53
Variance	0.00	2.33	0.00	0.00	0.33	3.00	0.33	0.33	0.00	1.33	0.81
<i>SUN 8/24/08 EXP 0600</i>											
Count	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	30.00
Sum	0.00	4.00	0.00	0.00	4.00	3.00	2.00	1.00	0.00	2.00	16.00
Average	0.00	1.33	0.00	0.00	1.33	1.00	0.67	0.33	0.00	0.67	0.53
Variance	0.00	2.33	0.00	0.00	0.33	3.00	0.33	0.33	0.00	1.33	0.81
<i>MON 8/11/08 CONT 0600</i>											
Count	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	30.00
Sum	22.00	11.00	22.00	25.00	29.00	18.00	22.00	19.00	11.00	12.00	191.00
Average	7.33	3.67	7.33	8.33	9.67	6.00	7.33	6.33	3.67	4.00	6.37
Variance	22.33	14.33	26.33	4.33	14.33	3.00	22.33	16.33	4.33	4.00	13.00
<i>MON 8/25/08 EXP 0600</i>											
Count	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	30.00
Sum	39.00	18.00	33.00	29.00	21.00	19.00	24.00	10.00	10.00	8.00	211.00
Average	13.00	6.00	11.00	9.67	7.00	6.33	8.00	3.33	3.33	2.67	7.03

Variance	112.00	9.00	151.00	36.33	19.00	22.33	16.00	5.33	4.33	1.33	37.07
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TUES
8/12/08
CONT
0600

Count	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	30.00
Sum	15.00	31.00	21.00	36.00	50.00	15.00	43.00	25.00	21.00	21.00	278.00
Average	5.00	10.33	7.00	12.00	16.67	5.00	14.33	8.33	7.00	7.00	9.27
Variance	4.00	9.33	31.00	37.00	40.33	7.00	44.33	2.33	37.00	3.00	29.51

TUES
8/26/08
EXP 0600

Count	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	30.00
Sum	27.00	28.00	29.00	29.00	27.00	35.00	33.00	22.00	23.00	13.00	266.00
Average	9.00	9.33	9.67	9.67	9.00	11.67	11.00	7.33	7.67	4.33	8.87
Variance	12.00	12.33	4.33	8.33	13.00	9.33	13.00	20.33	41.33	16.33	14.33

Total

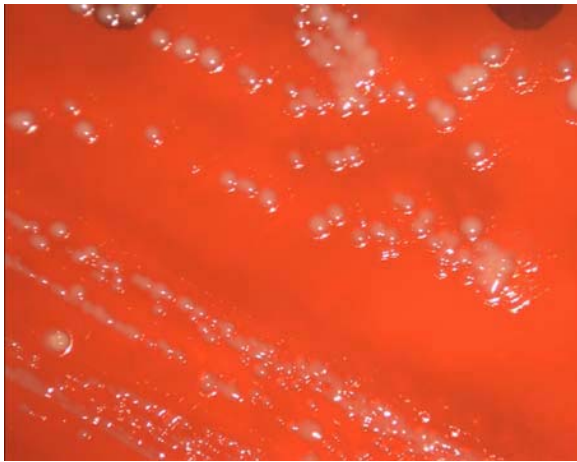
Count	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
Sum	106.00	99.00	108.00	122.00	138.00	96.00	129.00	81.00	68.00	61.00	1000.00
Average	5.89	5.50	6.00	6.78	7.67	5.33	7.17	4.50	3.78	3.39	33.33
Variance	39.16	18.03	43.06	32.30	37.76	18.24	35.91	14.97	18.65	7.66	100.00

