"In-use Test" of the Odorox® M.D.U. (Mobile Disinfecting Unit)

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ABSTRACT

The Odorox® M.D.U., manufactured by HGI Industries Inc. (West Palm Beach, FL), is a portable disinfection unit which sanitizes air by producing hydroxyl radicals (-OH). These radicals are produced inside of a chamber when UV light from two U-shaped UV light bulbs comes in contact with ambient humidity. Hydroxyl radicals exit the unit and interact with VOC's (volatile organic compounds), allergens, bacteria, mold and viruses on surfaces and in air. Independent laboratory testing by ATS Laboratory (Eagan, MN.) demonstrated that the unit effectively reduced 60.3% to 99.9% of bacteria on stainless steel and cotton fabric after a four hour exposure (1). The laboratory testing was performed in a sterile and sealed room with no airflow, furniture or human presence. The objective of the "in-use test" is to assess the instrument's effectiveness in a situation of actual use, where all the mentioned variables are not controlled. The sampling site for this study was the 3rd floor break room of the WTAMU Agriculture and Science Building which has medium traffic. Two sampling methods were used: surface sampling (cotton swab) and passive air sampling. Three experiments were set up, each consisting of two days, a background swab (day 1) and a swab 24 hours after exposure to the unit (day 2). Seven frequently-touched surfaces were selected for swabbing and seven sites throughout the room were selected to place open Tryptic Soy Agar (TSA) plates for 4, 8 and 12 hours. The TSA plates from both sampling methods were incubated at 37°C for 48 hours. Separately, two control experiments were set up following the same procedure but without the UV light bulbs to prevent hydroxyl production. Bacterial colonies on all plates were quantified using the Darkfield Quebec colony counter and 20 of the most common colonies were purified and identified using the BioMerieux VITEK® 2 identification system (Durham, NC) at the WTAMU Department of Agriculture. Results show that there were fewer bacteria obtained from surfaces and the air after running the Odorox ® M.D.U. for 24 hours.76% out of a hundred randomly chosen colonies from plates of both sampling methods were Gram positive, which

correlates with a study by Rintala *et al*. (3), which demonstrated that the microbial flora indoor was dominated by Gram-positive species which originated from the users of the building (3).

INTRODUCTION

Bacteria, mold and viruses are ubiquitous in the indoor and outdoor environment. Other particles like allergens and VOC's (volatile organic compounds) also represent a possible threat to human health (6). Most airborne organisms originate from natural sources as plants, human and animal activities and artificial sources such as sewage treatment, farming and agricultural activities, all of which release viable organisms into the air (4). Bacteria and fungi can become airborne by encapsulating themselves in tough sheets (becoming spores) which are carried by airflow that passes through these organisms. The ability to remain airborne has to do with special aerodynamic adaptations, durability and the substrate it may attach to (7). These organisms are not necessarily a threat to human health as we have innate and acquired mechanisms of defense that protect us from them. The problem arises for immunosuppressed persons, children and those with hypersensitivities. Also, many health-related places such as hospitals and nursing homes have to constantly evaluate the air and surfaces because of the amount of airborne pathogens that are possibly released and the threat they pose to individuals who are already sick. There is concern about the appearance of new strains of antibiotic-resistant bacteria such as Methicillin-resistant *Staphylococcus aureus* (MRSA),Vancomycin Resistant Enterococci (VRE), Extensive drug-resistant Tuberculosis (XDR TB) (2), etc. and the increasing infection rate in non-health related settings is a concern in the community. It is crucial to replace antimicrobial products in community settings with bactericidal compounds that will not induce bacterial resistance.

The Odorox M.D.U.:

The Odorox® M.D.U., manufactured by HGI Industries Inc. (West Palm Beach, FL), creates synthetic ultraviolet rays of multiple wavelengths which interact with ambient humidity in the chamber, forming hydroxyl radicals (Figure 1), which are among the most powerful naturally-occurring oxidizing agents in the environment. A free radical is an atom that contains one or more unpaired electrons, which alters the chemical reactivity of this atom making it more reactive, in other words attacking anything that it is next to (8). Free radicals bind to proteins,

phospholipid membranes and DNA, causing mutations and generalized cell damage (8)(9). Although these molecules are highly reactive, at the level produced by the Odorox® M.D.U., poses no threat to humans and animals because of antioxidants and vitamins present in many bodily secretions produced by our skin, mucous membranes and eyes (8). The purified air and hydroxyl radicals exit the unit and interact with volatile organic compounds (VOC's), bacteria, allergens, mold and viruses in air and on surfaces as seen below (Fig. 1).

