



LYLE
ENVIRONMENTAL

1507 Chambers Road, Columbus, Ohio 43212

614.488.1022

PREPARED FOR: CLEAN MY MAT

TEST SITE: 4800 Homer Ohio Ln, Groveport, OH 43125
SAMPLING / TESTING DATE: 08/19/2020

DISINFECTION CHALLENGE TESTING

PREPARED FOR:

CLEAN MY MAT

PHONE NUMBER: (604) 341-4240

EMAIL: CLEANMYMAT.MACHINE@GMAIL.COM

TEST LOCATION:

STABER INDUSTRIES

4800 HOMER OHIO LANE

GROVEPORT, OH 43125

CHAIN OF CUSTODY # 081920-A

Disinfection Challenge Testing

Various types of surfaces in our environment are subject to contamination by microorganisms responsible for disease. These organisms can be bacteria, viruses, yeasts, or molds. Microbiological analysis of surfaces, such as yoga mats, is one of the tools to control disease and to limit contamination risks. No regulation exists for surface microbial contamination. However, companies and industries tend to establish specifications to add value to their service or product and to limit contamination risks and reduce public health risks.

The purpose of this testing is to determine the efficacy of a yoga mat cleaning/disinfecting solution in a machine and to provide the procedure for validating this method and procedure for reducing the microbial load of various organisms on the surface of yoga mats.

Organisms listed and suggested by the United States Pharmacopeia (USP) for disinfection challenge testing are as follows:

ATCC #	Description of Organism
11229	<i>Escherichia coli</i> (prokaryote, bacterium)
6538	<i>Staphylococcus aureus</i> (prokaryote, bacterium)
10231	<i>Candida albicans</i> (eukaryote, fungus)

Procedure

Yoga mats are inoculated as follows:

Inoculum Spread on Yoga Mat	# of Mats Disinfected	# of Mats Not Disinfected
<i>Escherichia coli</i>	3	3
<i>Staphylococcus aureus</i>	3	3
<i>Candida albicans</i>	3	3
No Inoculum	3	3

1. Aseptically inoculate the surface of the appropriate yoga mat, using a sterile pipette, with a 2 ml inoculum of microorganism standardized to 1×10^8 to the 8^{th} per ml.
2. Distribute the inoculum evenly over the mat surfaces with a sterile glass applicator rod and allow to air dry. (This could take 15 to 20 minutes.)
3. Aseptically feed the yoga mats into the machine for disinfection treatment.
4. Aseptically remove the treated yoga mats from the machine as they exit and allow to air dry and cool.
5. Sample the yoga mats for recovery of organisms using the RODAC plate (Replicate Organism Detection and Counting) methodology. (Sampling must take place within 1 hour of the disinfecting process.)
6. Three RODAC sampling plates should be used per mat. The entire RODAC plate agar meniscus needs is pressed to the surface for 10 seconds. (Applied pressure and contact time need to be optimized.)

7. Incubate all TSA RODAC plates at 35 degrees Celsius and examine plates for growth at 24 and 48 hours. Count and record colony forming units at both time points. Incubate all SDA RODAC plates at 35 degrees Celsius and examine plates for growth at 24, 48, and 72 hours. Count and record colony forming units at all time points.
8. Report results as follows:

Colony Forming Units	Description/ Determination of Efficacy
0-5 colonies	None or very few colonies, efficacy considered excellent
6-15 colonies	Low number of colonies, efficacy considered good
16-30 colonies	Moderate, number of colonies, efficacy considered acceptable
31-50 colonies	Significant number of colonies, efficacy considered poor
> 50 colonies	Heavy contamination with many colonies, efficacy is considered unacceptable
TNTC	Number of colonies is "Too Numerous to Count" (TNTC), efficacy is considered unacceptable

TEST RESULTS OF CONTAMINATED MATS BEFORE TREATMENT

1. Mats inoculated with *Staphylococcus aureus*

Control Mat, No Treatment, 24 hour Reading

Colony Forming Units (<i>Staphylococcus aureus</i>)	Counts from the 3 Rodac Plates Tested per Mat	Description / Determination of Efficacy
TNTC	All plates were > 300cfu's and TNTC	Number of colonies is "Too Numerous to Count" (TNTC), efficacy is considered unacceptable.

Control Mat, No Treatment, 48 hour Reading

Colony Forming Units (<i>Staphylococcus aureus</i>)	Counts from the 3 Rodac Plates Tested per Mat	Description / Determination of Efficacy
TNTC	All plates were > 300cfu's and TNTC	Number of colonies is "Too Numerous to Count" (TNTC), efficacy is considered unacceptable.

2. Mats inoculated with *Escherichia coli*

Control Mat, No Treatment, 24 hour Reading

Colony Forming Units (<i>Escherichia coli</i>)	Counts from the 3 Rodac Plates Tested per Mat	Description / Determination of Efficacy
TNTC	All plates were > 300cfu's and TNTC	Number of colonies is "Too Numerous to Count" (TNTC), efficacy is considered unacceptable.

