MICROBIOTEST PROTOCOL

EFFICACY EVALUATION OF RESIDUAL SELF-SANITIZING ACTIVITY OF A COPPER ENHANCED HARD SURFACE SUPPLEMENTAL

Testing Facility
MICROBIOTEST
A Division of Microbac Laboratories, Inc.
105 Carpenter Drive
Sterling, VA 20164

Prepared for
Cupron Inc.
Suite 123
800 East Leigh Street
Richmond, VA 23219

January 3, 2012

AM

OBJECTIVE:

This test is designed to substantiate effectiveness claims for a substance containing copper with sanitizing claims intended to be registered with the Environmental Protection Agency as an inanimate hard surface other than those that come in contact with food or beverages. The test is consistent with the EPA Test Method for Residual Self-Sanitizing Activity of Copper Alloy Surfaces.

TESTING CONDITIONS:

Initial sets of test and control surfaces (four replicates each per microorganism) representing new surfaces will be inoculated with *Pseudomonas aeruginosa*, Methicillin Resistant *Staphylococcus aureus*, and *Escherichia coli* O157:H7, held for the stipulated contact time, transferred to a neutralizing solution and mixed. Dilutions of the neutralizer will be plated, incubated and observed for growth. The initial percent reduction of the new surfaces will be calculated. Two lots of the test surfaces will be evaluated.

Additional sets of test and control surfaces (four replicates each per microorganism) will be subjected to multiple wet and dry wears and reinoculation cycles. Following the multiple insults, the carriers will be inoculated with the challenge microorganisms, dried and held for the stipulated contact time, transferred to a neutralizing solution and mixed. Dilutions of the neutralizer will be plated, incubated and observed for growth. The percent reduction of the worn/insulted surfaces will be calculated to demonstrate the effectiveness of the surface as a sanitizer during normal use in between surface cleanings. Two lots of the test surfaces will be evaluated.

MATERIALS:

A. Test materials supplied by the sponsor: (see last page for details).

Test carriers:	25 mm x 25 mm
Control carriers:	25 mm x 25 mm (containing no active)

The test materials will be tested as supplied by the sponsor unless directed otherwise by written instructions. All operations performed on the materials such as specialized storage conditions must be specified by the sponsor before initiation of testing.

The sponsor assures MICROBIOTEST, a Division of Microbac Laboratories, Inc. (MICROBIOTEST) testing facility management that the materials have been appropriately tested for identity, strength, purity, stability, and uniformity as applicable.

MICROBIOTEST will retain all unused materials for at least three months after completion of the test, then return them to the sponsor of the study or discard them in a manner that meets the approval of the safety officer of the laboratory.

B. Materials supplied by MICROBIOTEST including but not limited to:

1. Challenge microorganisms, required by EPA and the sponsor:
   a. *Pseudomonas aeruginosa*, ATCC 15442
   b. Methicillin Resistant *Staphylococcus aureus* (MRSA), ATCC 33592
   c. *Escherichia coli* O157:H7, ATCC 35150

2. Media and reagents:
   a. Tryptic Soy Broth (TSB)
   b. Neutralizer: 2X Letheen Broth
   c. Tryptic Soy Agar (TSA)
   d. Heat-inactivated Fetal Bovine Serum (FBS)
   e. Triton X-100 solution (1% solution)
   f. 70-85% Isopropyl alcohol (IPA)
   g. Sterile deionized water

3. Miscellaneous laboratory equipment and supplies, including:
   a. Gardco Washability and Wear Tester – Paul N. Gardner Co., Inc., Model #D10V, Catalog # WA-2153
   b. Abrasion Boat and Weights for Washability and Wear Tester – Paul N. Gardner Co., Inc., Catalog #'s WA-2225, WA-2227, and WA2210/P01
   c. Polyurethane Foam Liner – Foam Wipe wiper, VWR Catalog # TW-TX704
   d. Cotton Cloth – TexWipe Clean Cotton Wipers, VWR Catalog # TW-TX309
   e. Preval sprayer (or equivalent)