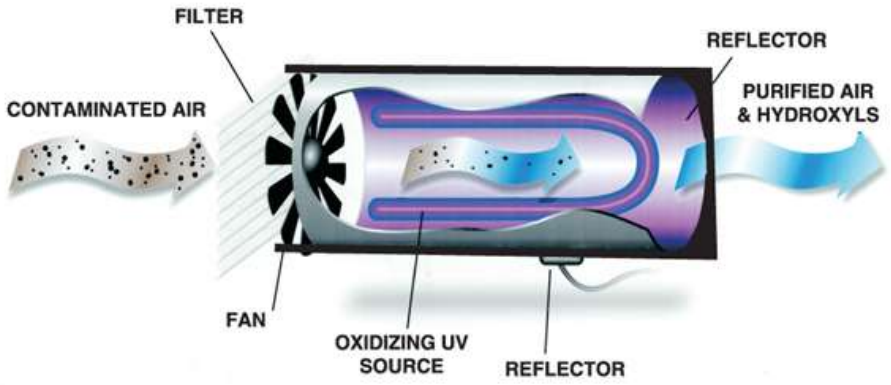

Figure 1. Hydroxyl radical formation and air decontamination in the Odorox® M.D.U. chamber (1).

The "in-use test":

We conducted an in-use test to assess the effectiveness of the Odorox M.D.U. in actual community conditions such as where the unit would be used. Independent laboratory testing was performed by ATS LABS (Boynton Beach, FL). Metal and fabric pieces were inoculated with representative Gram positive and Gram negative organisms (*Staphylococcus aureus*, *Streptococcus*, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Micrococcus*, *Pseudomonas aeruginosa*, *Escherichia coli*) and mold (*Aspergillus niger*). These objects were exposed to the unit for 4 to 96 hours depending on the organism. The room in which the laboratory testing was performed was sterilized and sealed, preventing human and airflow in and out of the room. These conditions are not realistic in a community setting. In the in-use test, we did not control the number of people or air flow in the room and there was no other disinfection method other than the Odorox® M.D.U.

MATERIALS AND METHODS

Surface and air sampling was done using swabs and open TSA plates, respectively. The sampling site was the 3rd floor break room of the WTAMU Agriculture and Science Building (Fig. 2) which has an area of xx ft². At the time of the sampling, there was human traffic of around 10 to 12 people in a weekday. Our study was conducted during the month of June when the room is used less frequently. The use of this room varies depending on the time of year and the day of the week. XX of the most common colonies were selected to be identified with the BioMerieux VITEK® 2 identification system (Durham, NC) at the WTAMU Department of Agriculture.

Figure 2. Sample room, 3rd floor break room of the Agriculture and Science Building. Numbers indicate swab sites.

Surface sampling:

Seven frequently-touched surfaces in the break room were selected. These sites were: 1) table 1, 2) back of a chair, 3) table 2, 4) metal cabinet door, 5) refrigerator handle, 6) microwave, and 7) microwave table. Three different experiments were conducted, each lasting two days. A background swab was performed on day one (before the Odorox® M.D.U. was turned on) and another swab sample was obtained after operating the unit for 24 hours. Sterile cotton swabs moistened with phosphate-buffered saline (PBS) were used to swab a 1 in^2 surface. The swabs were vortexed in 5 ml of PBS and plated in triplicate (1ml/plate) in 22 ml of TSA at 50°C. The

plates were incubated for 48 hours at 37^{o} C. Two separate control experiments were performed using the same procedure, in which the UV bulbs were removed from the unit to prevent hydroxyl radicals from being produced. Colonies were counted by hand using a Darkfield Quebec colony counter. The most common colonies (based on cultural characteristics) were isolated and Gram stained.

Passive air sampling:

Seven sites were selected in which to place open TSA plates, at different heights and distances from the Odorox® M.D.U. The TSA plates (63.61 cm² area each) were exposed for 4, 8 and 12 hours and then incubated for 48 hours at 37^{o} C. Colony quantification was performed as described in the surface sampling method, it is important to note that this method does not account for all the particles in the air, nor all bacterial colony forming units that have settled on the plates and that the volume of air from which the organisms originate is unknown. The colony numbers only reflect viable or live microorganisms that grew on exposed plates at 37°C.

RESULTS

The average colony counts obtained before and after 24-hr use of the Odorox® M.D.U. unit were averaged and compared, Tables 1 and 2 show these numbers. The colony counts from specific sites in the room, including airborne counts, were also compared. The average colony number from day one was compared to the average colony count obtained on day two and those numbers were compared through the following experiment days. There was at least a 50% reduction of bacterial colonies after running the Odorox ® M.D.U. for 24 hours. We attribute the decrease in colony counts to the action of the hydroxyl radicals and filters in the unit. In each of the three trials, the colony counts on the open plates from day one were fewer than the number of colonies from day two. For example, in Experiment One, 3-20 colonies were obtained in the background, whereas the number was reduced to 0-9 colonies after Odorox use for 24 hrs.

Figures 3 through 9 are a graphic representation of the average colony counts for each swab site through the different experiments and control experiments. Figure 10 is a comparison of average colony count for the passive air sampling.

