



### **C.diff. non-epidemic Spores**

The left plate is the control (no Sterilray treatment)

The right plate is 5 secs of Sterilray on high power  $\sim 100\text{mj}/\text{cm}^2$ .

The control has  $\sim 5\text{-}6$  logs of spores and the right plate  $\sim 2$  logs after treatment.

The spores are non-epidemic, 99.9% clean, bright phase (dormant, not germinated) spores in water.

A 4"x4" square area of the labtop was inoculated and the spore suspension was allowed to dry.

The area was touched with sterile gloves and then touched directly to pre-reduced C.diff agar plates and placed in the anaerobic chamber to incubate for 48 hours.



Photo is not for publication



Photos are not for publication



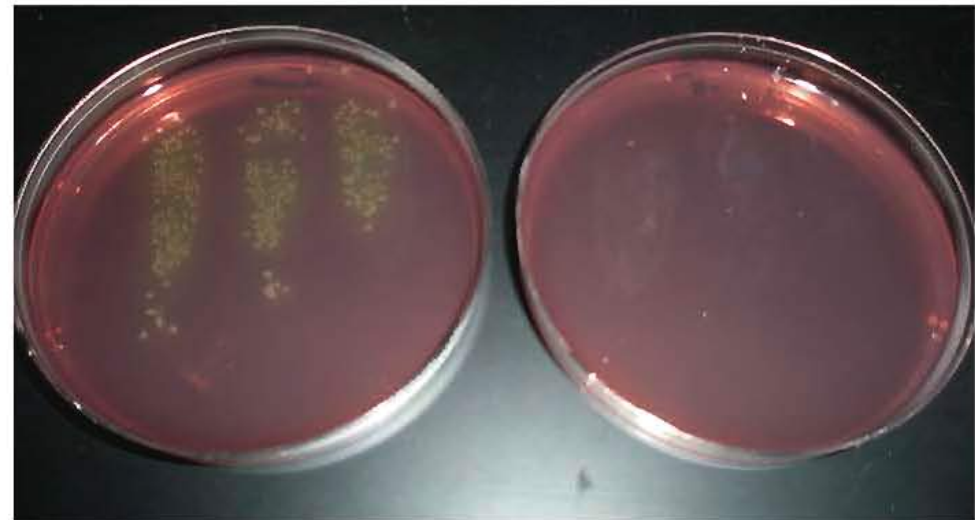
Left plate: control 5 logs of spores  
Right plate: Sterilray treated ~200 spores, 2.3 logs of spores



Left plate: control 4 logs of spores  
Right plate: Sterilray treated 60 spores, 1.8 logs of spores



Left plate: control 3 logs of spores  
Right plate: Sterilray 13 spores, 1.1 logs of spores



Left plate: control ~300 spores, 2.5 logs of spores  
Right plate: Sterilray 0 spores

# HEALTHY ENVIRONMENT INNOVATIONS

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## C.diff. NAP 1 epidemic Spores

The tops of each plate are the controls (no Sterilray treatment)

The bottoms of each plate is 5 secs of Sterilray on high power ~100mj/cm<sup>2</sup>.

The spores are NAP 1 epidemic, 99.9% clean, bright phase (dormant, not germinated) spores in water.

The spore suspension was allowed to dry on slides. Slides were then exposed to Sterilray.

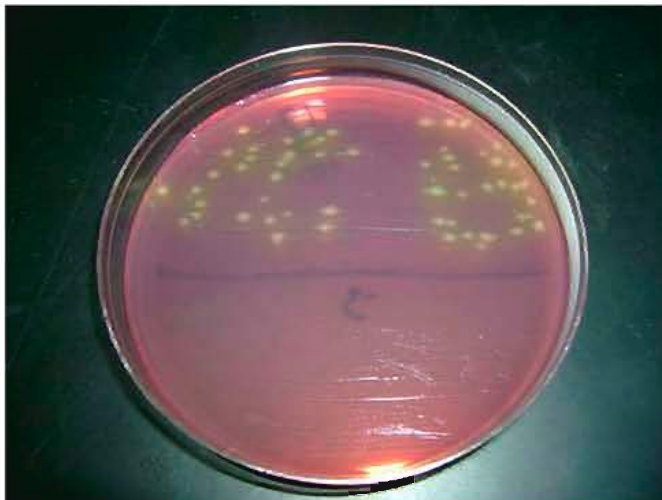
Cultured to pre-reduced C.diff agar plates and placed in the anaerobic chamber to incubate for 48 hours.



cdiff 17 -1.jpg



cdiff 17 -2.jpg



cdiff 17 -3.jpg



cdiff 17 -4.jpg

The samples (-1,-2,-3, -4) were serial diluted 1:10

Photos are not for publication

# HEALTHY ENVIRONMENT INNOVATIONS

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## Stool from a toxin positive patient treated for CDAD (c diff associated diarrhea)

The tops of each plate are the controls (no Sterilray treatment)

The bottoms of each plate is 5 secs of Sterilray on high power ~100mj/cm<sup>2</sup>.

