AMENDMENT TO GLP TEST PROTOCOL

ATS LABS

Amendment No.: 1

Effective Date: 6/13/13

Sponsor: Sherwin-Williams Diversified Brands
101 W. Prospect Ave.
Cleveland, OH 44115-1075

Test Facility: ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

Protocol Title: Test Method Three for Determining Efficacy

ATS Labs Protocol Number: SHE09021813.CRS.2

ATS Labs Project Number: A14703

Modifications to Protocol:

Per Sponsor's request, the protocol is amended to update the study title, product identity, and Sponsor address as follows:

Study Title: Original: "Test Method for Determining the Efficacy of Antimicrobial Coated Surfaces as Residual Self Sanitizers"
New: "Test Method Three for Determining Efficacy"

Product Identity: Original: "White Liquid SW2011-130: 79-87, 002XM2431"
New: "Sanitizer #1 Batches 2011-130:124, 2011-130:125, and 2011-130:126"

Previous Identity	New Identity
Untreated Lot 103-12 BTCNC	Untreated Lot 2011-130:109
Pos. Contl Lot G	2011-130:124
Pos. Contl Lot J	2011-130:125
Pos. Contl Lot M	2011-130:126

Original Sponsor Address: Sherwin Williams Breen Technical Center
601 Canal Road
Cleveland, OH 44113

New Sponsor address: Sherwin-Williams Diversified Brands
101 W. Prospect Ave.
Cleveland, OH 44115-1075

Changes to the protocol are acceptable as noted.

Matthew Sathe
Study Director

6-13-13
Date

EXACT COPY
INITIALS MS DATE 6-18-13

(For Laboratory Use Only)
ATS Labs Project # A14703
13

ATS LABS

PROTOCOL

Test Method for Determining the Efficacy of Antimicrobial Coated Surfaces as Residual Self Sanitizers

Test Organism:

Enterobacter aerogenes (ATCC 13048)

PROTOCOL NUMBER

SHE09021813.CRS.2

PREPARED FOR

Sherwin Williams Breen Technical Center
601 Canal Road
Cleveland, OH 44113

PERFORMING LABORATORY

ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

PREPARED BY

Matthew Sathe, B.S.
Senior Microbiologist

DATE

February 18, 2013

PROPRIETARY INFORMATION

THIS DOCUMENT IS THE PROPERTY OF AND CONTAINS PROPRIETARY INFORMATION OF ATS LABS. NEITHER THIS DOCUMENT, NOR INFORMATION CONTAINED HEREIN IS TO BE REPRODUCED OR DISCLOSED TO OTHERS, IN WHOLE OR IN PART, NOR USED FOR ANY PURPOSE OTHER THAN THE PERFORMANCE OF THIS WORK ON BEHALF OF THE SPONSOR, WITHOUT PRIOR WRITTEN PERMISSION OF ATS LABS.

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Test Method For Determining The Efficacy of Antimicrobial Coated Surfaces As Residual Self Sanitizers

SPONSOR: Sherwin Williams Breen Technical Center
601 Canal Road
Cleveland, OH 44113

TEST FACILITY: ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

PURPOSE

The purpose of this study is to evaluate the antimicrobial efficacy of coated surfaces as a residual self sanitizer against specific test organisms. The new (unworn) coated carriers used in this evaluation will represent the antimicrobial performance of a new or fresh paint coating. The challenged (worn) coated carriers used in this evaluation will have been exposed to a regime that simulates the worst-case situation of monthly cleaning of an interior wall paint coating over a 4 year period.

TEST SUBSTANCE CHARACTERIZATION

Test substance characterization as to content, stability, etc., (40 CFR, Part 160, Subpart F [160.105]) is the responsibility of the Sponsor. The test substance shall be characterized by the Sponsor prior to the experimental start date of this study. Pertinent information, which may affect the outcome of this study, shall be communicated in writing to the Study Director upon sample submission to ATS Labs.

SCHEDULING AND DISCLAIMER OF WARRANTY

Experimental start dates are generally scheduled on a first-come/first-serve basis once ATS Labs receives the Sponsor approved/completed protocol, signed fee schedule and corresponding test substance(s). Based on all required materials being received at this time, the proposed experimental start date is February 22, 2013. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of March 22, 2013. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at ATS Labs.