Twenty Gram positive organisms were selected randomly and identified using the VITEK® 2 bacterial identification unit. Ten of those organisms are listed below in table 3 with the place where they are commonly found. The other ten organisms were either an unsuccesful identification (slashline) or were identified as one of the ten organisms listed.

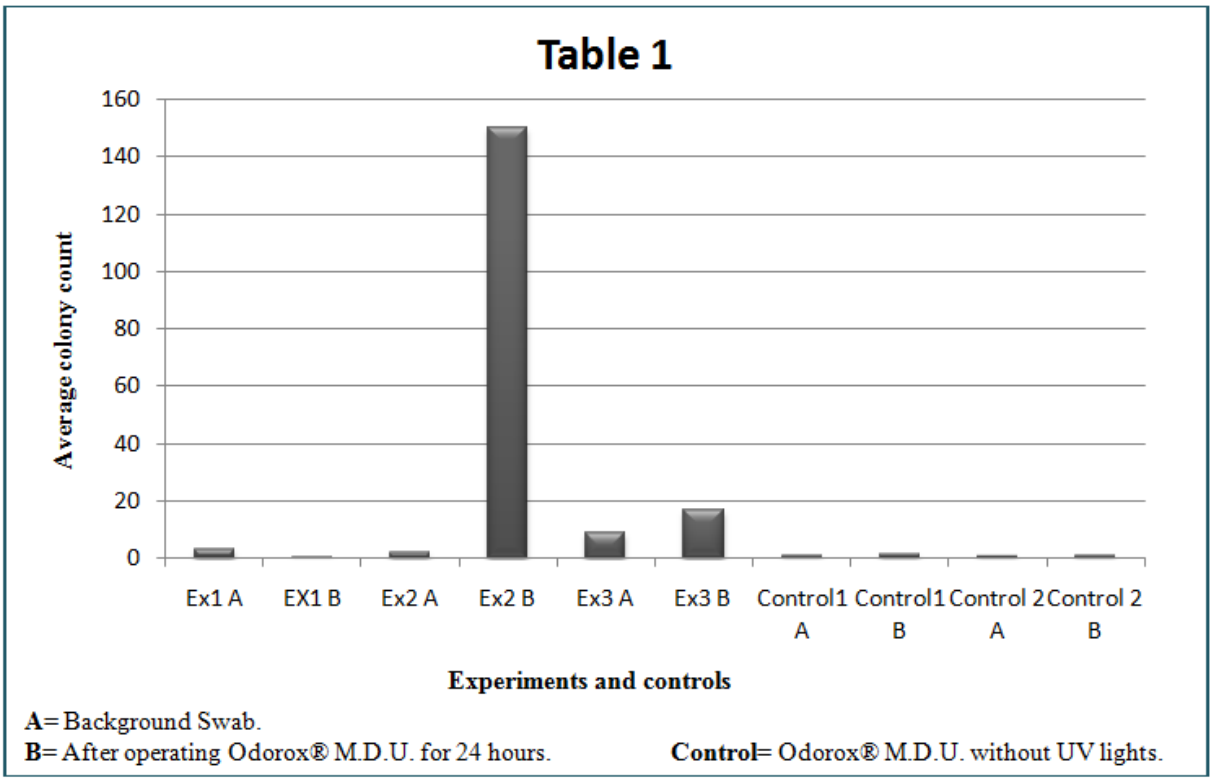

Figure 3. Comparison of average colony counts for table 1 (sample site #1).

