Laboratory Testing Report

Efficacy of ReSPR units with NCC technology at continuously reducing pathogens on surfaces in a controlled laboratory environment

PROPRIETARY & CONFIDENTIAL • FOR INTERNAL USE ONLY • NOT FOR DISTRIBUTION
© 2018 ReSPR Technology

Laboratory Testing Report

Sustained reduction of Microbial Burden on Surfaces through the Introduction of Photocatalytic Conversion technology

Executive Summary

A Healthcare Associated Infection (HAI) is an infection that is acquired in a hospital or other health care facility. In the United States, the Centers for Disease Control and Prevention estimated roughly 1.7 million hospital-associated infections, from all types of microorganisms, including bacteria and fungi combined, cause or contribute to 99,000 deaths each year (https://www.cdc.gov/hai/surveillance/index.html).
Surgical site infections (SSI) are caused by Microbial contamination and account for 21.8% of HAIs.. They threaten the lives of millions of patients each year and contribute to the spread of antibiotic resistance. If you have surgery, the chances of developing an SSI are about 2% to 5% (cosgrove 2015). In the United States, HAIs result in incremental avoidable costs of between $6.5B and $31.5B annually.

The contribution of surface contamination with pathogens to the development of HAI has increasingly been linked in recent research (1,).

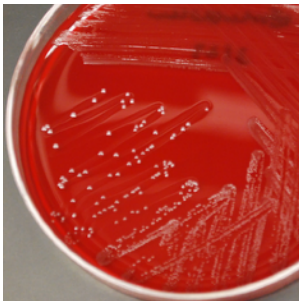
This study was conducted to determine whether the NCC® Technology could continuously reduce or eliminate microorganisms on surfaces in a controlled laboratory environment. Surface microorganisms are a major cause of Hospital Acquired Infections (HAI), including Surgical Site Infections (SSI), Central Catheter Related Blood Stream Infections (CLABSI), Catheter-associated Urinary Tract Infections (CAUTI) and Ventilator-associated Pneumonia.

Test Microorganism Information

The test microorganism(s) selected for this test:

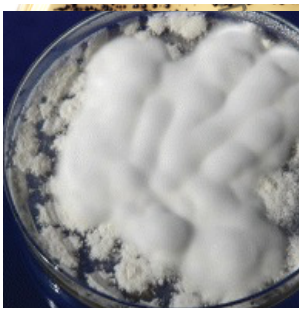
Staphylococcus aureus (MRSA) ATCC 33592

This bacteria is a Gram-positive, cocci shaped, aerobe which is resistant to the penicillin-derivative antibiotic methicillin. MRSA can cause troublesome infections, and their rapid reproduction and resistance to antibiotics makes them more difficult to treat. MRSA bacteria are resistant to drying and can therefore survive on surfaces and fabrics for an extended period of time and therefore makes this bacteria an excellent representative for antimicrobial efficacy testing on surfaces.



Enterococcus faecalis (VRE) ATCC 51299

This bacteria is a Gram-positive, spherical-shaped strain of Enterococcus faecalis that has developed resistance to the antibiotic vancomycin. E. faecalis (VRE) can cause a variety of local and systemic infections including endocarditis, bacteremia, and urinary tract infections, which are exceptionally difficult to treat because of this strain's acquired drug- resistance. Due to this bacterium's robust survival factors and resistance to commonly used antimicrobial agents, this bacterium is very challenging to disinfect.



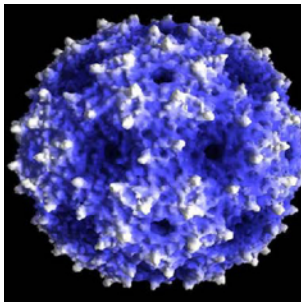
Trichophyton interdigitale ATCC 9533

Trichophyton interdigitale is a fungus that is part of a group known as dermatophytes. This fungus is known to cause a skin infection known as Dermatophytosis or Ringworm which appears on a person's skin as an inflamed circular pattern. This fungus produces spores which are difficult to eliminate via disinfection. Disinfection is especially important

for environments where Ringworm infections can occur and spread rapidly such as athletic facilities or schools.