If a test must be repeated, or a portion of it, due to failure by ATS Labs to adhere to specified procedures, it will be repeated free of charge. If a test must be repeated, or a portion of it, due to failure of internal controls, it will be repeated free of charge. "Methods Development" fees shall be assessed, however, if the test substance and/or test system require modifications due to complexity and difficulty of testing.

If the Sponsor requests a repeat test, they will be charged for an additional test.

Neither the name of ATS Labs or any of its employees are to be used in advertising or other promotion without written consent from ATS Labs.

The Sponsor is responsible for any rejection of the final report by the regulating agencies concerning report format, pagination, etc. To prevent rejection, Sponsor should carefully review the ATS Labs final report and notify ATS Labs of any perceived deficiencies in these areas before submission of the report to the regulatory agency. ATS Labs will make reasonable changes deemed necessary by the Sponsor, without altering the technical data.

JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM

Regulating agencies require that a specific antimicrobial claim for a residual self sanitizer be supported by appropriate scientific data demonstrating the efficacy of the sanitizer against the claimed test organism. This is accomplished in the laboratory by treating the target test organism with the antimicrobial surface (sanitizer) under conditions which simulate as closely as possible, the actual conditions under which the product is designed to be used. In this instance, the coating is intended to be applied to wall surfaces. The test system to be used in this study will follow modifications of the EPA approved protocols entitled "Test Method for Residual Self-Sanitizing Activity of Copper Alloy Surfaces" and "Test Method for Efficacy of Copper Alloy Surfaces as a Sanitizer". It should be noted that these protocols are typically reviewed on a case by case basis for regulatory compliance by the chosen regulating agencies. It is the Sponsor's responsibility to ensure compliance.

TEST PRINCIPLE

A layer of organism cells applied on the antimicrobial coated surface will be exposed for a specified exposure time. After exposure, the carriers will be neutralized and assayed for survivors. In order to demonstrate effective sanitization on a worn surface representative of end of use life, one set of material per lot will be subjected to wear procedure designed to simulate at least four years of quarterly washing of the painted wall surface. Appropriate controls including: culture purity, organic soil load sterility, initial suspension (inoculum count), neutralizer sterility, carrier sterility, stainless steel control (viability control), neutralization confirmation, and carrier quantitation will be performed.

TEST METHOD

Chart 1

Test Organisms (ATCC #)	Growth Medium	Recovery Medium	Incubation Parameters
Enterobacter aerogenes (ATCC 13048)	Synthetic Broth	Tryptic Soy Agar + 5% Sheep's Blood	25-30°C, aerobic

The test organism to be used in this study was obtained from the American Type Culture Collection (ATCC), Manassas, VA.

Carriers

The Sponsor will supply a minimum of two panels of treated test and untreated control materials. Prior to carrier preparation, a minimum of one panel of Sponsor provided treated test material per lot and Sponsor supplied untreated control samples will be subjected to a simulated wear procedure (see Simulated Wear section). The remaining panels of Sponsor supplied treated test material and Sponsor supplied untreated control samples will be held at room temperature.

Prior to testing, the worn and unworn panels will be cut into carriers approximately 1" x 1" in size. The carriers will be placed in the biosafety hood and exposed to UV light for 15±2 minutes on each side in order to decontaminate the surface. After decontamination, each carrier will be placed into a sterile plastic Petri dish matted with two pieces of sterile filter paper.

Stainless steel squares (1" x 1"), used as the organism viability control material, will be provided by ATS Labs. The stainless steel carriers will be prepared by removing the adhesive protective backing, if applicable. Each carrier will be cleaned by dipping in ethyl alcohol and rinsing thoroughly in deionized water. After cleaning, the carriers will be decontaminated by autoclave sterilization or by dipping in absolute ethanol and allowing the carriers to dry aseptically in a bio-safety hood. After decontamination, each carrier will be placed into a sterile plastic Petri dish matted with two pieces of sterile filter paper.