The spore suspension was allowed to dry on slides. Slides were then exposed to Sterilray.  
Cultured to pre-reduced C.diff agar plates and placed in the anaerobic chamber to incubate for 48 hours.



stool -0



stool -1



stool -2



stool -3

The samples  
(0,-1,-2,-3)  
were serial  
diluted 1:10

Photos are not for publication

# HEALTHY ENVIRONMENT INNOVATIONS

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## C.diff. non-epidemic Spores

The tops of each plate are the controls (no Sterilray treatment)

The bottoms of each plate is 5 secs of Sterilray on high power ~100mj/cm<sup>2</sup>.

The spores are non-epidemic, 99.9% clean, bright phase (dormant, not germinated) spores in water.

The spore suspension was allowed to dry on slides. Slides were then exposed to Sterilray.

Cultured to pre-reduced C.diff agar plates and placed in the anaerobic chamber to incubate for 48 hours.



cdiff 11 -1.jpg



cdiff 11 -2.jpg



cdiff 11 -3.jpg



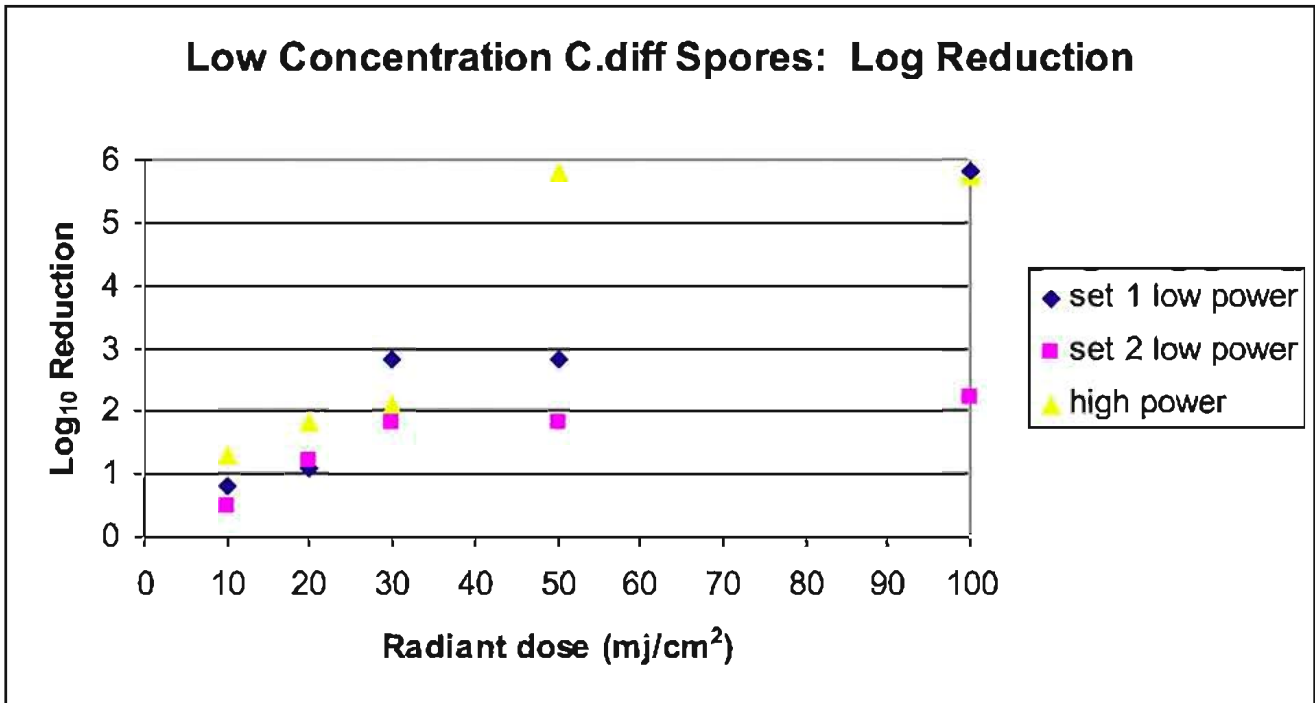
cdiff 11 -5.jpg

The samples (-1,-2,-3, -5) were serial diluted 1:10

Photos are not for publication



# Sterilray™ Disinfection Wand



100 mj/cm<sup>2</sup> can be achieved with less than 3 seconds exposure with a full power Sterilray lamp

Preliminary results of on going investigation of Sterilray at a VA Medical Center in Ohio. Photos and results are not for publication. For additional information please contact John Neister at Healthy Environment Innovations email:john.neister@he-innovations.com June 16, 2008





## Low Concentration C.diff Spores: Log Reduction

### Methods

Dormant, bright-phase, >99% clean NAP-1 *C. difficile* spores (17) diluted in sterile d.i. water. Plastic Petri dishes were prepared with 9 - 20 µl droplets. Petri dishes were prepared for control, 10, 20, 30, 50 and 100 radiant dose. Sterilray exposure was performed at low and high power and integrated to appropriate radiant dose per petri dish. For low doses of 10 and 20 a piece of cardboard was used to quickly expose the bacteria to Sterilray.

