

Final Report from The University of Michigan regarding the use of My-shield® products utilized by an authorized distributor (Gernfree Innovations, LLC)

University of Michigan Group Project # AWD019640

Date: 2/11/2022

EVALUATION

My-Shield® Broad Spectrum Disinfectant Persistent and Cumulative Antimicrobial Properties in a Lacrosse Locker Room at the University of Michigan

Submit to U-M Athletic Department and Germfree Innovations

Sample collection and analysis were performed by Dr. Jianfeng Wu, in Dr. Chuanwu Xi's Laboratory at University of Michigan
Protocol written by Daniel Kahl, GermFree Innovations, LLC

Data and report were reviewed and approved by Dr. Chuanwu Xi

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Study conducted by: Dr. Chuanwu Xi



_____ **Date: 4/9/2022** _____

INTRODUCTION:

This study was designed to evaluate the persistent and cumulative antimicrobial properties of the My-Shield® Broad Spectrum Disinfectant in a Lacrosse Locker Room during “real life” settings, taking into consideration persistent, microbial colony reduction on surfaces based on laboratory studies conducted on My-Shield® products using Zetrisil® technology. My-Shield® Broad Spectrum Disinfectant was tested against the daily application of the control, an EPA registered, hospital grade, 1% hydrogen peroxide and 5% benzyl alcohol solution. This facility was chosen based on the following criteria:

- Symmetrical conditions in the facility for both the control and test product areas of the facility were used during the study.
- The University of Michigan Lacrosse Locker Room has consistent introduction of microorganisms from the athletes and staff that use the facility as well as consistent treatment protocols.
- The Lacrosse Locker Room was selected because the team would use the locker room daily during the test period.
- The rate of exposure to sweat and microorganisms on the surfaces from the athletes would be higher during the test period.
- The tests in this facility could demonstrate the rate at which My-Shield® Broad Spectrum Disinfectant can reduce bacterial loads between treatments with continued persistent effect.
- The study would evaluate My-Shield® Broad Spectrum Disinfectant with Zetrisil® technology using the same standard daily sanitizing and cleaning protocols used with the hydrogen peroxide, benzyl alcohol product as the control.

Both the control and My-Shield® Broad Spectrum Disinfectant were assessed in consecutive weeks and presented on a table, a line graph presenting daily data, and bar graphs displaying compared CFU count averages for the procedural sections defined in the “Procedure for Application and Assessment of Product” section of the study. This was performed with similar testing timelines and with samples taken each day the locker room had athletes and staff using it. The total test period covered 31 days. The preparation of a comparative test summary chart, which includes test number, dates, temperature, and location of surface swab results, are recorded in the study.

The locker room was divided and labeled into section A, the control side, and section B, the My-Shield® Broad Spectrum Disinfectant side. The designated areas in section A were used as the control disinfectant and section B used My-Shield® Broad Spectrum Disinfectant. Both products were applied by the same staff members that normally applied the standard cleaning solutions and each followed the same product application protocol used for the control product. The application protocol was applied to both the control and My-Shield® Broad Spectrum Disinfectant in the facility. This trial took place over 31-days of exposure and with sample collections obtained from sections A and section B twice per day pre/post cleaning in the facility. A third sample was also taken at a random time each day of exposure.

All data was recorded in the charts and descriptions in the “RESULTS” section of this document. Notes and conclusions were recorded in the “CONCLUSIONS” section of this document.

OBJECTIVES

This product evaluation protocol of My-Shield® Broad Spectrum Disinfectant, formulated with Zetrisil® technology, will be tested against the daily application of the control to evaluate a cumulative and persistent antimicrobial effect. The Proposed testing will take place within specified areas in the University of Michigan Lacrosse Locker Room; Section A will be the control and Section B the test side. This study will evaluate the following:

1. The cumulative antimicrobial effect of repeated application of My-Shield® Broad Spectrum Disinfectant
2. The lasting persistent effect of application of My-Shield® Broad Spectrum Disinfectant.