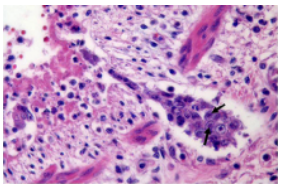
Aspergillus brasiliensis ATCC 9642

This fungi is a conidiophore, or a sexual spore generating aerobic fungus. A. brasiliensis, formerly listed as a strain of A. niger, is related to other Aspergillus species in that they produce spores which are highly resistant to chemical and environmental conditions. A. brasiliensis is commonly used as a benchmark fungus for antimicrobial fungicides and preservatives used in pharmaceutical and personal care products.



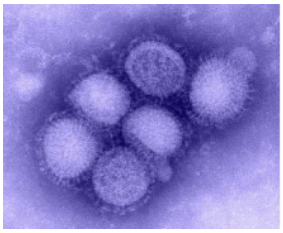
MS2 Bacteriophage (MS2), ATCC 15597-B1

This virus is a non-enveloped positive-stranded RNA virus of the bacteriophage family Leviviridae. Bacterial cells are the hosts for bacteriophages, and E. coli 15597 serves this purpose for MS2 bacteriophage. Its small size, icosohedral structure, and environmental resistance has made MS2 ideal for use as a surrogate virus (particularly in place of picornaviruses such as poliovirus and human norovirus) in water quality and disinfectant studies.
Permissive Host Cell System for MS2: *Escherichia coli*, 15597



Feline calicivirus (FCV), ATCC VR-782

This virus is a non-enveloped, positive-stranded RNA member of the genus Vesivirus, and a common cause of respiratory infections in cats. Symptoms of infection in felines include nasal discharge and mouth ulcers. As a member of the Caliciviridae viral family, FCV is closely related to human noroviruses, which cause acute gastroenteritis marked by nausea, vomiting, and diarrhea. Unlike human norovirus, however, a simple cell culture assay system is available for FCV. Therefore, feline calicivirus is the US EPA-approved surrogate microorganism for human norovirus label claims. Both FCV and human norovirus are able to remain viable on environmental surfaces for extended periods of time and are resistant to a number of disinfectant actives. **Permissive Host Cell Line Selected for FCV**: CRFK (Crandell-Rees Feline Kidney Cells), ATCC CCL-94



Influenza A (H1N1)

Influenza A virus is an enveloped, minus-stranded member of the family Orthomyxoviridae, and causative agent of the illness influenza (which is more widely recognized by the term 'flu'). Influenza is more serious than other seasonal mild, respiratory tract infections (e.g. the common cold) with symptoms that can last for upwards of several weeks. Young children and the elderly are particularly susceptible to severe illness and death due to infection. Influenza is readily transmitted via infective aerosols direct contact with infective respiratory secretions. Potential transmission by contaminated environmental surfaces (fomites) has increasingly become of interest, and Influenza virus is highly vulnerable to inactivation by drying and exposure to variety of disinfectant actives.

Permissive Host Cell Line Selected for Influenza A (H1N1): MDCK (Madin Darby Canine Kidney Cells), ATCC CCL-34

The results show material reduction of bacteria, fungi and viruses after the installation of the ReSPR units with NCC® Technology; significantly reducing the risk of infection from pathogens to those exposed in the hospital.

Individual results on surfaces within the test room showed significant reductions ranging from 91% to over 99.998%. The continuous disinfection from low levels of Hydrogen Peroxide were found to exert a significant reduction of the microbial burden compared to the control microorganisms non-exposed to the ReSPR NCC technology.

Materials and Methods

Summary of the Procedure

- The test microorganism is prepared, usually by growth in liquid culture medium or on an appropriate agar plate.
- The test culture may be supplemented with an artificial soil load, such as horse or fetal bovine serum, for one-step cleaner/sanitizer claims.
- Sterilized carriers are inoculated with a volume of the test culture. Inoculated slides are dried. Only completely dried carriers are used in the test.
- Test carriers are treated with the test device and incubated for the predetermined contact time.
- Control carriers are harvested at appropriate intervals to accurately represent any reduction during the contact time.
- At the conclusion of the contact time, test and control carriers are chemically neutralized.
- Dilutions of the neutralized test substance are evaluated using appropriate growth media to determine the surviving microorganisms at the respective contact time.
- The effect of the test substance is compared to the effect of the control substance in order to determine microbial reductions.