Droplets collected and serial diluted 1:10 from 10<sup>-1</sup> to 10<sup>-5</sup> in the anaerobic chamber. Dilutions were plated on *C.diff* plates and incubated 48 hours at 37°C for quantitative analysis

### Log<sub>10</sub> Reduction

radiant dose (mj/cm <sup>2</sup> )	set 1 low power	set 2 low power	high power
10	0.8	0.5	1.3
20	1.1	1.2	1.8
30	1.5	1.8	2.1
50	2.8	1.8	5.8
100	5.8	2.2	5.8

### Percentage of CFU/ml killed by radiant dose

radiant dose (mj/cm <sup>2</sup> )	set 1 low power	set 2 low power	high power
10	98.60	97.10	99.60
20	99.30	99.50	99.90
30	99.80	99.90	99.93
50	99.99	99.90	100.00
100	100.00	99.95	100.00

### CFU/ml counted from serial dilution plating method

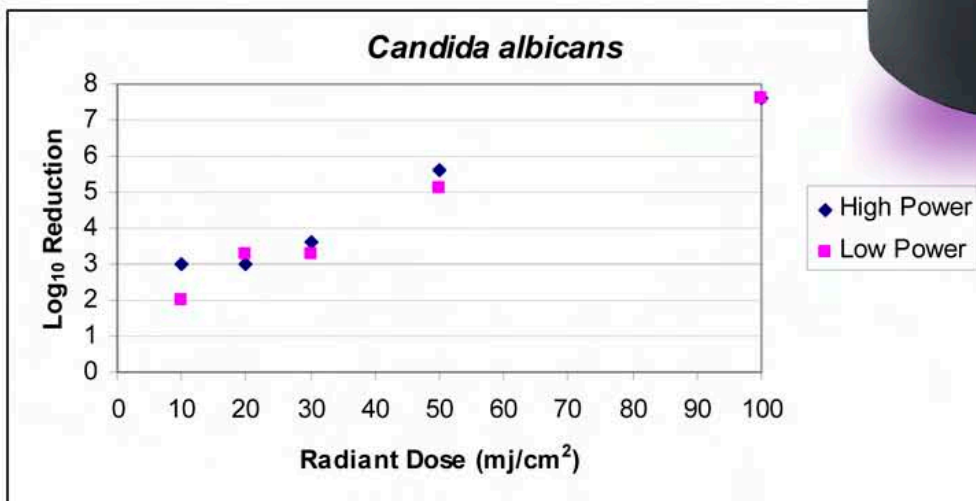
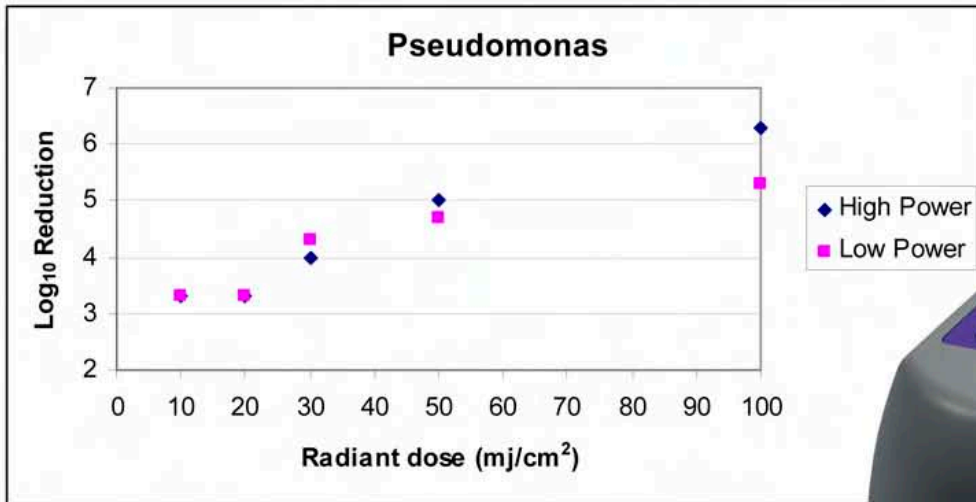
radiant dose (mj/cm <sup>2</sup> )	set 1 low power	set 2 low power	high power
Control (0)	7x10 <sup>5</sup>	7x10 <sup>5</sup>	7x10 <sup>5</sup>
10	1x10 <sup>5</sup>	2x10 <sup>5</sup>	3x10 <sup>4</sup>
20	5x10 <sup>4</sup>	4x10 <sup>4</sup>	1x10 <sup>4</sup>
30	2x10 <sup>4</sup>	1x10 <sup>4</sup>	5x10 <sup>3</sup>
50	1x10 <sup>3</sup>	1x10 <sup>4</sup>	0
100	0	4x10 <sup>3</sup>	0

