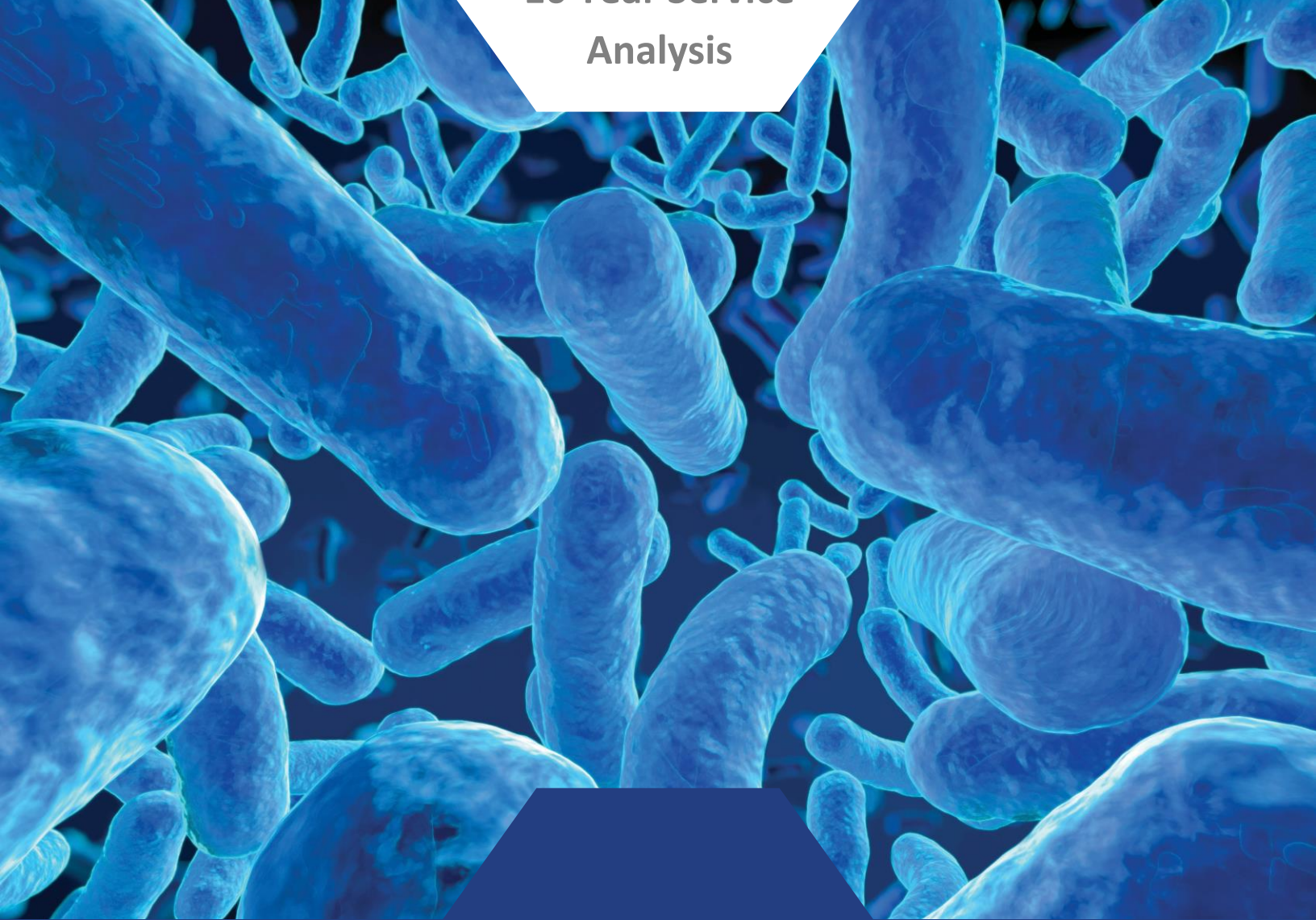




Nature's antimicrobial

University of Arizona
10 Year Service
Analysis



Agion antimicrobial compound suppresses the growth of bacteria, molds, fungi and other microbes, making it the go-to product for applications where antimicrobial protection is required or desired. Attached is the analysis of Agion coated steel in three such places after 10 years of service.

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Analysis of Agion® Coated Galvanized Steel Air Ducts in Woodward Academy Middle School after 10 Years of Service



Agion® antimicrobial treated galvanized steel was used to fabricate the air ducts for the Woodward Academy Middle School near Atlanta Georgia. Installation occurred in 2003 and the building has been in service for approximately 10 years.

The air duct surfaces were tested in 2014 by representatives of the University of Arizona to measure microorganism background levels as well as to determine if the coating continued to provide antimicrobial protection after 10 years. Results indicate that relative to adjacent bare galvanized the surfaces, the Agion coated ductwork exhibited a:

- 99.9% (i.e. “3 log”) reduction in total bacteria count
- 94% reduction in mold count
- 83% reduction in yeast count

In addition, efficacy evaluations conducted on the 10 year old ductwork indicate that within 48 hours of inoculation the Agion coated surfaces reduced:

- *Staphylococcus aureus* levels by 5 log (99.999%)
- *Enterobacter aerogenes* levels by 5.8 log (99.9998%)

Therefore the Agion coated ductwork surfaces not only had lower background microbial counts than adjacent bare galvanized steel but also ***continue to remain antimicrobially active after 10 years of service.***

***Disclaimer**

The information and data in this document are accurate to the best of our knowledge and belief, but are intended for general information only. Applications suggested for the materials are described only to help readers make their own evaluations and decisions, and are neither guarantees nor to be construed as express or implied warranties of suitability for these or other applications.

Data referring to material properties are the result of tests performed on specimens obtained from specific locations of the products in accordance with prescribed sampling procedures; the results thereof are limited to the values obtained at such locations and by such procedures. There is no warranty with respect to values of the materials at other locations.

The Agion® Antimicrobial is presently registered by the United States Environmental Protection Agency as a preservative and bacteriostatic for use in treated articles under 40 CFR 152.25a. This technical data is provided to substantiate the efficacy of the antimicrobial compound. However, the data are not intended to support or endorse public health claims for treated articles.

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the department of
Soil, Water and Environmental Science

THE UNIVERSITY OF ARIZONA

**AK Coatings
AIR DUCTS AT WOODWARD ACADEMY
IN COLLEGE PARK, GEORGIA**

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May 6, 2014

1.0 OBJECTIVE

AK Coatings, a subsidiary of AK Steel has manufactured a galvanized steel product with an antimicrobial coating for over ten years. The object of this study was to assess the performance of galvanized ductwork with the antimicrobial coating that had been in place for more than ten years in the Woodward Academy Middle School in College Park, Georgia.

2.0 AIR DUCTS AT THE WOODWARD ACADEMY

The Woodward Academy in College Park, Georgia has had AK Coatings Agion® treated steel air ducts in their Middle School since 2003. The coated rectangular ducts had bare galvanized steel access panels which allowed the researchers to make direct comparisons at the same locations. The goal was to determine if the levels of microorganisms after 10 years service were less on the treated vs. the untreated surfaces, and to assess the treated surfaces to determine if they still exhibited antimicrobial properties.