Simulated Wear Procedure
Simulated wear cycling is initiated after the final coat of paint on each test panel (Sponsor supplied treated material or Sponsor supplied untreated control) has been allowed to cure for at least 24 hours. The wear procedure is intended to simulate cleaning of a vertical surface; therefore, relatively low volumes of cleaner are applied to the test panels at a rate where the cleaner would not drip down to the floor if applied to a vertical surface.

A cleaning solution is prepared using Best Yet Citrus Cleaner™ (or similar multipurpose detergent) at the manufacturer's recommended dilution ratio. A common sponge is soaked in the prepared cleaner solution prior to the initial simulated wear cycle. The appropriate test or control panel is placed on the Gardco Washability Tester tray. The sponge is removed from the solution, wrung of excess solution and is positioned under the 1000±10 gram weigh boat. The sponge/weigh boat combination is then attached to the Gardco Washability Tester on top of the secured panel. The Gardco Washability Tester cycle counter is set to two. The cycle is started and two cycles are performed, representing one complete wear cycle. One complete wear cycle is equivalent to 4 washes or one month of cleaning. After the wear cycle is completed, remove the weigh boat and sponge. Place the sponge in the cleaner/water solution. Remove the panel from the Gardco Washability Tester and place on a horizontal surface to dry at room temperature. The panel is dried for ≥10 minutes before the next wear cycle is performed. Visually verify that the panel is dry before starting the next wear cycle. This procedure is repeated until a total of 50 wear cycles (total of 200 washes) have been performed on each panel. After completion of 50 wear cycles, carriers are prepared as previously described (see Carriers section).

Preparation of Test Organism
From a stock slant, an initial tube (10 mL) of culture broth will be inoculated as indicated in chart 1. This culture is termed the "initial broth suspension." From this initial broth suspension, a minimum of three daily transfers using 1 loopful (10 µL) of culture into 10 mL of culture media will be performed on consecutive days prior to use in testing procedure. For each test organism, the appropriate growth medium will be subcultured using a daily transfer (more than 3, but less than 18 transfers) of the test organism. Use 48±4 hour cultures as the inocula for this test.

Thoroughly mix each 48±4 hour culture on a "vortex" mixer and allow culture to settle for ≥15 minutes. Aspirate the upper two thirds of this suspension and use this as the inoculum for testing. An organic soil load and/or Triton X-100 (to aid in spreading of the inoculum) may be added to the test culture per Sponsor's request or if hydrophobicity is a concern. (Example: 0.25 mL serum + 0.05 mL Triton X-100 + 4.70 mL bacteria suspension.)

Inoculation of Treated Test and Untreated Control Carriers
Two sets of treated carriers per lot, representing both worn and unworn surfaces will be evaluated on the day of test. The unworn surfaces will be used to assess the initial sanitizing action of the surface. The worn surfaces will be used to assess the sanitizing action of the surface at the end of its usable life.

Inoculate each Sponsor supplied treated test and untreated control (either Sponsor provided or provided by ATS Labs) at staggered intervals with 40 µL of culture using a calibrated pipettor. At a low angle of incidence, slowly expel the inoculum across the surface of the carrier moving back and forth across the surface of the carrier to facilitate spreading of the inoculum. Care will be taken to avoid spreading of the inoculum over the edges of the carrier. If the inoculum is spread over the edges of the carrier the carrier will be discarded and a new carrier will be inoculated. Replace the lids on each Petri dish. Carrier inoculation for each individual carrier is completed in ≤20 seconds. For consistency, the exposure time will begin when the inoculum is first applied to the carrier. Hold at the Sponsor indicated conditions for the exposure time.

Test System Recovery
Following the Sponsor specified exposure time, transfer the Sponsor provided treated carriers to 20 mL of the appropriate neutralizer, following the same staggered intervals used during inoculation. Sonicate each neutralized vessel for 5 minutes to suspend any survivors from the carriers and rotate to mix. Prepare serial dilutions of the neutralized carriers. Plate 1.0 mL aliquots of 10^0 dilution and 0.10 mL aliquots of the $10^0 - 10^{-3}$ dilutions in duplicate using standard spread plate technique and appropriate agar.