### Actual radiant dose (mj/cm<sup>2</sup>)

radiant dose (mj/cm <sup>2</sup> )	set 1 low power	set 2 low power	high power
10	11.09	11.56	12.43
20	22.50	19.93	23.40
30	29.00	30.80	32.40
50	51.30	49.20	50.00
100	100.6	99.70	108.10

100 mj/cm<sup>2</sup> can be achieved with less than 3 seconds exposure with a full power Sterilray lamp

**Sterilray™**  
**Disinfection Wand**



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July 22, 2008





## **Sterilray™ Disinfection Wand**

### **Pseudomonas**

Radiant Dose (mj/cm <sup>2</sup> )	High Power	Low Power
Control 0	2x10 <sup>8</sup>	2x10 <sup>8</sup>
10	1x10 <sup>5</sup>	1x10 <sup>5</sup>
20	1x10 <sup>5</sup>	1x10 <sup>5</sup>
30	2x10 <sup>4</sup>	1x10 <sup>4</sup>
50	2x10 <sup>3</sup>	4x10 <sup>3</sup>
100	1x10 <sup>2</sup>	1x10 <sup>3</sup>

### **Candida albicans**

Radiant Dose (mj/cm <sup>2</sup> )	High Power	Low Power
Control 0	4x10 <sup>7</sup>	4x10 <sup>7</sup>
10	4x10 <sup>4</sup>	4x10 <sup>5</sup>
20	4x10 <sup>4</sup>	2x10 <sup>4</sup>
30	1x10 <sup>4</sup>	2x10 <sup>4</sup>
50	1x10 <sup>2</sup>	3x10 <sup>2</sup>
100	0	0

100 mj/cm<sup>2</sup> can be achieved with less than 3 seconds exposure with a full power Sterilray lamp

## **Methods**

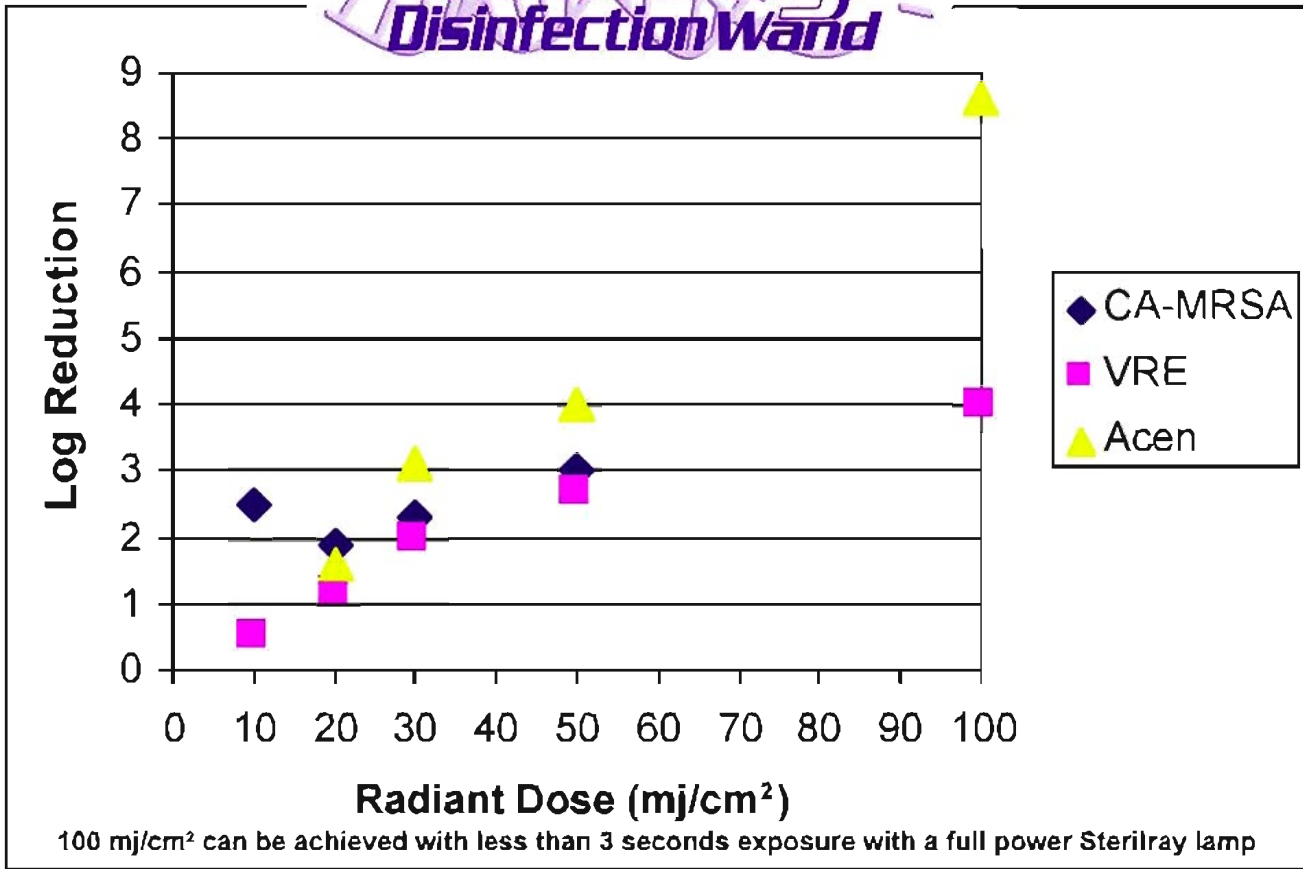
*Pseudomonas aeruginos* and *Candida albicans* were grown up over-night in appropriate nutrient media at 37°C. Cultures were spun to pellet and the supernatant was removed. Pellets were re-suspended in 1 ml sterile d.i. water. Again, the cultures were spun and the supernatant removed. The pellets were re-suspended in 10 ml of sterile d.i. water.

