Assessment of the Anti-microbial Activity of Radiant Shield

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Objective

The purpose of this study was to demonstrate the efficacy of “Radiant Shield” in inactivating bacteria and viruses. Radiant Shield uses a TiO_2 containing spray to coat surfaces and a lamp that gives off a wavelength activating the TiO_2 resulting in loss of viability of the microorganism.

Methods

All organisms used in this study were obtained from the American Type Culture Collection – ATCC (Manassas, VA).

Overnight cultures of Methicillin resistant *Staphylococcus aureus* (MRSA) ATCC 700698, *Clostridium difficile* ATCC 43593, *Escherichia coli* ATCC 25922, Vancomycin resistant *Enterococcus* (VRE) ATCC 51559 were grown in Trypticase Soy Broth (TSB) (Difco, Sparks, MD). Bacteria were prepared by centrifugation and resuspension in sterile physiological saline. This bacterial suspension was used as an inoculum on tile surfaces to be tested. *C. difficile* was grown overnight in TSB in an anaerobic jar to achieve a suspension of spores which was confirmed with microscopy.

Mycobacterium fortuitum ATCC 6841 was grown in Middlebrook 7H9 broth (Difco, Sparks, MD) at 37°C in a shaking water bath, Tween 80 was added as a surfactant to allow the dispersal of clumps during growth and ensure a homogenous bacterial suspension. Bacteria were then prepared by centrifugation to pellet the bacteria and resuspended in sterile physiological saline, this procedure was repeated twice. This bacterial suspension was used as an inoculum for the surfaces being tested.

Assay of coliphage MS-2 ATCC 15597-B1 was accomplished using the double-layer agar technique. A colony of *Escherichia coli* ATCC 15597 was transferred to TSB and grown for 3 hours at 37ºC in a shaking water bath to serve as host for the virus. The coliphage was produced by collecting it from an infected lawn of *E. coli* by addition of 10-20 mL of physiological saline and then removing it with a pipette after 1-2 hours. The suspension was then centrifuged at low speed to remove bacterial debris.

Acrylic plastic (Altuglas International, Philadelphia, PA) and glazed almond colored ceramic tiles (4x4 inch) (Dal-Tile Corporation, Dallas Texas) were purchased at a local building supply store in Tucson, Arizona. Vinyl, product # PLCX4 (Spralding, Pelham, AL) (1x1 inch squares were used) was purchased at a local sewing supply store in Tucson, Arizona.

Test Procedure

Efficacy – The test surfaces were inoculated with 100 μL of the bacterial suspension applied in 10 micro liter droplets using a micropipetter. After allowing the bacterial suspension to air dry for 20 minutes, Radiant Shield was applied to the surface. The product was applied in a sweeping motion and allowed to dry before testing. The Radiant Shield light bulb was allowed to warm up for 30 minutes and tiles were placed approximately 11 ½ inches from bulb. Control tiles were not treated or exposed to the light. After various periods of time the surfaces were swabbed using cotton-tipped applicator swabs pre-moistened in DE neutralizing broth (Remel, Lenexa, KS). The swabs were then placed into one mL of DE broth and vortexed for 30 seconds to recover the organisms. Bacteria were assayed by the spread plate method onto appropriate media for the organism and incubated according to the organisms requirements. After incubation, bacteria colonies were counted and log reduction was calculated ([Initial concentration/Final concentration] x log = log reduction).

Residual – Surfaces were sprayed with Radiant Shield and allowed to stand at room temperature for one day. To determine the residual effects of Radiant Shield, surfaces that had been treated with Radiant Shield one day before were then inoculated with 100 μL of the bacterial suspension applied in 10 micro liter droplets using a micropipetter. The Radiant Shield light bulb was allowed to warm up for 30 minutes and tiles were placed approximately 11 ½ inches from bulb. Control tiles were not treated or exposed to the light. At the designated times, surfaces were swabbed using cotton-tipped applicator swabs pre-moistened in DE neutralizing broth (Remel, Lenexa, KS). The swabs were then placed into one mL of DE broth and vortexed for 30 seconds to recover the organisms. Bacteria were assayed by the spread plate method onto the appropriate media.

MRSA was also tested on vinyl and *M. fortuitum* was also tested on plastic, both according to the above procedure.

Results

The efficacy of Radiant Shield against MS-2 on ceramic tiles is shown in Table 1. An initial sample was collected as soon as the surfaces were inoculated. A reduction of 99.98% of MS-2 occurred immediately after exposure to Radiant Shield. After one minute exposure to the light source the reduction exceeded. 99.995%.

Table 1. Die-off of MS-2 with Radiant Shield on Ceramic Tile

	Exposure time in minutes							
	Before light exposure	**1**	**5**	**10**	**20**	**30**	**40**	**50**
Log_{10} Reduction	3.88	4.38	4.63	5.07	4.44	4.09	4.47	4.47
Percent Reduction	99.98	99.996	99.998	99.9992	99.996	99.991	99.997	99.997

Table 2 shows the results obtained with *M. fortuitum* on ceramic tiles. A 77.77% reduction of the organism was observed initially increasing to greater than 99.9986% after 10 minutes.