ANOVA

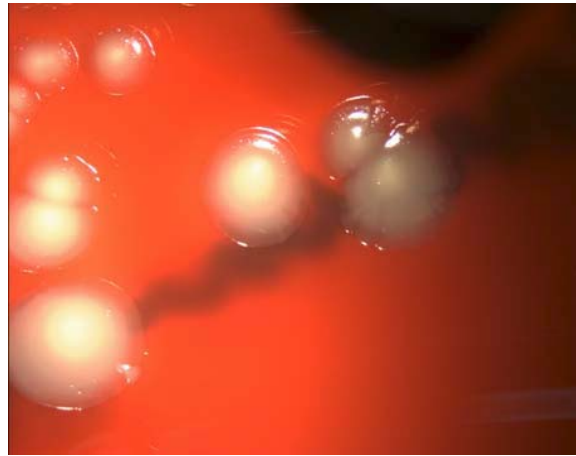
Source of Variation	SS	df	MS	F	P-value	F crit
Sample	2069.00	5.00	413.80	27.92	0.00	2.29
Columns	321.42	9.00	35.71	2.41	0.02	1.96
Interaction	670.11	45.00	14.89	1.00	0.48	1.47
Within	1778.67	120.00	14.82			
Total	4839.20	179.00				

In this investigation we studied the effect of 3000–3600 and Induct/Dux Deluxe Air Purifiers in reducing the microbial concentration in a research lab. The 3000-3600 air purifier was evaluated in the mycology room that is approximately 80 square feet, and two Induct/Dux Deluxe units were evaluated in the 960 square foot microbiology room. From the Graph 1A and 1B it is very clear that the number of bacterial and fungal colonies get reduced when the room air is exposed to the Induct/Dux Deluxe Air Purifiers.

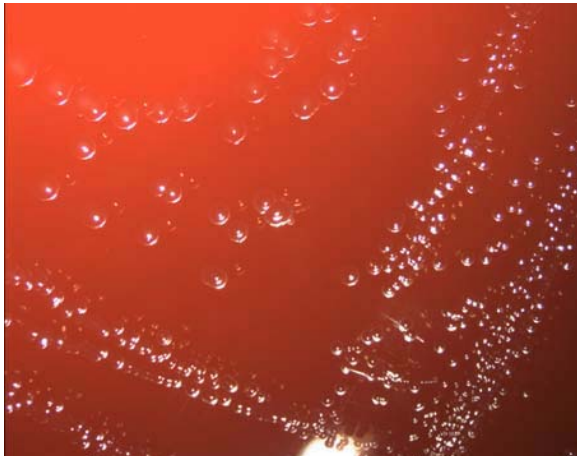
The following are the micrographs of the most common bacterial isolates:



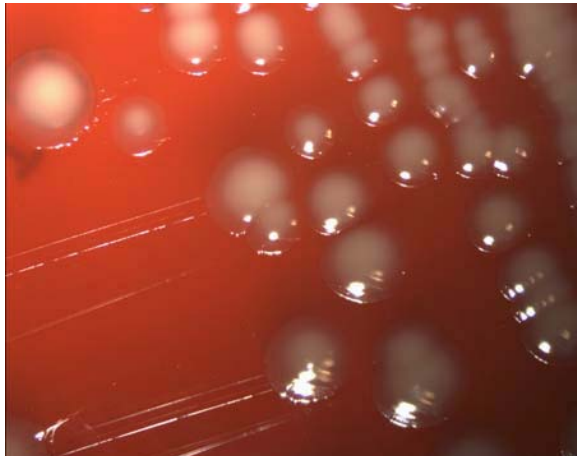
Common isolate 1



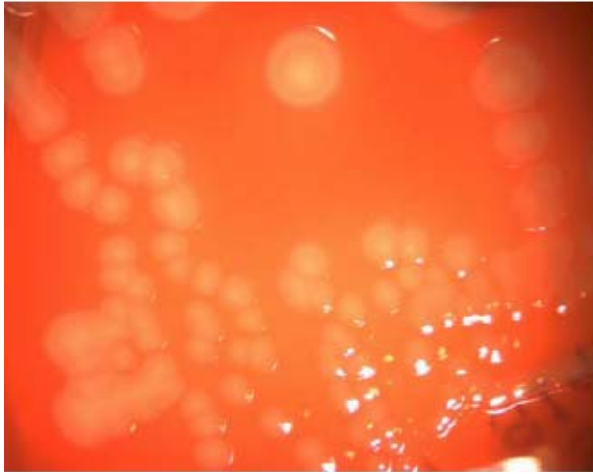
Common isolate 2



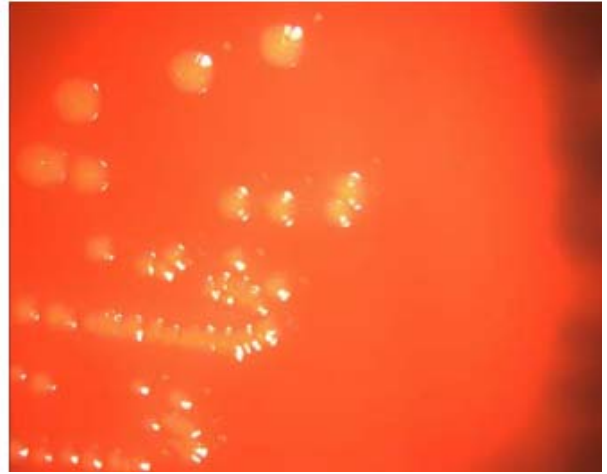
Common isolate 3



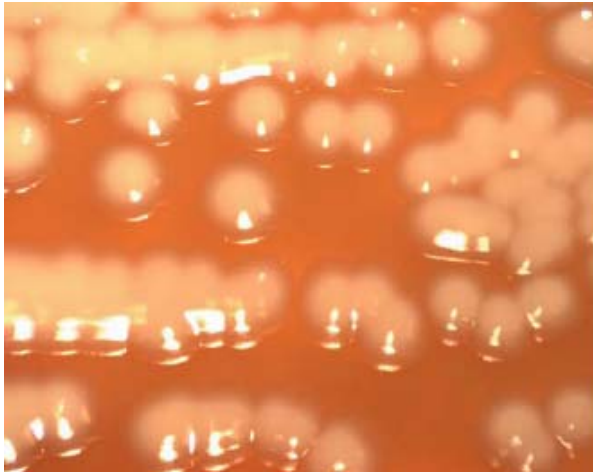
Common isolate 4



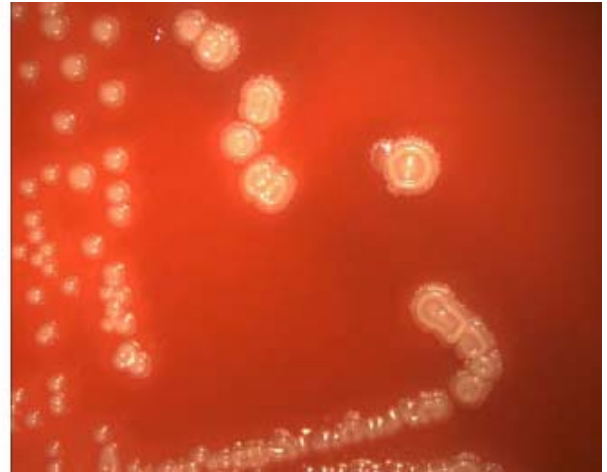
Common isolate 5



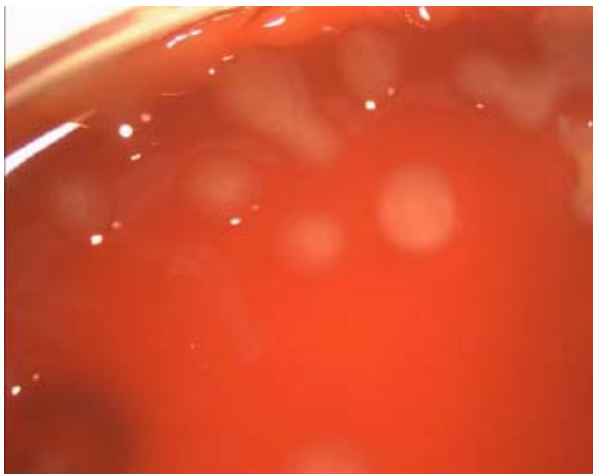
Common isolate 6



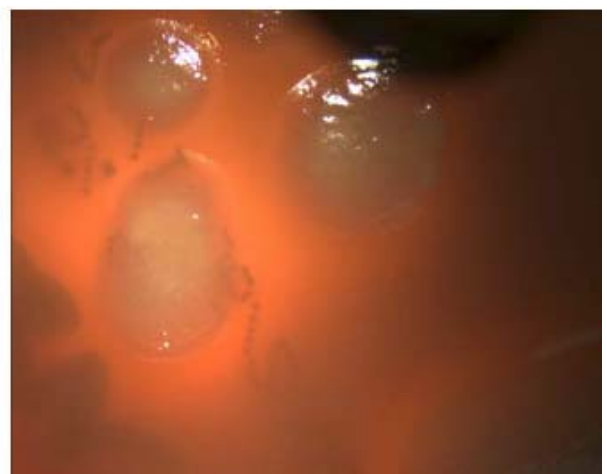
Common isolate 7



Common isolate 8



Common isolate 9



Common isolate 10

Legends for Figure 5:

Common isolate 1: *Coagulase negative Staphylococcus spp.*, Common isolates 2-8: *Coryneform (diphtheroids) spp.*, Common isolate 9: *Gram negative Bacillus, a mixture of Klebsiella, Enterobacter, or Serratia spp.*, Common isolate 10: *Bacillus spp.*

The majority of bacteria isolated from the room air exposure were gram positive bacilli such as *Bacillus spp* and *Coryneform (diphtheroids) spp*, coagulase negative *Staphylococcus spp*, *Micrococcus spp*, and encapsulated gram negative bacilli. No significant bacterial pathogens were isolated from the room air in the microbiology or fungus rooms.

By grouping the data by day for the control and the experimental data, the single factor analysis of variance it is definitely clear that the use of air purifier reduced the airborne pathogen in the laboratory (figure 4). Factors such as workload volume fluctuations, environmental contaminants due to hospital construction projects, and disruption of the negative pressure conditions of the microbiology and fungus rooms due to construction activities may have had significant effects on the results of this experiment.

In order to determine if net reduction in bacteria in room occurs with the operation of the 3000-3600 and the Induct/Dux Deluxe air purification systems, we used laboratory rooms of different sizes. In each and every case there was reduction in airborne pathogen. Although this experiment confirmed that infections such as *Staphylococcus aureus* infections, particularly MRSA, are not caused by airborne transmission, we devised a new technique to assess and evaluate the air purifiers in reducing the growth of the MRSA isolates.

The reduced numbers of gram negative bacteria from the isolates does suggest that the air purifiers do have a greater effect on this type of bacteria. Finally, safety measures and protective equipment used in a BSL-2 lab such as laminar flow hoods, disposable culture loops, and other protective measures may help reduce the number of potential bacterial pathogens from the room air. To culture the methicillin resistant *Staphylococcus aureus*, MRSA in both rooms, tryptic soy agar plates with 5% sheep blood were inoculated with the strain after serial dilutions as follows. 5 ml of initial