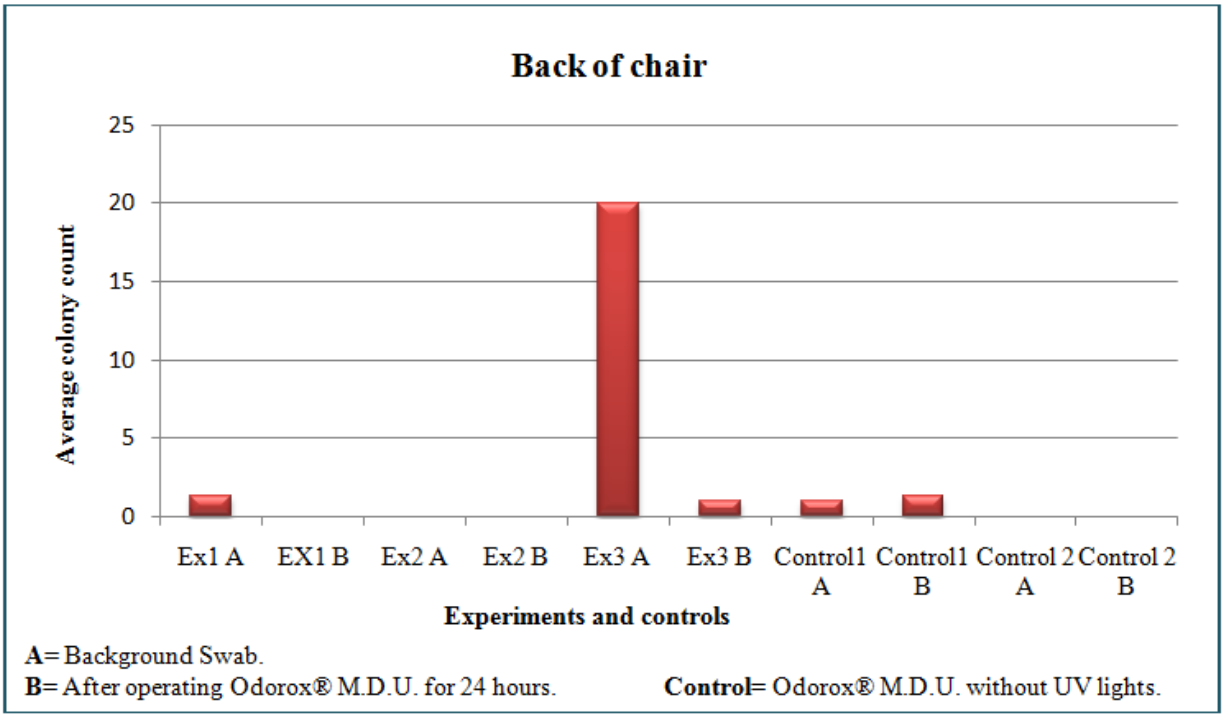

Figure 4. Comparison of average colony counts for back of a chair (sample site #2).

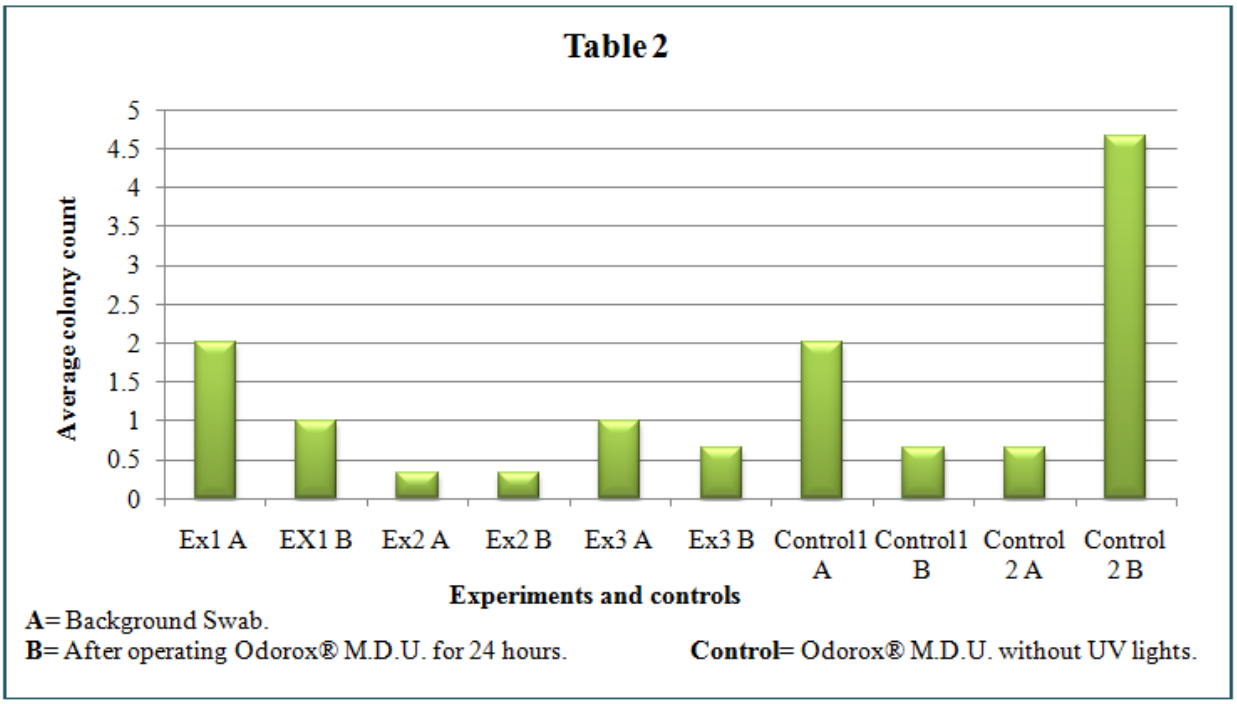

Figure 5. Comparison of average colony counts for table 2 (sample site #3).

