

# **University of Arizona**

*Department of Soil, Water and Environmental Science*

## **Hard-Surface Antimicrobial Barrier Tests**

### **MRSA Bacteria and MS-2 Virus (Norovirus Surrogate)**

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#### **1.0 Introduction and Background**

**UNELKO Corporation**, a Scottsdale, Arizona manufacturer of water repellents, surface treatments and protective coatings, asked the Department of Water, Soil & Environmental Science to measure both the initial antimicrobial efficacy and the residual antimicrobial benefits of their newly-patented **SANI-SHIELD®** 3-in-1 Surface Care. **SANI-SHIELD®** is claimed to clean, shield and protect surfaces in 1-Step with an antimicrobial barrier coating. (SANI-SHIELD is also marketed as Clean & Shield® for home use.)

Hard-surface sanitizers and disinfectants are proven efficacious when tested in the laboratory under carefully controlled conditions and employing "immersion-type" AOAC protocols using glass, stainless steel or plastic vessels with contact times that are dependent on specific organisms and the chemical compositions being tested. Sanitizers and disinfectants are judged effective when they provide at least a 3 to 6 log<sub>10</sub> reduction in the count/concentration of the test organisms.

Although immersion-type protocols can demonstrate the efficacy of a chemical composition, they cannot demonstrate the efficacy of the product as it is typically used on hard surfaces. Immersion protocols do not take into account the effect of a product's directions for use; including any required pre-cleaning or the identification of the organisms that are present in order to determine specific misting or flooding contact time required to deactivate them. Also, immersion protocols cannot measure any form of residual disinfectant activity. Assessment of both initial and extended hard surface antimicrobial activity is desirable since surfaces can become re-contaminated shortly after disinfection.

Accordingly, in conjunction with **UNELKO**, the Department of Soil, Water and Environmental Science developed a laboratory testing Protocol that replicates the "real world" use of hard-surface of cleaners, sanitizers and disinfectants and which measures both their initial efficacy and any residual antimicrobial benefits. As used herein, antimicrobial means a substance, surface condition or barrier (when formed on a surface) that inhibits the activity and growth of microbes such as bacteria, fungi, viruses or parasites and their transfer to uncontaminated animate and inanimate surfaces.

#### **2.0 Objective**

The goal of these tests was to measure the initial antimicrobial efficacy of SANI-SHIELD and determine its residual antimicrobial efficacy on everyday surfaces in keeping with the 1-Step use and performance claims made for the product.

The SANI-SHIELD "spray & wipe" product claims to "clean organic dirt and grime with the antiseptic power of hydrogen peroxide and simultaneously protect surfaces with an invisible water, soil & stain repellent barrier on which bacteria, mold & mildew will not grow and which reduces the adhesion and buildup of inorganic soil for easier next-time cleaning."

(The Clean & Shield “spray & wipe” product claims to “easily clean dirt, grease & everyday household spills & splatters and simultaneously provide a long-lasting invisible barrier on which bacteria, mold & mildew will not grow.”)

These product performance claims are distinguished from conventional sanitizers and disinfectants that require pre-cleaning of the surface, a level of misting or flooding of the surface for extended periods and their failure to provide instruction regarding some form of post-treatment in order to leave surfaces free of chemical residues.

### 3.0 Approach

The Protocol consists of two essential parts. In Part 1, the efficacy of the product was evaluated in terms of its ability to clean and remove/kill organisms on hard surfaces when such contaminated surfaces are “sprayed & wiped” with the product. As these are the most common techniques used when cleaning and/or decontaminating surfaces, any pre-cleaning steps, extended contact-times with the chemical compositions or post-treatment of the surfaces were specifically excluded.

In Part 2, the now cleaned and treated surfaces were stored (uncovered) at ambient conditions and were re-inoculated with test organisms at twenty-four (24) hour intervals (i.e. at 24, 48, 72, 96, 120, 144 & 168 hours) after the initial treatment. The inoculums were allowed to “dwell” from 15 minutes up to 6 hours before the surfaces were swab-tested for microbial activity.

### 4.0 Surfaces

The majority of hard surfaces in homes, public buildings and facilities are glass, porcelain, ceramics, stainless steel, chrome, acrylics, polycarbonates, laminated plastics, polished or lacquered wood, marble and granite. Since UNELKO Corporation, and through tests by various independent laboratories, has previously confirmed that the SANI-SHIELD barrier coating was substantive to this range of hard surfaces, for this study only 3”x 3” (9 sq. inches) glazed ceramic tiles were employed and tested.

The surfaces were sterilized (autoclaved) before being inoculated in Part 1.

