United States Environmental Protection Agency

Test Method for Residual Self-Sanitizing Activity of Copper Alloy Surfaces

Test Organisms: *Staphylococcus aureus* (ATCC 6538)
Enterobacter aerogenes (ATCC 13048)
Pseudomonas aeruginosa (ATCC 15442)
Methicillin Resistant *Staphylococcus aureus* MRSA (ATCC 33592)
Escherichia coli O157:H7 (ATCC 35150)

Sanitizer efficacy testing must be conducted against *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048), before additional organisms or claims (residual self-sanitizing activity and continuous reduction) are considered. Acceptable efficacy testing is required against *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048) as a non-food contact sanitizer before additional microorganisms or claims can be granted. For claims of Continuous Reduction and/or Residual Self-Sanitizing Activity, initial efficacy testing against *Staphylococcus aureus* and *Enterobacter aerogenes* is required before additional microorganisms are granted.

Test System

Preparation of Test Surfaces: Cut each copper alloy into individual 1" x 1" square carriers. Stainless steel carriers (1" x 1") must be incorporated into the test system. Copper alloy surfaces will be utilized as the test surfaces and stainless steel squares as control carriers for this assay. Prepare the 1" x 1" stainless steel control surfaces for pre-cleaning by removing adhesive protective backings. Clean all metal surfaces with alcohol and rinse thoroughly in deionized water and allow to air dry. Sterilize carriers prior to use in test. After sterilization, place each carrier into a plastic Petri dish matted with two pieces of filter paper using sterile forceps.

Preparation of Test Organisms

Staphylococcus aureus, Pseudomonas aeruginosa, Methicillin Resistant *Staphylococcus aureus*: From stock cultures, inoculate tubes of the appropriate broth with organism, and incubate for 24±2 hours at 35-37°C. Using a 4-mm inside diameter disposable sterile plastic transfer loop, transfer at least three consecutive daily cultures in appropriate broth prior to use as inoculum. Transfer two (2) loopfuls of culture into 10 ml broth medium. Transfers more than 15 days away from stock culture should not be used for the inocula for this test. Use 48±4 hour cultures on for the inocula on the day of testing. On the day of use, aspirate pellicle from the *Pseudomonas aeruginosa* culture.

Enterobacter aerogenes: From stock cultures, inoculate tubes of Tryptic Soy Broth and incubate for 24±2 hours of 25-30°C. Using a 4-mm inside diameter disposable sterile plastic transfer loop, perform at least three consecutive daily transfers of cultures in Tryptic Soy Broth prior to use as inoculum. Transfer two (2) loopfuls of culture into 10 ml broth medium. Transfers more than 15 days away from stock culture should not used for the inocula for this test.

a) For the initial sanitizer and final sanitizer inoculum, vortex mix a 48-54 hour culture and allow to stand for 15±1 minutes. Add a volume of serum to equal 5% organic soil load containing Triton X-100 (to aid in spreading of the inoculum), (0.25 ml serum + 0.05 ml Triton X-100 + 4.70 ml bacteria suspension). Decant the upper two thirds of this suspension, and transfer to a sterile tube for use in testing.
b) For the inoculation and reinoculations of the carriers used in the simulated wear tests, use an 18-24 hour culture to ensure that no culture is allowed to stand with organic soil for longer than eight hours. Vortex mix an 18-24 hour culture, and allow to stand for 15±1 minutes. Perform two (2) 0.1 ml to 9.9 ml serial dilutions and one final dilution of 5.0 ml to 5.0 ± 0.2 ml in sterile deionized water. Add a volume of serum to equal 5% organic soil containing Trition X-100 (0.25 ml serum + 0.05 ml Triton X-100 + 4.70 ml bacteria suspension). Vortex mix the suspension, and allow to stand 15 ± 1 minutes before being used to inoculate.

Antimicrobial Susceptibility Testing (if applicable): Antimicrobial susceptibility testing is required when utilizing a resistant organism. On the day of testing, verify the antimicrobial resistance pattern of Methicillin Resistant *Staphylococcus aureus* (MRSA). Subculture the organism onto a Blood Agar plate (BAP), and incubate for approximately 24 hours at 35-37°C. Following incubation, make a suspension of the test organism equal to 0.5 McFarland Standard in 0.85% sterile saline. Streak the suspension onto Mueller Hinton agar. Place an oxacillin disc in the center of the inoculated Mueller Hinton plate. Invert and incubate for ≥ 24 hours at 35-37°C. Following incubation, measure the zone of inhibition using a calibrated caliper. Concurrently run *Staphylococcus aureus* (ATCC 25923), a control organism, with the test organism to confirm the validity of the assay. The interpretation of the zone of inhibition is based on established National Committee for Clinical Laboratory Standards (NCCLS) performance standards.