Plastic Petri dishes were prepared with nine 20 µl droplets of each suspension. Petri dishes were prepared for control, 10, 20, 30, 50 and 100 (mj/cm<sup>2</sup>) radiant dose. Sterilray exposure was performed at low and high power and integrated to appropriate radiant dose per Petri dish. For high power doses of 10 and 20 mj/cm<sup>2</sup> a piece of cardboard was used to quickly expose the bacteria to Sterilray.

Droplets were collected and serial diluted 1:10 from 10<sup>-1</sup> to 10<sup>-12</sup> in PBS. Dilutions were plated on TSA blood plates and incubated overnight at 37°C for quantitative analysis.



# Sterilray™ Disinfection Wand



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**Sterilray™**  
**Disinfection Wand**

Percentage of CFU/ml killed by radiant dose

Radiant Dose (mj/cm <sup>2</sup> )	CA-MRSA	VRE	A. baumannii
10	99.7	97	not done
20	98.5	99.3	99.8
30	99.5	99.9	99.9
50	99.9	99.9	99.9
100	not done	99.9	100

CFU/ml counted from serial dilution plating method

Radiant Dose (mj/cm <sup>2</sup> )	CA-MRSA	VRE	A. baumannii
control	6x10 <sup>8</sup>	1x10 <sup>7</sup>	4x10 <sup>8</sup>
10	2x10 <sup>6</sup>	3x10 <sup>6</sup>	not done
20	9x10 <sup>6</sup>	7x10 <sup>5</sup>	9x10 <sup>6</sup>
30	3x10 <sup>6</sup>	1x10 <sup>5</sup>	3x10 <sup>5</sup>
50	9x10 <sup>5</sup>	2x10 <sup>4</sup>	4x10 <sup>4</sup>
100	not done	1x10 <sup>3</sup>	0

Actual Radiant dose

Radiant Dose (mj/cm <sup>2</sup> )	CA-MRSA	VRE	A. baumannii
control	0	0	0
10	9.05	10.31	not done
20	15.41	16.66	19.95
30	28.8	33.8	33.9
50	48.9	49.8	50.8
100	not done	95.3	103.6

**100 mj/cm<sup>2</sup> can be achieved with less than 3 seconds exposure with a full power Sterilray lamp**

**Methods**

*MRSA*, *VRE* and *A. baum* were grown up over-night in appropriate nutrient media at 37°C. Cultures spun to pellet and supernatant removed. Pellet re-suspended in 1 ml sterile d.i. water. Again, the cultures were spun and the supernatant removed. Pellet re-suspend in 1.2ml of sterile d.i. water.

Plastic Petri dishes were prepared with 9 - 20 µl droplets. Petri dishes were prepared for control, 10, 20, 30, 50 and 100 radiant dose. Sterilray exposure was performed at high power and integrated to appropriate radiant dose per petri dish. For low doses of 10 and 20 a piece of cardboard was used to quickly expose the bacteria to Sterilray. Droplets collected and serial diluted 1:10 from 10<sup>-1</sup> to 10<sup>-12</sup>. Dilutions were plated on TSA blood plates and incubated overnight at 37°C for quantitative analysis.

