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The Neutralization of Bioterrorism Agents: *Bacillus cereus* Spore Survival on Stainless Steel Surfaces in the Presence of Household Sanitizers

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Abstract

A study was conducted for the Department of Homeland Security and the National Center for Food Protection and Defense to evaluate the efficacy of various household sanitizers to inactivate spores of *Bacillus cereus* and prevent their germination on stainless steel surfaces. *Bacillus cereus* spores were used as a substitute for *Bacillus anthracis* spores in this experiment. Stainless steel coupons were inoculated with spore culture and allowed to dry and adhere to the stainless steel surfaces. Commercial sanitizer compounds that were purchased in a retail market were then tested at half and full strengths over varying time periods. Samples were neutralized and sonicated to remove the remaining spores from the surfaces, before being plated onto brain heart infusion (BHI) agar. Resultant colonies were compared with a positive control to determine the log reduction of spore populations and the resulting effectiveness of the sanitizers. Sanitizer solutions containing sodium hypochlorite in high concentrations were the most effective solutions for inactivating *Bacillus cereus* spores on stainless steel surfaces. Compounds containing strong acids (HCl) and topical solutions of hydrogen peroxide were also determined to reduce significant numbers of spores. However, topical solutions of hydrogen peroxide had the least effective sporicidal capabilities of the solutions tested. Further studies with food on surfaces will be required to test the efficiency of these compounds when exposed to large amounts of organic material, which have been shown to inactivate chlorine containing compounds.

I. Introduction

With the increasing threat of potential acts of bioterrorism, research is being conducted to evaluate the most effective methods for enhancing the safety of the general public should they come into contact with an infectious agent. Due to the recent threat to the American public and the subsequent publicity generated by the news media, *Bacillus anthracis* has been identified as a commonly used bioterrorism agent with devastating capabilities. This has triggered widespread investigation into all possible processes for the neutralization of these spores (*7*). To safely begin studying the inactivation of *Bacillus anthracis,* researchers have used the more prevalent and less dangerous food borne spores of *Bacillus cereus*, which possess a major spore coat protein common to *Bacillus anthracis* that is a target for spore inactivation (*4*). These spore coats provide spores with high resistance characteristics to chemical and physical extremes, such as a resistance to adverse temperatures. These are recognized by researchers to complicate the inactivation of potential bioterrorism agents in the home due to the hazards produced in generating the vast amounts of heat required to inactivate spores. However, studies which have focused on the chemical sterilization of spores have shown the ability of common sanitizers to successfully neutralize spore germination (*1,3,4,5,6*). With the ever increasing number of disinfectant products available to the public, it is important to test these products against more resistant bacterial spores to evaluate the general public's ability to neutralize possible bioterrorism threats. Although general suggestions for neutralization are available should a bioterrorism event occur, detailed guidelines for consumers are not yet prevalent. One of the goals of this study is to provide the

information required to establish a detailed procedure to supply to the general public, preparing them for potential bioterrorism attacks in the future.

II. Objectives

The purpose of this study is to develop an accurate and consistent method to assess the efficiency of commercial sanitizers to neutralize the spores of *Bacillus cereus* on stainless steel surfaces and prevent their germination. In the occurrence of a bioterrorism act involving foods, the results of this study and others could be published as guidelines for the decontamination of food contact surfaces at the consumer level.

III. Materials and Methods

Bacterial Cultures, Maintenance, and Spore Preparation: *Bacillus cereus* (33018 and 49064) spores were obtained from ATCC (American Type Culture Collection, Rockville, MD). Cultures were then grown aerobically in brain heart infusion broth (BHI) (Difco, Sparks, MD) for 24 hours at 35°C for two consecutive transfers prior to spore preparation. Spore preparation was initiated by serially diluting 1 mL of 24 hour culture in 9 mL of 0.9% NaCl (Sigma, St. Louis, MO). Spores were obtained using the method of Jagannath et al. (*2*), with modifications including: BHI substituted for TSA and differing centrifuge speeds. Plates contained 40 µg/mL of Manganese Sulfate Monohydrate and 100 µg/mL of Calcium Chloride Dihydrate (Sigma) to induce sporulation. After diluting 24 hour culture in 0.9% NaCl, plates were incubated aerobically at 35°C for up to 5 days to allow sporulation of the bacterial lawns. Sporulation progress was monitored using phase contrast microscopy. Once spore concentration was estimated at 90%, the bacterial lawn was physically removed from the plate and mixed in a 50 mL centrifuge tube (VWR, West Chester, PA) with 10 mL of

distilled water. The mixture was centrifuged for 15 minutes at 10,000 X g in a Biofuge 17R centrifuge (Baxter Scientific Products, West Chester, PA), followed by the removal of supernatant and pellet re-suspension in 10 mL of distilled water for three subsequent repetitions. Final spore pellets were suspended in 5 mL of distilled water and 5 mL of 95% ethanol (Sigma) for storage at 4°C prior to use.

Household Sanitizers and Dilution Strengths: Sanitizer compounds were purchased in a retail market and chosen based on use-type (cleaner, disinfectant, etc.) and anti-microbial specification; pH measurements were taken on each full strength compound. Table 1 relates the compounds studied with their manufacturer, active ingredient, concentration and pH.

Neutralizer Buffer Preparation and Testing: A neutralizer buffer solution was used to stop the action of active ingredients of the compounds at specified time points for each treatment. The neutralizer stock solution consisted of 40 g of lecithin (Sigma), 280 mL of polysorbate 80 (Tween 80) (Sigma), and 1.25 mL of phosphate buffer (PB) (68.0 g KH2PO4/L, pH=7.2 (Fisher Scientific, Fairlawn, NJ)) diluted with distilled water to 1 L and adjusted to pH 7.2 with 0.1 N NaOH (Fisher). Neutralizer working blanks were made by adding 100 ml of neutralizer stock solution, 25 mL of 0.25 M PB stock, and 1675 ml of distilled water; blanks were sterilized by autoclaving at 121°C for 15 minutes and stored at 4°C prior to use. The addition of sodium thiosulfate (6 g/L) (Sigma) was necessary to inactivate chlorine containing compounds. For hydrogen peroxide, catalase (source: *Micrococcus lysodeikticus*) (Sigma) (10,000 U/mL) was filter sterilized through a 0.2 μm cellulose acetate filter (Corning Inc., Corning, NY). The effectiveness of the neutralizer buffer on individual compounds was tested by aseptically transferring 0.1 mL

of spore cocktail, 0.9 mL of sanitizer, and 9.0 mL of neutralizer buffer. Samples were incubated at 25°C for 5 minutes, plated on BHI, and incubated for 24 hours at 35°C prior to enumeration.

Stainless Steel Coupon Preparation: Coupons were cleaned in a 95% ethanol solution, then scrubbed with soap and hot water, and laid flat to dry. Coupons were wrapped in a single layer of aluminum foil prior to autoclaving and not opened until immediately before use. Coupons with obvious damage or scratches were not used.

Spore Treatment and Plating: Spore stocks were cocktailed together in a sterile tube in a 1:1 ratio and vortexed for 15 seconds. From this mixture, 1.0 ml was pipetted into a 9.0 mL blank of 0.6% NaCl and vortexed vigorously. This mixture was considered the initial population and used to inoculate stainless steel coupons (0.1 mL per coupon). Coupons were placed on a sterile tray, covered with aluminum foil, and then allowed to dry for 1 hour in a 50°C incubator, after which the coupons appeared visibly dry. The coupons were then placed in a sterile empty Petri dish using sterile forceps. Sanitizer compounds were tested at full commercial concentration and 50% commercial concentration. Approximately 20 mL of sanitizer compound was poured into each Petri dish, covering the surface of the coupons. The Lysol Toilet compound was an exception to this as it removed the spores from the compound when poured at 20 mL due to high viscosity. For this compound, five drops of the solution were placed directly onto the coupons. The dish was covered during exposure times: 0, 1, 5, 10, 30, and 60 minute intervals up to 360 minutes for some compounds. After designated exposure time, coupons were transferred to a sterile beaker containing 9.9 mL of appropriate neutralizer buffer. The beakers were submerged to the level of the neutralizer buffer and sonicated

in a Branson Ultrasonicator 1510 (Branson Inc. Danbury, CT) for 5 minutes using a Fischer stand and two tube clamps to hold the beakers and assure viable repetitions. The beakers were then either diluted in 9 mL or 9.9 mL peptone and plated using a spiral plater at 10^{-3} and 10^{-4} dilutions (Don Whitley Scientific Limited, Yorkshire, UK). Due to the sensitivity of the spiral plater, 0.4 mL of hydrogen peroxide and 0.4 mL of Lysol with bleach at 50% commercial concentration were spread plated at 10^{-3} dilutions. Plates were incubated for 24 hours at 35°C before enumeration using a Protocol automatic plate counter (Synoptics Limited; Cambridge, UK). Resultant colonies from samples were compared with a positive control, noted as time 0 in the tables, to determine log reductions in populations.

IV. Results and Discussion

Figures 1 and 2 relate log survival to treatment time for full concentration strengths of all compounds showing significant inactivation. Figures 3 and 4 relate log survival to treatment time for 50% concentration strengths. Tables 2 and 3 relate the log count, in CFU/mL, recovered at various time points for each sanitizer compound at 50% strength and full strength, respectively. Means of replicate time points were calculated and used to determine the log reduction by comparison to the 0 min (control) time point. Standard deviations were calculated and revealed the amount of variation within the replications.

Sanitizer solutions containing sodium hypochlorite, NaOCl, in higher concentrations had more effective sporicidal capabilities against spores of *Bacillus cereus* on stainless steel surfaces. Compounds containing sodium hypochlorite at both concentrations inactivated a significant number of spores (3 or more logs) at 1, 5, and 10

minutes. Hydrogen peroxide and compounds containing HCl also showed inactivating potential, although hydrogen peroxide had the least effective sporicidal capabilities. The Works compound, which contains HCl, inactivated spores after a longer period of time than compounds containing sodium hypochlorite, taking as long as 60 minutes to reduce the spore population by 4.2 logs. At 100% concentration, the works compound was more effective than at 50% concentration reducing the same number of logs in only 10 minutes. Hydrogen peroxide took the longest to inactivate the spore populations, taking 3 hours at 50% concentration to inactivate 5.7 logs and 1 hour at 100% concentration to inactivate 1.5 logs. Although compounds containing sodium hypochlorite showed significant inactivating results, further studies with food on surfaces would be needed to test the efficiency of these compounds when exposed to large amounts of organic material, which is known to inactivate chlorine based compounds. This concern applies to HCl and hydrogen peroxide containing compounds as well.