Using a Sponge Stick (3M Company), 100 cm² samples were first collected from the inner surfaces of the AK Coatings treated air ducts (20 locations - Figure 1) and the non-treated access panels to the air ducts (20 locations – Figure 2) to analyze any variance in microbial counts. This is referred to below as the background.

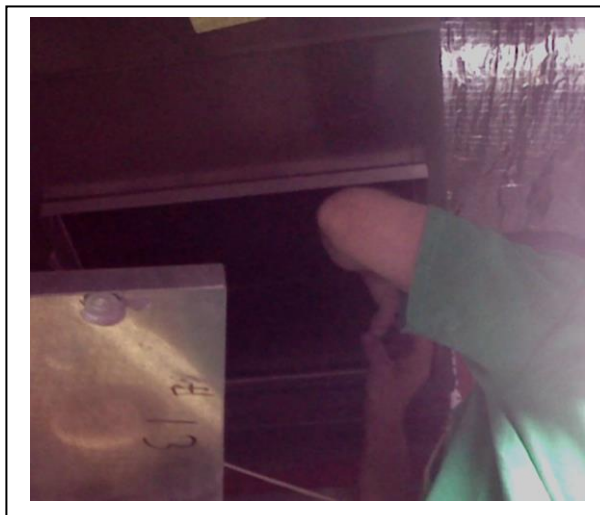


Figure 1. Swabbing coated air duct inner surface with Sponge Stick



Figure 2. Swabbing bare access panel with Sponge Stick

To determine the antimicrobial effectiveness of the AK Steel treated air ducts compared to the untreated access steel panels on the survival of two test bacteria, cultures of *Staphylococcus aureus* and *Enterobacter aerogenes* were grown overnight in trypticase soy broth (Difco, Detroit, MI) and then centrifuged to concentrate as a pellet. The broth was decanted and the cells resuspended in physiological saline and centrifuged again. This step was repeated two additional times.

S. aureus and *E. aerogenes* were pooled to make a single bacterial suspension and were then used to inoculate material in the same vicinity as the material that had been swabbed for the background sampling. The bacteria were applied “in situ” to the interior duct surfaces and access panels at adjoining locations so that separate samples could be

collected after 0, 24 and 48 hours. Each time point sample covered an area of 100 cm². After an approximate 30 to 60 second drying time the bacteria were removed using Sponge Sticks to get initial bacterial count (0 hour). Sponge Sticks were kept in a cooler with ice packs and were shipped overnight to the University of Arizona after each time collection point. Trypticase Soy Agar (Difco, Sparks, MD) amended with 5% sheep blood, 10 mg/L colistin, and 15 mg/L naladixic acid was used for the isolation of *S. aureus* (Figure 3) and MacConkey agar (Difco, Detroit, MI) was used for *E. aerogenes* (Figure 4). The agar plates were incubated at 35°C for 24-48 hours and colonies were counted and reported as colony forming units (CFU).



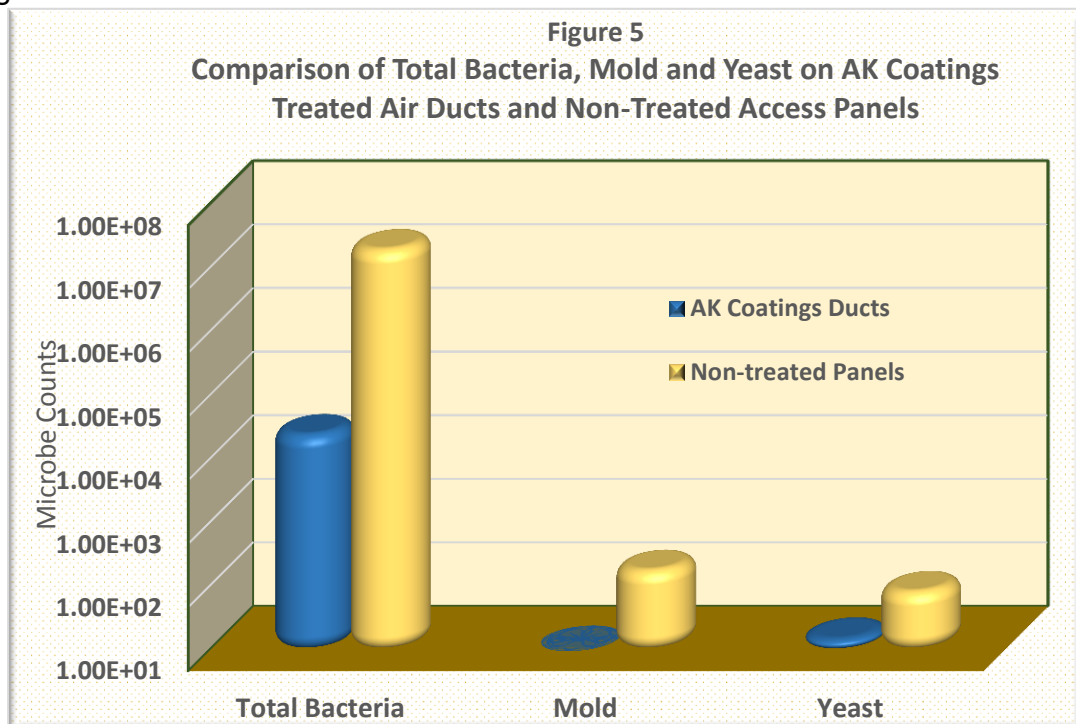
Figure 3 *S. aureus* on Blood agar



Figure 4 *E. aerogenes* on MacConkey agar

3.1 RESULTS

The background data per 100 cm² is shown in Figure 5. The non-treated steel air duct panels had 99.9% (3 logs) more total bacteria than what was found on AK Coatings material in the air duct system. Mold counts were 18 times greater on non-treated panels. Yeast had counts almost 6 times (5.53 logs) higher on non-treated panels than on AK Coatings antimicrobial steel.



Results on the inactivation of the *S. aureus* and *E. aerogenes* are shown in Tables 2 thru 4 and Figures 6 and 7.

Table 2. Inactivation of *S. aureus* on AK Coatings antimicrobial treated steel and non-treated steel in air ducts

<i>Staphylococcus aureus</i>							
AK Coatings panels	0 Hour	24 Hour	48 Hour	Non-treated panels	0 Hour	24 Hour	48 Hour
1	1.20E+05	1.70E+04	10	1	1.60E+06	2.60E+06	9.00E+03
2	1.80E+05	120	90	2	2.20E+05	10	20
3	2.10E+05	2.70E+05	10	3	1.10E+05	1.30E+04	20
4	1.40E+05	190	40	4	2.30E+04	1.10E+05	6.00E+05
5	7.50E+06	5.00E+05	40	5	1.60E+06	7.00E+03	1.30E+03
6	5.00E+06	180	10	6	1.50E+06	1.30E+02	3.00E+04
7	4.80E+06	2.30E+03	10	7	7.00E+04	2.10E+06	3.40E+04
8	2.70E+06	20	40	8	2.40E+04	1.80E+04	1.30E+03
9	3.20E+06	1.10E+05	10	9	3.70E+04	4.40E+04	1.00E+03
10	4.40E+05	2.10E+04	20	10	7.90E+05	2.60E+04	100
11	3.30E+06	2.00E+03	10	11	7.70E+05	10	7.50E+04
12	2.70E+06	1.30E+03	10	12	1.80E+05	4.70E+04	5.00E+04
13	1.80E+07	1.60E+03	10	13	8.70E+05	3.00E+04	2.30E+04
14	1.90E+07	1.70E+04	10	14	1.50E+06	7.80E+05	5.40E+04
15	2.50E+06	1.90E+04	10	15	1.40E+06	1.50E+03	200
16	3.30E+06	2.70E+04	10	16	1.40E+06	3.70E+03	2.00E+04
17	2.90E+06	40	60	17	1.80E+05	2.50E+03	2.00E+03
18	2.30E+06	2.20E+04	10	18	2.60E+05	3.00E+04	1.30E+04
19	1.70E+06	2.60E+04	10	19	1.30E+06	3.00E+04	100
20	2.10E+06	120	60	20	2.80E+05	7.00E+03	3.00E+03
Std dev	5.26E+06	1.22E+05	23		6.29E+05	7.29E+05	1.32E+05