Incubation and Observation
Incubate the subculture plates for 48±4 hours at the temperature indicated in Chart 1 prior to observation and enumeration. Alternate incubation conditions may be needed for certain organisms. The incubation conditions may be modified to suit the test organism if needed. If necessary, subculture plates can be stored for up to 3 days at 2-8°C prior to enumeration. Following incubation (or incubation and storage), the plates will be visually enumerated. Representative subcultures showing growth may be subcultured, stained and/or biochemically assayed to confirm or rule out the presence of the test organism. If possible, subcultures containing 30-300 colonies will be used for calculations.

TEST CONTROLS

Purity Control
A "streak plate for isolation" will be performed on the organism culture(s). The plate will be incubated as in the test and examined 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 Load Sterility Control
The solution(s) used for the soil load will be cultured, incubated as in the test, and visually examined. The acceptance criterion for this study control is lack of growth.

Initial Suspension Control (Inoculum Count)
Prepare and plate serial dilutions of the cultures used as the inocula. Incubate the resulting plates as in the test and then count the colonies to determine the number of organisms per milliliter of inoculum present at the start of the test. This control is for informational purposes only and therefore has no acceptance criterion.

Neutralizer Sterility Control
A representative sample of uninoculated neutralizing subculture medium will be incubated as in the test and visually examined. The acceptance criterion for this study control is lack of growth.

Stainless Steel Control (Viability Control)
Inoculated stainless steel control carriers will be used to verify the test organism's ability to survive on inert surfaces under the chosen test conditions. Following the completion of the exposure time, control carriers will be transferred to neutralizing subculture media and sonicated as in the test. Ten-fold serial dilutions of the neutralizing subculture medium will be prepared and 1.0 mL or 0.1 mL aliquots of the appropriate dilutions will be spread plated in duplicate to yield countable numbers. The plates will be incubated as in the test and enumerated. The acceptance criterion for this study control is a minimum geometric mean of 2.0×10^4 CFU/carrier.

Neutralization Confirmation
The neutralization of the Sponsor supplied treated treated carriers will be confirmed by neutralizing the test carrier as in the test procedure. (If multiple concentrations of treated test carriers are being evaluated, only the lot with the highest level of active ingredient needs to be evaluated in this control.) Transfer a 1.0 mL aliquot of a diluted suspension of the test organism to target approximately 100 CFU/mL of neutralized solution to the vessel. Sonicate each neutralized vessel containing the test carrier and test organism suspension for 5 minutes. Plate 1.0 mL of this mixed solution in duplicate.

Determine the concentration of inoculum used in the neutralization confirmation assay as a numbers control. Perform the numbers control utilizing untreated neutralizer. Transfer a 1.0 mL aliquot of a diluted suspension of the test organism to target approximately 100 CFU/mL of neutralized solution to the vessel. Mix the numbers control vessel (do not sonicate) and plate 1.0 mL of the mixed solution in duplicate. Incubate the resulting plates as in the test and enumerate. The acceptance criterion for this study control is growth within 1 $\log_{10}$ of the numbers control.

Note: If swarming is a concern, 0.1 mL aliquots may be spread plated. In this case, approximately 1000 CFU/mL will be targeted when adding organism to the neutralized solution.

Carrier Quantitation Control
Five inoculated Sponsor supplied untreated control carriers per wear group representing worn and unworn carriers will be used to determine the number of test organisms per carrier at each quantitative recovery time point. The Sponsor supplied untreated control carrier will be transferred to neutralizing subculture media and sonicated as in the test. Ten-fold serial dilutions of the neutralizing subculture medium will be prepared and 1.0 mL or 0.1 mL of the appropriate dilutions will be plated in duplicate to yield countable numbers. The plates will be incubated as in the test and enumerated. The results of this control will be used to calculate the reduction of test organism achieved by the Sponsor supplied test carriers. In the calculation the survivors on the worn carriers will be compared to the survivors on the worn control and the survivors on the unworn carriers will be compared to the survivors on the unworn control. There is no acceptance criterion for this study control.

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria
U.S. EPA efficacy data requirements state that a 99.9% reduction in numbers of the test organism(s) must be obtained as compared to the carrier quantitation control for both the unworn carriers (initial sanitizer) and the worn carriers (final sanitizer) for the product to be an effective residual self sanitizer.