# HEALTHY ENVIRONMENT INNOVATIONS

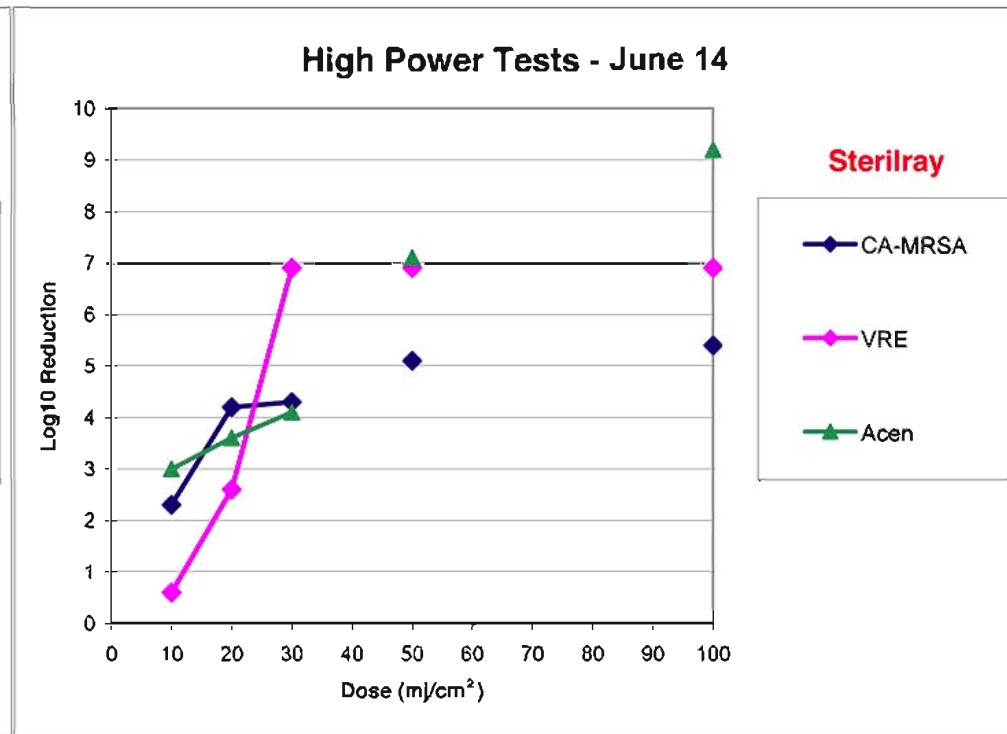
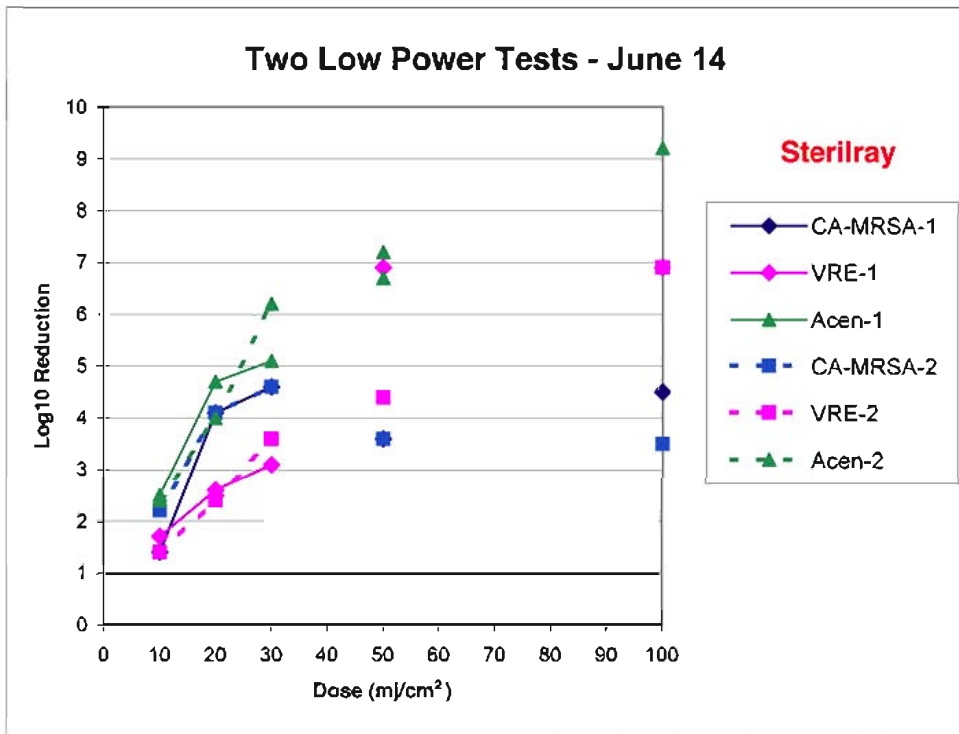
www.he-innovations.com



Preliminary results of on going investigation of Sterilray at a VA Medical Center in Ohio.  
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 For additional information please contact  
 John Neister at Healthy Environment Innovations  
 email:john.neister@he-innovations.com  
 June 14, 2008