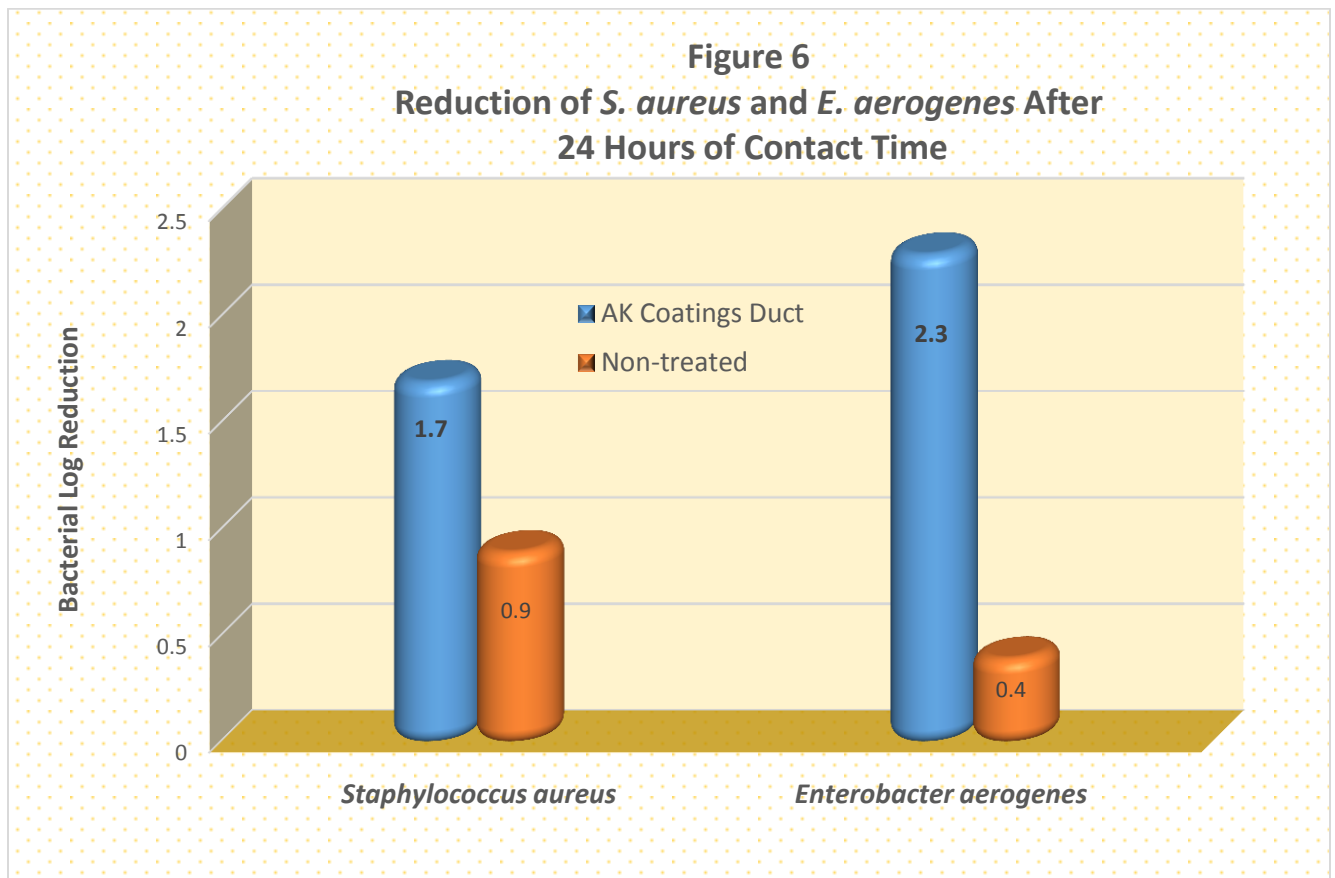
Table 3. Inactivation of *E. aerogenes* on AK Coatings antimicrobial treated steel and non-treated steel in air ducts

<i>Enterobacter aerogenes</i>							
AK Coatings panels	0 Hour	24 Hour	48 Hour	Non-treated panels	0 Hour	24 Hour	48 Hour
1	1.30E+07	10	10	1	1.50E+07	3.00E+07	2.00E+04
2	1.30E+07	10	10	2	1.50E+07	2.30E+04	10
3	1.20E+07	2.40E+05	10	3	1.80E+07	3.00E+06	10
4	1.20E+07	10	10	4	3.50E+07	1.90E+05	230
5	1.20E+07	4.00E+05	10	5	1.20E+07	3.60E+04	3.50E+04
6	1.40E+07	3.50E+03	10	6	1.30E+07	3.00E+03	4.70E+04
7	3.10E+07	10	10	7	1.20E+07	8.70E+07	5.70E+04
8	8.50E+07	10	10	8	1.60E+07	1.10E+06	2.90E+05
9	1.20E+07	1.90E+05	470	9	1.60E+07	1.40E+06	10
10	1.30E+07	8.70E+05	10	10	4.50E+07	3.50E+06	10
11	1.50E+07	1.70E+05	10	11	1.80E+07	1.10E+03	4.80E+05
12	1.30E+07	10	10	12	1.60E+07	3.70E+06	70
13	1.90E+07	2.30E+03	10	13	1.60E+07	1.90E+05	40
14	3.30E+07	10	10	14	1.60E+07	3.20E+04	10
15	1.70E+07	10	10	15	3.10E+07	10	2.70E+04
16	1.20E+07	10	10	16	1.80E+07	1.90E+07	6.00E+05
17	1.40E+07	10	10	17	1.30E+07	40	10
18	3.70E+07	10	10	18	1.20E+07	1.00E+07	1.00E+04
19	1.50E+07	10	10	19	1.10E+07	10	10.0
20	1.50E+07	10	10	20	3.50E+07	10	10.0
Std dev	1.70E+07	2.13E+05	103		9.44E+06	2.01E+07	1.72E+05

