BP51.3

Test Method for the Efficacy of Copper Alloy Surfaces as a Sanitizer

Issue 2 – January 2015

Amendments

Amendments are entered here when the method is to be reissued, and there have been changes to the content. Type refers to the amendment type which can be categorized as additions (A), deletions (D), Minor corrections (MC), or whole section revision (RW). Reason for amendment is defined as what the root cause of the revision is (i.e. legislation, standard revision, update, error). Summary of amendment should detail what the amendment entails.

None

Health & Safety

Please note that a risk assessment is associated with this method, and should be referred to in conjunction with the method. The method should not be carried out by anybody who has not read and understood the risk assessment, and the associated actions.

In addition, throughout the method, Health and Safety advice is indicated as demonstrated below:

If the text is shown in red (see below), then it is mandatory.

Wear safety glasses.

It is compulsory to follow the advice given, and the method should not be carried out unless this is possible.

Further hazard information is detailed within the method.

1. SCOPE AND FIELD OF APPLICATION

1.1. The method described is suitable for verifying the antimicrobial properties of copper and other metal alloy surfaces.

1.2. The scope of the method is suitable for any copper or other metal alloy surface.

2. REFERENCES

2.1. Test procedures from the United States Environmental Protection Agency: -.

Test Method for Efficacy of Copper Alloy Surfaces as a Sanitizer (Undated).

Test Method for the Continuous Reduction of Bacterial Contamination on Copper Alloy Surfaces (Undated).

Test Method for Residual Self-Sanitizing Activity of Copper Alloy Surfaces (Undated).

2.2. American Society for testing and materials international (ASTM) WK25874 - New Practice for Establishing the Efficacy of Solid, Non-Food, Contact Surfaces, as Self-Sanitizing Surfaces (Undated).

3. PRINCIPLE OF METHOD

3.1. Copper and certain other metal alloys have a natural ability to kill a wide range of microorganisms, i.e. these have antimicrobial properties. The use of copper and certain other metal alloy surfaces in area such as health care is proving of great interest in protecting public health.

This protocol will demonstrate the efficacy of copper, its alloys and other metals/alloys as a sanitizer by measuring the surviving bacteria on the metal/alloy after 2 hours exposure.

4. HAZARDS

4.1. Before carrying out this procedure, the associated risk assessment and safe working procedures should be read and understood.

4.2. The risk assessment for this procedure is RA BP51.3, and is located on the intranet

4.3. ALcontrol Laboratories ‘Safe Working Procedures’ should be followed. These are located on the intranet.

4.4. Be extremely careful when handling any cultures containing MRSA. See safe working procedure 200.

5. QUALITY CONTROL

NOTE: -Before any analysis or quality controls can begin, the test organism must be sufficiently prepared (See 9.2 Preparation of Test Organism).

5.1 Purity Control

5.1.1 Streak the organism that is to be tested onto Oxoid Columbia blood agar.

5.1.2 Incubate at 36 ± 1°C for 24 ± 2 hours

5.1.3 Examine the plate to ensure that you have a pure culture and that colony morphology is consistent. If not, then re-subculture to achieve a pure culture.

5.2 Carrier Sterility Control

5.2.1 The carrier sterility control test should always be carried out alongside sample analysis (see 9.5).

5.2.2 Add a representative uninoculated test, positive control (pure copper) and negative control (304L stainless steel) carriers to 20ml Letheen Broth neutralizing solution (6.7) in sterile 60ml pots (8.11) and leave for a period of 5 minutes prior to beginning the sonication step. The sterility controls are to be sonicated with the inoculated test samples.

5.2.3 Sonicate for 5 minutes ± 15seconds (50-60Hz, ~1.5 amp 230/240 Volts)

5.2.4 Culture, in duplicate, 250µl of the Letheen Broth neutralizing solution (6.7)

5.2.5 Incubate at 36 ± 1ºC for 48 ± 4 hours

5.2.6 Examine the plates. The sterility is confirmed if no bacterial growth is observed, should there be any bacterial growth present, the run will need to be repeated.