In this study, many protocol related difficulties were encountered. Bacterial spores lack the extensive glycocalex and protein network many vegetative bacteria use to form biofilms and adhere to surfaces. It was found that 1 hour of drying was needed to ensure the spore population had adequately adhered to the coupon. Also, dilution of spore populations in media other than 0.6 % NaCl resulted in early germination. Furthermore, the smallest dilution that could be made was 10^{-3}. When attempting to plate directly after sonication from the spore and neutralizer buffer solution, no growth was obtained. It is, therefore, suggested that spread plates, like those used for 50% hydrogen peroxide and 50% Lysol with bleach, be used to calculate growth at smaller dilutions. The method of pouring the sanitizer compound was determined to be the most likely

application by consumers. However, the spore population was removed upon application of Lysol Toilet due to the viscosity of that particular compound. In this case, it was necessary to apply 5 drops directly to the coupon to ensure that no spores were removed from the surface. Previous experiments led to variation in recovery from the sonicator due to human error resulting from variations in holding depth. To remedy this, an apparatus was constructed to hold the beakers at a consistent depth using a Fischer stand and two tube clamps. This significantly lowered the amount of variation obtained during the recovery of *Bacillus cereus* spores.

Table 1

Commercial Name	Manufacturer	Active Compound	Concentration	pH
Clorox Bleach	Clorox Company, Oakland, CA	NaOCl	6.00%	10.8
Tilex Mold & Mildew Remover	Clorox Company, Oakland, CA	NaOCl	2.40%	12.2
Lysol All Purpose w/ Bleach	Reckitt Benckiser Inc., Parsippany, NJ	NaOCl	2.00%	12.0
Lysol Toilet Bowl Cleaner	Reckitt Benckiser Inc., Parsippany, NJ	NaOCl	2.00%	11.9
The Works Drain Opener	HomeCare Labs, Lawrenceville, GA	HCl	20.00%	0.5
Hydrogen Peroxide	Kroger, Cincinnati, OH	H_2O_2	3.00%	4.2

Table 2

Compound (50% Strength)	Time	Mean Count (Log CFU/mL)	Standard Deviation	Log Reduction
*Hydrogen Peroxide	0	7.11	0.17	0
	1	7.49	0.21	0
	5	7.17	0.07	0
	10	7.45	0.27	0
	30	7.09	0.08	0.03
	60	6.68	0.30	0.43
	120	6.00	0.33	1.11
	180	1.40	0.11	5.71
	240	0	0	> 5.71
	300	0	0	> 5.71
	360	0	0	> 5.71
Lysol Toilet	0	7.08	0.06	0
	1	5.48	0.04	1.60
	5	5.59	0.01	1.49
	10	0	0	> 4.08
	30	0	0	> 4.08
	60	0	0	> 4.08
Works	0	7.28	0.20	0
	1	6.46	0.03	0.82
	5	6.51	0.03	0.77
	10	6.45	0.04	0.83
	30	5.98	0.09	1.30
	60	0	0	> 4.28
	120	0	0	> 4.28
*Lysol with Bleach	0	7.34	0.24	0
	1	6.93	0.09	0.41
	5	2.79	0.06	4.55
	10	0	0	> 4.55
	30	0	0	> 4.55
Tilex	0	7.60	0.35	0
	1	6.60	0.12	1.000
	5	0	0	> 4.60
	10	0	0	> 4.60
	30	0	0	> 4.60
July Expired Bleach	0	7.31	0.20	0
	1	0	0	> 4.31
	5	0	0	> 4.31
	10	0	0	> 4.31
Nov. Expired Bleach	0	7.27	0.08	0
	1	5.29	0.13	1.98
	5	0	0	> 4.27
	10	0	0	> 4.27

Table 2: Log count CFU/mL means, standard deviations, and log reductions for various time points for each sanitizer compound. Compounds designated with a "*" were spread plated due to spiral plater sensitivity.

Table 3

Compound (100% Strength)	Time	Mean Count (Log CFU/mL)	Standard Deviation	Log Reduction
Hydrogen Peroxide	0	7.27	0.20	0
	1	7.27	0.08	0
	5	7.22	0.11	0.04
	10	7.26	0.15	0.01
	30	6.83	0.10	0.44
	60	5.77	0.54	1.50
	120	0	0	> 4.27
	180	0	0	> 4.27
	240	0	0	> 4.27
	300	0	0	> 4.27
	360	0	0	> 4.27
Lysol Toilet	0	7.26	0.17	0
	1	5.84	0.09	1.42
	5	0	0	> 4.26
	10	0	0	> 4.26
	30	0	0	> 4.26
	60	0	0	> 4.26
Works	0	7.22	0.13	0
	1	6.31	0.02	0.92
	5	5.38	0.11	1.85
	10	0	0	> 4.22
	30	0	0	> 4.22
	60	0	0	> 4.22
	120	0	0	> 4.22
Lysol with Bleach	0	7.59	0.41	0
	1	4.45	0.21	3.14
	5	0	0	> 4.59
	10	0	0	> 4.59
	30	0	0	> 4.59
Tilex	0	7.89	0.23	0
	1	5.44	0.20	2.45
	5	4.60	0	3.29
	10	0	0	> 4.89
	30	0	0	> 4.89
July Expired Bleach	0	7.28	0.12	0
	1	0	0	> 4.28
	5	0	0	> 4.28
	10	0	0	> 4.28
Nov. Expired Bleach	0	7.26	0.16	0
	1	0	0	> 4.26
	5	0	0	> 4.26
	10	0	0	> 4.26

Table 3: Log count CFU/mL means, standard deviations, and log reductions for various time points for each sanitizer compound.

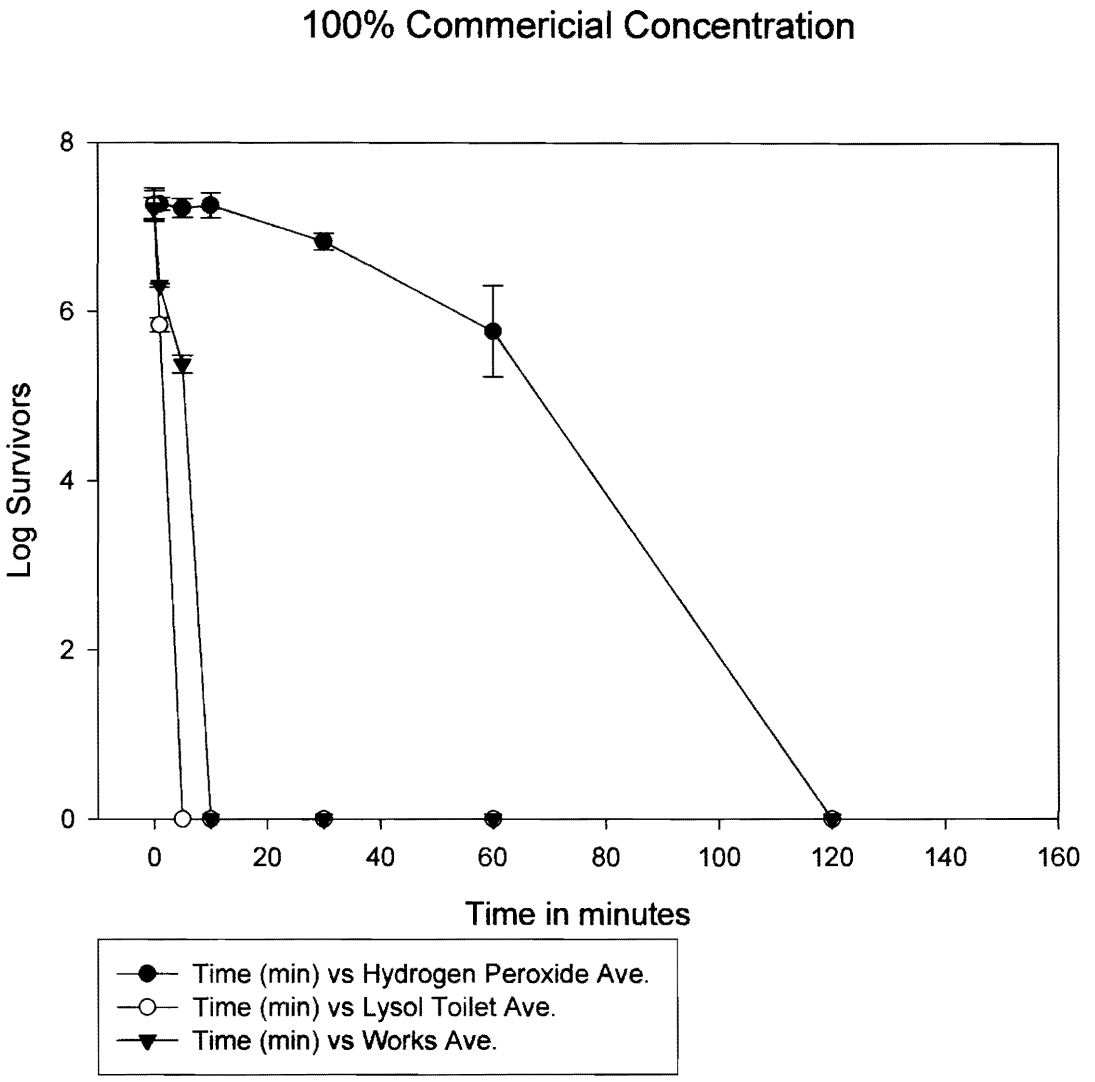

Figure 1: Inactivation of *Bacillus cereus* spores by sanitizers at full strength