culture was diluted to 10^{-4} . After an incubation at 37°C for 24 hours, the isolates were added to the petri plates containing medium (tryptic soy agar plates with 5% sheep blood) and allowed them to grow for 24 hours. One set consisting of 10 petri plates were kept at room temperature without any air purifier. The other set consisting of 10 petri plates were kept in front of the air purifiers with a constant distance from the air purifier 3000-3600. The same experiment was repeated with the constant distance from the air purifiers (6 feet) using Induct/Dux Deluxe air purifier. After 24, 48 and 72 hours the plates were secured over a numbered map of the plate marking on each plate where the top counter was positioned. The data were summarized in the following graph (figure 6) shows the reduction in the number of colonies produced in the inoculated TSA agar plates with 5% sheep blood when any of the air purifier was used. From the graph (Figure 6) and photograph of the agar plates (Figure 7 D) it can be concluded that the Induct/Dux Deluxe air purifier is more efficient than the 3000-3600 in reducing the bacterial colonies. Since the methicillin resistant *Staphylococcus aureus* did not grow on the agar plates in the original experimentation we plated them from the stock culture after high dilution (10^{-4}) to investigate the effect of the 3000-3600 and the Induct/Dux Deluxe air purifiers on the production of number of colonies of the methicillin resistant *Staphylococcus aureus*. Graph 6 shows that the air purifiers were effective in reducing the number of emerged colonies on agar plates at different intervals of 24, 48 and 72 hours. Induct/Dux Deluxe were the most effective ones, which was able to minimize the number of bacterial colonies. The 3000-3600 was the next effective one. Our research work presented at the World Allergy Congress 2007 on evaluation of 3000-3600 air purifier reported that the 3000-3600 air purifier was efficient in eradication of microflora over a longer period of time at the "high" setting by using the negative ion emission (2). In the present investigation we observed the reduction in the number of the bacterial colonies emerged on the inoculated agar plates because of the negative ion emission that has a negative effect on bacterial growth. Negative ions clean the air of impurities like dust, pollen, animal dander, mold spores, odors, smoke and even bacteria.

Due to these qualities negative ions are termed as the vitamins of air. Negative ions get attached to pollution particles (e.g. dust, pollen and other impurities in the air). When that happens, both the ions and the pollution particles tend to be swept out of the air by the electric field that exists naturally near the earth's surface. Hence, polluted or impure

surroundings tend to reduce the count of negative ions in the air (1) Bactericidal effects of negative air ions on airborne and surface *Salmonella enteritidis* from an artificially generated aerosol is well documented by the Agriculture Research Service, U.S. Department of Agriculture and other agencies (6,7).

We found that the colonies produced on the agar plates inoculated from the diluted culture could not grow further when subjected to the two air purification systems that we are evaluating because the negative ions generated by the air purifiers killed the bacterial population on agar plates restricting their further growth.

In our future research work we shall evaluate the efficiency of the 3000-3600 and Induct/Dux Deluxe air purifiers in reducing the odor and Volatile Organic Compounds in the air.