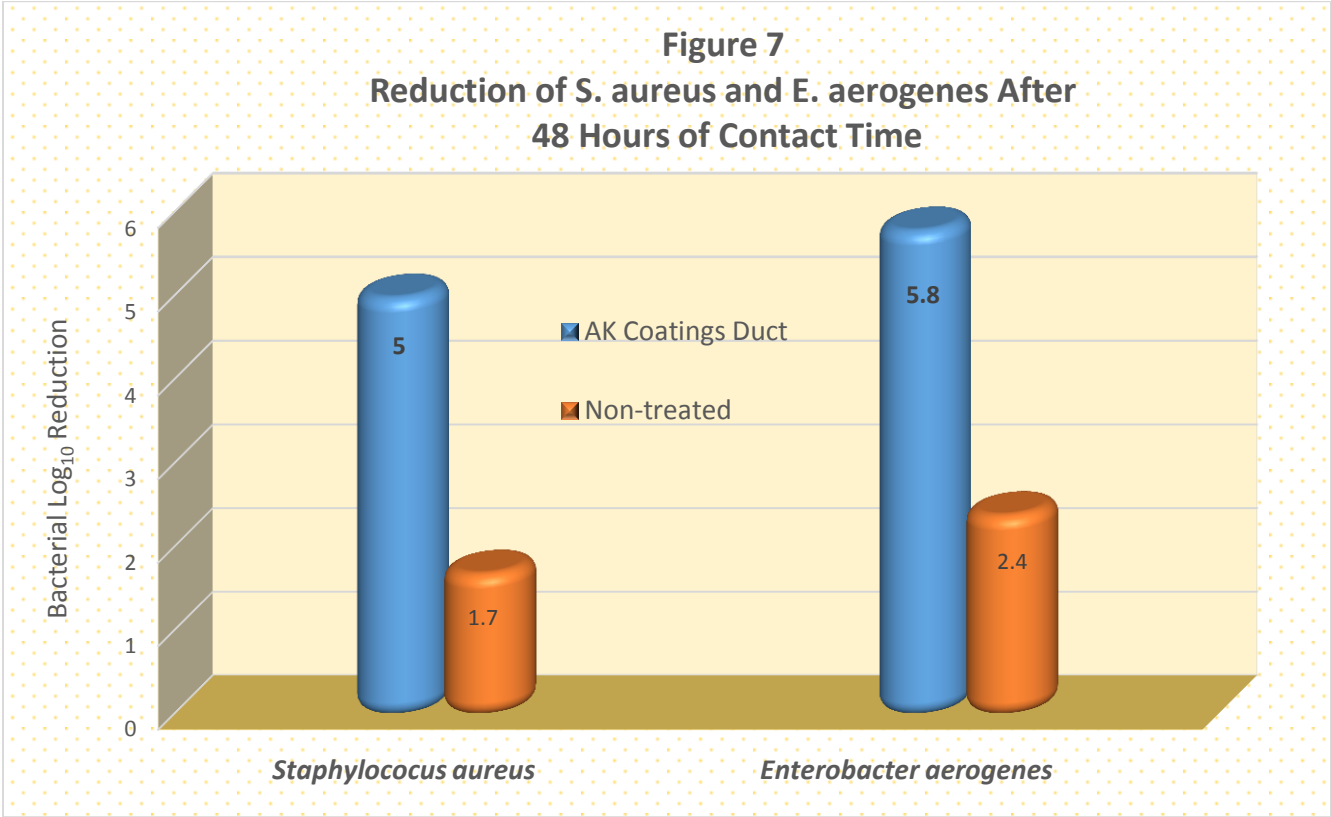
Table 4. Log reductions for *S. aureus* and *E. aerogenes* per 100 cm² for AK Coatings treated steel and non-treated steel after 24 and 48 hours

	24 Hour Log Reduction		48 Hour Log Reduction	
	AK Coatings Ducts	Non treated panel	AK Coatings Ducts	Non treated panel
<i>Staphylococcus aureus</i>	1.7	0.9	5	1.7
<i>Enterobacter aerogenes</i>	2.3	0.4	5.8	2.4

Figure 6 shows AK Coatings air duct material reduced *S. aureus* by 1.7 logs and the non-treated panels had a log reduction of 0.9 log after 24 hours of contact time. The *E. aerogenes* was reduced by AK Coatings treated material by 2.3 logs vs the non-treated panels with a 0.4 log reduction. After 48 hours of contact time (Figure 7), the AK Coatings air duct material reduced the *S. aureus* by 5 logs and the non-treated panels had a 1.7 log reduction.



For *E. aerogenes* at 48 hours, AK Coatings air ducts had a log reduction of 5.8 in contrast to non-treated panels with a 2.4 log reduction.



4.0 DISCUSSION

The AK Coatings Agion® antimicrobial treated air duct galvanized steel was found to have less total bacteria, molds and yeast than the bare, non-treated air duct access panels. Both *S. aureus* (Gram positive bacterium) and *E. aerogenes* (Gram negative bacterium) were inactivated more rapidly and to a greater degree on the AK Coatings treated air duct steel than the non-treated air duct steel. These results demonstrate that after ten years the AK Coatings treated air ducts still exhibit antimicrobial activity.

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Analysis of Agion® Coated Galvanized Steel Air Ducts in the Helford Clinical Research Hospital at City of Hope after 10 Years of Service



Agion® antimicrobial treated galvanized steel was used to fabricate the air ducts for the Helford Clinical Research Hospital at City of Hope in Duarte, California. Installation occurred in 2003 and the building has been in service for approximately 10 years.

The air duct surfaces were tested in 2014 by representatives of the University of Arizona to measure microorganism background levels as well as to determine if the coating continued to provide antimicrobial protection. Results indicate that after 10 years

- Bacteria counts were approximately 14X higher on bare samples than coated samples
- Mold was 2.5X higher on the bare samples
- Yeast was nearly 7X higher on the bare galvanized steel

In addition, efficacy evaluations conducted on the 10 year old ductwork indicate that within 24 hours of inoculation the Agion coated surfaces reduced:

- *Enterobacter aerogenes* levels by 5.3 log (99.9995%)
- *Staphylococcus aureus* levels by 3.8 log (99.984%)

Therefore the Agion coated ductwork surfaces not only had lower background microbial counts than adjacent bare galvanized steel but also ***continue to remain antimicrobially active after 10 years of service.***

*Disclaimer

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**AK COATINGS
EVALUATION OF AIR DUCTS IN THE HELFORD CLINICAL
RESEARCH HOSPITAL AT CITY OF HOPE AFTER 10 YEARS
SERVICE**

**Sheri Maxwell, B.S.
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February 28, 2014

1.0 OBJECTIVE

AK Coatings, a subsidiary of AK Steel has manufactured galvanized steel products with an antimicrobial coating for over ten years. The object of this study was to assess the performance of products with the Agion[®] antimicrobial coating that had been in place for more than ten years at the City of Hope located in Southern California.

2.0 AIR DUCTS AT THE CITY OF HOPE HOSPITAL

The Helford Clinical Research Hospital at the City of Hope in Duarte, California has used AK Coatings treated steel for most of their air duct work since the 1990's. Some sections of the hospital's duct work in adjoining buildings constructed before that time did not contain treated surfaces. The goal was to determine if the levels of microorganisms were less on the treated vs. the untreated surfaces, and to assess the treated surfaces to determine if they still exhibited antimicrobial properties.