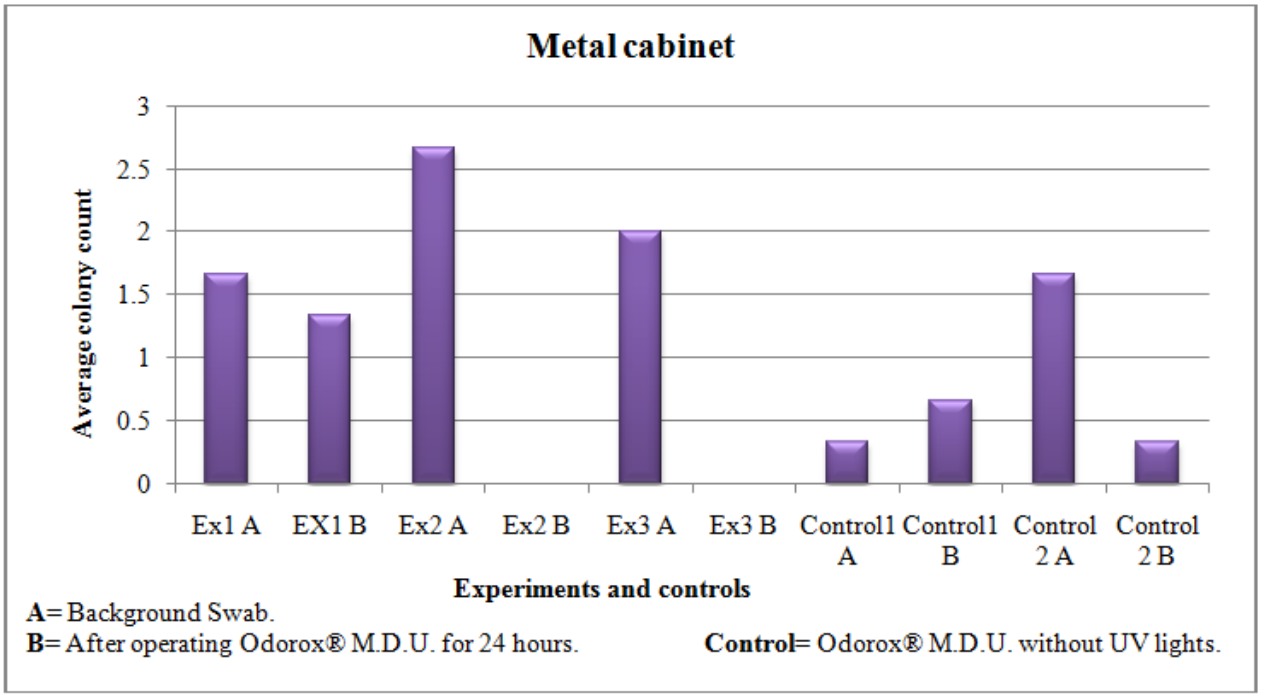

Figure 6. Comparison of average colony counts for a metal cabinet (sample site #4).