Control Acceptance Criteria
The study controls must perform according to the criteria detailed in the study controls description section. If any of the control acceptance criteria are not met, the test may be repeated under the current protocol number.

Timing Schematic for Antimicrobial Treated Surfaces as Residual Self Sanitizers

(This timing schematic is an example and assumes that one lab technician is testing one lot of product using a staggered interval of 30 seconds. Additional technicians may be utilized for extra lots and/or controls. Alternate staggered intervals may be utilized and documented in the raw data.)

Lot and Carrier #	Inoculation time	Subculture Time*
Lot A Carrier 1	0:00:00	2:00:00
Lot A Carrier 2	0:00:30	2:00:30
Lot A Carrier 3	0:01:00	2:01:00
Lot A Carrier 4	0:01:30	2:01:30
Lot A Carrier 5	0:02:00	2:02:00
Lot B Carrier 1	0:02:30	2:02:30
Lot B Carrier 2	0:03:00	2:03:00
Lot B Carrier 3	0:03:30	2:03:30
Lot B Carrier 4	0:04:00	2:04:00
Lot B Carrier 5	0:04:30	2:04:30
Lot C Carrier 1	0:05:00	2:05:00
Lot C Carrier 2	0:05:30	2:05:30
Lot C Carrier 3	0:06:00	2:06:00
Lot C Carrier 4	0:06:30	2:06:30
Lot C Carrier 5	0:07:00	2:07:00
Untreated Control Carrier 1	0:07:30	2:07:30
Untreated Control Carrier 2	0:08:00	2:08:00
Untreated Control Carrier 3	0:08:30	2:08:30
Untreated Control Carrier 4	0:09:00	2:09:00
Untreated Control Carrier 5	0:09:30	2:09:30
Stainless Steel Control Carrier 1	0:10:00	2:10:00
Stainless Steel Control Carrier 2	0:10:30	2:10:30
Stainless Steel Control Carrier 3	0:11:00	2:11:00

* The subcultures times listed represent the use of a 2 hour exposure time.

PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM

ATS Labs maintains Standard Operating Procedures (SOPs) relative to efficacy testing studies. Efficacy testing is performed in strict adherence to these SOPs which have been constructed to cover all aspects of the work including, but not limited to, receipt, log-in, and tracking of biological reagents including test organism strains for purposes of identification, receipt and use of chemical reagents. These procedures are designed to document each step of efficacy testing studies. Appropriate references to medium, batch number, etc. are documented in the raw data collected during the course of each study.

Additionally, each efficacy test is assigned a unique Project Number when the protocol for the study is initiated by the Study Director. This number is used for identification of the test subcultures, etc. during the course of the test. Test subcultures are also labeled with reference to the test organism, experimental start date, and test product. Microscopic and/or macroscopic evaluations of positive subcultures are performed in order to confirm the identity of the test organism. These measures are designed to document the identity of the test system.

METHOD FOR CONTROL OF BIAS: N/A

REPORT

The report will include, but not be limited to, identification of the sample, date received, initiation and completion dates, identification of the organism strains used, description of media and reagents, description of the methods employed, tabulated results and conclusion as it relates to the purpose of the test, and all other items required by 40 CFR Part 160.185.

PROTOCOL CHANGES

If it becomes necessary to make changes in the approved protocol, the revision and reasons for changes will be documented, reported to the Sponsor and will become a part of the permanent file for that study. Similarly, the Sponsor will be notified as soon as possible whenever an event occurs that may have an effect on the validity of the study.

Standard operating procedures used in this study will be the correct effective revision at the time of the work. Any minor changes to SOPs (for this study) or methods used will be documented in the raw data and approved by the Study Director.

TEST SUBSTANCE RETENTION

It is the responsibility of the Sponsor to retain a sample of the test substance. All unused test substance will be discarded following study completion unless otherwise indicated by Sponsor.

RECORD RETENTION

Study Specific Documents

All of the original raw data developed exclusively for this study shall be archived at ATS Labs. These original data include, but are not limited to, the following:

1. All handwritten raw data for control and test substances including, but not limited to, notebooks, data forms and calculations.
2. Any protocol amendments/deviation notifications.
3. All measured data used in formulating the final report.
4. Memoranda, specifications, and other study specific correspondence relating to interpretation and evaluation of data, other than those documents contained in the final study report.
5. Original signed protocol.
6. Certified copy of final study report.
7. Study specific SOP deviations made during the study.