5.3 Neutralisation Confirmation Control

5.3.1 The neutralisation confirmation control shall be run alongside sample analysis (see 9.5).

5.3.2 Place a sterile positive control (copper) carrier in 20ml of Letheen Broth neutralizing solution (6.7) in a 60 ml pot (8.11) and leave for a period of 5 minutes.

5.3.3 Place a sterile negative control (304L stainless steel numbers control) carrier in 20ml of Letheen Broth neutralizing solution (6.7) in a 60 ml pot (8.11) and leave for a period of 5 minutes.

5.3.4 Add 0.02ml (20ul) (the same amount that is added to the test carriers) of the test organism culture suspension which yields greater than 1.00×10^5 CFU/ml into the neutralising solution (not directly on to the carriers). Prepare serial dilutions of the inoculated neutralizing solutions. If either the Neutralization confirmation control or inoculum count is recorded to be lower than this value (10^5 CFU/ml) this control must be repeated with a new inoculum.

5.3.5 Sonicate both carriers for 5 minutes ± 15seconds (50-60Hz, ~1.5 amp 230/240 Volts)

5.3.6 Culture, in duplicate for both carriers, 250ul of the inoculated solution. Both cultures will need to be performed at dilutions from neat to 10^{-3}.

5.3.7 Incubate at 36 ± 1ºC for 48 ± 4 hours

5.3.8 Examine the plates; the acceptance is the neutralisation confirmation control is within one $\log_{10}$ of the inoculum count (See 5.4).

5.4 **Inoculum Count**

5.4.1 From the inoculated culture prepare serial dilutions from 10^{-1} to 10^{-6}. This is to be performed on the day of testing.

5.4.2 Culture, in duplicate, 100µl of each dilution onto Oxoid Columbia blood agar.

5.4.3 Incubate at 36 ± 1ºC for 48 ± 4 hours

5.4.4 Remove the plates from the incubator and manually count the number of colonies present at each dilution.

5.4.5 Records these results on the analysts' work record. This value is reported as CFU/ml.

5.5 **Carrier Quantitation Control.**

5.5.1 To be run alongside test samples. Inoculate five 304L stainless steel and five pure copper control carriers with 0.02ml (20µl) of the 48 ± 4 hour culture.

5.5.2 Transfer to 20ml of neutralizing subculture medium (Letheen Broth) in sterile 60ml pots (8.11).

5.5.3 Sonicate for 5 minutes

5.5.4 Prepare 10 fold dilutions from 10^{-1} to 10^{-4} of the neutralizer solution

5.5.5 Culture, in duplicate, 250µl of the neutralizing solution

5.5.6 Incubate at 36 ± 1ºC for 48 ± 4 hours

5.5.7 Examine the sub-culture medium. The acceptance criterion for the 304L stainless steel is that the mean recovery is greater than 2.00×10^6 CFU/ml. For the pure copper carriers the acceptance criterion is that there is no recovery of the target organism on any of the carriers.

6. REAGENTS

6.1. Nutrient Broth ~See Support Method SM086

6.2. Tryptic Soy Broth ~See Support Method SM087

6.3. Synthetic Broth ~See Support Method SM088

6.4. Organic soil load solution containing 1% Triton X-100 ~See Support Method SM089

6.5. Letheen Broth (Neutralising solution) ~See Support Method SM091

6.6. Columbia Blood Agar (Oxoid product code CM0331)

6.7. 1µg Oxacillin disc (Oxoid product code CT0159B)

6.8. 95% Ethanol

6.9. Sterile RO water ~See Support Method SM011

6.10. Acetone

7. CALIBRATION

7.1.1. All volumetric pipettes and pipetting aids used throughout the test procedure must be calibrated. See QMS/Pipettes for further reference. All incubators and weighing equipment used must be calibrated. See QMS records QMS/Thermocouples, QMS/Temperatures and QMS/Balances for reference.