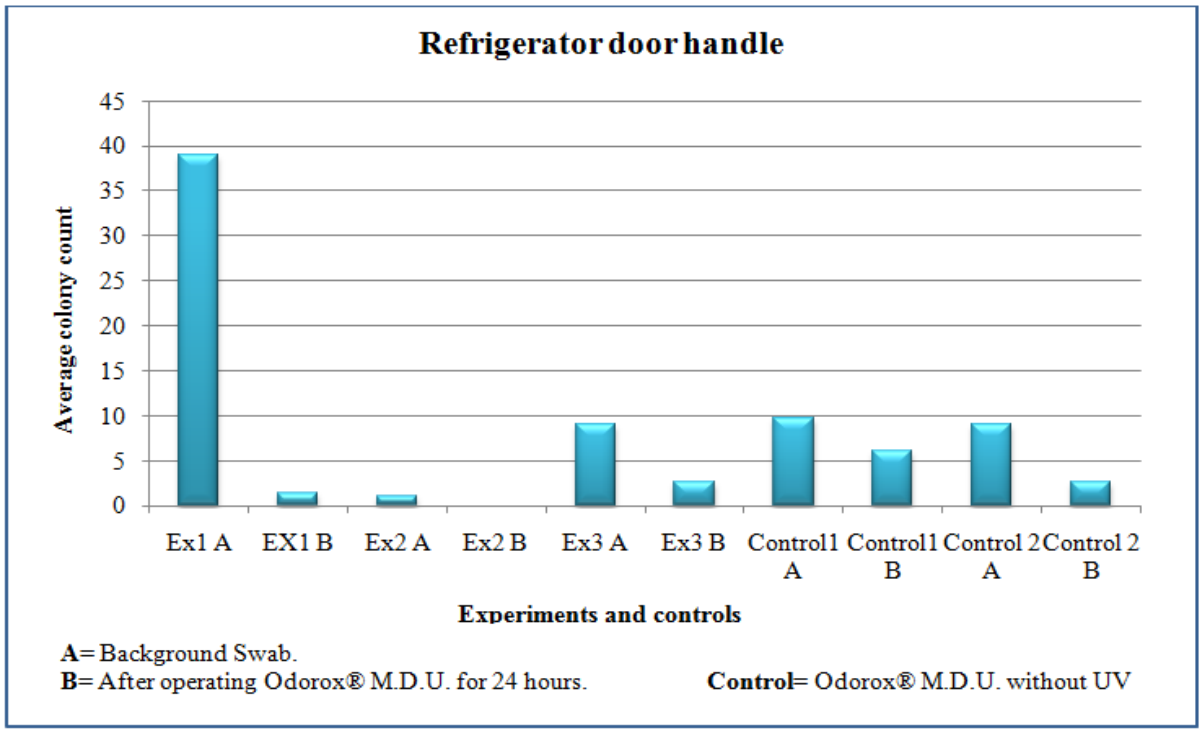

Figure 7. Comparison of average colony counts for refrigerator door handle (sample site # 5).

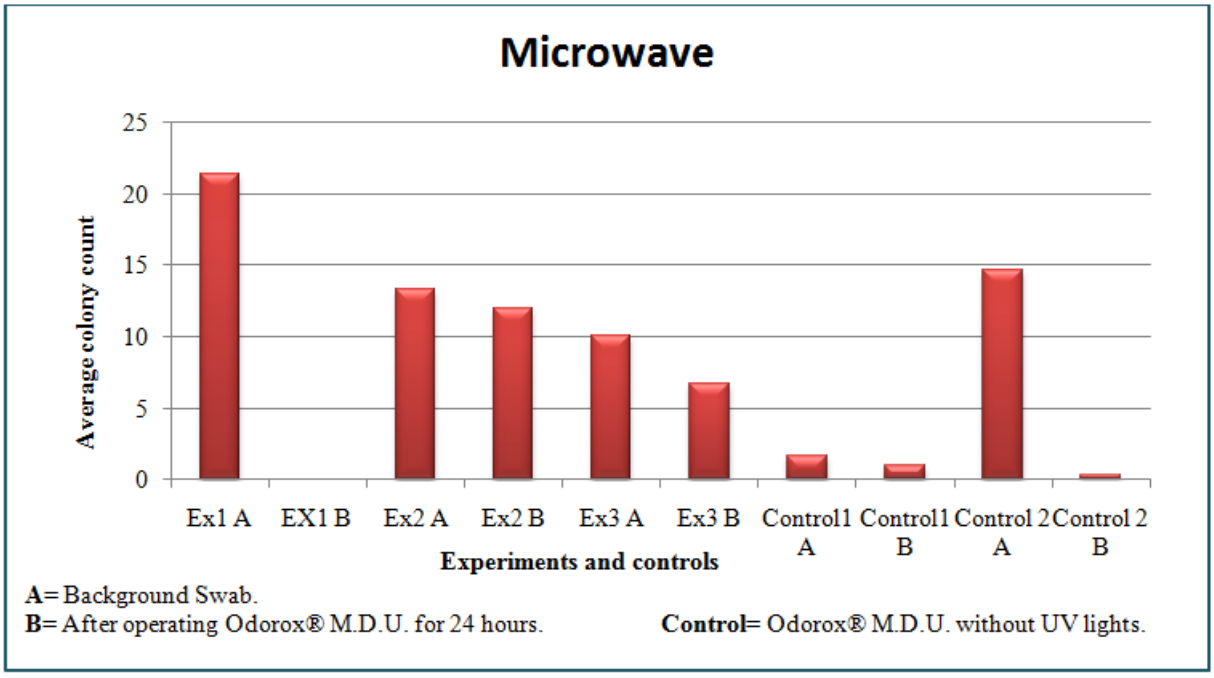

Figure 8. Comparison of average colony counts for microwave (sample site # 6).