A sponge Stick (3M Company) was used to collect samples from the surface 100 cm² of AK Coatings coated access panels (12) to the air ducts (see Figure 1) and non-treated access panels (6) to analyze any variance in microbial counts.

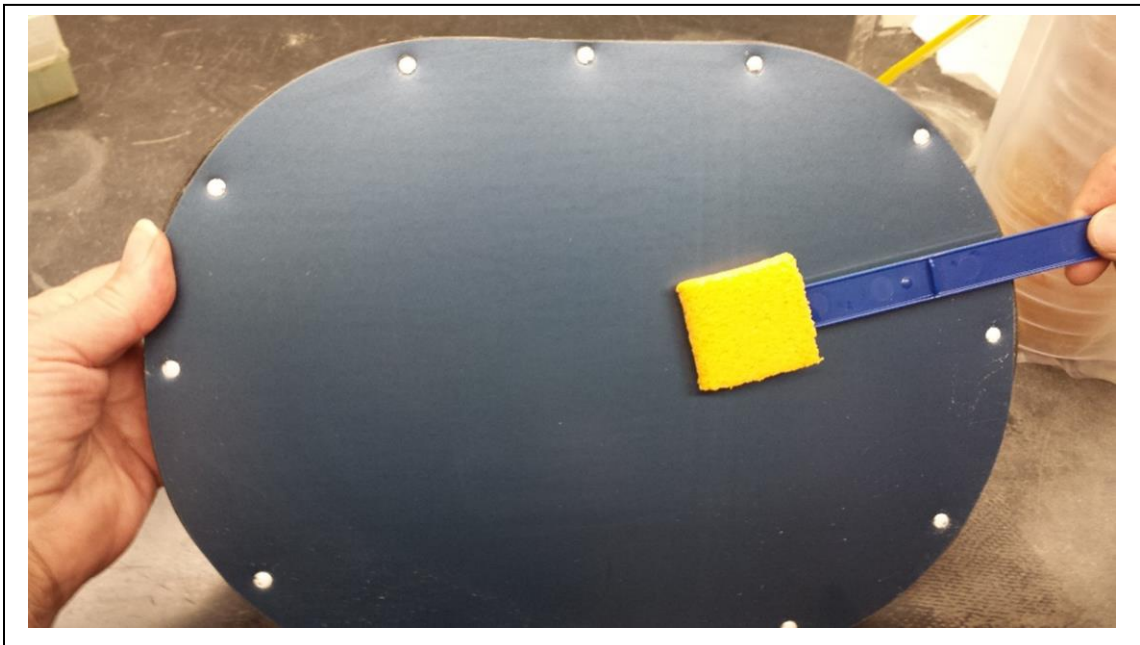


Figure 1. Swabbing Agion[®] treated air duct access panel with Sponge Stick

To determine the antimicrobial effectiveness of the material, two test bacteria cultures of *Staphylococcus aureus* and *Enterobacter aerogenes* were grown overnight in trypticase soy broth (Difco, Detroit, MI) and then centrifuged to concentrate as a pellet. The broth was decanted and the cells resuspended in physiological saline and centrifuged again. This step was repeated two additional times.

S. aureus and *E. aerogenes* were pooled to make a single bacterial suspension and were then used to inoculate the section of the access panels that had not been swabbed for the background sampling. The bacteria were applied to the access panels at different locations so that separate samples could be collected after 0, 4 and 24 hours. Each time point sample covered an area of 100 cm². After an approximate 30 to 60 second drying time the bacteria were removed using Sponge Sticks to get initial bacterial count. Sponge Sticks were kept in a cooler with ice packs and after the 4 hours contact time were shipped overnight to the University of Arizona. The same was done with the Sponge Sticks after the 24 hour contact time. Trypticase Soy Agar (Difco, Sparks, MD) amended with 5% sheep blood, 10 mg/L colistin, and 15 mg/L naladixic acid was used for the isolation of *S. aureus* (Figure 2) and MacConkey agar (Difco, Detroit, MI) was used for *E. aerogenes* (Figure 3). The agar plates were incubated at 35°C for 24-48 hours and colonies were counted and reported as colony forming units (CFU).