### 5.0 Organisms

The focus of this study was on Methicillin Resistant Staphylococcus Aureus (MRSA) and on Norovirus; using the pathogenic MS-2 Bacteriophage virus as a surrogate for Norovirus. The MS-2 coliphage was selected because it has been extensively used as a model for human viruses when testing disinfectants.

At least 1 million colony forming units (CFU) or plaque forming units (PFU) constituted the minimum inoculums tested.

### 6.0 Soil Loads

The organisms were suitably cultured and contained appropriate laboratory soil loads to maintain organism viability in keeping with standard AOAC standards for disinfectant testing.

Cultures were prepared using Trypticase Soy Agar with 5% Sheep Blood (BAP). Stock cultures were incubated at 35°C for 48 hours to obtain heavy growth for preparation of the inoculums. Tubes of sterile Butterfield’s buffer solution were inoculated with each type of organism to obtain a turbid culture. Aerobic

plate counts were performed on each tube of organism to determine the number of plaque forming units (PFU) or colony-forming units (CFU)/ml. The inoculums contained 5% fetal bovine serum as the soil load.

## 7.0 Controls

Part 1 employed two (2) controls to determine the comparative efficacy of the antimicrobial substance(s) being tested. One control was the standard “untreated” surface. The other control used distilled water to wash the surface using the same spray & wipe directions as for the SANI-SHIELD product being tested.

Part 2, in this study, employed only the “untreated” surface as a single control.

## 8.0 Application of Antimicrobial Products and Technologies

The SANI-SHIELD product was applied in accordance with the manufacturer’s use directions as stated on it’s and packaging.

The “directions” on the labels for SANI-SHIELD are: “To Clean, Shield & Protect Surfaces in 1-Step...Spray surface with SANI-SHIELD and *wipe up* with cloth, paper or MICROFIBER towel until surface is dry & polished. (For heavier soil...spray surface to saturate soil and *massage* with sponge or MICROFIBER cloth. Wipe up with dry cloth until polished. For electronic equipment and telephones, spray on cloth and wipe surface until clean and dry.)

## 9.0 Applicators – Wipes, Sponges & Scrubbers

Sterile cotton wipes were used to wash surfaces with distilled water and to wipe the sprayed SANI-SHIELD product until dry.

## 10.0 Tests of Surfaces and Applicators

Since the reduction of contamination and cross-contamination by microbes is one of the prime goals of hard surface sanitizers, disinfectants and other antimicrobial technologies, both the surfaces and the sterile cotton wipes used to wipe the surfaces were tested for microbial presence and product efficacy.

The test of the controls and the washed or “treated” antimicrobial surfaces was by swabbing techniques. Analysis of the swabs and wipes shall followed AOAC methods.

In Part 1 tests, the controls were washed and the antimicrobial product was applied no sooner than fifteen (15) minutes after inoculation with the test organism. After washing with distilled water or application of the antimicrobial product, the surfaces were swab-tested. The wipes and the swabs were cultured and tested for microbial contamination.

Part 2 tests for residual antimicrobial efficacy of a treated surface were performed in increments of 24 hours after the antimicrobial product was used on the test surface. After treatment, the test surfaces were stored uncovered at ambient room temperatures and conditions. At the selected 24-hour interval(s), the surfaces were contaminated with the test organism and the antimicrobial activity was assessed by swab-testing the surface 1 to 6 hours after inoculation.

Both the wipes used to wash the inoculated surfaces with distilled water and those used to clean and shield the inoculated surfaces were analyzed for residual, viable organisms. For the swab tests, a sterile cotton swab, pre-moistened in DE neutralizing broth (Remel, Lenexa, KS) was used to swab the surface of each tile after cleaning of the inoculums with the different products, i.e., distilled water and Sani-Shield. The swabs were placed into 1 mL of DE broth and vortexed for 30 seconds to release virus. The swabs were analyzed for viable bacteria using an aerobic plate count procedure.

Assay of bacterial virus MS-2 (ATCC 15597-B1 bacteriophage) was accomplished using the double-layer agar technique (Pepper and Gerba, 2005). Sample plates were incubated at 37°C for 24 hours. Plaque forming units (PFU) were counted and the log reduction calculated.

**11.0 Antimicrobial Test Results and Charts**

MRSA Study: - Table 1. Sterilized surfaces were inoculated with MRSA. After 15 minutes, they were cleaned/treated with DI water or **SANI-SHIELD** 3-in-1 Surface Care and tested.