Criteria for Scientific Defensibility of an ASTM E1153 Study

For Microchem Laboratory to consider an ASTM E1153 study to be scientifically defensible, the following criteria must be met:

1. Ordinary consistency between replicates must be observed for the control carriers.
2. Positive/Growth controls must demonstrate growth of appropriate test microorganism.
3. Negative/Purity controls must demonstrate no growth of test microorganism

NCC® Technology utilizes a revolutionary and doped hydrophilic photo catalytic coating, consisting of titanium dioxide with a proprietary combination of additional transition elements to enhance efficacy. Activated by multiple specific wavelengths of ultraviolet light, oxygen and humidity are extracted from the air to create a plasma of powerful hydroxyl oxidizers that targets air and surface pathogens. No ozone is produced. These oxidizers are extremely effective at destroying bacteria, viruses, fungi, volatile organic compounds (VOCs) and other environmental contaminants.
Most significantly, they are not harmful to humans, pets and plants, and are completely safe for indoor use in occupied spaces.

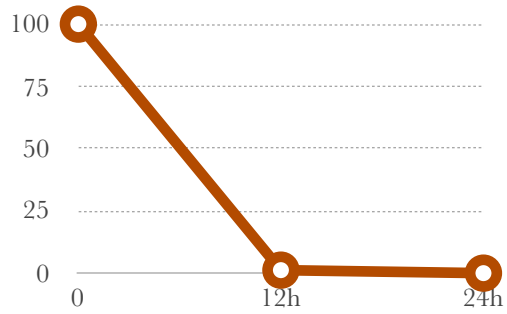
Results

The difference between the control samples and the samples exposed to the ReSPR technology shows a significant reduction of pathogens on surfaces independently of the pathogen tested.

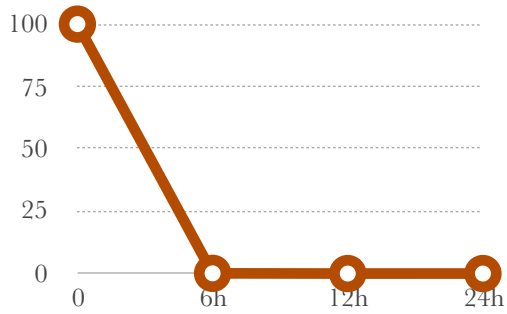
The following results show the percentages of reduction of microorganisms on surfaces, compared to a control which has not been exposed to the ReSPR technology.

Methicillin Resistant Staphylococcus aureus(MRSA)

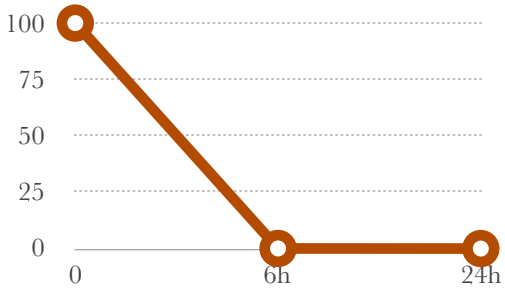
Enterococcus faecalis (VRE)



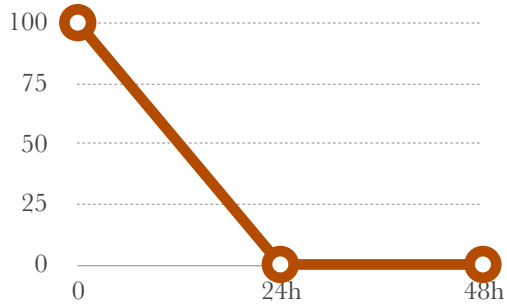
Pseudomonas aeruginosa



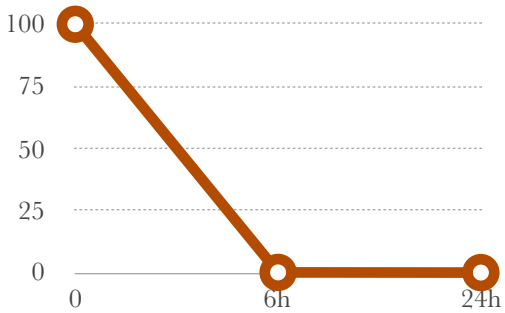
Feline Calicivirus



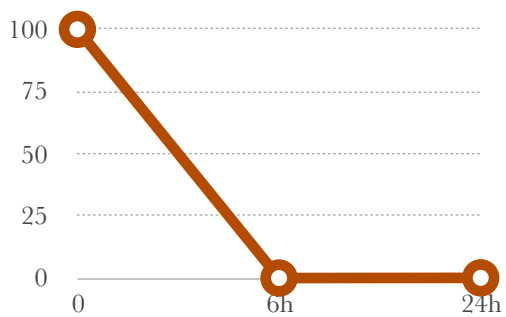
Trichophyton interdigitale



Influenza A (H1N1)