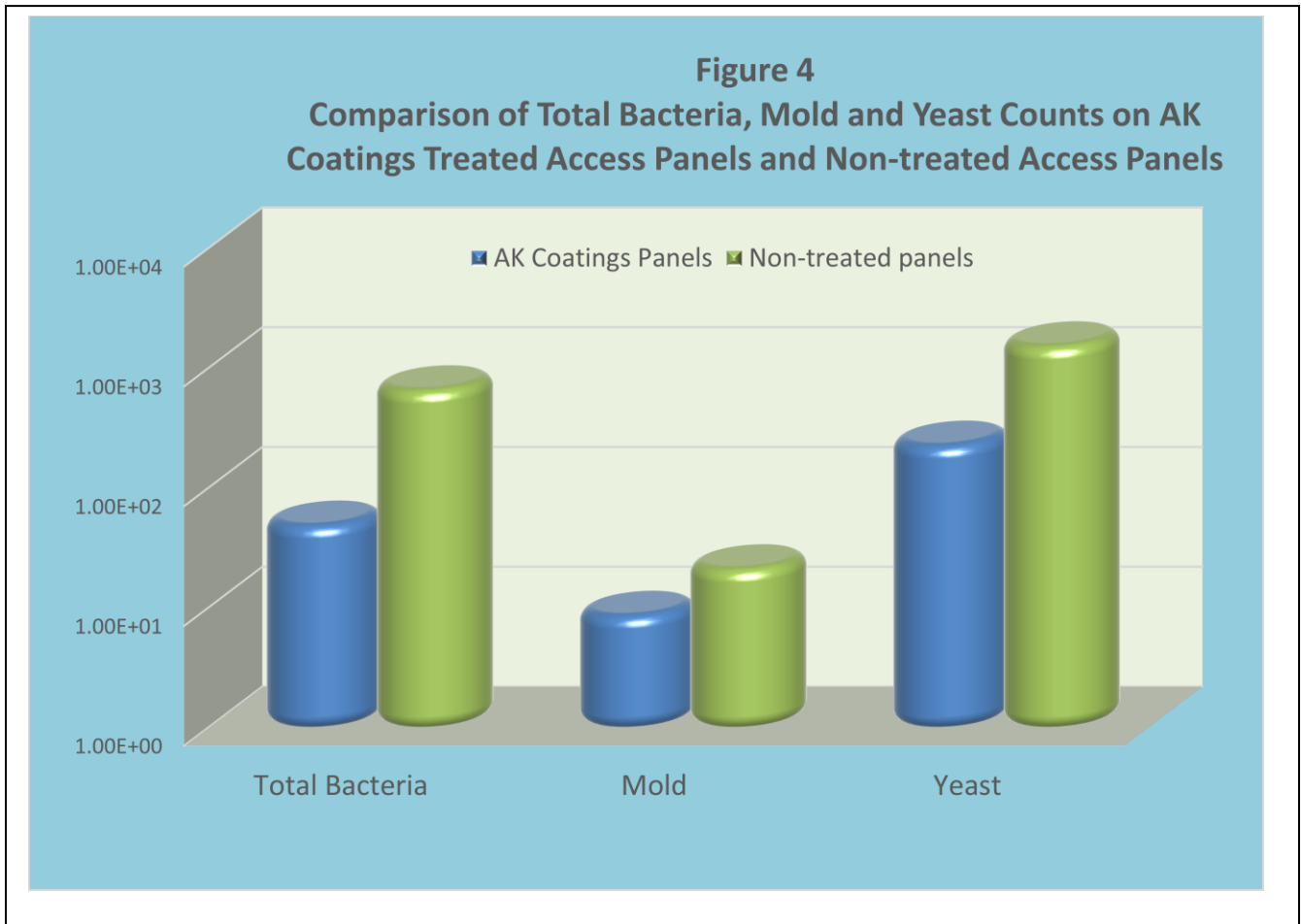
Figure 2. *S. aureus* on Blood agar



Figure 3. *E. aerogenes* on MacConkey agar

3.1 Results

The background data per 100 cm² is shown in Figure 4. The total bacteria on non-treated steel air duct panels were almost 14 times (13.47) greater than on AK Coatings panels and mold counts were 2.5 times greater on non-treated panels. Yeast, although not intentionally isolated, but grew on the media used for mold isolation and had counts almost 7 times (6.75) higher on non-treated panels than on AK Coatings antimicrobial steel panels.



Results on the inactivation of the *S. aureus* and *E. aerogenes* antimicrobial treated and untreated panels are shown in Tables 2 thru 4 and Figure 5. The 4 hour contact time had too many colonies to count on the appropriate dilutions indicating no significant reduction. Table 2 shows the log reduction results for only the 24 hour sample. *S. aureus* was reduced by 0.7 log (63%) more on the AK Steel panels than non-treated panels, AK Steel panels reduced *E. aerogenes* 1.97 log (99.5%) more than non-treated panels after 24 hours.

Table 2. Inactivation of *S. aureus* on AK Coatings antimicrobial treated and non-treated air duct panels collected at different sites

<i>S. aureus</i>					
AK Coatings panels	0 Hour	24 Hour	Non-treated panels	0 Hour	24 Hour
1	9.50E+04	30	1	1.95E+04	480
2	18	10	2	5.75E+05	370
3	63	10	3	1.10E+03	35
4	1.35E+03	10	4	1.03E+05	140
5	9.00E+04	140	5	6.00E+04	110
6	1.08E+05	60	6	1.95E+04	260
7	3.00E+04	190	7	8.50E+04	30
8	1.48E+05	80	8	1.50E+05	460
9	3.50E+04	100	9	2.70E+05	270
10	1.03E+05	10	10	1.15E+05	360
11	6.50E+04	10			

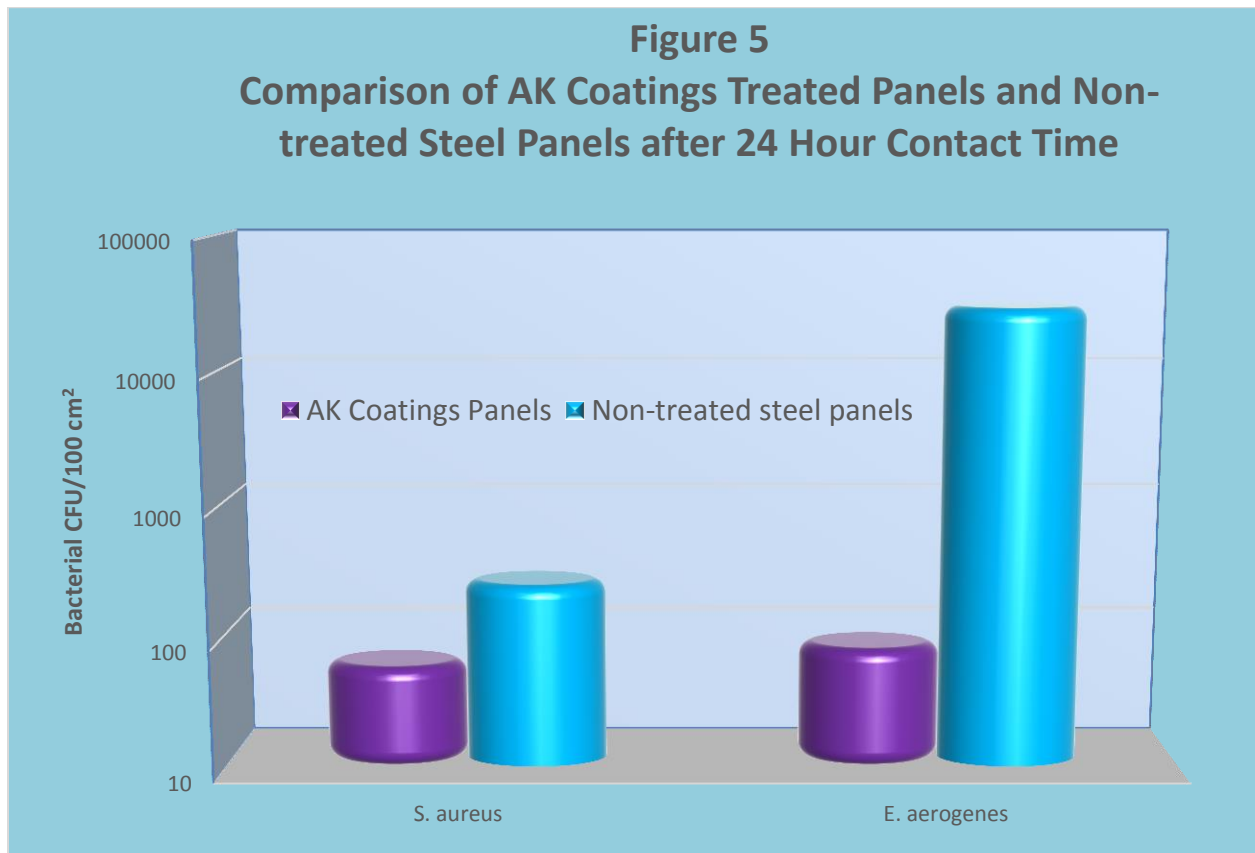
Table 3. Inactivation of *E. aerogenes* on AK Coatings panels and non-treated panels at different sites

<i>E. aerogenes</i>					
AK Coatings panels	0 Hour	24 Hour	Non-treated panels	0 Hour	24 Hour
1	640	0	1	1.60E+05	0
2	6.80E+03	0	2	5.30E+07	3.00E+05
3	2.30E+05	0	3	7.50E+04	0
4	10	0	4	4.30E+07	0
5	100	0	5	2.80E+07	0
6	2.30E+05	0	6	3.70E+07	250
7	1.00E+03	0	7	4.70E+07	0
8	7.30E+03	0	8	4.40E+07	850
9	330	0	9	5.40E+07	108
10	3.60E+07	900	10	4.10E+04	575
11	2.10E+03	0			

Table 4. Log reductions for *S. aureus* and *E. aerogenes* per 100 cm² for AK Coatings panels and non-treated steel panels after 24 hours

Contact Time Points	<i>Staphylococcus aureus</i>			<i>Enterobacter aerogenes</i>		
	*0 Hour	24 Hour	Log Reduction	*0 Hour	24 Hour	Log Reduction
AK Coatings panel	3.48E+05	59	3.8	1.63E+07	82	5.3
Non-treated Steel panel	3.48E+05	259	3.1	1.63E+07	3.02E+04	2.7

* 0 Hour counts for *S. aureus* and *E. aerogenes* is an average from all 21 sites



4.0 DISCUSSION

The treated galvanized steel HVAC access plates were found to have less total bacteria, molds and yeast than the bare, untreated galvanized steel access plates. The difference was greater for total bacteria than molds and yeast. Both *S. aureus* (Gram positive bacterium) and *E. aerogenes* (Gram negative bacterium) were inactivated more rapidly on the treated material than the untreated material. *E. aerogenes* was reduced by over 99.5% more than the untreated plates after 24 hours and *S. aureus* by 63%. Gram positive bacteria like *S. aureus* are usually more resistant to inactivation by metals than Gram negative bacteria such as *E. aerogenes*. These results demonstrate that after ten years the antimicrobial coated galvanized steel surfaces still exhibit antimicrobial activity.