	Test of Treatment Wipes	15 Minutes After Treatment	1 Hour After Treatment	4 Hours After Treatment
<b>SANI-SHIELD</b>	>1			
DI Water	4.80E+03			
		Swab Test of Surface	Swab Test of Surface	Swab Test of Surface
Control		3.60E+04	1.40E+04	2.50E+04
<b>SANI-SHIELD</b>		>1	>1	>1
DI Water		260	180	230

The data in Table 1 showed an instant kill of MRSA by **SANI-SHIELD** since there was hardly any MRSA on the treatment wipes or on the surface after treatment.

MRSA Study: Table 2. **SANI-SHIELD** antimicrobial coatings were applied to surfaces and the treated surfaces were stored under ambient conditions. After storage for 7 days, they were inoculated with MRSA and tested at the intervals shown.

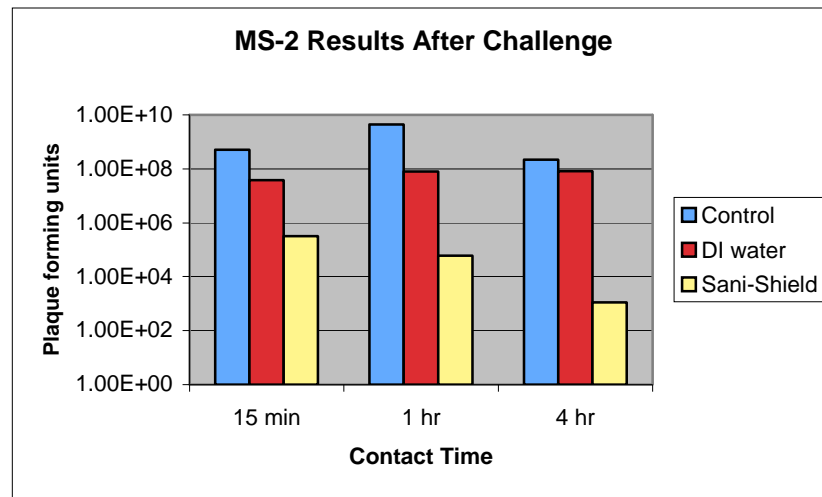
	7 Days After Treatment			
	MRSA Inoculate Deposited	15 Minutes After Inoculation	1 Hour After Inoculation	4 Hours After Inoculation
Control		3.60E+04	1.40E+04	2.50E+04
<b>SANI-SHIELD</b>		29	32	>1

MS-2 Virus Study: - Table 1. Sterilized surfaces were inoculated with MS-2 virus, then cleaned/treated with DI water or SANI-SHIELD 3-in-1 Surface Care (with antimicrobial barrier) and tested.

	Test of Treatment Wipe	Surface Test 15 Min After Treatment	Surface Test 1 Hour After Treatment	Log Reduction	Percent Reduction	Surface Test 1 Hour After Treatment	Log Reduction	Percent Reduction
<b>SANI-SHIELD</b>	180 Million							
DI water	650 Million							
Control		510 Million	4.4 Billion	-0.9	N/a	220 Million	0.37	56.86
<b>SANI-SHIELD</b>		<b>320,000</b>	<b>62,000</b>	<b>0.71*</b>	<b>80.63*</b>	<b>1,100</b>	<b>2.5</b>	<b>99.66</b>
DI water		38 Million	81 Million	-0.3	N/a	83 Million	-0.3	N/a

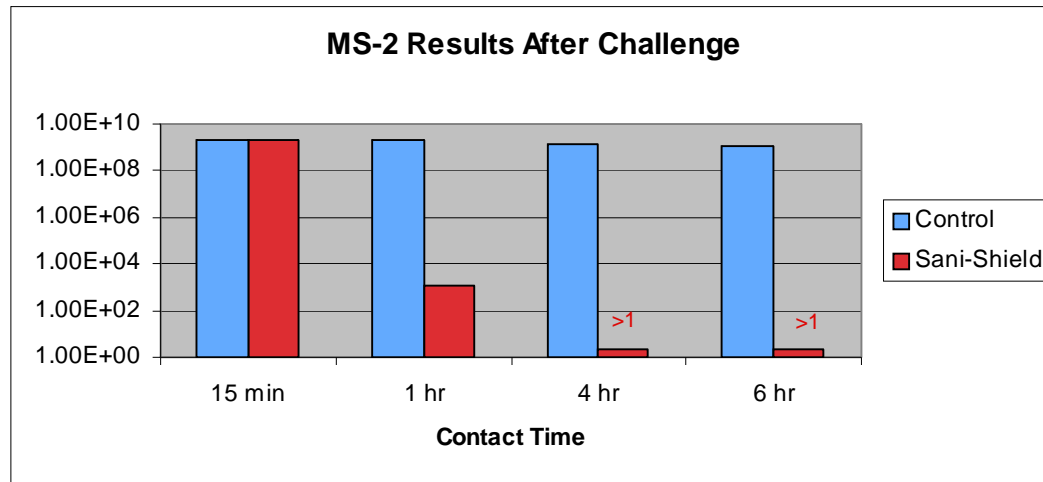
N/a = not applicable

\*Please note that SANI-SHIELD had a 2.93 log reduction over the average of the control and the DI water at the initial hour, which may indicate an instant kill.