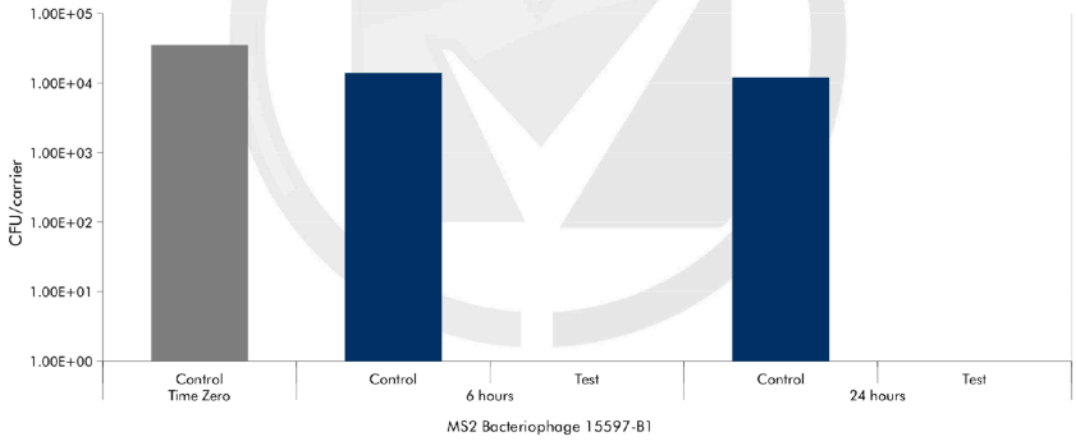


MS2 Bacteriophage (MS2)

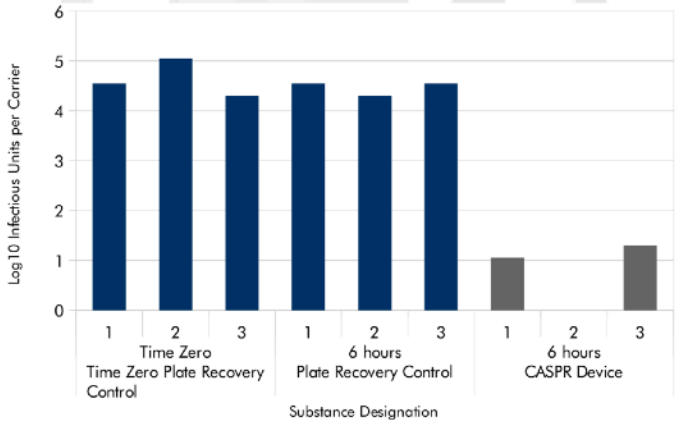


Test Microorganism	Contact Time	Run Type	CFU/carrier	Percent Reduction vs. Parallel Control	Log_{10} Reduction vs. Parallel Control
MS2 Bacteriophage ATCC 15597-B1	Time Zero	Control	3.50E+04	N/A	
	6 hours	Control	1.40E+04	N/A	
		Test	<1.00E+00	>99.993%	>4.15
	24 hours	Control	1.19E+04	N/A	
		Test	<1.00E+01	>99.92%	>3.08

Note: the limit of detection for the 6-hour contact time was 1.00E+00 CFU/carrier and for the 24-hour contact time was 1.00E+01 CFU/carrier. Values observed below the limit of detection are listed as <1.00E+00 or <1.00E+01 in the table and as zero in the graph.