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Analysis of Agion® Coated Stainless Steel and Galvanized Steel Surfaces in the Concept Home after 10 Years of Service



The Concept Home was built in 2003 in the Simi Valley, California and was the nation's first home to include extensive use of antimicrobial surfaces. The home features products made from AK Coatings carbon and stainless steels with coatings containing the AgION® antimicrobial compound. Antimicrobial-coated steels reduce bacteria, mold and fungus growth. In total, the Concept Home incorporated about 200,000 pounds of steel, including 35,000 pounds of steel coated with the antimicrobial compound.

Surfaces were swabbed after 10 years of service to determine bacteria levels. In general the numbers of bacteria were lower than found in a typical home. In addition, the physical condition of the coated surfaces was unaffected after 10 years. No peeling, flaking or discoloration was noted.

Efficacy tests conducted on a 10 year old door push plate removed from the home was tested for efficacy using *Enterobacter aerogenes* and *Pseudomonas aeruginosa*. Results indicate that the test organisms were reduced vs. the untreated control by 94% to 97%. This demonstrates that the material continues to exhibit antimicrobial activity after 10 years of service.

***Disclaimer**

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The Agion® Antimicrobial is presently registered by the United States Environmental Protection Agency as a preservative and bacteriostatic for use in treated articles under 40 CFR 152.25a. This technical data is provided to substantiate the efficacy of the antimicrobial compound. However, the data are not intended to support or endorse public health claims for treated articles.

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AK COATINGS EVALUATION OF SURFACES AT THE CONCEPT HOME AFTER 10 YEARS SERVICE

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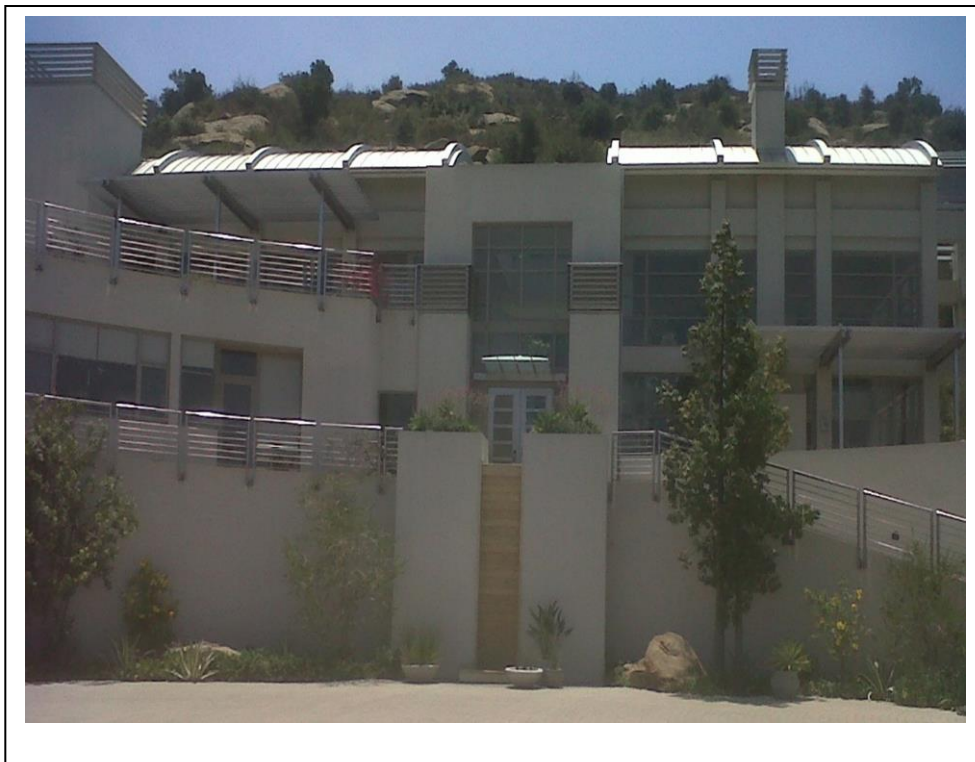
February 28, 2014

1.0 OBJECTIVE

AK Coatings, a subsidiary of AK Steel has manufactured galvanized steel and stainless steel products with an antimicrobial coating for over ten years. The object of this study was to assess the performance of various surfaces with the antimicrobial coating that had been in place for more than ten years in the Concept Home located in Southern California.

2.0 THE CONCEPT HOME

The Concept Home was built in 2003 in the Simi Valley, California and was the nation's first home to include extensive use of antimicrobial surfaces. The home features products made from AK Coatings carbon and stainless steels with coatings containing the AgION™ antimicrobial compound. Antimicrobial-coated steels reduce bacteria, mold and fungus growth. In total, the Concept Home incorporated about 200,000 pounds of steel, including 35,000 pounds of steel coated with the antimicrobial compound.



In the kitchen, antimicrobial-coated stainless steels were utilized on appliances, cabinets and even ceiling panels. Throughout the home, “high-touch” features such as door handles utilize antimicrobial steel. In addition, the heating, ventilation and air conditioning system is made with galvanized steel coated with an antimicrobial.

On June 12, 2013 the coated and uncoated surfaces were sampled throughout the home to determine comparative microbial load after ten years since construction. Sample areas were tested for total bacterial number (heterotrophic bacteria counts or HPC) and one site for coliform/*Escherichia coli* bacteria. Samples were taken from an approximately 100 cm² surface area using a Sponge Stick (3M Company, St. Paul, MN) and immediately shipped overnight to the University of Arizona and processed for total bacteria, coliform bacteria and *E. coli*.

2.1 Results

If one excluded the sink, which usually has high numbers of bacteria because of the presence of food particles and water, the numbers of bacteria were similar on treated and untreated surfaces (Table 1). In general the numbers of bacteria were lower than found in a typical home with children (Rusin et al, 1998). We can say that the numbers are lower than the typical home, but because only two persons inhabit the household it is difficult to make firm conclusions. Also, a maid is available and the home is probably regularly cleaned more than the typical home.

3.0 DISCUSSION

Total bacterial numbers found on the surfaces in the Concept home were generally low compared to typical homes probably because only two persons are inhabiting the home and the availability of a maid. This probably accounts as to why there are no differences in the numbers seen in treated vs. untreated surfaces.