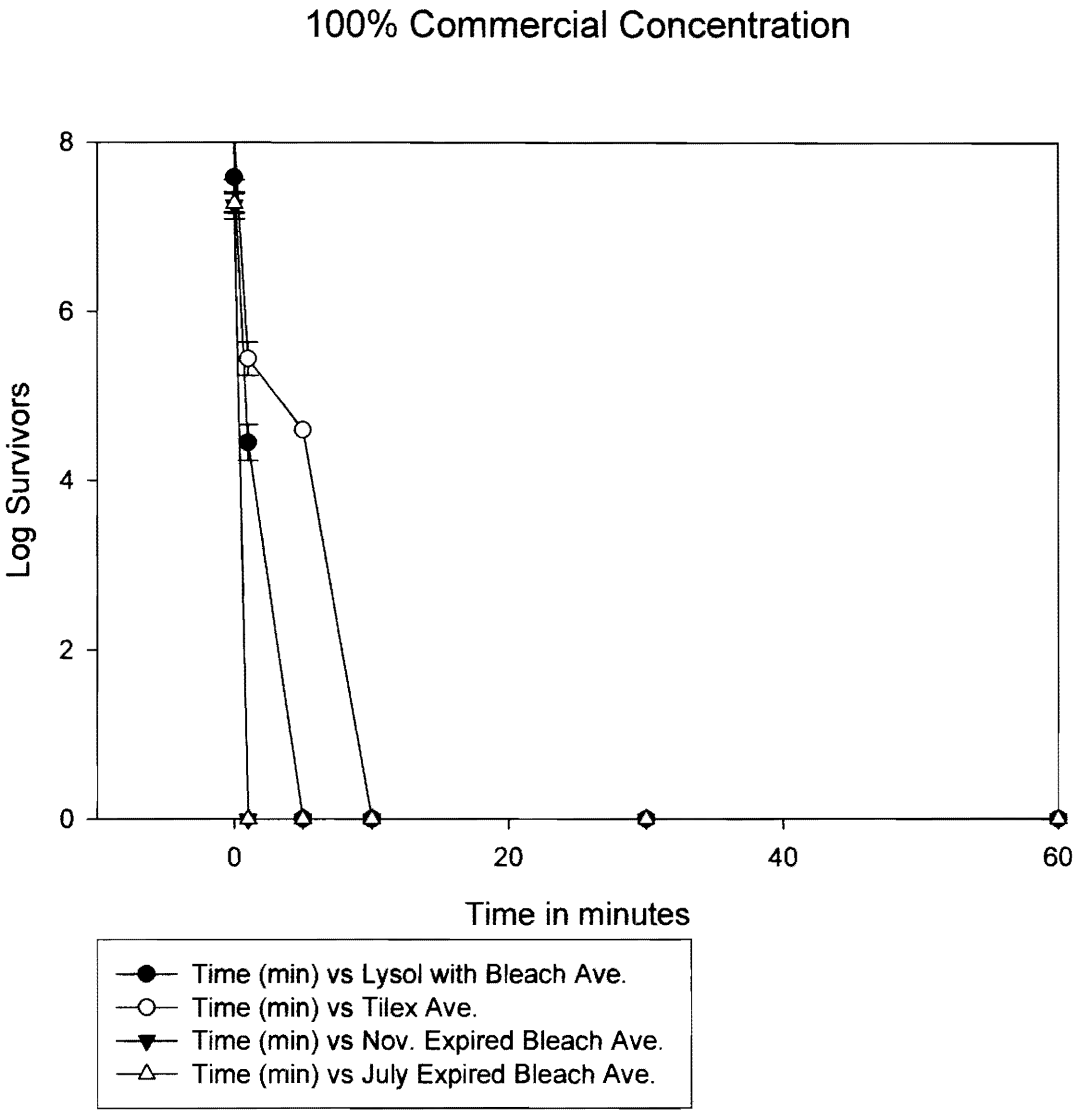

Figure 2: Inactivation of *Bacillus cereus* spores by sanitizers at full strength

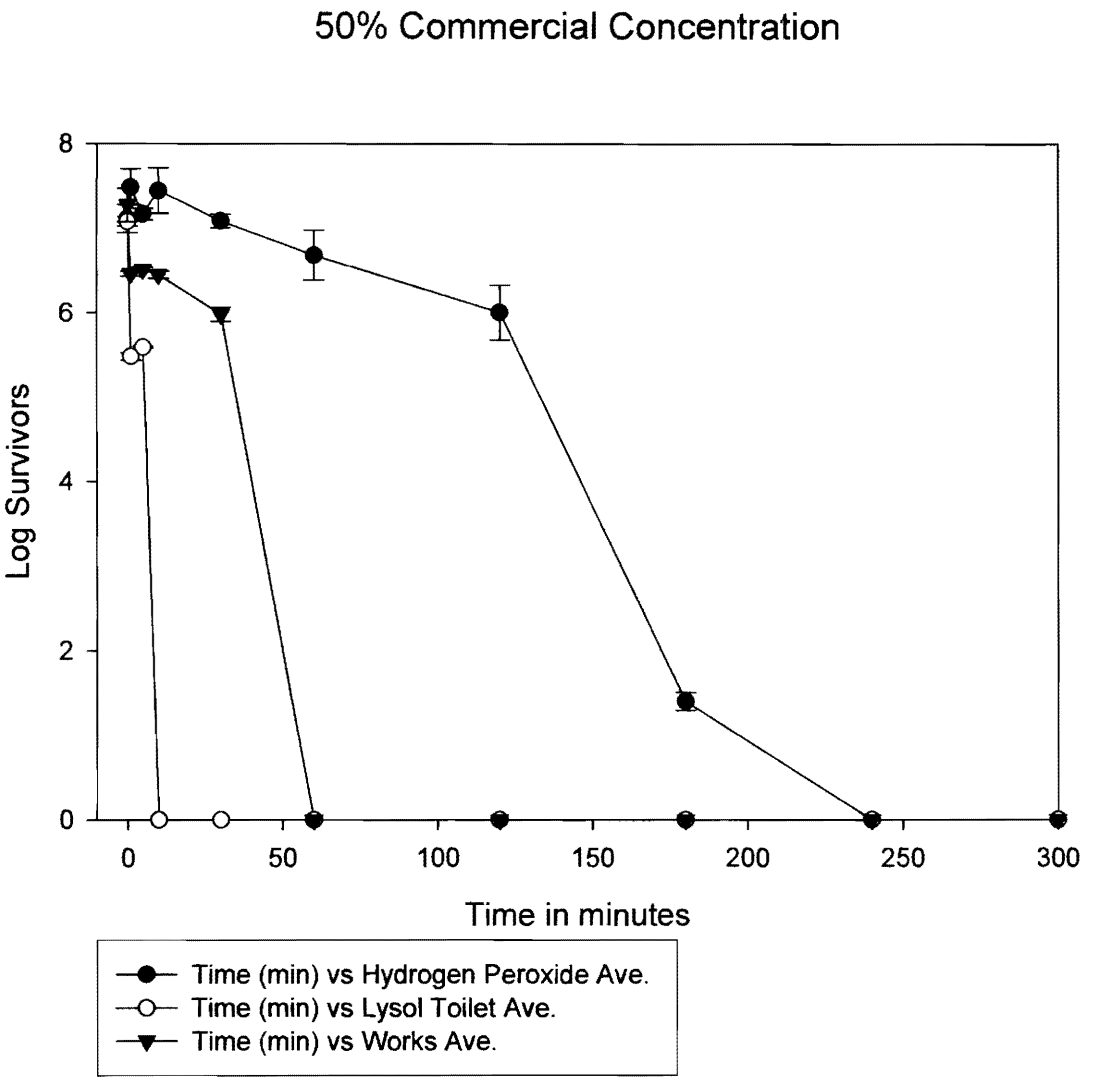

Figure 3: Inactivation of *Bacillus cereus* spores by sanitizers at 50% strength

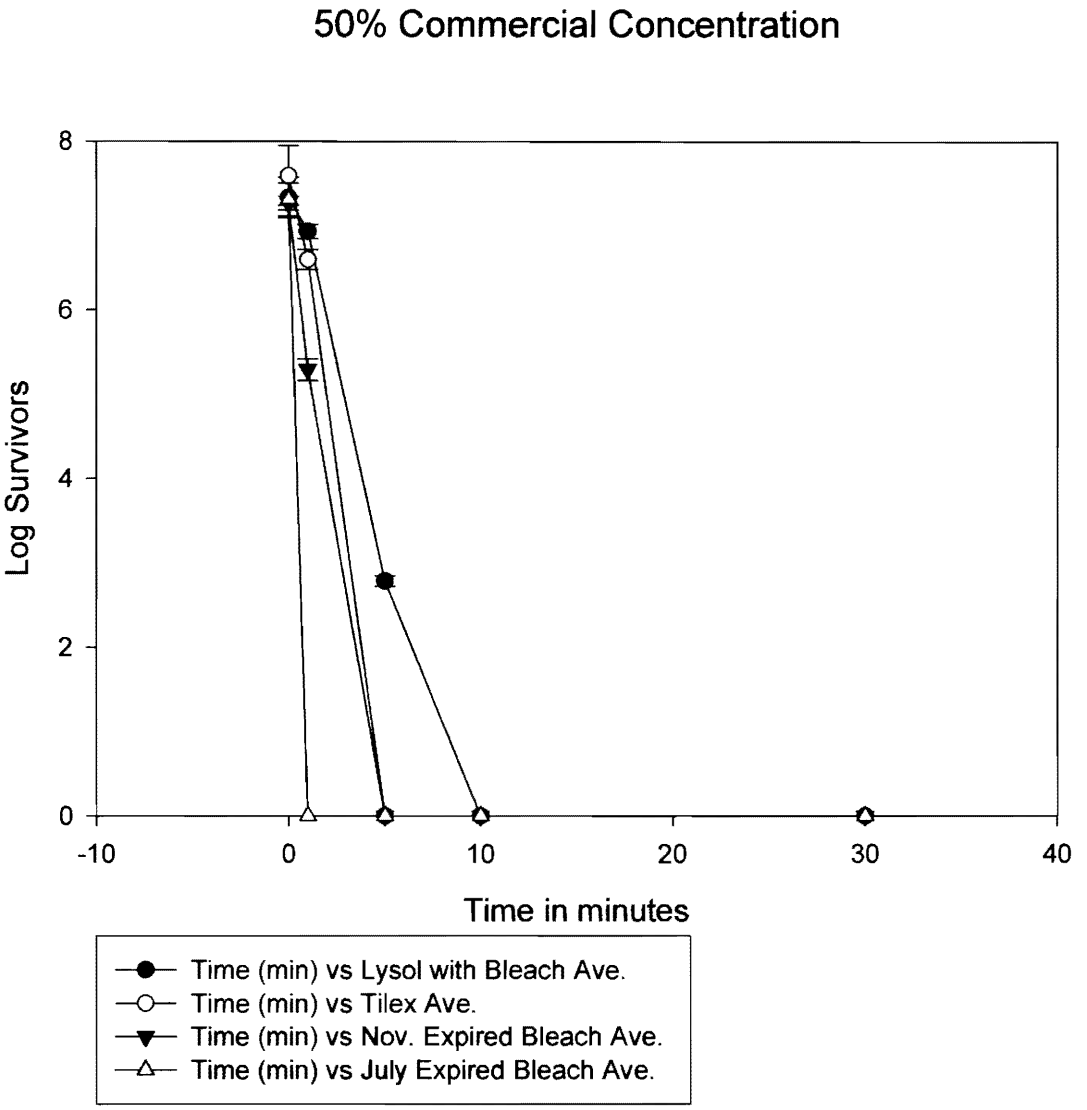

Figure 4: Inactivation of *Bacillus cereus* spores by sanitizers at 50% strength

References

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Synbiosis ProtoCOL Colony Counter 3.15

Study Name	NEW Bacillus Spores on Stainless Steel Surface
Batch Name	July Expired Clorox Bleach 50%
Type	Spiral Plate
Based on	
Comment	

System Parameters		Time	Means	SD
Video State	Inverted	0	7.31	0.198158
Selected Lamp	Top Lamp On	1	0	0
Camera Shutter	1/250 sec	5	0	0
Contrast Setting	Medium	10	0	0
Dish Diameter	88 mm			
Sample Volume	1 ml			
Area Limits	Off			
Spiral Size	Spiral 90 mm			
Spiral Frame	Two Sectors			
Spiral Plater	wasp100ul			
Minimum Spiral Count Rule	20			
Maximum Spiral Count Rule	75			
Calibration Factor	0.1615 mm \ Pixel	Created:	10:12 AM	8/2/2006