8. EQUIPMENT

8.1. Petri Dish (Diameter 55 mm)

8.2. Sterile Filter Paper

8.3. Tweezers

8.4. Disposable 10µl loop

8.5. Vortex

8.6. Sterile Pasteur Pipettes

8.7. Calliper

8.8. Spreader

8.9. Sonicator (50-60Hz, 3amp 230/240 volts)

8.10. Sterile universals

8.11. 60 ml sterile pots suitable for receiving the 25 mm square metal coupons

8.12. Pipettes (100 µl, 250 µl and 1000 µl)

8.13. Incubators capable of maintaining 22 ºC, 30ºC and 36ºC (All with a tolerance of ±1^{o}C)

8.14. Calibrated timer

8.15. 100 ml beakers

9. ANALYTICAL PROCEDURE

9.1 Good Laboratory Practice

9.1.1 Before analysis can begin, all contaminated materials, including samples from the previous analysis must be removed from the laboratory in autoclave bags or other suitable containers. All work surfaces must be cleaned with a suitable disinfectant. The disinfectant must be used at an appropriate working dilution. Air monitoring (settle) plates should be placed at all work stations in the laboratory.

9.1.2 Clean laboratory coats, designated only for microbiology analysis should be worn throughout the procedure and stored separately from other areas of contamination. These must be changed weekly or if they become contaminated.

9.2 Preparation of Test Organism

9.2.1 From the stock culture transfer approximately 10 µl of cells into a 20 ml tube containing 10 ml of the appropriate broth:

synthetic Broth for both *staphylococcus aureus* NCTC 10788 and Meticillin Resistant Staphylococcus aureus (MRSA) NCTC 13143

9.2.2 Incubate the tube at 36 ± 1ºC for 24 ± 2 hours.

9.2.3 From this broth daily transfers must be carried out. The transfers must be carried out **for a minimum of three consecutive days,** before the culture broth can be used as the inoculum. To ensure sterility of technique a blank tube containing the same broth must also be transferred daily and cultured on the same day of testing.

9.2.4 To perform a daily transfer, use a sterile 10µl disposable loop, transfer 1 loopful of culture into 10 ml of the appropriate broth medium (see 9.2.1), vortex the tube and incubate at 36 ± 1ºC for 24 ± 2 hours.

9.2.5 Cultures older than 15 days from the initial subculture step should ***not*** be used. On the day of testing the inoculum that is 48 ± 4 hours old is to be used.

9.3 Antimicrobial Susceptibility Testing (applicable if using Methicillin Resistant *Staphylococcus aureus*)

9.3.1 Prior to the day of testing, subculture strains of *Staphylococcus aureus* and Methicillin Resistant *Staphylococcus aureus* onto Oxoid Columbia blood agar.

9.3.2 Incubate the plates at 36 ± 1ºC for 24 ± 2 hours.

9.3.3 From the culture plates prepare a suspension equal to 0.5 McFarland Standard in 0.85% m/V sterile saline.

9.3.4 Spread 100µl of the suspension on to Columbia blood agar.

9.3.5 Place an Oxacillin disc in the centre of the inoculated plates.

9.3.6 Invert and incubate at 36 ± 1ºC for a minimum of 24 hours

9.3.7 Remove the plate from the incubator and measure the zone of inhibition using a calibrated calliper.

9.3.8 Using the manufacturers guide measure the zone of inhibition, the test or organism should show resistance while the control should remain susceptible. If either of these two does not meet the requirements the susceptibility testing must be performed again.

9.4 **Cleaning of Carriers**

9.4.1 Prior to testing, clean the coupons by sonicating for 2 min in ~ 50 mL of acetone in a 100 ml beaker.

9.4.2 Immediately rinse the carriers in RO water and allow to dry.

9.4.3 After cleaning, sterilize the coupons using ethanol (6.8) immersion and Bunsen flaming, and then transfer to an individual lidded sterile Petri dish prior to inoculation to prevent contamination. Coupons remain in the Petri dish during each exposure test.

