

Evaluation of the effect of MicroShield 360 on biofilms of *Listeria monocytogenes*

Scott M. Russell, Ph.D.
Professor
Poultry Science Department
The University of Georgia

Introduction:

Scientists have recently discovered that bacteria can communicate with each other. When confronted with environmental conditions that are unfavorable, such as refrigeration, they signal each other. Imagine riding in a convertible with some of your friends and it begins to rain. One passenger would say “let’s put the top up”. Bacteria do the exact same thing. They signal one another to begin forming a protective coating called a “biofilm”. For the food processor, this is disastrous because once the bacteria form this protective coating, it is nearly impossible to kill them. In fact, studies have demonstrated that when spraying sanitizer on bacterial colonies encased in biofilms, only 60% of the bacteria die. This is less than a 1 log reduction. Where this becomes a problem is in foods that are fully cooked or ready to eat. In coolers, freezers, and on processing belts and other equipment surfaces used to process or store chicken nuggets, chicken patties, hot dogs, cheese, frozen pizza, deli meats, and many other foods, if these bacteria are allowed to form biofilms, then a major problem occurs. By visiting the USDA-Food Safety Inspection Service’s website (<http://www.fsis.usda.gov/OA/recalls/recdb/rec2000.htm>), it is easy to see how many recalls were performed in the year 2000 (this is the most current year they have listed). It is astonishing to see the number of companies affected by *Listeria*, *E. coli* O157:H7, and

Salmonella recalls. These recalls, in some cases, involve hundreds of thousands of pounds of meat products. In fact, the second case listed on this site refers to a recall of Cargill's ready to eat turkey and chicken because of *Listeria* contamination. In this single instance, a total of 16,895,000 lbs. were recalled at a market value average of \$6-\$7 per pound (**\$109,817,500**). This is one example of many pages of recalls listed on this page of the website. In all cases, *Listeria* contamination of meats involves a process by which *Listeria* from drains or floors is transmitted to cooling units in a cooler or freezer or to equipment surfaces as the result of aerosolization of the bacteria when employees use high pressure hoses to spray floors. The bacteria then attach to the surfaces of the cooling units or equipment, signal each other, and begin to form biofilms. When this happens, the sanitation crew cannot fully clean and sanitize the plant because their sanitizers are not able to penetrate the biofilm and get down to the bacteria. When fully cooked, ready to eat products are then produced the next day, *Listeria* are blown onto them in the cooler or freezer from the cooling units or are transferred by contact with a contaminated belt. The product then is evaluated by a laboratory, found to be positive, and the product is recalled or someone gets sick and 30% of these individuals will die as a result of this contamination. Scientists have demonstrated, using the most high tech methods available, that *Listeria* is almost impossible to eliminate from a processing plant once it is established.

MS360 is an antimicrobial that controls bacteria not by poisoning the cell, but by puncturing the cell wall on a microscopic level unlike chemical sanitizers. The MS360 technology relies on an electrically-charged spiked molecular structure. In nature, most microbes carry the opposite electrical charge from the molecule in MS360. Therefore, the

bacteria are physically and irresistibly drawn into contact with MS360 pointed molecules, to the point that the cell walls are punctured and destroyed. MS360 does not leach or mutate. MS360 is EPA registered. Once it is sprayed on the surface, it bonds and retains its effectiveness indefinitely unless painted, covered, or worn off in high-traffic areas. The coating makes surfaces easier to clean and is based on the same technology as Rain-X or Dr. Sholl's Odor Destroyers. It is important that, before application of MS360, the surface should be cleaned. And once applied, the surface should be kept as clean as possible with mPerial and the microfiber cloth. Theoretically, application of MS360 to a surface, such as the stainless steel wall of a cooler would greatly assist in preventing *Listeria monocytogenes* producing a biofilm on the surface and causing a post-cook contamination scenario.

The purpose of this study was to determine if electrostatic application of MS360 to stainless steel coupons could prevent the formation of a biofilm of *Listeria monocytogenes* on that surface. If successful, this technology would be extremely useful for poultry and red meat producers to prevent massive product recalls.