STUDY PROTOCOL AND RESULTS OUTLINE

Facility location... 2540 S State St, Ann Arbor, MI 48104

Test Product... My-Shield® Broad Spectrum Disinfectant

Control Product... A hospital grade, 1% H₂O₂, 5% benzyl alcohol solution that is a standard solution used for athletic and medical facilities.

Lacrosse player level... 50 players

Evaluation start date ... November 15, 2021

Evaluation completion date... Jan 18, 2022

Report date... February 11, 2022

Sample collection method... wet swab

Sample collection surfaces... locker room bench surface; vinyl seat

Sample collection temperature... 22°C

Incubation and growth media data... 30°C, sheep's blood agars

Colony formation description... Isolated colonies had a light yellow, smooth surface, and smooth edge. A small portion of them are white, smooth surfaces.

CFU counting method... manual count

SAMPLE CULTURE METHOD

Sheep's blood agar was used as the culture median. All good laboratory practices were incorporated in relation to:

- Transportation & storage of sample: ice
- Sterilization of all glassware
- Sterilization of grown medium
- Incubation: 48hrs @ 30 degrees Celsius

Counting Colonies: Manual colony counter to according to best laboratory practices. To calculate the number of bacteria per mL of diluted sample was used according to the following equation: 1x 10⁻⁸ dilution plate you plated 0.1 mL of the diluted cell suspension and counted 200 bacteria, then the calculation would be: 200/0.1 mL x 10⁻⁸ or 200/10⁻⁹ or 2.0 x 10¹¹ bacteria per mL.

PROCEDURE FOR APPLICATION AND ASSESSMENT OF TEST AND CONTROL PRODUCTS

Protocol for Treatment

1. The control product, was applied to side A using the same daily protocol for all 31 days during the test period.
2. My-Shield® Broad Spectrum Disinfectant, was applied daily for the first seven days beginning on November 15 and ending November 23.
3. After the first 7 days, weeks 2 & 3, My-Shield® Broad Spectrum Disinfectant was applied every other day for days 8-20 beginning on November 24 and ending December 10
5. Weeks 4 & 5, My-Shield® Broad Spectrum Disinfectant was applied once per week on days 21-31, beginning on January 4th and ending January 18th
6. The locker room was not treated, occupied, or tested on weekends. The room was also not treated, tested, or occupied on November 25th – November 28th due to vacation. The locker room was also not occupied or treated on December 11th – January 2nd; however, samples were collected on December 20th from both sides of the locker room.

Sample Collection Protocol

1. **Collection Frequency:** Sample collection was Monday through Friday on days the locker room was occupied and treated throughout this study, with one day of sample collection on December 20th during holiday break, while the room was neither treated with either product, or occupied.
2. **Days 1 - 31:** Swabs were taken from random locations within the designated areas before facility use (cleaned). Swabs were taken after facility use but prior to the next scheduled cleaning. Random swab samples were taken from Sections A and B at a varying time during the day.
3. Nominated surfaces were swabbed with laboratory swabs.

RESULTS

As detailed in the attached protocol, a Lacrosse Locker Room at University of Michigan Athletic Department, was selected as the testing site. U-M Athletic Department Janitorial staff performed the application of the product according to the schedule. The level of viable

bacterial cells at random three locations from the control side (A: standard sanitation protocol) and the treatment side (B: My-Shield[®] product), respectively, was determined and expressed as Colony forming units (CFU) per area. The graphs below show the CFU number during the testing period with four application scenarios: (1, once every day; 2, once every other day; 3, Holiday break; 4, once per week) (Figure 1). The top panel shows the CFU in the absolute value and the bottom panel shows the CFU in the log-transformed value. The original data was amended below in the report. Average CFU/100 cm² during the different application frequencies were also plotted (Figures 2-4).

The data showed a range of 93%-100% reduction in the viable bacterial counts with the application of the My-Shield product once every day, compared to the standard sanitation protocol and a range of 95%-100% reduction with the application once every other day with 100% reduction on days 6-10 and 14-16. When the application frequency moved to once every week, a range of 98% to -251% reduction was observed; however, the average during this 2 week test period of once per week was a 70% reduction. Readings of 53% and 251% higher than that of the control were observed at this application frequency. The exceptional higher counts may be due to heavy shedding from athletes right before the sampling event.