Initial Sanitizer Evaluation: Inoculate four (4) sterile carriers of each copper alloy and stainless steel control carrier with a 10 µl aliquot of the 48-54 hour "sanitizer" organism suspension at staggered intervals. Spread the inoculum to within ⅛ inch of the edge using a bent inoculating needle, and allow to dry for 30-40 minutes at 35-37°C, at a 38-42% relative humidity.

Immediately after drying, the 120 minute exposure period will begin at ambient temperature.

After the 120 minute exposure period, using sterile forceps, transfer the test or control surfaces, at the same staggered intervals used for inoculation, to 30 ml of Letheen broth (or appropriate neutralizer broth) in jars. Repeat this until all the test surfaces and control surfaces have been transferred.

Following the transfer, sonicate the neutralized samples for 20 ± 2 seconds in a sonicating waterbath. Mix the samples on an orbital shaker for 3-4 minutes at 250 rpm.

Serial dilute the test and control samples in 9.0 ± 0.1 ml of sterile deionized water. Serial dilutions (10^{-2} through 10^{-4} dilutions for the control samples and 10^{0} to 10^{-2} dilutions for the test samples) were made in duplicate and plated within approximately one hour of their transfer to the neutralizer broth.

Incubate plates at 35-37°C for *S. aureus* (other test microorganisms) and 25-30°C for *E. aerogenes* for 48±4 hours prior to evaluation. If possible, count and record the plates containing between 30 and 300 CFU. Determine the number of surviving organisms per carrier of each test and control sample by multiplying the number of recovered test organisms by the dilution factor and multiplying by 30 (to account for broth volume) and divide by the volume plated. The control plates must have minimum of 2×10^{4} CFU/carrier for a valid test.

Inoculation, Simulated Wear and Reinoculation of the Test and Control Surfaces:

Prior to carrier inoculation, set the abrasion tester to a speed of 2.25 to 2.5 for a total surface contact time of approximately 4-5 seconds for one complete cycle. Measure the speed with a calibrated stopwatch. Calibrate the machine's cycles by adjusting the number counter to 1, 5, 10, 20 and verifying cycle time. Provide one pass on the abrasion tester with the surfaces for a contact time of approximately 2 seconds. A wear cycle equals one pass to the left and a return pass to the right.

A minimum of fifteen minutes after the wear cycle, reinoculate each carrier as described above and dry at ambient temperature for at least 30 minutes.

Decontaminate the surface holder on the Gardner apparatus with absolute ethanol between each set of surface wears to prevent carryover contamination. Allow the alcohol to completely evaporate before proceeding. Replace the foam liner and the cotton cloth between each set of surface wears.

Alternate the wet-wears with the dry-wears. For the wet wear cycles, the boat assembly should include a new foam liner and dry cotton cloth sprayed with sterile deionized water, using a Preval sprayer, from a distance of 75±1 cm for not more than one second. Following the moistening of the cloth, place the abrasion boat on the scrubber and perform a wear cycle (one pass to the left and a return pass to the right).

The following table provides an overview of an example wear and reinoculation procedure, which includes 12 wear cycles. At least 24 hours pass between the initial inoculation and final sanitizer.

"Wear" and Re-inoculation Procedure
1. Initial inoculation with test organism
2. Wear cycle** with dry cloth (wear #1)
3. Reinoculation with test organism
4. Wear cycle with moist cloth (wear #2)
5. Reinoculation with test organism
6. Wear cycle with dry cloth (wear #3)
7. Reinoculation with test organism
End of first day
8. Wear cycle with moist cloth (wear #4)
9. Reinoculation with test organism
10. Wear cycle with dry cloth (wear #5)
11. Reinoculation with test organism
12. Wear cycle with moist cloth wear (wear #6)
13. Repeated until 12 wear cycles are completed (Day 2 ended after the 9th reinoculation)
14. Sanitizer test performed after the 12th wear cycle and 2 days after the initial inoculation

Final Sanitizer Test: Following the last wear cycle, inoculate each copper alloy and stainless steel control with a 10 µl aliquot of the 48-54 hour "sanitizer" organism suspension at staggered intervals. Spread the inoculum to within ⅛ inch of the edge using a bent inoculating needle, and allow to dry for 30-40 minutes at 35-37°C and 38-42% relative humidity.

Immediately after drying, the 120 minute exposure period begins at ambient temperature.

After the 120 minute exposure period, use sterile forceps to transfer the test or control surfaces, at the same staggered intervals used for inoculation, to 30 ml of Letheen broth (or appropriate neutralizer broth) in jars. Repeat this until all the test surfaces and control surfaces have been transferred.