Plate Id	Count per Frame	Log count per ml	Sector used	Dilution Factor	Spiral Plater	Flags	Time	Date	Comment	User
50%July Control P1	106	7.33	Total	1 in 10000	wasp100ul	LU	10:13 AM	8/2/2006		
50%July Control P2	67	7.13	Total	1 in 10000	wasp100ul	LU	10:13 AM	8/2/2006		
50%July Control P3	19	7.58	Total	1 in 100000	wasp100ul	LU	10:13 AM	8/2/2006		
50%July Control P4	8	7.2	Total	1 in 100000	wasp100ul	LU	10:13 AM	8/2/2006		
50%July 0min P1	86	7.24	Total	1 in 10000	wasp100ul	LU	10:14 AM	8/2/2006		
50%July 0min P2	73	7.16	Total	1 in 10000	wasp100ul	LU	10:14 AM	8/2/2006		
50%July 0min P3	11	7.34	Total	1 in 100000	wasp100ul	LU	10:14 AM	8/2/2006		
50%July 0min P4	9	7.26	Total	1 in 100000	wasp100ul	ELU	10:15 AM	8/2/2006		
50%July 1min P1	0	0	Total	1 in 1000	wasp100ul	ELU	10:16 AM	8/2/2006		
50%July 1min P2	0	0	Total	1 in 1000	wasp100ul	ELU	10:16 AM	8/2/2006		
50%July 5min P1	0	0	Total	1 in 1000	wasp100ul	ELU	10:16 AM	8/2/2006		
50%July 5min P2	0	0	Total	1 in 1000	wasp100ul	ELU	10:16 AM	8/2/2006		
50%July 10min P1	0	0	Total	1 in 1000	wasp100ul	ELU	10:16 AM	8/2/2006		

50%July 10min P2		0	0	Total	1 in 1000	wasp100ul ELU	10:16 AM	8/2/2006

Time	Log		Average	Standard Deviation
	0	7.33		
	0	7.13		
	0	7.58		
	0	7.2	7.31	0.198158
	1	0		
	1	0	0	0
	5	0		
	5	0	0	0
	10	0		
	10	0	0	0

Synbiosis ProtoCOL Colony Counter 3.15

Study Name	NEW Bacillus Spores on Stainless Steel Surface
Batch Name	July Expired Clorox Bleach 100%
Type	Spiral Plate
Based on	
Comment	

System Parameters		Time	Means	SD
Video State	Inverted	0	7.2775	0.117296
Selected Lamp	Top Lamp On	1	0	0
Camera Shutter	1/250 sec	5	0	0
Contrast Setting	Medium	10	0	0
Dish Diameter	88 mm			
Sample Volume	1 ml			
Area Limits	Off			
Spiral Size	Spiral 90 mm			
Spiral Frame	Two Sectors			
Spiral Plater	wasp100ul			
Minimum Spiral Count I	20			
Maximum Spiral Count	75			
Calibration Factor	0.1609 mm \ Pixel	Created:	9:59 AM	8/1/2006

Plate Id	Count per Frame	Log count per ml	Sector used	Dilution Factor	Spiral Plater	Flags	Time	Date	Comment	User
100%July Control P1	80	7.2	Total	1 in 10000	wasp100ul	LU	10:00 AM	8/1/2006		
100%July Control P2	72	7.16	Total	1 in 10000	wasp100ul	LU	10:01 AM	8/1/2006		
100%July Control P3	11	7.34	Total	1 in 100000	wasp100ul	LU	10:01 AM	8/1/2006		
100%July Control P4	13	7.41	Total	1 in 100000	wasp100ul	LU	10:01 AM	8/1/2006		
100%July 0min P1	96	7.28	Total	1 in 10000	wasp100ul	LU	10:02 AM	8/1/2006		
100%July 0min P2	98	7.29	Total	1 in 10000	wasp100ul	ELU	10:02 AM	8/1/2006		
100%July 0min P3	14	7.45	Total	1 in 100000	wasp100ul	LU	10:02 AM	8/1/2006		
100%July 0min P4	12	7.38	Total	1 in 100000	wasp100ul	ELU	10:03 AM	8/1/2006		
100%July 1min P1	0	0	Total	1 in 1000	wasp100ul	LU	10:03 AM	8/1/2006		
100%July 1min P2	0	0	Total	1 in 1000	wasp100ul	LU	10:03 AM	8/1/2006		
100%July 5min P1	0	0	Total	1 in 1000	wasp100ul	LU	10:03 AM	8/1/2006		
100%July 5min P2	0	0	Total	1 in 1000	wasp100ul	LU	10:03 AM	8/1/2006		
100%July 10min P1	0	0	Total	1 in 1000	wasp100ul	LU	10:04 AM	8/1/2006		

100%July 10min P2	0	0	Total	1 in 1000	wasp100ul LU	10:04 AM	8/1/2006

Time	Log	Average	Standard Deviation
0	7.2		
0	7.16		
0	7.34		
0	7.41	7.2775	0.117296
1	0		
1	0	0	0
5	0		
5	0	0	0
10	0		
10	0	0	0

Synbiosis ProtoCOL Colony Counter 3.15

Study Name	NEW Bacillus Spores on Stainless Steel Surface
Batch Name	Nov. Expired Clorox Bleach 50%
Type	Spiral Plate
Based on	
Comment	

System Parameters		Time	Means	SD
Video State	Inverted	0	7.2675	0.082209
Selected Lamp	Top Lamp On	1	5.29	0.127279
Camera Shutter	1/250 sec	5	0	0
Contrast Setting	Medium	10	0	0
Dish Diameter	88 mm			
Sample Volume	1 ml			
Area Limits	Off			
Spiral Size	Spiral 90 mm			
Spiral Frame	Two Sectors			
Spiral Plater	wasp100ul			
Minimum Spiral Count I	20			
Maximum Spiral Count	75			
Calibration Factor	0.1612 mm \ Pixel	Created:	2:12 PM 8/16/2006	

Plate Id	Count per Frame	Log count per ml	Sector used	Dilution Factor	Spiral Plater	Flags	Time	Date	Comment	User
50%Nov Control P1	71	7.15	Total	1 in 10000	wasp100ul	LU	2:12 PM	8/16/2006		
50%Nov Control P2	95	7.28	Total	1 in 10000	wasp100ul	LU	2:12 PM	8/16/2006		
50%Nov Control P3	10	7.3	Total	1 in 100000	wasp100ul	ELU	2:13 PM	8/16/2006		
50%Nov Control P4	11	7.34	Total	1 in 100000	wasp100ul	LU	2:13 PM	8/16/2006		
50%Nov 0min P1	80	7.2	Total	1 in 10000	wasp100ul	LU	2:13 PM	8/16/2006		
50%Nov 0min P2	86	7.24	Total	1 in 10000	wasp100ul	LU	2:14 PM	8/16/2006		
50%Nov 0min P3	14	7.45	Total	1 in 100000	wasp100ul	ELU	2:14 PM	8/16/2006		
50%Nov 0min P4	13	7.41	Total	1 in 100000	wasp100ul	LU	2:14 PM	8/16/2006		
50%Nov 1min P1	8	5.2	Total	1 in 1000	wasp100ul	ELU	2:15 PM	8/16/2006		
50%Nov 1min P2	12	5.38	Total	1 in 1000	wasp100ul	ELU	2:15 PM	8/16/2006		
50%Nov 5min P1	0	0	Total	1 in 1000	wasp100ul	LU	2:15 PM	8/16/2006		
50%Nov 5min P2	0	0	Total	1 in 1000	wasp100ul	LU	2:15 PM	8/16/2006		
50%Nov 10min P1	0	0	Total	1 in 1000	wasp100ul	LU	2:15 PM	8/16/2006		

50%Nov 10min P2 0 0 Total 1 in 1000 wasp100ul LU 2:15 PM 8/16/2006

Time	Log	Average	Standard Deviation
0	7.15		
0	7.28		
0	7.3		
0	7.34	7.2675	0.082209
1	5.2		
1	5.38	5.29	0.127279
5	0		
5	0	0	0
10	0		
10	0	0	0

Synbiosis ProtoCOL Colony Counter 3.15

Study Name	NEW Bacillus Spores on Stainless Steel Surface
Batch Name	Nov. Expired Clorox Bleach 100%
Type	Spiral Plate
Based on	
Comment	

System Parameters	
Video State	Inverted
Selected Lamp	Top Lamp On
Camera Shutter	1/250 sec
Contrast Setting	Medium
Dish Diameter	88 mm
Sample Volume	1 ml
Area Limits	Off
Spiral Size	Spiral 90 mm
Spiral Frame	Two Sectors
Spiral Plater	wasp100ul
Minimum Spiral Count	20
Maximum Spiral Count	75
Calibration Factor	0.1612 mm \ Pixel

Created: 10:05 AM 8/1/2006

Time	Means	SD
0	7.255	0.161348
1	0	0
5	0	0
10	0	0

Plate Id	Count per Frame	Log count per ml	Sector used	Dilution Factor	Spiral Plater	Flags	Time	Date	Comment	User
100%Nov Control P1	55	7.04	Total	1 in 10000	wasp100ul	LU	10:06 AM	8/1/2006		
100%Nov Control P2	85	7.23	Total	1 in 10000	wasp100ul	LU	10:06 AM	8/1/2006		
100%Nov Control P3	13	7.41	Total	1 in 100000	wasp100ul	ELU	10:07 AM	8/1/2006		
100%Nov Control P4	11	7.34	Total	1 in 100000	wasp100ul	LU	10:07 AM	8/1/2006		
100%Nov 0min P1	101	7.31	Total	1 in 10000	wasp100ul	LU	10:07 AM	8/1/2006		
100%Nov 0min P2	95	7.28	Total	1 in 10000	wasp100ul	LU	10:08 AM	8/1/2006		
100%Nov 0min P3	14	7.45	Total	1 in 100000	wasp100ul	LU	10:08 AM	8/1/2006		
100%Nov 0min P4	12	7.38	Total	1 in 100000	wasp100ul	LU	10:08 AM	8/1/2006		
100%Nov 1min P1	0	0	Total	1 in 1000	wasp100ul	LU	10:08 AM	8/1/2006		
100%Nov 1min P2	0	0	Total	1 in 1000	wasp100ul	LU	10:08 AM	8/1/2006		
100%Nov 5min P1	0	0	Total	1 in 1000	wasp100ul	LU	10:08 AM	8/1/2006		
100%Nov 5min P2	0	0	Total	1 in 1000	wasp100ul	LU	10:08 AM	8/1/2006		
100%Nov 10min P1	0	0	Total	1 in 1000	wasp100ul	LU	10:08 AM	8/1/2006		

100%Nov 10min P2 0 0 Total 1 in 1000 wasp100ul LU 10:08 AM 8/1/2006

Time	Log	Average	Standard Deviation
0	7.04		
0	7.23		
0	7.41		
0	7.34	7.255	0.161348
1	0		
1	0	0	0
5	0		
5	0	0	0
10	0		
10	0	0	0