4. Media, reagents and supplies for Antimicrobial Susceptibility Testing of MRSA:
   a. TSA containing 5% defibrinated sheep's blood (TSA+)
   b. 0.85% NaCl (SS)
   c. Mueller Hinton Agar (MHA)
   d. Control microorganism: *Staphylococcus aureus*, ATCC 25923
   e. 0.5% McFarland Standard
   f. Caliper measuring device
   g. 1 µg Oxacillin disc

TEST SYSTEM IDENTIFICATION:

All test and control tube racks will be labeled with microorganism, test agent (if applicable) and project number prior to initiation of the study and during incubation. Petri dishes will be labeled with microorganism prior to initiation of the study and microorganism and project number during incubation.

EXPERIMENTAL DESIGN:

A. Inocula preparation:

Bacteria from stock cultures will be transferred into TSB and incubated at 35-37°C for 24±2 hours. Daily transfers will be made for at least three consecutive days (but no more than 10 days). For each transfer, tubes containing 10 mL of TSB will be inoculated using two loopfuls (4-mm inside diameter) of inoculum for each tube.

The pellicle formed in the *Pseudomonas aeruginosa* culture will be aspirated before use.

For all cultures: transfers more than 15 days away from the stock cultures will not be used for the inocula for the test.

For the initial and final sanitizer tests inoculum:

For each challenge microorganism, a 48-54 hour culture will be mixed on a vortex and allowed to stand for 15±1 minutes.

Addition of organic load: a 0.25 mL aliquot of FBS plus 0.05 mL1% Triton X-100 solution to 4.70 mL of bacteria suspension to yield a 5% FBS and 0.01% Triton X-100 soil load.

The upper two-thirds of each culture will be aspirated and used as the inoculum.

For the inoculation/reinoculations of the carriers used in the simulated wears tests:

For each challenge microorganism, an 18-24 hour culture will be mixed on a vortex and allowed to stand for 15±1 minutes. The upper two-thirds of each culture will be aspirated and used as the inoculum. Two 1:100 dilutions of the culture will be made using sterile deionized water (two 0.1 mL to 9.9 mL serial dilutions) and one final dilution of 5.0 mL of the diluted suspension to 5.0 mL of sterile deionized water.

Addition of organic load: a 0.25 mL aliquot of FBS plus 0.05 mL1% Triton X-100 solution to 4.70 mL of bacteria suspension to yield a 5% FBS and 0.01% Triton X-100 soil load.

Note: No culture will be allowed to stand with organic load longer than eight hours.

B. Test and Control Carrier preparation:

The test and control surfaces (carriers) will be cleaned by submersion in 70-85% in Isopropyl alcohol, rinsed with sterile deionized water, and allowed to air dry. After drying completely, the carriers will be steam sterilized for 15 minutes at 121°C. The carriers will be allowed to cool and held at ambient room temperature until use. Prior to use, each carrier will be aseptically transferred into plastic Petri dishes (one dish for each carrier) matted with two pieces of filter paper using sterile forceps.

For each lot of the test material, per microorganism, two sets of with four replicate carriers per set will be prepared along with two sets per microorganism of the control material with four replicate carriers each for the primary aspects of the test. Additional surfaces will be prepared as required for remaining controls.

C. Initial Sanitizer Evaluation Test:

For each lot of the test surface, per microorganism, four carriers and four control surface carriers (per microorganism) will be inoculated at staggered intervals with 10 µL (0.01 mL) of the prepared initial sanitizer inoculum using a calibrated pipette. The inoculum will be spread to within approximately 1/8" of the edge of the carrier and the carriers will be allowed to dry for 30-40 minutes at 35-37°C, at a 38-42% relative humidity (RH).