Table 2. Die-off of *M. fortuitum* with Radiant Shield on Ceramic Tile

	Exposure time in minutes				
	Before light exposure	**10**	**20**	**40**	**60**
Log_{10} Reduction	0.653	4.85	5.16	4.77	5.43
Percent Reduction	77.77	99.9986	99.9993	99.998	99.9996

Radiant Shield reduced *M. fortuitum* on plastic by 99.96% immediately after the initial application on plastic. After ten minutes of exposure to the light source, *M. fortuitum* was reduced by 99.9954%. No *M. fortuitum* could be detected after twenty minutes of contact (>99.9981% reduction)(Table 3).

Table 3. Die-off of *M. fortuitum* with Radiant Shield on Plastic

	Exposure time in minutes				
	Before light exposure	**10**	**20**	**40**	**60**
Log_{10} Reduction	3.43	4.34	>4.73	>4.97	>4.94
Percent Reduction	99.96	99.995	>99.998	>99.9989	>99.9988

MRSA was reduced by 99.967% immediately after initial application and by >99.997% after 30 minutes exposure to Radiant Shield (Table 4).

Table 4. Die-off of MRSA with Radiant Shield on Ceramic Tile

	Exposure time in minutes					
	Before light exposure	**10**	**20**	**30**	**40**	**60**
Log_{10} Reduction	3.48	3.87	3.53	>4.62	>4.25	>4.31
Percent Reduction	99.97	99.986	99.97	>99.997	>99.994	>99.995

Table 5 shows the results of testing against MRSA on a vinyl surface. A 99.1% reduction was observed increasing to 99.999% after 10 minutes.

Table 5. Die-off of MRSA with Radiant Shield on vinyl

	Exposure time in minutes				
	Before light exposure	**10**	**20**	**40**	**60**
Log_{10} Reduction	1.88	5.0	5.1	4.8	5.4
Percent Reduction	99.1	99.999	99.9992	99.998	99.9996

VRE was reduced by 99.9995% after 10 minutes exposure to the light source and >99.99962% after 20 minutes exposure to Radiant Shield (Table 6).

Table 6. Die-off of VRE with Radiant Shield on Ceramic Tile

	Exposure time in minutes			
	Before light exposure	**10**	**20**	**40**
Log_{10} Reduction	0.189	5.30	>5.43	>5.55
Percent Reduction	35.3	99.9995	>99.9996	>99.9997

The efficacy of Radiant Shield against *C. difficile* on ceramic tiles is shown in Table 7. After 20 minutes of exposure to Radiant Shield, *C. difficile* was reduced by greater than 99.92%.

Table 7. Die-off of *C. difficile* with Radiant Shield on Ceramic Tile

	Exposure time in minutes		
	Before light exposure	**10**	**20**
Log_{10} Reduction	1.47	2.11	>3.07
Percent Reduction	96.66	99.23	>99.92

Radiant Shield reduced *E. coli* on ceramic tiles by 99.90% immediately after the initial application. After ten minutes of exposure to the light source, *E. coli* was reduced by almost 99.99933% (Table 8).

Table 8. Die-off of *E.coli* with Radiant Shield on Ceramic Tile

	Exposure time in minutes			
	Before light exposure	**10**	**20**	**40**
Log_{10} Reduction	3.00	5.17	5.22	5.42
Percent Reduction	99.90	99.99933	99.9994	99.9996

After one day post application of Radiant Shield, *E. coli* was reduced by almost 99.85% after forty minutes (Table 9), demonstrating that the product was still active.

Table 9. Residual Action of Radiant Shield against *E. coli* on Ceramic Tile

	Exposure time in minutes			
	Before light exposure	**10**	**20**	**40**
Log_{10} Reduction	0.34	0.90	0.75	>2.81
Percent Reduction	54.3	87.5	81.9	>99.85

A summary of the efficacy of Radiant Shield against the tested bacteria and MS-2 after 10 minutes exposure on ceramic tiles is shown in Table 10. Overall, Radiant Shield resulted at least a 99.99% reduction for all bacteria and virus tested except *C. difficile* (99.2%) after 10 minutes exposure.

Table 10. Summary of the efficacy of Radiant Shield with Ceramic Tile

Bacteria	**% reduction before exposure to light source**	**% reduction after 10 minutes exposure to light source**
MS-2	99.98	99.9992
Mycobacterium fortuitum	77.77	99.9986
MRSA	99.967	99.986
C. difficile	96.66	99.2
E. coli	99.90	99.9993
VRE	35.29	>99.9995

Discussion

All of the organisms tested demonstrated a 99% or more reduction on the surfaces onto which the Radiant Shield had been applied before being exposed to the light source, except *C. difficile.* This demonstrated that Radiant Shield was also effective before exposure to the light. MS-2*, E. coli* and MRSA were the most rapidly inactivated, while *C. difficile* required a longer exposure, but still was inactivated by 99.9% after 20 minutes of exposure to the light source. Both mycobacterium and *C. difficile* are know to be more resistant to disinfectants that most other common pathogenic bacteria.

M. fortiutum was inactivated more rapidly on plastic than ceramic tiles and MRSA was inactivated more rapidly on vinyl than ceramic tiles. Ceramic tiles may be somewhat more porous than the other surfaces which may explain the slower inactivation rates on these surfaces. Still the organisms where inactivated by more than 99.99% on all the surfaces on which they were tested.