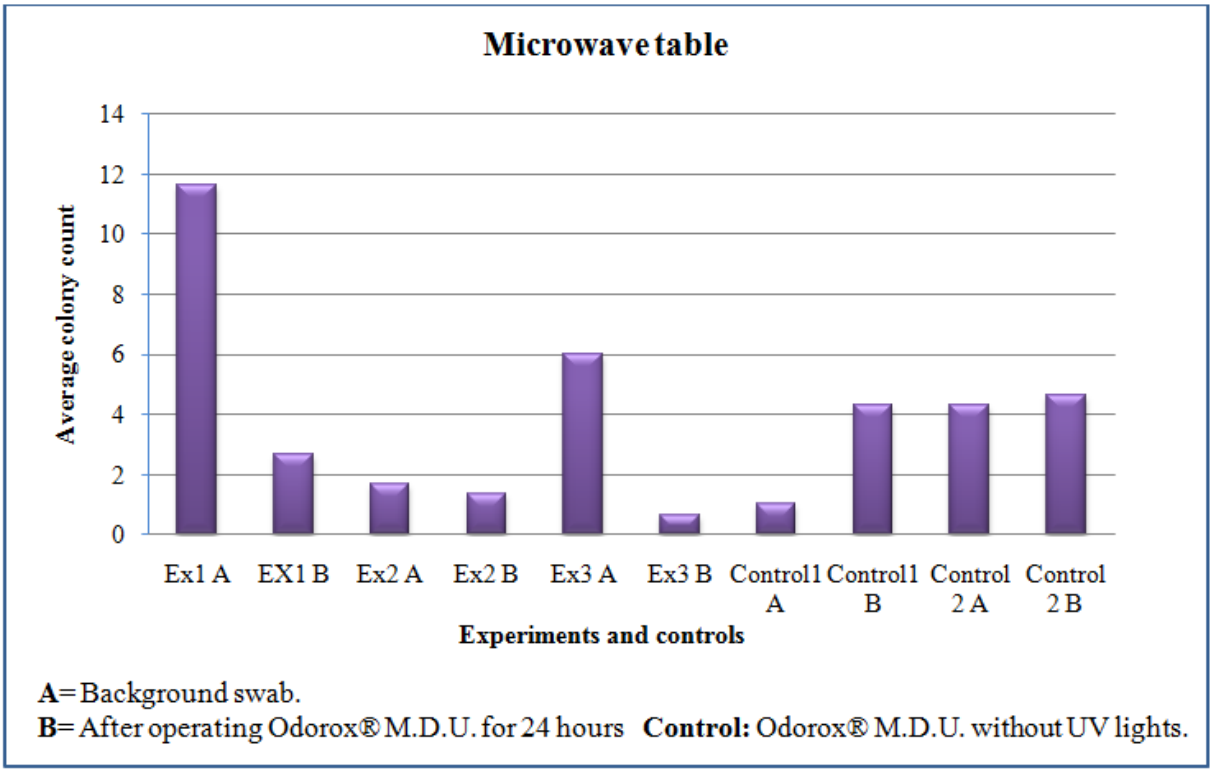

Figure 9. Comparison of average colony counts for microwave table (sample site # 7).

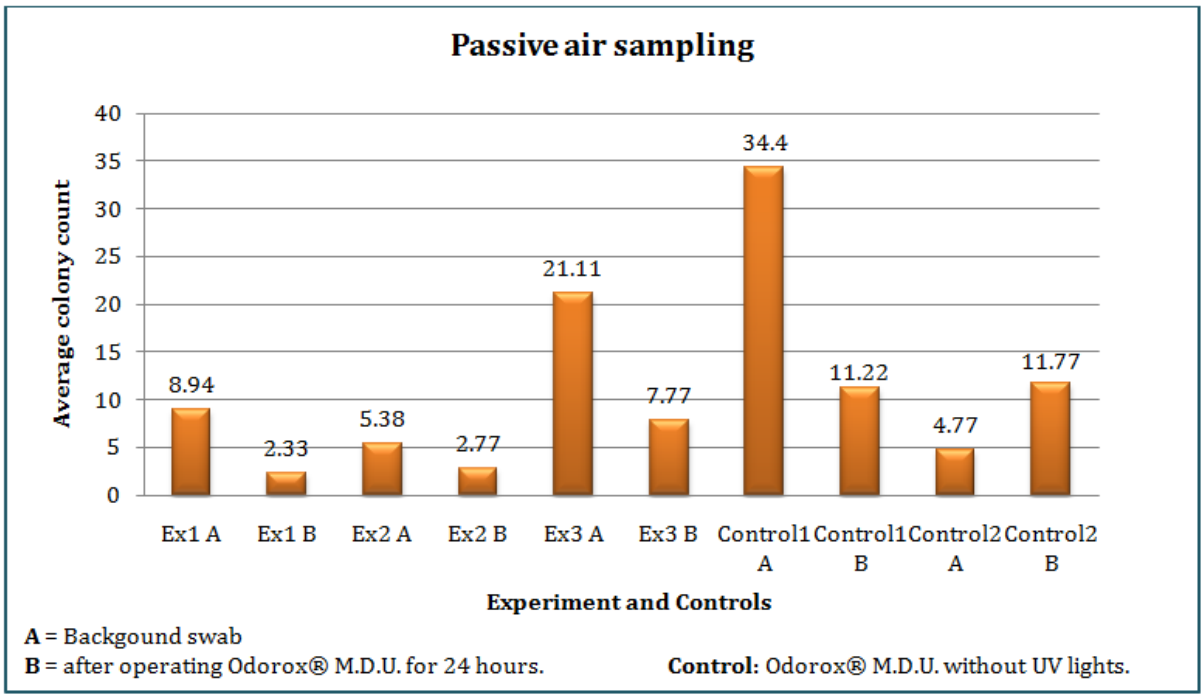

Figure 10. Comparison of average colony counts for passive air sampling.