Immediately after drying, the 120 minute contact time (exposure period) will begin at ambient temperature.

At the conclusion the 120 minute contact time, each carrier will be transferred to a jar containing 30 mL of neutralizer at the appropriate staggered intervals. Each jar will be sonicated for 20±2 seconds. The samples will then be mixed on an orbital shaker for 3-4 minutes at 250 rpm. Within one hour after sonication, serial dilutions will be prepared using sterile deionized water (10^{-1} – 10^{-4}). Duplicate 1.0 mL aliquots from each jar/dilution (10^{0} – 10^{-2}) will be plated using TSA pour plates. Duplicate 1.0 mL aliquots from each jar/dilution (10^{1} – 10^{-4}) for the control carriers will be plated using TSA pour plates.

Note: All dilutions and plating for each replicate carrier will be performed within one hour of the transfer into the neutralizer.

All test plates will be incubated for 48±4 hours at 35-37°C, colonies will be counted and CFU/carrier calculated.

D. Simulated Wear and Reinoculation:

Prior to inoculation, the abrasion tester will be set to a speed of 2.25 – 2.50 for a total surface contact time of approximately 4-5 seconds for one complete cycle. The speed will be measured with a calibrated stopwatch. The machine's cycle will be calibrated by adjusting the number counter to 1, 5, 10, and 20 and verifying cycle time. It will be set so that one pass on the abrasion tester with the surfaces is equal to a contact time of approximately 2-seconds.

A wear cycle will equal one pass to the left and a return pass to the right on the Gardner scrubber with an abrasion boat fitted with a foam liner and dry cotton cloth. The fully-assembled abrasion boat will consist of two weights, a foam liner and a cotton cloth. It will be assembled in an aseptic manner. The weight of the fully-assembled weight boat will be verified to weigh 1084 ±1g prior to use.

For each lot of the test surface, per microorganism, four carriers will be inoculated at staggered intervals with 10 µL (0.01 mL) of the prepared simulated wears inoculum using a calibrated pipette. The inoculum will be spread to within approximately 1/8" of the edge of the carrier and the carriers will be allowed to dry for 30-40 minutes at 35-37°C. These inoculation and drying procedures will be designated as "reinoculated and drying".

To initiate the wear cycles, each carrier will be subjected to a dry wear cycle using the Gardco Washability and Wear Tester and the fully-assembled weight boat.

At least 15 minutes after the initial wear cycle; each carrier will be reinoculated and dried as previously described.

Each carrier will then be subjected to a wet wear cycle using the Gardco Washability and Wear Tester and the fully-assembled weight boat. The fully-assembled weight boat is sprayed for one second with sterile deionized water using a Preval sprayer (or equivalent) from a distance of 75±1 cm for not more than one second.

At least 15 minutes after this secondary wear cycles, each carrier will be reinoculated and dried, and subjected to alternating dry and wet wears until a total of 11 reinoculations and 12 wear cycles have been performed in accordance with the procedures and timeline outlined in Table 1 on the following page.

Note: The surface holder on the Gardner apparatus will be decontaminated with 70% IPA between each set of surface wears to prevent carryover contamination. The IPA will be allowed to completely evaporate before proceeding. The foam liner and the cotton cloth will be replaced between each set of surface wears.

Table 1: Wear and Reinoculation Procedure
1. Initial inoculation and drying
2. Wear cycle with dry cloth (wear #1)
3. Reinoculation and drying
4. Wear cycle with moist cloth (wear #2)
5. Reinoculation and drying
6. Wear cycle with dry cloth (wear #3)
7. Reinoculation and drying
End of first day
8. Wear cycle with moist cloth (wear #4)
9. Reinoculation and drying
10. Wear cycle with dry cloth (wear #5)
11. Reinoculation and drying
12. Wear cycle with moist cloth (wear #6)
13. Reinoculation and drying
14. Wear cycle with dry cloth (wear #7)
15. Reinoculation and drying
16. Wear cycle with moist cloth (wear #8)
17. Reinoculation and drying
18. Wear cycle with dry cloth (wear #9)
19. Reinoculation and drying
20. Wear cycle with moist cloth (wear #10)
21. Reinoculation and drying
22. Wear cycle with dry cloth (wear #11)
23. Reinoculation and drying
24. Wear cycle with moist cloth (wear #12)
Final Sanitizer Evaluation is performed after the 12th wear cycle and <u>two days</u> after the initial inoculation