Procedures:

- 1) 15 stainless steel coupons were treated by electrostatic application of MS360, coating the entire coupon on both sides. 15 stainless steel coupons were not treated to serve as controls.
- 2) These coupons were allowed to remain in a bag for 3 weeks before any testing was done to determine if the MS360 had an antimicrobial impact after 3 weeks time as advertised.
- 3) All 30 coupons were dipped into actively growing *Listeria monocytogenes* (multiple strain mixture)
- 4) The *Listeria* were encouraged to form a biofilm by placing the coupons (individually in different containers) into minimal media. This signaled the

Listeria “you have no food and are in danger” but did not kill them. The *Listeria* then began to produce the biofilms.

5) After 12 hours, the coupons were removed from the minimal medium and the biofilms (if present) were recovered.

6) The coupons were placed into sterile urine sample cups with sterile glass beads and shaken to remove the biofilms from the surface.

7) This sample was plated onto Modified Oxford Agar to recover *Listeria*.

8) The *Listeria* was counted and the groups (treated with MS360 vs. controls) were evaluated statistically.

9) The experiment was conducted 3 times. (3 Repetitions)

Results:

The ability of MS360 to prevent the attachment and biofilm formation by *Listeria monocytogenes* on stainless steel three weeks after being applied to the coupons was impressive. Photos 1 and 2 show turbidity and a *Listeria* biofilm covered coupon for the untreated sample. Photos 3 and 4 show the stainless steel coupon in the minimal medium with no visible biofilm on the MS360 treated coupon and no turbidity in the medium, indicating little if any growth.

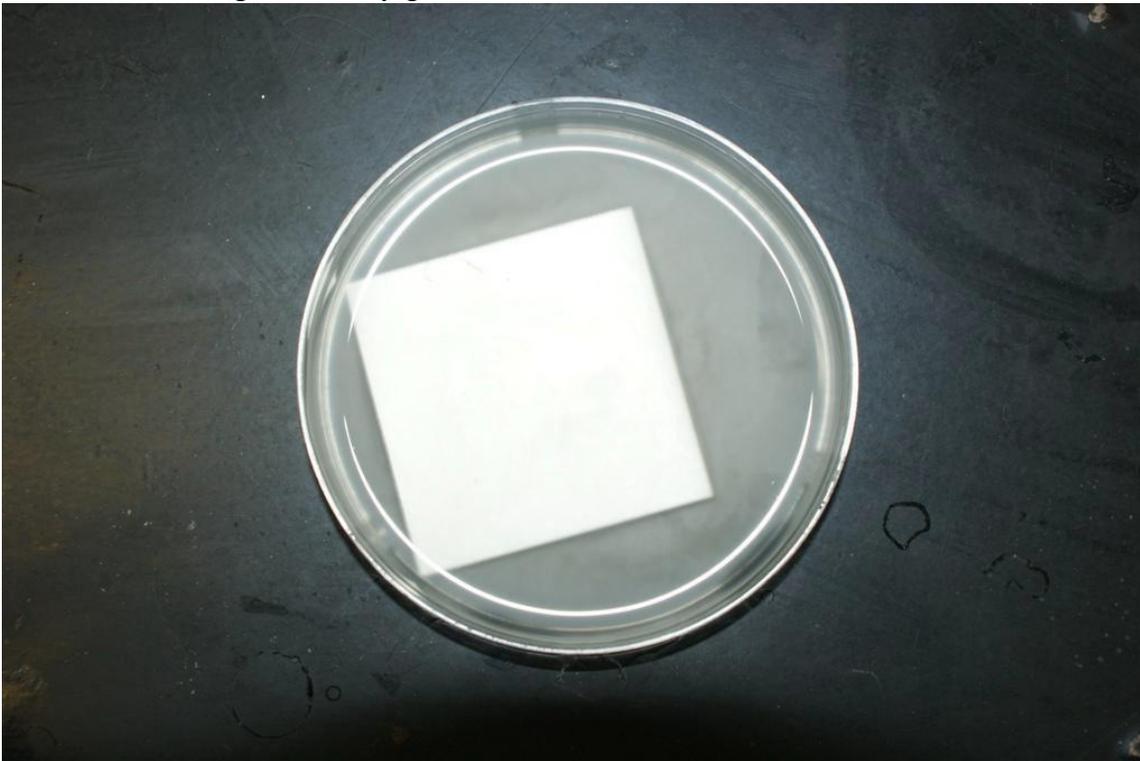


Photo 1. Stainless steel coupon control (untreated), showing significant *Listeria* growth and biofilm formation.