Synbiosis ProtoCOL Colony Counter 3.15

Study Name	NEW Bacillus Spores on Stainless Steel Surface
Batch Name	Lysol with Bleach 50%
Type	Spiral Plate
Based on	
Comment	

System Parameters		Time	means	sd	
Video State	Inverted	0	7.335	0.241178	
Selected Lamp	Top Lamp On	1	6.93	0.084853	
Camera Shutter	1/250 sec	5	2.39	3.37997	*used spread plates
Contrast Setting	Medium	10	0	0	
Dish Diameter	88 mm	30	0	0	
Sample Volume	1 ml				
Area Limits	Off				
Spiral Size	Spiral 90 mm				
Spiral Frame	Two Sectors				
Spiral Plater	wasp100ul				
Minimum Spiral Count Rule	20				
Maximum Spiral Count Rule	75				
Calibration Factor	0.1618 mm \ Pixel	Created:	9:49 AM	7/27/2006	

Plate Id	Count per Frame	Log count per ml	Sector used	Dilution Factor	Spiral Plater	Flags	Time	Date	Comment	User
50%LB Control P1	68	7.13	Total	1 in 10000	wasp100ul	LU	9:50 AM	7/27/2006		
50%LB Control P2	69	7.14	Total	1 in 10000	wasp100ul	LU	9:50 AM	7/27/2006		
50%LB Control P3	14	7.45	Total	1 in 100000	wasp100ul	LU	9:50 AM	7/27/2006		
50%LB Control P4	21	7.62	Total	1 in 100000	wasp100ul	LU	9:51 AM	7/27/2006		
50%LB 0min P1	68	7.13	Total	1 in 10000	wasp100ul	LU	9:51 AM	7/27/2006		
50%LB 0min P2	83	7.22	Total	1 in 10000	wasp100ul	LU	9:51 AM	7/27/2006		
50%LB 0min P3	13	7.41	Total	1 in 100000	wasp100ul	LU	9:51 AM	7/27/2006		
50%LB 0min P4	17	7.53	Total	1 in 100000	wasp100ul	LU	9:52 AM	7/27/2006		
50%LB 1min P1	45	6.99	4c	1 in 1000	wasp100ul	2	9:52 AM	7/27/2006		
50%LB 1min P2	55	6.87	4b	1 in 1000	wasp100ul	2	9:53 AM	7/27/2006		
50%LB 5min P1	0	0	Total	1 in 1000	wasp100ul	LU	9:53 AM	7/27/2006		
50%LB 5min P2	3	4.78	Total	1 in 1000	wasp100ul	LU	9:53 AM	7/27/2006		
50%LB 10min P1	0	0	Total	1 in 1000	wasp100ul	LU	9:53 AM	7/27/2006		

50%LB 10min P2	0	0	Total	1 in 1000	wasp100ul LU	9:53 AM	7/27/2006
50%LB 30min P1	0	0	Total	1 in 1000	wasp100ul LU	9:53 AM	7/27/2006
50%LB 30min P2	0	0	Total	1 in 1000	wasp100ul LU	9:53 AM	7/27/2006

Time	Log		Average	Standard Deviation	
0	7.13				
0	7.14				
0	7.45				
0	7.62		7.335	0.241178	
1	6.99				
1	6.87		6.93	0.084853	
5	0				
5	4.78		2.39	3.37997	*used spread plates/ not these
10	0				
10	0		0	0	
30	0				
30	0		0	0	

Synbiosis ProtoCOL Colony Counter 3.15

Study Name	NEW Bacillus Spores on Stainless Steel Surface
Batch Name	Lysol with Bleach 100%
Type	Spiral Plate
Based on	
Comment	

		Time	Means	SD
System Parameters		0	7.59	0.411582
Video State	Inverted	1	4.45	0.212132
Selected Lamp	Top Lamp On	5	0	0
Camera Shutter	1/250 sec	10	0	0
Contrast Setting	Medium	30	0	0
Dish Diameter	88 mm			
Sample Volume	1 ml			
Area Limits	Off			
Spiral Size	Spiral 90 mm			
Spiral Frame	Two Sectors			
Spiral Plater	wasp100ul			
Minimum Spiral Count F	20			
Maximum Spiral Count I	75			
Calibration Factor	0.1615 mm \ Pixel	Created:	9:46 AM 7/26/2006	

Plate Id	Count per Frame	Log count per ml	Sector used	Dilution Factor	Spiral Plater	Flags	Time	Date	Comment	User
100% LB Control P1	13	7.41	Total	1 in 100000	wasp100ul	ELU	9:46 AM	7/26/2006		
100% LB Control P2	14	7.45	Total	1 in 100000	wasp100ul	ELU	9:47 AM	7/26/2006		
100% LB Control P3	1	7.3	Total	1 in 10^6	wasp100ul	ELU	9:47 AM	7/26/2006		
100% LB Control P4	8	8.2	Total	1 in 10^6	wasp100ul	ELU	9:48 AM	7/26/2006		
100% LB 0min P1	73	7.16	Total	1 in 10000	wasp100ul	LU	9:54 AM	7/26/2006		
100% LB 0min P2	89	7.25	Total	1 in 10000	wasp100ul	LU	9:54 AM	7/26/2006		
100% LB 0min P3	5	7	Total	1 in 100000	wasp100ul	ELU	9:54 AM	7/26/2006		
100% LB 0min P4	9	7.26	Total	1 in 100000	wasp100ul	ELU	9:54 AM	7/26/2006		
100% LB 1min P1	2	4.6	Total	1 in 1000	wasp100ul	ELU	9:55 AM	7/26/2006		
100% LB 1min P2	1	4.3	Total	1 in 1000	wasp100ul	ELU	9:55 AM	7/26/2006		
100% LB 5min P1	0	0	Total	1 in 1000	wasp100ul	LU	9:56 AM	7/26/2006		
100% LB 5min P2	0	0	Total	1 in 1000	wasp100ul	LU	9:56 AM	7/26/2006		
100% LB 10min P1	0	0	Total	1 in 1000	wasp100ul	LU	9:56 AM	7/26/2006		

100% LB 10min P2	0	0	Total	1 in 1000	wasp100ul LU	9:56 AM 7/26/2006
100% LB 30min P1	0	0	Total	1 in 1000	wasp100ul LU	9:56 AM 7/26/2006
100% LB 30min P2	0	0	Total	1 in 1000	wasp100ul LU	9:56 AM 7/26/2006

Time	Log		Average	Standard Deviation
0	7.41			
0	7.45			
0	7.3			
0	8.2	7.59	0.411582	
1	4.6			
1	4.3	4.45	0.212132	
5	0			
5	0	0	0	
10	0			
10	0	0	0	
30	0			
30	0	0	0	

Synbiosis ProtoCOL Colony Counter 3.15

Study Name	NEW Bacillus Spores on Stainless Steel Surface
Batch Name	Lysol Toilet 50%
Type	Spiral Plate
Based on	
Comment	

		Time	Means	SD
System Parameters		0	7.08	0.057155
Video State	Inverted	1	5.48	0.042426
Selected Lamp	Top Lamp On	5	5.59	0.014142
Camera Shutter	1/250 sec	10	0	0
Contrast Setting	Medium	30	0	0
Dish Diameter	88 mm	60	0	0
Sample Volume	1 ml			
Area Limits	Off			
Spiral Size	Spiral 90 mm			
Spiral Frame	Two Sectors			
Spiral Plater	wasp100ul			
Minimum Spiral Coun	20			
Maximum Spiral Coun	75			
Calibration Factor	0.1612 mm \ Pixel	Created:	9:44 AM	8/1/2006

Plate Id	Count per Frame	Log count per ml	Sector used	Dilution Factor	Spiral Plater	Flags	Time	Date	Comment	User
50%LT Control P1	68	7.13	Total	1 in 10000	wasp100ul	LU	9:44 AM	8/1/2006		
50%LT Control P2	65	7.11	Total	1 in 10000	wasp100ul	LU	9:45 AM	8/1/2006		
50%LT Control P3	5	7	Total	1 in 100000	wasp100ul	LU	9:45 AM	8/1/2006		
50%LT Control P4	6	7.08	Total	1 in 100000	wasp100ul	LU	9:45 AM	8/1/2006		
50%LT 0min P1	68	7.13	Total	1 in 10000	wasp100ul	LU	9:46 AM	8/1/2006		
50%LT 0min P2	75	7.18	Total	1 in 10000	wasp100ul	LU	9:46 AM	8/1/2006		
50%LT 0min P3	11	7.34	Total	1 in 100000	wasp100ul	ELU	9:46 AM	8/1/2006		
50%LT 0min P4	7	7.15	Total	1 in 100000	wasp100ul	ELU	9:47 AM	8/1/2006		
50%LT 1min P1	16	5.51	Total	1 in 1000	wasp100ul	LU	9:47 AM	8/1/2006		
50%LT 1min P2	14	5.45	Total	1 in 1000	wasp100ul	LU	9:47 AM	8/1/2006		
50%LT 5min P1	20	5.6	Total	1 in 1000	wasp100ul	LU	9:47 AM	8/1/2006		
50%LT 5min P2	19	5.58	Total	1 in 1000	wasp100ul	LU	9:48 AM	8/1/2006		
50%LT 10min P1	0	0	Total	1 in 1000	wasp100ul	LU	9:48 AM	8/1/2006		

50%LT 10min P2	0	0	Total	1 in 1000	wasp100ul LU	9:48 AM	8/1/2006
50%LT 30min P1	0	0	Total	1 in 1000	wasp100ul LU	9:48 AM	8/1/2006
50%LT 30min P2	0	0	Total	1 in 1000	wasp100ul LU	9:48 AM	8/1/2006
50%LT 60min P1	0	0	Total	1 in 1000	wasp100ul LU	9:48 AM	8/1/2006
50%LT 60min P2	0	0	Total	1 in 1000	wasp100ul LU	9:48 AM	8/1/2006

Time	Log	Average	Standard Deviation
0	7.13		
0	7.11		
0	7		
0	7.08	7.08	0.057155
1	5.51		
1	5.45	5.48	0.042426
5	5.6		
5	5.58	5.59	0.014142
10	0		
10	0	0	0
30	0		
30	0	0	0
60	0		
60	0	0	0

Synbiosis ProtoCOL Colony Counter 3.15

Study Name	NEW Bacillus Spores on Stainless Steel Surface
Batch Name	Lysol Toilet 100%
Type	Spiral Plate
Based on	
Comment	

		Time	Means	SD
System Parameters		0	7.2625	0.170563
Video State	Inverted	1	5.84	0.084853
Selected Lamp	Top Lamp On	5	0	0
Camera Shutter	1/250 sec	10	0	0
Contrast Setting	Medium	30	0	0
Dish Diameter	88 mm	60	0	0
Sample Volume	1 ml			
Area Limits	Off			
Spiral Size	Spiral 90 mm			
Spiral Frame	Two Sectors			
Spiral Plater	wasp100ul			
Minimum Spiral Count R	20			
Maximum Spiral Count R	75			
Calibration Factor	0.1609 mm \ Pixel	Created:	9:55 AM 7/27/2006	