E. Final Sanitizer Evaluation: (Performed at least two days after the initial inoculation to the Simulated Wear and Reinoculation procedures)

For each lot of the test surface, per microorganism, four carriers and four control surface carriers (per microorganism) will be inoculated at staggered intervals with 10 µL (0.01 mL) of the prepared final sanitizer inoculum using a calibrated pipette. The inoculum will be spread to within approximately 1/8" of the edge of the carrier and the carriers will be allowed to dry for 30-40 minutes at 35-37°C, at a 38-42% relative humidity (RH).

Immediately after drying, the 120 minute contact time (exposure period) will begin at ambient temperature.

At the conclusion the contact time, each carrier will be transferred to a jar containing 30 mL of neutralizer at the appropriate staggered intervals. Each jar will be sonicated for 20±2 seconds. The samples will then be mixed on an orbital shaker for 3-4 minutes at 250 rpm. Within one hour after sonication, serial dilutions will be prepared using sterile deionized water ($10^{-1} - 10^{-4}$). Duplicate 1.0 mL aliquots from each jar/dilution ($10^{0} - 10^{-2}$) for the test carriers will be plated using TSA pour plates. Duplicate 1.0 mL aliquots from each jar/dilution ($10^{1} - 10^{-4}$) for the control carriers will be plated using TSA pour plates.

Note: All dilutions and plating for each replicate carrier will be performed within one hour of the transfer into the neutralizer.

All test plates will be incubated for 48±4 hours at 35-37°C, colonies will be counted and CFU/carrier calculated.

For *Enterobacter aerogenes*: Plates will be incubated for 48±4 hours at 25-30°C, colonies will be counted and CFU/carrier calculated.

F. Controls:

1. Culture purity control:

Each prepared culture will be streaked for isolation using TSA (initial and final sanitizer inocula preparations as well as each Simulated Wear and Reinoculation inocula (two, one for each day of the two day regimen)). All plates will be incubated with the test plates. The isolated cultures will be observed for purity.

2. Organic soil sterility control:

Duplicate 1.0 mL aliquots of the prepared organic soil will be plated in TSA pour plates. This will be performed on each of the following days of the assay: the initial and final sanitizer days as each of the two day Simulated Wear and Reinoculation procedures. The plates will be incubated with the test plates and observed for growth or no growth.

3. Inoculum confirmation counts control:

Each prepared inoculum will be serially diluted using PBS and selected dilutions will be plated in duplicate using TSA pour plates. This will be performed on each of the following days of the assay: the initial and final sanitizer days and each of the two day Simulated Wear and Reinoculation procedures. All plates will be incubated with the test plates.

4. Neutralizer sterility control:

A single jar of containing the neutralizer will be incubated for 48±4 hours at 35-37°C. The neutralizer will be observed for growth or no growth.

5. Carrier sterility control:

An uninoculated test (per lot) and control carrier will be subcultured into independent jars containing the neutralizer and incubated for 48±4 hours at 35-37°C. The neutralizer will be observed for growth or no growth.

6. Carrier viability control:

For each challenge microorganism, a single inoculated control carrier will be subcultured into a jar containing the neutralizer and incubated in the same with the test plates (this control will be done for both the initial and final sanitizer test days). The neutralizer jars will be observed for growth or no growth.