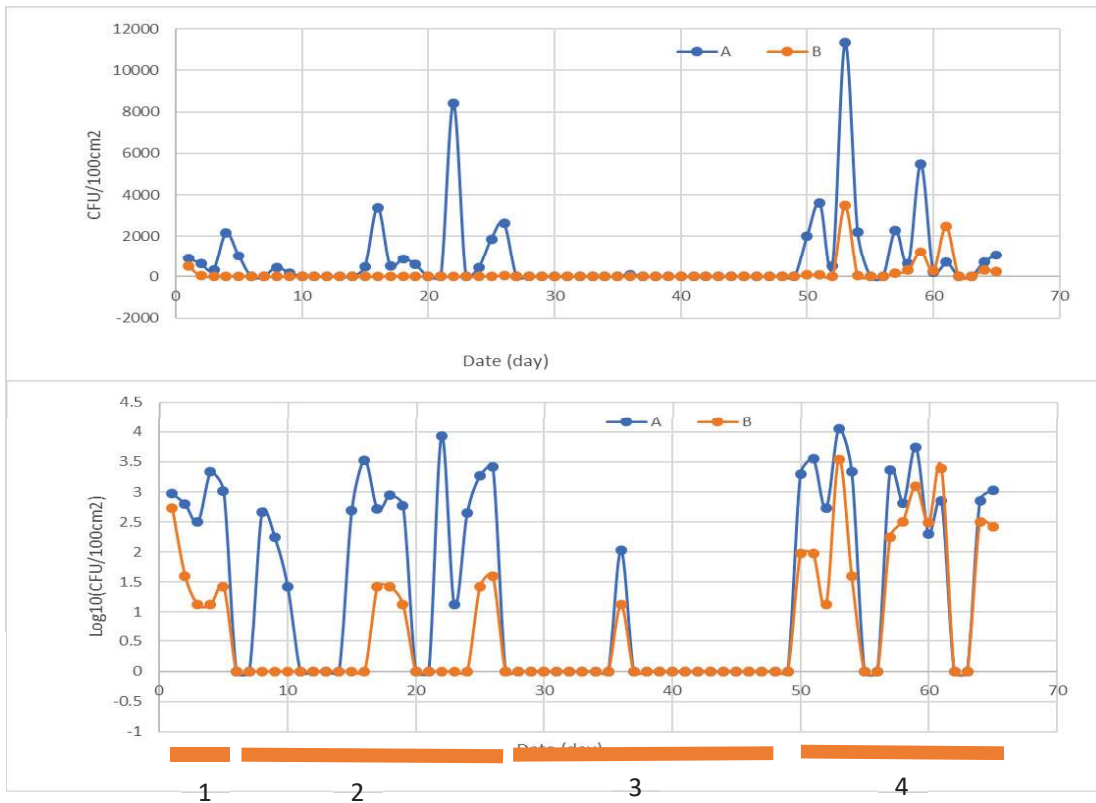


Figure 1: Daily bacterial load on the bench surfaces in a Lacrosse Locker Room at U-M prior to the routine sanitation with two different products