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9.5 **Sample Analysis – Start Test – Inoculation and Evaluation**

9.5.1 Using flame sterilised tweezers ensure that the surface specified to be tested is facing up.

9.5.2 Before inoculation of the carriers, the Organic soil load containing 1% m/V Triton X-100 (SM089 Para 5.2) is to be added to the inoculated broth. To perform this, add 0.30ml of this mixture to 4.7ml of the 48 hour old inoculated broth to make a 0.01% m/V Triton X-100 solution. It is from this solution that the overall inoculum count is calculated (see 5.4).

9.5.3 Inoculate each carrier, at staggered intervals of 15 - 30 seconds, with 0.02ml (20 µl) of the inoculated broth containing the organic soil load.

9.5.4 Spread the inoculum using the pipette tip, to within 1/8 inch of the edges of the carrier.

9.5.5 Dry the carriers at 22 ± 1^{o}C for 30 ± 5 minutes with the Petri dish lid ajar in an incubator at 22 ± 1^{o}C

9.5.6 Carrier 1 is inoculated at 00 h:00 min:00 sec and recovered at 02:30:00. The process will be repeated at staggered intervals of 30 seconds (carrier 2 will be inoculated at 00:00:30 and recovered at 02:30:30 etc.).

9.5.7 After the 30 ± 1 minute drying period, the exposure period begins. The carriers will be exposed for 120 ± 5 minutes. at 22 ± 1°C

9.5.8 Following the 120 min exposure period transfer the carriers in sequence to 20ml of Neutralizer solution (Letheen Broth) contained in a 60 ml pot (8.11) and leave for a period of 5 ± 1 minute prior to sonicating.

9.5.9 Sonicate each 60 ml pot containing the carrier and neutralizing solution for 5 ± 0.5 minutes to suspend any survivors from the carriers. If possible sonicate all the carriers at the same time.

9.5.10 Within 1 hour after sonication, prepare serial dilutions from 10^0 to 10^{-3} of the neutralized solution.

9.5.11 In duplicate, pipette 250µl of each dilution onto Oxoid Columbia blood agar.

9.5.12 Using a sterile spreader, spread the solution over the agar ensuring that all the moisture is absorbed into the agar.

9.5.13 Incubate the plates for 48 ± 4 hours at the appropriate temperature.

36 ± 1ºC for *S.* aureus, Methicillin Resistant *S. aureus*

9.6 Examination

9.6.1 Following incubation, samples are removed from the incubator and all colonies are manually counted (with the use of a magnifying lens, if required). Any plates with a count greater than 300 CFU/plate are to be considered too numerous to count and will be recorded as TNTC and will not be used in the calculations for result reporting.

9.7 Calculations

9.7.1 The following calculation should be applied in order to report the percentage reduction:

9.7.2 Number of Organisms Surviving per Carrier:

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

9.7.3 Geometric mean number of organisms surviving on control carrier:

$$\text{Geometric mean} = \frac{\text{Antilog of } (\text{Log}_{10}X_1 + \text{Log}_{10}X_2 + \text{Log}_{10}X_3 + \text{Log}_{10}X_4 + \text{Log}_{10}X_5)}{5}$$

Where X = equals CFU/control carrier

9.7.4 Geometric mean number of organisms surviving on test carrier:

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

Where Y = CFU/test carrier

9.7.5 Percent reduction:

% reduction = [(a-b)/a x 100]

Where a = geometric mean of the number or organisms surviving on the inoculated control carrier

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

9.7.6 Neutralisation Verification

Recovery Log_{10} Difference = (Log_{10} Numbers Control) – (Log_{10} Test Results).

10 **EXPRESSION OF RESULTS**

10.1 Results are entered directly into the Laboratory LIMS system