7. Neutralizer effectiveness control:

The neutralization efficacy will be evaluated for each challenge microorganism concurrently with the testing. Using sterile forceps, sterile carriers (one replicate for each of the three test lots and one replicate of the control surface) will be transferred into jars containing 30 mL of neutralizer. At time intervals after each surface addition, an aliquot of the bacterial suspension (to yield approximately 1,000 CFU) will be added and the jars will be mixed. At 5±1 minutes, a 1.0 mL aliquot will be removed from each jar and plated using TSA pour plates.

These procedures will be repeated using additional dilutions (to yield approximately 500 CFU and 250 CFU).

All plates will be incubated with the initial sanitizer test plates.

9. Antimicrobial Susceptibility Testing of MRSA:

The prepared MRSA culture will be subcultured onto a TSA+ plate and the plate will be incubated for approximately 24 hours at 35-37°C. Following incubation, a suspension will be prepared by suspending growth from the TSA+ culture in SS to yield equivalent turbidity to a 0.5 McFarland Standard. This prepared suspension will be streaked onto MHA plate in a cross-hatch pattern and a 1 µg Oxacillin disc will be placed onto the center of the plate. The plate will be inverted and incubated for ≥ 24 hours at 35-37°C.

The same procedures will be conducted concurrently using the control microorganism, *Staphylococcus aureus*, ATCC 25923 to confirm the validity of the assay.

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The interpretation of the zone of inhibitions (ZOI) will be based on established National Committee for Clinical Laboratory Standards (NCCLS) performance standards. As currently published, (NCCLS standard M100-S21) ZOI breakpoints must be ≤ 10 mm (rounded to the nearest whole mm) confirms resistance, 11-12 mm is considered intermediate resistance, and ≥ 13 mm confirms susceptibility.

8. Microorganism confirmation procedures:

A randomly selected colony from the carrier quantitation control plates, and if applicable, a randomly selected colony from a test plate will be confirmed by colony morphology and Gram stain according to extant SOPs. The same procedures will be performed using the culture purity control plates and the result regarding purity will be documented as well.

TEST ACCEPTANCE CRITERIA:

The test will be acceptable for evaluation of the test results if the neutralizer is effective and non-toxic. The study director may consider other causes that may affect test reliability and acceptance. There are no proposed statistical methods for this test.

- The average recovery for the control surfaces must be at least 2.0×10^4 CFU/carrier for both the Initial and Final Sanitizer Test evaluations.
- The CFU recovered for the test surfaces and the control surfaces for the neutralizer effectiveness controls (per concentration of inoculum evaluated) should be within 1.0 $\log_{10}$.
- The carrier sterility controls must exhibit no growth.
- The carrier viability controls must exhibit growth.
- The purity controls must demonstrate pure cultures.
- The organic soil sterility controls must exhibit no growth.
- The neutralizer sterility controls must exhibit no growth.
- For the Antimicrobial Susceptibility Testing: the test MRSA strain must exhibit resistance and the *Staphylococcus aureus* control strain (ATCC 25923) must exhibit susceptibility to Oxacillin.

PRODUCT EVALUATION CRITERIA:

According to EPA guidelines, the test agent meets effectiveness requirements, if the test results exhibit a bacterial reduction of at least 99.9% over the Carrier Quantitation Control for both the Initial and Final Sanitizer evaluations.

DATA PRESENTATION:

The final report will include the following information in tabular form:

- The average colony-forming units (CFU)/carrier and percent reduction for each evaluation.
- The results for all the controls.

CONFIDENTIALITY:

All data generated at MICROBIOTEST are held in strictest confidence and are available only to the sponsor. In turn, no reference to the work, data, or MICROBIOTEST may be made public without the written consent of MICROBIOTEST.