Following the transfer, sonicate the neutralizer samples for 20 ± 2 seconds in a sonicating waterbath. Mix the samples on an orbital shaker for 3-4 minutes at 250 rpm.

Serial dilute the test and control samples in 9.0 ± 0.1 ml of sterile deionized water. Perform serial dilutions (10^{-2} through 10^{-4} dilutions for the control samples and 10^{0} to 10^{-2} dilutions for the test samples) in duplicate, and plate within approximately one hour of their transfer to the neutralizer broth.

Incubate plates at 35-37°C for *S. aureus* and 25-30°C for *E. aerogenes* for 48±4 hours prior to evaluation. Following incubation and incubation and storage, visually examine the subculture plates by counting plates containing between 30 and 300 CFU, if possible, and record.

Determine the number of surviving organisms per carrier of each test and control sample by multiplying the number of recovered test organisms by the dilution factor and multiplying by 30 (to account for broth volume) and dividing by the volume plated. The control plates must have minimum of 2×10^4 CFU/carrier for a valid test.

Study Controls

Inoculum Population Controls: Determine the concentration of the sanitizer test inoculum and each 24-hour re-inoculum by serially diluting in sterile deionized water and plating using standard microbiological techniques in duplicate to agar medium plates. Incubate the plates at 35-37°C for *S. aureus* and 25-30°C for *E. aerogenes* for 48±4 hours.

Purity Controls: Perform a "streak plate for isolation" on each organism culture and following incubation examine in order to confirm the presence of a pure culture. The acceptance criterion for this study control is a pure culture demonstrating colony morphology typical of the test organism.

Organic Soil Sterility Control: Culture, incubate, and visually examine the serum used for soil load. The acceptance criterion for this study control is lack of growth.

Carrier Sterility Control: Add a representative uninoculated test and control carrier to the neutralizing subculture medium. Incubate and examine the subculture medium containing each carrier. The acceptance criterion for this study control is lack of growth.

Neutralizing Subculture Medium Sterility Control: Incubate and visually examine a representative sample of uninoculated neutralizing subculture medium. The acceptance criterion for this study control is lack of growth.

Viability Control: Add a representative inoculated control carrier to the subculture medium. Incubate and visually examine the subculture medium containing the carrier for growth. The acceptance criterion for this study control is growth.

Neutralization Confirmation Control: Conduct neutralization efficacy concurrently with testing. Using sterile forceps, transfer sterile carriers (both control and test) to individual bottles containing 30 ml of the sterile neutralizer broth. At time intervals after each surface addition, add a volume of the bacterial suspension (approximately 1000 organisms) to the bottles and mix. At 5 ± 1 minutes, remove 1.0 ± 0.1 ml from each bottle and plate onto appropriate agar. This control is performed with multiple dilutions of the test organism. Evaluate plates after incubation at 35-37°C for *S. aureus* and 25-30°C for *E. aerogenes* for 48±4 hours. Recovery of colonies on the plate indicates the copper test surface has been adequately neutralized by the neutralizer broth. Recoveries from the test surface suspensions should be similar to the controls recovered from the control surface suspensions. The acceptance criterion for this study control is growth within ±1 $\log_{10}$ for the test and control suspensions.

Study Acceptance Criteria

Test Substance Performance Criteria

To be defined as a residual self-sanitizer, the test material must reduce the total number of organisms by at least 99.9% on the surface within the prescribed exposure time.

Control Acceptance Criteria

The study controls must perform according to the criteria detailed in the study controls description section.

Data Analysis

Calculations

The geometric mean of the number of organisms surviving on four control surfaces of four test surfaces was determined by the following equation:

Geometric Mean= $\frac{\text{Antilog of } (\text{Log}_{10}X1 + \text{Log}_{10}X2 + \text{Log}_{10}X3 + \text{Log10}X4}{4}$

Where X equals the number of organisms surviving per carrier

The percent reduction of organisms surviving on test surfaces over organisms surviving on parallel control surfaces was determined by the following equation:

%Reduction = $\frac{\text{Geometric mean of control survivors – geometric mean of test survivors}}{\text{Geometric mean control survivors}} \times 100$

Recovery Log_{10} Difference =

(Log_{10} Neutralization Confirmation Numbers Control) – (Log_{10} Neutralizer Control Growth)

Statistical Methods

Geometric Mean and Percent Reduction. Three digits were used when reporting Log, Average Log, Geometric Mean, and Percent Reduction values.

Label Claims Supported By the Protocol.

This surface kills greater than 99.9% of bacteria* for 24 hours.

*Includes list of tested organisms.