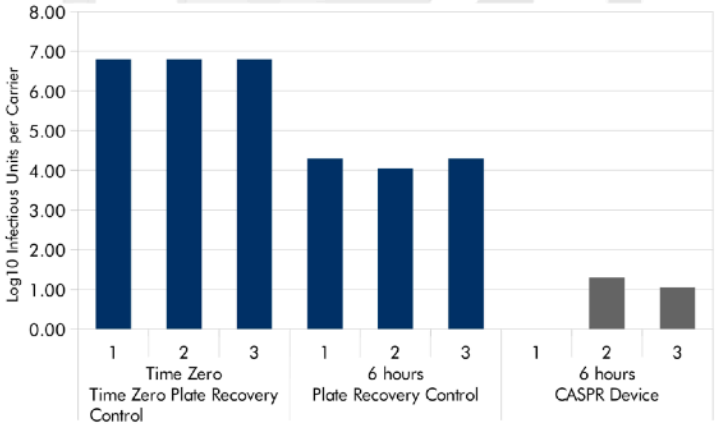
Test Microorganism	Substance Designation	Contact Time	Replicate	Log_{10} Infectious Units Per Carrier	Average Log_{10} Infectious Units Per Carrier	Average Log_{10} Reduction Infectious Units Per Carrier Compared to Plate Recovery Control	Average Percent Reduction Infectious Units Per Carrier Compared to Plate Recovery Control
Feline Calicivirus (EPA-approved human norovirus surrogate), ATCC VR-782	Time Zero Plate Recovery Control	Time Zero	1	4.55	4.63	N/A	
			2	5.05			
			3	4.30			
	Plate Recovery Control	6 hours	1	4.55	4.47		
			2	4.30			
			3	4.55			
	CASPR Device	6 hours	1	1.05	≤1.05	≥3.42	≥99.96%
			2	≤0.80			
			3	1.30			



Note: The limit of detection for this assay was 0.80 log_{10} $TCID_{50}$ per carrier. Observations below this limit are presented as ≤0.80 in the table and zero in the graph above.

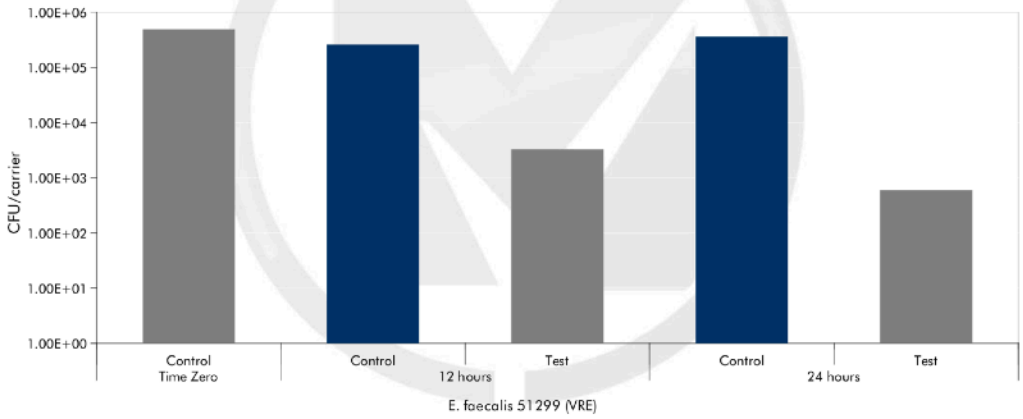
Results for Influenza A (H1N1), ATCC VR-1469:

Test Microorganism	Substance Designation	Contact Time	Replicate	Log_{10} Infectious Units Per Carrier	Average Log_{10} Infectious Units Per Carrier	Average Log_{10} Reduction Infectious Units Per Carrier Compared to Plate Recovery Control	Average Percent Reduction Infectious Units Per Carrier Compared to Plate Recovery Control
Influenza A (H1N1), ATCC VR-1469	Time Zero Plate Recovery Control	Time Zero	1	6.80	6.80	N/A	
			2	6.80			
			3	6.80			
	Plate Recovery Control	6 hours	1	4.30	4.22		
			2	4.05			
			3	4.30			
	CASPR Device	6 hours	1	≤0.80	≤1.05	≥3.17	≥99.93%
			2	1.30			
			3	1.05			



Results of the Study *E. faecalis* ATCC 51299 (VRE)

