

Test Method for the Continuous Reduction of Bacterial Contamination on 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

Carrier Surfaces and Preparation: Cut 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 carriers and stainless steel squares as control carriers for this assay. Clean carriers with alcohol, rinse with 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. Test five (5) test carriers per material per organism per time point.

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. On the day of use, aspirate pellicle from the *Pseudomonas aeruginosa* culture.

Enterobacter aerogenes: From stock cultures, inoculate tubes of Tryptic Soy broth (TSB) 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 be used for the inocula for this test. Use the 24-54 hour cultures for the inocula in this test.

For each test organism, thoroughly mix the culture on a “vortex” mixer and allow to settle. Aspirate the upper two thirds of this suspension and use as the inoculum for testing. Add an organic soil load containing Triton X-100 (to aid in spreading the inoculum) to the test culture.

Addition of Organic Soil Load: Add 0.25 ml aliquot of serum + 0.05 ml Triton X-100 to 4.70 ml bacteria suspension to yield a 5% fetal bovine serum and 0.01% Triton X-100.

Antimicrobial Susceptibility Testing (if applicable): Antimicrobial susceptibility testing is required when utilizing a resistant organism. Before testing, verify the antimicrobial resistance pattern of Methicillin Resistant *Staphylococcus aureus* (MRSA). Subculture the organism onto a Tryptic Soy agar (TSA) plates (or 5% sheep blood agar plates (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.

Inoculation of Carriers: Use five (5) sets of five test carriers and five sets of three control carriers in this study. Inoculate all carrier sets at time zero. Inoculate each sterile test carrier at staggered intervals with 5 µl of culture using a calibrated pipette. Spread the inoculum on the surface of the carriers. Dry the carriers at ambient conditions for the duration of the exposure. The exposure period begins with the initial inoculation of the carrier.

Re-inoculate carrier sets not removed for quantitative recovery as follows at 3, 6, 9, 12, 15, 18, and 21 hours (as described above).

Neutralization and Subculture: Remove sets of test and control carriers for quantitative recovery at 2, 6, 12, 18 and 24 hours. These carriers were inoculated 1, 2, 4, 6 and 8 times, respectively. At each recovery time, transfer the carriers into individual containers with 20 ml of the Lethen broth (or appropriate neutralizer). Sonicate each neutralizer container for five minutes to suspend any survivors from the carriers, and rotate to mix. Within one hour after sonicating the carriers, prepare serial dilutions (10^{-1} - 10^{-4}) of the neutralized solution from each of the containers and plate in duplicate for survivors using standard spread plate technique and TSA plates (5% sheep blood agar plates).

Incubation and Observation: Incubate the plates at 35-37°C for 48±4 hours (Incubate *E. aerogenes* plates at 25-30°C for 48±4 hours) prior to observation for number of colonies. Visually enumerate the plates. Stain and/or biochemically assay to confirm or

deny the presence of the test organism. If possible, use subcultures containing 30-300 colonies for calculations.

Study Controls

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 for growth 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: Perform the neutralization confirmation control to demonstrate the neutralizer’s ability to inactivate the test carrier. The neutralization of the test carriers is confirmed by using sterile test carriers and neutralizing as in the test procedure. A 1.0 ml aliquot of a diluted suspension of the test organism yielding ≤ 100 CFU/ml of neutralizing subculture medium is transferred to the jar and mixed. A 1.0 ml aliquot of this mixed solution is plated in duplicate. A numbers control is performed utilizing sterile stainless steel control carriers. The resulting plates are incubated as in the test and enumerated. The acceptance criterion for this study is growth within $1 \log_{10}$ of the numbers control.

Inoculum Count: Serial dilutions of the cultures used as the inocula are prepared and plated. TSA plates (or 5% sheep blood agar plates) are used for all organisms. Incubate the resulting plates for 48 ± 4 hours at $35-37^{\circ}\text{C}$, and then count the colonies to determine the number of organisms per milliliter of inoculum present at the start of the test.

Carrier Quantitation Control: Use three (3) inoculated stainless steel control carriers to determine the number of test organisms per carrier at each quantitative recovery time point. Transfer the control carriers to neutralizing subculture media and sonicate as in the test. Prepare ten-fold serial dilutions of the neutralizing subculture medium and plate

1.0 ml of the appropriate dilutions in duplicate to yield countable numbers. Incubate and enumerate the plates as in the test. The acceptance criterion for this study control is a minimum geometric mean of 2.0×10^4 CFU/carrier.

Study Acceptance Criteria

Test Substance Performance Criteria

To support a claim for continuously reducing bacteria on a copper alloy surface, a minimum of a 90% reduction in numbers of the test organism(s) on the test surface compared to the number of test organism(s) on the control surface must be achieved at all recovery times over the 24 hour inoculation and exposure period.

Control Acceptance Criteria

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

Data Analysis

Calculations

Number of Organisms Surviving per Carrier

CFU/carrier= $\frac{\text{(average number colonies/plate @ dilution)} \times \text{(dilution factor)} \times \text{(volume neutralized solution)}}{\text{(volume plated)}}$

The carrier population was calculated and reported using data from the most appropriate dilution(s).

Geometric Mean Number of Organisms Surviving on Control Carrier

Geometric Mean= Antilog of $\frac{\text{Log}_{10}X_1 + \text{Log}_{10}X_2 + \text{Log}_{10}X_3}{3}$

Where X equals CFU/control carrier

Geometric Mean of Number of Organisms Surviving on Test Carrier

Geometric Mean= Antilog of $\frac{\text{Log}_{10}Y_1 + \text{Log}_{10}Y_2 + \text{Log}_{10}Y_3 + \text{Log}_{10}Y_4 + \text{Log}_{10}Y_5}{5}$

Where Y equals CFU/test carrier

Percent Reduction

% reduction = $[(a-b) / a] \times 100$

Where:

a= geometric mean of the number of organisms surviving on the inoculated control carriers

b= geometric mean of the number of organisms surviving on the test carriers.

Recovery Log₁₀ Difference = (Log₁₀ Numbers Control) – (Log₁₀ Test Results)

Used to calculate the neutralization confirmation control

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.

1. This surface continuously reduces bacterial* contamination.
2. This surface provides continuous/ongoing/persistent antimicrobial action even with repeated exposures.
3. This surface continuously kills over 90% of bacteria* after repeated exposures during a day.
4. This surface prevents the buildup of disease-causing bacteria*.
5. This surface delivers continuous, long-lasting antibacterial* activity.

* Includes list of tested organisms.

Claims are limited to indoor, hard, non-porous surfaces where cleaning practices are consistent

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. Health 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 plan 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 or 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.

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