Required Label Language

The use of a Copper Alloy surface is a supplement to and not a substitute for standard infection control practices; user must continue to follow all current infection control practices, including those practices related to cleaning and disinfection of environmental surfaces. The Copper Alloy surface material has been shown to reduce microbial contamination, but does not necessarily prevent cross contamination.

Proper Care and Use of Antimicrobial Copper Alloys: The use of Antimicrobials Copper Alloys does not replace standard infection control procedures and good hygienic practices. Antimicrobial Copper Alloys surfaces must be cleaned and sanitized according to standard practice. Heath care facilities must maintain the product in accordance with infection control guidelines; users must continue to follow all current infection control practices, including those practices related to disinfection of environmental surfaces.

Cleaning Directions: Routine cleaning to remove dirt and filth is necessary for good sanitization and to assure the effective antibacterial performance of the Antimicrobial Copper Alloy surface. Cleaning agents typically used for traditional touching surfaces are permissible; the appropriate cleaning agent depends on the type of soiling and the measure of sanitization required.

This product must not be waxed, painted, lacquered, varnished, or otherwise coated.

The following are a listing of Conditions of Registration for Antimicrobial Copper Alloy registrations and associated labeling issues:

Condition 1

The registrant will prepare and implement an Antimicrobial Copper Alloy Stewardship plant to support the responsible use of antimicrobial copper products. The Plan will be submitted for EPA review and approval within two months after the registration date. If EPA determines at any time after 18 months following registration that the Plan is not being adequately or timely implemented or that implementation of the Plan is not effectively ensuring the proper sale, distribution, or use of antimicrobial copper alloy products, the registration may be automatically canceled by the Agency by order with opportunity for a hearing but only after notification to the Registrant and an opportunity to meet with the Director of the Office of Pesticide Programs.

The Plan will include, at a minimum, the following elements:

(a) Outreach to the infection control community, including,

(i) A goal of educating and reinforcing, for infection control professional and other product users, the proper use of Antimicrobial Copper Alloys.

(ii) Written (including electronic) communications directed to associations of infection control professionals, including at least the APIC, ASHES, and any other relevant organizations identified by CDA or EPA, and State Departments of Health.

(iii) Outreach communications will be sent within six months after the date of registration and within one year after the date of registration, and then annually thereafter on the anniversary of the date of registration unless more frequent outreach is deemed necessary.

(iv) The content of the outreach communications will include statements explaining the registered claims and applications of Antimicrobial Copper Alloys, as well as their proper use. The communications also will inform the recipients about (1) the Antimicrobial Copper Alloy Working Group, and invite participation; (2) other sources of information on Antimicrobial Copper Alloys, including the Stewardship Website. Additional content of outreach efforts will be developed as part of the Working Group activities.

(b) Development of Website

(i) The website will serve as a resource for conveying accurate information to the public about the efficacy and proper use of Antimicrobial Copper Alloys.

(ii) The website will include information on proper labeling and claims (including advertising); supporting science; applications; maintenance; and federal and state regulations and statutory requirements.

(iii) A question and answer of Frequently Asked Questions (FAQs) section will be incorporated to address common issues or questions raised with regard to Antimicrobial Copper Alloys.

(iv) The website also serves as a forum to correct any false or misleading third party statements or publications, including scientific papers, concerning antimicrobial Copper Alloys. Any such false or misleading third party statements of publications will be corrected promptly after the registrant becomes aware of such and the responsive website update will be incorporated promptly thereafter. The registrant will inform EPA within 30 calendar days following its receipt of any such false or misleading third party statements or publications and at the same time provide the Agency with a copy of such statement or publication along with a hard copy of the Website entry correcting such statement or publication.

(v) The registrant will arrange for and establish links between the website and the websites of appropriate infection control organization, including but not limited to APIC and ASHES.

(c) Establishment/Participation in Antimicrobial Copper alloy Working Group

(i) Invited participants will include alloy manufacturers, component makers, and representatives from the infection control community, including appropriate trade associations (e.g. APIC and ASHES) and State Departments of Health.

(ii) The Working Group will meet at least twice a year, either in person or by live video conferencing or teleconference.

(iii) The Working Group will serve as a forum to expand educational efforts, develop outreach communications, and address any questions or concerns from the public and infection control community.

(iv) The registrant will provide the Agency with minutes of any such meetings within 60 days of the end of any such meeting.

Condition 2

For at least the first 24 months after registration or until the Agency terminates this conditions, whichever is later, the registrant will submit to EPA sample advertising materials. Advertising materials will be representative of advertisements intended for use in the marketplace.

Point of Contact: Tajah Blackburn, Ph.D., (703)-308-0372