REPORT FORMAT:

MICROBIOTEST employs a standard report format for each test design. Each final report provides the following information:

- Sponsor identification
- Test agent identification
- Type of test and project number
- Interpretation of results and conclusions
- Test results in tabular form
- Methods and evaluation criteria
- Quality Assurance and Compliance Statements

PERSONNEL AND TESTING FACILITIES:

A study director will be assigned prior to initiation of the test. Resumes for the technical personnel are maintained and are available on request. This study will be conducted in the Applied Microbiology Laboratory at MICROBIOTEST, 105 Carpenter Drive, Sterling, Virginia 20164.

RECORDS TO BE MAINTAINED:

All raw data, protocol, protocol modifications, test agent records, final report, and correspondence between MICROBIOTEST and the sponsor will be stored in the archives at MICROBIOTEST, 105 Carpenter Drive, Sterling, Virginia 20164 or in a controlled facility off site.

All changes or revisions to this approved protocol will be documented, signed by the study director, dated and maintained with this protocol. The sponsor will be notified of any change, resolution, and impact on the study as soon as practical.

The proposed experimental start and termination dates; additional information about the test agent; challenge microorganism used; media and reagent identification; and the type of neutralizers employed in the test will be addressed in a project sheet issued separately. The date the study director signs the protocol will be the initiation date. All project sheets will be forwarded to the study sponsor.

MISCELLANEOUS INFORMATION:

The following information is to be completed by sponsor before initiation of study:

A. Name and address: Cupron Inc.
Suite 123
800 East Leigh Street
Richmond, VA 23219

B. Test surface name*: CUPRON ENHANCED EOS HARD SURFACE GREY

Active ingredient: Copper oxide

Lot No. 1: _____

Lot No. 2: _____

Contact time: 120 minutes
Note: the same contact time will be used for the Initial and Final Sanitizer evaluations

Exposure temperature: Ambient room temperature 20±1C

*Note: the sponsor will also provide control surfaces that will not contain any antimicrobial active ingredient (Cupron Control Hard Surfaces).

C. Organic load – serum added to achieve 5% in the inoculum: ■ yes ☐ no

D. Precautions/storage – MSDS or certificate of analysis provided: ☐ yes ■ no

REPORT HANDLING: The sponsor intends to submit this information to: ■ US EPA ☐ US FDA ☐ Health Canada ☐ CAL DPR ☐ ARTG ☐ other: Internal Purposes

STUDY CONDUCT: ■ GLP ☐ non-GLP

PROTOCOL APPROVAL:

Sponsor Signature: _____ Date: 1/27/12
Alastair B. Monk, PhD

Study Director Signature: _____ Date: 02/29/12
Angela L. Hollingsworth

Date Issued: 03/12/12 Project Sheet No. 1 Page No. 1 Laboratory Project Identification No. 619-114

STUDY TITLE: EFFICACY EVALUATION OF RESIDUAL SELF-SANITIZING ACTIVITY OF A COPPER ENHANCED HARD SURFACE - SUPPLEMENTAL	**STUDY DIRECTOR:** Angela L. Hollingsworth Signature 03/12/12 Date		
TEST AND CONTROL ARTICLES:	**LOT NO:**	**DATE RECEIVED:**	**DS NO.:**
Cupron Enhanced EOS Hard Surface Grey	05012064	03/07/12	C133
Cupron Enhanced EOS Hard Surface Grey	05112024	03/07/12	C134
Cupron Control Hard Surface	Not applicable	03/02/12 & 03/07/12	C122
PERFORMING DEPARTMENT(S): Applied Microbiology Laboratory	**STORAGE CONDITIONS:** Location: F4 ■ Dark ■ Ambient Room Temperature ☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:		

PROTECTIVE PRECAUTION REQUIRED: MSDS ☐Yes / ■ No

PHYSICAL DESCRIPTION: ■Solid ☐ Liquid ☐ Aerosol ☐ Other:

PURPOSE: See attached protocol. **AUTHORIZATION:** See client signature.