Facility Specific Documents

The following records shall also be archived at ATS Labs. These documents include, but are not limited to, the following:

1. SOPs which pertain to the study conducted.
2. Non study specific SOP deviations made during the course of this study which may affect the results obtained during this study.
3. Methods which were used or referenced in the study conducted.
4. QA reports for each QA inspection with comments.
5. Facility Records: Temperature Logs (ambient, incubator, etc.), Instrument Logs, Calibration and Maintenance Records.
6. Current curriculum vitae, training records, and job descriptions for all personnel involved in the study.

REFERENCES

1. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, Efficacy Data Requirements Sanitizer Test (for inanimate, non-food contact surfaces), DIS/TSS-10, January 7, 1982.
2. U.S. Environmental Protection Agency Pesticide Assessment Guidelines, Subdivision G, Section 91-2; Item j Sanitizers (for non-food contact surfaces).
3. ASTM Test Method, Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces, E1153.
4. Association of Official Analytical Chemists (AOAC) Official Method 961.02 Germicidal Spray Products as Disinfectants. In Official Methods of Analysis of the AOAC, 2009 Edition.
5. EPA Protocol: "Test Method for Efficacy of Copper Alloy Surfaces as a Sanitizer." Website link: *http://www.epa.gov/oppad001/pdf_files/test_method_copper_alloy_surfaces.pdf*
6. EPA Protocol: "Test Method for Residual Self-Sanitizing Activity of Copper Alloy Surfaces." Website link: *http://www.epa.gov/oppad001/pdf_files/test_meth_residual_surfaces.pdf*

CALCULATIONS

Initial Suspension

$$\text{CFU/mL} = \frac{\text{(average number of colonies/plate@dilution) x (dilution factor)}}{\text{(volume plated in mL)}}$$

Number of Organisms Surviving per Carrier

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

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

Geometric Mean of Number of Organisms Surviving on the Treated Test or Untreated Control Carriers

$$\text{Geometric Mean} = \text{Antilog of } \frac{\text{Log}_{10}X_1 + \text{Log}_{10}X_2 + \ldots \text{Log}_{10}X_N}{N}$$

where X equals CFU/carrier and N equals the number of replicates tested

Percent Reduction Achieved by the Test Carriers

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

where:

a = geometric mean of the number of organisms surviving on the Sponsor supplied untreated control carriers
b = geometric mean of the number of organisms surviving on the Sponsor provided treated test carriers

Log_{10} Reduction Achieved by the Test Carriers

(Average Log_{10} Sponsor supplied untreated control carriers) – (Average Log_{10} Sponsor provided treated test carriers)

Neutralization Control Recovery Log_{10} Difference = (Log_{10} Numbers Control) – (Log_{10} Neutralization Results)
Used for the neutralization confirmation control only.

Statistical Analysis
None used.

Three digits will be used when calculating Log, Average Log, Geometric Mean and Percent Reduction values.

STUDY INFORMATION

(All sections must be completed prior to submitting protocol)

Test Substance (Name and Batch Number - exactly as it should appear on final report):
SEE ATTACHMENT

Carriers are: ☐ Pre-cut to _____ (approximate size) or ☑ Shall be cut to 1" x 1" (approximate size)
NOTE: Recommended carrier size is 1" x 1" to accommodate neutralization

Sponsor Supplied Untreated Lot (charged as an additional lot)
☑ Untreated Lot 103-12 BTC NC
☐ Not Applicable

☑ Perform calculations based on untreated lot
☐ Perform calculations based on stainless steel control

Expiration Date: N/A

Test Substance Active Concentration (upon submission to ATS Labs): Barquat MB-80, n-Alkyl (approximately C14-50%, C12-40%, C16-10%) Dimethyl Benzyl-Ammonium Chloride.