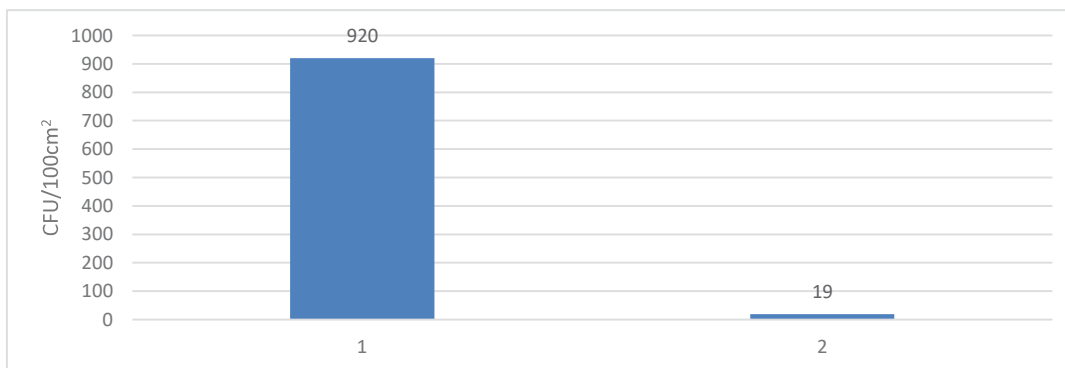


Figure 2: Average CFU/100cm² counts for daily treatment with the control were 920 with a standard deviation of 659 (1) and daily treatment with My-Shield® Broad Spectrum Disinfectant were 19 with a standard deviation of 15 (2)

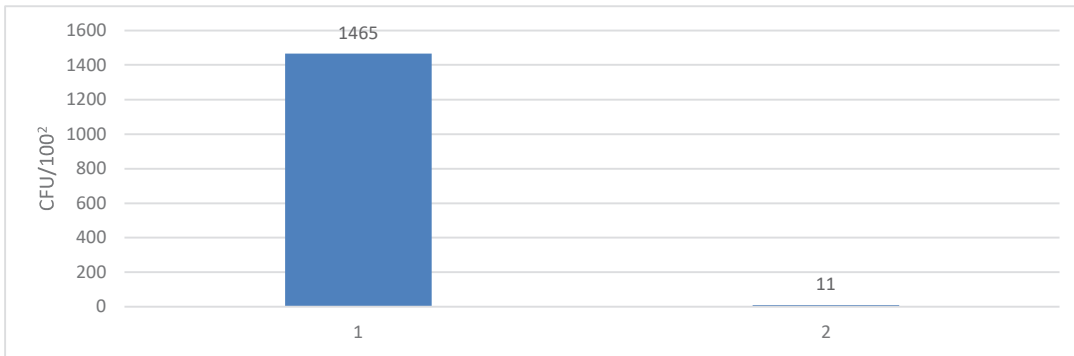


Figure 3: Average CFU/100cm² counts for daily treatment with the control were 1,465 with a standard deviation of 2,405 (1) and treatments every 2nd day with My-Shield[®] Broad Spectrum Disinfectant were 11 with a standard deviation of 15 (2)

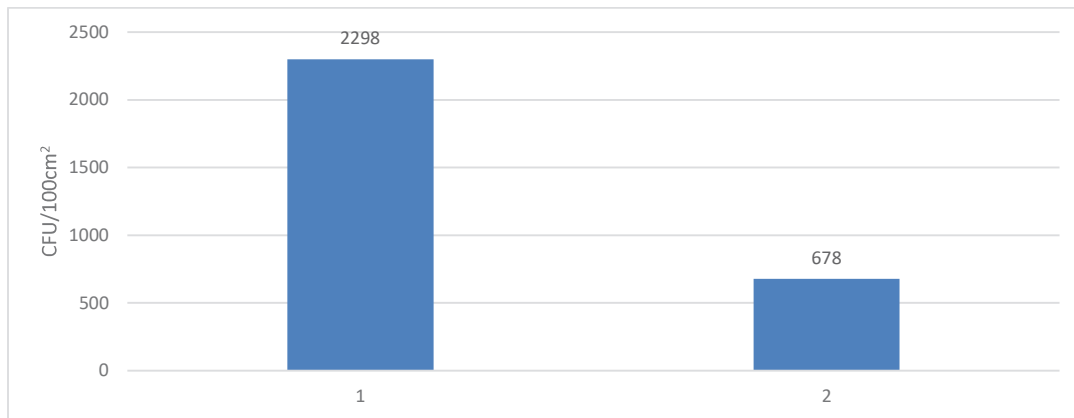


Figure 4: Average CFU/100cm² counts for daily treatment with the control were 2298 with a standard deviation of 3,084 (1) and once per week treatment with My-Shield[®] Broad Spectrum Disinfectant were 678 with a standard deviation of 1,117 (2)