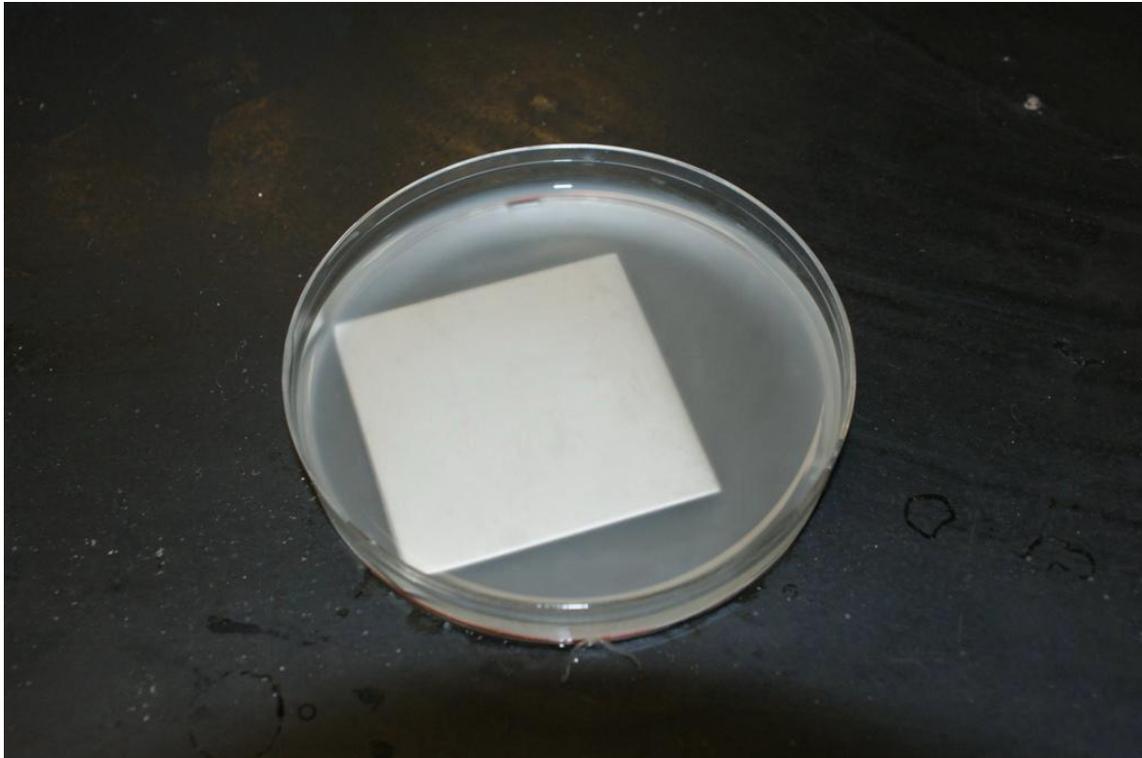


Photo 2. Stainless steel coupon control (untreated), showing significant *Listeria* growth and biofilm formation.