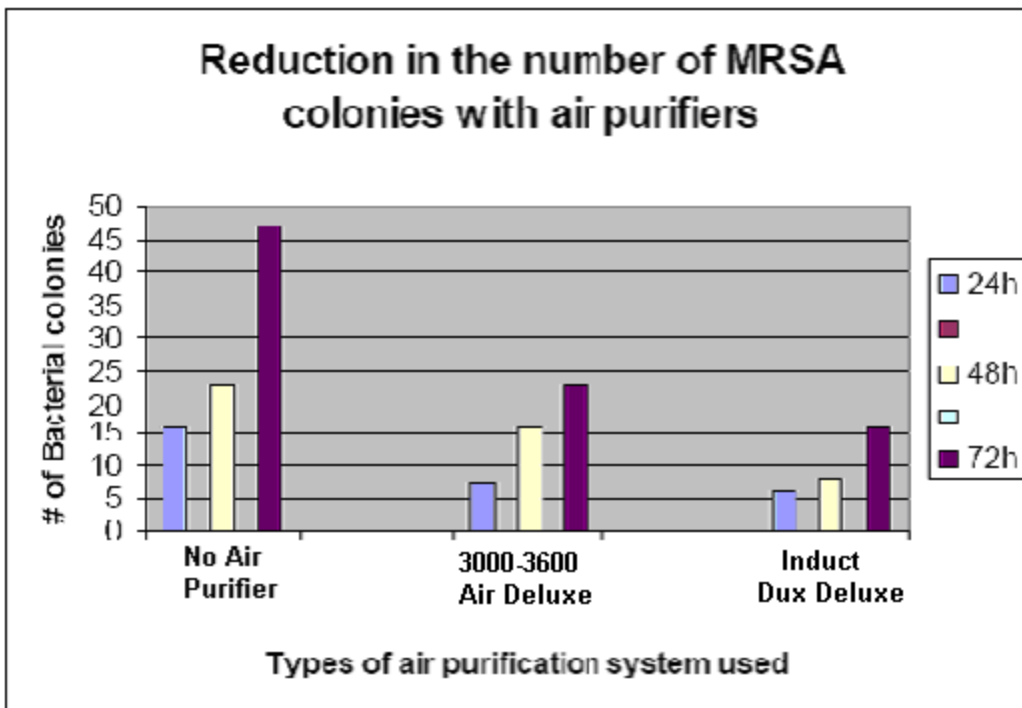
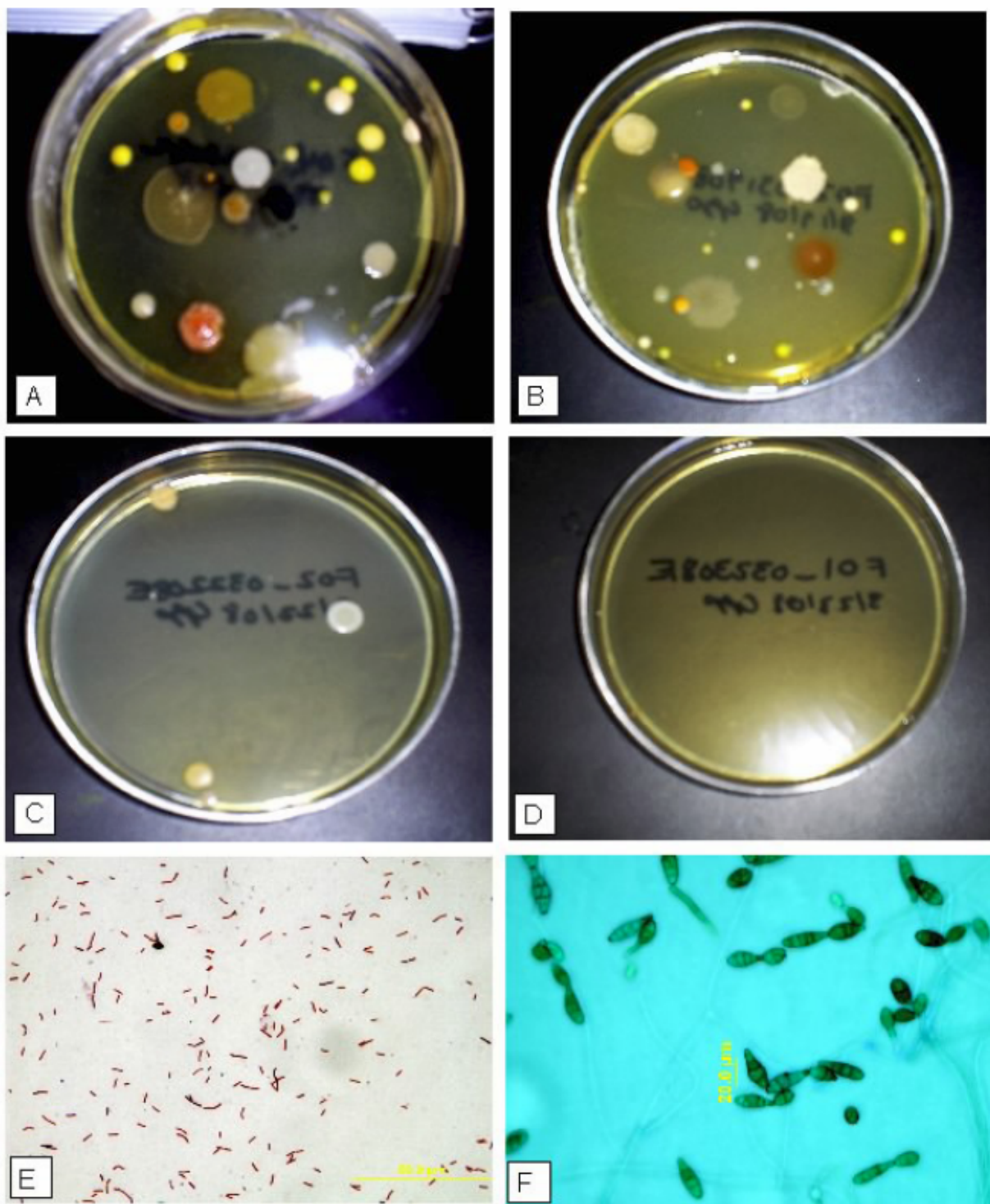