4.0 REFERENCES

Rusin, P., P., Orosz-Coughlin and C. Gerba. 1998. Reduction of faecal coliform, coliform and heterotrophic plate count bacteria in the household kitchen and bathroom by disaffection with hypochlorite cleaners. *Appl. Microbiol* 85:819-828.

Table 1. Number of bacteria (colony forming units) detected on various surfaces tested in the Concept Home

Concept Home					
Swab #	Location	Comments	Total Bacteria per site (100 sq. cm.)	Coliform Bacteria	<i>E. coli</i> Bacteria
1	Drain in kitchen sink	Non-Coated	3,000	2,160	310
2	Door handle on Oven	Non-Coated	10	0	0
3	Door Panel on Refrigerator	Coated	10	0	0
4	Handle on Refrigerator	Non-Coated	30	0	0
5	Faucet on kitchen sink	Non-Coated	10	0	0
6	Dishwasher panel	Coated	<10	0	0
7	Door Push Plate	Coated	10	0	0
8	Inside door handle on front Door	Coated	30	0	0
9	HVAC diffuser grill side of fireplace	Coated	220	0	0
10	HVAC diffuser grill kitchen	Coated	10	0	0
11	Entry handle bathroom door	Coated	20	0	0
12	Wine rack	Coated	100	0	0
13	Inside handle on door to patio	Coated	20	0	0
14	HVAC diffuser grill laundry room	Coated	50	0	0
15	HVAC duct behind kitchen diffuser	Coated	20	0	0
16	Ice-O-Matic machine	Coated	70	0	0
17	Elevator Plate	Coated	<10	0	0
18	Wall in kitchen near sink	Non-Coated	10	0	0
19	Wall in bathroom above sink	Non-Coated	10	0	0
20	Metal handle on kitchen cabinet	Non-Coated	70	0	0
21	Splash wall behind stove	Coated	<10	0	0

Assessment of Anti-microbial Activity of AK Coatings Push Plate after 10 years

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Purpose: The goal of these tests was to assess the anti-microbial activity of anti-microbial push plates which had been in use in the Concept house for 10 years. These plates were collected from a door entering the kitchen from the living rooms. They were used in this testing. Control untreated stainless steel plates provided to our laboratory were used as controls.

Material and Methods: Three different bacteria were selected for testing. *Staphylococcus aureus* (Gram positive) and *Pseudomonas aeruginosa* are standard test bacteria used by the U.S. Environmental Protection Agency (USEPA) for registration of hard surface disinfectants. *Enterobacter aerogenes* is one of the test organisms used in the USEPA test for registration of self-sanitizing surfaces.

Test bacteria: Three different types of test bacteria were used: *Enterobacter aerogenes*, *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*. All of the test bacteria were obtained from the American Type Culture Collection (Bethesda, MD). They were grown overnight in trypticase soy broth (Difco, Detroit, MI) and then centrifuged to concentrate as a pellet. The broth was decanted and the cells resuspended in physiological saline and centrifuged again. This step was repeated two additional times.

Methods: Each inoculum was spread evenly over the surface of each panel using a sterile cotton-tipped applicator. The inocula were allowed to air dry at room temperature at a relative humidity of 30% (Fisher Hygrometer, Pittsburgh, PA). At one, four and 24 h, concentrations of surviving bacteria were determined in the following manner: a sterile applicator was moistened in one mL of D/E neutralizing broth. Excess D/E broth was firmly pressed out and the applicator was rubbed over the entire surface of a panel by moving back and forth in closely spaced rows. The applicator was then rotated 180 degrees and rubbed over the entire surface of the panel in a 90-degree angle from the original direction of movement. The swab was then placed in one mL of D/E broth. The surviving bacteria were enumerated using 10-fold dilutions in sterile saline and the spread plate method on trypticase soy agar (Difco). All testing was performed in duplicate or in triplicate.

Results: The test organisms were reduced vs. the untreated control by 1.2 to 1.4 log (Table 1), indicating that they still exhibit antimicrobial activity.

Table 2 and 3 show results of *S. aureus* and *Escherichia coli* conducted 10 years ago using coated stainless steel pans. I don't know if these were the same coatings, but the two enteric bacteria (*E. aerogenes* and *E. coli* are closely related), were about the same. The MRSA seems more resistant, but I don't know if this was the identical material.

Table 1. Reduction of test bacteria on push plate collected from the Concept Home at room temperature.

Organism	Sample	Colony Forming Units	Log₁₀ Reduction vs. Control
<i>Enterobacter aerogenes</i>	0 hour	4.1 X 10 ⁵	
	24 hours control	8.0 X 10 ⁴	
	24 hours antimicrobial* plate	4.0 X 10 ³	1.3 (95%**)
<i>Staphylococcus aureus</i> (MRSA)	0 hour	3.7 X 10 ⁵	
	24 hours control	3.6 X 10 ⁵	
	24 hours antimicrobial plate	5.1 X 10 ⁴	0.86 (14%)
<i>Pseudomonas aeruginosa</i>	0 hour	3.5 X 10 ⁶	
	24 hours control	6.1 X 10 ⁴	
	24 hours antimicrobial plate	5.6 A 10 ³	1.03 (92%)

*Push plate collected from concepts house. **percent reduction

Table 2 Survival of *Staphylococcus aureus*,(MRSA) (CFU), on control stainless steel panels and on stainless steel panels with 2.5% Ag/14% Zn zeolite pan coatings incubated at room temperature and 30% relative humidity.

	Inoculum Placed on Panels	Survival of <i>S. aureus</i> after:			Log ₁₀ Reduction after 24 hours
		1 h	4 h	24 h	
Test 1					
Controls	8.7x10 ⁴	5.5x10 ⁴	1.8x10 ⁴	3.3x10 ³	
		2.8x10 ⁴	8.6x10 ³	1.1x10 ³	
Ag/Zn Coating	8.7x10 ⁴	1.3x10 ²	<1.0x10 ¹	<1.0x10 ¹	2.1
		1.7x10 ²	2.0x10 ¹	<1.0x10 ¹	
Test 2					
Controls	3.9x10 ⁵	8.2x10 ⁴	1.2x10 ⁵	2.4x10 ³	
		3.1x10 ⁵	9.8x10 ⁴	1.7x10 ³	
		1.4x10 ⁵	7.6x10 ⁴	4.2x10 ³	
Ag/Zn Coating	3.9x10 ⁵	8.1x10 ¹	<1.0x10 ¹	<1.0x10 ¹	2.44
		2.2x10 ²	1.0x10 ¹	<1.0x10 ¹	
		4.1x10 ²	4.0x10 ¹	<1.0x10 ¹	

Table 3. Reduction of *E. coli* O157:H7 in saline at 37°C in stainless steel pans with and without silver/zinc coatings.

Time (h)	<i>E. coli</i> O157:H7		
	Ag/Zn	Control	Log ₁₀ Reduction vs. Control
Inoculum ^a	1.4 x 10 ⁵	1.4 x 10 ⁵	
1 ^b	1.3 x 10 ⁴	2.4 x 10 ⁴	0.27
4	1.1 x 10 ⁴	1.5 x 10 ⁴	0.13
24	1.7 x 10 ¹	3.7 x 10 ³	1.27

^a CFU/ml used in inoculum

^b Average CFU/ml of triplicate tests.



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