PROPOSED EXPERIMENTAL START DATE: 03/16/12 **TERMINATION DATE:** 03/20/12

CONDUCT OF STUDY: ☐ FDA ■ EPA ☐ R&D ■GLP ☐ GCP ☐ Other:

SPONSOR:	Cupron Inc. 800 East Leigh Street, Suite 123 Richmond, VA 23219	**CONTACT PERSON:** Phone: E-mail:	Alastair B. Monk, PhD 804-381-5514 amonk@cupron.com

TEST CONDITIONS:

Challenge organism(s):	*Pseudomonas aeruginosa*, ATCC 15442 Methicillin Resistant *Staphylococcus aureus* (MRSA), ATCC 33592 *Escherichia coli* O157:H7, ATCC 35150
Active ingredient(s):	Copper oxide
Neutralizer(s):	Letheen Broth – 2X
Contact Time(s):	120 minutes (Initial & Final Sanitizer Evaluations)
Contact Temperature(s):	Ambient (20±1°C)
Organic Load:	■ Yes / ☐ No (Per the protocol)
Incubation Time(s):	48±4 hours
Incubation Temperature(s):	35-37°C

Comments: The Miscellaneous Information section of the protocol did not include the specific lot numbers for the test and control articles. These identifiers are outlined above.

Date Issued: 03/17/12 Project Sheet No. 2	Page No. 1 Laboratory Project Identification No. 619-114		
STUDY TITLE: EFFICACY EVALUATION OF RESIDUAL SELF-SANITIZING ACTIVITY OF A COPPER ENHANCED HARD SURFACE - SUPPLEMENTAL	**STUDY DIRECTOR:** Angela L. Hollingsworth Signature 03/17/12 Date		
TEST AND CONTROL ARTICLES:	**LOT NO:**	**DATE RECEIVED:**	**DS NO.:**
Cupron Enhanced EOS Hard Surface Grey	05012064	03/07/12	C133
Cupron Enhanced EOS Hard Surface Grey	05112024	03/07/12	C134
Cupron Control Hard Surface	Not applicable	03/02/12 & 03/07/12	C122
PERFORMING DEPARTMENT(S): Applied Microbiology Laboratory	**STORAGE CONDITIONS:** Location: F4 ■ Dark ■ Ambient Room Temperature ☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:		
CONDUCT OF STUDY: ☐ FDA ■ EPA ☐ R&D ■GLP ☐ GCP ☐ Other:			
SPONSOR: Cupron Inc. 800 East Leigh Street, Suite 123 Richmond, VA 23219	**CONTACT PERSON:** Alastair B. Monk, PhD Phone: 804-381-5514 E-mail: amonk@cupron.com		

EXPLANATION:

Protocol Amendment(s):

1. In reference to the plating procedures outlined in Section C (Initial Sanitizer Evaluation Test) and Section E (Final Sanitizer Evaluation) of the protocol. To clarify, after the samples were mixed on the orbital shaker for 3-4 minutes at 250 rpm, serial dilutions were prepared using sterile deionized water. Duplicate 1.0 mL aliquots from each jar/dilution ($10^0 – 10^{-2}$) for the test carrier samples were plated using TSA plates. For the control carriers, duplicate 1.0 mL aliquots from the $10^{-2} – 10^{-4}$ dilutions were plated using TSA plates. In addition, the samples were diluted and plated within one of the transfer into the neutralizer; the reference to within one hour of sonication should be disregarded.

2. In reference to Section F (Controls), part 3 (Inoculum Confirmation Counts) section of the protocol. The diluent defined in this section inadvertently indicates the use of PBS whereas the procedure requires the use of sterile deionized water, the same diluent used for all of the test and remaining control procedures.

3. In reference to Section E (Final Sanitizer Evaluation) of the protocol. Incubation parameters for *Enterobacter aerogenes* are inadvertently defined. This reference should be disregarded.