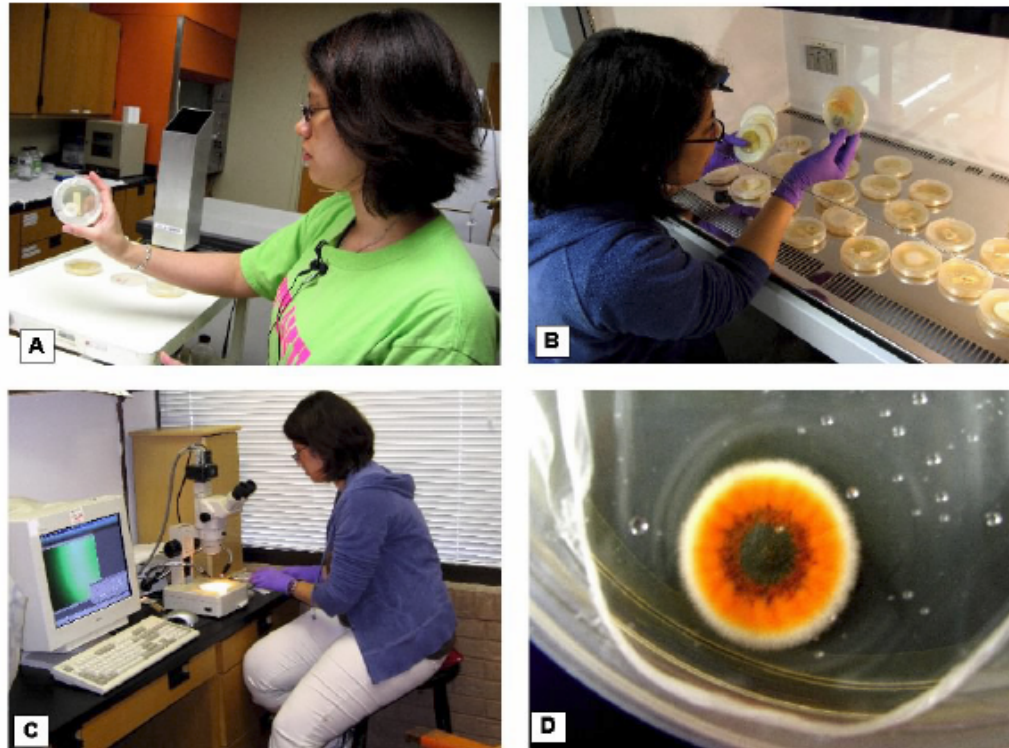
Figure 6: Graph showing the reduction in the number of colonies produced in the inoculated TSA agar plates with 5% sheep blood.

Figure: 7



Gradual reduction of Aeroallergen by the use of Air Purifier Induct/Dux Deluxe and 3000-3600. Figs. A and B are showing the microbial colonies grew on the TSA plates in the Control Sets after 120 hours (A) and 72 hours (B) with no air purification system. Figs. C and D showing the reduction in the number of colonies after 72 hours (C) and 120 hours (D) of treatments with Induct/Dux Deluxe air purifier. Fig. E showing Gram negative Bacilli and F Alternaria codia from petri dishes exposed in the room air for 120 hours without the air purifier (Control Set).

Figure 8:



Figures A, B, C and D are showing the steps of working on evaluation on microbial growth. A. set up experiment with air purifier. B. Working under Laminar Hood. C. Working with SZ-40 Stereo scope. D. Stereograph of a colony.

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