Plate Id	Count per Frame	Log count per ml	Sector used	Dilution Factor	Spiral Plater	Flags	Time	Date	Comment	User
100%LT Control P1	77	7.19	Total	1 in 10000	wasp100ul	LU	9:56 AM	7/27/2006		
100%LT Control P2	100	7.3	Total	1 in 10000	wasp100ul	LU	9:56 AM	7/27/2006		
100%LT Control P3	6	7.08	Total	1 in 100000	wasp100ul	LU	9:56 AM	7/27/2006		
100%LT Control P4	15	7.48	Total	1 in 100000	wasp100ul	ELU	9:57 AM	7/27/2006		
100%LT 0min P1	78	7.19	Total	1 in 10000	wasp100ul	LU	9:57 AM	7/27/2006		
100%LT 0min P2	93	7.27	Total	1 in 10000	wasp100ul	LU	9:57 AM	7/27/2006		
100%LT 0min P3	11	7.34	Total	1 in 100000	wasp100ul	LU	9:57 AM	7/27/2006		
100%LT 0min P4	11	7.34	Total	1 in 100000	wasp100ul	LU	9:57 AM	7/27/2006		
100%LT 1min P1	40	5.9	Total	1 in 1000	wasp100ul	LU	9:58 AM	7/27/2006		
100%LT 1min P2	30	5.78	Total	1 in 1000	wasp100ul	LU	9:58 AM	7/27/2006		
100%LT 5min P1	0	0	Total	1 in 1000	wasp100ul	ELU	9:58 AM	7/27/2006		
100%LT 5min P2	0	0	Total	1 in 1000	wasp100ul	ELU	9:59 AM	7/27/2006		
100%LT 10min P1	0	0	Total	1 in 1000	wasp100ul	LU	9:59 AM	7/27/2006		

100%LT 10min P2	0	0	Total	1 in 1000	wasp100ul LU	9:59 AM	7/27/2006
100%LT 30min P1	0	0	Total	1 in 1000	wasp100ul LU	9:59 AM	7/27/2006
100%LT 30min P2	0	0	Total	1 in 1000	wasp100ul LU	9:59 AM	7/27/2006
100%LT 60min P1	0	0	Total	1 in 1000	wasp100ul LU	9:59 AM	7/27/2006
100%LT 60min P2	0	0	Total	1 in 1000	wasp100ul LU	9:59 AM	7/27/2006

Time	Log	Average	Standard Deviation
0	7.19		
0	7.3		
0	7.08		
0	7.48	7.2625	0.170563
1	5.9		
1	5.78	5.84	0.084853
5	0		
5	0	0	0
10	0		
10	0	0	0
30	0		
30	0	0	0
60	0		
60	0	0	0

Synbiosis ProtoCOL Colony Counter 3.15

Study Name	NEW Bacillus Spores on Stainless Steel Surface
Batch Name	Tilex 50%
Type	Spiral Plate
Based on	
Comment	

System Parameters	
Video State	Inverted
Selected Lamp	Top Lamp On
Camera Shutter	1/250 sec
Contrast Setting	Medium
Dish Diameter	88 mm
Sample Volume	1 ml
Area Limits	Off
Spiral Size	Spiral 90 mm
Spiral Frame	Two Sectors
Spiral Plater	wasp100ul
Minimum Spiral Count	20
Maximum Spiral Count	75
Calibration Factor	0.1615 mm \ Pixel

Time	Means	SD
0	7.595	0.353789
1	6.595	0.120208
5	0	0
10	0	0
30	0	0

Created: 9:36 AM 7/26/2006

Plate Id	Count per Frame	Log count per ml	Sector used	Dilution Factor	Spiral Plater	Flags	Time	Date	Comment	User
50%Tilex Control P1	9	7.26	Total	1 in 100000	wasp100ul	ELRU	9:38 AM	7/26/2006		
50%Tilex Control P2	11	7.34	Total	1 in 100000	wasp100ul	ELU	9:39 AM	7/26/2006		
50%Tilex Control P3	3	7.78	Total	1 in 10^6	wasp100ul	LU	9:39 AM	7/26/2006		
50%Tilex Control P4	5	8	Total	1 in 10^6	wasp100ul	ELU	9:39 AM	7/26/2006		
50%Tilex 0min P1	83	7.22	Total	1 in 10000	wasp100ul	LU	9:40 AM	7/26/2006		
50%Tilex 0min P2	84	7.23	Total	1 in 10000	wasp100ul	LU	9:40 AM	7/26/2006		
50%Tilex 0min P3	6	7.08	Total	1 in 100000	wasp100ul	ELU	9:40 AM	7/26/2006		
50%Tilex 0min P4	9	7.26	Total	1 in 100000	wasp100ul	ELU	9:41 AM	7/26/2006		
50%Tilex 1min P1	36	6.68	4b	1 in 1000	wasp100ul	2	9:41 AM	7/26/2006		
50%Tilex 1min P2	40	6.51	4a	1 in 1000	wasp100ul	2	9:42 AM	7/26/2006		
50%Tilex 5min P1	0	0	Total	1 in 1000	wasp100ul	LU	9:42 AM	7/26/2006		
50%Tilex 5min P2	0	0	Total	1 in 1000	wasp100ul	LU	9:42 AM	7/26/2006		
50%Tilex 10min P1	0	0	Total	1 in 1000	wasp100ul	LU	9:42 AM	7/26/2006		

50%Tilex 10min P2	0	0	Total	1 in 1000	wasp100ul LU	9:42 AM 7/26/2006
50%Tilex 30min P1	0	0	Total	1 in 1000	wasp100ul LU	9:42 AM 7/26/2006
50%Tilex 30min P2	0	0	Total	1 in 1000	wasp100ul LU	9:42 AM 7/26/2006

Time	Log	Average	Standard Deviation
0	7.26		
0	7.34		
0	7.78		
0	8	7.595	0.353789
1	6.68		
1	6.51	6.595	0.120208
5	0		
5	0	0	0
10	0		
10	0	0	0
30	0		
30	0	0	0

Synbiosis ProtoCOL Colony Counter 3.15

Study Name	NEW Bacillus Spores on Stainless Steel Surface
Batch Name	Tilex 100%
Type	Spiral Plate
Based on	
Comment	

System Parameters		Time	Means	SD
Video State	Inverted	0	7.8925	0.227358
Selected Lamp	Top Lamp On	1	5.44	0.19799
Camera Shutter	1/250 sec	5	4.6	0
Contrast Setting	Medium	10	0	0
Dish Diameter	88 mm	30	0	0
Sample Volume	1 ml	60	0	0
Area Limits	Off			
Spiral Size	Spiral 90 mm			
Spiral Frame	Two Sectors			
Spiral Plater	wasp100ul			
Minimum Spiral Count Rul	20			
Maximum Spiral Count Rul	75			
Calibration Factor	0.1615 mm \ Pixel	Created:	9:14 AM 7/25/2006	

Plate Id	Count per Frame	Log count per ml	Sector used	Dilution Factor	Spiral Plater	Flags	Time	Date	Comment	User
Control P P1	21	7.62	Total	1 in 100000	wasp100ul	LU	9:16 AM	7/25/2006		
Control P P2	31	7.79	Total	1 in 100000	wasp100ul	LU	9:16 AM	7/25/2006		
Control P P3	6	8.08	Total	1 in 10^6	wasp100ul	LU	9:16 AM	7/25/2006		
Control P P4	6	8.08	Total	1 in 10^6	wasp100ul	ELU	9:17 AM	7/25/2006		
100%Tilex 0min P1	55	7.04	Total	1 in 10000	wasp100ul	LU	9:18 AM	7/25/2006		
100%Tilex 0min P2	70	7.15	Total	1 in 10000	wasp100ul	LU	9:18 AM	7/25/2006		
100%Tilex 0min P3	3	6.78	Total	1 in 100000	wasp100ul	LU	9:18 AM	7/25/2006		
100%Tilex 0min P4	16	7.51	Total	1 in 100000	wasp100ul	LU	9:19 AM	7/25/2006		
100%Tilex 1min P1	10	5.3	Total	1 in 1000	wasp100ul	LU	9:20 AM	7/25/2006		
100%Tilex 1min P2	19	5.58	Total	1 in 1000	wasp100ul	LRU	9:20 AM	7/25/2006		
100%Tilex 5min P1	2	4.6	Total	1 in 1000	wasp100ul	LU	9:21 AM	7/25/2006		
100%Tilex 5min P2	0	0	Total	1 in 1000	wasp100ul	LRU	9:22 AM	7/25/2006		
100%Tilex 10min P1	0	0	Total	1 in 100000	wasp100ul	LU	9:22 AM	7/25/2006		

100%Tilex 10min P2	0	0	Total	1 in 100000	wasp100ul LU	9:22 AM	7/25/2006
100%Tilex 30min P1	0	0	Total	1 in 100000	wasp100ul LU	9:22 AM	7/25/2006
100%Tilex 30min P2	0	0	Total	1 in 100000	wasp100ul LU	9:22 AM	7/25/2006
100%Tilex 60min P1	0	0	Total	1 in 100000	wasp100ul LU	9:22 AM	7/25/2006
100%Tilex 60min P2	0	0	Total	1 in 100000	wasp100ul LU	9:22 AM	7/25/2006

Time	Log	Average	Standard Deviation
0	7.62		
0	7.79		
0	8.08		
0	8.08	7.8925	0.227358
1	5.3		
1	5.58	5.44	0.19799
5	4.6		
5	0	4.6	0
10	0		
10	0	0	0
30	0		
30	0	0	0
60	0		
60	0	0	0

Synbiosis ProtoCOL Colony Counter 3.15

Study Name	NEW Bacillus Spores on Stainless Steel Surface
Batch Name	Works 50%
Type	Spiral Plate
Based on	
Comment	

System Parameters		Time	Means	SD
Video State	Inverted	0	7.275	0.1974
Selected Lamp	Top Lamp On	1	6.46	0.028284
Camera Shutter	1/250 sec	5	6.51	0.028284
Contrast Setting	Medium	10	6.45	0.042426
Dish Diameter	88 mm	30	5.98	0.084853
Sample Volume	1 ml	60	0	0
Area Limits	Off	120	0	0
Spiral Size	Spiral 90 mm			
Spiral Frame	Two Sectors			
Spiral Plater	wasp100ul			
Minimum Spiral Count R	20			
Maximum Spiral Count F	75			
Calibration Factor	0.1606 mm \ Pixel	Created:	9:50 AM	8/1/2006