Log Reduction		Low Power Tests				
dose	CA-MRSA-1	VRE-1	Acen-1	CA-MRSA-2	VRE-2	Acen-2
10	1.4	1.7	2.5	2.2	1.4	2.4
20	4.1	2.6	4.7	4.1	2.4	4
30	4.6	3.1	5.1	4.6	3.6	6.2
40						
50	3.6	6.9	7.2	3.6	4.4	6.7
60						
70						
80						
90						
100	4.5	6.9	9.2	3.5	6.9	9.2

Log Reduction		High Power Tests		
dose	CA-MRSA	VRE	Acen	
10	2.3	0.6	3	
20	4.2	2.6	3.6	
30	4.3	6.9	4.1	
40				
50	5.1	6.9	7.1	
60				
70				
80				
90				
100	5.4	6.9	9.2	



100 mj/cm<sup>2</sup> can be achieved with less than 3 seconds exposure with a full power Sterilray lamp

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 June 14,2006

### LOW POWER TEST O1

Percentage of CFU/ml killed by radiant dose

	CA-MRSA	VRE	Acen
10	99.6	99.8	99.99
20	99.99	99.99	99.99
30	99.99	99.99	99.99
50	99.99	100	99.99
100	99.99	100	100

Log reduction by radiant dose

	CA-MRSA	VRE	Acen
10	1.4	1.7	2.5
20	4.1	2.6	4.7
30	4.6	3.1	5.1
50	3.6	6.9	7.2
100	4.5	6.9	9.2

Actual radiant dose

	CA-MRSA	VRE	Acen
10	9.58	13.29	8.46
20	19.89	19.11	18.67
30	31.9	30.3	28.2
50	50.2	50.8	48.8
100	97.1	97.7	98.2

CFU/ml counted from serial dilution plating method

	CA-MRSA	VRE	Acen
105x10 <sup>6</sup>	1.6x10 <sup>5</sup>	5x10 <sup>6</sup>	
201x10 <sup>4</sup>	2x10 <sup>4</sup>	3x10 <sup>4</sup>	
303x10 <sup>3</sup>	6x10 <sup>3</sup>	1.2x10 <sup>4</sup>	
503x10 <sup>4</sup>	0	1x10 <sup>2</sup>	
1004x10 <sup>3</sup>	0	0	

### LOW POWER TEST O2

Percentage of CFU/ml killed by radiant dose

	CA-MRSA	VRE	Acen
10	99.94	99.6	99.96
20	99.99	99.99	99.99
30	99.99	99.99	99.99
50	99.99	99.99	99.99
100	99.99	100	100

Log Reduction by Radiant Dose

	CA-MRSA	VRE	Acen
10	2.2	1.4	2.4
20	4.1	2.4	4
30	4.6	3.6	6.2
50	3.6	4.4	6.7
100	3.5	6.9	9.2

Actual Radiant Dose

	CA-MRSA	VRE	Acen
10	11.35	9.8	10.37
20	21.04	21.79	19.6
30	30.2	32.1	29.9
50	48.7	50	50.1
100	100.3	100.9	99.6

CFU/ml counted from serial dilution plating method

	CA-MRSA	VRE	Acen
10	8x10 <sup>5</sup>	3x10 <sup>5</sup>	7x10 <sup>6</sup>
20	1.6x10 <sup>4</sup>	3x10 <sup>4</sup>	1.5x10 <sup>5</sup>
30	6x10 <sup>3</sup>	2x10 <sup>3</sup>	1.1x10 <sup>3</sup>
50	2x10 <sup>3</sup>	3x10 <sup>2</sup>	3x10 <sup>2</sup>
100	4x10 <sup>4</sup>	0	0

### HIGH POWER TEST O1

Percentage of CFU/ml killed by radiant dose

	CA-MRSA	VRE	Acen
10	99.95	75	99.99
20	99.99	99.98	99.99
30	99.99	100	99.99
50	99.99	100	99.99
100	99.99	100	100

Log Reduction by radiant dose

	CA-MRSA	VRE	Acen
10	2.3	0.6	3
20	4.2	2.6	3.6
30	4.3	6.9	4.1
50	5.1	6.9	7.1
100	5.4	6.9	9.2

Actual Radiant Dose

	CA-MRSA	VRE	Acen
10	10.37	7.12	11.4
20	18.68	21.57	21.37
30	30.6	25.3	26.9
50	51.1	50	51.5
100	97.4	97.3	108.6

CFU/ml counted from serial dilution plating method

	CA-MRSA	VRE	A. baum
control	1.2x10 <sup>8</sup>	8x10 <sup>6</sup>	1.5x10 <sup>9</sup>
10	7x10 <sup>5</sup>	2.1x10 <sup>6</sup>	1.8x10 <sup>6</sup>
20	8x10 <sup>3</sup>	2x10 <sup>4</sup>	4x10 <sup>5</sup>
30	6x10 <sup>3</sup>	0	1.2x10 <sup>5</sup>
50	1x10 <sup>3</sup>	0	1x10 <sup>2</sup>
100	5x10 <sup>2</sup>	0	0