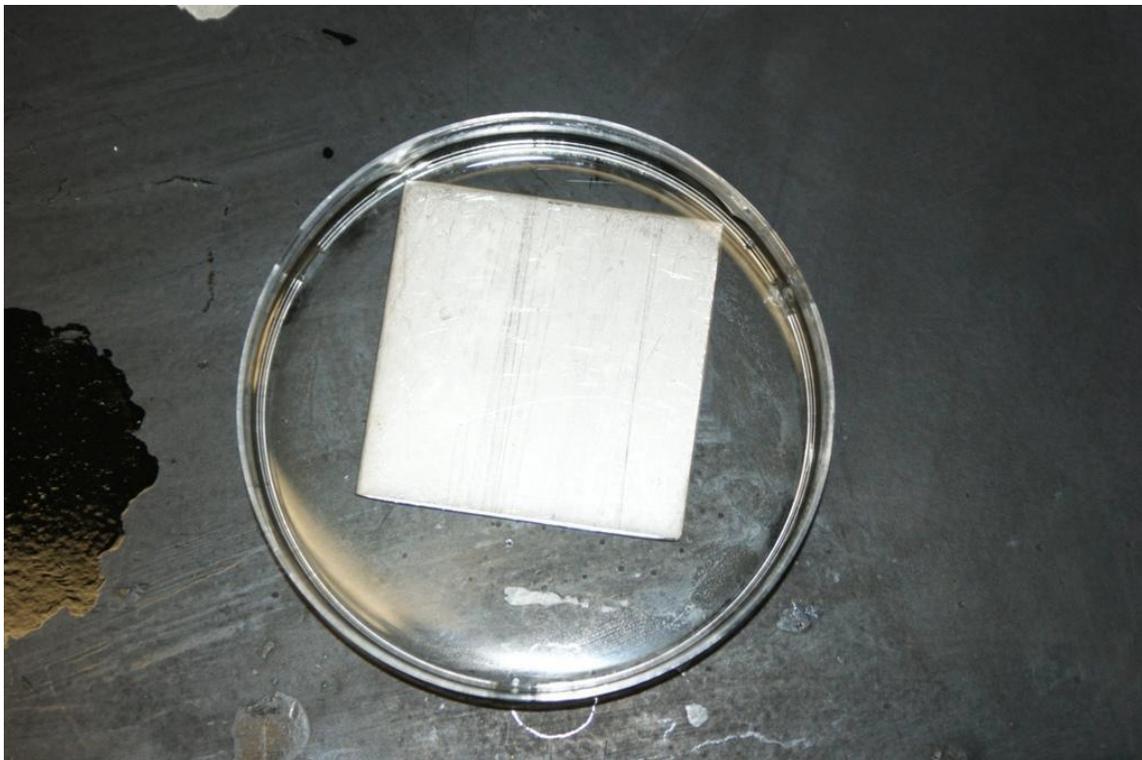


Photo 3. Stainless steel coupon treated with MS360, showing no *Listeria* growth or biofilm formation.