CUF/100cm2							mean		stdev		
Date	Control	Control	Control	My-Shield	My-Shield	My-Shield	ControlClick or tap here to enter text.	My-Shield	Control	My-Shield	Reduction%
15-Nov	520	1640	640	280	600	720	933	533	615	227	42.9
16-Nov	280	360	1240	0	0	120	627	40	533	69	93.6
17-Nov	160	440	360	0	40	0	320	13	144	23	95.8
18-Nov	240	4840	1360	0	40	0	2147	13	2399	23	99.4
19-Nov	240	1120	1760	0	40	40	1040	27	763	23	97.4
20-Nov											
21-Nov											
22-Nov	440	600	360	0	0	0	467	0	122	0	100.0
23-Nov	0	320	200	0	0	0	173	0	162	0	100.0
24-Nov	0	0	80	0	0	0	27	0	46	0	100.0
25-Nov											
26-Nov											
27-Nov											
28-Nov											
29-Nov	720	240	520	0	0	0	493	0	241	0	100.0
30-Nov	480	1280	8400	0	0	0	3387	0	4360	0	100.0
1-Dec	840	80	640	0	80	0	520	27	394	46	94.9
2-Dec	1000	960	720	0	80	0	893	27	151	46	97.0
3-Dec	840	480	440	0	40	0	587	13	220	23	97.7
4-Dec											
5-Dec											
6-Dec	240	120	24840	0	0	0	8400	0	14238	0	100.0
7-Dec	0	40	0	0	0	0	13	0	23	0	100.0
8-Dec	0	240	1080	0	0	0	440	0	567	0	100.0
9-Dec	4280	480	840	80	0	0	1867	27	2098	46	98.6
10-Dec	1320	2240	4320	0	120	0	2627	40	1537	69	98.5
11-Dec											
12-Dec											
13-Dec											
14-Dec											
15-Dec											
16-Dec											
17-Dec											
18-Dec											
19-Dec											
20-Dec	40	280	0	0	40	0	107	13	151	23	87.5
21-Dec											
22-Dec											
23-Dec											
24-Dec											
25-Dec											
26-Dec											
27-Dec											
28-Dec											

29-Dec											
30-Dec											
31-Dec											
1-Jan											
2-Jan											
3-Jan	520	2480	3000	80	200	0	2000	93	1308	101	95.3
4-Jan	6880	3120	800	240	0	40	3600	93	3068	129	97.4
5-Jan	800	200	600	0	0	40	533	13	306	23	97.5
6-Jan	7200	14400	12400	720	8400	1320	11333	3480	3717	4271	69.3
7-Jan	560	1200	4800	0	80	40	2187	40	2286	40	98.2
8-Jan											
9-Jan											
10-Jan	640	4920	1280	80	440	0	2280	173	2309	234	92.4
11-Jan	400	1480	40	920	40	0	640	320	749	520	50.0
12-Jan	800	8400	7200	1320	80	2280	5467	1227	4086	1103	77.6
13-Jan	240	120	240	0	80	840	200	307	69	464	-53.3
14-Jan	1680	280	160	120	40	7280	707	2480	845	4157	-250.9
15-Jan											
16-Jan											
17-Jan	920	600	640	0	760	200	720	320	174	394	55.6
18-Jan	1600	80	1520	120	80	600	1067	267	855	289	75.0

Conclusion

The results of the study of the My-Shield product testing applications every day for week 1, every other day for weeks 2 and 3, and once per week in weeks 4 and 5 demonstrated the following: 1) A building antimicrobial effect as demonstrated by the consistent reduction in detected CFU's, and 2) a lasting antimicrobial effect after application.

Based on the results of this study there is evidence to suggest that when the My-Shield® Broad Spectrum Disinfectant product is applied once per week, every other day, or daily, it provides more effective antimicrobial protection over the daily application of the hospital grade, 1% hydrogen peroxide and 5% benzyl alcohol control in a locker room setting. This may also apply to other environments as well.