Product Description:
☐ Copper ☐ Silver ☑ Other SW2011-130:79-87, 002X M2431

Sample Preparation:
☐ Pre-cleaning needed: _____
☑ No pre-cleaning needed

Sample Decontamination:
☑ UV sterilization
☐ Dipping in ethanol and drying aseptically
☐ Autoclave sterilization (dry cycle)
☐ No sterilization is necessary

Neutralization/Subculture Broth:
☐ _____
☑ ATS Labs' Discretion. By checking, the Sponsor authorizes ATS Labs, at their discretion, to perform neutralization confirmation assays at the Sponsor's expense prior to testing to determine the most appropriate neutralizer. (See Fee Schedule).

Storage Conditions:
☑ Room Temperature
☐ 2-8°C
☐ Other: _____

Hazards:
☑ None known: Use Standard Precautions
☐ Material Safety Data Sheet, Attached for each product
☐ As Follows: _____

Test Organism(s):
☑ *Enterobacter aerogenes* (ATCC 13048)

Carrier Number: 5 treated, 5 untreated, and 3 stainless steel control carriers

Detergent to be Used for Simulated Washing: Best Yet All Purpose Neutral Cleaner

Detergent Dilution Instructions: 2 oz/gal. (1:64)

Number of wear cycles: 50 **Exposure Temperature for Wear Cycles:** Ambient

Number of wear cycle passes: 2 (1 cycle will pass over the carrier twice – over and back.)

Exposure Time: 120 Minutes (Time period following final carrier inoculation, prior to subculture)

Exposure Temperature (for Sanitizer Test): 25±2°C and 60±2% RH

Organic Soil Load:
☑ Minimum 5% Organic Soil Load (Fetal Bovine Serum) and a final concentration of 0.01% Triton X-100
☐ Minimum 5% Organic Soil Load (Fetal Bovine Serum)
☐ Final concentration of 0.01% Triton X-100 only, to aid in spreading of the test organism
☐ No Organic Soil Load Required
☐ Other _____

TEST SUBSTANCE SHIPMENT STATUS

☐ Has been used in one or more previous studies at ATS Labs .
☐ Has been shipped to ATS Labs (but has not been used in a previous study).
Date shipped to ATS Labs:_____ Sent via *overnight* delivery? ☐ Yes ☐ No
☑ Will be shipped to ATS Labs.
Date of expected receipt at ATS Labs: 2/25/2013
☐ Sender (if other than Sponsor):_____

COMPLIANCE

Study to be performed under EPA Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures.
☑ Yes
☐ No (Non-GLP Study)

PROTOCOL MODIFICATIONS

☐ Approved without modification
☑ Approved with modification
A carrier sterility control will also be performed by; Representative uninoculated Sponsor provided treated test and untreated control (either Sponsor provided or provided by ATS Labs) carriers from both the worn and unworn samples will be added to the neutralizing subculture medium. A 1.0 mL sample will be plated onto an appropriate agar and will be incubated and examined. This control is for informational purposes only and therefore, has no acceptance criterion.

PROTOCOL ATTACHMENTS

Supplemental Information Form Attached - ☑ Yes ☐ No

APPROVAL SIGNATURES

SPONSOR:

NAME: Mrs. Stephanie Magnani TITLE: Chemist II
SIGNATURE: Stephanie Magnani DATE: 2/22/2013
PHONE: (216) 566 - 3235 FAX: (216) 515 - 5824 EMAIL: stephanie.magnani@sherwin.com

For confidentiality purposes, study information will be released only to the sponsor/representative signing the protocol (above) unless other individuals are specifically authorized in writing to receive study information.

Other individuals authorized to receive information regarding this study: ☐ See Attached

ATS Labs:

NAME: Matthew Sathe
Study Director
SIGNATURE Matthew Sathe DATE: 2-25-13
Study Director

RESID RED	PRODUCT ID :	White Liquid SW2011-130: 79-87, 002XM2431
PROTOCOL	SAMPLE	LABEL
SHE09021813.CRS.2	Untreated Lot	103-12 BTCNC
SHE09021813.CRS.2	Positive 1	Pos. Contl Lot G
SHE09021813.CRS.2	Positive 2	Pos. Contl Lot J
SHE09021813.CRS.2	Positive 3	Pos. Contl Lot M

Per Sponsor, All lots ≥ 60 days old MS 2-25-13

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