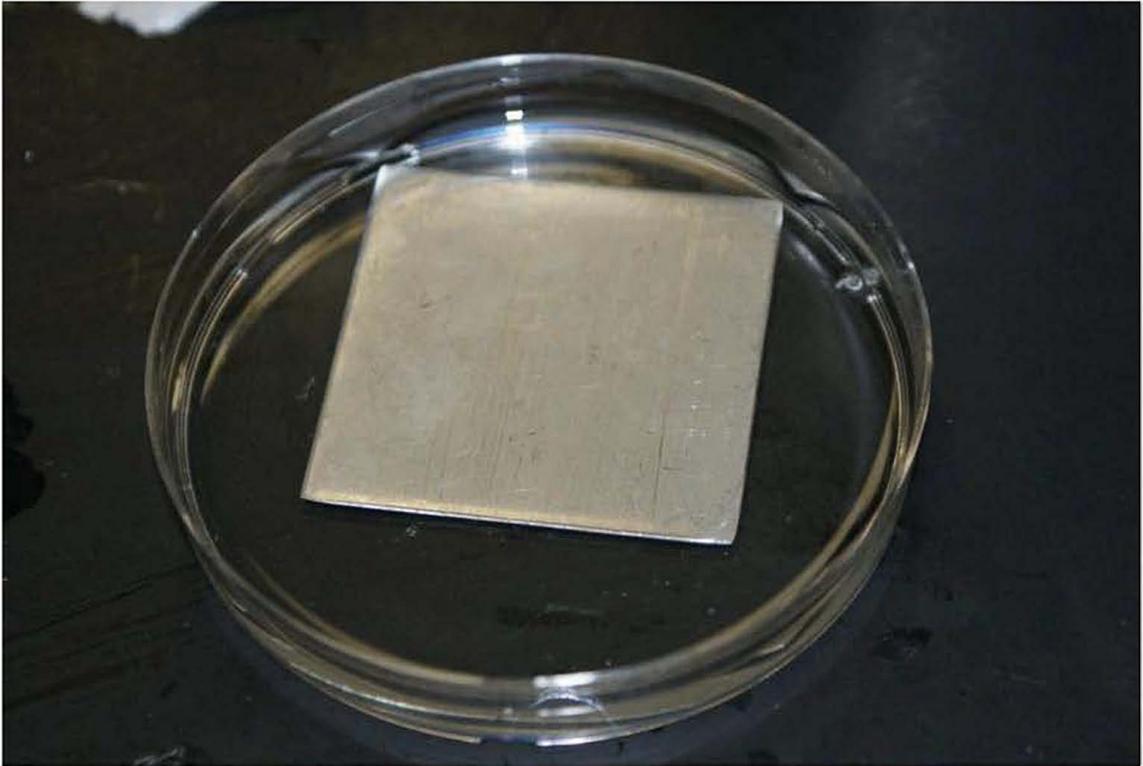
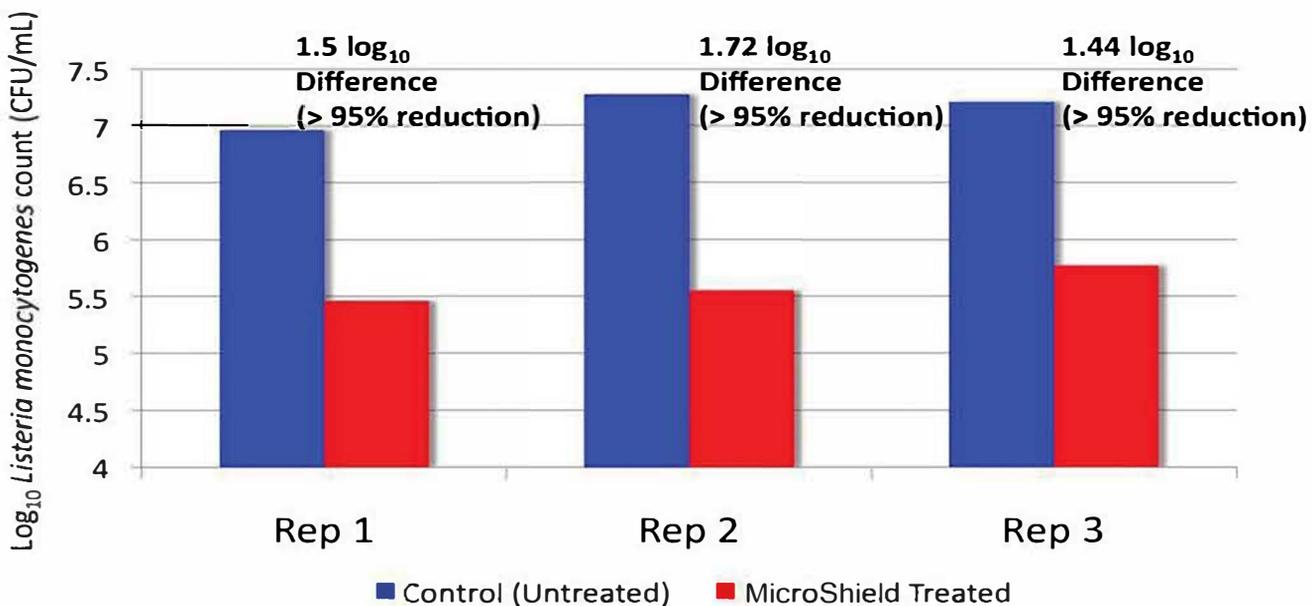


Photo 4. Stainless steel coupon treated with MS360, showing no *Listeria* growth or biofilm formation.

The data obtained in this study may be found in Appendix A (raw data) and summarized in Figure 1.

The effect of coating steel coupons with MS360 on the ability of *Listeria monocytogenes* for form biofilms



In repetition 1, coating the stainless steel coupon significantly ($P \leq 0.05$) lowered *Listeria monocytogenes* numbers recovered after attempting to form a biofilm by 1.5 \log_{10} (>95%) colony forming units (CFU)/mL of recovery rinse. In repetition 2, *Listeria* was lowered by 1.72 \log_{10} (>95%) and in repetition 3, *Listeria* was lowered by 1.44 \log_{10} (~95%). It is important to note that in this experimental trial, extremely high numbers of *Listeria* were used to challenge the ability of MS360 to perform (~1,000,000 cells/0.1 mL of inoculum placed into solution with each coupon). In the real world, only a few *Listeria* cells would be traveling on a fomite (such as a dust particle) and would be transmitted to the surface. Theoretically, the effect of MS360 would be much greater in terms of preventing biofilm formation by *Listeria* on stainless steel surfaces when the bacterial challenge is much lower than was used in this experiment. For a study such as this, a reduction of 1 \log_{10} would be considered of practical significance and would be highly useful in an industrial setting for prevention of *Listeria* biofilms in food processing facilities and, thereby, preventing extremely costly recalls.