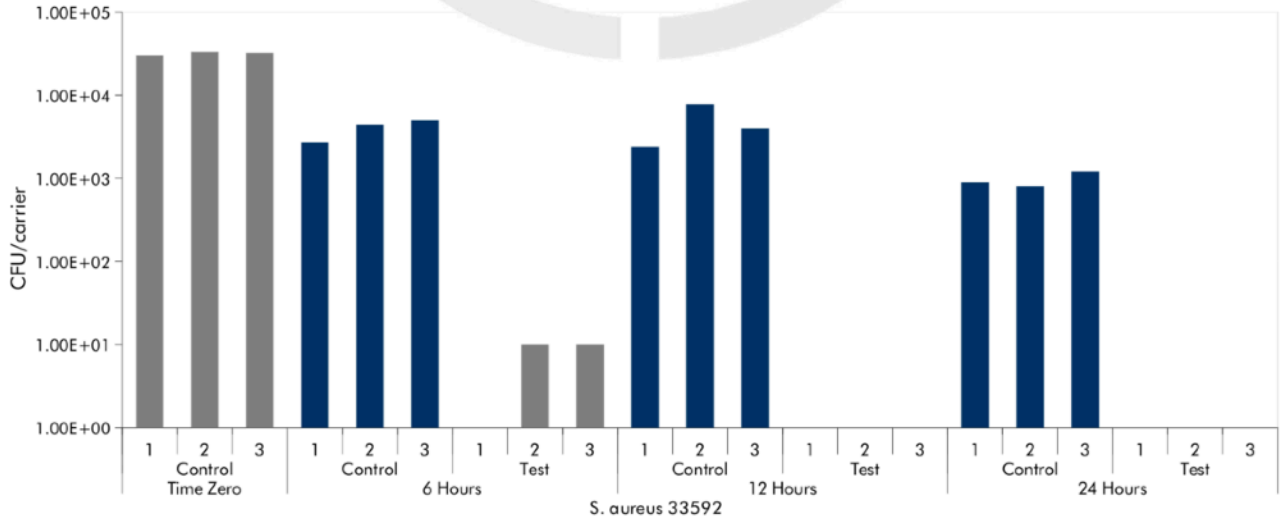
Test Microorganism	Contact Time	Run Type	CFU/carrier	Percent Reduction vs. Parallel Control	Log_{10} Reduction vs. Parallel Control
E. faecalis ATCC 51299 (VRE)	Time Zero	Control	4.90E+05	N/A	
	12 hours	Control	2.60E+05	N/A	
		Test	3.30E+03	98.73%	1.90
	24 hours	Control	3.60E+05	N/A	
		Test	6.00E+02	99.83%	2.78



Results of the Study *S. aureus* ATCC 33592 (MRSA)

Test Microorganism	Contact Time	Run Type	Replicate	CFU/carrier	Average CFU/carrier	Average Percent Reduction vs. Parallel Control	Average Log_{10} Reduction vs. Parallel Control
S. aureus (MRSA) ATCC 33592	Time Zero	Control	1	3.00E+04	3.17E+04	N/A	
			2	3.30E+04			
			3	3.20E+04			
	6 Hours	Control	1	2.70E+03	4.03E+03	N/A	
			2	4.40E+03			
			3	5.00E+03			
		Test	1	<1.00E+01	<1.00E+01	>99.75%	>2.61
			2	1.00E+01			
			3	1.00E+01			
	12 Hours	Control	1	2.40E+03	4.73E+03	N/A	
			2	7.80E+03			
			3	4.00E+03			
		Test	1	<1.00E+00	<1.00E+00	>99.98	>3.68
			2	<1.00E+00			
			3	<1.00E+00			
	24 Hours	Control	1	9.00E+02	9.67E+02	N/A	
			2	8.00E+02			
			3	1.20E+03			
		Test	1	<1.00E+00	<1.00E+00	>99.90%	>2.99
			2	<1.00E+00			
			3	<1.00E+00			

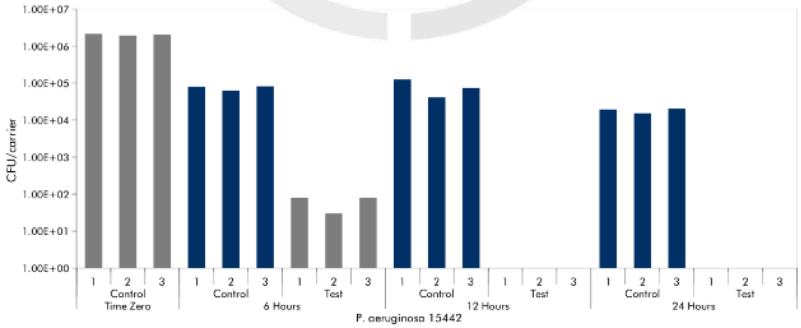
Note: the limits of detection for the 6-hour contact time was 1.00E+01 CFU/carrier and for the 12 and 24-hour contact times was 1.00E+00 CFU/carrier. Values observed below the limit of detection are listed as <1.00E+01 and <1.00E+00 in the table and as zero in the graph.