Control Mat, No Treatment, 48 hour Reading

Colony Forming Units (<i>Escherichia coli</i>)	Counts from the 3 Rodac Plates Tested per Mat	Description / Determination of Efficacy
TNTC	All plates were > 300cfu's and TNTC	Number of colonies is "Too Numerous to Count" (TNTC), efficacy is considered unacceptable.

3. Mats inoculated with *Candida albicans*

Control Mat, No Treatment, 24 hour Reading

Colony Forming Units (<i>Candida albicans</i>)	Counts from the 3 Rodac Plates Tested per Mat	Description / Determination of Efficacy
TNTC	All plates were > 300cfu's and TNTC	Number of colonies is "Too Numerous to Count" (TNTC), efficacy is considered unacceptable.

Control Mat, No Treatment, 48 hour Reading

Colony Forming Units (<i>Candida albicans</i>)	Counts from the 3 Rodac Plates Tested per Mat	Description / Determination of Efficacy
TNTC	All plates were > 300cfu's and TNTC	Number of colonies is "Too Numerous to Count" (TNTC), efficacy is considered unacceptable.

TEST RESULTS OF CONTAMINATED MATS AFTER TREATMENT

1. Mats inoculated with *Staphylococcus aureus*

Control Mat, Disinfecting Treatment , 24 hour Reading

Mat #	Colony Forming Units (<i>Staphylococcus aureus</i>)	Counts from the 3 Rodac Plates Tested per Mat	Description / Determination of Efficacy
Mat # 1	0-5 colonies	1	None or very few colonies, efficacy considered excellent EXCELLENT, > 99% reduction of microbes
		1	
		0	
Mat # 2	0-5 colonies	0	None or very few colonies, efficacy considered excellent EXCELLENT, > 99% reduction of microbes
		1	
		0	
Mat # 3	0-5 colonies	1	None or very few colonies, efficacy considered excellent EXCELLENT, > 99% reduction of microbes
		0	
		0	

Control Mat, Disinfecting Treatment , 48 hour Reading

Mat #	Colony Forming Units (<i>Staphylococcus aureus</i>)	Counts from the 3 Rodac Plates Tested per Mat	Description / Determination of Efficacy
Mat # 1	0-5 colonies	1	None or very few colonies, efficacy considered excellent EXCELLENT, > 99% reduction of microbes
		1	
		0	
Mat # 2	0-5 colonies	0	None or very few colonies, efficacy considered excellent EXCELLENT, > 99% reduction of microbes
		1	
		0	
Mat # 3	0-5 colonies	1	None or very few colonies, efficacy considered excellent EXCELLENT, > 99% reduction of microbes
		0	
		0	

2. Mats inoculated with *Escherichia coli*

Control Mat, Disinfecting Treatment , 24 hour Reading

Mat #	Colony Forming Units (<i>Escherichia coli</i>)	Counts from the 3 Rodac Plates Tested per Mat	Description / Determination of Efficacy
Mat # 1	0-5 colonies	1	None or very few colonies, efficacy considered excellent EXCELLENT, > 99% reduction of microbes
		1	
		0	
Mat # 2	0-5 colonies	1	None or very few colonies, efficacy considered excellent EXCELLENT, > 99% reduction of microbes
		1	
		0	
Mat # 3	0-5 colonies	1	None or very few colonies, efficacy considered excellent EXCELLENT, > 99% reduction of microbes
		0	
		2	

Control Mat, Disinfecting Treatment , 48 hour Reading

Mat #	Colony Forming Units (<i>Escherichia coli</i>)	Counts from the 3 Rodac Plates Tested per Mat	Description / Determination of Efficacy
Mat # 1	0-5 colonies	1	None or very few colonies, efficacy considered excellent EXCELLENT, > 99% reduction of microbes
		1	
		0	
Mat # 2	0-5 colonies	1	None or very few colonies, efficacy considered excellent EXCELLENT, > 99% reduction of microbes
		1	
		0	
Mat # 3	0-5 colonies	1	None or very few colonies, efficacy considered excellent EXCELLENT, > 99% reduction of microbes
		0	
		2	

3. Mats inoculated with *Candida albicans*

Control Mat, Disinfecting Treatment , 24 hour Reading

Mat #	Colony Forming Units (<i>Candida albicans</i>)	Counts from the 3 Rodac Plates Tested per Mat	Description / Determination of Efficacy
Mat # 1	0-5 colonies	3	None or very few colonies, efficacy considered excellent EXCELLENT, > 99% reduction of microbes
		1	
		1	
Mat # 2	0-5 colonies	1	None or very few colonies, efficacy considered excellent EXCELLENT, > 99% reduction of microbes
		1	
		2	
Mat # 3	0-5 colonies	2	None or very few colonies, efficacy considered excellent EXCELLENT, > 99% reduction of microbes
		2	
		1	

