



## **METHODS:**

A novel copper technology will be brought into contact with a known population of microorganisms for a specified period of time at a specific temperature. Sampling intervals for this experiment will be at 0, 1 and 3 hours post inoculum drying on surfaces. Surviving microorganisms will be enumerated.

### **Organism Preparation:**

Isolates to be evaluated will be retrieved from -80°C Freezer and plated on a Blood Agar Plate (BAP) manufactured by Remel Inc. Lenexa, KS. The plates will be incubated at 36°C in ambient air for 24 hours. Post incubation, 3 medium-sized colonies will be inoculated into 5 mL of Tryptic Soy Broth (TSB) manufactured by Remel Inc. Lenexa, KS. The tubes will incubate for 24 hours at 36°C.

### **Test Surface Preparation:**

2 inch diameter testing discs of copper product, LDPE control and autoclavable control disc (non-product related) will be used for testing. Discs will be cleaned with alcohol, rinsed with sterile deionized water and allowed to air dry. (EPA reference)

### **Inoculum Suspension Preparation and Determination of Microbial Population:**

200 µL of TSB + test organism solution will be added to 1 mL of 0.9% saline. The turbidity will be assessed by visual comparison to a 0.5 McFarland standard ( $\sim 1.5 \times 10^8$  CFU/mL) and the suspension adjusted as needed. Serial dilutions will be set up from the McFarland adjusted solutions. 1.0 mL of the suspension will be pipetted into 7.9 mL of 0.9% saline, 1.0 mL BSA and 0.1 mL Triton X-100 (EPA Reference) mixed by vortexing to achieve a  $\sim 10^7$  CFU/mL suspension of the organism to be evaluated.

All "test solutions" of the organisms will be used immediately. BAPs will be inoculated immediately before use to determine accuracy/purity of suspensions in triplicate. The plates will be incubated at 36°C for 24 hrs. The number of colonies will be counted and appropriate dilution factors will be applied.

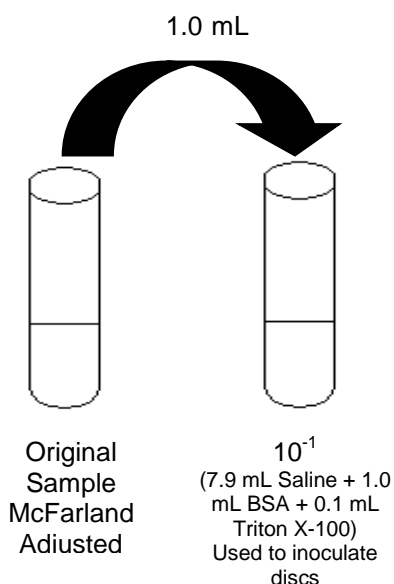
Testing discs made of autoclavable plastic, discs covered with LDPE and discs covered with copper film will be inoculated with the bacterial suspension. 100 µL of  $\sim 10^7$  CFU/mL inoculum will be pipetted into the center of each disk and spread over surface with a sterile inoculating loop. Each test disc will be allowed to air dry. The testing disks will be sampled at 0, 1 and 3 hours. Samples will be obtained by swabbing the area with a pre-moistened swab. (BBL CultureSwab with Liquid Stewart Medium manufactured by Becton, Dickinson and Company Sparks, MD)

### **Specimen Processing:**



Samples will be processed immediately. Each swab will be vortexed for 20 seconds in 1 mL of sterile 0.9% saline. An aliquot will be plated onto a BAP after serial dilution. Aliquots will be one plate with 0.5 mL, one plate with 0.1 mL of original (dilution factor of 10), and one plate with 0.1 mL of a 1:10 dilution (dilution factor of 100). An approximate count will be obtained by counting colonies on the incubated plates.

Diagram of how to make inoculum for testing material:



Results:

No MRSA was recovered from the copper testing disc at any time point.