Table 3. Organisms identified with VITEK® 2 and where they are commonly found (9).

Organism	Common location
Micrococcus luteus	Skin and nasal membranes of humans.
Staphylococcus haemolyticus	Vertebrate skin.
Enterococcus cecorum	Environment, human intestines and feces.
Staphylococcus warneri	Skin and mucous membranes of warm-blooded organisms, dust, water and food.
Alloicoccus otitis	Vertebrate middle ear.
Staphylococcus epidermidis	Human skin flora, mucous membranes and also found in animals.
Granulicatella adiacens	Mouth flora, can cause infective endocardiatis.
Kocuria varians	Non-pathogenic commensals of the skin and mucous membrane and oropharynx
Staphylococcus auricularis	Skin and mucous membranes of deer, dogs and humans.
Staphylococcus aureus	Skin and nasal membranes of humans.

DISCUSSION

Higher effectiveness of this product may be seen when combined with other disinfection methods and regular cleaning practices.

Microorganisms present in the room are less depended on outside air in the Agriculture and Science building since only 20% of the circulating air is taken from outside, while the other 80% is recycled air (1). There are 48 different filters between the outside and inside air in the ANS building (1). Studies show that people are responsible for doubling the atmospheric particle concentration in a room (2) more than air units and particles already existing in the room. This explains why it is important to test this unit in actual community settings where varying numbers of people are present.

Organisms recovered were on the most part Gram positive organisms that are usually present in the environment and are form part of the normal flora of vertebrates (R).

It is not expected to find the same bacterial count each day, but overall the counts were reduced after using the unit for 24 hrs.

We did not quantify molds or viruses.

ACKNOWLEDGEMENT

I sincerely thank Dr. Bouma for overseeing this project and for her incredible patience and help thoughout my research. I thank Glenn Norris, Dr. Stephen Norris and Eldin Norris for the use of the Odorox® M.D.U., devoting their time and putting their trust in me to perform this testing. I also want to thank the Attebury Honors Program for funding the organism identification for this project. A special thanks to Rebecca McCarthy for donating materials and time to perform this testing.

FOR FURTHER INFORMATION

About the in-use test please contact Cynthia Reinoso at reinoso.cynthia@yahoo.com and Dr. Carolyn Bouma at cbouma@wtamu.edu. More information about the Odorox M.D.U. can be found in www.odorox.com.

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Table 1. Surface sampling results for all sampled sites.

*Average colony number of three plates per experiment site. **A:** Background swab. **B:** After operating Odorox® M.D.U. for 24 hours.
Control: Odorox® M.D.U. without UV lights.

Ex*/ Location	Ex 1 A	Ex 1 B	Ex 2 A	Ex 2 B	Ex 3 A	Ex3 B	Control 1 A	Control 1 B	Con
#1	3	0.33	2	150.3	9	16.66	1	1.33	0
#2	1.33	0	0	0	20	1	1	1.33	
#3	2	1	0.33	0.33	1	0.66	2	0.66	0
#4	1.66	1.33	2.66	0	2	0	0.33	0.66	1
#5	39	1.33	1	0	9	2.66	1.66	6	
#6	21.33	0	13.33	12	10	6.66	1.66	1	14
#7	11.66	2.66	1.66	1.33	6	0.66	1	4.33	4

Table 2 Passive air sampling results for all sampled sites.

*Average colony number of three plates per experiment site. **A:** Background swab. **B:** After operating Odorox® M.D.U. for 24 hours.
Control: Odorox® M.D.U. without UV lights.

Ex*/ Location	Ex 1 A			Ex 1 B			Ex 2 A			Ex 2 B			Ex 3 A			Ex 3 B			Control 1 A			Cont	
Time(hrs)	**4**	**8**	**12**	**4**	**8**	**12**	**4**	**8**	**12**	**4**	**8**	**12**	**4**	**8**	**12**	**4**	**8**	**12**	**4**	**8**	**12**	**4**	**8**
#1	8	7	13	0	3	0	4	6	1	1	2	5	14	14	225	3	3	8	1	5	25	1	2
#2	9	7	10	1	3	6	2	9	7	0	4	0	10	7	10	6	22	12	23	26	36	7	7
#3	7	12	20	1	1	9	3	8	1	2	3	3	1	5	7	5	8	13	38	40	35	10	1
#4	3	7	15	1	1	5	0	34	4	2	2	6	4	12	30	5	9	8	28	50	52	5	9
#5	8	5	11	0	2	2	5	3	6	1	0	7	3	4	10	6	5	7	42	32	63	14	8
#6	4	7	17	0	2	3	3	1	1	2	6	3	5	5	12	4	10	6	38	33	53	16	9
average / day	8.94			2.33			5.38			2.77			21.11			7.77			34.4			11.	