MS-2 Virus Study -Table 2. Surfaces were treated with SANI-SHIELD 3-in-1 Surface Care (with antimicrobial barrier), then inoculated with MS-2 virus and tested. Results are in plaque forming units (PFU)

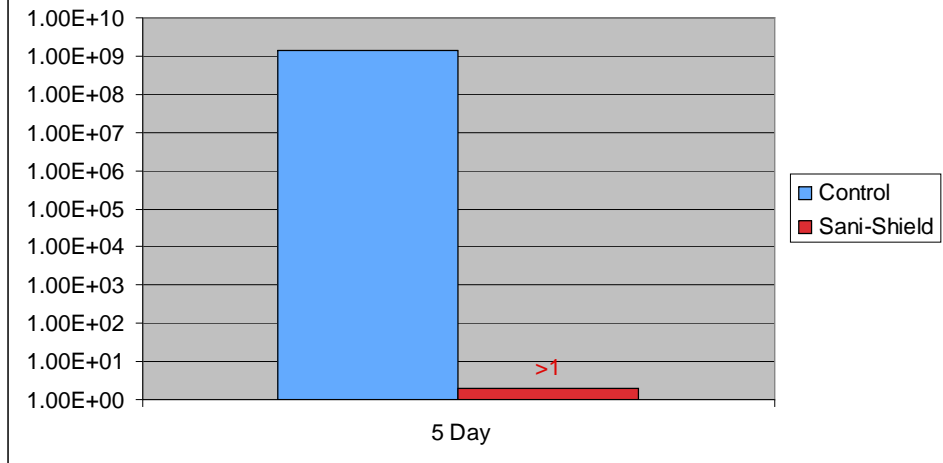
	15 Minutes After Inoculation	1 Hour After Inoculation	Log Reduction	Percent Reduction	4 Hours After Inoculation	Log Reduction	Percent Reduction	6 Hours After Inoculation	Log Reduction	Percent Reduction
Control	2 Billion	2 Billion	0	N/a	1.4 Billion	0.15		1.2 Billion	0.22	
SANI - SHIELD	2.2 Billion	1,100	6.3	99.999999	>1	>9.34	99.999999	>1	>9.34	99.999999



MS-2 Virus Study: - Table 3. Sani-Shield antimicrobial barrier coatings were applied to surfaces and the treated surfaces were stored under ambient conditions. After storage for 5 days, the surfaces were inoculated with MS-2 virus and were tested after 6 hours contact time with the MS-2 inoculates. Log reduction and Percent reduction for Sani-Shield are calculated based on the numbers of PFU on the control.

	5 Days After Treatment	Log Reduction	Percent Reduction
Control	1.4 Billion		
SANI-SHIELD	>1	>9.15	99.999999

### MS-2 Results After 5 Days 6 Hour Contact Time



## 12.0 Conclusion and Remarks

Both the initial and the residual results of the tests performed validate the efficacy of SANI-SHIELD to clean/remove/kill at least 99.9% of the MRSA bacteria and the MS-2 virus and to provide an invisible barrier on which these organisms did not grow when the surfaces were re-inoculated after a period of 5 days in-between cleaning. In fact, the test results demonstrated that 99.9% of the organisms were killed by the antimicrobial barrier.

Thus, for the broad range of surfaces and objects that cannot be cleaned and sanitized by “immersion” – or saturated by misting or flooding for extended time periods – the SANI-SHIELD “spray & wipe” composition represents a viable alternative in terms of killing at least 99.9% of any organisms on the surface and protecting the now treated surface with an antimicrobial barrier that inhibits the spread and impact of re-contamination.

Since the invisible, pathogenic surface-contact organisms are invariably deposited with some form of organic soil that is visible, by cleaning the soil with the antiseptic SANI-SHIELD composition, the organisms are automatically inactivated. And by simultaneously protecting the surface with the antimicrobial barrier, cross-contamination is virtually eliminated in-between cleaning.

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