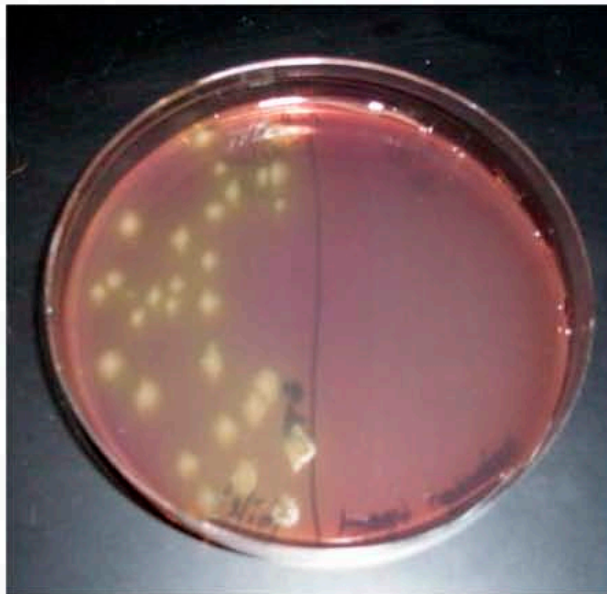
100 mj/cm<sup>2</sup> can be achieved with less than 3 seconds exposure with a full power Sterilray lamp



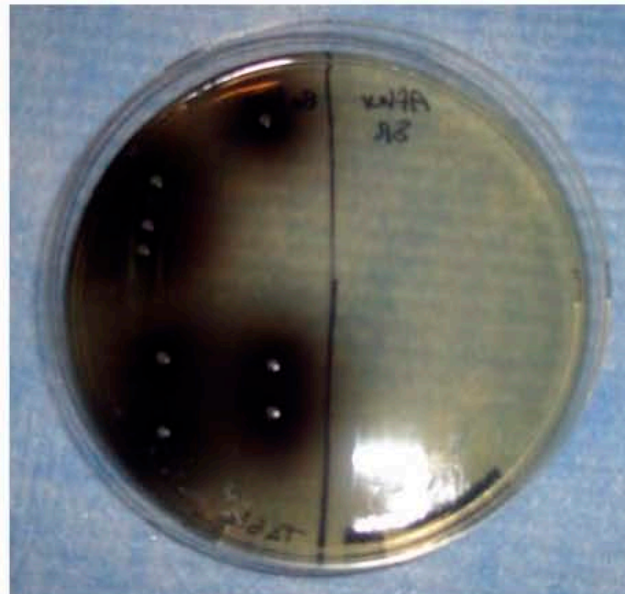
## Patient Rooms

Below are some photos of examples of cleaning patient rooms with Sterilray. So far, Sterilray reduced C.diff by 96% and 100% of VRE in patient rooms. We cultured small areas, half was swabbed as a before culture and half was swabbed after 5 secs of Sterilray treatment. For example, the call button (6"x3") was split in half and the right side was swabbed before treatment and the left side was swabbed after treatment. The drawback is that we assume a homogenous area of contamination. The probability of always having one side positive and one negative is nearly impossible, so the more rooms we do, the more significant the data becomes.

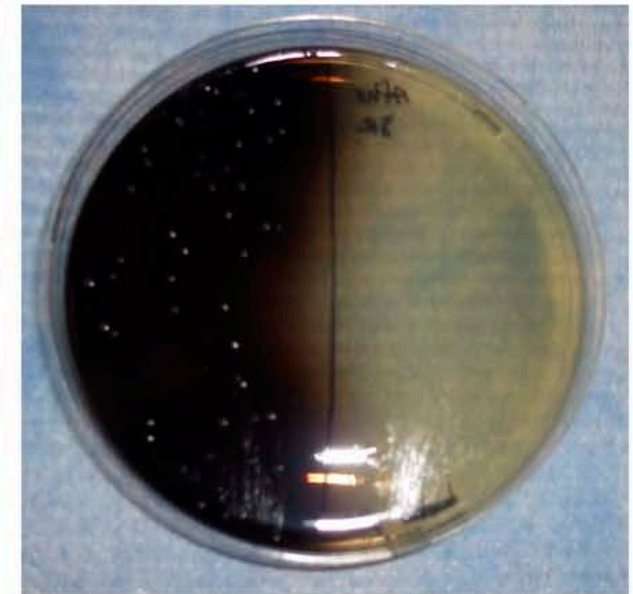
We have done 28 rooms for C.diff and 20 rooms for VRE.



**Sterilray Toilet 122-1 day 1  
Before and After Sterilray**



**Sterilray VRE table ex. 2 in room  
Before and After Sterilray**



**Sterilray VRE table in room  
Before and After Sterilray**

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June 23, 2008**

**Photos and results are not for publication.**