Results of the Study *P. aeruginosa* ATCC 15442

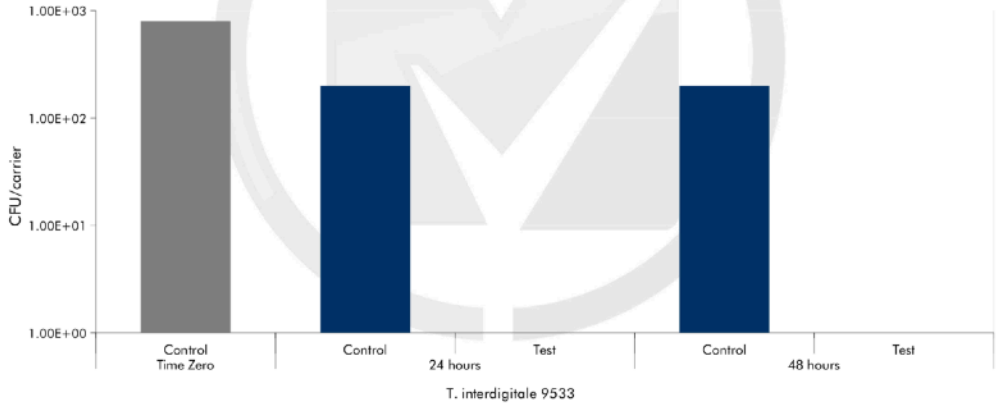
Test Microorganism	Contact Time	Run Type	Replicate	CFU/carrier	Average CFU/carrier	Average Percent Reduction vs. Parallel Control	Average Log_{10} Reduction vs. Parallel Control
P. aeruginosa ATCC 15442	Time Zero	Control	1	2.15E+06	2.04E+06	N/A	
			2	1.93E+06			
			3	2.03E+06			
	6 Hours	Control	1	7.90E+04	7.40E+04	N/A	
			2	6.20E+04			
			3	8.10E+04			
		Test	1	8.00E+01	6.33E+01	99.91%	3.07
			2	3.00E+01			
			3	8.00E+01			
	12 Hours	Control	1	1.25E+05	8.00E+04	N/A	
			2	4.10E+04			
			3	7.40E+04			
		Test	1	<1.00E+00	<1.00E+00	>99.998%	>4.90
			2	1.00E+00			
			3	<1.00E+00			
	24 Hours	Control	1	1.93E+04	1.82E+04	N/A	
			2	1.51E+04			
			3	2.03E+04			
		Test	1	<1.00E+00	<1.00E+00	>99.995%	>4.26
			2	<1.00E+00			
			3	<1.00E+00			

Note: the limit of detection for this study was 1.00E+00 CFU/carrier. Values observed below the limit of detection are listed as <1.00E+00 in the table and as zero in the graph



Test Microorganism	Contact Time	Run Type	CFU/carrier	Percent Reduction vs. Parallel Control	Log_{10} Reduction vs. Parallel Control
T. interdigitale ATCC 9533	Time Zero	Control	8.00E+02	N/A	
	24 hours	Control	2.00E+02	N/A	
		Test	<1.00E+01	>95.00%	>1.30
	48 hours	Control	2.00E+02	N/A	
		Test	<1.00E+01	>95.00%	>1.30

Note: the limit of detection for this study was 1.00E+01 CFU/carrier. Values observed below the limit of detection are listed at <1.00E+01 in the table and as zero in the graph.



Conclusion

Testing results indicate that the ReSPR units with NCC® Technology materially reduces or eliminates microorganisms on surfaces in a controlled environment. Results showed Bacteria was reduced over 99.998%, Fungi was reduced over 95%, Methicillin Resistant Staphylococcus (MRSA) was reduced over 99.98%, Influenza A (H1N1) was reduced over 99.93% and MS2 (virus) was reduced over 99.993%, on surfaces during the test period.

The ReSPR NCC® Technology was very effective in eliminating hospital indigenous pathogens (including MRSA) upon contact on all surfaces.

Full laboratory reports available on request.