Plate Id	Count per Frame	Log count per ml	Sector used	Dilution Factor	Spiral Plater	Flags	Time	Date	Comment	User
50%Works Control P1	52	7.02	Total	1 in 10000	wasp100ul	LU	9:51 AM	8/1/2006		
50%Works Control P2	83	7.22	Total	1 in 10000	wasp100ul	LU	9:52 AM	8/1/2006		
50%Works Control P3	13	7.41	Total	1 in 100000	wasp100ul	LU	9:52 AM	8/1/2006		
50%Works Control P4	14	7.45	Total	1 in 100000	wasp100ul	ELU	9:52 AM	8/1/2006		
50%Works 0min P1	96	7.28	Total	1 in 10000	wasp100ul	LU	9:53 AM	8/1/2006		
50%Works 0min P2	103	7.31	Total	1 in 10000	wasp100ul	LU	9:53 AM	8/1/2006		
50%Works 0min P3	13	7.41	Total	1 in 100000	wasp100ul	LU	9:54 AM	8/1/2006		
50%Works 0min P4	17	7.53	Total	1 in 100000	wasp100ul	LU	9:54 AM	8/1/2006		
50%Works 1min P1	38	6.48	4a	1 in 1000	wasp100ul	2	9:55 AM	8/1/2006		
50%Works 1min P2	138	6.44	Total	1 in 1000	wasp100ul	LU	9:55 AM	8/1/2006		
50%Works 5min P1	155	6.49	Total	1 in 1000	wasp100ul	LU	9:55 AM	8/1/2006		
50%Works 5min P2	170	6.53	Total	1 in 1000	wasp100ul	U	9:55 AM	8/1/2006		
50%Works 10min P1	150	6.48	Total	1 in 1000	wasp100ul	LU	9:56 AM	8/1/2006		

50%Works 10min P2	131	6.42	Total	1 in 1000	wasp100ul LU	9:56 AM	8/1/2006
50%Works 30min P1	42	5.92	Total	1 in 1000	wasp100ul LU	9:56 AM	8/1/2006
50%Works 30min P2	55	6.04	Total	1 in 1000	wasp100ul LU	9:56 AM	8/1/2006
50%Works 60min P1	1	4.3	Total	1 in 1000	wasp100ul LU	9:56 AM	8/1/2006
50%Works 60min P2	0	0	Total	1 in 1000	wasp100ul ELU	9:57 AM	8/1/2006
50%Works 120min P1	0	0	Total	1 in 1000	wasp100ul ELU	9:57 AM	8/1/2006
50%Works 120min P2	0	0	Total	1 in 1000	wasp100ul ELU	9:57 AM	8/1/2006

Time	Log	Average	Standard Deviation
0	7.02		
0	7.22		
0	7.41		
0	7.45	7.275	0.1974
1	6.48		
1	6.44	6.46	0.028284
5	6.49		
5	6.53	6.51	0.028284
10	6.48		
10	6.42	6.45	0.042426
30	5.92		
30	6.04	5.98	0.084853
60	0		
60	0	0	0
120	0		
120	0	0	0

Synbiosis ProtoCOL Colony Counter 3.15

Study Name	NEW Bacillus Spores on Stainless Steel Surface
Batch Name	Works 100%
Type	Spiral Plate
Based on	
Comment	

System Parameters		Time	Means	SD
Video State	Inverted	0	7.2225	0.125266
Selected Lamp	Top Lamp On	1	6.305	0.021213
Camera Shutter	1/250 sec	5	5.375	0.106066
Contrast Setting	Medium	10	0	0
Dish Diameter	88 mm	30	0	0
Sample Volume	1 ml	60	0	0
Area Limits	Off			
Spiral Size	Spiral 90 mm			
Spiral Frame	Two Sectors			
Spiral Plater	wasp100ul			
Minimum Spiral Count Rule	20			
Maximum Spiral Count Rule	75			
Calibration Factor	0.1612 mm \ Pixel	Created:	10:18 AM	8/2/2006

Plate Id	Count per Frame	Log count per ml	Sector used	Dilution Factor	Spiral Plater	Flags	Time	Date	Comment	User
100%Works Control P1	74	7.17	Total	1 in 10000	wasp100ul	LU	10:19 AM	8/2/2006		
100%Works Control P2	73	7.16	Total	1 in 10000	wasp100ul	LU	10:19 AM	8/2/2006		
100%Works Control P3	7	7.15	Total	1 in 100000	wasp100ul	ELU	10:19 AM	8/2/2006		
100%Works Control P4	13	7.41	Total	1 in 100000	wasp100ul	ELU	10:19 AM	8/2/2006		
100%Works 0min P1	72	7.16	Total	1 in 10000	wasp100ul	LU	10:20 AM	8/2/2006		
100%Works 0min P2	89	7.25	Total	1 in 10000	wasp100ul	LU	10:20 AM	8/2/2006		
100%Works 0min P3	9	7.26	Total	1 in 100000	wasp100ul	ELU	10:20 AM	8/2/2006		
100%Works 0min P4	11	7.34	Total	1 in 100000	wasp100ul	ELU	10:20 AM	8/2/2006		
100%Works 1min P1	98	6.29	Total	1 in 1000	wasp100ul	LU	10:21 AM	8/2/2006		
100%Works 1min P2	105	6.32	Total	1 in 1000	wasp100ul	LU	10:21 AM	8/2/2006		
100%Works 5min P1	10	5.3	Total	1 in 1000	wasp100ul	LU	10:21 AM	8/2/2006		
100%Works 5min P2	14	5.45	Total	1 in 1000	wasp100ul	LU	10:21 AM	8/2/2006		
100%Works 10min P1	0	0	Total	1 in 1000	wasp100ul	ELU	10:24 AM	8/2/2006		

100%Works 10min P2	0	0	Total	1 in 1000	wasp100ul ELU	10:24 AM	8/2/2006
100%Works 30min P1	0	0	Total	1 in 1000	wasp100ul ELU	10:24 AM	8/2/2006
100%Works 30min P2	0	0	Total	1 in 1000	wasp100ul ELU	10:24 AM	8/2/2006
100%Works 60min P1	0	0	Total	1 in 1000	wasp100ul ELU	10:24 AM	8/2/2006
100%Works 60min P2	0	0	Total	1 in 1000	wasp100ul ELU	10:24 AM	8/2/2006

Time	Log	Average	Standard Deviation
0	7.17		
0	7.16		
0	7.15		
0	7.41	7.2225	0.125266
1	6.29		
1	6.32	6.305	0.021213
5	5.3		
5	5.45	5.375	0.106066
10	0		
10	0	0	0
30	0		
30	0	0	0
60	0		
60	0	0	0

Synbiosis ProtoCOL Colony Counter 3.15

Study Name	NEW Bacillus Spores on Stainless Steel Surface
Batch Name	Hydrogen Peroxide 50%
Type	Spiral Plate
Based on	
Comment	

System Parameters	
Video State	Inverted
Selected Lamp	Top Lamp On
Camera Shutter	1/250 sec
Contrast Setting	Medium
Dish Diameter	88 mm
Sample Volume	1 ml
Area Limits	Off
Spiral Size	Spiral 90 mm
Spiral Frame	Two Sectors
Spiral Plater	wasp100ul
Minimum Spiral Count Ru	20
Maximum Spiral Count Ru	75
Calibration Factor	0.1612 mm \ Pixel

Time (min)	Means	SD	
0	7.1125	0.165605	
1	7.4875	0.213756	
5	7.165	0.067577	
10	7.445	0.27	
30	7.085	0.080623	
60	6.6825	0.295113	
120	6.0025	0.325615	
180	3.79	2.535639	*used spread plates
240	0	0	
300	0	0	
360	0	0	

Created: 1:49 PM 8/16/2006

Plate Id	Count per Frame	Log count per ml	Sector used	Dilution Factor	Spiral Plater	Flags	Time	Date	Comment	User
50%H2O2 Control P1	98	7.29	Total	1 in 10000	wasp100ul	LU	1:49 PM	8/16/2006		
50%H2O2 Control P2	75	7.18	Total	1 in 10000	wasp100ul	LRU	1:50 PM	8/16/2006		
50%H2O2 Control P3	6	7.08	Total	1 in 100000	wasp100ul	ELU	1:51 PM	8/16/2006		
50%H2O2 Control P4	4	6.9	Total	1 in 100000	wasp100ul	LU	1:50 PM	8/16/2006		
50%H2O2 0min P1	95	7.28	Total	1 in 10000	wasp100ul	LU	1:51 PM	8/16/2006		
50%H2O2 0min P2	88	7.25	Total	1 in 10000	wasp100ul	LU	1:52 PM	8/16/2006		
50%H2O2 0min P3	10	7.3	Total	1 in 100000	wasp100ul	LU	1:52 PM	8/16/2006		
50%H2O2 0min P4	14	7.45	Total	1 in 100000	wasp100ul	ELU	1:52 PM	8/16/2006		
50%H2O2 1min P1	82	7.21	Total	1 in 10000	wasp100ul	LU	1:53 PM	8/16/2006		
50%H2O2 1min P2	137	7.44	Total	1 in 10000	wasp100ul	LU	1:53 PM	8/16/2006		
50%H2O2 1min P3	25	7.7	Total	1 in 100000	wasp100ul	LU	1:53 PM	8/16/2006		
50%H2O2 1min P4	20	7.6	Total	1 in 100000	wasp100ul	ELU	1:53 PM	8/16/2006		
50%H2O2 5min P1	87	7.24	Total	1 in 10000	wasp100ul	LU	1:54 PM	8/16/2006		