Date Issued: 03/28/12 Project Sheet No. 3 Page No. 1 Laboratory Project Identification No. 619-114			
STUDY TITLE: EFFICACY EVALUATION OF RESIDUAL SELF-SANITIZING ACTIVITY OF A COPPER ENHANCED HARD SURFACE - SUPPLEMENTAL	**STUDY DIRECTOR:** Angela L. Hollingsworth Signature 03/28/12 Date		
TEST AND CONTROL ARTICLES:	**LOT NO:**	**DATE RECEIVED:**	**DS NO.:**
Cupron Enhanced EOS Hard Surface Grey	05012064	03/07/12	C133
Cupron Enhanced EOS Hard Surface Grey	05112024	03/07/12	C134
Cupron Control Hard Surface	Not applicable	03/02/12 & 03/07/12	C122
PERFORMING DEPARTMENT(S): Applied Microbiology Laboratory	**STORAGE CONDITIONS:** Location: F4 ■ Dark ■ Ambient Room Temperature ☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:		
CONDUCT OF STUDY: ☐ FDA ■ EPA ☐ R&D ■GLP ☐ GCP ☐ Other:			
SPONSOR: Cupron Inc. 800 East Leigh Street, Suite 123 Richmond, VA 23219	**CONTACT PERSON:** Alastair B. Monk, PhD Phone: 804-381-5514 E-mail: amonk@cupron.com		
CO-SPONSOR: EOS Surfaces, L.L.C. PO BOX 4146 Portsmouth, VA 23701	**CONTACT PERSON:** Kenneth G. Trinder, II Phone: 757-393-3671, ext. 4 E-mail: kgt@eos-surfaces.com		

EXPLANATION:

Protocol Amendment(s):

4. At the request of the original sponsor, Cupron Inc., a co-sponsor, EOS Surfaces, L.L.C. will be added for reporting purposes. EOS Surfaces, L.L.C. will be identified in the final report however all authorizations affiliated with the protocol (Protocol Amendment(s) and/or Deviation(s)), with the exception of this Amendment will be approved by Alastair Monk, PhD of Cupron Inc.

Date Issued: 03/31/12 Project Sheet No. 4 Page No. 1 Laboratory Project Identification No. 619-114			
STUDY TITLE: EFFICACY EVALUATION OF RESIDUAL SELF-SANITIZING ACTIVITY OF A COPPER ENHANCED HARD SURFACE - SUPPLEMENTAL	**STUDY DIRECTOR:** Angela L. Hollingsworth Signature 03/31/12 Date		
TEST AND CONTROL ARTICLES:	**LOT NO:**	**DATE RECEIVED:**	**DS NO.:**
Cupron Enhanced EOS Hard Surface Grey	05012064	03/07/12	C133
Cupron Enhanced EOS Hard Surface Grey	05112024	03/07/12	C134
Cupron Control Hard Surface	Not applicable	03/02/12 & 03/07/12	C122
PERFORMING DEPARTMENT(S): Applied Microbiology Laboratory	**STORAGE CONDITIONS:** Location: F4 ■ Dark ■ Ambient Room Temperature ☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:		
CONDUCT OF STUDY: ☐ FDA ■ EPA ☐ R&D ■GLP ☐ GCP ☐ Other:			
SPONSOR: Cupron Inc. 800 East Leigh Street, Suite 123 Richmond, VA 23219	**CONTACT PERSON:** Alastair B. Monk, PhD Phone: 804-381-5514 E-mail: amonk@cupron.com		
CO-SPONSOR: EOS Surfaces, L.L.C. PO BOX 4146 Portsmouth, VA 23701	**CONTACT PERSON:** Kenneth G. Trinder, II Phone: 757-393-3671, ext. 4 E-mail: kgt@eos-surfaces.com		

EXPLANATION:

Protocol Amendment(s):

5. In reference to Section B on Page 5 of the protocol regarding the statement of "two sets of with four carriers per set will be prepared". This statement should have indicated "two sets with four carriers per set will be prepared".