It is important to note that three weeks elapsed between the time when the MS360 was coated onto the coupons and when the coupons were exposed to bacteria, demonstrating a powerful residual effect. Also, MS360 was not only able to control *Listeria* growth and biofilm formation on the coupon, but was also able to prevent growth of *Listeria* in the surrounding medium, as indicated by the photos. MS360 represents a significant step forward in terms of allowing food processors to meet the zero tolerance requirement of USDA-FSIS for fully cooked foods such as deli meats, hot dogs, and chicken nuggets. This product would be very useful for companies that wish to prevent *Listeria* biofilm formation in their facilities.

coupons after allowing them to form biofilms for treated (MS360) versus untreated (controls).

MS360 Coupon Coating Study

Sample	Rep	Count	Dilution	Actual Count	Log10
C1	1	61	5.00	6100000	6.785329835
C2	1	66	5.00	6600000	6.819543936
C3	1	50	5.00	5000000	6.698970004
C4	1	88	5.00	8800000	6.944482672
C5	1	56	5.00	5600000	6.748188027
C6	1	92	5.00	9200000	6.963787827
C7	1	46	5.00	4600000	6.662757832
C8	1	29	6.00	29000000	7.462397998
C9	1	15	6.00	15000000	7.176091259
C10	1	21	6.00	21000000	7.322219295
					6.958376868
C11	2	116	5.00	11600000	7.064457989
C12	2	15	6.00	15000000	7.176091259
C13	2	24	6.00	24000000	7.380211242
C14	2	57	6.00	57000000	7.755874856
C15	2	24	6.00	24000000	7.380211242
C16	2	101	5.00	10100000	7.004321374
C17	2	29	6.00	29000000	7.462397998
C18	2	84	5.00	8400000	6.924279286
C19	2	16	6.00	16000000	7.204119983
C20	2	25	6.00	25000000	7.397940009
					7.274990524
C21	3	15	6.00	15000000	7.176091259
C22	3	16	6.00	16000000	7.204119983
C23	3	90	5.00	9000000	6.954242509
C24	3	33	6.00	33000000	7.51851394
C25	3	20	6.00	20000000	7.301029996
C26	3	16	6.00	16000000	7.204119983
C27	3	20	6.00	20000000	7.301029996
C28	3	24	6.00	24000000	7.380211242
C29	3	69	5.00	6900000	6.838849091
C30	3	15	6.00	15000000	7.176091259
					7.205429926

Sample	Rep	Count	Dilution	x20 CM sq	Count	Log10
T1	1	17	3.00		340	340000 5.531478917
T2	1	18	3.00		360	360000 5.556302501
T3	1	52	2.00	NA		5200 3.716003344
T4	1	18	3.00		360	360000 5.556302501
T5	1	22	3.00		440	440000 5.643452676
T6	1	18	3.00		360	360000 5.556302501
T7	1	24	3.00		480	480000 5.681241237
T8	1	26	3.00		520	520000 5.716003344
T9	1	48	3.00		960	960000 5.982271233
T10	1	25	3.00		500	500000 5.698970004 5.463832826
T11	2	161	3.00	NA		161000 5.206825876
T12	2	19	3.00		380	380000 5.579783597
T13	2	60	3.00	NA		60000 4.77815125
T14	2	30	3.00		600	600000 5.77815125
T15	2	12	3.00		240	240000 5.380211242
T16	2	40	3.00		800	800000 5.903089987
T17	2	21	3.00		420	420000 5.62324929
T18	2	14	3.00		280	280000 5.447158031
T19	2	40	3.00		800	800000 5.903089987
T20	2	42	3.00		840	840000 5.924279286 5.55239898
T21	3	42	3.00		840	840000 5.924279286
T22	3	49	3.00		980	980000 5.991226076
T23	3	55	3.00	NA		55000 4.740362689
T24	3	29	3.00		580	580000 5.763427994
T25	3	25	3.00		500	500000 5.698970004
T26	3	48	3.00		960	960000 5.982271233
T27	3	44	3.00		880	880000 5.944482672
T28	3	36	3.00		720	720000 5.857332496
T29	3	32	3.00		640	640000 5.806179974
T30	3	52	3.00		1040	1040000 6.017033339 5.772556576

This document was provided by Clearstream Technologies LLC as an accurate representation of the performance of the proprietary active ingredient in MicroShield 360