Control Mat, Disinfecting Treatment , 48 hour Reading

Mat #	Colony Forming Units (<i>Candida albicans</i>)	Counts from the 3 Rodac Plates Tested per Mat	Description / Determination of Efficacy
Mat # 1	0-5 colonies	3	None or very few colonies, efficacy considered excellent EXCELLENT, > 99% reduction of microbes
		1	
		1	
Mat # 2	0-5 colonies	1	None or very few colonies, efficacy considered excellent EXCELLENT, > 99% reduction of microbes
		1	
		2	
Mat # 3	0-5 colonies	2	None or very few colonies, efficacy considered excellent EXCELLENT, > 99% reduction of microbes
		2	
		1	

Control Mat, Disinfecting Treatment , 72 hour Reading

Mat #	Colony Forming Units (<i>Candida albicans</i>)	Counts from the 3 Rodac Plates Tested per Mat	Description / Determination of Efficacy
Mat # 1	0-5 colonies	3	None or very few colonies, efficacy considered excellent EXCELLENT, > 99% reduction of microbes
		1	
		1	
Mat # 2	0-5 colonies	1	None or very few colonies, efficacy considered excellent EXCELLENT, > 99% reduction of microbes
		1	
		2	
Mat # 3	0-5 colonies	2	None or very few colonies, efficacy considered excellent EXCELLENT, > 99% reduction of microbes
		2	
		1	

4. Untreated Virgin Yoga Mats not Inoculated with a Microorganism

Control Mat, No Treatment, 24 hour Reading

Mat #	Colony Forming Units	Counts from the 3 Rodac Plates Tested per Mat	Description / Determination of Efficacy
Mat # 1	6-15 colonies	7	Low number of colonies, efficacy considered good
		6	
		3	
Mat # 2	6-15 colonies	9	Low number of colonies, efficacy considered good
		7	
		3	
Mat # 3	6-15 colonies	2	Low number of colonies, efficacy considered good
		5	
		5	

5. Treated Virgin Yoga Mats not Inoculated with a Microorganism

Control Mat, Disinfecting Treatment , 24 hour Reading

Mat #	Colony Forming Units	Counts from the 3 Rodac Plates Tested per Mat	Description / Determination of Efficacy
Mat # 1	0-5 colonies	All mats demonstrated 0 CFU's	None or very few colonies, efficacy considered excellent
Mat # 2	0-5 colonies	All mats demonstrated 0 CFU's	None or very few colonies, efficacy considered excellent
Mat # 3	0-5 colonies	All mats demonstrated 0 CFU's	None or very few colonies, efficacy considered excellent

6. Efficacy of stand alone UV light treatment on contaminated mats with no silver ion / ozone machine disinfection

Microbe	UV Exposure 0 Minutes	UV Exposure 1Minute	UV Exposure 5 Minutes	UV Exposure 10 Minutes	UV Exposure 20 Minutes
<i>Escherichia coli</i>	TNTC in CFU's	TNTC in CFU's	TNTC in CFU's	33 CFU's	No Growth Observed
<i>Staphylococcus aureus</i>	TNTC in CFU's	TNTC in CFU's	35 CFU's	No Growth Observed	No Growth Observed
<i>Candida albicans</i>	TNTC in CFU's	TNTC in CFU's	TNTC in CFU's	75 CFU's	No Growth Observed

* CFU = Colony forming units

* TNTC = Too numerous to count

NOTES:

A. pH of the silver ion/ozone water solution used for treatment in the yoga mat disinfecting machine was between 2.2 and 2.8. Also, the solution was examined and tested for the presence of microbes both before and after the disinfection procedure of yoga mats. No microbes (cfu's) were observed with the plate count (pour plate) method either before or after the cleaning and disinfecting procedure.

B. Gram stains were used to determine if CFU's observed on the RODAC plates were gram positive cocci or gram negative rods.

C. Microscopy was used to determine the morphology of and hence the presence of *Candida albicans*.

COMMENTS

Rodac plates (RODAC = Replicate Organism Detection And Counting) are used world wide for microbiological control of all kinds of surfaces. For example, textiles, tables, mats, conveyor belts, trolleys, containers and more. Rodac plates are suitable for use in the industrial cleaning arena and they are a simple and inexpensive way to check surfaces of all kinds for microbial contamination and the hygienic status of the surface.

The U. S. Pharmacopoeia is a compendium of information published for the United States. It outlines quality standards for testing methodologies along with standards for food and dietary ingredients. The U. S. Pharmacopoeia also recommends organisms to be used in efficacy testing of products and surfaces.

Results from the machine disinfection procedure indicate that yoga mats contaminated with certain bacterial and fungal organisms can be readily disinfected with this silver ion and ozone machine treatment. Bacterial disinfection for *Staphylococcus aureus* and *Escherichia coli* was greater than 99%, while *Candida albicans* fungal disinfection was greater than 98%. This is considered excellent disinfection.

Louise Karl

08/26/2020

Louise Karl, Ph.D.

Date

Dept. Manager

Mycologist / Microbiologist

manager@lylelabs.com

ELECTRONICALLY CERTIFIED