50%H2O2 5min P2	78	7.19 Total	1 in 10000	wasp100ul LU	1:54 PM	8/16/2006
50%H2O2 5min P3	6	7.08 Total	1 in 100000	wasp100ul LU	1:54 PM	8/16/2006
50%H2O2 5min P4	7	7.15 Total	1 in 100000	wasp100ul ELU	1:54 PM	8/16/2006
50%H2O2 10min P1	85	7.23 Total	1 in 10000	wasp100ul LU	1:55 PM	8/16/2006
50%H2O2 10min P2	84	7.23 Total	1 in 10000	wasp100ul LU	1:55 PM	8/16/2006
50%H2O2 10min P3	31	7.79 Total	1 in 100000	wasp100ul LU	1:55 PM	8/16/2006
50%H2O2 10min P4	17	7.53 Total	1 in 100000	wasp100ul LU	1:55 PM	8/16/2006
50%H2O2 30min P1	52	7.02 Total	1 in 10000	wasp100ul LU	1:55 PM	8/16/2006
50%H2O2 30min P2	55	7.04 Total	1 in 10000	wasp100ul LU	1:56 PM	8/16/2006
50%H2O2 30min P3	8	7.2 Total	1 in 100000	wasp100ul ELU	1:56 PM	8/16/2006
50%H2O2 30min P4	6	7.08 Total	1 in 100000	wasp100ul ELU	1:56 PM	8/16/2006
50%H2O2 60min P1	41	6.91 Total	1 in 10000	wasp100ul LU	1:57 PM	8/16/2006
50%H2O2 60min P2	42	6.92 Total	1 in 10000	wasp100ul ELU	1:57 PM	8/16/2006
50%H2O2 60min P3	2	6.6 Total	1 in 100000	wasp100ul ELU	1:57 PM	8/16/2006
50%H2O2 60min P4	1	6.3 Total	1 in 100000	wasp100ul ELU	1:58 PM	8/16/2006
50%H2O2 120min P1	73	6.16 Total	1 in 1000	wasp100ul LU	1:58 PM	8/16/2006
50%H2O2 120min P2	112	6.35 Total	1 in 1000	wasp100ul LU	1:58 PM	8/16/2006
50%H2O2 120min P3	2	5.6 Total	1 in 10000	wasp100ul ELU	1:59 PM	8/16/2006
50%H2O2 120min P4	4	5.9 Total	1 in 10000	wasp100ul ELU	1:59 PM	8/16/2006
50%H2O2 180min P1	6	5.08 Total	1 in 1000	wasp100ul LU	1:59 PM	8/16/2006
50%H2O2 180min P2	3	4.78 Total	1 in 1000	wasp100ul LU	1:59 PM	8/16/2006
50%H2O2 180min P3	1	5.3 Total	1 in 10000	wasp100ul ELU	2:00 PM	8/16/2006
50%H2O2 180min P4	0	0 Total	1 in 10000	wasp100ul LU	2:00 PM	8/16/2006
50%H2O2 240min P1	0	0 Total	1 in 1000	wasp100ul LU	2:00 PM	8/16/2006
50%H2O2 240min P2	0	0 Total	1 in 1000	wasp100ul LU	2:00 PM	8/16/2006
50%H2O2 240min P3	0	0 Total	1 in 10000	wasp100ul LU	2:00 PM	8/16/2006
50%H2O2 240min P4	0	0 Total	1 in 10000	wasp100ul LU	2:00 PM	8/16/2006
50%H2O2 300min P1	0	0 Total	1 in 1000	wasp100ul LU	2:00 PM	8/16/2006
50%H2O2 300min P2	0	0 Total	1 in 1000	wasp100ul ELU	2:01 PM	8/16/2006
50%H2O2 300min P3	0	0 Total	1 in 10000	wasp100ul LU	2:01 PM	8/16/2006
50%H2O2 300min P4	0	0 Total	1 in 10000	wasp100ul LU	2:01 PM	8/16/2006
50%H2O2 360min P1	0	0 Total	1 in 1000	wasp100ul LU	2:01 PM	8/16/2006
50%H2O2 360min P2	0	0 Total	1 in 1000	wasp100ul LU	2:01 PM	8/16/2006
50%H2O2 360min P3	0	0 Total	1 in 10000	wasp100ul LU	2:01 PM	8/16/2006
50%H2O2 360min P4	0	0 Total	1 in 10000	wasp100ul LU	2:01 PM	8/16/2006

Time	Log		Average	Standard Deviation	
	0	7.29			
	0	7.18			
	0	7.08			
	0	6.9	7.1125	0.165605	
	1	7.21			
	1	7.44			
	1	7.7			
	1	7.6	7.4875	0.213756	
	5	7.24			
	5	7.19			
	5	7.08			
	5	7.15	7.165	0.067577	
	10	7.23			
	10	7.23			
	10	7.79			
	10	7.53	7.445	0.27	
	30	7.02			
	30	7.04			
	30	7.2			
	30	7.08	7.085	0.080623	
	60	6.91			
	60	6.92			
	60	6.6			
	60	6.3	6.6825	0.295113	
	120	6.16			
	120	6.35			
	120	5.6			
	120	5.9	6.0025	0.325615	
	180	5.08			
	180	4.78			
	180	5.3			
	180	0	3.79	2.535639	*used spread plates/ not these
	240	0			
	240	0			
	240	0			
	240	0	0	0	

300	0		
300	0		
300	0		
300	0	0	0
360	0		
360	0		
360	0		
360	0	0	0

Synbiosis ProtoCOL Colony Counter 3.15

Study Name	NEW Bacillus Spores on Stainless Steel Surface
Batch Name	Hydrogen Peroxide 100%
Type	Spiral Plate
Based on	
Comment	
System Parameters	
Video State	Inverted
Selected Lamp	Top Lamp On
Camera Shutter	1/250 sec
Contrast Setting	Medium
Dish Diameter	88 mm
Sample Volume	1 ml
Area Limits	Off
Spiral Size	Spiral 90 mm
Spiral Frame	Two Sectors
Spiral Plater	wasp100ul
Minimum Spiral Count I	20
Maximum Spiral Count	75
Calibration Factor	0.1612 mm \ Pixel

Time	Means	SD
0	7.265	0.199583
1	7.2725	0.078049
5	7.2225	0.109659
10	7.2575	0.149304
30	6.83	0.098995
60	5.7675	0.539838
120	0	0
180	0	0
240	0	0
300	0	0
360	0	0

Created: 10:01 AM 8/2/2006

Plate Id	Count per Frame	Log count per ml	Sector used	Dilution Factor	Spiral Plater	Flags	Time	Date	Comment	User
100%H2O2 Control P1	66	7.12	Total	1 in 10000	wasp100ul	LU	10:02 AM	8/2/2006		
100%H2O2 Control P2	76	7.18	Total	1 in 10000	wasp100ul	LU	10:02 AM	8/2/2006		
100%H2O2 Control P3	8	7.2	Total	1 in 100000	wasp100ul	LU	10:02 AM	8/2/2006		
100%H2O2 Control P4	18	7.56	Total	1 in 100000	wasp100ul	ELU	10:03 AM	8/2/2006		
100%H2O2 0min P1	80	7.2	Total	1 in 10000	wasp100ul	LU	10:03 AM	8/2/2006		
100%H2O2 0min P2	90	7.26	Total	1 in 10000	wasp100ul	LU	10:03 AM	8/2/2006		
100%H2O2 0min P3	11	7.34	Total	1 in 100000	wasp100ul	LU	10:03 AM	8/2/2006		
100%H2O2 0min P4	10	7.3	Total	1 in 100000	wasp100ul	ELU	10:04 AM	8/2/2006		
100%H2O2 1min P1	79	7.2	Total	1 in 10000	wasp100ul	LU	10:04 AM	8/2/2006		
100%H2O2 1min P2	81	7.21	Total	1 in 10000	wasp100ul	LU	10:04 AM	8/2/2006		
100%H2O2 1min P3	11	7.34	Total	1 in 100000	wasp100ul	ELU	10:05 AM	8/2/2006		
100%H2O2 1min P4	11	7.34	Total	1 in 100000	wasp100ul	ELU	10:05 AM	8/2/2006		
100%H2O2 5min P1	58	7.06	Total	1 in 10000	wasp100ul	LU	10:05 AM	8/2/2006		

100%H2O2 5min P2	93	7.27	Total	1 in 10000	wasp100ul LU	10:05 AM	8/2/2006
100%H2O2 5min P3	10	7.3	Total	1 in 100000	wasp100ul LU	10:06 AM	8/2/2006
100%H2O2 5min P4	9	7.26	Total	1 in 100000	wasp100ul LU	10:06 AM	8/2/2006
100%H2O2 10min P1	72	7.16	Total	1 in 10000	wasp100ul LU	10:06 AM	8/2/2006
100%H2O2 10min P2	78	7.19	Total	1 in 10000	wasp100ul LU	10:06 AM	8/2/2006
100%H2O2 10min P3	8	7.2	Total	1 in 100000	wasp100ul LU	10:06 AM	8/2/2006
100%H2O2 10min P4	15	7.48	Total	1 in 100000	wasp100ul ELU	10:07 AM	8/2/2006
100%H2O2 30min P1	29	6.76	Total	1 in 10000	wasp100ul LU	10:07 AM	8/2/2006
100%H2O2 30min P2	40	6.9	Total	1 in 10000	wasp100ul LU	10:07 AM	8/2/2006
100%H2O2 30min P3	0	0	Total	1 in 100000	wasp100ul LU	10:07 AM	8/2/2006
100%H2O2 30min P4	0	0	Total	1 in 100000	wasp100ul LU	10:07 AM	8/2/2006
100%H2O2 60min P1	85	6.23	Total	1 in 1000	wasp100ul LU	10:08 AM	8/2/2006
100%H2O2 60min P2	86	6.24	Total	1 in 1000	wasp100ul LU	10:08 AM	8/2/2006
100%H2O2 60min P3	1	5.3	Total	1 in 10000	wasp100ul ELU	10:26 AM	8/2/2006
100%H2O2 60min P4	1	5.3	Total	1 in 10000	wasp100ul ELU	10:26 AM	8/2/2006
100%H2O2 120min P1	0	0	Total	1 in 1000	wasp100ul ELU	10:26 AM	8/2/2006
100%H2O2 120min P2	0	0	Total	1 in 1000	wasp100ul ELU	10:26 AM	8/2/2006
100%H2O2 120min P3	0	0	Total	1 in 10000	wasp100ul ELU	10:26 AM	8/2/2006
100%H2O2 120min P4	0	0	Total	1 in 10000	wasp100ul ELU	10:26 AM	8/2/2006
100%H2O2 180min P1	0	0	Total	1 in 1000	wasp100ul ELU	10:26 AM	8/2/2006
100%H2O2 180min P2	0	0	Total	1 in 1000	wasp100ul ELU	10:26 AM	8/2/2006
100%H2O2 240min P1	0	0	Total	1 in 1000	wasp100ul ELU	10:27 AM	8/2/2006
100%H2O2 240min P2	0	0	Total	1 in 1000	wasp100ul ELU	10:27 AM	8/2/2006
100%H2O2 300min P1	0	0	Total	1 in 1000	wasp100ul ELU	10:27 AM	8/2/2006
100%H2O2 300min P2	0	0	Total	1 in 1000	wasp100ul ELU	10:27 AM	8/2/2006
100%H2O2 360min P1	0	0	Total	1 in 1000	wasp100ul ELU	10:27 AM	8/2/2006
100%H2O2 360min P2	0	0	Total	1 in 1000	wasp100ul ELU	10:27 AM	8/2/2006

Time	Log	Average	Standard Deviation
0	7.12		
0	7.18		
0	7.2		
0	7.56	7.265	0.199583
1	7.2		
1	7.21		
1	7.34		
1	7.34	7.2725	0.078049

5	7.06		
5	7.27		
5	7.3		
5	7.26	7.2225	0.109659
10	7.16		
10	7.19		
10	7.2		
10	7.48	7.2575	0.149304
30	6.76		
30	6.9		
30	0		
30	0	6.83	0.098995
60	6.23		
60	6.24		
60	5.3		
60	5.3	5.7675	0.539838
120	0		
120	0		
120	0		
120	0	0	0
180	0		
180	0	0	0
240	0		
240	0	0	0
300	0		
300	0	0	0
360	0		
360	0	0	0